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A plausible radiobiological model of cardiovascular disease at low or fractionated doses

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Abstract

Atherosclerosis is the main cause of coronary heart disease and stroke, the two major causes of death in developed society. There is emerging evidence of excess risk of cardiovascular disease at low radiation doses in various occupationally-exposed groups receiving small daily radiation doses. Assuming that they are causal, the mechanisms for effects of chronic fractionated radiation exposures on cardiovascular disease are unclear. We outline a spatial reaction-diffusion model for atherosclerosis, and perform stability analysis, based wherever possible on human data. We show that a predicted consequence of multiple small radiation doses is to cause mean chemo-attractant (MCP-1) concentration to increase linearly with cumulative dose. The main driver for the increase in MCP-1 is monocyte death, and consequent reduction in MCP-1 degradation. The radiation-induced risks predicted by the model are quantitatively consistent with those observed in a number of occupationally-exposed groups. The changes in equilibrium MCP-1 concentrations with low density lipoprotein cholesterol concentration are also consistent with experimental and epidemiologic data. This proposed mechanism would be experimentally testable. If true, it also has substantive implications for radiological protection, which at present does not take cardiovascular disease into account. The Japanese A-bomb survivor data implies that cardiovascular disease and cancer mortality contribute similarly to radiogenic risk. The major uncertainty in assessing the low-dose risk of cardiovascular disease is the shape of the dose response relationship, which is unclear in the Japanese data. The analysis of the present paper suggests that linear extrapolation would be appropriate for this endpoint.

Quantification of γ-H2AX foci in Jurkat cells following irradiation with α-particles and γ-rays

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Abstract

Objectives: Phosphorylation of histone H2AX occurs at sites flanking DNA double-strand breaks (DSBs) and can provide a measure of the number of DSBs within a cell. We investigated whether the mean intensity and the mean number of radiation-induced γ-H2AX foci per cell vary as a function of radiation quality and dose.

Materials and methods: Jurkat cells were irradiated with different doses of either low linear energy transfer (LET) ^{137}Cs γ-rays or high LET ^{241}Am α-particles. The γ-H2AX foci were detected using immunocytochemistry and quantified by measuring the mean signal intensity of γ-H2AX foci per cell using flow cytometry and by counting the number of γ-H2AX foci with a fluorescence microscope.

Results: The mean numbers of γ-H2AX foci per cell increase with dose and they are fairly identical at 1 Gy for both investigated radiation qualities. The mean intensity of γ-H2AX foci per cell after α-irradiation is significantly increased when compared to γ-irradiation at the same radiation dose. A more advanced flow cytometry analysis reveals that the percentage of γ-H2AX-negative cells as well as the distribution of single γ-H2AX fluorescence signals depend on the radiation quality.

Conclusion: The mean signal intensity, but not the mean number, of γ-H2AX foci per cell depends on the LET in Jurkat cells. When comparing the induction of γ-H2AX foci in Jurkat cells after exposure to γ-rays and α-particles, the analysis by flow cytometry is more appropriate than the microscopical quantification by eye, considering the LET-dependence of foci-size and -intensity, the cell cycle dependence of γ-H2AX frequencies and the distributions of single γ-H2AX fluorescence signals.

Radiation-induced transgenerational instability and genetic risk

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Abstract

Mutation induction in the directly exposed cells is currently regarded as the main component of the genetic risk of ionizing radiation. However, recent data on the delayed effects of exposure to ionizing radiation represent a new challenge to the existing paradigm. The results of numerous studies show that ionizing radiation can not only induce mutations in the directly exposed cells, but can also lead to delayed effects, with new mutations arising many cell divisions after irradiation.

Apart from the studies on mutation rates in somatic cells, considerable progress has been made in the analysis of radiation-induced instability in the mammalian germline, where the effects of radiation exposure were investigated among the offspring of irradiated parents. Our results show that mutation rates at tandem repeat DNA loci and protein-coding genes are substantially elevated in the germline and somatic tissues of non-exposed offspring of irradiated male mice. According to our data, the transgenerational destabilization can be attributed to the presence of a subset of endogenous DNA lesions. We have recently shown that paternal treatment by the alkylating agent ethylnitrosourea also results in the transgenerational effects, thus implying that this phenomenon is most probably triggered by a stress-like response to a generalized DNA damage.

Our data imply that instability detected in the non-exposed offspring is caused by some DNA-dependent signal transmitted from the irradiated father and implicate an epigenetic mechanism for the transgenerational instability. The potential implication of these results for the estimates of genetic risks for humans will be discussed.

Low doses of irradiation on nervous cells impairs neurite outgrowth and causes neuronal degeneration

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Abstract
Brain damage induced by prenatal irradiation is a major concern and an important issue in radiation protection. The embryonic stage of brain development is known to be highly sensitive to radiations, since most of the concerned cells are still mitotic. Thus, an acute irradiation during pregnancy could selectively damage cells, which at that particular time of exposure, are proliferating or migrating. Data collected from the epidemiological studies on the children that were irradiated in utero during Hiroshima and Nagasaki's A-Bombing, showed an increase of some mental and/or cognitive disorders. Depending on the received radiation dose, the severity of the damage occurs at crucial embryonic stages for neuronal survival, proliferation, migration, and/or neural network formation. In this study, we investigate the main cellular and molecular mechanisms involved in radiation-induced neurite outgrowth and apoptosis. Various time points and irradiation doses were compared at different neuronal maturation stages. Image analysis of immuno stained control compared to irradiated neuron cells with low and moderates doses (0.1, 0.2 and 0.5 Gy), showed a clear negative radiation effect on neurite outgrowth depending on the maturation stage of neurons. In particular, the main radiation-induced morphological effects were: shortened neurite length, decreased number of neurite per neuron and reduced synapse branching. These effects could lead to a defect in neural network formation and consequently to possible cognitive disorders at the adult age. The molecular and biochemical events will be further analysed using transcriptomic, proteomic and metabolomic technologies in order to identify the compromised molecular and signaling pathways in neuron cells after exposure to moderate and low doses of X-rays. Molecular strategies (overexpression or silencing) would help to validate the identified pathways.

The European Radiobiological Archives: online access to data from radiobiological experiments

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Abstract

Today's research is providing us more and more with the opportunity to quantify radiation risks at the individual level. New approaches allow the re-analysis of old data using new techniques. Thus, the retrospective analysis of earlier studies is an important resource for modelling and evaluating risk parameters. The European Radiobiology Archives (ERA), together with corresponding Japanese and American databases, hold data from nearly all experimental animal radiation studies carried out between the 1950s and the 1990s, involving more than 400,000 animals. These experiments were performed on different species with the aim of understanding the effects of irradiation. This mass of information has led to the requirement for a computerized database to store, organize and index this data. The previously existing ERA archive has now been transferred to a web-based database to maximise its usefulness to the scientific community and bring data coding and structure of this legacy database into congruence with currently accepted semantic standards for anatomy and pathology. The accuracy of the primary data input was assessed and improved. The original rodent pathology nomenclature was recoded to Mouse Pathology (MPATH) and Mouse Anatomy (MA) ontology terms. A pathology panel sampled histopathological slide material and compared the original diagnoses with currently accepted diagnostic criteria. The mean non-systematic error rate was low with only 1.7%. Detected errors were corrected. The majority of the original pathology terms have been translated into a combination of MPATH and MA ontology terms. The database is accessible online at *http://era.bfs.de*. It has the potential of becoming a world-wide radiobiological research tool for numerous applications, such as the re-analysis of existing data and using the database as an information resource for planning future animal studies.

A large-scale gene expression database on the effect of low doses of radiation: a systems biology approach

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Abstract

Current radiation protection regulatory limits are based on the linear non-threshold theory using epidemiological data from atomic bombing survivors. Recent studies sparked debate on the validity of the theory, especially at low doses. Recent advances in molecular biology have shown that different genes and pathways might be triggered by low doses and by high doses of ionising radiation. Identifying the genes and pathways involved in the response to low doses is therefore critical for a better prediction of a possible clinical outcome following radiation exposure.

The increasing interest in the effect of low doses of radiation coincides with the breakthrough in the development of new high-throughput technologies in molecular biology. The microarray technology allows simultaneous measurement of the expression of thousands of genes. As gene expression is the predominant level controlling cell functioning, this technology has become a powerful tool to measure the impact of low doses of radiation.

A major challenge is how to integrate all data resulting from a wide range of experiments and use them to improve our global understanding of the general molecular response of a cell after radiation exposure to low doses. Therefore, we organized into one microarray compendium all radiation expression studies performed within our laboratory. In total, our compendium comprises 233 arrays representing 89 different biological conditions. Applying a systems biology approach on these data will shed light on the relationship between gene expression and different parameters like dose, tissue and organism. Additionally, identification of pathways triggered upon low doses of radiation might open new opportunities towards biodosimetry and appropriate regulation regarding medical exposure and intrinsic radiation sensitivity.

Introduction

The last decade, molecular biology has been revolutionized by the development of many high-throughput technologies *e.g.* in DNA sequencing, gene expression analysis, electrophoresis, and mass spectrometry. A major advancement herein was the invention

of microarrays allowing the transcriptional analysis of thousands of genes in a single experiment. This technological progress now allows analyzing simultaneously the expression of genes at a genome-wide scale. The usage of this technology is certainly not new, as different research groups – including ours - already used this technology to study the influence of radiation on different tissues like blood, brain, liver, kidney, thyroid and others (Amundson et al. 2000, Franco et al. 2005, Paul and Amundson 2008, Pawlik et al. 2009, Tachiiri et al. 2006, Taki et al. 2009, Zhao et al. 2006).

However, to our knowledge it is the first time that a systematic approach is developed to analyse a large and diverse compendium of microarray data using a systems biology approach. Over the last three years, a large number of microarray experiments using Affymetrix technology were performed within the radiobiology group of the Belgian Nuclear Research Center (SCK•CEN), exposing different tissues from human and mouse origins to various doses of ionising radiation (ranging from 0.025 Gy to 8 Gy of X-rays). This resulted in a total of 233 arrays, representing 89 different conditions. The aim of this study is to combine the knowledge present in all these data sets and explore the influence of different parameters (species, tissue, dose) on the genome-wide transcription profiles. In a first stage, a high-level analysis of the data is performed, applying traditional data mining techniques, and exploring the clustering of the different microarray data in the light of the here above mentioned parameters. In a second stage, the data are analysed at the functional level by classifying them based on their annotation, and mapping the gene expression data on existing pathways in order to identify new pathways triggered upon ionizing radiation.

Material and methods

Data sets

All samples from various organisms (mice or men) and various tissues (blood, thyroid, brain and embryo) were irradiated using X-rays ranging from 0.025 Gy to 8 Gy with a Pantak HF420 RX machine operating at 250 keV, 1.5 or 15 mA, 1 mm Cu filtration and a dose rate of 0.375 Gy/min. Dosimetry was performed on a regular basis with a 0.6 cm^3 ionisation chamber (NE 2571), which was connected to a dosemeter (Farmer dosemeter 2570). The chamber was placed in parallel to the irradiated mouse cages. Dose homogeneity was evaluated as being <1.5%. From all samples, RNA was extracted and converted to biotin-labelled cDNA, which was hybridized to GeneChip® Human Gene 1.0 ST Array and Mouse Gene 1.0 ST Array (Affymetrix, Santa Clara, USA) for human and mouse samples respectively (Table 1).

Table 1. Overview of microarray data.

Species	Tissue	Doses (Gy)	# Arrays	# Conditions
Human	Blood	0.1, 1	60	30
Human	Thyroid	0.0625, 0.5, 4	12	4
Mouse	Thyroid	0.025, 0.05, 0.0625, 0.1, 0.5, 1, 4, 8	86	30
Mouse	Embryonic brain	0.1, 0.2, 0.5, 1	37	10
Mouse	Whole embryo (early gastrula)	0.2, 0.4	38	15
		Total	**233**	**89**

Data preprocessing

Raw Affymetrix data were preprocessed using the "Affy" package (version 1.22.0) in BioConductor (version 2.4) as follows: 1) background correction based on the Robust Multichip Average (RMA) convolution model (Irizarry et al. 2003), 2) quantile normalization to make expression values from different arrays more comparable (Bolstad et al. 2003), 3) summarization of multiple probe intensities for each probeset to one expression value per gene using RMA (Irizarry et al. 2003). To test for differential expression between the different irradiated conditions and the reference conditions (no irradiation) we used the Bayesian adjusted *t*-statistics as implemented in the "LIMMA" package (version 2.18.0) (Smyth 2004). P-values were corrected for multiple testing using the Benjamini and Hochberg's method to control the false discovery rate (Benjamini and Hochberg 1995).

Statistical and data mining applications

All statistical and data mining analyses like principal component analysis, correlation analysis and hierarchical clustering were performed with "Stats" package within the statistical software R (version 2.9.0).

Differentially expressed genes and overlapping gene sets

In order to identify differentially expressed genes, a fixed cutoff on the fold change of 1.5 was used. Statistical significance of the overlap of differentially expressed genes between different experiments was calculated using the hypergeometric distribution. This distribution allows calculating the overlap of genes between two conditions, taking into account the number of differentially expressed genes in both conditions separately.

Functional enrichment

A gene set enrichment analysis (GSEA) determines to which extent a specific gene set is associated with a particular pathway or biological function. In order to calculate the statistically significant enrichment of a set of differentially expressed genes towards a specific pathway or biological process, the KEGG (Kanehisa et al. 2010) and Gene Ontology (Ashburner et al. 2000) databases were used respectively. This analysis was performed using the "GOstats" package (version 2.10.0) (Falcon and Gentleman 2007) of BioConductor (version 2.5)

Results

Data preprocessing

All data were preprocessed as explained in the "Material and methods" section. The relative expression of genes was measured by comparing the gene expression in irradiated tissues with gene expression in control samples of the same tissue. This prevented that we would only find tissue-specific genes when comparing different experiments, which would not be related to irradiation but rather to the intrinsic function of the cells in specific tissues. By recalculating the expression levels to fold changes (*i.e.* the ratio of the expression level in irradiated tissues over the expression level in control non-irradiated samples), the number of conditions was reduced from 89

to 63 experiments. By applying a variation filter, all genes showing minimal variation across different experiments were excluded.

Classification of radiation expression profiles

Using Principal Component Analysis (PCA), a first approach focused on the general similarities or dissimilarities between transcription profiles of different experiments. PCA is an algorithm that reduces the dimensionality of the data while retaining most of the variation in the data set. Experiments grouping closely together in a PCA biplot can be considered as sharing a similar transcriptional response after irradiation.

The extreme position of two thyroid irradiation experiments is a first emerging pattern (see Figure 1). The position of these experiments on the PCA plot could be more easily explained by the experimental setup, than by an effect of dose or tissue. Indeed, in contrast to other irradiation experiments where messenger RNA (mRNA) was isolated at most a few hours after irradiation, the mice in those experiments were irradiated at a specific time point, and the mRNA of the thyroid was isolated after 9 or 18 months respectively.

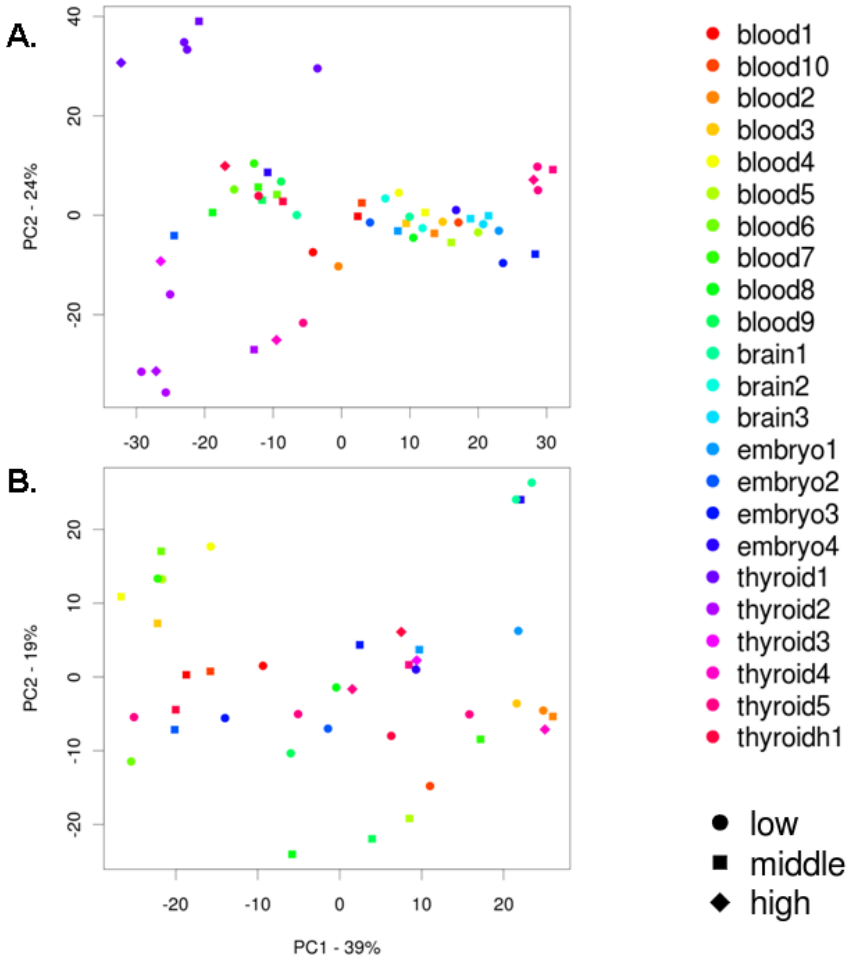

Fig. 1. PCA biplot of A) all irradiation experiments. B) all irradiation experiments with exception of long-term thyroid experiment (mRNA extraction after 9 [thyroid1] and 18 months [thyroid2]). Colour codes represent the different experiments, symbols indicate the radiation dose: low (<0.2Gy), middle (0.2Gy – 1Gy) and high (> 1Gy)

As a second observation, no tight clustering was found for the parameter of dose. This is remarkable as the data set included samples irradiated over a wide dose range, varying from low doses as 0.025 Gy up to doses as high as 8 Gy. The same conclusion could be drawn concerning the species-effect: no clear distinction could be made between the gene expression profiles originating from human or mouse samples. On the contrary, all microarray data seemed to cluster together rather on the type of experiment, even when irradiated with largely different radiation doses. For example considering the arrays measuring the gene expression after irradiation of the thyroid followed by immediate extraction of mRNA, all arrays cluster tightly together regardless irrespective of the irradiation dose (0.1 or 4Gy). Such effect could hardly be explained by an artefact resulting from a batch effect, since experiments performed with the same settings but at time points lying at least one year from each other, tend to cluster together as observed in the irradiated human blood samples.

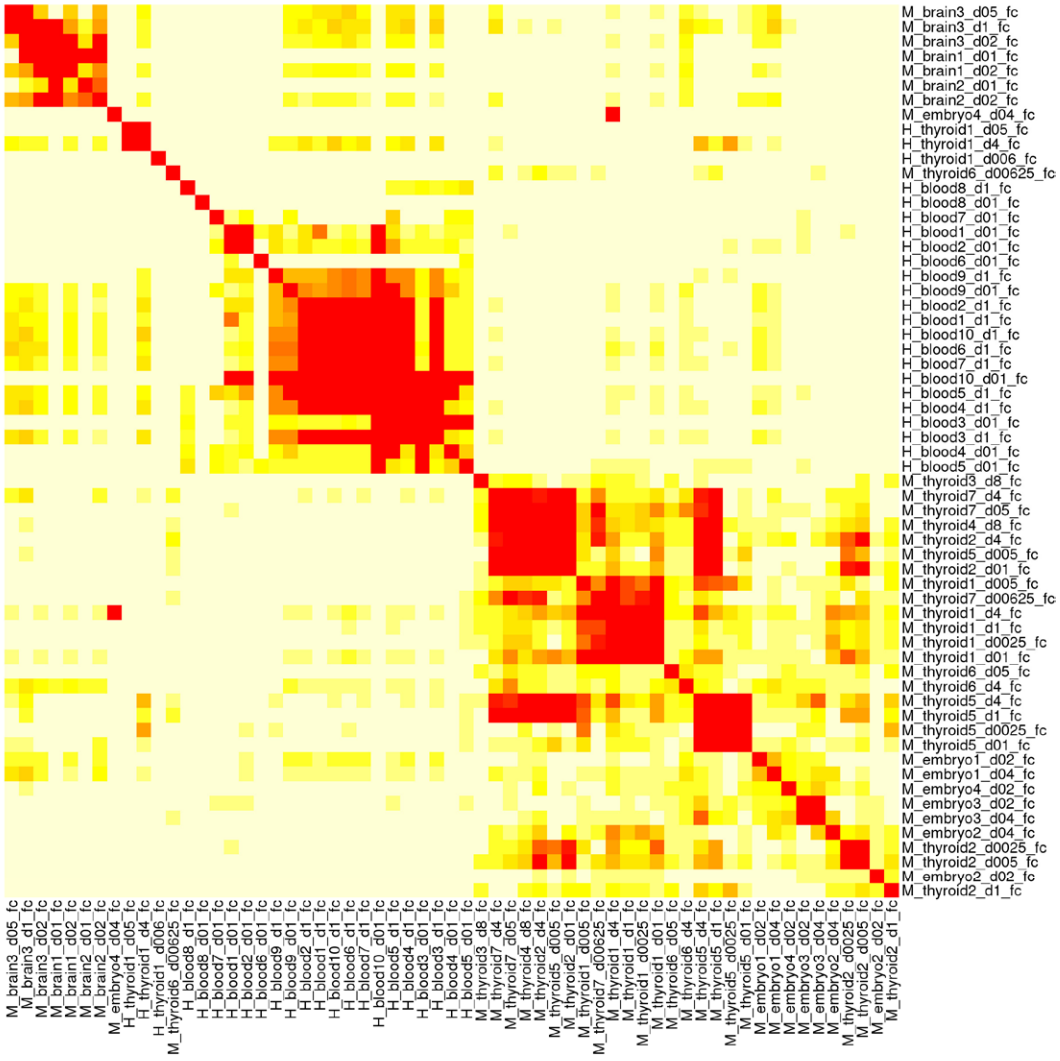

Fig. 2. Clustering of 63 experiments based on the statistical significance (P-value) of the number of overlapping genes shared between two experiments. The red colour indicates a highly significant overlap, the white colour means no significant overlap.

Differentially expressed genes

Whereas previous high-level analysis was based on simultaneous analysis of thousands of genes with the highest variance over the different conditions, analysis of the differentially expressed genes within the various conditions allows interpretation from an alternative angle. Taking into account the number of upregulated genes with a cutoff on the fold change of 1.5, the highest number of differentially expressed genes was observed for the thyroid irradiated samples with late mRNA extraction (after 9 and 18 months respectively) and for a subset of irradiated embryo (gastrula stage) samples. The former observation is in agreement with the PCA results reported in the previous paragraph. When taking into account the overlap of differentially expressed genes between various conditions, clustering based on the statistical significance of the overlap was done according to the tissue type (blood, brain, thyroid, and embryo) (Figure 2). These results suggest the presence of a tissue-specific transcriptional response towards radiation. From a species-specific point of view, the number of overlapping genes between human and mouse irradiated thyroid is very limited.

Focussing on the blood samples, a significant increase in the number of differentially expressed genes is observed when increasing the radation dose from low (0.1 Gy) to high (1 Gy). Moreover, taking into account the overlap of differentially expressed genes between all blood samples, a tight grouping of the 1 Gy samples is observed, while a less robust overlapping set of genes is observed for the lower dose (0.1 Gy).

Functional interpretation of radiation response

Using a gene set enrichment strategy, functional enrichment of the differentially expressed genes in all irradiation experiments was performed using the KEGG pathway database (Kanehisa et al. 2010) and the Gene Ontology classification system.

No KEGG pathway or GO biological process could be found to be commonly enriched in all (or a majority of) experiments. This corresponds with the observation of differentially expressed genes being tissue specific, as discussed in previous paragraph. As such, clustering based on functional enriched categories results in grouping of the experiments based on tissue.

The most significantly enriched KEGG pathways (cut-off on p-value on 0.05) were the p53 signalling (KEGG:04115), the nucleotide excision repair (KEGG:03420) and the cell cycle (KEGG:04110) pathways, enriched respectively in 28, 22 and 13 out of the 63 experiments. The first pathway was significantly enriched in roughly all irradiated blood samples (0.1 Gy and 1 Gy) and almost all irradiated embryonic brain samples (0.2 Gy and 0.4 Gy) (Figure 3.A). With regard to the blood samples, a clear dose dependency could be observed. The other two pathways could not be assigned to a specific subset of experiments. Remarkably, the apoptosis pathway (KEGG: 04210) was only enriched in four experiments. However, zooming in onto the individual expression values of the genes belonging to this pathway revealed an interesting pattern: a consistent set of genes was found to be upregulated within the thyroid experiments where extraction of mRNA was performed after several months compared with the non-irradiated sample at the same time (Figure 3.B).

Similarly, the most enriched GO biological processes were related to DNA damage and repair (e.g. GO:0006974, GO:0006281) and cell cycle (e.g. GO:0051726,

GO:0007067), all enriched in at least 14 out of the 63 experiments, with a maximum of 24 for response to DNA damage stimulus (GO:0006974). The response to DNA damage stimulus was mostly enriched in the irradiated blood samples, as well as in a subset of the embryonic experiments.

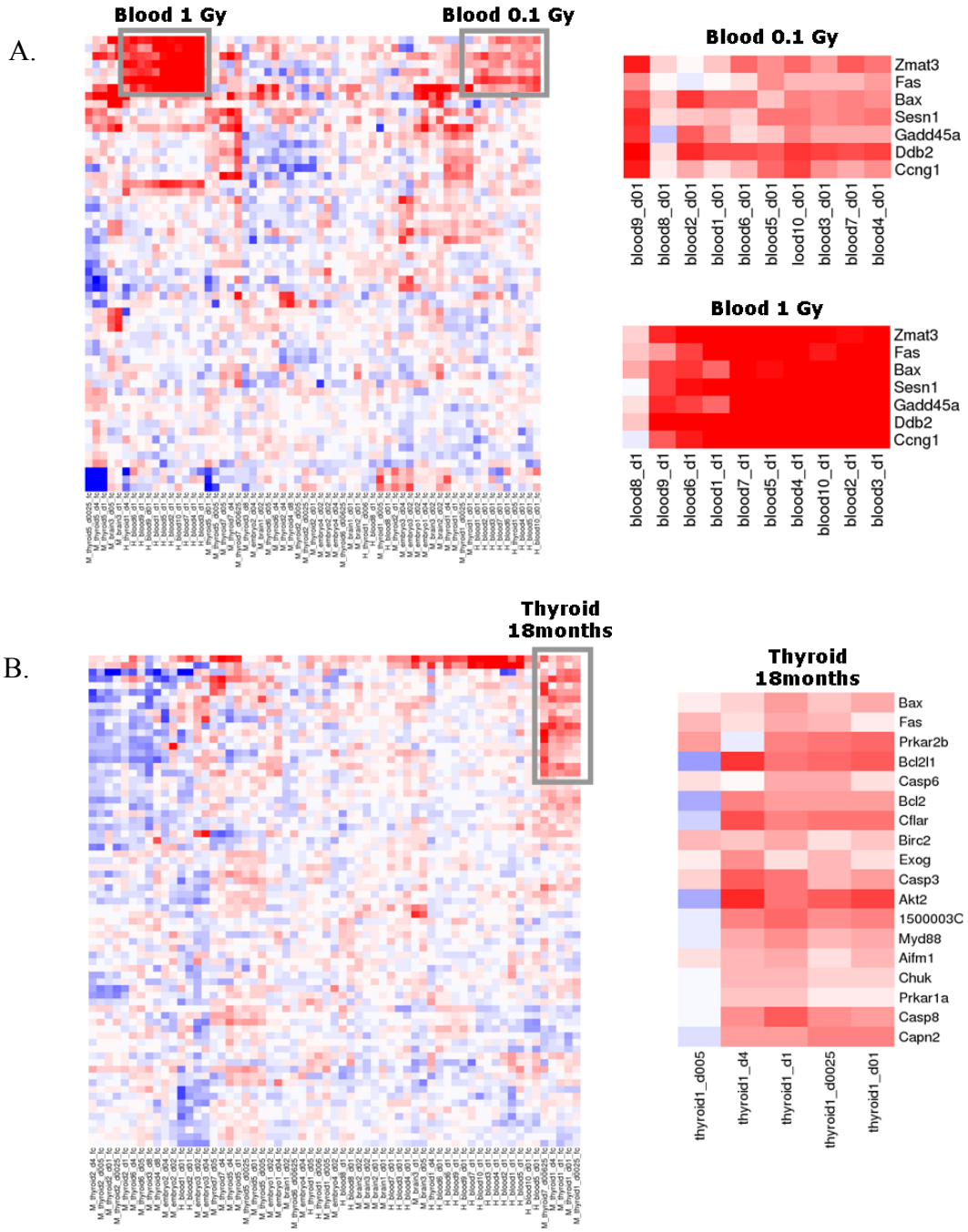

Fig. 3. Heatmap of expression of all genes belonging to A) the p53 signalling (KEGG:04115) and B) the apoptosis (KEGG:04210) pathways. For the p53 signalling pathway, a subset of genes appears to be upregulated in a dose dependent manner in the blood samples. For the apoptosis pathway, a set of genes is upregulated in the long-term experiment with mRNA extraction 18 months after irradiation with low and high doses.

Discussion

The advances in high-throughput technologies like microarrays have revolutionized the field of molecular biology. This microarray technology has already been used extensively to study the effect of low and high doses of ionising radiation at the transcriptional level. However, until now most studies have focussed on the impact of radiation on single specific tissues. In this paper, a more systematic approach was applied to study the radiation effect over various parameters.

A first remarkable observation in the PCA analysis was the aberrant transcriptional profile of two long-term experiments where mRNA was extracted from mouse thyroid tissue several months after low and high doses of irradiation. This deflected transcriptional behaviour was confirmed by the increasing number of upregulated genes in this long-term experiment compared to the acute stress experiment. These data obtained on mouse thyroid tissues are in line with the observation of Fält *et al.* (2003) who noticed that the number of differentially expressed genes increased dramatically with increasing culture time. In their experiment, primary human lymphocytes were irradiated, and mRNA was extracted at different time points (7, 17 and 55 days), showing a clear increase with the time in the number of modulated genes. Moreover, while the changes in the pattern of gene expression reported by Fält *et al.* (2003) occurred after a high radiation dose (3 Gy), the observations in the present study allow to extend their conclusions to doses as low as 0.05 and 0.025 Gy. In contrast with their study in which a limited overlap of genes was found between the immediate and long-term data, our results point towards a significant overlap with the acute response based on the number of upregulated genes shared between short- and long-term experiments. Interestingly, one of these long-term experiments (18 months) showed a significant enrichment of apoptosis related genes when compared to gene expression in a non-irradiated mouse of the same age.

Based on analysis by PCA, irradiation experiments are grouped together according to the experiments rather than by the irradiation dose. Accordingly, clustering based on the number of overlapping upregulated genes shows clear links with the tissue origin. As such, the gene expression in irradiated biological material seems to be tissue-specific rather than dose-specific. A similar, small scale analysis comparing two sets of differentially expressed genes after irradiation of kidney and brain with a high dose of 10 Gy, basically resulted in the same conclusion: only a very limited number of overlapping genes could be identified (Zhao et al. 2006), also pointing towards a tissue-specific response. Similarly, Pawlik *et al.* (2009) found that the expression profile of irradiated liver tissue correlated only to a small extent to the one in other tissues.

As a consequence of the tissue specificity of the transcriptional response after irradiation, no common pathway could be identified yet over different experiments or doses. However, certain pathways e.g. related to p53 signalling, cell cycle, DNA damage and repair were activated in a wide range of experiments, but mainly in the irradiated blood samples. This manifest activation of pathways related to DNA repair and cell cycle is probably related to the increased sensitivity of haematopoietic cells to radiation. Moreover, for the p53 signalling pathway, a dose specific transcriptional response was observed within irradiated blood samples, which is in agreement with similar results by other research groups aiming at identifying a potential set of radiation responsive biomarkers in blood (Dressman et al. 2007, Paul and Amundson 2008).

However, a new trend in disease classification also incorporates pathway information in order to classify diseases based on the activity of entire pathways instead of expression levels of individual genes (Lee et al. 2008, Svensson et al. 2006). Therefore, based on this and previous studies, the p53 signalling cascade might be a good candidate to be used as pathway-based biodosimetry classifier. However, the absence of a non-perturbed basal expression profile of this pathway in biodosimetry application is still a challenging issue (Paul and Amundson 2008).

Conclusions

First, results presented here point towards an important tissue-specific aspect in response to ionzing radiation. Secondly, gene expression results confirm the importance of performing studies on the long-term effect of radiation, as differential expression increased with longer post-irradiation times.

In this paper, the data are only interpreted from a bird's-eye view. However, a more thorough analysis is necessary and currently under progress. Generally, our studies point towards subtle but undeniable changes in gene expression. Therefore, an important future challenge is extracting these subtle changes in a statistically significant way by exploiting additional systems biology tools.

Acknowledgements

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A combined analysis of three European studies on the joint effects of radon exposure and smoking on lung cancer risk among uranium miners

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Abstract

Objectives: Three case-control studies nested in the French, German and Czech cohorts of uranium miners were conducted in the frame of Alpha-Risk, a European research project on the quantification of health risks associated with multiple radiation exposures. These studies aimed at investigating the joint effects of radon and smoking on lung cancer risk. A combined analysis of individual data of the three studies is presented.

Methods: The combined data set includes 1476 cases and 3389 matched controls. Cumulated radon exposure during employment was obtained from measurements or a job exposure matrix. Smoking habits were determined from medical archives and questionnaires. Analysis was performed by conditional logistic regression using a linear excess relative risk model.

Results: Smoking status was established for 1046 cases and 2492 controls. Ninety four percent of cases and 76% of controls were ex- or current smokers. Mean five-year lagged cumulated radon exposure was 335 Working Level Months (WLM) for cases and 211 WLM for controls. The excess relative risk per WLM adjusted for smoking was 0.79% (95% confidence-interval: 0.44 –1.41%). Lung cancer excess relative risk per unit exposure was about two times higher among non-smokers than among smokers and the results suggest a sub-multiplicative interaction between smoking and radon exposure on lung cancer mortality risk.

Conclusions: This collaborative study is the largest uranium miners case-control study on lung cancer with smoking information in Europe. It confirms the persistence of radon effect on lung cancer risk when smoking is taken into account and allows investigating the interaction between radon and smoking.

Cerebrovascular diseases in the cohort of Mayak PA nuclear workers

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Abstract

Incidence of and mortality from cerebrovascular diseases (CVD) have been studied in a cohort of 12210 workers first employed at one of the main plants of the Mayak nuclear facility during 1948–1958 and followed up to 31 December 2000. Information on external gamma doses was available for virtually all of these workers (99.9%); the mean (± standard deviation, SD) total external gamma dose was 0.91±0.95 Gy (99% percentile 3.9 Gy) for men and 0.65±0.75 Gy (99% percentile 2.99 Gy) for women. In contrast, plutonium body burden was measured only for 30% of workers; amongst those monitored, the mean (± SD) absorbed cumulative liver dose was 0.40±1.15 Gy (99% percentile 5.88 Gy) for men and 0.81±4.60 Gy (99% percentile 15.95 Gy) for women. 4418 disease cases and 753 deaths from CVD were identified in the study cohort. Having adjusted for non-radiation factors and internal alpha exposure from incorporated plutonium-239, there was statistically significant increasing trend in CVD incidence with total external gamma dose; the excess relative risk per Gy (ERR/Gy) was 0.464 (95% CI 0.360, 0.567). Much of the evidence for the raised incidence in relation to external dose arose for workers with cumulative doses above 1 Gy, although the data are consistent with a linear trend in risk with external dose. There was no statistically significant trend in CVD mortality risk with external dose or differences in mortality rates between categories for external dose. Having adjusted for non-radiation factors and external gamma exposure, there was statistically significant increasing trend in CVD incidence with total absorbed internal liver dose; the ERR/Gy was 0.155 (95% CI 0.075, 0.235). CVD incidence was statistically significantly higher among workers with a plutonium liver dose above 0.1 Gy, although the trend estimates differed between workers at different plants. There was a statistically significant increased risk of CVD mortality among workers exposed to internal alpha exposure with total liver dose of 0.1–0.5 Gy as compared with workers exposed to lower doses. There was no statistically significant trend in CVD mortality risk with absorbed internal liver dose. The risk estimates for external radiation are generally compatible with those from other large occupational studies, although the incidence data point to higher risk estimates compared to those from the Japanese A-bomb survivors.

Introduction

Ischemic heart disease and cerebrovascular diseases (CVD) are currently among the main death causes in developed countries. CVD are multifactorial diseases, and different endogenous (genetic predisposition, gender, age, hypertension etc.) and exogenous (smoking, emotional stress etc.) factors contribute to their development. Over the last two decades several studies have examined the possible effects of ionizing radiation exposure on circulatory diseases, including CVD. Most of the studies (McGeoghegan et al 2008; Kreuzer et al 2006; Muirhead et al 2009; Shimizu et al 2010; Vrijheid et al 2007) focused on CVD mortality, whereas several studies (Yamada et al 2004; De Bruin et al 2009; Ivanov et al 2006) focused on CVD incidence.

This study was aimed at estimating risks of both CVD incidence and mortality in the cohort of Mayak PA nuclear workers first employed at one of the main plants (reactors, radiochemical, or plutonium) during 1948-1958 in relation to external gamma and internal alpha exposures, taking non-radiation factors into account.

Material and methods

Mayak PA began operations in 1948 as the first and largest nuclear weapons facility in the former Soviet Union and included all the plants necessary for weapon-grade plutonium production, namely, reactors, radiochemical plant, plutonium production plant and auxiliary plants.

From the first days of Mayak PA operation, the special system of personnel medical observation included an obligatory pre-employment medical examination and routine medical examinations of all the workers based on a common standard program. After quitting their job at Mayak, if the former worker stayed in Ozyorsk, he/she was examined at the same specialized medical hospital based on the same standard program. This system of medical observation of Mayak PA personnel health allowed a unique archive of primary medical data to be accumulated and formed the basis for establishment of the unique “Clinic” medical-dosimetry database for the Mayak PA workers cohort (Azizova et al 2008).

The study cohort included 12210 Mayak PA workers, 3552 (29.1%) of whom were women, first employed at one of the main plants from the start of operations through the end of 1958 independently of gender, age, nationality, occupation, and other characteristics. The method of identifying this cohort has been described previously elsewhere (Koshurnikova et al 1988, 1998a, 1998b, 1999).

Vital status as of 31 December 2000 was known for 88.4% of cohort members; of these workers, 52.7% were known to have died and 47.3% were known to be alive as of that time. 53.7% of the 12210 members of the study cohort were known to have left Ozyorsk by 31 December 2000. For persons who continued to be residents of Ozyorsk, vital status was known for all but one person (99.98%). Cause of death was known for 93.5% of deceased cohort members. Morbidity data for the whole period of follow-up were collected for 11597 (95.0%) workers in the study cohort. Only for 5.0% of workers could information not be collected, owing to the loss of their medical documentation. It should be noted that the CVD incidence analysis was restricted to the period of residence in Ozyorsk.

Data on vital status and on dates and causes of death for those workers who migrated from Ozyorsk were provided by the SUBI Epidemiology Laboratory; data on

date and causes of death for those Ozyorsk residents whose medical cards and/or case histories had been lost, were provided by the SUBI Occupational Health Laboratory.

Individual dosimetric control of external exposure was performed at Mayak PA from the beginning of operations there. Regular monitoring of internal exposure among those who might have been exposed to plutonium-239 began later, during the 1960s (Vasilenko et al 2007a; Khokhryakov et al 2000). The results of individual monitoring of external and internal exposure were recorded in individual dosimetric cards and journals. The data contained in these documents formed the basis for the establishment of the dosimetric database of Mayak PA workers (Vasilenko et al 2007a, 2007b; Bess et al 2007; Smetanin et al 2007a, 2007b; etc).

Dose estimates from the *Mayak-Doses 2005* dosimetric system were used in this study. Annual doses of external gamma exposure were available practically for all persons in the study cohort (99.9%). The mean (± SD) total gamma dose for the whole period of work at Mayak was 0.91±0.95 Gy (99% percentile 3.9 Gy) for men and 0.65±0.75 Gy (99% percentile 2.99 Gy) for women. The range of total gamma doses was very wide, with 32.6% having a total gamma dose greater than 1 Gy.

Plutonium body burden was measured only for 30.0% of workers. Among workers monitored for plutonium exposure, absorbed dose to liver from alpha radiation was used as a surrogate for the dose to muscle; this latter dose is likely to be similar to the dose to blood vessels and the chambers of the heart. Although doses to the liver and muscle would differ, they should be highly correlated with each other. Consequently, the liver dose can be used to look for any *dose–response* relationship between plutonium exposure and circulatory disease. Amongst those who were monitored, the mean (± SD) total liver dose was 0.40±1.15 Gy (99% percentile 5.88 Gy) for men and 0.81±4.60 Gy (99% percentile 15.95 Gy) for women.

Data on occupational histories and external gamma exposures for the study cohort were provided by the Mayak PA Radiation Safety Department; data on internal alpha exposure from incorporated plutonium-239 were provided by the SUBI Internal Dosimetry Department.

This study focuses on incidence of and mortality from CVD (ICD-9 codes: 430–438). Follow-up started on the date of first employment at one of the main plants and continued until the earliest of: date of the first diagnosis of CVD (for the incidence analysis) or the date of death from whatever cause (for both the mortality analysis and the incidence analysis); 31 December 2000 for those known to be alive at that time; the recorded date of departure from Ozyorsk (for the incidence analysis); and the date of "last medical information" in the case of unknown vital status. Comparisons were performed within the Mayak PA workers cohort first employed during 1948–1958.

The analyses included the calculation of relative risks (RRs) for categories of one or more factors, having adjusted for other variables. The relative risks for these categorical analyses were calculated by maximum likelihood, using the AMFIT module of EPICURE (Preston et al 1993). 95% confidence intervals for the RRs and p-values from tests of statistical significance were obtained via likelihood-based methods, using AMFIT. Attention was initially directed to non-radiation factors, following which measures of radiation exposure were analysed with adjustment (through stratification) for non-radiation factors. Analyses of internal radiation exposures were restricted to workers known to have been monitored for possible plutonium exposure.

In addition to the categorical analyses, models for trends in disease rates with level of radiation exposures were also fitted to the data, using Poisson regression methods. These models again were fitted using the AMFIT module in EPICURE. In particular, the excess relative risk (ERR) (ie. the relative risk minus 1) was modeled by a linear trend with external or internal dose, with adjustment for non-radiation factors.

In these main analyses, adjustments were made – through stratification – for gender, attained age, calendar period, period of first employment at the main plants of Mayak PA, plant, smoking and alcohol consumption.

Sensitivity analyses were conducted to examine the impact of: a) modifying the set of non-radiation factors (extra adjustment for hypertension, body mass index, employment duration) for which adjustment was made in the analyses of radiation factors; b) restricting the mortality follow-up (like that of incidence) to Ozyorsk, because some migrants were lost to follow-up and because of lower autopsy rates among those who left the city; c) adjusting for internal dose in analyses of external dose and vice versa; d) using various lag periods for external and internal doses. Furthermore, examination was made of how radiation risks might vary by gender, or between plants at Mayak or by attained age.

To allow for the possibility that radiation might affect CVD risk by modifying levels of blood pressure (Preston et al 2003; Ivanov et al 2001) and body mass index (Telnov 1985), the level of these factors at the time of preliminarily medical examination (before employment at Mayak PA) was considered, in order to avoid systematic errors that might arise through adjusting for values of these factors at later times. In contrast, smoking and alcohol consumption were classified at the time of last information (for the mortality analysis) or at the time of last information prior to the first diagnosis of CVD (for the incidence analysis).

Results

By 31 December 2000, 4418 disease cases of CVD were diagnosed during 197344 person-years of follow-up and 753 death cases from CVD were identified during 443350 person-years of follow-up.

Non-radiation factors

It is known that CVD are multifactorial diseases, therefore analyses of incidence and mortality risks in relation to non-radiation factors were performed first. Our analyses revealed statistically significant effects of well-known factors such as gender, age, hypertension, body mass index and smoking, which were taken into account in the analyses of radiation risks, either in the main analysis or in sensitivity analyses.

Radiation factors

External gamma exposure: Table 1 shows that CVD incidence was statistically significantly higher among workers chronically exposed to external gamma exposure with a total dose above 0.5 Gy as compared with workers externally exposed to lower doses.

Table 1. RRs and ERR (95% CI) for CVD incidence and mortality in relation to total dose of external gamma exposure (main analysis based on a zero year lag period).

	RRs (vs. <0.5 Gy)		ERR/Gy
	0.5-1.0 Gy	>1.0 Gy	
Incidence	1.137 (1.036, 1.248)	1.599 (1.465, 1.746)	0.464 (0.360, 0.567)
Mortality	1.150 (0.923, 1.434)	0.994 (0.799, 1.237)	-0.022 (-0.117, 0.073)

Sensitivity analyses (results not shown) revealed that when comparing gamma doses of 0.5-1.0 Gy with lower doses, statistically significantly raised risks of CVD incidence were seen among radiochemical plant workers and among females; in other sub-groups, the findings were very close to statistical significance. CVD incidence was statistically significantly higher among workers with a gamma dose above 1.0 Gy relative to workers with a gamma dose below 0.5 Gy, irrespective of the lag period used, whether additional adjustment was made for other non-radiation factors and internal exposure as well as whether analysis was restricted to workers at different plants or by gender.

There was a statistically significant increasing trend in CVD incidence with increasing external dose (Table 1). Sensitivity analyses (results not shown) revealed that adjustments for other non-radiation factors and internal exposure as well as restriction to workers at different plants or by gender had little impact on this finding. There was also no evidence of variation in the ERR per Gy by attained age.

There was no statistically significant trend in CVD mortality risk with external dose, and for the most part mortality rates did not differ to a statistically significant extent between categories for external dose.

Internal alpha exposure: Table 2 shows that CVD incidence was statistically significantly higher among workers exposed to total absorbed dose to liver from internal alpha exposure above 0.1 Gy, as compared with workers exposed to lower doses. This finding held irrespective of the lag period used, whether adjustment was made for other non-radiation factors and external exposure as well as whether analysis was restricted to workers at different plants or by gender (results not shown).

Table 2. RRs and ERR (95% CI) for CVD incidence and mortality in relation to total absorbed liver dose from internal alpha exposure (main analysis based on a zero year lag period).

	RRs (vs. <0.1 Gy)		ERR/Gy
	0.1-0.5 Gy	>0.5 Gy	
Incidence	1.233 (1.126, 1.350)	1.576 (1.346, 1.844)	0.155 (0.075, 0.235)
Mortality	1.401 (1.020, 1.924)	1.047 (0.609, 1.800)	0.120 (-0.116, 0.356)

There was a statistically significant increasing trend in CVD incidence with increasing internal liver dose from alpha exposure. The ERR/Gy for CVD incidence increased with increasing lag period, with values of 0.155 (95% CI 0.075, 0.235), 0.218 (95% CI 0.111, 0.326) and 0.330 (95% CI 0.174, 0.487), 0.508 (95% CI 0.269, 0.747) and 0.853 (95% CI 0.443, 1.262) based on 0, 5, 10, 15 and 20 year lags respectively.

There was a non-linearity in the trend based on zero lag, although the evidence for non-linearity appeared to be weaker when a 5-year lag was used. Adjustments for arterial hypertension, body mass index, duration of work and external exposure had little impact on the estimated trend (results not shown). Much of the evidence for this trend appeared to arise at attained ages of 50 years or more, but the variation in the ERR/Gy by attained age was not statistically significant. Furthermore, the evidence for a dose trend came mainly from males rather than females, as well as from workers at the radiochemical plant rather than plutonium production plant workers.

CVD mortality was statistically significantly raised among workers exposed to internal alpha exposure in total liver doses of 0.1–0.5 Gy as compared with workers exposed to lower doses irrespective of lag period and whether adjustment was made for other non-radiation factors as well as whether follow-up was restricted to Ozyorsk and whether adjustment was made for external exposure. However, when comparing internal doses of >0.5 Gy with doses <0.1 Gy, statistically significantly raised risks were not seen for CVD mortality, possibly reflecting the relatively small number of deaths in this subgroup.

There was no statistically significant trend in CVD mortality in relation to total absorbed liver dose from internal alpha exposure.

Discussion

Our analyses of CVD incidence and mortality revealed statistically significant effects of well-known factors such as gender, age, hypertension, body mass index and smoking, which are consistent with findings of other studies. In contrast, our analyses did not reveal any statistically significant effect of alcohol consumption on CVD incidence or mortality, either for males and females, whereas some studies (Donahue et al 1986; etc) indicated an increase in stroke mortality associated with excess alcohol consumption.

The evidence for associations with radiation in this study relates mainly to CVD incidence. For CVD mortality, the data did not show a statistically significantly increasing trend with either external or internal dose, and the estimates of these trends were lower than those for CVD incidence. There were far fewer deaths from CVD than cases in this study. A complication to interpretation is the lack of knowledge as to those tissues or organs for which radiation exposure might increase the risk of CVD, which is particularly problematic in the case of plutonium intakes. For this analysis, liver dose has been used as a surrogate for the dose to muscle, which is likely to be similar to the dose to blood vessels and the chambers of the heart. Furthermore, the liver and muscle doses should be highly correlated with each other. However, there is uncertainty about which tissue or organ dose is appropriate for this type of analysis. A further complication relates to uncertainties in estimates for internal doses for Mayak workers. In addition, whilst the results for external doses appeared to be broadly consistent with a linear *dose-response* (albeit with uncertainties in estimates at relatively low doses), the results for internal doses appeared to show some degree of non-linearity. Also, whilst the trend estimates in relation to external exposure were similar across sub-groups of workers, the trend in CVD incidence with internal dose differed not only between males and females but also between workers at the radiochemical plant and the plutonium production plant. It should be noted that – because the dose to the liver from intakes of plutonium would be greater than that to the circulatory system – the ERR/Gy

estimated here based on liver dose would be lower than that based on dose to blood vessels and the chambers of the heart. At present information is not available from other studies of populations exposed to plutonium that would allow comparison of risk estimates for CVD in relation to such exposures. For these reasons, the findings in relation to internal exposure need to be interpreted with caution.

Comparison with other studies

Table 3 presents estimates of the ERR per Gy from the current study and from other studies of groups exposed to external low-LET radiation in which this trend was estimated.

Table 3. Estimates of ERR per Gy (95% CI) for CVD following exposure to external low-LET radiation.

Cohort	Mean total dose (Gy)	Mortality or incidence?	Lag period (years)	No. of deaths or cases	ERR/Gy
Japanese A-bomb survivors: Life Span Study (Shimizu et al 2010)	0.20	Mortality	5	9622	0.09 (95% CI 0.01, 0.17)
Japanese A-bomb survivors: Adult Health Study (Yamada et al 2004)	0.57	Incidence	13	729	0.07 (95% CI -0.08, 0.24)
Mayak PA workers (this study)	0.84	Mortality	10	744	-0.02 (95% CI -0.12, 0.08)
Mayak PA workers (this study)	0.84	Incidence	10	3840	0.45 (95% CI 0.34, 0.56)
Nuclear workers (international) (Vrijheid et al 2007)	0.018	Mortality	10	1224	0.88 (95% CI -0.67, 3.16)
BNFL workers (UK) (McGeoghegan et al 2008)	0.053	Mortality	15	1018	0.43 (90% CI -0.10, 1.12)
UK National Registry for Radiation Workers (Muirhead et al 2009)	0.025	Mortality	10	1817	0.16 (95% CI -0.42, 0.99)
German uranium miners (Kreuzer et al 2006)	0.041	Mortality	5	1297	0.09 (95% CI -0.6, 0.8)
Chernobyl recovery operations workers (Ivanov et al 2006)	0.109	Incidence	–	12832	0.45 (95% CI 0.11, 0.80)

Whilst the estimated ERR/Gy for mortality among Mayak workers is consistent with the A-bomb survivor findings, some of the occupational studies point towards higher risk estimates. The statistically precise estimate for CVD incidence among Mayak workers is higher than that for the A-bomb survivors but is consistent with the incidence estimate for Chernobyl recovery operation workers and the mortality estimates from the international and BNFL worker studies. The central estimates for the ERR/Gy from the UK National Registry for Radiation Workers and the German uranium miner study are not statistically significantly different from zero and are close

to the estimates from the Mayak mortality data, but the confidence intervals also encompass the Mayak incidence findings.

Conclusions

Having adjusted for non-radiation factors and internal alpha exposure, there was an increasing trend in CVD incidence – but not mortality – among Mayak PA workers with total external gamma dose. Much of the evidence for a raised risk of CVD incidence in relation to external dose arises for workers with cumulative doses above 1 Gy. Although the dose response is consistent with linearity, the statistical power to detect non-linearity at external doses below 1 Gy was low. Having adjusted for non-radiation factors and external gamma exposure, there was an increasing trend in CVD incidence among Mayak PA workers with total internal alpha dose to liver. CVD incidence was statistically significantly higher among workers with a plutonium liver dose above 0.1 Gy, although the trend estimates differed between workers at different plants. There was statistically significant increased risk of CVD mortality among workers exposed to internal alpha exposure with total liver dose of 0.1–0.5 Gy as compared with workers exposed to lower doses. There was no a statistically significant trend of CVD mortality risk with absorbed internal liver dose or difference in mortality rates between categories for internal dose. The risk estimates for external radiation are generally compatible with those from other large occupational studies, although the incidence data point to higher risk estimates compared to those from the Japanese A-bomb survivors.

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Ischemic heart disease in the cohort of Mayak PA nuclear workers

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Abstract

Incidence of and mortality from ischemic heart disease (IHD) have been studied in a cohort of 12210 workers first employed at one of the main plants of the Mayak nuclear facility during 1948–1958 and followed up to 31 December 2000. Information on external gamma doses was available for virtually all of these workers (99.9%); the mean (± standard deviation, SD) total external gamma dose was 0.91±0.95 Gy (99% percentile 3.9 Gy) for men and 0.65±0.75 Gy (99% percentile 2.99 Gy) for women. In contrast, plutonium body burden was measured only for 30% of workers; amongst those monitored, the mean (± SD) absorbed cumulative liver dose was 0.40±1.15 Gy (99% percentile 5.88 Gy) for men and 0.81±4.60 Gy (99% percentile 15.95 Gy) for women. 3751 disease cases and 1495 deaths from IHD were identified in the study cohort. Having adjusted for non-radiation factors, there was statistically significant increasing trend in IHD incidence with total external gamma dose; the excess relative risk per Gy (ERR/Gy) was 0.109 (95% CI 0.049, 0.168). This trend with external dose was little changed after adjusting for internal dose. Much of the evidence for the raised incidence in relation to external gamma dose arose for workers with cumulative doses above 1 Gy, although the data are consistent with a linear trend in risk with external dose. The trend with external dose in IHD mortality was not statistically significantly greater than zero, but was consistent with the corresponding trend in IHD incidence even once adjustment for internal dose was made. There was stronger evidence for an association with internal alpha dose to liver in the IHD mortality data than in the corresponding incidence data, although the associated confidence intervals overlap. Furthermore, the estimated trend in IHD mortality with internal dose was lower and not statistically significant once adjustment was made for external dose. The risk estimates for IHD in relation to external radiation are generally compatible with those from other large occupational studies and the Japanese A-bomb survivors.

Introduction

The first evidence of excess mortality from heart disease following radiation exposure came from the study of Japanese A-bomb survivors, who received single whole-body

doses in the range 0-4 Gy; the most recent findings are described by Shimizu et al (2010). However, the corresponding data on ischemic heart disease (IHD) incidence in a subset of these survivors provided less evidence of an association (Yamada et al 2004). An increased risk of cardiovascular diseases has also been observed in patients who underwent radiotherapy for breast cancer or for Hodgkin lymphoma with doses of 40 Gy or more to mantle or limited fields (UNSCEAR 2006). In contrast, studies of radiation workers have yielded mixed findings. McGeoghegan et al (2008) reported an association between mortality from non-cancer causes of death, particularly circulatory disease, and external exposure to ionizing radiation in a cohort of nuclear industry workers with a mean cumulative external dose of 0.053 Sv and a 99% percentile of 0.59 Sv. However, a study of radiation workers from 15 countries did not find a statistically significant association between circulatory disease mortality and radiation dose (mean cumulative radiation dose of 0.019 Sv, with some workers receiving more than 0.5 Sv), although – in common with other studies of nuclear workers – the power of this analysis was low (Vrijheid et al 2007).

This study was aimed at estimating risks of both IHD incidence and mortality in the cohort of Mayak PA nuclear workers first employed at one of the main plants (reactors, radiochemical, or plutonium) during 1948-1958 in relation to external gamma and internal alpha exposures, taking non-radiation factors into account.

Material and methods

Mayak PA began operations in 1948 as the first and largest nuclear weapons facility in the former Soviet Union and included all the plants necessary for weapon-grade plutonium production, namely, reactors, radiochemical plant, plutonium production plant and auxiliary plants.

From the first days of Mayak PA operation, the special system of personnel medical observation included an obligatory pre-employment medical examination and routine medical examinations of all the workers based on a common standard program. After quitting their job at Mayak, if the former worker stayed in Ozyorsk, he/she was examined at the same specialized medical hospital based on the same standard program. This system of medical observation of Mayak PA personnel health allowed a unique archive of primary medical data to be accumulated and formed the basis for establishment of the unique “Clinic” medical-dosimetry database for the Mayak PA workers cohort (Azizova et al 2008).

The study cohort included 12210 Mayak PA workers, 3552 (29.1%) of whom were women, first employed at one of the main plants from the start of operations through the end of 1958 independently of gender, age, nationality, occupation, and other characteristics. The method of identifying this cohort has been described previously elsewhere (Koshurnikova et al 1988, 1998a, 1998b, 1999).

Vital status as of 31 December 2000 was known for 88.4% of cohort members; of these workers, 52.7% were known to have died and 47.3% were known to be alive as of that time. 53.7% of the 12210 members of the study cohort were known to have left Ozyorsk by 31 December 2000. For persons who continued to be residents of Ozyorsk, vital status was known for all but one person (99.98%). Cause of death was known for 93.5% of deceased cohort members. Morbidity data for the whole period of follow-up were collected for 11597 (95.0%) workers in the study cohort. Only for 5.0% of

workers could information not be collected, owing to the loss of their medical documentation. It should be noted that the CVD incidence analysis was restricted to the period of residence in Ozyorsk.

Data on vital status and on dates and causes of death for those workers who migrated from Ozyorsk were provided by the SUBI Epidemiology Laboratory; data on date and causes of death for those Ozyorsk residents whose medical cards and/or case histories had been lost, were provided by the SUBI Occupational Health Laboratory.

Individual dosimetric control of external exposure was performed at Mayak PA from the beginning of operations there. Regular monitoring of internal exposure among those who might have been exposed to plutonium-239 began later, during the 1960s (Vasilenko et al 2007a; Khokhryakov et al 2000). The results of individual monitoring of external and internal exposure were recorded in individual dosimetric cards and journals. The data contained in these documents formed the basis for the establishment of the dosimetric database of Mayak PA workers (Vasilenko et al 2007a, 2007b; Bess et al 2007; Smetanin et al 2007a, 2007b; etc).

Dose estimates from the *Mayak-Doses 2005* dosimetric system were used in this study. Annual doses of external gamma exposure were available practically for all persons in the study cohort (99.9%). The mean (± SD) total gamma dose for the whole period of work at Mayak was 0.91±0.95 Gy (99% percentile 3.9 Gy) for men and 0.65±0.75 Gy (99% percentile 2.99 Gy) for women. The range of total gamma doses was very wide, with 32.6% having a total gamma dose greater than 1 Gy.

Plutonium body burden was measured only for 30.0% of workers. Among workers monitored for plutonium exposure, absorbed dose to liver from alpha radiation was used as a surrogate for the dose to muscle; this latter dose is likely to be similar to the dose to blood vessels and the chambers of the heart. Although doses to the liver and muscle would differ, they should be highly correlated with each other. Consequently, the liver dose can be used to look for any *dose–response* relationship between plutonium exposure and circulatory disease. Amongst those who were monitored, the mean (± SD) total liver dose was 0.40±1.15 Gy (99% percentile 5.88 Gy) for men and 0.81±4.60 Gy (99% percentile 15.95 Gy) for women.

Data on occupational histories and external gamma exposures for the study cohort were provided by the Mayak PA Radiation Safety Department; data on internal alpha exposure from incorporated plutonium-239 were provided by the SUBI Internal Dosimetry Department.

This study focuses on incidence of and mortality from IHD (ICD-9 codes: 410–414). Follow-up started on the date of first employment at one of the main plants and continued until the earliest of: date of the first diagnosis of CVD (for the incidence analysis) or the date of death from whatever cause (for both the mortality analysis and the incidence analysis); 31 December 2000 for those known to be alive at that time; the recorded date of departure from Ozyorsk (for the incidence analysis); and the date of "last medical information" in the case of unknown vital status. Comparisons were performed within the Mayak PA workers cohort first employed during 1948–1958.

The analyses included the calculation of relative risks (RRs) for categories of one or more factors, having adjusted for other variables. The relative risks for these categorical analyses were calculated by maximum likelihood, using the AMFIT module of EPICURE (Preston et al 1993). 95% confidence intervals for the RRs and p-values

from tests of statistical significance were obtained via likelihood-based methods, using AMFIT. Attention was initially directed to non-radiation factors, following which measures of radiation exposure were analysed with adjustment (through stratification) for non-radiation factors. Analyses of internal radiation exposures were restricted to workers known to have been monitored for possible plutonium exposure.

In addition to the categorical analyses, models for trends in disease rates with level of radiation exposures were also fitted to the data, using Poisson regression methods. These models again were fitted using the AMFIT module in EPICURE. In particular, the excess relative risk (ERR) (ie. the relative risk minus 1) was modeled by a linear trend with external or internal dose, with adjustment for non-radiation factors.

In these main analyses, adjustments were made – through stratification – for gender, attained age, calendar period, period of first employment at the main plants of Mayak PA, plant, smoking and alcohol consumption.

Sensitivity analyses were conducted to examine the impact of: a) modifying the set of non-radiation factors (extra adjustment for hypertension, body mass index, employment duration) for which adjustment was made in the analyses of radiation factors; b) restricting the mortality follow-up (like that of incidence) to Ozyorsk, because some migrants were lost to follow-up and because of lower autopsy rates among those who left the city; c) adjusting for internal dose in analyses of external dose and vice versa; d) using various lag periods for external and internal doses. Furthermore, examination was made of how radiation risks might vary by gender, or between plants at Mayak or by attained age.

To allow for the possibility that radiation might affect IHD risk by modifying levels of blood pressure (Preston et al 2003; Ivanov et al 2001) and body mass index (Telnov 1985), the level of these factors at the time of preliminarily medical examination (before employment at Mayak PA) was considered, in order to avoid systematic errors that might arise through adjusting for values of these factors at later times. In contrast, smoking and alcohol consumption were classified at the time of last information (for the mortality analysis) or at the time of last information prior to the first diagnosis of IHD (for the incidence analysis).

Results

By 31 December 2000, 3751 disease cases of IHD were diagnosed during 205249 person-years of follow-up and 1495 death cases from IHD were identified during 443350 person-years of follow-up.

Non-radiation factors

Our analyses revealed statistically significant effects of well-known factors such as gender, age, hypertension, body mass index and smoking, which were taken into account in the analyses of radiation risks, either in the main analysis or in sensitivity analyses.

Radiation factors

External gamma exposure: Table 1 shows that IHD incidence was statistically significantly higher among workers chronically exposed to external gamma exposure

with a total dose above 1.0 Gy as compared with workers externally exposed to doses below 0.5 Gy.

Table 1. RRs and ERR (95% CI) for IHD incidence and mortality in relation to total dose of external gamma exposure (main analysis based on a zero year lag period).

	RRs (vs. <0.5 Gy)		ERR/Gy
	0.5-1.0 Gy	>1.0 Gy	
Incidence	1.017 (0.919, 1.126)	1.198 (1.087, 1.320)	0.109 (0.049, 0.168)
Mortality	0.918 (0.778, 1.084)	1.115 (0.959, 1.295)	0.065 (-0.017, 0.148)

Sensitivity analyses (results not shown) revealed that IHD incidence was statistically significantly higher among workers with a gamma dose above 1.0 Gy relative to workers with a gamma dose below 0.5 Gy, irrespective of the lag period used, whether additional adjustment was made for other non-radiation factors and internal exposure. The RRs above 1 Gy for IHD incidence were statistically significant only among men as well as reactor or plutonium plant workers.

There was a statistically significant increasing trend in IHD incidence with increasing external gamma dose (Table 1). Sensitivity analyses (results not shown) revealed that using different lag periods, adjustments for other non-radiation factors and internal exposure had little impact on this finding. Much of the evidence of the raised risk related to men as well as reactor and plutonium plant workers. The findings were too imprecise to judge whether the relative risk in relation to external dose varied by attained age.

There was no statistically significant trend in IHD mortality risk with external dose, and for the most part mortality rates did not differ to a statistically significant extent between categories for external dose.

Internal alpha exposure: Table 2 shows that IHD incidence and mortality were statistically significantly higher among workers with a total absorbed dose to liver from internal alpha exposure of either 0.1-0.5 Gy or above 0.5 Gy, as compared with workers exposed to doses below 0.1 Gy.

Table 2. RRs and ERR (95% CI) for IHD incidence and mortality in relation to total absorbed liver dose from internal alpha exposure (main analysis based on a zero year lag period).

	RRs (vs. <0.1 Gy)		ERR/Gy
	0.1-0.5 Gy	>0.5 Gy	
Incidence	1.171 (1.060, 1.295)	1.225 (1.036, 1.447)	0.032 (-0.014, 0.079)
Mortality	1.332 (1.082, 1.640)	1.591 (1.158, 2.185)	0.276 (0.050, 0.501)

Table 2 shows that the estimates of the RRs and of the ERR/Gy were higher in the mortality data than in the corresponding incidence data. These findings were maintained when different lag periods were used (with a tendency for the estimated ERR/Gy to increase with increase lag), when (for the mortality analysis) follow-up was restricted to Ozyorsk, and – for the most part – when additional adjustment was made for other non-

radiation factors (results not shown). For IHD mortality, the trend in risk with internal liver dose was statistically significantly greater than zero, but the estimated ERR/Gy was lower and was not statistically significant once adjustment was made for external dose. Overall, there was no statistically significant trend in IHD incidence with internal liver dose, either with or without adjustment for external dose, although the ERR estimate was greater for males than for females and the difference between the gender-specific values was statistically significant. The findings were too imprecise to judge whether the relative risk in relation to internal dose varied by attained age.

Discussion

Our analyses of IHD incidence and mortality revealed statistically significant effects of well-known factors such as gender, age, hypertension, body mass index and smoking, which are consistent with findings of other studies. In contrast, our analyses did not reveal any statistically significant effect of alcohol consumption on IHD incidence or mortality, either for males and females.

There was a statistically significant increasing trend in IHD incidence with total external gamma dose. Much of the evidence for this trend in IHD incidence appeared to relate to external doses in excess of 1 Gy. Adjustments to other non-radiation factors as well as internal exposure did not have much effect on this trend. The trend with external dose in IHD mortality, whilst not statistically significantly greater than zero, was consistent with the statistically significantly trend estimate in IHD incidence. Among workers with internal liver doses there was stronger evidence for an association with these doses in the IHD mortality data than in the corresponding incidence data, although the associated confidence intervals overlap. Furthermore, the estimated trend in IHD mortality with internal dose was maintained when adjustment was made for non-radiation factors, but was lower and not statistically significant once adjustment was made for external dose.

A complication to interpretation is the lack of knowledge as to those tissues or organs for which radiation exposure might increase the risk of IHD, which is particularly problematic in the case of plutonium intakes. For this analysis, liver dose has been used as a surrogate for the dose to muscle, which is likely to be similar to the dose to blood vessels and the chambers of the heart. Furthermore, the liver and muscle doses should be highly correlated with each other. However, there is uncertainty about which tissue or organ dose is appropriate for this type of analysis. A further complication relates to uncertainties in estimates for internal doses for Mayak workers. It should be noted that – because the dose to the liver from intakes of plutonium would be greater than that to the circulatory system – the ERR/Gy estimated here based on liver dose would be lower than that based on dose to blood vessels and the chambers of the heart. At present information is not available from other studies of populations exposed to plutonium that would allow comparison of risk estimates for IHD in relation to such exposures. For these reasons, the findings in relation to internal exposure need to be interpreted with caution.

Comparison with other studies

Table 3 presents estimates of the ERR per Gy from the current study and from other studies of groups exposed to external low-LET radiation in which this trend was

estimated. It can be seen that the estimates from the various studies are mostly consistent. In particular, the incidence findings for Mayak PA workers are consistent with incidence and mortality results for the Japanese atomic studies. The wide confidence interval from the 15-country nuclear worker study means that these results are not greatly informative, whilst the morbidity data reported by Chernobyl clean-up workers – although yielding a trend estimate higher than that found here – are consistent with the Mayak findings. The only finding that is inconsistent with the Mayak results arises for workers at BNFL in the UK, whereas a larger study of UK radiation workers that included BNFL workers yielded a lower ERR estimate.

Table 3. Estimates of ERR per Gy (95% CI) for IHD following exposure to external low-LET radiation.

Cohort	Mean total dose (Gy)	Mortality or incidence?	Lag period (years)	No. of deaths or cases	ERR/Gy
Japanese A-bomb survivors: Life Span Study (Shimizu et al 2010)	0.20	Mortality	5	3252	0.02 (95% CI 0.10, 0.15)
Japanese A-bomb survivors: Adult Health Study (Yamada et al 2004)	0.57	Incidence	13	1546	0.05 (95% CI 0.05, 0.16)
Mayak PA workers (this study)	0.84	Mortality	10	1461	0.07 (95% CI 0.02, 0.15)
Mayak PA workers (this study)	0.84	Incidence	10	3133	0.12 (95% CI 0.05, 0.19)
Nuclear workers (international) (Vrijheid et al 2007)	0.018	Mortality	10	5821	-0.01 (95% CI 0.59, 0.69)
BNFL workers (UK) (McGeoghegan et al 2008)	0.053	Mortality	15	3567	0.70 (95% CI 0.33, 1.11)
UK National Registry for Radiation Workers (Muirhead et al 2009)	0.025	Mortality	10	7168	0.26 (95% CI 0.05, 0.61)
Chernobyl recovery operations workers (Ivanov et al 2006)	0.109	Incidence	–	10942	0.41 (95% CI 0.05, 0.78)

Conclusions

Having adjusted for non-radiation factors, there was statistically significant increasing trend in IHD incidence with total external gamma dose. This trend with external dose was little changed after adjusting for internal dose. Much of the evidence for the raised incidence in relation to external gamma dose arose for workers with cumulative doses above 1 Gy, although the data are consistent with a linear trend in risk with external dose. The trend with external dose in IHD mortality was not statistically significantly greater than zero, but was consistent with the corresponding trend in IHD incidence even once adjustment for internal dose was made. There was stronger evidence for an association with internal alpha dose to liver in the IHD mortality data than in the corresponding incidence data, although the associated confidence intervals overlap.

Furthermore, the estimated trend in IHD mortality with internal dose was lower and not statistically significant once adjustment was made for external dose. The risk estimates for IHD in relation to external radiation are generally compatible with those from other large occupational studies and the Japanese A-bomb survivors.

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Risk of thyroid cancer among Chernobyl liquidators

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Abstract

Aims: While the increased risk of thyroid cancer is well demonstrated in children exposed to radioactive iodines in the most contaminated areas around the Chernobyl power plant, the effect of exposure on adults remains unclear. The objective of the study was to evaluate the radiation-induced risk of this disease among Chernobyl liquidators.

Methods: A collaborative case-control study of thyroid cancer was conducted, nested within cohorts of Belarussian, Russian and Baltic liquidators of the Chernobyl accident. The liquidators were mainly exposed to external radiation, although substantial dose to the thyroid from iodine isotopes may have been received by those who worked in May – June 1986 and who resided in the most contaminated territories of Belarus. Individual doses to the thyroid from external radiation and from iodine-131 were estimated for each subject.

Findings: 107 case patients and 423 matched controls were included in the study. Median age at the moment of first exposure was 37 years. Most subjects received low

doses (median 69 mGy). The doses were much higher for women (median 196 mGy) than for men (median 64 mGy).

A significantly elevated Odds Ratio was seen at doses of 300 mGy and above. The overall Excess Relative Risk (ERR) per 100 mGy was 0.38 (95% confidence interval (CI): 0.10, 1.09). Risk estimates were similar for iodine-131 and external exposure – ERR per 100 mGy was 0.45 (95% CI: 0.10, 1.61) and 0.38 (95% CI: -0.11, 2.07), respectively.

Conclusions: Although higher than risk estimated from a-bomb survivors exposed as adults, the significantly elevated risk observed in the present study is similar to that obtained in the recent studies of thyroid cancer following exposure to iodine-131 in childhood in the areas contaminated after the Chernobyl accident. The increased risk appears to be related to iodine-131 exposure, as well as to doses from external radiation.

Lens opacities among physicians occupationally exposed to radiation

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Abstract

We estimated the prevalence of lens opacities in physicians occupationally exposed to radiation.

Based on a nationwide registry of 1312 physicians, mostly radiologists with occupational exposure to ionising radiation, 120 subjects were invited to participate and 59 (49%) consented. The inclusion criteria included age 45-70 years, cumulative recorded radiation dose >10 mSv, and duration of work with dose monitoring >15 years. The participants filled in a questionnaire regarding occupational history and other risk factors for lens opacities. A full ophthalmological examination was performed. Lenticular changes were graded using the Lens Opacities Classification System, version II (LOCS II).

Any lens opacities were detected in 42% (95% confidence interval [CI] 29-56) of the 57 physicians without prior cataract surgery. Nuclear opacities were found in 11% (95% CI 4-22), cortical 8% (95% CI 2-19) and posterior subcapsular in 5% (95% CI 1-15) of the subjects. The prevalence of lens opacities increased with age, smoking and cumulative recorded radiation dose. After controlling for age, sex and smoking, cumulative radiation dose was positively associated with any lens opacities (p=0.06). Intervention radiologists and cardiologists had higher rates of cortical and posterior lens opacities than the other physicians.

Our preliminary results show posterior subcapsular lens opacities in physicians exposed to occupational radiation, consistent with recent studies on low dose radiation exposure. A full study with an unexposed reference group for risk estimation is warranted.

Introduction

The prevalence of lens opacities varies by age and diagnostic criteria used. The aetiology of cortical cataract is related to ultraviolet radiation and diabetes, whereas established risk factors for nuclear cataract include nutritional factors and smoking (Taylor et al. 1988, West et al. 1989, Klein et al. 1999). Other factors associated with

cataracts include diabetes, steroid use, ocular injury, myopia and cardiovascular disease (Taylor et al. 1988, Leske et al. 1991, Delcourt et al. 2000).

Radiation cataract is a well established effect of high doses shown in experimental studies and also in humans among survivors of the Hiroshima and Nagasaki atomic bombs (Otake v1992, 1996, Minamoto et al. 2004, Nakashima et al. 2006). Radiation induces changes in the lens epithelium, typically in the posterior subcapsular part of the lens. Radiation-related cataract has been regarded as a deterministic effect, which occurs only after high doses, with a threshold of approximately 1 Gy.

However, this view has been challenged by recent studies, which have shown increased risk of lens opacities even after low dose exposure (Ainsbury et al. 2009). Re-analyses of atomic bomb survivor data have demonstrated that the findings are compatible both with threshold (about 1.5 Sv) and linear models (Otake et al. 1992, 1996, Minamoto et al. 2004, Nakashima v2006). Besides posterior subcapsular opacities, dose-dependence in frequency of cortical cataracts has been shown among A-bomb survivors. Posterior subcapsular opacities were also more frequent among Ukrainian children affected by the fallout from the Chernobyl nuclear power plant accident (with a prevalence of 1%) than in controls (Day et al. 1995). More than 50% of Icelandic airline pilots exposed to cosmic radiation had some lens opacities, and 7-8% of them had posterior subcapsular cataracts (Rafnsson et al. 2005).

We conducted ophthalmological examinations among radiologists and other physicians exposed to occupational radiation in order to determine the frequency of lens opacities, especially posterior subcapsular ones, and evaluated the relation of lens changes to occupational factors.

Material and methods

The population at risk was identified from the national occupational radiation exposure registry maintained by STUK - Radiation and Nuclear Safety Authority, a governmental institution responsible for radiation protection in Finland. At the time of the study, the registry covered a total of 1312 physicians monitored for radiation exposure.

Eligible subjects were physicians included in the occupational exposure registry aged 45-70 years, history of dose monitoring for 15 years or more, and a recorded cumulative effective dose exceeding 10 mSv. The information for assessment of eligibility was obtained from the dose registry. No separate dose estimates for the lens were available. For logistic reasons, the subjects had to be current residents of the Uusimaa region. All 120 subjects who fulfilled these criteria were invited to participate.

The year of start and duration of dose monitoring, as well as the recorded cumulative dose were obtained from the registry. The recorded doses are based on film dosimeters worn outside the lead apron at work and therefore they overestimate the effective dose by at least a factor of 10. The relation of the recorded dose to the exposure to the eye is complex and it is unclear if measurements above or below the lead apron are better indicators of ocular doses. Furthermore, the registry covers only doses exceeding the recording threshold, which was decreased over time from 3.0 mSv to 0.1 mSv in a three-month monitoring period (3.0 mSv in 1964 until 1974, 1.5 mSv in 1975-1979, 0.5 mSv in 1980-1988, 0.3 mSv in 1989-1997 and finally 0.1 mSv from 1998 onward). Recorded doses were not available before 1969 and earlier doses were missing. The frequency of missing dosimeter readings was very low (<0.1%).

The study was approved by the ethical committee of the Helsinki University Central Hospital (tracking no. 352/E9/06) and followed the tenets of the Helsinki Declaration.

All the 120 eligible subjects were approached by mail and invited to participate in the study. The radiological society was contacted prior to the study and it informed its members in advance. The letter explained the purpose and procedures of the study and included also the study questionnaire for collection of information on medical and work history, use of radiation shielding, and smoking. The 59 subjects who gave their consent were asked to fill in the questionnaire. Based on questionnaire data and ophthalmological examinations, two subjects with prior cataract surgery were excluded from the study.

The study subjects underwent a comprehensive eye examination at Department of Ophthalmology, Helsinki University Hospital after pupillary dilatation. The Lens Opacities Classification System, version II (LOCS II), was used to grade lens changes (Chylack et al. 1989). LOCS II utilises a set of standard slit-lamp and retroillumination color transparencies for grading different degrees of nuclear, cortical, and subcapsular cataract. The system has four grades for nuclear opalescence and colour, five grades for cortical, and four subcapsular opacities. If the two eyes differed in grade, the worse grade was recorded.

The 95% confidence intervals for prevalences were calculated using the binomial distribution. Frequencies of categorical variables were compared using the chi square test and for small frequencies the Fisher's exact test. Differences in mean age, cumulative dose and duration of work career between subjects with and without lens opacities were evaluated with the non-parametric Kolmogorov-Smirnov test (due to the skewed distribution). Logistic regression analysis was conduced with presence versus absence of lens opacities as the binary response variable. The main results are based on any lens opacities (LOCS grade I-II nuclear, cortical and posterior subcapsular opacities), but a separate analysis was also conducted for posterior and cortical changes combined (excluding nuclear opacities).

Results

The mean age at examination of the 57 physicians in the final analysis (28 men and 29 women) was 58 years (median 60, range 46-70). Of them, 42 were radiologists (including 9 interventional radiologists), 14 cardiologists and a surgeon. The mean duration of radiation work was 24 years (median 25, range 4-45). Eleven subjects (19%) reported having used protective eyewear (lead glass spectacles), but only six (11%) had used them regularly.

Thirty-three (58%) of the 57 examined physicians were free of any lens changes, while a lens opacity (LOCS II grade 1-2) was found in 24 (42%, 95% CI 29-56%; Table 1). Nuclear opacities were most common (six subjects; prevalence 11%, 95% CI 4-22%), followed by cortical changes (4 cases with LOCS grade 1-2, prevalence 8% 95% CI 2-19; with 7 additional subjects showing cortical traces 19%, 95% CI 10-32%). The physicians with any lens opacities were older (mean age 64 versus 54 years among subjects without any lens changes, $p<0.001$) and more commonly smokers than those without such eye changes. They also had had a longer career (30 versus 20 years, $p>0.001$) and a higher cumulative radiation dose (84 vs. 42 mSv, $p=0.02$).

Posterior subcapsular opacities were found in two subjects (4%, 95% CI % 1-15). One had bilateral changes and the other a grade II opacity. The two physicians with posterior subcapsular opacities were aged 68 and 70 years, and had radiation work histories of 31 and 36 years with cumulative doses of 11 mSv and 22 mSv. One was a radiologist and the other an intervention radiologist. In addition, an intervention radiologist with a previously operated cataract in one eye had a posterior subcapsular opacity in the other, non-operated eye.

All lens opacities combined, as well as posterior and cortical changes combined were associated with increasing age. Smoking was significantly associated with all lens opacities combined, but not with cortical and posterior lens changes. Starting radiation work prior to 1975 was associated with all types of lens changes, as well as cortical and posterior changes combined. Having been monitored for radiation exposure for at least 20 years was associated with all lens opacities and working with radiation on a daily basis with cortical and posterior changes combined.

Age and smoking status were associated with significantly increased prevalence of lens opacities in the logistic regression analysis even after mutual adjustment. Duration of radiation work was associated with the presence of any lens opacities in the bivariate analysis, but the association was no longer significant after adjusting for age.

When nuclear changes were excluded, the cumulative radiation dose was not associated with cortical and posterior opacities. Intervention radiologists and cardiologists had higher risk of cortical and posterior lens changes compared with other specialists (mainly other radiologists).

Discussion

Lens opacities were common among Finnish physicians exposed to ionising radiation, but the majority of them were nuclear opacities, which generally are not thought to be related to ionising radiation. Posterior subcapsular opacities, which classically are associated with ionising radiation, were found in 4% of the participants. Cortical opacities were found in 7% subjects, but another 12% had cortical traces, and if these findings are included the proportion of subjects with cortical opacities is as high as 19%, exceeding the number of nuclear findings. None of the subjects had a cataract that would require surgery. However, two had already undergone cataract surgery.

There was some indication for an association of lens opacities with the cumulative radiation dose, though the relation was no longer significant after adjustment for age. Further, this association was not observed when the analysis was restricted to cortical and posterior opacities only (the types of changes associated with radiation in earlier studies). The main limitations of our study are the small size and lack of an unexposed reference group.

Overall, the exposure levels were well below those traditionally thought as the threshold for cataract induction in the entire study group (mean 60 mSv, maximum 300 mSv), as well as those with the posterior subcapsular and cortical opacities (mean 65 mSv, maximum 72 mSv). However, recent studies have suggested increased cataract rates among subjects with occupational, environmental and medical radiation exposures with similar dose levels.

Radiation exposure varies substantially between radiologists. Those who perform interventional procedures such as catheterisations may receive doses approaching the

annual dose limits (currently, 20 mSv), whereas others are subjected to almost no radiation during their entire career. We selected the study subjects among those with the highest recorded doses, long work history and tasks with potential for radiation exposure. Yet, detailed information on frequency of performing various procedures was not collected. Nevertheless, those working with fluoroscopic intervention procedures (intervention radiologists and cardiologists) had a higher frequency of cortical and posterior opacities combined than the other physicians.

We had access to results of dose monitoring based on personal dosimeters. However, their readings are not a direct indicator doses for the eye. The recorded doses are likely to substantially overestimate ocular doses, because the dosimeters were worn at the chest level outside the lead apron. The radiation dose is primarily due to scatter from the patients and is highly non-uniform (Martin 2009). The dose to the eyes depends heavily on the amount of radiation delivered to the patient, proximity of the physician to the x-ray tube and use of protective devices such as lead spectacles or ceiling-mounted shields.

Infrequent use of eye protection may reflect a prevailing perception that risk for cataract is minimal or irrelevant. In our study, only one in five exposed physicians used any eye protection at all and only one in ten used them regularly.

Few previous studies have evaluated occurrence of lens opacities among medical personnel. Case reports indicate lens injuries following exposures substantially exceeding the dose limits (Vano et al. 1998). A recent study, without dosimetric information, showed a significantly increased frequency of cataracts among 35,705 U.S. radiologic technologists (Chodick et al. 2008). Similarly, an Italian showed increased prevalence of lens changes among radiologists and radiological technicians compared with unexposed medical workers (Milacic 2009). Further, posterior subcapsular cataracts in 8% and smaller dot-like lens opacities in 37% of the 59 participants of the annual meeting of the Society of Interventional Radiology (RSNA 2004).

Conclusions

Radiologists with any signs of cataract had a longer history of radiation work and a larger cumulative radiation dose than those not affected. Yet, risk of cataracts is strongly age-dependent and also cumulative dose and career length increase with age. After adjustment for age, sex and smoking the effect of dose was of borderline significance and duration of radiation work was no longer significant. Yet, a higher frequency of cortical and posterior lens changes was associated with interventional procedures even after adjustment for other factors. The study indicates the need for a more comprehensive study on radiologists to assess the effect of radiation on the lens of the eye.

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Occupational cataracts and lens opacities in interventional cardiology: The O'Cloc study

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Abstract

Interventional cardiologists are repeatedly and acutely exposed to scattered ionizing radiation (X-rays) during their diagnostic or therapeutic procedures. These exposures may cause damages to the eye lens and induce early cataracts known as radiation-induced cataracts. The O'CLOC study is a French epidemiological study designed to test the hypothesis of an increased risk of cataracts among interventional cardiologists as compared with unexposed cardiologists. This multicenter cross-sectional study will include a total of 300 cardiologists aged > 40 years: a group of interventional cardiologists (approx. 2/3 of coronary interventionists and 1/3 of electrophysiologists) and a group of unexposed cardiologists (clinicians or echocardiographists), matched for age, sex and place of work. Individual information, including risk factors of cataracts (age, diabetes, myopia, etc. ...), will be collected during a telephone interview. For the exposed group, a specific section of the questionnaire is focusing on their occupational history, the procedures description (type, frequency, use of radiation protection tools) and will be used to classify "comparable exposure level" groups according to their estimated cumulative dose. For all participants, clinical eye examinations will be performed to specifically detect cataracts even at the early stages (lens opacities, LOCS according to the international standard classification). The overall analysis will provide an estimation of the risk of cataract in interventional cardiology comparatively to not-exposed reference group, taking into account other risk factors. A complementary comparative analysis of risks according to the level of exposure is also planned. This epidemiological study will provide further knowledge on the potential risk of occupational radiation-induced cataracts in interventional cardiology and will contribute to the awareness of cardiologists in radiation protection.

Cardiovascular disease and radiation – review and meta-analysis of epidemiological evidence at low doses

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Abstract

Although the link between high doses of ionizing radiation and damage to the heart and coronary arteries has been well established for some time, the association between lower dose exposures and late occurring cardiovascular disease has only recently begun to emerge, and is still controversial. In this paper, we extend an earlier systematic review by Little and colleagues on the epidemiological evidence for associations between low and moderate doses of ionizing radiation exposure and late occurring blood circulatory system disease. Excess relative risks per unit dose in epidemiological studies vary over at least two orders of magnitude, possibly a result of confounding and effect modification by well known (but unobserved) risk factors, and there is statistically significant ($p < 0.00001$) heterogeneity between the risks. This heterogeneity is reduced, but remains significant, if adjustments are made for the effects of fractionated delivery or if there is stratification by endpoint (cardiovascular disease vs stroke, morbidity vs mortality). One possible biological mechanism is damage to endothelial cells and subsequent induction of an inflammatory response, although it seems unlikely that this would extend to low dose and low dose-rate exposure. A recent paper of Little et al. proposed an arguably more plausible mechanism for fractionated low-dose effects, based on monocyte cell killing in the intima. Although the predictions of the model are consistent with the epidemiological data, the experimental predictions made have yet to be tested. Further epidemiological and biological evidence will allow a firmer conclusion to be drawn.

Co-exposure to radiation and methyl mercury during critical phase of neonatal brain development in mice enhances developmental neurobehavioral defects

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Abstract
Organisms, including man, are continuously exposed to low doses of ionizing radiation as well as persistent and nonpersistent chemicals in the environment. Hence, in the process of developing numerical limits for environmental protection, there is a strong need to consider interactive effects between radiation and other environmental stressors. It is known that ionizing radiation, as well as methyl mercury, can give rise to neurotoxicological and neurobehavioural effects in mammals and that developmental neurotoxic effects can be seen after exposure during gestation. However, there is a lack of knowledge concerning effects and consequences from low-dose exposure during critical phases of perinatal and/or neonatal brain development and the combination of ionizing radiation and environmental chemicals. Epidemiological studies of patients with haemangioma have indicated that radiation exposures to the brain during infancy might deteriorate cognitive ability in adulthood. Ten-day old neonatal NMRI male mice were exposed to a single oral dose of MeHg (0.40 or 4.0 mg/kg bw). Four hours after the MeHg exposure the mice were irradiated with 60Co gamma radiation at doses of 0,2 and 0,5 Gy. The animals were subjected to a spontaneous behaviour test at the ages of 2- and 4-months, and the water maze test at the age of 5 months. Neither the single dose of MeHg (0.4 mg/kg bw) nor the radiation dose of 0.2 Gy affected the spontaneous behavior, but the co-exposure to radiation and MeHg caused developmental neurotoxic effects. These effects were manifested as disrupted spontaneous behavior, lack of habituation, and impaired learning and memory functions. Studies are continuing to verify the effects ant to elucidate possible underlying mechanisms.

DNA-Triplex-forming-oligonucleotides as a tool to target specific DNA sequences

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Abstract

Purpose: Triplex-forming-oligonucleotides (TFOs) are able to bind complementary DNA sequences in a sequence specific manner and are therefore a promising tool to manipulate genes or gene regulatory units in a cellular environment. TFOs might have also therapeutic potential e.g. as a carrier for Auger-Electron-Emitter (AEE) to target DNA of tumour cells. A main obstacle is the access of the TFOs to their targets in the cell nucleus. Thus we studied the intracellular biokinetics of TFOs with the focus in the intracellular transfer from the cytoplasm into the nucleus.

Methods: TFOs specific for the genes *cdkn2a*, *bcl2*, *brca1*, *chk2*, *cdk4* were designed using TFO Target Sequence Search (Univ. of Texas). DNA-Triplex-formation was confirmed by electrophoretic-mobility-shift assay (EMSA) in vitro. For biokinetic studies SCL-II cells were transfected by electroporation with Alexa488-labelled TFOs. Transfected cells were subsequently cultured for 1 h, 6 h, 12 h, 18 h, 24 h, 30 h, 48 h and 72 h and TFO signal intensity was determined in single cells and in isolated cell nuclei by flow cytometry (FACS-Canto II, BD) at each time point.

Results: Sequence design of TFOs by TFO Target Sequence Search (Univ. of Texas) for the desired genes is generally not suitable to predict DNA-Triplex-formation in vitro as could be demonstrated by EMSA. The desired Triplex-DNA-formation could be confirmed for 57 % of all tested TFOs by EMSA. The biogenetic studies showed that TFO-Alexa488 positive cells were detectable as soon as 1 h after transfection and the signal intensity remained constant for at least 30 h. 72 h after transfection the signal was less intense but still detectable. A substantial loss of Alexa488-labelled TFO positive cell nuclei was observed within the first 6 h post-transfection followed by a significant increase up to 18 h post-transfection.

Conclusions: Stable Triplex-DNA-formation in vitro can not be predicted by the sequence of TFOs only. TFOs initially located in the cytoplasm are re-located to the cell nucleus within 12 h after delivery of the TFOs probably during cell division.

Introduction

Triplex-forming-oligonucleotides (TFO) are short oligodeoxyribonucleotides, able to bind complementary DNA sequences in a sequence specific manner. They are major groove binding ligands which are associated to the DNA duplex via hydrogen bonds known as Hoogsteen-Bonds. Triplexes can only be formed with homopurine-homopyrimidine regions of the DNA (Kamenetskii 1995). The orientation to the target duplex can be parallel and antiparellel depending on the base composition of the TFO. While the bases thymine, cytosine and guanine are able to bind in both orientations, triplexes with adenine as the third base can only form a triplex in antiparallel conformation. TFOs containing cytosine form triplexes only under acidic conditions as the cytosine has to be protonated (Praseuth et al. 1988).

Regions containing homopurine-homopyrimidine sequences are quite common in the human genome as could be demonstrated by Goni et al. 2003. The presence of a third strand in the major groove can influence the behaviour of the DNA, changing its ability to recognize specific proteins and consequently altering mechanisms controlling DNA function. Despite the direct interaction of TFO and DNA it can also serve as a strong tool, for example as a carrier system in biotechnological and biomedical applications (Vekhoff et al. 2008).

During the decay of 125I a shower of low-energy Auger-electrons is emitted leading to the deposition of very high energies in a rather small volume (Pomplun et al. 1987). Panyutin et al. 1994 showed that 125I-labelled TFOs binding to DNA lead to a significant increase of double strand breaks. Though the energy deposition of the Auger electrons is limited to a 10 to 100 nm sphere at the decay site, the damage to the cellular environment will be only minor, even at high activity concentrations of 125I (Sedelnikova et al. 1998, Kriehuber et al. 2004). On the contrary, the decay of 125I close to its target will induce significant damage which is difficult to repair (Sedelnikova et al. 1998). Referring to these characteristics the TFO can serve as a tool to silence genes very specifically without necessarily killing the cell itself. This application could be useful for Antigene Radiotherapy (Panyutin 2003), which is the generic term for damaging of selected genes by a high dose of radiation from radionuclides delivered to this gene by specific DNA-binding molecules.

One of the main problems, despite the binding of the TFO to its target sequence, is the delivery of the TFO to the point of action, usually the cell nucleus. Alexa488-labelled functional TFOs were used to examine the dispersion and distribution of TFOs in cells and isolated cell nuclei after transfection of tumour cells by electroporation employing flow cytometry at different time points post-transfection. Viability and apoptosis induction was analyzed in parallel.

Material and methods

Oligonucleotides and TFO Labeling

TFOs and biotinylated oligonucleotides were designed employing TFO Target Sequence Search (Univ. of Texas) and synthesized by Metabion (Martinsried) in standard quality. Alexa488-labelled TFOs were purchased by TIB Molbiol (Berlin). Labeling of TFOs with ^{125}I was performed employing the Primer Extension Method. Therefore the preTFOs (TFOs used for the Primer Extension reaction) and template

(200 pmol each) were annealed in 1 x Klenow buffer (500 mM Tris-HCl, pH 8.0, 50 mM $MgCl_2$, 10 mM DTT) for 10 min incubation at 90 °C followed by slow cooling (1 °C/min) to room temperature. Primer extension reaction was carried out in the presence of 10 µCi ^{125}IdCTP (Perkin Elmer, Rodgau) by 2.5 units of exonuclease-free Klenow fragment (Fermentas, Leon-Rot) in a total volume of 10 µl. After 15 min at 37 °C the reaction was stopped by heating to 70 °C for 10 min. The duplex-DNA product was purified by ethanol precipitation and resuspended in TEN_{100}-Binding Buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 100 mM NaCl). The labelled duplex-DNA was bound to Streptavidin Magnetic Particles (Roche, Mannheim) by incubation for 10 min at room temperature followed by two washing steps with TEN_{1000} Wash Buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 M NaCl). The magnetic particles were resuspended in TES Buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 150 mM NaCl). The duplex-DNA was denatured by addition of NaOH to a final concentration of 200 mM for 5 min. The magnetic particles were then removed with a magnet and the supernatant containing the ^{125}I-TFOs was collected, ethanol precipitated and resuspended in nuclease free water (Qiagen, Hilden). At this step the amount of incorporated ^{125}I-dC was calculated by activity measurement on a PerkinElmer 1480 Automatic Gamma-Counter. Labelled TFOs were stored at -70 °C.

Amplification of Target DNA-Fragments from DNA isolates of SCL-II cells

PCR Primer sets were designed for the amplification of DNA fragments containing the specific target-sequence for the labelled TFOs. The primers were synthesized by Metabion in standard quality. The sequence lengths varied from 200 bp to 2000 bp and the amplification was carried out on a TProfessional Gradient Cycler (Biometra, Goettingen). After the amplification the DNA fragments were ethanol precipitated, resuspended in water and stored at -20 °C.

In Vitro Triplex Formation and Binding Assay

^{125}I-labelled TFO (~ 0.6 µCi) were mixed with the amplificated DNA fragment (~ 10 µg) containing the specific TFO target sequence and TFO-binding-buffer (10 mM Tris-HCl, pH 5.8 and pH 7.5, 10 mM $MgCl_2$, 1 mM Spermidine) in a total volume of 24 µl. The mixture was incubated at 37 °C for 24 h. After the incubation the triplex was frozen for at least 30 days (0.5 half-life of ^{125}I) at -70 °C for decay accumulation. For *in vitro* verification of triplex formation an electrophoretic mobility shift assay (EMSA) was performed. Aliquots of the samples were loaded on a 12% native polyacrylamid gel containing 10 mM $MgCl_2$ and ran at 150 V for 20 h in TBE buffer (Tris-Base 89 mM, pH 8.0, boric acid 89 mM, EDTA 2 mM). Afterwards the gel was silver stained for visualization. Triplex formation was detected by band shift in silver stained gels and additionally verified by exposition to Fujifilm MS Imaging Plates BAS-MS3543 and visualization with a FLA-5000 Imaging System (Fujifilm, Düsseldorf).

Cell Line and Culture Conditions

SCL-II (squamous carcinoma cell line II) cells were grown in minimum essential medium Eagle (MEM, PAA Laboratories GmbH, Cölbe) with L-Glutamine, supplemented with 16 % fetal bovine serum (FBS , PAA Laboratories GmbH, Cölbe) in

a water-saturated atmosphere of 5 % CO_2 at 37° C as previously described (Kriehuber et al. 2004) and cultivated at standard conditions.

Transfection experiments

Cells were grown in cell culture flasks (PAA Laboratories GmbH, Cölbe) to 70-80 % confluency, trypsinized and resuspended in MEM. The required number of cells ($2x10^6$) was centrifuged for 10 min at 90 x g and resuspended in 100 µl Nucleofector Solution (Lonza AG, Cologne). 5 µl of Alexa488-labelled TFO [20 pmol/µl] (TIB Molbiol, Berlin) was added and the electroporation reaction was performed on the Amaxa Nucleofector Device I (Lonza AG, Cologne) employing transfection program L-013. Immediately after the reaction 500 µl of MEM was added and the cell solution was transferred into a 6-well plate at a cell concentration of $3x10^5$ cells/well and, after adding 2 ml MEM, incubated at 37 °C.

Flow cytometric Analysis of Transfected Cells

SCL-II cells transfected with Alexa488-labelled TFO were incubated at 37 °C. After 1 h, 6 h, 12 h, 18 h, 24 h, 30 h, 48 h and 72 h at each time samples were trypsinized and washed two times in PBS buffer. For analysis of cell nuclei the cells were further processed following a nucleic isolation protocol. Cells were incubated with Cell-Extraction buffer (Sucrose 320 mM, Hepes 10 mM, $MgCl_2$ 5 mM, Triton X-100 1%, pH 7.4) for 10 min on ice followed by two wash steps with Nucleic-Wash buffer (Sucrose 320 mM, Hepes 10 mM, $MgCl_2$ 5 mM, pH 7.4). Finally, cell nuclei were resuspended in Nucleic-Wash buffer. According to the samples, controls transfected with non-labelled TFOs and without any TFO were performed according to the above protocol. Transfected cells and isolated cell nuclei were analyzed in a flow cytometer (BD, Canto II) for presence of Alexa488 signal. Apoptosis induction was quantified employing non-labelled TFOs using the Annexin-V-FITC/PI-assay (BD, Heidelberg) according to the manufacturer's recommendation.

Results

Triplex formation *in vitro*

A variety of oligonucleotides (Table 1.) were analyzed for their ability of triplex formation with their specific DNA-target *in vitro*, employing electrophoretic mobility shift assay (EMSA). About 43 % of the tested oligonucleotides (not all displayed), all having a suitable sequence to form a Triplex-DNA with their specific DNA-targets, failed to form a DNA-triplex in the EMSA *in vitro* (Fig.1, Lanes 2, 17 and Table 1.). Triplex formation *in vitro* can therefore not be predicted on TFO and DNA-target sequence only. Target sequences for the functional TFOs were located in the genes *cdkn2a*, *bcl2*, *brca1*, *chk2* and *cdk4* (Table 1.). Non-complementary TFOs (Non-sense TFOs) failed in all cases, as expected, to form DNA-triplexes (Fig.1, lanes 3, 6, 9, 12, 15, 18, 22, 25).

Triplex formation of ^{125}I-labelled TFO with its specific target sequence could be demonstrated and verified using an autoradiographic assay (Fig.2 a; red arrow). The lower band (green arrow) represents an enrichment of fragments, resulting from the special gel running conditions and characteristics of a low pH electrophoresis (pH 5.8)

(Osafune 2004). After verification of triplex formation, samples were frozen at -70°C for decay accumulation.

Biokinetic of TFOs in human cells *in vitro*

The flow cytometric analysis showed that immediately after transfection Alexa488-labelled TFOs were distributed throughout the cells as TFOs could be detected 1 h post-transfection in whole cell samples and in isolated nuclei (Fig.3). A transfection efficiency of ~ 80 % in whole cells and ~ 60 % in isolated nuclei was observed. Within the first 6 h post-transfection a substantial loss of TFO positive cells and cell nuclei (~ 65 % and ~ 15 % respectively) occurred, which corresponded to an increase of apoptotic cells (Fig.3, black line), followed by a significant increase of TFO-positive cells and nuclei up to 18 h post-transfection (~ 85 % and ~ 25 % respectively) where it remained constant for further 6 h. Between 30 h and 72 h after transfection a smooth decline of TFO positive cells and cell nuclei was detected. The percentage of apoptotic cells reached its maximum 12 h post-transfection and constantly declined to control level at 72 h post-transfection.

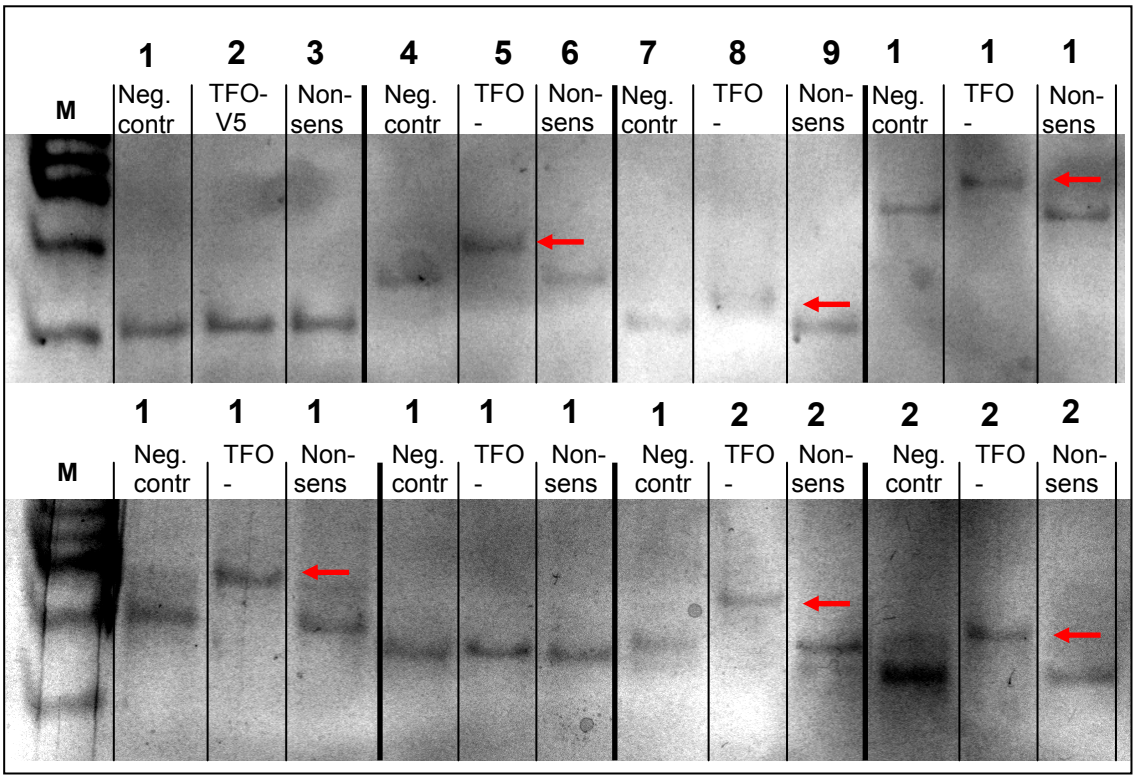

Fig. 1. Triplex formation *in vitro* visualized with electrophoretic mobility shift assay. A band shift shows successful triplex formation (red arrow; lane 5, 8, 11, 14, 20 and 24); TFO-V5 and TFO-V11 failed to form a DNA-triplex (lane 2 and lane 17). Native polyacrylamid gel; silver staining; Neg. contr.: Controls containing the dsDNA target fragment only (lane 1, 4, 7, 10, 13, 16, 19 and 23). TFO: Triplex forming oligonucleotides with their specific target sequence (lane 2, 5, 8, 11, 14, 17, 20 and 24). Non-sense.; Non-sense samples containing the target sequence with a non-specific (non-sense) TFO; TFO-V4 (lane 3, 6, 9, 12, 15, 18, 22 and 25).

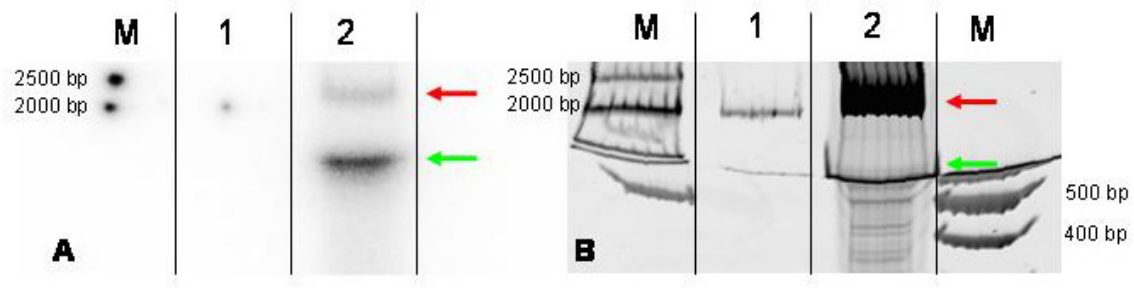

Fig. 2. Triplex formation of ^{125}I-labelled TFO-V13 with its specific target on a 1910 bp dsDNA fragment *in-vitro*. Electrophoresis in 10 % native Polyacrylamide gel (pH 5.8). (A) Autoradiographic scan. M; marker. Visualization of labelled TFO bound to its target (red arrow). Concentration of shorter fragments (green arrow) in lane 2. (B) Same gel stained with ethidium bromide. M: marker. Lane 1: dsDNA target fragment. Lane 2: TFO with high concentrated target fragments (red arrow). Concentration of shorter fragments (green arrow).

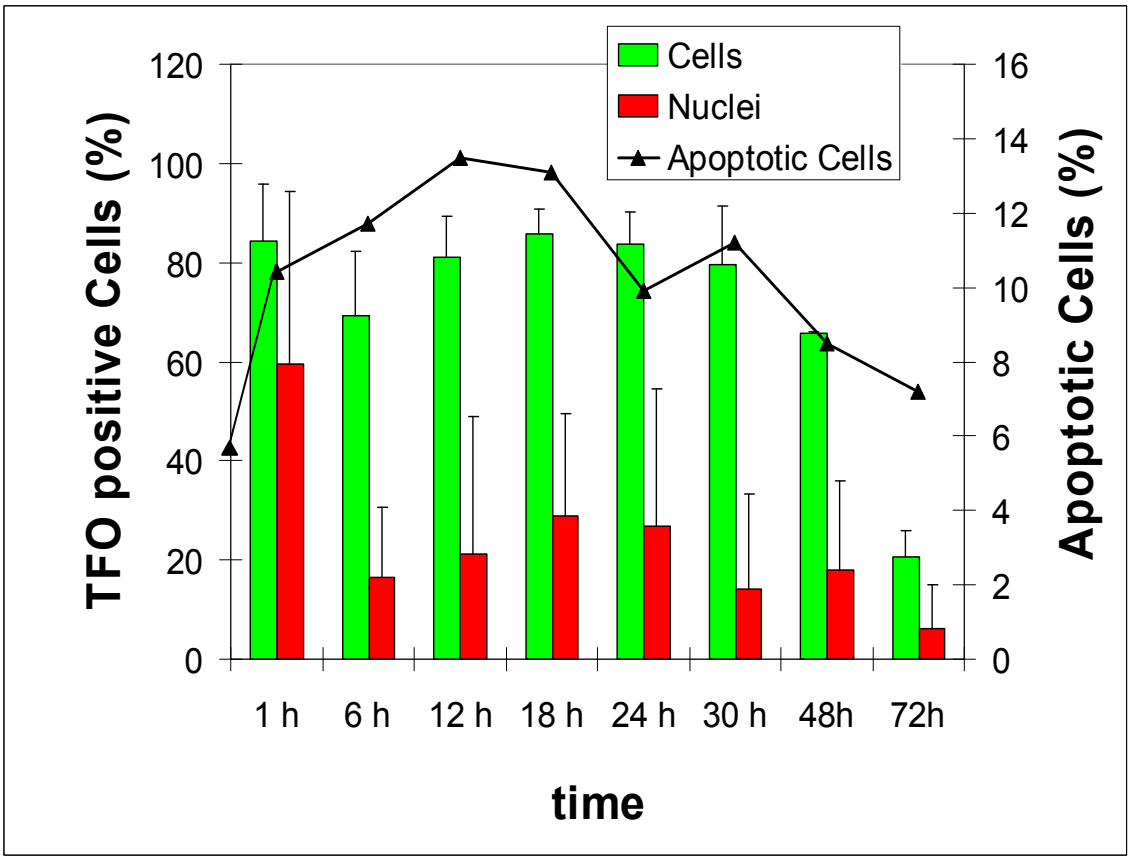

Fig. 3. Flow cytometric analysis of SCL-II cells after transfection with Alexa488-labelled TFO-V2 (N=4). Cells were transfected by electroporation with 100 pmol of Alexa488-labelled TFO-V2 and incubated at 37 °C. Cell and nucleic samples were prepared for flow cytometric analysis after different time points (1 h – 72 h). Percent of TFO positive cells (green bars, left axis) and cell nuclei (red bars, left axis) plotted against time after transfection. Cell samples transfected with non-labelled TFO-V2 were additionally analyzed for apoptotic cell death (black line); percent of apoptotic cells (right axis) plotted against time after transfection.

Table 1. Nucleotide sequence of binding TFOs and corresponding target sequence. Marked in red and bold-typed the nucleotide which were used for ^{125}I-labelling. Asterisks (*) indicates no triplex formation could be detected for these TFOs.

Name	TFO-Sequence (5´- 3´)	Target-Sequence (5´- 3´)	Gene (locus)
TFO-V2	**ttt gtt ggg tgg tgg gtt ggt tgt gtt**	**aag aga agg aag gga gga ggg aag aaa**	*cdkn2a* (21969523)
TFO-V5*	**gtt gtg tgt gtg tgt gtg**	**ctt ctc tct ctc tct ctc**	*survivin* (76219872)
TFO-V6	**ttg tgg gtg tgg tgg gct tt**	**aaa ggg gag gag agg gag aa**	*cdk4* (58148139)
TFO-V7	**gtt gtg gtt gtg gtt ggg**	**ggg aag gag aag gag aag**	*cdk4* (58148122)
TFO-V8	**ggt gtt ggt tgg ttg ttt tgg tgg tgg gc**	**ggg gag gag gaa aag aag gaa gga aga gg**	*bcl2* (60874077)
TFO-V9	**gtg ttt gtt ttt gtt ggg tgg tgt ggg gc**	**ggg gga gag gag gga aga aaa aga aag ag**	*bcl2* (60796002)
TFO-V10	**tgg gtg tgt gtt ggt gtg ttg ttc c**	**gga aga aga gag gaa gag aga ggg a**	*brca1* (41203138)
TFO-V11*	**tgg gtg gtg ggt gtt tgt tgt g**	**g aga aga aag agg gat gag gga**	*brca2* (32932129)
TFO-V12	**ggg tgg tgt ggg tgg gtt ttt ttt**	**aaa aaa aag gga ggg aga gga ggg**	*chk2* (29133020)
TFO-V13	**ggg ttt ttt gtg ggg gtg ttc**	**gaa gag ggg gag aaa aaa ggg**	*chk2* (29099560)

Discussion

The TFO Target Sequence Search (Univ. of Texas) proposed functional TFO sequences, which did not form DNA triplexes *in vitro* with their specific target sequence (Fig.1 and Table 1). Although all TFOs were designed according to the following special criteria: - minimum length of 15 bp, - guanine content minimum 50 %, - no pyrimidine interrupts allowed, - default settings for TFO-target binding at near-physiological conditions (pH 7.2, 37°C, 10 mM $MgCl_2$), it could be clearly demonstrated that every single TFO has to be confirmed in EMSA.

To obtain sufficient Triplex formation with ^{125}I-labelled cytosines the pH of the binding buffer had to be adjusted to pH 5.8 to protonate the cytosine in order to get proper Hoogsteen hydrogen bonds. However, pre-testing confirmed triplex formation for non-labelled TFOs with a maximum of two cytosines under physiological conditions, which is consistent to the findings of Duca et al. 2008.

The biokinetic studies confirmed that the applied Amaxa transfection protocol caused an initial efficient transfer of TFOs into the cell nucleus (Fig.3.). The substantial loss of TFO-positive nuclei (- 40 %) within the first 6 hours is probably caused by the chosen transfection procedure, by which a direct initial transfer of TFO into the cell nucleus occurs (Hamm et al. 2002). After transfection, a pronounced increase of

apoptotic cells was recorded. Though this effect is not described in the literature for TFO after electroporation, it might be assumed that the high TFO concentration led to the observed increased apoptotic cell death by a nucleotoxic effect, as described by Shen et al. 2003 in human lung carcinoma cells transfected with TFOs by a liposome-based transfection method.

The increase of TFO positive cell nuclei 6 – 24 h post-transfection reflects either an active transfer of TFOs into the cell nucleus by still unknown processes or a passive relocation of TFOs into the cell nucleus during mitosis. The observed transfer to the cell nucleus is in good agreement with the data published by Sedelnikova et al. 1998 and 1999, showing a rapid relocation of radionuclide-labelled TFOs into the nucleus within 3 h after delivery to the cytoplasm using DMRIE-C liposomes.

The observed significant decrease of TFO-positive cells 48 h post-transfection is probably due to dilution caused by ongoing cell division. Degradation processes cannot be generally excluded but are unlikely since Sedelnikova et al. 1999 could confirm triplex stability in HeLa cells for at least 48 h and Kadenbach et al. 2005 showed non-degradation of TFO by FRET in SCL-II cells for at least 24 h post-delivery.

Conclusions

TFOs, either non-labelled, fluorochrome-labelled or ^{125}I-labelled, are suitable to form stable DNA-triplexes with their specific target sequences *in vitro*. It can be concluded from our results that the prediction of DNA-triplex-formation shall not be based on the TFO sequence alone, but has to be approved by additional experimental methods, like the electrophoretic mobility shift assay. Within the first 18 h after transfection a fraction of TFOs was relocated from the cytoplasm into the nucleus, probably by the break-down of the nuclear envelope during cell division. TFOs can be detected in considerable amounts in the cytoplasm and in the cell nucleus at least 48 h post-transfection. Electroporation is a suitable method to deliver TFOs in cells and cell-lines, although the used transfection procedure induced enhanced apoptotic cell death in the transfected cells, especially in cells, where TFOs were initially directly transfected into the cell nucleus.

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Molecular, metabolic and genomic response of TPC-1 cells to external X-irradiation

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Abstract

The release of radioactive iodine isotopes into the atmosphere in the wake of the Chernobyl disaster and the subsequent increase in the cases of thyroid cancer amongst children provided us with solid evidence of the link between irradiation and thyroid cancer. The link between the radiation dose and the adverse effects on human health has been established and a linear correlation drawn. However, that correlation only holds for doses higher than a certain threshold, below which there is lack of unanimity in the scientific community. We attempted to subject TPC-1, a cell line of human papillary thyroid carcinoma origin to a range of X-ray doses from low to high (0.0625 to 4 Gy) and test their effect on cellular proliferation, apoptosis and molecular signaling to uncover the effect of low doses of radiation on them. We observed a decrease in proliferation concomitant with a decrease in cells in the S phase of the cell cycle even at the lowest dose. On the other hand, there was no significant increase in apoptotic or necrotic cells at the same time point. On the protein level, an increase in p53 and some of its targets (e.g. p21 and mdm-2) was observed whereas an absence of p73 was apparent. An up-regulation of anti-apoptotic proteins such as Bcl-2 and Akt was also noted. A microarray run was performed and revealed an up-regulation in the p53 pathway whereas one gene, RFLP-1, appeared to be consistently up-regulated at all doses tested. Further research into this gene are underway. In addition, analysis of the metabolic profile of control and irradiated cells will be done on the supernatants of these cells. Metabolomics is a relatively new high throughput technique and is sensitive enough to hopefully measure the effect of low doses of radiation. Finally, analysis of the changes in microRNAs in irradiated versus control cells is currently underway.

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Irradiation of mouse zygotes and genomic instability: influence of genes involved in DNA repair, cell cycle regulation and apoptosis

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Abstract

Studies under way in our laboratory aim at investigating how heterozygous mutations in important genes might influence the radiation sensitivity of early embryos. We mainly focus on the zygote (first day of gestation in mammals), which occurs while women cannot be aware of pregnancy. Moreover, x-irradiation of wild-type zygotes has been reported to result in the development of a genomic instability in two different mouse strains. The results presented here concern the development of such effect after x-irradiation of zygotes of other strains, carrying a mutation in either the p53 gene or the PARP gene. P53+/- embryos were obtained by mating CF1 p53+/+ females with CF1 p53-/- males, while PARP+/- embryos were obtained by mating PARP-/- females/males (C57BL genetic background) with C57BL males/females. Females showing a vaginal plug were x-irradiated 2 h after fertilization (0.2 or 0.4 Gy) and sacrificed on day 8 of gestation. Their gastrulas were collected and cultured for 7 h in rat serum supplemented with colchicine and their cells were fixed for chromosome analysis. The frequencies and types of chromosome anomalies did not differ between control p53+/- and p53+/+ cells or between control PARP+/- and PARP+/+ cells. Anomalies were essentially of the chromatid-type, with a majority of chromatid gaps. No increase of chromosome damage was found after irradiation of p53 +/- or PARP +/- zygotes and, again, chromatid-type anomalies largely predominated over chromosome-type anomalies. These results differ from those obtained by others in wild-type embryos irradiated with higher doses. Moreover, they suggest that the presence of one mutated allele for two important genes would not result into an increased risk of developing a chromosomal instability after irradiation with moderate doses at the zygote stage. Similar studies are under way with other important DNA responses genes (supported by a contract between the SCK•CEN and the Federal Agency for Nuclear Control).

Individual sensitivity in targeted and non-targeted effects of radiation – ATM as a model for characterizing individual susceptibility

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Abstract

The aim of this study is to investigate the role of genetic heterogeneity in the *ATM* gene with respect to the individual variation of response to ionizing radiation. The issues of targeted and non-targeted effects particularly at low doses will be addressed. ATM is a major activator of cellular response to DNA-double strand breaks. *ATM* germ line mutations cause ataxia-telangiectasia (AT), a rare resessive autosomal disorder. Female carriers are phenotypically normal but there is evidence of increased risk of breast cancer among these women. We hypothesize that cells from *ATM* mutations carriers are more susceptible to bystander signals than ATM-proficient cells. The experimental system is based on co-culture technique where irradiated cells communicate through media with unirradiated cells. The co-culture system will be established by using human lymphoblastoid cell line TK6 cells that are irradiated by X-rays with doses of 0.01, 0.1, 1, and 2 Gy and co-cultured with unirradiated bystander cells for 1 hour and 18 hours. The induction of direct and bystander effects of radiation will be analyzed using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] cell viability assay, apoptosis and chromosomal aberration assays. In addition to results from TK6, corresponding data from experiments using cell lines with different *ATM* mutations will be evaluated against the wildtype *ATM* gene.

Influence of genetic polymorphisms on the yield of chromosomal aberrations among Estonian Chernobyl cleanup workers

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Abstract

The frequency of chromosomal aberrations (CAs) in peripheral blood lymphocytes has been applied as a biomarker for biodosimetry of radiation. Individual variability in CA frequency is known to be caused by several factors such as age and various kinds of exposures as well as genetic factors. The aim of the present study is to evaluate the effect of individual susceptibility to the amount of CAs in Estonian Chernobyl cleanup workers. The data- and biobank of a study cohort of 4832 men contains detailed information of, e.g., recorded doses, smoking habits, medical radiation exposure, and health history. From this cohort, 300 men with doses of 48–300 mSv were chosen to the present study. In addition, 100 unexposed Estonian men were used as a control group. CAs were analyzed in peripheral blood lymphocytes using the fluorescence in situ hybridization (FISH) technique. In order to investigate the generic susceptibility, we studied single nucleotide polymorphisms (SNPs) in DNA repair genes, i.e., ATM (Phe858Leu, Pro1054Arg, Asp1853Asn, intron 5, rs228599 and intron 61, rs664143), LIG4 (Thr9Ile, Asp568Asp), XRCC1 (Arg194Trp, Arg280His, and Arg399Gln), XRCC3 (Thr241Met), XRCC5 (intron, rs3835 and 5' near gene, rs11685387), and XRCC6 (Gly593Gly and 5' near gene, rs2267437) and in an apoptosis gene, i.e., CASP8 (Asp302His and 5' near gene, rs6747918). The individual yield of CAs (particularly translocations) will be statistically analysed with respect to the SNPs, recorded dose, and other confounders. The results will bring more knowledge to the underlying mechanisms of individual variation in the induction of CAs. The information is also essential in the context of the well-established association between high CA frequency and increased cancer incidence.

The frequency of the chromatid breaks in G2 peripheral blood lymphocytes as an *in vitro* indicator of human individual sensitivity to ionizing radiation

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Abstract

Individuals differ in their response to ionizing radiation related to human individual radiosensitivity. This radiosensitivity has been linked with the sensitivity of human cells to radiation and other DNA-damaging agents. The radiosensitivity of human lymphocytes is commonly assessed by the frequency of micronuclei or chromatid breaks after *in vitro* irradiation of peripheral blood lymphocytes in the G_0- or G_2-phase of the cell cycle.

This study was performed to test the ability of these predictive indicators of intrinsic radiosensitivity for the identification in the normal population of individuals with increased radiosensitivity. The study was carried out with blood samples taken from 29 healthy donors and irradiated with 1 Gy of X rays to induce DNA damage converted into micronuclei or chromatid breaks scored in cultured peripheral blood lymphocytes at interphase (G_o assay) or metaphase (G_2 assay).

The results obtained indicate that chromatid breaks in peripheral blood lymphocyte can be the indicator of choice for the assessment of individual radiosensitivity because they are sensitive and specific to radiation and have low background frequency.

Introduction

Individuals differ in their response to ionizing radiation related to human individual radiosensitivity. There are radiosensitive sub-groups with hypersensitivity to carcinogenic risks (stochastic effects) and hypersensitivity to deterministic effects (Hoeller *et al*.,2003). Identification of individuals with increased inherent radiosensitivity is of relevance for their protection from the adverse effects of radiation after occupational or environmental exposure and after radiotherapy in oncology.Both situations require, however, a good indicator of individual radiosensitivity.

Radiation can induce a large spectrum of DNA damage in human cells. In general, damage located on one DNA strand is more effectively repaired than that affecting both strands. Misrepaired or non-repaired double strand breaks (DSB) are

considered as the main lesions involved in cellular radiosensitivity and predisposition to cancer (Pichierri *et al.*,2000). In this regard, radiosensitivity would result from mutations or polymorphism of cancer predisposition genes, which are involved, in the repair of radiation-induced DSBs or their conversion in chromosome damage, which is linked to the signaling of the DSB to the checkpoint mechanism (Terzoudi *et al.,*2009). If the chromosomal radiosensitivity in human cells has been attributed to low DSB repair activity or to an impaired cell cycle control, increased yield of chromosomal damage after in vitro cell irradiation, can be a predictive indicator of radiosensitive individuals and patients. To date two such indicators are commonly used for predicting individual radiosensitivity. These are: micronuclei scored at interphase after the in vitro irradiation of human peripheral blood lymphocytes in the G_o-phase of the cell cycle (Champion *et al.,* 1995) and chromatid breaks scored at first metaphase following in vitro G_2-phase lymphocyte irradiation (Terzoudi *et al.,* 2006, Terzoudi *et al.,* 2009). Micronuclei and chromatid breaks are considered to result from non- or misrepaired DSBs (Natarajan, 1993). They are known to be repaired by the two processes non-homologous end joining (NHEJ) and homologous recombination (HR) that depends on the phase of the cell cycle. While NHEJ was shown to be active in all cell phases, HR is involved only in late S and G2 phase (Bentomane, 2004; Jeggo, 1990). In cultured peripheral blood lymphocytes DSBs induced in G_o-phase are processed during G_1 to S-phase transition so that the yield of residual lesions visualized and quantified at interphase cells as micronuclei reflects the G_1 checkpoint potential to facilitate recognition and repair of radiation induced DSBs before entering the S phase. Consequently, the yield of G_2 chromatid breaks represents the effect of the G_2 checkpoint during G_2 to M- phase transition of cultured peripheral blood lymphocytes. A variation in the yield of micronuclei or chromatid breaks between individuals has been correlated to variations in individual radiosensitivity (IR). Since inter-individual variation in radiosensitivity obtained for examined individuals are described by normal distribution with a mean value (MV) and a standard deviation of the mean (SD), individuals may be classified as normal when IR=MV±SD, resistant when IR< MV-SD and sensitive when IR> MV+SD (Terzoudi *et al.*, 2006).

Both predictive assays for individual radiosensitivity were adapted at the CLOR for monitoring of occupation health and safety. The aim of this study was to test the ability of these assays for the identification of individuals with increased radiosensitivity in order to direct to more relevant screening for cancer susceptibility.

Material and methods

Subjects and blood donors

The subjects of this study comprised 29 healthy female and male donors employed at CLOR. Informed consent was obtained from each of the donor after an explanation of the objective of the study.

Whole blood samples (about 5 ml) were collected into heparinized tubes. Half of blood was irradiated with 1 Gy of X-rays for inter-individual analysis of variations in radiosensitivity measured using G_0- and G_2 -assays, while the other half was kept as a non-irradiated control. Peripheral blood lymphocytes were cultured adding 0.5 ml of whole blood to 4.5 ml PB-MAX Karyotyping Medium (Gibco). This medium was

composed of liquid PRMI-1640 basal medium supplemented with 10% Fetal Bovine Serum, 1% phytohemagglutinin (PHA), 1% L-glutamine, gentamicin sulfate at 35 mcg/ml and 1% heparin (Sigma).

Irradiation

Irradiation of whole blood or proliferating peripheral blood lymphocytes was performed at room temperature using a PANTAK X-ray machine operating at 243,0 kV, 10 mA with additional filtration by 1,62 mm Cu and 4 mm Al. The average energy of X-rays was 122 kV. The measured dose rate at the distance of 50 cm from a focus, which was a point of irradiation, was 0,35 Gy/min. Before every irradiation the standard value of dose was measured by PTW-UNIDOS electrometer with 0,6 cc volume ionization chamber.

G_o-assay

G_o-assay was performed according to the cytochalasin-B-blocked micronucleus assay described by Fenech *et al.,* 1986. Briefly, immediately after irradiation duplicate lymphocyte cultures were incubated for 72 hours at 37°C in a humidified incubator in an atmosphere of 5% $C0_2$ and 95% air. After 48 h of stimulation with PHA, cells were treated for 24 h with 6µl/ml of cytochalasin-B (Sigma) in order to block cytokinesis. Thereafter, cells were submitted to hypotonic stress (0.075M KCl) for 20 minutes and fixed three times in 3:1(v/v) methanol/acetic acid. Fixed cells were pooled and dropped on preclined wet slides, air-dried for at least 24 h and stained with 2 % Giemsa solutions for 10 minutes. Micronuclei were scored in binucleated cells with well-preserved cytoplasm with a light microscope (Nikon) using a 1000x magnification. 1000 binucleated cells from various slides were scored for each donor and at a given dose. Micronuclei were considered if their dimension did not exceed one third of the diameter of the main nucleus, with the exception micronuclei touching the main nuclei as well as free-lying nuclei.

G_2-assay

G_2-assay was carried out as described by Terzoudi and Pantelias, (2006). Duplicate lymphocyte cultures were incubated for 69-70 hours at 37°C in a humidified incubator in an atmosphere of 5% $C0_2$ and 95% air before their use for radiosensitivity measurements. Half of proliferating cells were then irradiated with 1 Gy and incubated for 30 minutes at 37°C to allow division of cells irradiated at mitosis. Lymphocytes were than blocked in metaphase by the addition of 0.15µl/ml Colcemid (Gibco) to the cell cultures (Gibco) for 60-90 min. Further preparations of metaphase arrested cell was similar to that described for the G_o-assay. However, for better scoring of chromatide beaks an additional wash with 5% acetic acid was added after the hypotonic stress. The aim of this modification was to produce metaphases with well spread chromosomes as free as possible from the surrounding cytoplasm or nucleoplasm. For each donor and dose, 250 metaphase cells were scored for chromatid breaks. Chromatid gaps were scored as breaks only when they were longer than a chromatid width (Fig.2.).

Results

To classify healthy individuals according to their radiosensitivity, we analysed the frequency of chromatid breaks in peripheral blood lymphocytes from 19 donors. The mean age in this group was equal to 41 years and the range of age was between 25 and 64 years. Only for one donor we found 2 spontaneous chromosome breaks in 250 scored cells that was substracted to obtain the radiation-induced yield of aberrations. The mean, standard deviation of the mean, median, lowest and highest values of chromatid breaks frequency in control and irradiated cells are sumarized in Table 1. When data were analysed using a normal distribution, the mean value of the frequency of radiation-induced chromatid breaks was determined to be 0.237 ± 0.073 per lymphocytes. The ratio of highset to lowest values is also given and indicates the range of individual variability. The ratio is 2,61. In Table 1 are also presented the mean, lowest and highest values of the micronuclei frequency for both spontaneous and radiation induced micronuclei. Results were obtained in our preliminary experiments with the chromosomal sensitivitiy of human lymphocytes. Blood samples were taken from 10 healthy donors in the age between 22 and 61 years. The mean micronucleus frequency was determined to be 0.019±0.013 (ratio 0,004-0,048) per cell for spontaneous micronuclei and 0.061±0.011(ratio 0,042-0,076) for radiation-induced ones. The ratios of highest/lowest values for spontaneous and induced micronuclei are 12 and 1,81, respectively. This indicates that a significant variation exists between individuals in the frequency of spontaneous micronuclei, which reflects the level of cumulative damage occuring during the live time of lymphocytes. Radiation-induced micronuclei that reflect chromosomal radiosensitivity of human limphocytes also vary between individuals. However, our results indicate that the variability of radiation-induced micronuclei among the10 donors appears to be about one and half fold lower that found for radiation-induced chromatid breaks (Table 1).

The inter-individual variation in radiosensitivity obtained using chromosomal radiosensitivity determined in G_{2}- phase lymphocytes shows Figure 1. The theoretical clasification (Terzoudi *et al*., 2006) in resistant (< MV-SD), normal (MV±SD) and sensitive (> MV+SD) results in this case in 16 normal and 3 sensitive donors. Figure 3 shows the inter-individual variation in chromosomal radiosensitivity in G_0-phase lymphocytes. According to above criteria 1 of 10 donors was classified as resistant, 7 as normal and 2 as sensitive.

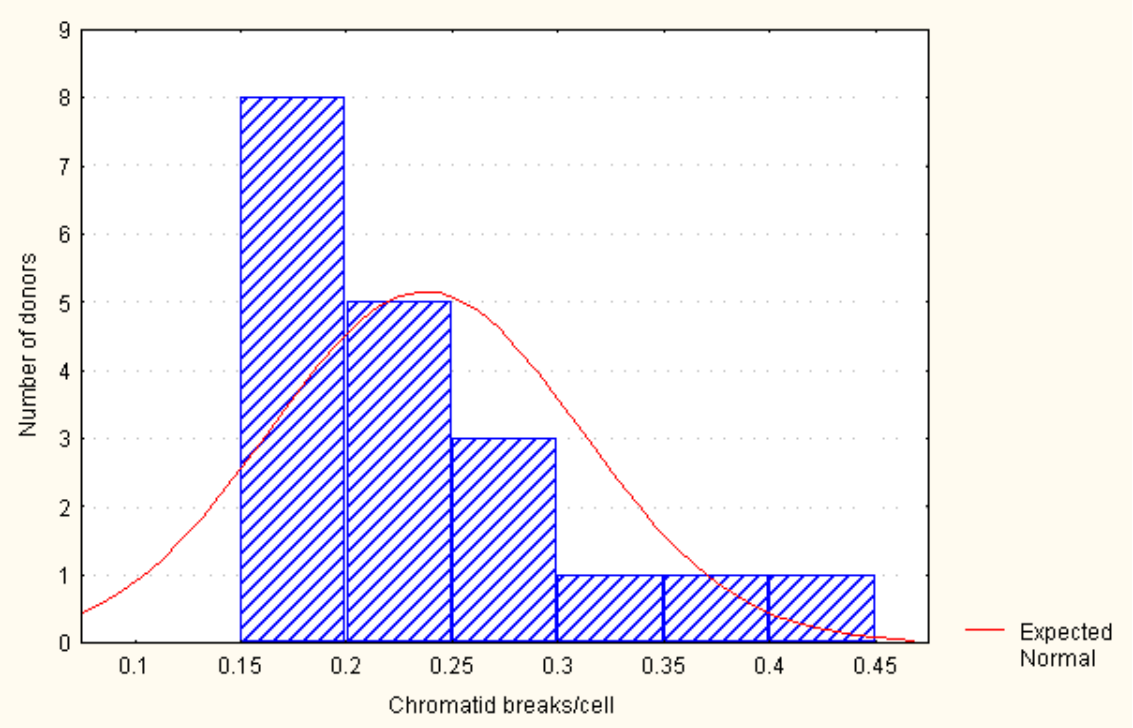

Fig. 1. Inter-individual variation in radiosensitivity obtained from 19 healthy blood donors using the G_2 assay.

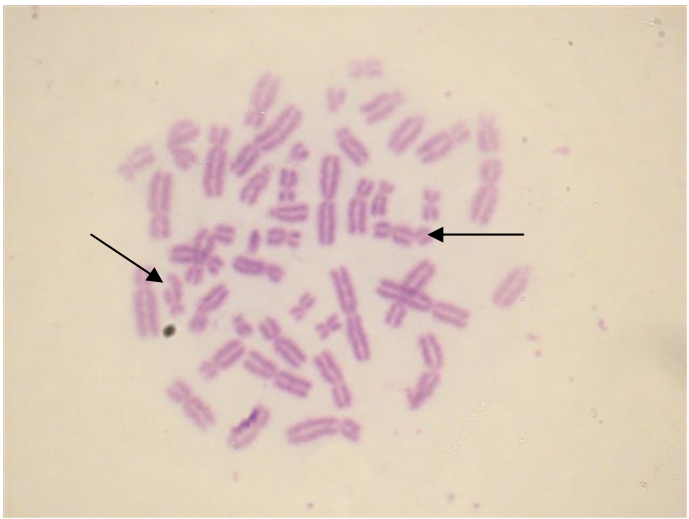

Fig. 2. Chromatid brekas after 1 Gy of X-ray irradiation as visualized at methaphase pheripheral blood lymphocyte from a healthy donor using G_2 assay. Notice two chromatid breaks.

Table 1. Mean, standard deviation of the mean, median, lowest and highest values of micronucleus (MN) frequency in 10 healthy donors and chromatid breaks(CB) frequency in 19 healthy donors.

Variables	Chromatid breaks/cell		Micronuclei/cell	
	sponatneous	induced	sponatneous	induced
No. of donors	19	19	10	10
Mean	0.0004	0.237	0.019	0.061
SD	0.0018	0.073	0.013	0.011
Median	0,000	0.209	0.017	0.061
Lowest	0.000	0.164	0.004	0.042
Highest	0.008	0.428	0.048	0.076
Highest/Lowest	-	2.61	12	1.81
Mean-SD	-	0.164	-	0.050
Mean+SD	-	0.310	-	0.072

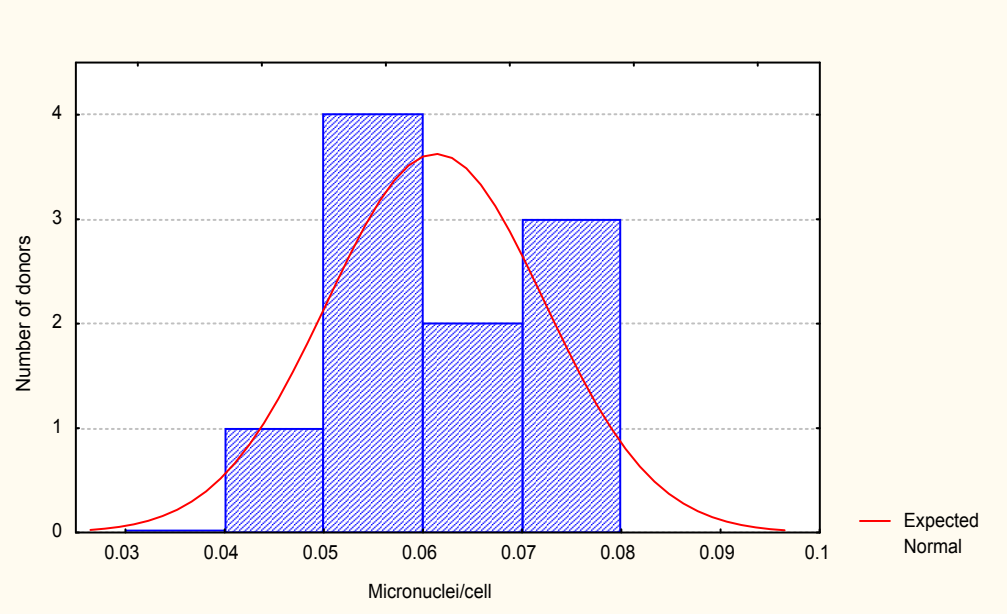

Fig. 3. Inter-individual variation in radiosensitivity obtained from10 healthy blood donors using.

Conclusions

In conclusion: micronuclei are less specific to ionising radiation than chromatid breaks because they detect not only radiation-induced chromosome breaks but also chromosome loss induced by chemical agents (Fenech *et al.,* 1986). They are also less sensitive than chromatid breaks (MV=0,061 vs. MV=0. 0.237), and have high and variable background frequency (range 4 - 48 per 1000 cells). Therefore, the increased frequency of radiation-induced chromatid breaks in peripheral blood lymphocyte can be the indicator of choice for screening a large number of individuals for hypersensitivity or for the assessment of individual radiosensitivity in radiation therapy.

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Comparative cytogenetic analysis of chromosomal aberrations and premature centromere division in persons exposed to radionuclides

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Abstract

The aim of this research is determining correlation between the frequency of premature centromere division (PCD) in metaphases in persons professionally exposed to radionuclides and noted chromosome aberrations (CA). Biological dosimetry was performed by conventional cytogenetic technique. The presence of PCD was confirmed by fluorescent in situ hybridization (FISH). The assay of pL1.84a repetitive DNA for chromosome 18 was used for detection of centromeric region. The analysis included 50 subjects from the Clinical Centre of Serbia (C) (the average age of 45.24±1.18 and the average exposition time 17.96±1.15), and 40 subjects from the control group (K) (the average age of 44.40±0.98 and the average years of 19.67±0.98), which were not exposed to genotoxic agents in their workplaces. The results showed that frequencies of CA and PCD are statistically significantly higher in subject exposed to radionuclides than in the control group (Mann-Whitney U test, P<001). The number of isochromatid breaks was statistically significantly higher in group of exposed to radionuclides (smokers compared to non-smokers) (Spearman's test, r=0.32; P<0.05). Analysis showed that there was a positive correlation between PCD and dicentrics, acentric fragments and chromatide breaks (Spearman's test, r=0.49, 0.54, 0.29, P<0.05). Applying the FISH method showed the presence of PCD in metaphases and interphase nuclei in subjects exposed to radionuclides. Considering PCD as manifestation of genomic instability, our research suggest that this phenomenon could be used as a possible cytogenetic biomarker for proffesional exposure to ionizing radiation.

Key words: chromosome aberrations, ionizing radiations, lymphocytes of peripheral blood, premature centromere division

Introduction

In the occupational pathology, discovery of radiation damages is extremely important, especially in providing the proof that certain changes in health status of an patient may be the consequence of chronicle exposure to ionizing radiation. A number of researchers have examined the impact of small radiation doses among the people employed by health institutions, and it was concluded that there was an increase in incidence chromosome aberrations in comparison to the control group (Ghiassi-Nejad et al 2002, Jovićić et al 2009, Milačić 2005, Garaj-Vrhovac et al 2006).

Beside the classical researches related to the impact of ionizing radiation to lymphocytes in peripheral blood in the exposed persons, attention has been recently paid to new approaches and manners of analyzing necessary for definition of cytogenetic markers, all aimed at recognition of harmful effects of ionizing radiation. In addition to classical analysis of chromosome aberrations (CA), the attention is focused to the detection of premature centromere division (PCD).

This phenomenon, found in lymphocytes of peripheral blood, is characterized by divided chromatides of chromosomes already in metaphase of cell cycle. However, if premature division of constitute heterochromatin occurs at centromere level (PCD), then we may observe the phenomenon of aneuploidy, which has been confirmed in large number of papers (Vig et al 1974).

Disturbance of centromere in the form of premature centromere splitting (Eng-centromere splitting, centromere spreading), centromere puffing, actually presents manifestation of disturbance in spatial and time mechanisms of mitosis process with various types of neoplasia, which may further lead to genetic instability (Litmanović et al 1998). The answer to the question whether PCD causes aneuploidia in carcinogenic cells is not known, but it is sure that there are indications today which support the thesis which pertains to correlation between PCD and aneuploidia. The aim of our research was focused on determination of PCD prevalence, as well as on determination of correlation between PCD and chromosome aberrations among people who are occupationally exposed to radionuclides. In addition, the idea whether PCD could be observed as a possible cytogenetic biomarker among people occupationally exposed to radiation was also taken into consideration. In order to confirm presence of PCD at certain chromosome in metaphases and interphase nuclei and in order to answer the question at which stage of cell cycle centromere regions split, we have applied fluorescent *in situ* hybridization (FISH).

Material and methods

Our research included a group of 50 patients employed by Clinical Centre of Serbia, who are occupationally exposed to radionuclides (C) and 40 patients from the control group (K), who have never been exposed to physical or chemical agents in their workplaces (Table 1). The effective radiation doses were measured by thermoluminiscent dosimeter (TLD) once a month during their occupational exposure. Biological dosimetry was carried out by the means of modified micromethod for lymphocytes of peripheral blood and conventional cytogenetic technique for the analysis of chromosome aberration (Moorhead et al 1983, IAEA: Biological dosimetry 1986). The analysis of CA and PCD was examined in 200 metaphase cells. In order to confirm the results of premature segregation of centromere regions, FISH was applied.

The results in this paper were processed by applying Statistics 5 (StatSoft, Inc) and SAS 6.12 software for PS.

Table 1. General characteristics of the exposed and control patients groups

		Control (N=40)	Exposed (N=50)	*P*-value
Age	Mean±SE	44.40±0.98	45.24±1.18	0.599
	Min-Max	34-60	27-59	
Sex				
Male	N	16	23	0.568
Female	N	24	27	
Smoking status				
Smokers	N	12	22	0.173
Non-smokers	N	28	28	
LT-labour time	Mean±SE	19.67±0.98	21.94±1.13	0.144
	Min-Max	13-36	5-35	
ERS-exposition	Mean±SE	/	17.96±1.15	
	Min-Max	/	5-35	
Effective dose (2008) (mSv)	Mean±SE	/	2.19±0.27	
	Min-Max	/	0-10.10	
Effective dose (2004-2008) (mSv)	Mean±SE	/	9.87±1.31	
	Min-Max	/	0.16-47.38	

Results and discussion

The results obtained in the researches of CA and PCD in persons occupationally exposed to the low doses of ionizing radiation are provided in Table 1. On the basis of statistic data (Mann-Whitney U test), we have reached the conclusion that frequency of CA and PCD is more significant in lymphocytes of peripheral blood of people from the exposed group than of those from the control one ($p>0.05$), Table 2. We may pose a question whether frequency of PCD may be related to artifacts, or presence of PCD really may be seen as biological response of a cell to the effects of ionizing radiation. Some authors suggest that presence of PCD in human kariotype is not accidental (Bühler et al 1987).

If the presence of PCD was accidental, than such presence should be recorded in the control group as well. However, frequency of PCD is far lower in the control than in the exposed group. Presence of PCD recorded to a smaller extent in the control group implies a conclusion that PCD may have occurred there as a consequence of the effects of outer environmental factors.

Table 2. Frequency of CA and PCD in limphocytes of the exposed groups and control ones (ACF - acentric fragments, HB – chromatide breaks, iHB – isochromatide breaks, tPCD – metaphases with more than 10 PCD)

		Control	Exposed	*P*-value
No of aberrated cells	Mean±SE	0.85±0.16	4.06±0.15	0.000
	Min-Max	0-4	2-7	
Dicentrics	Mean±SE	0.13±0.05	0.66±0.11	0.001
	Min-Max	0-1	0-2	
Ring	Mean±SE	/	0.12±0.05	0.334
	Min-Max	/	0-1	
ACF	Mean±SE	0.37±0.09	2.64±0.17	0.000
	Min-Max	0-2	0-5	
HB	Mean±SE	0.33±0.07	0.68±0.07	0.004
	Min-Max	0-1	0-1	
iHB	Mean±SE	0.17±0.06	0.5±0.07	0.008
	Min-Max	0-1	0-1	
tPCD	Mean±SE	0.25±0.09	2.12±0.25	0.000
	Min-Max	0-2	0-7	
PCD 1-5	Mean±SE	4.05±0.25	8.5±0.41	0.000
	Min-Max	1-7	2-14	
PCD 5-10	Mean±SE	1.72±0.22	4.64±0.23	0.000
	Min-Max	0-5	2-9	

Statistic data have shown that there was no difference in frequency of CA and PCD between smokers and non-smokers in the exposed group. Our researches are partially in accordance with the authors' researches (Chung et al 1996, Lovreglio et al 2006) who have established that smoking does not affect chromosome aberrations, although there are studies which proove opposite (Sierra-Torres et al 2004). However, further statistic analysis has shown that there is a significant correlation between smoking and frequency of isochromatide breaks in the exposed group of patients (Spearman's test, r=0.32; $P<0.05$). Among the patients from the control group, statistic data indicate that frequency of chromatide breaks is significantly higher among smokers than among non-smokers (means±SE: smokers 0.67±0.14 vs. non-smokers 0.18±0.07). In addition, it was established that frequency of acentric fragments was significantly higher among women than among men in the control patients group (women 0.54±0.12 vs. men 0.12±0.09).

It is important to emphasize that frequency of tPCD among the occupationally exposed people was positively correlated with the dicentric frequency, acentric fragments and chromatide abruptions (Spearman's test, r=0.49, 0.54, 0.29, $P<0.05$).

Further analysis has established that there was no positive correlation between the measured effective doses among patients and frequency of chromosome breaks and PCD (Andreassi et al 2009).

Applying the FISH method we have established that centromere division occurs already in the cell cycle interphase, i.e. in G2 phase of the cell cycle. In addition, we have confirmed the presence of PCD in metaphases and interphase nuclei of patients exposed to radionuclei (Table 3).

Table 3. Distribution of fluorescent signals for centromere region of chromosome 18 in metaphases and in interphase nuclei by applying DNA repetitive probe, both to the exposed and control groups

	INTERPHASE NUCLEI		
Patients	**Number of analyzed nuclei**	**Without PCD**	**With PCD**
1.	83	70 (84.337%)	13 (15.663%)
2.	102	95 (93.137%)	7 (6.863%)
	METAPHASES		
	Number of analyzed metaphases	**Without PCD**	**With PCD**
1.	28	14 (50%)	14 (50%)
2.	24	24 (100%)	0 (0%)

Conclusions

Our researches suggest the possibility to use PCD as possible cytogenetic biomarker in persons occupationally exposed to radionuclides. It is necessary to emphasize that these results include preliminary researches in relatively small sample. Our future researches will include larger number of patients in combination with *in vitro* researches, which will surely contribute to better understanding of this phenomenon.

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Population study of chromosomal aberrations in aircrew members compared to a control group

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Abstract

Aircrew was included as an occupational exposed radiation group in the last report of the International Commission for Radiation Protection (ICRP 90).

Chromosome translocations are a biomarker of cumulative exposure to external ionising radiations better than chromosome dicentrics, we analysed the frequency of translocations from peripheral blood of a group of aircrew members and controls matched for the age, smoking habits and gender.

Metaphases spreads obtained after incubation of peripheral blood lymphocytes in a PHA enriched culture medium for 48 hours at 37ºC (laboratory routine procedures). Chromosomes 1 and 2 painted red and a pancentromeric probe used simultaneously to coloured all centromers green; to distinguish translocations and dicentrics, this procedure gives an efficiency of 28% when extrapolating to the full genome. There was analysed around 2000 metaphases per person, which means 560 cell equivalents.

Our results do not show statistically significance differences in translocations frequency between both populations.

Introduction

The presence of chromosomal aberrations in peripheral blood lymphocytes provides a good biomarker of exposure to ionizing radiation. The analysis of dicentric chromosomes is the "gold standard" for estimating doses in cases of accidental overexposure to ionizing radiation. The main limitation of this type of dosimeter is its instability, because of altered morphological involved, cells carrying dicentric fail its mitosis and these alterations are not transmitted to daughter cells, so the post exposure frequency of dicentric decreases over time.

The translocations are another type of chromosomal aberration induced by ionizing radiation by the same mechanism as dicentric chromosomes, but do not lead to morphological changes that hinder the cell mitosis, so its frequency will remain constant throughout the time and also because of its potential accumulation, allow the identification of chronic exposures.

The development of hybridization techniques with specific DNA probes has facilitated "paint" chromosome translocations to identify quickly and easily, allowing

the analysis of a large number of cells and thus calculating the frequency of translocations routinely in biological dosimetry studies.

To study the biological impact of ionizing radiation on chronically exposed populations, the most appropriate method is fluorescence in situ hybridization (FISH) as it has proven that it can detect an increase in the frequency of translocations due to such exposure.

With increasing altitude increases exposure to ionizing radiation from solar and cosmic origin, so many countries has included aircrew as occupationally exposed, as recommended by the UNSCEAR 2000 (United Nations Scientific Committee on the effects of atomic radiation). Ionizing radiations at altitudes used in long haul flights are mainly from neutron and gamma radiation but are also detected protons, alpha particles and heavy nuclei. It has estimated that 60% of crew exposure to cosmic radiation is due to radiation of high linear energy transfer (LET), especially from neutrons generated by its interaction with the atmosphere.

Because of the proven relationship between the frequency of chromosome aberrations and increased cancer risk, is necessary to perform frequency studies in populations exposed to ionizing radiation, among which are the air crews.

This report describes the work done at the Biological Dosimetry laboratory in Hospital General Universitario Gregorio Marañón, on the collaboration agreement between the Foundation for Biomedical Research at University General Hospital Gregorio Marañón, Iberia Airlines of Spain, and Co-Muprespa Fraternity.

Material and methods

Population Description

To determine the increased risk of a given population associated with a factor, it is important to compare it with another population with similar characteristics but not exposed to that factor. Therefore, since the objective of this work is to determine whether there is an increased risk of disease associated with exposure to ionizing radiation of cosmic origin, between the flight crew in Iberia, this work includes the comparison of two group's persons:

- Aircrew members for intercontinental flights above 9000 meters, which must meet the following requirements:
  Being active
  At least 10 years of continuous service with full schedules.
  At least 5 years of continuous service in long-haul fleets, preferably A-340
- Iberia ground staff.

Matching populations for age, gender and smoking habits

The grounds for exclusion to be part of the study are:

- Being younger than 45 years at the time of blood collection
- Radiological studies in the last year TGI type, barium enema, urography descending, thoracoabdominal CT, and interventional radiology.
- Previous diagnosis of cancer or other disease treated with chemotherapy and / or radiotherapy
- Work history in professions associated with cancer risk
- Substance abuse

All individuals who are part of the work were selected by The Iberia Medical Service, which has also obtain and complete the necessary information and informed consent in addition to blood collection and shipment to Biological Dosimetry Laboratory at HGUGM.

LABORATORY METHODOLOGY

Sample consists of three tubes of blood per person, sent to the laboratory together with the informed consent document and the name. Manipulation of samples performed as appropriate to the laboratory culture and processing protocols.

Fluorescence in situ hybridization FISH

Hybridization is performed on 3 consecutive days following the standardized protocol in the laboratory which allows the hybridization of chromosomes 1 and 2 with rhodamine labelled probes, a FITC pancentromeric labelled probe, and a DAPI counterstained.

Analysis of metaphases

The analysis done blind, ie the slides coded with a number assigned by the laboratory so that the analyst does not know the origin and characteristics of the individual studied.

The analysis performed by fluorescence microscope (Leica DM5000B) with appropriate filters for visualization of rhodamine, FITC and DAPI. We analyzed about 2000 metaphases per individual, discarding people who could not be analyzed more than 600 metaphases.

There have photographed all chromosome abnormalities found but for the study only account translocations in stable cells, ie those without dicentric and acentric fragments or rings, and selected only cells where there are close to 46 chromosomes and in which all material paint is present.

Results

The study done in a total number of 80 people, 38 controls and 42 aircrews, analysed 67.297 metaphases from control samples and 69.690 from aircrew samples.

We analysed translocations involving chromosomes 1 and 2 which constitute about 16% (16.32 in males or 16.05 in women) of the cellular genome, we calculate the genomic frequency of translocations using the formula described by Lucas Group 1992, for our technique we obtain an approximate efficiency of 28% (27.99% to 27.62% for men and women)

The following tables show the data obtained for each population: 38 controls and 42 crew (tables 1 and 2 respectively), as were sent to the Health Protection Agency (HPA, Chilton Didcot Oxford, UK) for statistical analysis.

Table 1. Control population data. 38 people analysed, genome equivalent = metaphases analysed x f. % translocations to genome = translocations / genome equivalent x 100. Smoking habits: S = smokers. NS = non smokers, included people who gave up smoking at least 10 years ago.

Nº	Gender	f	analysed met.	genome equivalent	GE (gender)	Translo-cations	%trans. to genome	Age	smoking habit
13	M	0,27996	1424	398,72	398,66	1	0,251	57	NS
44	F	0,27622	2069	579,32	571,49	0	0,000	50	NS
45	M	0,27996	2103	588,84	588,76	3	0,509	58	NS
46	M	0,27996	2009	562,52	562,44	2	0,356	52	S
47	M	0,27996	2001	560,28	560,20	1	0,178	52	NS
51	M	0,27996	2003	560,84	560,76	2	0,357	45	S
52	F	0,27622	2171	607,88	599,67	1	0,165	50	S
53	F	0,27622	2005	561,4	553,81	0	0,000	53	NS
54	F	0,27622	1998	559,44	551,88	0	0,000	59	S
55	M	0,27996	2010	562,8	562,72	0	0,000	60	S
56	M	0,27996	2011	563,08	563,00	3	0,533	49	NS
57	M	0,27996	2015	564,2	564,12	4	0,709	59	NS
58	M	0,27996	2007	561,96	561,88	2	0,356	53	S
61	F	0,27622	2086	584,08	576,19	0	0,000	53	NS
63	M	0,27996	2043	572,04	571,96	2	0,350	56	S
64	M	0,27996	2001	560,28	560,20	1	0,178	59	NS
65	F	0,27622	1603	448,84	442,78	0	0,000	58	NS
66	M	0,27996	1624	454,72	454,65	2	0,440	56	NS
67	M	0,27996	1995	558,6	558,52	5	0,895	51	S
68	M	0,27996	2153	602,84	602,75	3	0,498	47	NS
72	F	0,27622	1851	518,28	511,28	2	0,386	54	NS
73	F	0,27622	2049	573,72	565,97	5	0,872	54	NS
74	M	0,27996	2002	560,56	560,48	3	0,535	45	NS
75	M	0,27996	1942	543,76	543,68	1	0,184	62	NS
76	M	0,27996	2050	574	573,92	1	0,174	55	NS
78	M	0,27996	2017	564,76	564,68	2	0,354	48	NS
113	M	0,27996	1094	306,32	306,28	1	0,326	49	NS
116	M	0,27996	1185	331,8	331,75	3	0,904	57	S
118	M	0,27996	982	274,96	274,92	0	0,000	55	S
119	M	0,27996	1529	428,12	428,06	2	0,467	49	S
120	M	0,27996	2010	562,8	562,72	2	0,355	58	NS
121	M	0,27996	2023	566,44	566,36	2	0,353	48	S
122	F	0,27622	822	230,16	227,05	0	0,000	48	NS
124	M	0,27996	1065	298,2	298,16	3	1,006	48	NS
131	F	0,27622	654	183,12	180,65	2	1,092	48	NS
132	F	0,27622	1335	373,8	368,75	0	0,000	45	NS
133	F	0,27622	1338	374,64	369,58	3	0,801	54	NS
134	F	0,27622	2018	565,04	557,40	1	0,177	52	NS

Table 2. Aircrew data. 42 people analysed, genome equivalent = metaphases analysed x f. % translocations to genome = translocations / genome equivalent x 100. Smoking habits: S = smokers. NS = non smokers, included people who gave up smoking at least 10 years ago.

n	Gender	f	analysed metaphases	genome equivalent	GE (gender)	translocations	% transl to genome	Age	smoking habit
17	F	0,27622	1144	320,32	315,99	0	0,000	54	NS
18	M	0,27996	2049	573,72	573,64	7	1,220	54	NS
24	F	0,27622	1430	400,4	394,99	1	0,250	47	NS
25	F	0,27622	2342	655,76	646,90	0	0,000	47	NS
26	M	0,27996	1438	402,64	402,58	2	0,497	58	S
27	M	0,27996	2024	566,72	566,64	3	0,529	54	S
28	M	0,27996	625	175	174,97	2	1,143	54	S
29	M	0,27996	1677	469,56	469,49	3	0,639	54	S
30	F	0,27622	2023	566,44	558,79	2	0,353	54	NS
31	M	0,27996	2012	563,36	563,28	1	0,178	59	NS
37	F	0,27622	2000	560	552,43	1	0,179	59	NS
42	F	0,27622	2239	626,92	618,45	1	0,160	51	S
60	M	0,27996	2041	571,48	571,40	0	0,000	54	NS
62	F	0,27622	2048	573,44	565,69	5	0,872	59	NS
82	M	0,27996	1215	340,2	340,15	0	0,000	59	NS
84	M	0,27996	769	215,32	215,29	0	0,000	53	NS
85	F	0,27622	588	164,64	162,42	0	0,000	57	NS
86	M	0,27996	863	241,64	241,61	1	0,414	58	S
87	M	0,27996	2017	564,76	564,68	0	0,000	51	NS
88	F	0,27622	2070	579,6	571,77	2	0,345	59	NS
90	M	0,27996	708	198,24	198,21	0	0,000	56	NS
91	F	0,27622	2040	571,2	563,48	6	1,050	54	NS
92	F	0,27622	2086	584,08	576,19	1	0,171	45	S
96	M	0,27996	2014	563,92	563,84	3	0,532	52	NS
97	F	0,27622	2024	566,72	559,06	4	0,706	48	NS
98	F	0,27622	2038	570,64	562,93	2	0,350	45	S
100	M	0,27996	1997	559,16	559,08	3	0,537	45	NS
101	M	0,27996	2000	560	559,92	2	0,357	54	S
102	F	0,27622	1562	437,36	431,45	1	0,229	50	S
103	M	0,27996	2001	560,28	560,20	3	0,535	52	NS
104	M	0,27996	1821	509,88	509,81	1	0,196	57	S
105	F	0,27622	479	134,12	132,31	1	0,746	51	NS
107	M	0,27996	2008	562,24	562,16	2	0,356	57	S
108	F	0,27622	891	249,48	246,11	1	0,401	54	S
109	F	0,27622	1005	281,4	277,60	0	0,000	52	NS
110	F	0,27622	2002	560,56	552,99	3	0,535	48	S
111	F	0,27622	1935	541,8	534,48	1	0,185	49	NS
112	M	0,27996	2012	563,36	563,28	3	0,533	53	S
117	F	0,27622	1369	383,32	378,14	1	0,261	49	S
123	F	0,27622	1785	499,8	493,05	3	0,600	52	S
125	M	0,27996	2066	578,48	578,40	3	0,519	46	NS
135	M	0,27996	1233	345,24	345,19	0	0,000	46	NS

The following tables 3 and 4 show the statistics of the results obtained.

First two columns correspond to the expected frequencies due to age. These data are used to calculate excess of translocations observed, shown in the next column. Excess of translocations is less than expected; except for those marked with yellow, and both populations have the same number of cases with excess. Apparently, there is a difference between both populations but, when use t test we obtain a p value of 0.065 (when using only baseline due to sex and age) or 0.063 (when using risk rates estimated by the group of Sigurdson). These data imply that, although there is a difference between the two populations, is not statistically significant.

Table 3. Control population statistics.

	Background for age			Adjusted rate ratio				
IB-n	Per 100	Per GE	Excess translocations	age	smoking	gender	RR	Excess
13	0,93	3,71	-2,71	1,37	1,00	1,00	3,22	-2,22
44	1,06	6,06	-6,06	1,25	1,00	0,92	4,27	-4,27
45	0,93	5,48	-2,48	1,37	1,00	1,00	4,76	-1,76
46	0,83	4,67	-2,67	1,25	1,19	1,00	4,94	-2,94
47	0,83	4,65	-3,65	1,25	1,00	1,00	4,13	-3,13
51	0,92	5,16	-3,16	1,27	1,19	1,00	5,00	-3,00
52	1,06	6,36	-5,36	1,25	1,19	0,92	5,33	-4,33
53	1,06	5,87	-5,87	1,25	1,00	0,92	4,14	-4,14
54	0,87	4,80	-4,80	1,37	1,19	0,92	5,38	-5,38
55	1,14	6,41	-6,41	1,78	1,19	1,00	7,03	-7,03
56	0,92	5,18	-2,18	1,27	1,00	1,00	4,22	-1,22
57	0,93	5,25	-1,25	1,37	1,00	1,00	4,56	-0,56
58	0,83	4,66	-2,66	1,25	1,19	1,00	4,93	-2,93
61	1,06	6,11	-6,11	1,25	1,00	0,92	4,31	-4,31
63	0,93	5,32	-3,32	1,37	1,19	1,00	5,50	-3,50
64	0,93	5,21	-4,21	1,37	1,00	1,00	4,53	-3,53
65	0,87	3,85	-3,85	1,37	1,00	0,92	3,63	-3,63
66	0,93	4,23	-2,23	1,37	1,00	1,00	3,68	-1,68
67	0,83	4,64	0,36	1,25	1,19	1,00	4,90	0,10
68	0,92	5,55	-2,55	1,27	1,00	1,00	4,52	-1,52
72	1,06	5,42	-3,42	1,25	1,00	0,92	3,82	-1,82
73	1,06	6,00	-1,00	1,25	1,00	0,92	4,23	0,77
74	0,92	5,16	-2,16	1,27	1,00	1,00	4,20	-1,20
75	1,14	6,20	-5,20	1,78	1,00	1,00	5,71	-4,71
76	0,93	5,34	-4,34	1,37	1,00	1,00	4,64	-3,64
78	0,92	5,20	-3,20	1,27	1,00	1,00	4,23	-2,23
113	0,92	2,82	-1,82	1,27	1,00	1,00	2,30	-1,30
116	0,93	3,09	-0,09	1,37	1,19	1,00	3,19	-0,19
118	0,93	2,56	-2,56	1,37	1,19	1,00	2,64	-2,64
119	0,92	3,94	-1,94	1,27	1,19	1,00	3,82	-1,82
120	0,93	5,23	-3,23	1,37	1,00	1,00	4,55	-2,55
121	0,92	5,21	-3,21	1,27	1,19	1,00	5,05	-3,05
122	0,87	1,98	-1,98	1,27	1,00	0,92	1,72	-1,72
124	0,92	2,74	0,26	1,27	1,00	1,00	2,23	0,77
131	0,87	1,57	0,43	1,27	1,00	0,92	1,37	0,63
132	0,87	3,21	-3,21	1,27	1,00	0,92	2,80	-2,80
133	1,06	3,92	-0,92	1,25	1,00	0,92	2,76	0,24
134	1,06	5,91	-4,91	1,25	1,00	0,92	4,17	-3,17
		Average	-2,99					-2,41
		SD	1,81					1,77
		Var	3,28					3,12

Table 4. Air crew population statistics.

	Background for age			Adjusted rate ratio				
IB-n	Per 100	Per GE	Excess translocations	age	smoking	gender	RR	Excess
17	1,06	3,35	-3,35	1,25	1,00	0,92	2,17	-2,17
18	0,83	4,76	2,24	1,25	1,00	1,00	4,23	2,77
24	0,87	3,44	-2,44	1,27	1,00	0,92	2,76	-1,76
25	0,87	5,63	-5,63	1,27	1,00	0,92	4,52	-4,52
26	0,93	3,74	-1,74	1,37	1,19	1,00	3,87	-1,87
27	0,83	4,70	-1,70	1,25	1,19	1,00	4,97	-1,97
28	0,83	1,45	0,55	1,25	1,19	1,00	1,54	0,46
29	0,83	3,90	-0,90	1,25	1,19	1,00	4,12	-1,12
30	1,06	5,92	-3,92	1,25	1,00	0,92	3,84	-1,84
31	0,93	5,24	-4,24	1,37	1,00	1,00	4,55	-3,55
37	0,87	4,81	-3,81	1,37	1,00	0,92	4,16	-3,16
42	1,06	6,56	-5,56	1,25	1,19	0,92	5,06	-4,06
60	0,83	4,74	-4,74	1,25	1,00	1,00	4,21	-4,21
62	0,87	4,92	0,08	1,37	1,00	0,92	4,26	0,74
82	0,93	3,16	-3,16	1,37	1,00	1,00	2,75	-2,75
84	0,83	1,79	-1,79	1,25	1,00	1,00	1,59	-1,59
85	0,87	1,41	-1,41	1,37	1,00	0,92	1,22	-1,22
86	0,93	2,25	-1,25	1,37	1,19	1,00	2,32	-1,32
87	0,83	4,69	-4,69	1,25	1,00	1,00	4,17	-4,17
88	0,87	4,97	-2,97	1,37	1,00	0,92	4,31	-2,31
90	0,93	1,84	-1,84	1,37	1,00	1,00	1,60	-1,60
91	1,06	5,97	0,03	1,25	1,00	0,92	3,88	2,12
92	0,87	5,01	-4,01	1,27	1,19	0,92	4,79	-3,79
96	0,83	4,68	-1,68	1,25	1,00	1,00	4,16	-1,16
97	0,87	4,86	-0,86	1,27	1,00	0,92	3,91	0,09
98	0,87	4,90	-2,90	1,27	1,19	0,92	4,68	-2,68
100	0,92	5,14	-2,14	1,27	1,00	1,00	4,19	-1,19
101	0,83	4,65	-2,65	1,25	1,19	1,00	4,91	-2,91
102	1,06	4,57	-3,57	1,25	1,19	0,92	3,53	-2,53
103	0,83	4,65	-1,65	1,25	1,00	1,00	4,13	-1,13
104	0,93	4,74	-3,74	1,37	1,19	1,00	4,90	-3,90
105	1,06	1,40	-0,40	1,25	1,00	0,92	0,91	0,09
107	0,93	5,23	-3,23	1,00	1,19	1,00	3,95	-1,95
108	1,06	2,61	-1,61	1,37	1,19	0,92	2,21	-1,21
109	1,06	2,94	-2,94	1,25	1,00	0,92	1,91	-1,91
110	0,87	4,81	-1,81	1,27	1,19	0,92	4,60	-1,60
111	0,87	4,65	-3,65	1,27	1,00	0,92	3,73	-2,73
112	0,83	4,68	-1,68	1,25	1,19	1,00	4,94	-1,94
117	0,87	3,29	-2,29	1,27	1,19	0,92	3,14	-2,14
123	1,06	5,23	-2,23	1,25	1,19	0,92	4,04	-1,04
125	0,92	5,32	-2,32	1,27	1,00	1,00	4,33	-1,33
135	0,92	3,18	-3,18	1,27	1,00	1,00	2,59	-2,59
		Average	-2,40					-1,83
		SD	1,62					1,57
		Var	2,62					2,47
control vs aircraft:		T-test p	0,065					0,063

Conclusions

The frequency of translocations has not statistically significant differences, although there was a slight increase in the frequency of translocations in the crew population that does not exceed observed frequencies in other populations described in other publications.

It would be interesting to extend the study with a larger number of people, as this would allow us to clarify if the trend continues and / or wide, although noted that the frequencies observed in our study are consistent with published for different population groups.

In the individual analysis, it was found that 6 people have a higher frequency of translocations than expected, but if you look at the data shows, that the number of analyzed genomic equivalent in these cases is low, so even if they can take into account the population study, individual study would require testing more metaphases to allow any conclusion.

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Assessment of dose response for chromosome aberrations due to internal alpha-radiation

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Abstract

Chromosome slides with Chromosome 5 painted using mBAND were analyzed for 40 employees of the Mayak Production Association. Mean frequency of stable intra-chromosomal aberrations per total cells analyzed was 0.005±0.007. Correlation analysis indicated the lack of a significant correlation between external dose and the yield of intra-chromosomal aberrations (r = -0.02). A linear dependence of the yield of intra-chromosomal aberrations on absorbed dose of internal exposure to incorporated ^{239}Pu was revealed: $Y = (0.0303 \pm 0.0065)D_{\alpha}$, where: Y is the yield of intra-chromosomal aberrations (in % per 100 cells); D_{α} is the absorbed dose to bone marrow due to internal alpha-radiation (cGy).

Introduction

The most sensitive and well-known bioindicator of radiation is the yield of chromosome aberrations in lymphocytes from peripheral blood in human (IAEA, 2001). The peripheral blood lymphocytes are a simple and unique model to study radiation-induced mutagenesis. A low spontaneous level of chromosome aberrations in lymphocytes from peripheral blood of healthy donors and high radiosensitivity allow detecting an excess in the yield of radiation-induced chromosome aberrations over spontaneous rate even at low doses (IAEA, 2001; Tucker JD, 2001; Balakrishnan S and Rao SB, 1999).

Many studies have demonstrated that radiation-induced chromosome aberrations are long-lasting in human and can be used as a biological indicator of radiation exposure Aberrations imply the DNA double-strand breaks and repair defects. Stable intra-chromosomal aberrations (e.g. para- and pericentric inversions) are produced by misrepair of pairs of chromosome breaks (DNA double-strand breaks) within a single chromosome; intra-chromosomal aberrations may be contrasted with inter-chromosomal aberrations, produced by misrepair of pairs of chromosome breaks in different chromosomes (Brenner DJ, Sachs RK, 1994).

The theoretical background relates to the observation that densely-ionizing radiations, such as alpha-particles or neutrons, produce uniquely elevated intra-chromosomal aberrations in contrast with almost all other mutagens, such as gamma-rays or general aging processes.

This implies that alpha-particles or neutrons produce multiple chromosome breaks in each of a small number of chromosomes, as compared to X-rays or chemicals, or

endogenous aging processes, where a comparable exposure (i.e. yielding the same total number of chromosome breaks) results in fewer breaks in larger numbers of chromosomes, predominantly inter-chromosomal aberrations.

The objective is assessment of dose response for the yield of intra-chromosomal aberrations and internal dose from alpha-radiation.

Material and methods

A cytogenetic analysis of the peripheral blood lymphocytes was performed for 40 workers of the Mayak PA, who were exposed to external and internal radiation. Most individuals analyzed were males (77.5%). External doses from gamma-rays ranged from 0.021 up to 5.726 Gy; ^{239}Pu body burden was 0-10.5 kBq. External doses to red bone marrow (RBM) from gamma-rays ranged within 0.130-4.262 Gy; absorbed dose from alpha-radiation due to incorporated ^{239}Pu was 0.003-0.538 Gy.

For the purposes of cultivation, whole blood samples were drawn from ulnar vein by a heparinized syringe, 5 ml. The lymphocytic pellet and 10 ml of PB-MAX medium were placed in a culture flask. The flask was then incubated in the CO_2 incubator at 37°C for 48 to 68 hrs. Four hours prior to fixation, 0.01 μg/ml colchicine was added in the flask. The pellet was resuspended in 0.075 M KCl and placed in a 37°C water bath for 15 minutes. The pellet was then resuspended in fixative (3:1 ethanol: glacial acetic acid, cooled to 0^0C). The cell suspension was placed on slides and allowed to air dry.

Hybridization of chromosome slides using mBAND was performed in compliance with the XCyte Lab Manual (Metasystems, Germany). The slides were denatured in 0.07 N NaOH for 1 minute at room temperature. mBAND probes were denatured in parallel with denaturation of the slides. The slides were placed in a humidified chamber in a 37°C incubator for 48 hrs. For detection of hybridization signals, a mixture of blocking buffer and detection solution was used. DAPI/antifade was applied as a counterstain.

The slides were analyzed using a fluorescence imaging microscope (Leica DM RA2) with filter sets for DAPI, FITC, Texas Red, Spectrum Orange, DEAC, Cy5. Images were captured using the MetaSystems camera. Although using mBAND only two chromosomes were colored, whole metaphases were also captured to observe any translocations. Karyotyping was performed using Isis 4 software (MetaSystems, Germany). Statistical analysis was performed by standard linear regression methods using Statistica software (Borovikov V, 2003).

Results

Chromosome slides from 40 Mayak workers were analyzed using mBAND fluorescence *in situ* hybridization for chromosome 5. The slides were analyzed to account for stable intra-chromosomal aberrations, such as para- and pericentric inversions, terminal and interstitial deletions, and insertions. Aberrations were scored per 100-150 metaphases. Total 4,825 cells were analyzed. The mean yield of stable intra-chromosomal aberrations per total number of analyzed cells was 0.005 ± 0.007.

A high yield of intra-chromosomal aberrations (4.1 per 100 cells) was found for one worker. Analysis of medical records indicated malignant neoplasm for this individual. Thus, results of cytogenetic analysis based on chromosome slides from this worker were excluded from the statistical analysis.

As demonstrated earlier, the yield of intra-chromosomal aberrations in a group of workers at the reactor complex did not actually differ from that in the control group (Hande MP et al, 2003; Mitchell CR et al, 2004). In addition, no correlation of the yield of intra-chromosomal aberrations with age was found. The correlation analysis revealed no significant correlation between the yield of intra-chromosomal aberrations and external dose (r = -0.02). The analysis indicated a linear dependence of the yield of stable intra-chromosomal aberrations in the peripheral blood lymphocytes on absorbed dose to red bone marrow (RBM) from internal exposure to alpha-radiation from incorporated ^{239}Pu:

$$Y = (0.0303 \pm 0.0065)D_{\alpha}, \quad (1)$$

where:
Y is the yield of intra-chromosomal aberrations (in percentage per 100 cells);
D_{α} is absorbed dose from internal dose to RBM from alpha-radiation (Gy).

A coefficient, b = 0.0303, specifying the growth rate of aberrations per unit dose (%/cGy), showed high statistical reliability with increased absorbed dose ($p < 0.05$). The regression equation in general was significant by Fisher's ratio test ($p < 0.05$). The correlation coefficient (r = 0.43) was also significant ($p < 0.05$).

Fig. 1 illustrates Dependence (1) with the 95% CI and empirical points.

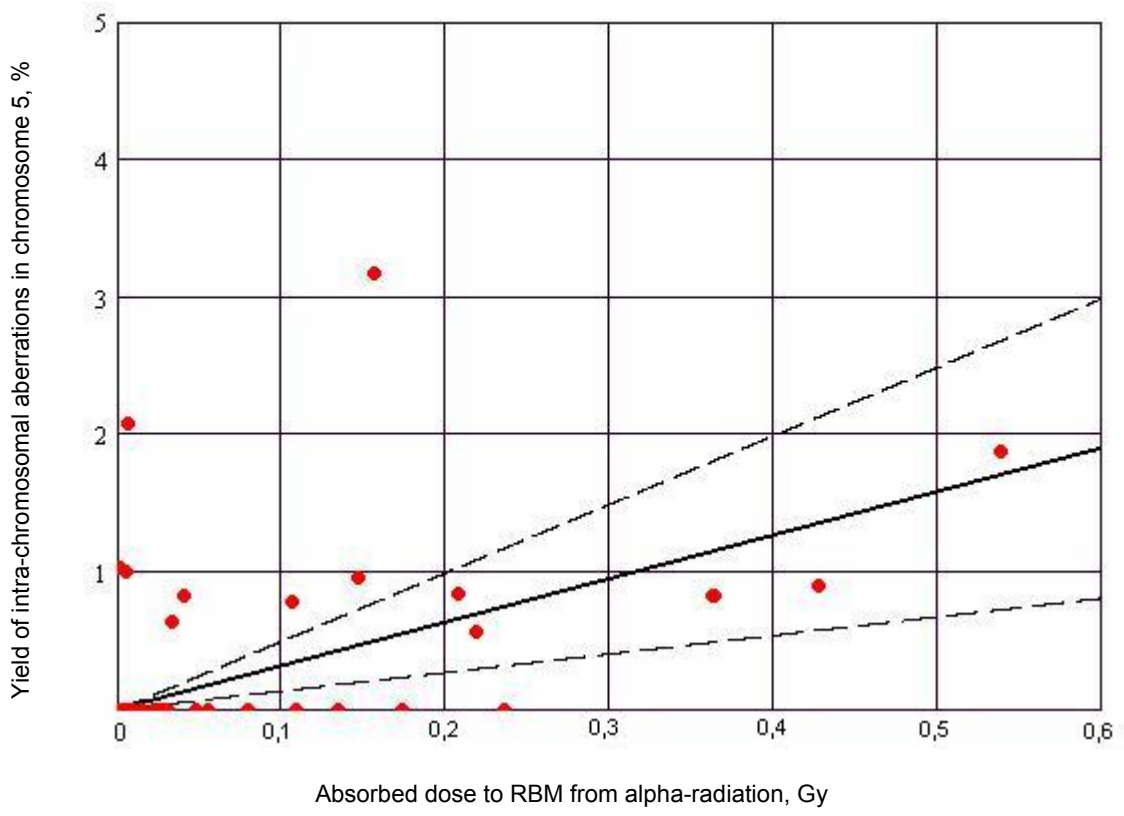

Fig. 1.1. Dependence of the yield of stable intra-chromosomal aberrations on absorbed dose to RBM from alpha-radiation.

Discussion

Our choice of chromosome 5 for this analysis was based on the findings by Mitchell et al., who had analyzed the yield of intra-chromosomal aberrations in three large chromosomes (1, 2, and 5) and demonstrated a higher intra-chromosomal yield per unit DNA length for chromosome 5 (12.7 ± 2.1 /10^5 Mb) than either chromosome 1 (5.6 ± 1.4 /10^5 Mb) or chromosome 2 (4.5 ± 2.1 /10^5 Mb).

The derived linear dependence allows for the approximated assessment of absorbed dose from internal exposure based on the yield of stable intra-chromosomal aberrations. However, a small sample size (40 individuals) did not allow obtaining any statistically significant linear correlation coefficient. In the future, the derived dependence will be refined with the increased statistical data size.

Conclusions

The preformed analysis revealed a linear dependence of the yield of stable intra-chromosomal aberrations in the peripheral blood lymphocytes in human on absorbed dose to red bone marrow from internal exposure to alpha-radiation from ^{239}Pu in workers of the Mayak PA.

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Cytogenetic damage in cells exposed to ionizing radiation under conditions of a changing dose-rate

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Abstract
The current international paradigm on radiation effects is mainly based on the effects of dose with some consideration for the dose rate. No allowance has been made for the influence of a changing dose rate (second derivative of dose) and biological effects of exposing cells to changing dose-rates have never been analyzed. Here, we provide evidence that radiation effects on cells may depend on temporal changes in the dose-rate. In the experiments cells were moved to and from a source of radiation. The speed of movement, the time of irradiation and temperature during exposure were controlled. Here we report the results of first experiments with TK6 cells which were exposed to a constant, to an increasing and to a decreasing dose-rate. The average dose rate and the total dose were same for all samples. Micronuclei were scored as the endpoint. The results show that the level of cytogenetic damage was higher in cells exposed to a decreasing dose-rate as compared both to an increasing and a constant dose-rate. This finding may suggest that the second derivative of dose may influence radiation risk estimates and it is expected to trigger further studies on this issue.

The effect of dimethyl sulfoxide on radiation induced chromosome aberrations in cultured CHO cells

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Abstract

The radioprotector properties of dimethyl sulfoxide (DMSO) are rather attractive for fundamental and applied implication in radiation biology and medicine. The cultured CHO cell line is a suitable model that allows estimation cell survival and chromosome aberration yield in mammalian cells. The goal of the current study was to investigate the redaction of radiation induced chromosome aberration yield in mammalian cells treated by DMSO. The CHO-K1 wild type cells were used as in vitro experimental model. The cells were grown in DMEM media containing 10% bovine serum albumine and antibiotics in monolayer culture. The cell samples with or without DMSO were irradiated to 1.0, 2.0, 3.0 and 4.0 Gy at a dose rate of 0.3 Gy/min, using 60-Co source. The DMSO was added to samples five min before irradiation in concentration of 0.5 and 1 M. The synchronized (G-1 phase) and logarithmic growing (cycling) cells were investigated. After exposure, cell were washed and fresh 10% bovine serum albumine was added to culture. The cells were fixed at 5, 7 and 9 h after irradiation. Approximately, 600 metaphases for chromosome-type aberrations were scored from each dose sample. It was found that DMSO treatment results in a 40–50% reduction of chromosomal aberrant cells as compared with non-treated cells irradiated to the same dose. The radiation-induced frequency of chromosomal aberrations decreased 2-fold having clear dose dependence. This reduction in chromosome aberration frequency is thought to be the result of a free radical scavenging and, perhaps, a suppressing radiation induced bystander effects in CHO culture. The experimental study was accompanied by theoretical Monte Carlo computer simulation of indirect action to DNA of cells by free water radical including OH scavenging in DMSO. The match of experimental and theoretical results is discussed.

The relative biological effectiveness of accelerated 73 MeV protons determined by CHO cell assay

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Abstract

The purpose of the study was to estimate relative biological effectiveness (RBE) of 73 MeV protons using CHO-K1 cells assay. The cells were grown at 37 0Ñ in DMEM media containing 10% bovine serum albumine and antibiotics in suspension. The synchronized (G-1 phase) cells were investigated. The cell samples were irradiated with single doses: 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 4.0 Gy, using pulsed proton beam. Gamma-radiation of 60-Co at a dose rate of 0.5 Gy/min was used as a reference source. After exposure cell were washed and fresh 10% bovine serum albumine was added to culture. The cells were fixed at 18, 19 and 21 h after irradiation. Approximately, 500 metaphases for chromosome-type aberrations were scored from each dose sample using traditional Giemsa stain and light microscope. The experiment was carried out twice in a period of a year. The RBE values were determined by comparing the doses needed to obtain the same yield of chromosomal aberrations as with the reference 60-Co gamma source. It was found that the yield of dicentrics per 100 cells follows linear-quadratic model: Y=1.56D+2.48D**2 and Y=6.88D+2.11D**2 (where D – absorbed dose in Gy) for 60-Co rays and 73 MeV proton irradiation, respectively. Therefore, protons were somewhat more biologically effective than 60-Co gamma-rays according to criterion of chromosomal aberrations. The proton RBE values obtained by regression fitting ranged 1.1-2.6 for absorbed doses from 6.0 to 0.5 Gy respectively. The data obtained at other proton acceleration centers agree with our results. Although the RBE value for protons was found to be rather small, it could be of radiotherapy importance. Accurate determinations of the RBE are necessary when extra therapeutic dose is inadmissible.

Effect of low dose gamma-radiation upon antioxidant parameters in heart and skeletal muscle of chick embryo

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Abstract

This study was performed to investigate the effect of low-dose gamma-irradiation upon activity of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT) and level of glutathione (GSH) and lipid peroxidation (TBARS) in heart and skeletal muscle of chick embryo and newly hatched chicks. Fertilised chicken eggs were irradiated with the dose of 0.3 Gy gamma radiation (source ^{60}Co) on the 19th day of incubation. The antioxidant parameters were measured in breast muscle (*m.pectoralis superficialis*), thigh muscle (*m. biceps femoris*) and heart of chick embryos on 1, 3, 6, 24 and 72 h after egg irradiation. All parameters were measured spectrophotometrically. On the 1st hour after irradiation lipid peroxidation significantly decreased in all type of muscles. At the same time GSH level and CAT activity were significantly decreased in the breast and thigh muscle of chick embryo while SOD activity was significantly decreased in thigh muscle. On the other side on the 1st hour after irradiation GSH-Px activity was significantly increased in thigh muscle. On the 3rd, 6th and 24th hour of experiment there were no significantly changes in the investigated parameters between the groups. On the 72nd hour after irradiation results showed decreased activity of SOD in thigh muscle; decreased activity of CAT in the breast and heart muscle; decreased TBARS level in thigh muscle; increased level of GSH in the breast and thigh muscle; and increased GSH-Px activity in the breast muscle. The obtained results suggest that acute irradiation of chicken eggs on the 19th day of incubation with the dose of 0.3 Gy gamma radiation causes an oxidative stress in all types of muscles immediately after irradiation. However, the results of GSH-Px activity and GSH level on the one-day old chicks (72nd hour after irradiation) in skeletal muscle suggest probably a stimulating effect of low dose irradiation.

Influence of the ionizing radiation on the individual variability of the antioxicant status indices

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Abstract
The variability of the different parameters of the physicochemical regulatory system of the lipid peroxidation (LPO) in tissues of the laboratory mice (white outbreed and SHK) is studied in norm and under the action of radiation at the low doses and within the wide range of dose rate. It is shown that the different variability and the ability to normalization of the antioxidant activity and composition of lipids, intensity of LPO, the peroxide content of lipids and their antiperoxide activity in tissues are due to the antioxidant status of tissues and the radiosensitivity of mice. It was revealed that the radiation dose and dose rate result to the indefinite changes in the variability of the studied parameters. The data obtained allow us to conclude that the high variability of the LPO regulatory system parameters in the murine tissues plays the important role for the development of the biological consequences after the radiation action depending on the dose and dose rate.

Introduction
At present it is established that the lipid peroxidation (LPO) processes play the important role in the regulation of the cell metabolism [10]. The steady-state of the LPO in tissues of intact animals is provided by the physicochemical regulatory system, which is functioning not only at the membrane level [3], but the cellular and organ levels [13]. Among parameters of this regulatory system in the last case are the antioxidant (AOA) and antiperoxide (APA) activities and composition of lipids; the initial content of the peroxide in lipids, stipulated by their nonsaturation degree; the lipid oxidizability, which may be estimated as the ratio of sums of the more easily (EO) to the more poorly oxidizable (PO) fractions of phospholipids ($\sum$EOPL/$\sum$POPL); the LPO intensity and other. The biological consequences of the radiation action are due to the level of the AOA of the tissue lipids, the activities of the antioxidant defense enzymes and the LPO intensity in organs and tissues of animals and also their ability to repair and normalization after the radiation injury [4, 5, 12, 18].

The aim of this work is to reveal the variability of the different parameters of the antioxidant status in tissues of mice possessing different radiosensitivity both in norm and under the action of radiation at the low doses and within the wide range of dose rate.

Materials and methods

The experiments were carried out on 140 mice SHK (males, 17 – 23 g), 54 white outbreed mice (females, 23 – 30 g) and 20 mice SHK (females, 20 – 25 g). The γ-irradiation of mice SHK (males) was performed by a GUT-Co-400 apparatus (a source of γ -60Co). Mice SHK (males) were divided in 4 groups as follows:

1. The γ-irradiation at the dose of 15 cGy, the dose rate was 0,01 cGy/min (group 1; 30 mice); the irradiation interval was 25 h;
2. The γ-irradiation at the dose of 15 cGy, the dose rate was 0.25 cGy/min (group 2; 30 mice); the irradiation interval was 1 h;
3. The γ-irradiation at the dose of 15 cGy, the dose rate was 9 cGy/min (group 3; 30 mice); the irradiation interval is 100 s.
4. Intact mice from the same party (group 4, 20 mice) serve as biological control.

Mice were irradiated by storage of the food and the drinking-water (group 1) or only the drinking-water (group 2) in cells for animals per 10 mice. Mice of the group 3 were irradiated in the special container where the each mouse could easily drive.

The single acute X-irradiation of mice (females, 10 mice) at the dose of 16 cGy was performed by a RUT-200-20-3 apparatus in the same special container at the following conditions: current strength – 15 mA; filter 0.5 mm Cu; the dose rate is 44 cGy/min; the total irradiation interval 22 s.

Decapitation of mice after γ-irradiation was done within 1, 7 and 30 days and 1 month after X-irradiation. Decapitation of intact mice was performed simultaneously with the experimental group per 6 – 10 animals. As known, the lipid AOA level and the LPO intensity have the season variability [4, 9, 12]. It is need to note that experiments are performed on the mice SHK (males) in April and May, on the white outbreed mice in September and October, on the mice SHK (female) in October and November. All parameters of intact mice were determined for each animal. Mice in experimental groups were divided in subgroups per 1 – 4 animals. After decapitation of mice, blood was collected in test tubes treated by 5% solution of sodium citrate. The erythrocytes from the blood plasma were separated by centrifugation. The murine liver and brain was placed in ice-cooled weighting bottles immediately after decapitation.

Lipids were isolated by the method of Blay and Dyer in the Kates modification [8]. The AOA of lipids was determined by using the methyl oleate oxidation model [4] as the ratio of the differences in the induction period of methyl oleate oxidation in presence and absence of lipids to the concentration of the added lipid. Preliminary methyl oleate was purified by vacuum distillation. The lipid concentration in methyl oleate was 20 mg/ml. Oxidation was carried out in a temperature-controlled chamber at a constant temperature 37±0.1ºC by blowing air through at a rate providing oxidation in a kinetic range. In detail the lipid AOA analysis was described in [14]. The peroxide content in lipids was determined iodometrically. The antiperoxide activity (APA) of lipids, that is their ability to decompose peroxides, was evaluated as the ratio of the difference in the peroxide concentrations of methyl oleate in absence and presence of lipids to the amount of the added lipid [16].

The qualitative and quantitative composition of phospholipids (PL) was determined by thin-layer chromatography [2]. It was used type G or H silica gel (Sigma, USA), glass plates 9×12 cm and mixture of solvents chloroform : methanol : glacial acetic acid : water (25:15:4:2) as a mobile phase. All the solvents were of specially pure

or chemically pure grade. The development of chromatograms was performed by iodine vapour. For the colour reaction for phosphorus we used ammonium molybdate and ascorbic acid (Serva, FRG) and perchloric acid of chemically pure grade. The amount of inorganic phosphorus was judged from the optical density of the solution at the wavelength 810 nm with Beckman Du-50 (Austria) or KFK-3 (Russia) instruments. The sterol content was determined spectrophotometrically by the method cited in [17]. In addition to quantitative analysis of the different fractions of PL and sterols the following parameters of lipid composition were also evaluated: the molar ratio of [sterols]/[PL]; the PL proportion in the total lipid composition (% PL); the ratio of the phosphatidyl choline to phosphatidyl ethanolamine content in PL (PC/PE) and the ratio of the sums of the more easily to the more poorly oxidizable fractions of PL (∑EOPL/∑POPL). The last value was calculated by the formula: (PI + PS + PE +CL + PA)/(LPC + SM + PC), where PI is phosphatidylinositol, PS is phosphatidylserine, CL is cardiolipin, PA is phosphatidic acid, LPC are lysoforms of PL, SM is sphingomielin.

The content of LPO products which have reacted with 2-thiobarbituric acid (TBA-reactive substances, TBA-RS) was determined spectrophotometrically by the method described in [1] with adding 10 µl of 0.01% 4-methyl-2,6-di*tert*.butylphenol (BHT) solution in ethanol. The diene conjugates and ketodiene amount in the liver and brain lipids were judged from the optical density of the lipid solutions in hexane (the range of concentration from 0.02 to 0.2 mg/ml) as their ratio at the wavelengths 230±2 nm and 270±2 nm to 205±3 nm, correspondingly [7] used UV-3101 PC (Japan) instrument. Protein was determined according [6].

The statistic treatment of the results were performed using standard method of the variational statistics. The variability of indices was evaluated as the ratio of mean square error of average mean to average mean for group expressed as a percentage.

Results

Earlier it is shown the existence of the reverse correlation between the lipid AOA value and the initial peroxide amount in the spleen lipids of the CBA mice [18]. The high ability to the peroxyl radical formation is also revealed by γ-irradiation of mice SHK at the dose of 15 cGy with the different dose rate for the spleen and erythrocyte lipids [11]. It is need to note that the lipids from these tissues characterize the prooxidant activity in the autooxidation reactions [16]. Besides, the highest heterogeneity of the peroxide content in the murine lipids within the experimental groups were namely established for the spleen and erythrocytes lipids of mice SHK after γ-irradiation at the low doses.

Usually the organ of the intact mice in accordance with their lipid AOA may be presented by following consequences: liver > brain > spleen [4, 12, 16]. The different initial level of these parameters is due to the differences in the sensitivity to the radiation injury another indices of the LPO regulatory system. Thus, the least changes of the LPO intensity which is usually evaluated by the TBA-reactive substances content in a complex biological system [7] were revealed in the liver lipids of mice SHK under their γ-irradiation at the dose of 15 cGy, especially at the 0.25 cGy/min dose rate [11]. Besides, the greatest changes of this parameter were obtained by the radiation of mice SHK at the low intensity dose rate (0.01 cGy/min) in the blood erythrocytes. The substantial dependence of the change scale on the dose rate was obtained for the lipid

composition of liver, despite the fact that lipids of this organ characterize the most high level of AOA. The relative changes of generalized parameters in the liver lipids under γ-irradiation of mice with the different dose rate are presented in Fig. 1. It is seen the absence of the normalization practically for the all studied indices value within 1 month after irradiation. Besides, the more substantial changes are revealed for the molar [sterols]/[PL] ratio at the all dose rate. Although the PC/PE ratio is a more stable as compared with the another parameters there are the reliable changes in the relative content of both PC and PE in the PL liver of the irradiated mice especially at the dose rate of 0.01 cGy/min.

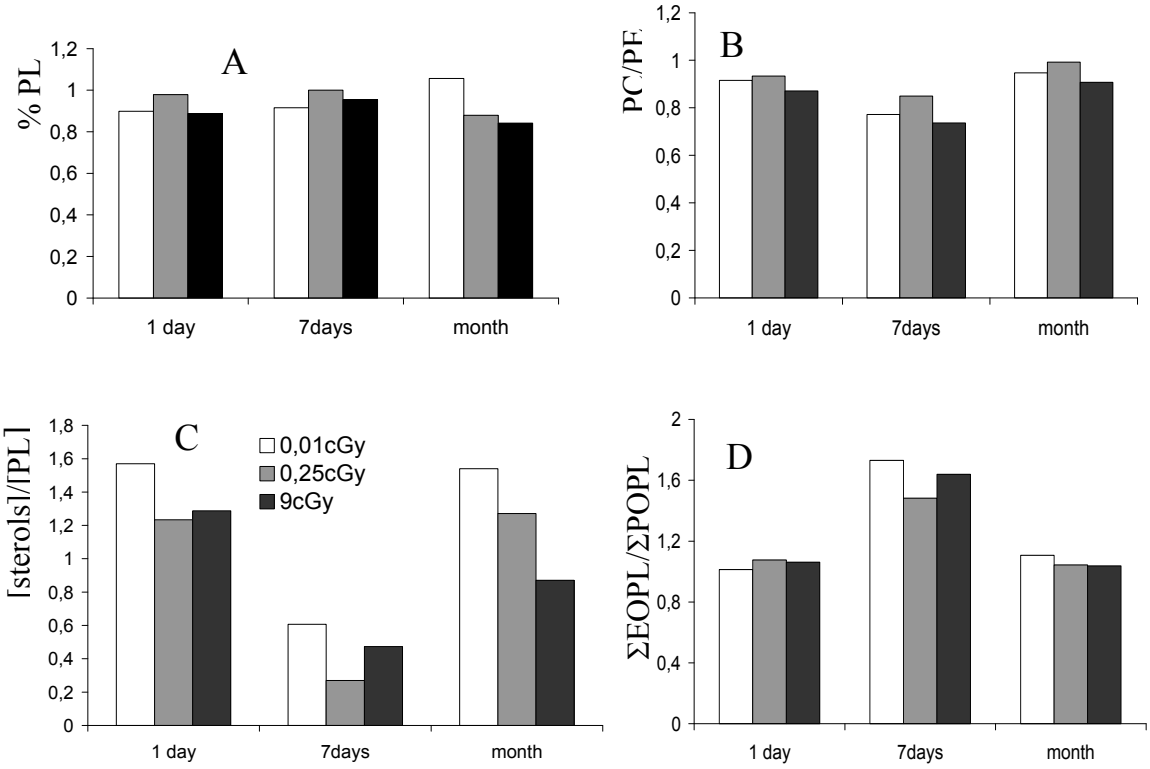

Fig. 1. Influence of the dose rate on the relative changes of the generalized parameters of the liver lipid composition under γ-irradiation of mice at the dose of 15 cGy.

Within 1 month after the acute X-radiation of mice SHK at the dose 16 cGy the reliable difference of the PC/PE and ΣEOPL/ΣPOPL ratio in the liver PL are obtained between the average values for the control and experimental groups. While the more high variability there is within the irradiated mice as compared with control group for the [sterols]/[PL] ratio, the another generalized parameters have a more limit of variance for the control mice. The data which are presented in Table are confirmed this conclusion.

Table. Limits of variations of the generalized parameters of the lipid composition in liver of mice SHK (females) within 1 month after X-radiation at the dose of 16 cGy.

Variant of experience	Parameters of the lipid composition			
	% PL	PC/PE	ΣEOPL/ΣPOPL	[sterols]/[PL]
Intact biological control X-irradiation at the dose of 16 cGy	10,7 – 60,8 21.3 – 83.6	1.19 – 2.28 2.01 – 2.39	0.30 – 1.06 0.43 – 0.53	0.54 – 1.09 0.58 – 0.53

It is necessary to note that the LPO intensity, the diene conjugate (DC) and ketodiene (KD) amount in the liver and brain lipids changes at mice of the different age (Fig. 2 and Fig. 3). As seen, the individual variability of these parameters differs both in the AOA of lipids and the analyzed parameter. So, the greatest variability for the TBA-reactive substance content in brain is revealed for the young mice (11.8 %), but in liver for the mice 18 weeks age (12.7 %). The DC content also has the highest individual variability in the brain lipids of the young mice (21.6 %) and in the liver lipids for mice 18 weeks age (35.5 %). The individual variability of the KD content decreases in the brain lipids from 13.6 % to 8.3 % at the aging of mice. Besides, this parameters has the most variability in liver lipids of the 18 week mice (29.0 %).

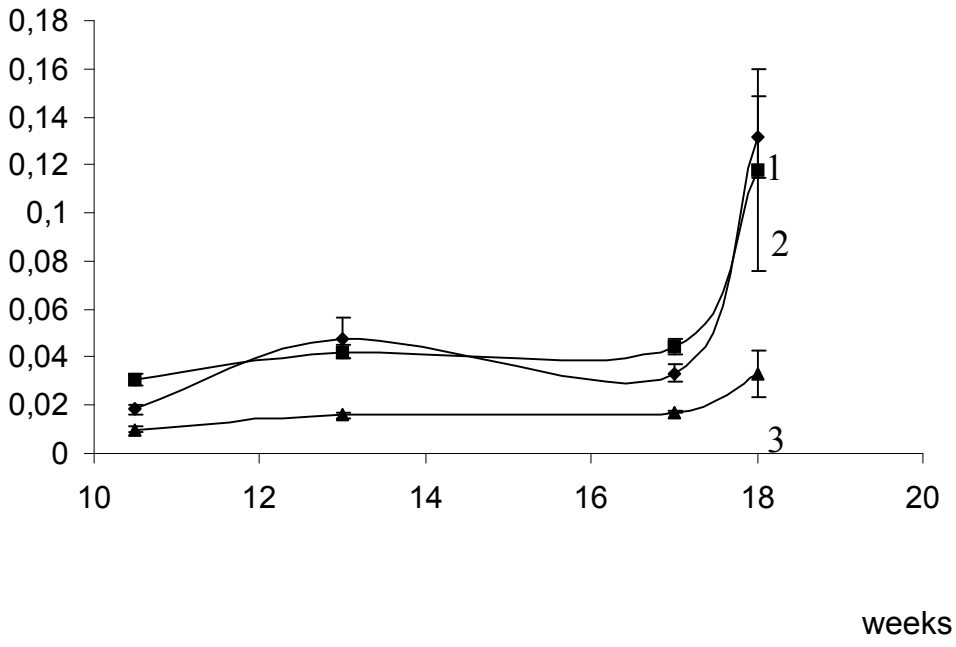

Fig. 2. The aging changes of the TBA-reactive substances (1, nmol/mg of protein), diene conjugate (2, a.u.) and ketodiene (3, a.u.) content in the liver lipids.

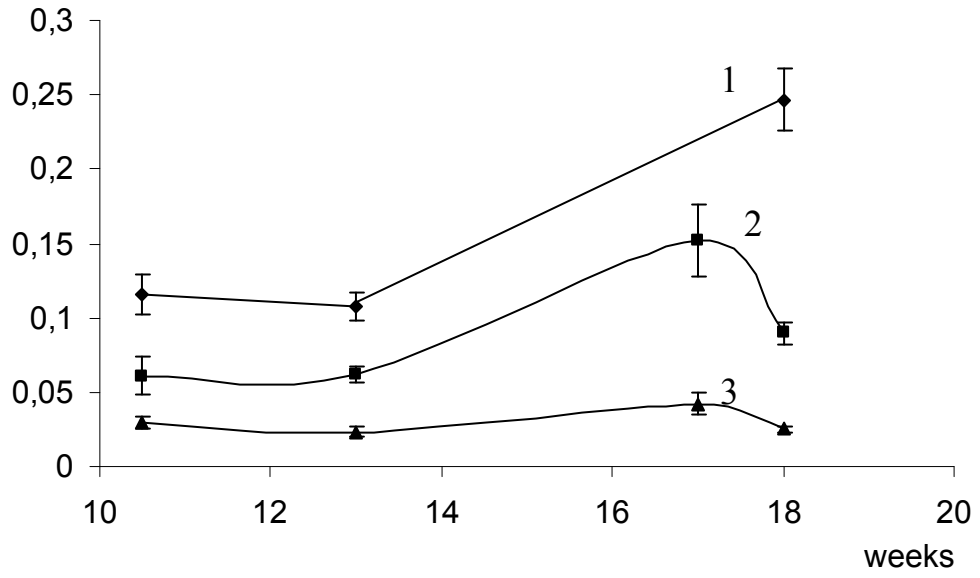

Fig. 3. The aging changes of the TBA-reactive substances (1, nmol/mg of protein), diene conjugate (2, a.u.) and ketodiene (3, a.u.) content in the brain lipids.

Conclusions

As early established, the oxidative processes in lipids have to influence on forming of the biological effects under the acute X-radiation of the laboratory animals which characterize the different radiosensitivity [18]. As also shown, the relationship of membrane and DNA damage with lipid peroxidation under the low doses of some technogenic factors [15]. Besides, the lipid AOA play the important role for the development of the biological consequences under the radiation action at the low doses [13]. The high variability of the physicochemical characteristics and the composition of lipids in norm has in influence on the forming of the biological effects in organs of the irradiated mice. The scale of changes are due to the radiation dose and dose rate and the antioxidant status of tissues. The data obtained allow us to conclude that the individual variability of the antioxidant activity and composition of lipids, intensity of LPO, the peroxide content of lipids and their antiperoxide activity in tissues takes into account for the development of the biological consequences after the radiation action on organism.

Acknowledgement

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STORE – Sustaining access to Tissues and data frOm Radiobiological Experiments

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Abstract

Sharing of data and biomaterials from publicly-funded experimental radiation science will yield substantial scientific rewards through re-analysis and new investigations. To that end, the STORE Consortium will create a platform for the storage and dissemination of both data and biological materials from past, present and future radiobiological research, a so-called Data Warehouse. STORE will provide a single online portal to radiobiological information that is presently distributed over scientific centres worldwide, and it will provide the necessary standard operating procedures (SOP) for the evaluation of archived tissue usability. The final goal of the project will be to propose viable financial models for the long-term sustainability of both material and data emerging from radiobiological research.

Fig. 1. Logo of STORE (http://www.fp7store.eu).

The strategy to achieve these goals is multi-level: 1) to provide a "one-stop-shop" portal integrating international databases and other repositories currently active, such that the user can find material and data held remotely; 2) to archive primary (raw) data or pointers to data in public databases, from radiobiological experiments and studies, while this resource will be open to individual investigators and to funding agencies as a potential central repository for data sharing; 3) to physically archive threatened material resources which are considered to be a valuable resource to the Community, and whose state of preservation is consistent with STORE benchmarks; 4) to provide a single point of access to the integrated biomaterial resources through standardised request procedures.

The provision of a central portal is expected to help in the dissemination of awareness of the existence of these resources, many of which are, anecdotally, underused because their availability and existence is unknown. The generation of benchmarks for sample preservation and usability by preparation of SOPs will also help to disseminate formal standards by which the usefulness of archive material of this type can be assessed.

Introduction

Since the late 1950s, valuable data have been collected on the effects of experimental, accidental and medical irradiation of humans and animals. Sharing and re-use of data and materials from publicly funded experimental radiation science and clinical research adds huge value to the original investment and can yield substantial scientific rewards through re-analysis and investigation, but such activity requires an infrastructural resource in order to be realised.

High-throughput screening techniques, so called "omics", have been developing in recent years with a breathtaking speed. The question arises which modern techniques are suitable to be used with the FFPE tissue (Tapio et al 2008a). Finding novel methods to re-evaluate the archive material will provide us with a vast amount of research data, the concept of data banking becoming increasingly important. Combined with information on individual exposure, disease diagnostics, and available biomaterial this data become extremely valuable.

The principal aim of data sharing is to maximise the return from the scientific endeavour and make the best use of research and funding. Although opportunities and developments in technology allow rapid, efficient and independent dissemination of vast amounts of information and despite the existence of guidelines on access to data, good practice is not widespread (Schofield et al). Open access to quality-controlled data in interoperable formats would allow better use of the original investment in collecting the data. Re-analysis of experimental results could be used to validate and adjust previous results and, in an interdisciplinary setting, also open up new research avenues well beyond the initial context in which the data were collected. Consequently, current hypothesis could be tested and new hypothesis could be advanced.

STORE, a 3-year project running from 2009 to 2012 and supported by the European Commission, currently works to realise the vision of an open access to data gained from radiobiological studies on animals. The main challenge here is finding financial support for maintenance and development of data resources to best serve the scientific community (Chandras et al).

STORE wants to a) improve the quality of and accessibility to radiobiological data and to promote the awareness of the importance of data sharing and b) create a platform for the storage and dissemination of both data and biological materials from past, present and future radiobiological research. The STORE platform will consist of a combined "Data Warehouse" and physical repository that will enable the sharing of experimental data sets and materials. STORE will provide a single portal to radiobiological information that is presently distributed over scientific centres worldwide. STORE will provide the necessary Standard Operating Procedures (SOPs) for the evaluation of archived tissue usability. The project will put forward feasible financing models for long-term support of data and bio resources in the radiobiology.

Methods

Within STORE two databases will be developed and maintained. The primary database (Data Warehouse) will form a public repository for radiobiological data and will provide links to other relevant databases. The second one will be a smaller bioresource database containing information about the biological samples in the STORE. Both data bases will be linked to each other.

Data Warehouse

The Data Warehouse will contain a) primary (raw) datasets from legacy experiments and new investigations, input by users or curators, b) summary information and links to datasets from individual projects already submitted to other public databases and c) a portal to other existing databases, such as ERA, the Chernobyl Tissue Bank, and the Northwestern Janus database, mediated through the generation of a BIOMART. BIOMART is an open-source application which takes data tables from distant databases and transforms them into a standard structure to allow queries of the same form to be made across all the databases.

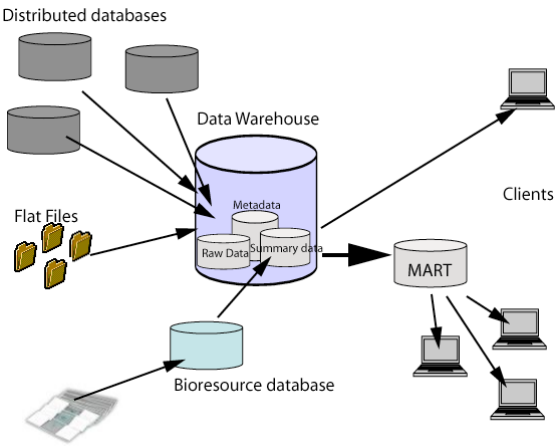

Fig. 2. Scheme of the database.

The Data Warehouse will be a database with the primary data object being the "Study". Key metadata, such as SPECIES, RADIATION QUALITY, ENDPOINT, will be attached to the data and study. Where appropriate, the structure of primary data will

be transformed into a standardised form. The data organisation and metadata will be based on the growing group of accepted data structure standards and ontologies for functional genomics and biological and biomedical investigations to enhance interoperability with other databases.

Bioresource database

A second database and curation facility will be established to deal with the physical archiving issues. Current good practice guidelines for biological resource databases have already been described by several agencies, most recently by the NCI (http://biospecimens.cancer.gov/practices/) and within the caBIG project in the USA (http://biospecimens.cancer.gov/practices/cabig_tools/). Such recommendations are a gold standard as they are designed for human clinical material; the degree to which they are implemented in STORE will depend on the results of the investigations on the practice and regulatory issues surrounding tissue retention and international transfer. We suggest that the system developed here will be sample-centric, each sample possessing its own unique identifier corresponding to the individual animal and the study. Interactions with the Data Warehouse to source detailed experimental information on the samples will be accomplished through the individual codes. Where samples have been processed, for example into RNA and DNA, the database will support quality control, aliquoting, bar-coded containers and processes. Tracing and internal audit will be possible so as to identify end users and obtain feedback, and to comply with regulatory requirements.

Regulatory Issues

The regulations concerning archiving and transfer of animal and human tissue across international borders will be a major focus of the project. As we expect that biomaterial will be transported across European but also international boarders during the lifetime of the project, it is one of the main goals to facilitate exchange of samples between remote archives offering researchers advice and support. Based on international regulations on transfer of human and animal biomedical data and biological material a respective set of recommendations will be developed. A particular question important for the radiobiology community is the transport of radioactive material and will be given special attention (see below). To facilitate the comparison of international regulations links to relevant institutions in the US and in Japan have been made.

Sustainability and accessibility of the biological archives

STORE wants to develop operational parameters for a sustainable and accessible radiobiological tissue archive. For that purpose, questions concerning the current status and ownership of biological samples and their documentations have already been investigated within an FP6 project Promotion and Update of the European Radiobiological Archives (ERA-PRO; Tapio et al 2008b) A further task is to prepare a concept for sustainability and accessibility to the biological archive material and documentation in consultation with other international archives. Finally, evaluation of the radiological issues involved in the storage, transport and use of the archive materials is of great importance. Specifically, STORE will give recommendations on how to handle material arising from experiments using long-lived internal emitters. In this case,

some radioactivity coming from radionuclides in the tissue has to be taken into consideration when handling the sample.

Establishment of Standard Operation Procedures

The biological material in physical repositories has to be used with greatest care to avoid damage due to its irreplaceable nature.

It has to be borne in mind that most techniques available at the moment were optimised for fresh/frozen tissue. FFPE tissue has recently gained considerable interest as an alternative to fresh/frozen tissue in retrospective biomarker discovery. However, macromolecules such as DNA, RNA and proteins undergo degradation and in the case of proteins also cross-linking during the fixation and aging process, making the applicability of conventional analysis problematic. There is an obvious need for methodological optimisation for FFPE material. Furthermore, as different storing times and conditions and the length of fixation influence the quality of various macromolecules in a different manner, these parameters will be tested using a common tissue model.

Taken together, STORE aims to establish Standard Operating Procedures (SOP) for evaluating quality of radiobiological archive tissue and defining test systems describing the usefulness of such material. Given that successful analysis of biological material isolated from archived tissue is feasible, the procedures performing best in terms of different endpoints compared to the fresh/frozen control tissue, will be defined as SOPs. At the end of the project, the SOPs will be validated by a STORE member not involved in their development.

Discussion

Sharing research data avoids its recollection by unnecessarily repeating animal experiments and enables their reanalysis. The vast amounts of data generated by previous and modern research will be valuable long after the experiments have been finished. It is less clear how data and databases will be maintained in order to guarantee the future access to these resources. The maintenance of databases is particularly at risk of falling victim to the constraints of traditional grant-based funding. We suggest that entirely different criteria have to be applied to judge the value of a database. For example, is it a unique and essential community resource? How are the data curated and validated? How comprehensive, detailed and interconnected are the results?

In many cases, researchers collect ancillary data that extends the primary research outputs: survey data, lab notebooks, spreadsheets, and annotations just to name a few. Much of this ancillary data (and some of the primary data as well) is not currently digitised, e.g. lab notebooks or handwritten specimen labels. Some of this additional data is maintained; much of it is lost, either because it is difficult or impossible to locate, or because it is simply deleted or destroyed. Research data (from old and newly performed experiments) in a digitalised form can be easily transported, worked on with new tools, merged with other data, categorised in new ways and stored in vast volumes. Research data are valuable long-term resources and the way to ensure their potential value is *to share* and *make them accessible*.

There is a need to collect, store, curate and distribute radiobiological data in a standardised form to ensure interoperability within a central database and databases

linked to it. Action is urgently required for funding of data and biomaterial repositories on a stable, long-term basis as uncertainty about the future funding and hosting of these resources will be a disadvantage for the whole radiobiological research community.

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Malignant blood diseases and tumours in acute radiation sickness survivors following the Chernobyl accident

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Abstract

In the result of Chernobyl accident acute radiation sickness (ARS) developed in 134 patients, including 28 ones who had died in 1986 due to severe radiation damages. At the end of 1986 we started follow-up study on oncological pathology development in 91 ARS survivors (doses up to 7.1 Gy) and 99 patients with doses below 1 Gy. All patients were residents of Ukraine. During 24 post-accidental years malignant diseases of hematopoiesis were observed in 5 patients and solid tumours in 15 ones. Oncohematological pathology consisted of hypoplasia of hematopoiesis (1 case; 1986), acute myelomonoblastic leukaemia (1 case; 1998) and myelodisplastic syndrome (3 cases; 1993, 1995, 1996). All diseases brought patients to death. Patients' mean age was 46.3±7.5 at the moment of irradiation, 54.0±8.1 on disease onset and 55.5±6.8 years at person's death. Leukaemia and myelodisplastic syndromes arose only in ARS survivors. Their latent period was 9.6±2.1 years. The first malignant tumour, sarcoma of hip soft tissues, was diagnosed in 1992. This patient died in 1993. Before 2009 it was revealed as far back as 14 malignant neoplasm of different localization: cancers of colon (3 cases; 1997, 1999, 2001), stomach (2 cases; both in 2004), thyroid gland (2 cases; both in 2000), throat (1 case; 2000), kidney (1 case; 2000), lung (1 case; 2001), prostate gland (1 case 2001), basal cell carcinoma of head (1 case; 2006), urinary bladder (1 case; 2008), and neurinoma of lower jaw that had a malignant transformation (2003). Amongst persons suffered from cancer, 7 patients were successfully treated but the rest ones died. Totally, malignant tumours were diagnosed in 6 ARS survivors (6.6%) and 9 persons from group of comparison (9.1%). At the moment of death from malignant tumors the patients' age was 66.2±9.8 years. The period from irradiation to neoplasm development averaged 15.9±3.1 years.

Introduction

Malignant tumours and leukaemia are considered the stochastic effects of radiation. The majority of radiogenic tumours have no dose threshold (Beebe 1984; WHO 2005) and a risk of cancer development increases with radiation dose. Studying consequences of the Chernobyl accident some researches came to the conclusion of oncological morbidity

rate increase in victims during post-accidental period (Demina 2002; Bespalchuk et al. 2003; Ivanov et al. 2001; Kirillov et al. 2001) and experimental animals as well, who was keeping in Chernobyl zone (Serkiz 1991). Other authors have the opinion that the accident at the Chernobyl nuclear power plant (CNPP) didn't result in essential growth of cancer and mortality from malignant tumours in exposed persons in comparison with the rest population (Buldakov 2002; Ganul et al. 1991; Ivanov et al. 2001; Moysich et al. 2002; Tukov et al. 1998).

Such contradictory results of numerous investigations possibly connected with the fact that CNPP clean-up staff are characterised by wide radiation dose range. The opinion is existed that doses 0.1 Sv and higher are able to cause statistically significant excess risk of cancer incidence (Beebe 1984; Filyushkin et al. 2000; UNSCEAR 2000). This situation makes the study of oncological and oncohematological pathology in persons exposed to irradiation in doses upper 0.1 Sv, especially ARS survivors, are very actual.

Material and methods

After the Chernobyl accident 91 ARS survivors were under follow-up in the hospital of Research Centre for Radiation Medicine (RCRM). From this number 38 patients survived ARS grade 1 (mild), 41 ARS grade 2 (moderate) and 12 ARS grade 3 (severe). As a control group 99 patients with whole-body radiation doses lower 1 Gy (ARS 0 group) were examining during entire post-accidental period (table 1). Patients' groups didn't differ significantly by age

Table 1. Age-specific and dosimetric characteristic of the examined patients

Variables	ARS 0	ARS grade 1	ARS grade 2	ARS grade 3
Number of persons	99	38	41	12
Gender: M / F	89 / 10	36 / 2	40 / 1	12 / 0
Age at exposure, years mean ± SD min - max. 95% confidence interval	 35.9±10.3 18.4-60.3 33.8-38.2	 33.2±8.2 17.6-56.3 30.5-35.8	 40.9±16.5 17.9-79.3 34.1-42.9	 36.6±12.5 20.4-72.6 30.4-51.3
Persons with known dose	15	30	37	11
Dose of irradiation, Gy mean ± SD min – max 95% confidence interval	 0.4±0.3 0.1-1.0 0.2-0.5	 1.0±0.6 0.1-3.3 0.8-1.3	 2.4±0.9 0.5-4.9 2.1-2.7	 4.5±1.4 2.9-7.1 3.6-5.5

All patients had a hospital examination and treatment at the average once per 2 years.

The check-up protocols included full blood count, biochemical tests (basic metabolic panel, chemical pathology and liver function tests), immunological profile (humoral and cellular indicators), thyroid tests (ultrasound and TSH, FT_4), urinalysis, full physical examination, and various instrumental tests as necessary (electrocardiogram (ECG), electroencephalogram, ophthalmological tests, gastro- or colonoscopy, chest x-ray, functional lung test etc. When necessary, patients were consulted by specialists (ophthalmologist, endocrinologist, pulmonologist, cardiologist,

gastroenterologist, haematologist, dermatologist, neuropathologist, psychiatrist, urologist or gynaecologist). Further, more complex diagnostic methods were applied as necessary, including CT scans, biopsy, cardio-stress-ECG, serological tests for specific causes, e.g. hepatitis A, B or C viruses, bone marrow tests etc.

Results

The first case of malignant hypoplasia of haematopoiesis was revealed in August 1986 in a patient, who worked several days in the CNPP zone in May and July 1986. His haematological status is characterised by essential leukocytopenia and slight anaemia. The onset of oncohematologic pathology was mistakably estimated as ARS grade 1 manifestation. However the following disease course dispelled any doubts against blood system pathology. The bone marrow aspiration confirmed the oncohematological diagnosis.

Subsequently two ARS grade 3 and one ARS grade 1 survivors developed myelodisplastic syndrome, which had the lethal outcome for all patients (table 2). One more patient, who had survived ARS grade 2, fell ill with acute myelomonoblastic leukaemia after 11.8 years passed from irradiation and died in spite of treatment.

Table 2. Cases of malignant blood disease in ARS survivors and control group

No	Diagnosis	ARS grade	First diagnosed	Age at (years)		Outcome
				diagnosis	death	
1	Hypoplasia of hemopoiesis	0	Aug 1986	48.6	49.3	Died on Apr 1987
2	Myelodisplastic syndrome	1	Sept 1996	45.0	50.7	Died on May 2002
3	Myelodisplastic syndrome	3	Mar 1993	52.1	52.2	Died on Apr 1993
4	Myelodisplastic syndrome	3	Oct 1995	64.6	64.7	Died on Dec 1995
5	Acute myelomonoblastic leukaemia	2	Feb 1998	59.9	60.7	Died on Dec 1998

All patients with malignant blood diseases were male. Their mean age at the exposure was 46.3±7.5 years, at the diagnosis 54.0±8.1 and at death 55.5±6.8 years. The latent period from exposure to first signs of disease was 9.6±2.1 years. Today the relative number of patients, who suffered from oncohematological pathology and died, is 2.9% of 190 persons under follow-up or 4.4% of ARS survivors.

The first case of malignant tumour was revealed in patients of ARS 0 group in 1992 (table 3). During examination of 34 years old man there was found subcutaneous, solid and tender formation on the right hip with dimensions as a pigeon egg. The patient was sent in a city oncological hospital but he refused from examination without clear explanations. When he was admitted in the RCRM hospital next year, it was found several solid subcutaneous nodes. The patients refused from oncological examination again and at the end of 1993 he died. Autopsy proved the supposed diagnosis sarcoma of hip soft tissues.

The second case of oncological pathology as cancer of sigmoid colon was diagnosed in 1997. A patient (ARS grade 1 survivor) only complained on bloody

discharges from anus during defecation. The adenocarcinoma in situ was diagnosed after colonoscopy and proved by histological examination.

Table 3. Cases of oncological pathology in ARS survivors and control group

No	Diagnosis	ARS grade	First diagnosed	Age at (years)		Outcome
				diagnosis	death	
1	Sarcoma of hip soft tissues	0	Apr 1992	34.1	34.8	Died in 1993
2	Cancer of colon	1	Mar 1997	54.5		Operated on Jun 1997
3	Leiomyosarcoma of shin Cancer of colon	0	Aug 1998 Jun 1999	72.5 73.4		Operated on Sep 1998 Operated on Jun 1999
4	Cancer of larynx	0	Jul 2000	59.3	59.9	Died on Feb 2001
5	Cancer of thyroid gland	2	Nov 2000	37.6		Operated on Nov 2000
6	Cancer of thyroid gland	2	Dec 2000	42.9		Operated on Jan 2001
7	Cancer of kidney	0	Dec 2000	44.4		Operated on Jan 2001
8	Cancer of colon	0	Nov 2001	63.6	67.5	Died on Oct 2005
9	Cancer of prostate	0	May 2001	71.7	73.3	Died in 2003
10	Cancer of lung	0	Feb 2001	46.1		Operated on Jun 2003
11	Neurinoma of lower jaw	2	Oct 2003	52.7	53.9	Died on Dec 2004
12	Cancer of stomach	0	May 2004	77.5	78.1	Died on Jan 2005
13	Cancer of stomach	0	Jul 2004	65.7	65.9	Died on Sep 2004
14	Basal cell carcinoma of head	1	Nov 2006	51.9		Irradiation
15	Cancer of urinary bladder	2	Jun 2008	66.8	66.9	Died on Aug 2008

It is necessary to note that all subsequent cases of solid tumour either had no clinical symptoms, being a result of causal findings during the routine examination (endoscopy, ultrasound scanning), or coursed with minimum non-specific complaints (so called "the syndrome of minor signs"). In the majority of cases the tumours were revealed owning to physicians having oncologic alarm. However the events took place when patients ignored doctor's opinion and refused informative diagnostic procedure and treatment as well. An operable stomach cancer with clear clinical symptoms and evident endoscopic picture was diagnosed in ARS 0 patient. The patient refused a surgical treatment and died over 3 months.

During 18 years after the first sarcoma diagnosis 14 cancers of different location were revealed: 3 cancers of colon, 2 ones of stomach, 2 thyroid gland, and a cancer of larynx, lung, kidney, urinary bladder, prostate, as well as basal cells carcinoma of head and neurinoma of lower jaw that was undergone malignant transformation. In a patient of ARS 0 group cancer of colon followed leiomyosarcoma of left shin. He was operated with subsequent fractionated X-ray irradiation of shin in total dose of 52 Gy.

Two patients developed cancer of stomach and larynx that had rapid course. The period from diagnosis to lethal outcome was 3 and 6 months, respectively. The first patient was undergone endoscopic procedure in Feb 2004 without any changes of stomach mucous membrane but in July 2004 he was admitted in RCRM hospital with

clinical and endoscopic signs of stomach cancer. Cancer of larynx $T_3N_1M_0$ was diagnosed in the second patient during routine follow-up but a year before the patient had no any complaints. Surgical treatment didn't stop metastatic disease with following lethal outcome.

All malignant tumours were diagnosed in male patients. Their mean age at the moment of exposure was 40.9±12.1 years, at the tumour diagnosis 57.9±17.1 and at the moment of death 66.2±9.8 years. The tumours latent period was 15.9±3.9 years. The overall number of patients with oncological pathology is 7.9%, amongst ARS survivors 7.6%, and in ARS 0 group 9.1%.

Statistical analysis showed that tumour incidence and their frequency rate didn't depend on ARS grade and ARS presence or absence (table 4).

Table 2. Relation between cancer incidence and radiation factor

Radiation factor	Spearman's rank correlation		χ2-test	
	r	p	F	p
ARS grade	-0.07	0.36	2.69	0.44
Presence or absence of ARS	-0.083	0.28	1.2	0.27

Discussion

As it was showed above the malignant blood diseases in ARS survivors preceded solid tumours and are not characterised by wide diversity of nosological form (3 myelodisplastic syndromes, acute leukaemia and hypoplasia of haematopoiesis) whereas in A-bombing victims of Hiroshima and Nagasaki from 1950 to 1987 there was diagnosed various form of acute and chronic leukaemia, Hodgkin's and non-Hodgkin's lymphomas, and multiple myelomas (Mabuchi et al. 1995; Preston et al. 1994). The peak of leukaemia incidence fell on 5^{th}-8^{th} year after irradiation (Mabuchi et al. 1995).

Analysing leukaemia incidence in hibakusha and persons, who was exposed to medical irradiation, E. Hall (1989) concluded that leukaemia latent period is 5 year. Other authors considered it shorter, 2-3 years (Rudnev 1992), or longer, 7-10 years (Nenot et al. 1995). The last term is better corresponded our data: all 4 cases of oncohematological pathology, except the first, appeared 7 years later the exposure. Hypoplasia of hematopoiesis in the patient No. 1 (table 2) developed in improbably short term after the initial contact with ionizing radiation. It doesn't enable to consider this case caused by radiation. Most likely the disease set in early but primary clinical symptoms appeared during his work in the CNPP zone.

From 1992 to 2000 myelodisplastic syndrome developed in 112 from 240800 CNPP clean-up workers, including 3 cases in ARS survivors. According to I. Sekine (2003), amongst 93700 A-bomb victims during the 50-year period only 13 cases of myelodisplastic syndrome developed that is absolutely and relatively less than in Chernobyl victims. Both high frequency rate and the latent period of 6-14 years enable to suspect radiation in this disease incidence. To prove this suggestion the additional study has to be set up.

Cancers that developed in CNPP clean-up staff during the first years after the accident, according A. Romanenko et al. (2000), might be the result of existing cancer promotion but not of the new cancer induction. The peak of radioinduced cancer should expect in 15-25 years after irradiation. The first cancer was revealed on 11th year after the exposure in ARS grade 1 survivor. This fact as well as following cases of malignant tumours onset proves the data that minimal latent period for solid tumours has to be 10 years and more (Kato et al. 1995). After this their incidence will increase constantly.

Cancers is a non-threshold radiation effect. After the whole-body short-term radiation exposure with low linear-energy transfer in dose 0.1 Sv the risk of cancer mortality increases on 0.5-1.4% from basal level (Fabrikant 1981) but if the dose 1 Sv it elevates to 9% in men and 13% in women (UNSCEAR 2000). Basing on this data it should expect higher cancer incidence in ARS survivors in comparison with persons exposed to radiation in doses lower 1 Gy. However our study showed that frequency of cancer was higher in control group (9.1%) than in ARS survivors (7.6%) with insignificant difference. There was not found significant difference of cancer incidence between patients with various ARS grades.

Amongst all oncological cases in ARS survivors and persons exposed in doses lower 1 Gy it is impossible to discriminate what tumours are caused by irradiation or developed spontaneously. These cohorts are too small for epidemiological investigations. The data of Japanese survivors showed that amongst 86572 individuals in the Life Span Study cohort there were 7578 deaths from solid tumours during 1950-1990. Of those cancer death, 334 (4.4%) could be attributed to radiation exposure. However this data cannot be extrapolated on relatively small group of ARS survivors or less irradiated persons.

ARS survivors and control group patients more frequently fell ill with cancer of colon (3 cases), stomach (2 cases) and thyroid gland (2 cases). According to results of hibakusha follow-up (1958-1987) their overall tumours included the first two types of cancer for whom the significant excess risks of morbidity and mortality was determined (Ron et al. 1994). The cancer of lung and urinary bladder, which were diagnosed in a patient of control group and ARS grade 2 survivor respectively, also belong to the tumours with the high mortality risk. There was revealed the significant increment of nonmelanoma skin cancer in the incidence data, but not in the mortality series. No significant radiation effect was seen for cancers of the pharynx, rectum, gallbladder, pancreas, nose, larynx, uterus, prostate or kidney in either series.

Sarcomas are the malignant tumours derived from mesenchyme whereas cancers descended from endothelium. No one investigation dedicated to A-bombing Japanese survivors contained data of sarcomas morbidity increase connected with irradiation. Our investigation revealed only 2 sarcoma cases in patients without ARS in anamnesis. This cohort size is not enough for statistical analysis.

WHO concluded (2005, 2008) that several last decades various organs cancer ranks as second having ahead cardiovascular diseases as mortality reasons in European countries including Ukraine. During 24 post-accidental years amongst restricted group of ARS survivors and persons exposed in doses lower 1 Gy 13 persons died due to oncological and oncohematological pathology, 12 ones of cardiovascular diseases and 11 patients of other reasons including traumas and accidents.

The patients' mean age was 56.4±12.4 years when malignant blood diseases and solid tumours were diagnosed. Thus 4 patients belonged to persons of young and mature age (20-44 years), 8 ones to middle age (45-59 years) and 8 patients to elderly and old age (60 and more years). The life span of 13 patients, who died from oncohematological pathology and tumours, was 59.8±11.5 years and didn't reach the average population level that according to WHO data (WHO 2005, 2008) was 62.3 year for male in Ukraine in 2001-2003 but decreased to 61.6 years in 2005. Therefore more than a half of the patients, who died, didn't live till very low average population life span in Ukraine (e.g. in Finland this index was 75.8 years in 2005). This fact enables to consider that the oncohematologic situation in ARS survivors and control group patients is disturbed. The way out of this is the early diagnostic and a radical cure. The localization of neoplasm (blood, digestive tract, thyroid gland, larynx, kidney, lung, prostate, skin) in followed up patients enables to reveal it on the early stage of development by routine and non-invasive diagnostic methods as blood analysis, x-ray examination, ultrasound, endoscopy, examining by otolaryngologist, gynaecologist, urologist, endocrinologist, pulmonologist, haematologist etc.

The second problem, which associates with successful diagnostic, is early patient's visit to physician for medical care. Due to low sanitary and general culture some patients didn't go to physician even if on the background of old complaints a new ones appeared and health changed for the worse. Some patients visited "folk healer" instead of physician or ignored medical recommendations. Therefore the obligatory annual examination of ARS survivors and other Chernobyl victims has to be combined with community health and medical staff oncologic alarm.

Our investigations had showed that malignant tumours appeared in the Chernobyl victims on the background of chronic non-specific diseases of internal organ systems, which didn't threaten patients' life themselves but as any pathological process increased the probability of cell mutagenesis. So a treatment of these chronic diseases is the one more way to decrease the risk of malignant tumours incidence.

Conclusions

During 24 years after the Chernobyl accident malignant blood diseases and tumours ranked as first by mortality reasons in ARS survivors and other exposed persons in doses lower 1Gy. They left behind even cardiovascular diseases. More than 50% of fallen ill with oncological pathology and died of them didn't reach the level of average population life span in Ukraine, which is 20% less the analogous index in economically developed European countries.

Effective treatment of patients depends on both early diagnostic and patients' readiness to follow doctors' recommendations.

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Radiation-epidemiological estimation of thyroid pathology risk

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Abstract

The main source of irradiation of the population after the Chernobyl accident was ^{131}I and its other short-lived isotopes that effected practically the whole population of Belarus. More than 30 % of children aged up to 2 years old received doses over 1 Gy.

The aim of study is the retrospective analysis of clinical data depending on thyroid exposure level among people aged 0–3 at the moment of the Chernobyl accident.

Out of people observed in State registry, we formed 2 groups which are annually examined on thyroid pathology.

1. Basic group including 1004 residents of Gomel oblast aged 0–3 at the moment of the Chernobyl accident who were exposed to short-lived iodine radioisotopes.
2. Control group including 2020 persons born in 1987–1988 in Gomel region and who are observed in State Registry.

As one would expect, the highest estimates of the relative risk were received on thyroid cancer. Even at rather low absorbed thyroid doses, the attributive fraction was more than 94%. In group with high doses, practically all the cases were radiation-induced (AF = 98,5%).

At the same time, the analysis results showed that radiation component was the predominant one at realization of practically all the spectrum of thyroid pathology among subjects irradiated in early childhood. Evident growth of OR was observed in patients with all nodular forms of goiter with increase of thyroid dose. In subjects with thyroid dose above 1 Gy, the attributive fraction of different forms of nodular pathology varied from 60 to 98%.

Statistically significant estimates of relative risk were calculated on all forms of thyroid nodular pathology.

Relative risk of any goiter nodular form in persons affected in early childhood was 3,7 (AF= 71,1%).

These results suggest that considerable part (up to 71%) of goiter nodular form diseases among residents of Gomel region, affected in early childhood, can be referred to thyroid exposure.

Acute myocardial infarction in the cohort of Mayak PA nuclear workers

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Abstract

Incidence of and mortality from acute myocardial infarction (AMI) have been studied in a cohort of 12210 workers first employed at one of the main plants of the Mayak nuclear facility during 1948–1958 and followed up to 31 December 2000. Information on external gamma doses was available for virtually all of these workers (99.9%); the mean (± standard deviation, SD) total external gamma dose was 0.91±0.95 Gy (99% percentile 3.9 Gy) for men and 0.65±0.75 Gy (99% percentile 2.99 Gy) for women. In contrast, plutonium body burden was measured only for 30% of workers; amongst those monitored, the mean (± SD) absorbed cumulative liver dose was 0.40±1.15 Gy (99% percentile 5.88 Gy) for men and 0.81±4.60 Gy (99% percentile 15.95 Gy) for women. 683 disease cases and 338 deaths from AMI were identified in the study cohort. Having adjusted for non-radiation factors, AMI mortality data showed a statistically significantly increasing trend with external dose, excess relative risk per Gy (ERR/Gy) was equal to 0.265 (95% CI 0.004, 0.526), but the evidence for this trend was greatly reduced if adjustment was made for internal liver plutonium dose. In contrast to the mortality findings, there was very little evidence of an association between AMI incidence and external dose, ERR/Gy was equal to 0.029 (95% CI -0.076, 0.134). Among workers with internal liver doses from plutonium exposures, AMI incidence and mortality were raised at 0.1–0.5 Gy relative to lower doses; there was still borderline evidence of such differences after adjusting for external dose. The trends in AMI incidence or mortality with internal dose were not statistically significant. Whilst the data on AMI incidence and mortality were consistent when analysing internal doses, the same was not true when analysing external doses.

Introduction

Acute myocardial infarction (AMI) is one of the most severe forms of the ischemic heart disease with the highest lethality and high percent of complications resulting in long disability, which reduce the quality of life.

The cause of AMI in the overwhelming majority (93-98.5%) of cases is the atherosclerosis of coronary vessels, which is a multifactorial disease, and different endogenous (genetic predisposition, gender, age, hypertension etc.) and exogenous (smoking, emotional stress etc.) factors contribute to its development. Over the last two decades several studies have examined the possible effects of ionizing radiation exposure on circulatory diseases, including AMI. Most of the studies (McGeoghegan et al 2008; Kreuzer et al 2006; Muirhead et al 2009; Shimizu et al 2010; Vrijheid et al 2007) focused on CVD mortality, whereas several studies (Yamada et al 2004; De Bruin et al 2009; Ivanov et al 2006) focused on CVD incidence.

This study was aimed at estimating risks of both AMI incidence and mortality in the cohort of Mayak PA nuclear workers first employed at one of the main plants (reactors, radiochemical, or plutonium) during 1948-1958 in relation to external gamma and internal alpha exposures, taking non-radiation factors into account.

Material and methods

Mayak PA began operations in 1948 as the first and largest nuclear weapons facility in the former Soviet Union and included all the plants necessary for weapon-grade plutonium production, namely, reactors, radiochemical plant, plutonium production plant and auxiliary plants.

From the first days of Mayak PA operation, the special system of personnel medical observation included an obligatory pre-employment medical examination and routine medical examinations of all the workers based on a common standard program. After quitting their job at Mayak, if the former worker stayed in Ozyorsk, he/she was examined at the same specialized medical hospital based on the same standard program. This system of medical observation of Mayak PA personnel health allowed a unique archive of primary medical data to be accumulated and formed the basis for establishment of the unique “Clinic” medical-dosimetry database for the Mayak PA workers cohort (Azizova et al 2008).

The study cohort included 12210 Mayak PA workers, 3552 (29.1%) of whom were women, first employed at one of the main plants from the start of operations through the end of 1958 independently of gender, age, nationality, occupation, and other characteristics. The method of identifying this cohort has been described previously elsewhere (Koshurnikova et al 1988, 1998a, 1998b, 1999).

Vital status as of 31 December 2000 was known for 88.4% of cohort members; of these workers, 52.7% were known to have died and 47.3% were known to be alive as of that time. 53.7% of the 12210 members of the study cohort were known to have left Ozyorsk by 31 December 2000. For persons who continued to be residents of Ozyorsk, vital status was known for all but one person (99.98%). Cause of death was known for 93.5% of deceased cohort members. Morbidity data for the whole period of follow-up were collected for 11597 (95.0%) workers in the study cohort. Only for 5.0% of workers could information not be collected, owing to the loss of their medical documentation. It should be noted that the CVD incidence analysis was restricted to the period of residence in Ozyorsk.

Data on vital status and on dates and causes of death for those workers who migrated from Ozyorsk were provided by the SUBI Epidemiology Laboratory; data on

date and causes of death for those Ozyorsk residents whose medical cards and/or case histories had been lost, were provided by the SUBI Occupational Health Laboratory.

Individual dosimetric control of external exposure was performed at Mayak PA from the beginning of operations there. Regular monitoring of internal exposure among those who might have been exposed to plutonium-239 began later, during the 1960s (Vasilenko et al 2007a; Khokhryakov et al 2000). The results of individual monitoring of external and internal exposure were recorded in individual dosimetric cards and journals. The data contained in these documents formed the basis for the establishment of the dosimetric database of Mayak PA workers (Vasilenko et al 2007a, 2007b; Bess et al 2007; Smetanin et al 2007a, 2007b; etc).

Dose estimates from the *Mayak-Doses 2005* dosimetric system were used in this study. Annual doses of external gamma exposure were available practically for all persons in the study cohort (99.9%). The mean (± SD) total gamma dose for the whole period of work at Mayak was 0.91±0.95 Gy (99% percentile 3.9 Gy) for men and 0.65±0.75 Gy (99% percentile 2.99 Gy) for women. The range of total gamma doses was very wide, with 32.6% having a total gamma dose greater than 1 Gy.

Plutonium body burden was measured only for 30.0% of workers. Among workers monitored for plutonium exposure, absorbed dose to liver from alpha radiation was used as a surrogate for the dose to muscle; this latter dose is likely to be similar to the dose to blood vessels and the chambers of the heart. Although doses to the liver and muscle would differ, they should be highly correlated with each other. Consequently, the liver dose can be used to look for any *dose–response* relationship between plutonium exposure and circulatory disease. Amongst those who were monitored, the mean (± SD) total liver dose was 0.40±1.15 Gy (99% percentile 5.88 Gy) for men and 0.81±4.60 Gy (99% percentile 15.95 Gy) for women.

Data on occupational histories and external gamma exposures for the study cohort were provided by the Mayak PA Radiation Safety Department; data on internal alpha exposure from incorporated plutonium-239 were provided by the SUBI Internal Dosimetry Department.

This study focuses on incidence of and mortality from AMI (ICD-9 codes: 410). Follow-up started on the date of first employment at one of the main plants and continued until the earliest of: date of the first diagnosis of AMI (for the incidence analysis) or the date of death from whatever cause (for both the mortality analysis and the incidence analysis); 31 December 2000 for those known to be alive at that time; the recorded date of departure from Ozyorsk (for the incidence analysis); and the date of "last medical information" in the case of unknown vital status. Comparisons were performed within the Mayak PA workers cohort first employed during 1948–1958.

The analyses included the calculation of relative risks (RRs) for categories of one or more factors, having adjusted for other variables. The relative risks for these categorical analyses were calculated by maximum likelihood, using the AMFIT module of EPICURE (Preston et al 1993). 95% confidence intervals for the RRs and p-values from tests of statistical significance were obtained via likelihood-based methods, using AMFIT. Attention was initially directed to non-radiation factors, following which measures of radiation exposure were analysed with adjustment (through stratification) for non-radiation factors. Analyses of internal radiation exposures were restricted to workers known to have been monitored for possible plutonium exposure.

In addition to the categorical analyses, models for trends in disease rates with level of radiation exposures were also fitted to the data, using Poisson regression methods. These models again were fitted using the AMFIT module in EPICURE. In particular, the excess relative risk (ERR) (ie. the relative risk minus 1) was modeled by a linear trend with external or internal dose, with adjustment for non-radiation factors.

In these main analyses, adjustments were made – through stratification – for gender, attained age, calendar period, period of first employment at the main plants of Mayak PA, plant, smoking and alcohol consumption.

Sensitivity analyses were conducted to examine the impact of: a) modifying the set of non-radiation factors (extra adjustment for hypertension, body mass index, employment duration) for which adjustment was made in the analyses of radiation factors; b) restricting the mortality follow-up (like that of incidence) to Ozyorsk, because some migrants were lost to follow-up and because of lower autopsy rates among those who left the city; c) adjusting for internal dose in analyses of external dose and vice versa; d) using various lag periods for external and internal doses. Furthermore, examination was made of how radiation risks might vary by gender, or between plants at Mayak or by attained age.

To allow for the possibility that radiation might affect stroke risk by modifying levels of blood pressure (Preston et al 2003; Ivanov et al 2001) and body mass index (Telnov 1985), the level of these factors at the time of preliminarily medical examination (before employment at Mayak PA) was considered, in order to avoid systematic errors that might arise through adjusting for values of these factors at later times. In contrast, smoking and alcohol consumption were classified at the time of last information (for the mortality analysis) or at the time of last information prior to the first diagnosis of stroke (for the incidence analysis).

Results

By 31 December 2000, 683 disease cases of AMI were diagnosed during 248030 person-years of follow-up and 338 death cases from stroke were identified during 443350 person-years of follow-up.

Non-radiation factors

Analyses of non-radiation factors revealed statistically significant effects of well-known factors such as gender, age, smoking, hypertension, which were taken into account in the analyses of radiation risks, either in the main analysis or in sensitivity analyses.

Radiation factors

External gamma exposure: Table 1 shows that AMI mortality was statistically significantly higher among workers exposed to external gamma rays in total dose above 1 Gy as compared with workers exposed in doses below 0.5 Gy.

Sensitivity analysis (results not shown) revealed that AMI mortality was statistically significantly higher among workers with a gamma dose above 1.0 Gy relative to workers with a gamma dose below 0.5 Gy, irrespective of lag period used, whether additional adjustment was made for other non-radiation factors and internal exposure. AMI mortality was raised among males and radiochemical plant workers.

There was a statistically significant increasing trend of AMI mortality with increasing external gamma dose (Table 1), but the evidence for this trend was greatly reduced if adjustment was made for internal liver plutonium dose.

In contrast to the mortality findings, there was very little evidence of an association between AMI incidence and external dose.

Table 1. RRs and ERR (95% CI) for AMI incidence and mortality in relation to total dose of external gamma exposure (main analysis based on a zero year lag period).

	RRs (vs. <0.5 Gy)		ERR/Gy
	0.5-1.0 Gy	>1.0 Gy	
Incidence	0.912 (0.710, 1.172)	1.034 (0.823, 1.298)	0.029 (-0.076, 0.134)
Mortality	1.104 (0.766, 1.592)	1.693 (1.229, 2.334)	0.265 (0.004, 0.526)

Internal alpha exposure: Table 2 shows that Both AMI incidence and mortality were statistically significantly raised among workers with cumulative absorbed dose to the liver from plutonium of less than 0.1-0.5 Gy as compared with workers exposed to lower internal liver doses. However, sensitivity analysis (results not shown) revealed that these findings were sensitive once different lag periods were used, whether additional adjustment was made for other non-radiation factors. However, there was still borderline evidence of such differences after adjusting for external dose.

Neither for AMI incidence nor for mortality was there a statistically significant trend in risk with internal dose with or without adjustment for external exposure.

Table 2. RRs and ERR (95% CI) for AMI incidence and mortality in relation to total absorbed liver dose from internal alpha exposure (main analysis based on a zero year lag period).

	RRs (vs. <0.1 Gy)		ERR/Gy
	0.1-0.5 Gy	>0.5 Gy	
Incidence	1.279 (1.026, 1.595)	1.255 (0.878, 1.794)	0.078 (-0.084, 0.241)
Mortality	1.854 (1.216, 2.827)	1.488 (0.743, 2.981)	0.211 (-0.171, 0.594)

Discussion

Our analyses of AMI incidence and mortality revealed, as expected, statistically significant effects of well-known factors such as gender, age, hypertension, smoking, which are consistent with findings of other studies. In contrast, our analyses did not reveal any statistically significant effect of alcohol consumption or body mass index on AMI incidence or mortality, either for males and females.

Having adjusted to non-radiation factors there was statistically significant trend in AMI mortality with increasing external gamma exposure, but the evidence for this trend was greatly reduced if adjustment was made for internal liver plutonium dose. In contrast to the mortality findings, there was very little evidence of an association between AMI incidence and external dose

Among workers with internal liver doses from plutonium exposures, AMI incidence and mortality were raised at 0.1–0.5 Gy relative to lower doses; there was still

borderline evidence of such differences after adjusting for external dose. However, trend in AMI incidence and mortality with internal liver dose were not statistically significant.

A complication to interpretation is the lack of knowledge as to those tissues or organs for which radiation exposure might increase the risk of stroke, which is particularly problematic in the case of plutonium intakes. For this analysis, liver dose has been used as a surrogate for the dose to muscle, which is likely to be similar to the dose to blood vessels and the chambers of the heart. Furthermore, the liver and muscle doses should be highly correlated with each other. However, there is uncertainty about which tissue or organ dose is appropriate for this type of analysis. A further complication relates to uncertainties in estimates for internal doses for Mayak workers. It should be noted that – because the dose to the liver from intakes of plutonium would be greater than that to the circulatory system – the ERR/Gy estimated here based on liver dose would be lower than that based on dose to blood vessels and the chambers of the heart. For these reasons, the findings in relation to internal exposure need to be interpreted with caution.

In addition, information is not currently available from other studies of populations exposed to external low-LET radiation or plutonium that would allow comparison of risk estimates for AMI taking non-radiation factors such as smoking or alcohol into account in relation to such exposures.

Conclusions

There was a statistically significantly increasing trend in AMI mortality with external dose, having adjusted for non-radiation factors. Much of the evidence for a raised risk concerned workers with cumulative external gamma doses above 1 Gy. The evidence for this trend was greatly reduced if adjustment was made for internal liver plutonium dose. Among workers with internal liver doses from plutonium exposures, AMI incidence and mortality were raised at 0.1–0.5 Gy relative to lower doses; there was still borderline evidence of such differences after adjusting for external dose. The trends in AMI incidence or mortality with internal dose were not statistically significant. Whilst the data on AMI incidence and mortality were consistent when analysing internal doses, the same was not true when analysing external doses.

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Stroke in the cohort of Mayak PA nuclear workers

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Abstract

Incidence of and mortality from stroke have been studied in a cohort of 12210 workers first employed at one of the main plants of the Mayak nuclear facility during 1948–1958 and followed up to 31 December 2000. Information on external gamma doses was available for virtually all of these workers (99.9%); the mean (± standard deviation, SD) total external gamma dose was 0.91±0.95 Gy (99% percentile 3.9 Gy) for men and 0.65±0.75 Gy (99% percentile 2.99 Gy) for women. In contrast, plutonium body burden was measured only for 30% of workers; amongst those monitored, the mean (± SD) absorbed cumulative liver dose was 0.40±1.15 Gy (99% percentile 5.88 Gy) for men and 0.81±4.60 Gy (99% percentile 15.95 Gy) for women. 665 disease cases and 404 deaths from stroke were identified in the study cohort. Having adjusted for non-radiation factors there were no statistically significant trends in either incidence or mortality from stroke with either total external gamma dose or total absorbed internal liver dose; and stroke incidence or mortality rates did not differ significantly between categories for external or internal dose.

Introduction

Cerebrovascular diseases are multifactorial diseases, and different endogenous (genetic predisposition, gender, age, hypertension etc.) and exogenous (smoking, emotional stress etc.) factors contribute to their development. Over the last two decades several studies have examined the possible effects of ionizing radiation exposure on circulatory diseases, including CVD. Most of the studies (McGeoghegan et al 2008; Kreuzer et al 2006; Muirhead et al 2009; Shimizu et al 2010; Vrijheid et al 2007) focused on CVD mortality, whereas several studies (Yamada et al 2004; De Bruin et al 2009; Ivanov et al 2006) focused on CVD incidence.

This study was aimed at estimating risks of both stroke incidence and mortality in the cohort of Mayak PA nuclear workers first employed at one of the main plants (reactors, radiochemical, or plutonium) during 1948-1958 in relation to external gamma and internal alpha exposures, taking non-radiation factors into account.

Material and methods

Mayak PA began operations in 1948 as the first and largest nuclear weapons facility in the former Soviet Union and included all the plants necessary for weapon-grade plutonium production, namely, reactors, radiochemical plant, plutonium production plant and auxiliary plants.

From the first days of Mayak PA operation, the special system of personnel medical observation included an obligatory pre-employment medical examination and routine medical examinations of all the workers based on a common standard program. After quitting their job at Mayak, if the former worker stayed in Ozyorsk, he/she was examined at the same specialized medical hospital based on the same standard program. This system of medical observation of Mayak PA personnel health allowed a unique archive of primary medical data to be accumulated and formed the basis for establishment of the unique "Clinic" medical-dosimetry database for the Mayak PA workers cohort (Azizova et al 2008).

The study cohort included 12210 Mayak PA workers, 3552 (29.1%) of whom were women, first employed at one of the main plants from the start of operations through the end of 1958 independently of gender, age, nationality, occupation, and other characteristics. The method of identifying this cohort has been described previously elsewhere (Koshurnikova et al 1988, 1998a, 1998b, 1999).

Vital status as of 31 December 2000 was known for 88.4% of cohort members; of these workers, 52.7% were known to have died and 47.3% were known to be alive as of that time. 53.7% of the 12210 members of the study cohort were known to have left Ozyorsk by 31 December 2000. For persons who continued to be residents of Ozyorsk, vital status was known for all but one person (99.98%). Cause of death was known for 93.5% of deceased cohort members. Morbidity data for the whole period of follow-up were collected for 11597 (95.0%) workers in the study cohort. Only for 5.0% of workers could information not be collected, owing to the loss of their medical documentation. It should be noted that the CVD incidence analysis was restricted to the period of residence in Ozyorsk.

Data on vital status and on dates and causes of death for those workers who migrated from Ozyorsk were provided by the SUBI Epidemiology Laboratory; data on date and causes of death for those Ozyorsk residents whose medical cards and/or case histories had been lost, were provided by the SUBI Occupational Health Laboratory.

Individual dosimetric control of external exposure was performed at Mayak PA from the beginning of operations there. Regular monitoring of internal exposure among those who might have been exposed to plutonium-239 began later, during the 1960s (Vasilenko et al 2007a; Khokhryakov et al 2000). The results of individual monitoring of external and internal exposure were recorded in individual dosimetric cards and journals. The data contained in these documents formed the basis for the establishment of the dosimetric database of Mayak PA workers (Vasilenko et al 2007a, 2007b; Bess et al 2007; Smetanin et al 2007a, 2007b; etc).

Dose estimates from the *Mayak-Doses 2005* dosimetric system were used in this study. Annual doses of external gamma exposure were available practically for all persons in the study cohort (99.9%). The mean (± SD) total gamma dose for the whole period of work at Mayak was 0.91±0.95 Gy (99% percentile 3.9 Gy) for men and

0.65±0.75 Gy (99% percentile 2.99 Gy) for women. The range of total gamma doses was very wide, with 32.6% having a total gamma dose greater than 1 Gy.

Plutonium body burden was measured only for 30.0% of workers. Among workers monitored for plutonium exposure, absorbed dose to liver from alpha radiation was used as a surrogate for the dose to muscle; this latter dose is likely to be similar to the dose to blood vessels and the chambers of the heart. Although doses to the liver and muscle would differ, they should be highly correlated with each other. Consequently, the liver dose can be used to look for any *dose–response* relationship between plutonium exposure and circulatory disease. Amongst those who were monitored, the mean (± SD) total liver dose was 0.40±1.15 Gy (99% percentile 5.88 Gy) for men and 0.81±4.60 Gy (99% percentile 15.95 Gy) for women.

Data on occupational histories and external gamma exposures for the study cohort were provided by the Mayak PA Radiation Safety Department; data on internal alpha exposure from incorporated plutonium-239 were provided by the SUBI Internal Dosimetry Department.

This study focuses on incidence of and mortality from stroke (ICD-9 codes: 430–432, 434, 436). In the literature, the term "stroke" is sometimes used as a synonym of a wider category of cerebrovascular diseases. In this study the term "stroke" relates to the following group of diseases: subarachnoidal hemorrhage (ICD-9 codes: 430), other and non-specified intracranial hemorrhage (ICD-9 codes: 432), intracerebral hemorrhage (ICD-9 codes: 431), cerebral infarction (ICD-9 codes: 434) and stroke not specified as either cerebral hemorrhage or cerebral infarction (ICD-9 codes: 436). ICD-9 code 435 corresponds to transient cerebral ischemic attack and is not stroke *per se*.

Follow-up started on the date of first employment at one of the main plants and continued until the earliest of: date of the first diagnosis of stroke (for the incidence analysis) or the date of death from whatever cause (for both the mortality analysis and the incidence analysis); 31 December 2000 for those known to be alive at that time; the recorded date of departure from Ozyorsk (for the incidence analysis); and the date of "last medical information" in the case of unknown vital status. Comparisons were performed within the Mayak PA workers cohort first employed during 1948–1958.

The analyses included the calculation of relative risks (RRs) for categories of one or more factors, having adjusted for other variables. The relative risks for these categorical analyses were calculated by maximum likelihood, using the AMFIT module of EPICURE (Preston et al 1993). 95% confidence intervals for the RRs and p-values from tests of statistical significance were obtained via likelihood-based methods, using AMFIT. Attention was initially directed to non-radiation factors, following which measures of radiation exposure were analysed with adjustment (through stratification) for non-radiation factors. Analyses of internal radiation exposures were restricted to workers known to have been monitored for possible plutonium exposure.

In addition to the categorical analyses, models for trends in disease rates with level of radiation exposures were also fitted to the data, using Poisson regression methods. These models again were fitted using the AMFIT module in EPICURE. In particular, the excess relative risk (ERR) (ie. the relative risk minus 1) was modeled by a linear trend with external or internal dose, with adjustment for non-radiation factors.

In these main analyses, adjustments were made – through stratification – for gender, attained age, calendar period, period of first employment at the main plants of Mayak PA, plant, smoking and alcohol consumption.

Sensitivity analyses were conducted to examine the impact of: a) modifying the set of non-radiation factors (extra adjustment for hypertension, body mass index, employment duration) for which adjustment was made in the analyses of radiation factors; b) restricting the mortality follow-up (like that of incidence) to Ozyorsk, because some migrants were lost to follow-up and because of lower autopsy rates among those who left the city; c) adjusting for internal dose in analyses of external dose and vice versa; d) using various lag periods for external and internal doses. Furthermore, examination was made of how radiation risks might vary by gender, or between plants at Mayak or by attained age.

To allow for the possibility that radiation might affect stroke risk by modifying levels of blood pressure (Preston et al 2003; Ivanov et al 2001) and body mass index (Telnov 1985), the level of these factors at the time of preliminarily medical examination (before employment at Mayak PA) was considered, in order to avoid systematic errors that might arise through adjusting for values of these factors at later times. In contrast, smoking and alcohol consumption were classified at the time of last information (for the mortality analysis) or at the time of last information prior to the first diagnosis of stroke (for the incidence analysis).

Results

By 31 December 2000, 665 disease cases of stroke were diagnosed during 249530 person-years of follow-up and 404 death cases from stroke were identified during 443350 person-years of follow-up.

Non-radiation factors

It is known that stroke is a multifactorial disease, therefore analyses of incidence and mortality risks in relation to non-radiation factors were performed first. Our analyses revealed statistically significant effects of well-known factors such as gender, age, hypertension, body mass index, which were taken into account in the analyses of radiation risks, either in the main analysis or in sensitivity analyses.

Radiation factors

External gamma exposure: Table 1 shows that neither for incidence nor for mortality from stroke was there a statistically significant trend in risk with total external gamma dose, nor did incidence or mortality rates differ to a statistically significant extent between categories for external dose. Sensitivity analyses (results not shown) revealed that this finding held irrespective of the lag period used, whether additional adjustment was made for other non-radiation factors and internal exposure, as well as whether analysis was restricted to workers at different plants, or according to gender or attained age.

Internal alpha exposure: Table 2 shows that neither for incidence nor for mortality from stroke was there a statistically significant trend in risk with total internal alpha dose to liver, nor did incidence or mortality rates differ to a statistically significant extent between categories for internal dose. Sensitivity analyses (results not shown) revealed

that this finding held irrespective of the lag period used, whether additional adjustment was made for other non-radiation factors and internal exposure as well as whether analysis was restricted to workers at different plants, or according to gender or attained age.

Table 1. RRs and ERR (95% CI) for stroke incidence and mortality in relation to total dose of external gamma exposure (main analysis based on a zero year lag period).

	RRs (vs. <0.5 Gy)		ERR/Gy
	0.5-1.0 Gy	>1.0 Gy	
Incidence	1.278 (0.999, 1.636)	1.237 (0.977, 1.567)	0.054 (-0.063, 0.170)
Mortality	1.246 (0.911, 1.703)	1.106 (0.816, 1.498)	0.016 (-0.128, 0.160)

Table 2. RRs and ERR (95% CI) for stroke incidence and mortality in relation to total absorbed liver dose from internal alpha exposure (main analysis based on a zero year lag period).

	RRs (vs. <0.1 Gy)		ERR/Gy
	0.1-0.5 Gy	>0.5 Gy	
Incidence	1.001 (0.800, 1.252)	1.080 (0.751, 1.554)	0.017 (-0.097, 0.131)
Mortality	1.401 (1.020, 1.924)	1.047 (0.609, 1.800)	0.120 -0.116, 0.356)

Discussion

Our analyses of stroke incidence and mortality revealed statistically significant effects of well-known factors such as gender, age, hypertension, body mass index, which are consistent with findings of other studies. In contrast, our analyses did not reveal any statistically significant effect of smoking and alcohol consumption on stroke incidence or mortality, either for males and females.

For both incidence and mortality from stroke, there was no evidence for associations with either external or internal radiation in this study.

A complication to interpretation is the lack of knowledge as to those tissues or organs for which radiation exposure might increase the risk of stroke, which is particularly problematic in the case of plutonium intakes. For this analysis, liver dose has been used as a surrogate for the dose to muscle, which is likely to be similar to the dose to blood vessels and the chambers of the heart. Furthermore, the liver and muscle doses should be highly correlated with each other. However, there is uncertainty about which tissue or organ dose is appropriate for this type of analysis. A further complication relates to uncertainties in estimates for internal doses for Mayak workers. It should be noted that – because the dose to the liver from intakes of plutonium would be greater than that to the circulatory system – the ERR/Gy estimated here based on liver dose would be lower than that based on dose to blood vessels and the chambers of the heart. For these reasons, the findings in relation to internal exposure need to be interpreted with caution.

In addition, information is not currently available from other studies of populations exposed to external low-LET radiation or plutonium that would allow

comparison of risk estimates for stroke (defined by the ICD-9 codes 430-432, 434, 436) in relation to such exposures.

Conclusions

Having adjusted for non-radiation factors there was no statistically significant trends in both stroke incidence and mortality risk with either total external gamma dose or total absorbed internal liver dose; and stroke incidence or mortality rates did not differ between categories for external or internal dose.

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Uranium health effects: results from the French cohort of uranium processing workers

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Abstract

A cohort of 2709 male workers (72786 person-years) employed at the AREVA uranium processing plant (1960–2005) in France was constructed to investigate the risk of cancer and non-cancer mortality in relation to occupational exposure to uranium. Cause-specific mortality in the cohort was compared to the national and regional mortality rates (1968–2005) by computing standardized mortality ratios. Exposure to natural and reprocessed uranium compounds, classified by their solubility, was assessed trough the plant-specific job-exposure matrix. Internal comparison between exposed and unexposed subjects was then done to estimate relative risk of mortality in regards of each type of exposure. Cox regression models adjusted for age, calendar period and socioeconomic status were used for analyses. At the end of the follow-up, 15% of the cohort was deceased. From 193 cancer deaths, 48 lung cancers and 18 lympho-hematopoietic cancers, considered as uranium target organs, were observed, as well as 101 deaths from cardiovascular diseases. For none of these causes, mortality among the cohort was significantly increased comparatively to the reference mortality rates. Internal comparison showed an elevated risk of mortality from considered causes among workers exposed to reprocessed uranium-bearing compounds. Mortality risk tended to increase with decreasing solubility of uranium compounds. This cohort study provides original results on cancer and non-cancer mortality related to protracted low-dose uranium exposure. Results suggest that effects on mortality differ by type and solubility of uranium compounds. Availability of data on associated exposure to carcinogenic chemical is another interest of this cohort. The cohort is still young but its further follow-up with extension to other AREVA plants will increase statistical power of the future analyses. Further investigation will focus on cardiovascular risk in dose-response analyses including biological parameters into the models.

Leukaemia risk among European uranium miners in dependence on doses from radon, external gamma, and long lived radionuclides

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Abstract

The study presents recent findings based on leukaemia mortality (69 cases) in three European cohort studies of uranium miners, including 9979 Czech, 3271 French, and 34 994 German miners. The risk is analyzed in relation to cumulated equivalent dose from radon and its progeny, from external gamma radiation, and from inhaled long lived alpha radionuclides. Exposures were estimated from measurements of radon, external gamma, and gross alpha activity in the aerosol. The earlier exposure estimates were based on uranium content in the ore and aerosol measurements in mines. The annual absorbed doses to the red bone marrow from exposure to radon gas, its progeny, long-lived radionuclides, and from exposure to external gamma radiation have been calculated for each miner from the first year of employment to the end of follow-up using the ICRP dosimetric and biokinetic models. The mean cumulated absorbed doses in the entire study are 38 mGy from external gamma, 2.1 mGy from radon and its progeny, and 0.9 mGy from long lived radionuclides. In terms of equivalent dose (using a radiation weighting factor 20 for alpha radiations), about 42% is from radon and its progeny, 39% is due to gamma radiation, and 19% is due to inhalation of uranium and its decay products. The risk coefficients (excess relative risk per sievert) were estimated using Poisson regression analysis. For each separate component, the risk coefficient was significant with p-values: 0.010 (gamma), 0.008 (radon), and 0.020 (long lived radionuclides). The estimated risk coefficient for the combined dose was 3.7 (90%CI: 1.1–8.8). Although the estimated risk is subject to some uncertainty due to small numbers and the dose uncertainty, its magnitude is consistent with estimates from other studies.

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Ionizing radiation and mortality from stomach cancer – Results of the German uranium miners cohort study, 1946–2003

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Abstract

Introduction: In the German uranium miners cohort study a 1.15fold statistically significantly increased risk of mortality from stomach cancer compared to the general male population was observed. The aim of the present analyses is to investigate the influence of ionizing radiation.

Methods: The cohort includes 58,987 men who had been employed for at least six months between 1946 and 1990 at the former Wismut uranium mining company in East Germany. By 2003 a total of 20,920 deaths occurred, among them 595 deaths from stomach cancer. Information on exposure to radon progeny in Working Level Months (WLM), external gamma radiation (mSv) and long-lived radionuclides (kBqh/m^3) is based on a detailed job-exposure matrix. Internal Poisson regression models were used to estimate the excess relative risk (ERR) per unit of cumulative exposure and its 95% confidence limits (CI).

Results: There was a statistically significant increase of death from stomach cancer in relation to either cumulative exposure to radon (ERR/100 WLM = 0.022; 95% CI: 0.001–0.043), external gamma radiation (ERR/Sv=1.83; 95% CI: 0.41–3.25) or long-lived radionuclides (ERR/kBqh/m^3=0.018; 95% CI: 0.0044–0.032), respectively. A statistically significantly increased relative risk was observed at exposure categories above 1,500 WLM (RR=1.74; 95% CI: 1.06–1.43) and above 300 mSv external gamma radiation (RR=2.22: 95% CI: 1.07–3.37).

Conclusion: The present preliminary analyses suggest that occupational exposure to ionizing radiation may increase the risk for stomach cancer among uranium miners. In a next step stomach doses based on dosimetric models will be calculated and the corresponding risk estimates presented. Moreover, potential confounder like exposure to arsenic, fine or quartz fine dust will be considered.

Case-control study of lung cancer incidence under combine occupational and domestic radon exposure

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Abstract

Case-control study of lung cancer incidence for the population of Lermontov city (Caucasus, Russia) was performed. Near the territory of Lermontov city the uranium mining was conducted from 1947 to 1990. The considerable part of male population of the city worked in uranium mining. In the Lermontov city three main districts with different kinds of houses exist: multi-storey buildings constructed after 1970; one – three storey buildings constructed before 1970; one-storey rural type houses. The average indoor radon concentrations in these districts are 170, 540 and 910 Bq/m^3 respectively. The lung cancer rate considerably above the average regional level was observed for the population of Lermontov city. The indoor radon concentrations and occupation radon exposure levels in the group of 121 lung cancer cases and in control group composed of 196 peoples were analyzed. Both archive and contemporary indoor radon measurements in combination with the retrospective detectors were used for reconstruction of domestic exposure. The temperature normalization approach was used for assessment of average annual radon concentration in dwellings from the results of radon measurements by charcoal absorbers. The average radon exposure reconstruction period was 38 years for case group and 34 years for control group. The occupation expose information was obtained from medical-sanitary department of former uranium mining facility. It was shown that in average 32% of total radon exposure for uranium miners was due to domestic indoor exposure. It means that in epidemiological studies of uranium miners the domestic radon exposure also should be taken into account. The excess relative risk of lung cancer incidence in case of combine occupational and domestic radon exposure was ERR=0.011 (– 0.003 ÷ 0.029) WLM^{-1}. After the excluding of uranium miners from analysis (46 men from case group and 9 from control group) the excess relative risk value was estimated as ERR=0.0040 (– 0.003 ÷ 0.034) WLM^{-1}.

Introduction

To estimate the influence of radon exposure on lung cancer incidence the case-control studies on uranium miner cohorts were conducted in various countries (Lubin et.al. 1994, Tomasek 2002, Grosche et.al. 2006). The results of these studies were used for estimation radon exposure hazards both for occupational and indoor exposure (BEIR VI 1999). Unfortunately in the analysis of dose-effect response in uranium miners studies the contribution of domestic indoor exposure was not taken into account. As a result the considerable uncertainties in radiation risk assessment can take place because of the indoor radon exposure in uranium rich regions can be comparable with the occupational exposure.

To estimate the radiation risk under the combine occupation and domestic indoor radon exposure the case-control study for the population of Lermontov city (Caucasus, Russia) was performed. Near the territory of Lermontov city the uranium mining was conducted from 1947 to 1991. The considerable part of male population of the city worked on uranium mining facility.

Material and methods

The main cohort in the study was formed from the peoples diagnosed as lung cancer patients from 1995 to 2004. The diagnoses were verified by instrumental and morphological methods. Practically all diagnosed lung cancer patients were included in the case group. The control group was formed from the Lermontov city inhabitants by random approach. The control group corresponded to the gender, age and professional distribution of the adult population of the Lermontov city. The size of case group was 121 and control group – 196 peoples.

The radon exposure estimation was made by the combination of the archive results of the measurements conducted by Regional Department of Federal Medical Biological Agency from 1992 and the measurements conducted by the nuclear track detectors during the our studies. The measurements made by the Regional Department in general were performed by charcoal absorbers sometimes in the combination with the nuclear track detectors. The average charcoal absorber exposure duration has been 4.7 days. More than 3200 archive results of radon measurements were used during the analysis. In the Lermontov city three main districts with different kinds of houses exist: multi-storey buildings constructed after 1970; one – three storey buildings constructed before 1970; one-storey rural type houses. The average indoor radon concentrations in these districts are 170, 540 and 910 Bq/m^3 respectively.

To estimate the average annual radon concentrations in the dwellings of the case and control groups the archive measurement data were analyzed using the temperature normalization approach. The archive meteorological data on the outdoor temperature on the period 1992 – 2004 were used for analysis. For the different kinds of dwellings in which the multiply measurements were carried out the typical dependence of radon concentration relative change on outdoor temperature was obtained. The example of such dependence for one-storey rural type houses is presented on Fig. 1.

For some flats the direct measurements of radon concentrations were not conducted. In this case the results of the measurements performed in the neighbouring flat in the same house were used. In some cases the coefficient connecting the radon concentration between the ground and upper floors was applied (Fig. 2).

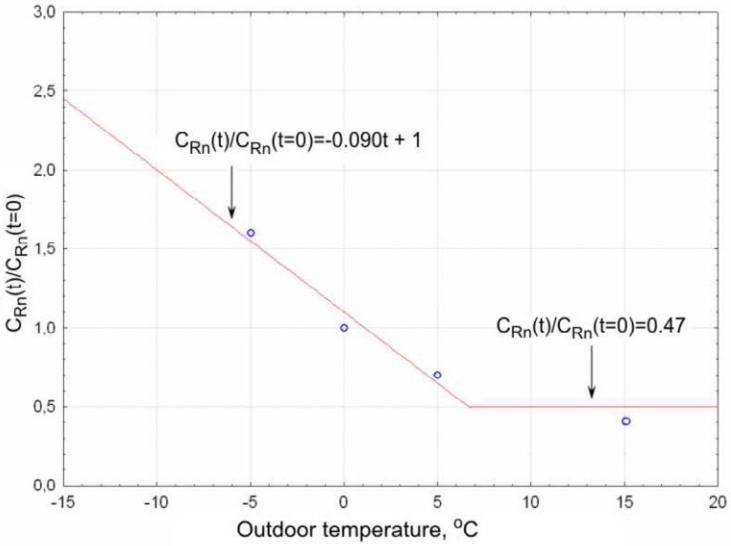

Fig. 1. The dependence of radon concentration on outdoor temperature for one-storey rural type houses in Lermontov city.

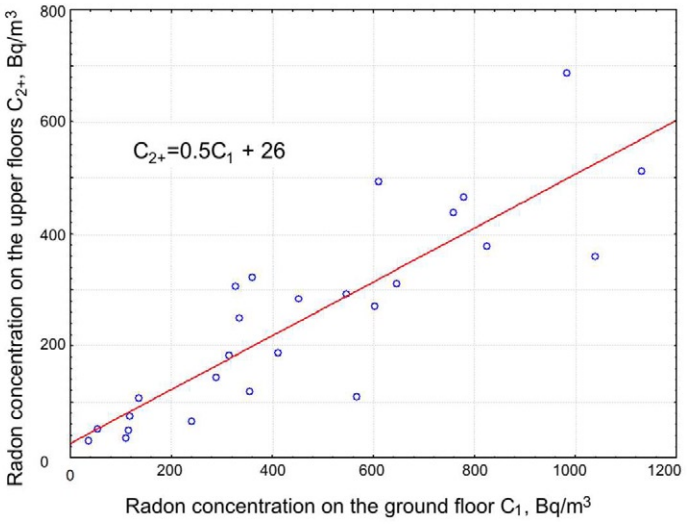

Fig. 2. The dependence of radon concentration between the ground and upper floors for multi-storey houses in Lermontov city.

The correctness of temperature normalization approach for the radon exposure assessment was verified by the use of retrospective surface trap radon detectors. The multilayer LR-115 track detectors designed in our laboratory (Bastrikov, Zhukovsky 2004, Bastrikov et.al. 2006) were placed in the dwellings on the mirrors or furniture glasses. Such comparative measurements have been conducted in 27 dwellings. The age of the objects used for retrospective measurements of radon concentrations was in the range from 13 to 68 years (31 year in average). The good correlation has been found between the values of the average radon concentrations measured by retrospective surface trap detectors and calculated by the temperature normalization approach with the use of archive results of charcoal absorber radon measurements (Fig. 3).

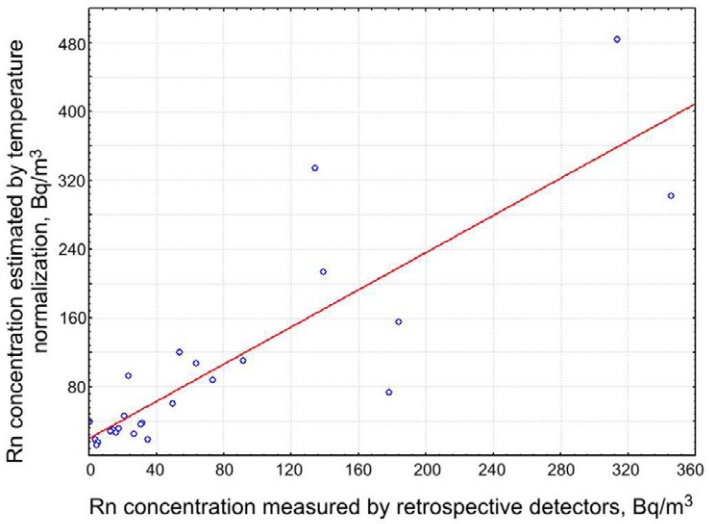

Fig. 3. Correlation between the values of the average radon concentrations measured by retrospective surface trap detectors and calculated by the temperature normalization approach.

Results

The radon concentration distribution parameters for the case and control groups in Lermontov city are presented in the Table 1. For each member of investigated groups the levels of domestic radon exposure have been calculated taking into account the radon levels in the dwellings, duration of the living in the last and in the previous dwellings and the average value of equilibrium factor F=0.44 estimated for the Lermontov city by the direct measurements of equilibrium shift between radon decay products. The average radon exposure reconstruction period was 38 years for case group and 34 years for control group. The last five years before the lung cancer diagnosing or including into control group were not considered during cumulative radon exposure calculations. The parameters of domestic radon exposure distributions are presented in the Table 2.

Table 1. Radon concentration distribution parameters for case and control groups in Lermontov city.

Group	Arithmetic mean, Bq/m^3	Geometric mean, Bq/m^3	σ_{LN}
Case	277	64	1.12
Control	243	73	0.89
All groups	257	70	0.99

Table 2. Parameters of domestic cumulative radon exposure distributions.

Group	Geometric mean, WLM	Minimum, WLM	Maximum, WLM	σ_{LN}
Case	23	0.7	499	1.19
Control	21	0.3	281	1.19
All groups	22	0.3	499	1.19

On the next step of our work the levels of occupational radon exposure were reconstructed. In one of uranium mine near Lermontov city the average radon concentrations decreased from 278 in 1947 to 3.0 kBq/m^3 in 1975. In other uranium mine radon levels decreased from 7.0 to 1.5 kBq/m^3 in period from 1960 to 1991. After 1991 the uranium mining in Lermontov city has been ceased. The levels of individual occupational exposure were reconstructed on the base of monitoring data and information about duration of work in the mines or on the territory of facility logged in workbooks. The occupational exposure was calculated for 60 members of case group and 31 members of control group. Among the professionally exposed peoples 48 miners worked directly in underground mines. The parameters of occupational radon cumulative exposure distributions are presented in the Table 3. The parameters of total radon exposure distribution are given in the Table 4. It was found that for uranium miners in average 32% of total radon exposure was due to domestic indoor exposure. It means that in epidemiological studies for uranium miners the domestic indoor exposure should be taken into account as well as occupation radon exposure.

Table 3. Parameters of occupational cumulative radon exposure distributions.

Group	Geometric mean, WLM	Minimum, WLM	Maximum, WLM	σ_{LN}
Case	42	3	603	1.56
Control	26	3	1352	1.78
All groups	35	3	1352	1.65

Table 4. Parameters of total cumulative radon exposure distributions.

Group	Geometric mean, WLM	Minimum, WLM	Maximum, WLM	σ_{LN}
Case	41	0.7	628	1.34
Control	24	0.3	1388	1.34
All groups	30	0.3	1388	1.36

For radiation risk assessment the odds ratios of lung cancer incidence for combined occupational and domestic radon exposure were calculated. The results are presented in the Table 5.

Table 5. Odds ratio of lung cancer incidence for combined occupational and domestic radon exposure.

Range of Rn exposure, WLM	Peoples in case group	Peoples in control group	Odds ratio	90% confidence interval
0 – 5	8	23	1.00	-
5 – 40	47	102	1.32	0.64 – 2.76
40-140	43	56	2.21	1.04 – 4.68
> 140	23	15	4.41	1.85 – 10.5

The dose-effect dependence can be described by the equation:

$$OR(P_{tot}) = 0.011(P_{tot} - P_0) + 1 , \quad (1)$$

where P_{tot} – cumulative total (occupational and domestic) radon progeny exposure, WLM; P_0=2 WLM – radon progeny exposure for which the OR=1 assumed. The 90 % confidential interval of the slope factor is in the range from – 0.003 to 0.029 WLM^{-1}.

Odds ratios were also calculated only for situation of domestic indoor exposure. The uranium miners were excluding from analysis (46 men from case group and 9 from control group). The odds ratios for reduced groups exposed only indoors presented in the Table 6.

Table 5. Odds ratio of lung cancer incidence for domestic radon exposure.

Range of Rn exposure, WLM	Peoples in case group	Peoples in control group	Odds ratio	90% confidence interval
0 – 3	4	13	1.00	-
3 – 30	39	100	1.27	0.47 – 3.40
30 – 140	29	68	1.39	0.51 – 3.79
> 140	3	6	1.63	0.37 – 7.22

The dose-effect dependence for domestic radon progeny exposure can be described by the equation:

$$OR(P_{ind}) = 0.004(P_{ind} - P_0) + 1 , \quad (2)$$

where P_{ind} – cumulative domestic radon progeny exposure, WLM; P_0=5 WLM – radon progeny exposure for which the OR=1 assumed. The 90 % confidential interval of the slope factor is in the range from – 0.003 to 0.034 WLM^{-1}.

Discussion

The most detailed studies of radon-induced lung cancer for uranium miners were presented in the works (BEIR VI 1999, Grosche et.al. 2006). The excess relative risk of lung cancer estimated in these works was 0.0076 (0.0041 – 0.014) WLM^{-1} (BEIR VI 1999) and 0.0021 (0.0018 – 0.0024) WLM^{-1} (Grosche et.al. 2006) respectively. The

values of the slope factors obtained in our paper 0.011 (– 0.003 ÷ 0.029) WLM^{-1} for combine exposure and 0.004 (– 0.003 ÷ 0.034) WLM^{-1} for domestic exposure in general are in good agreement with the published data. The differences in the risk assessment for combine occupational and domestic exposure and for indoor exposure in dwellings can be explained by different kinds of uncertainties in occupational and indoor exposure assessments and not very high statistical power of this epidemiological study.

Conclusions

The case-control study is conducted in the Lermontov city (Caucasus, Russia) to establish the connection between lung cancer incidence and combine occupational and domestic radon exposure. The temperature normalization approach has been developed to estimate the cumulative indoor radon exposure on the base of archive radon measurements by charcoal absorbers and track detectors. The good correlation has been found between the values of the average radon concentrations measured by retrospective surface trap detectors and calculated by the temperature normalization approach. The levels of individual occupational exposure were reconstructed on the base of monitoring data and information about duration of work in the mines or on the territory of facility. It was found that for uranium miners in average 32% of total radon exposure is due to domestic indoor exposure. The excess relative risk of lung cancer incidence in case of combine occupational and domestic radon exposure is 0.011 (– 0.003 ÷ 0.029) WLM^{-1}. After the excluding of uranium miners from analysis (46 men from case group and 9 from control group) the excess relative risk value was estimated as 0.0040 (– 0.003 ÷ 0.034) WLM^{-1}. The obtained results are in good agreement with BEIR VI data.

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Late effects of radioactive and chemical contamination

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Abstract

The influence of the polluted environment on health of the population has been studied for a decades in industrial countries. It is established that polluted environment can cause great variety of diseases in all categories of inhabitants. Most vulnerable groups are children and pregnant women because of possible genetic or teratogenic defects in the newborns. To prevent this situation, control of the environment is established. Besides the usual industrial pollutions, during the NATO air strikes on Serbia in 1999, many industrial facilities were destroyed and harmful, toxic substances were released in concentrations even ten thousand times higher than maximum permissible. Additionally, depleted uranium ammunitions were deployed in south of Serbia. In the years after, health surveillance of the soldiers who served in DU contaminated regions in Kosovo were organized and increased cancer incidence is noticed as well as non-specific diseases (cardiovascular, rheumatic) and psychological disturbances, including PTSP, alcohol abuse and social problems. According to EUROCAT protocol their children born after the strikes were followed-up too, and higher rate of congenital (skeletal, cardio-vascular) and chromosomal anomalies, immunological disorders and endocrine diseases were noticed. Similar results were found in the inhabitants of the most polluted regions.

Mortality due to gastrointestinal cancers in northern part of East-Ural Radioactive Trace

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Abstract

The objective of the study was to evaluate the register of causes of death, created using deaths certificates issued by rural municipalities at northern part of EURT, as a source of information for analysis of mortality caused by radiation induced gastrointestinal cancers. In the register the case (6158) and control (4844) groups were formed according to assessments of initial contamination of municipalities by Sr-90 – above and bellow 0.1 Ci per square kilometer respectively. Average colon dose 0.07 Gy and 440 gastrointestinal cancer deaths was observed in case group. The analysis included comparison of gastrointestinal cancers contribution to total number of deaths in the groups and estimation of excess number of deaths for sex, age and time since accident categories. To assess years of life lost (YLL) due to excess cancer cases the life durations were compared. By results of estimation total number of excess cancer deaths is 57±39 (with 90% CI) and total YLL is 893±279 for period from 1967 to 2000.

Introduction

Accidental explosion of waste storage tank at former Soviet Union plutonium production plant "Mayak" in 1957 had released considerable radioactivity to atmosphere and caused contamination of the environment (East-Ural Radioactive Trace, EURT). Available data on the radioactive contamination allowed the assessment of post-accident population radiation exposure. For analysis of radiation induced health effects the rural population of Kamensky rajon (Sverdlovsk oblast, Russia) was evaluated. That administrative district is situated in northern part of EURT, as far as 80-130 km from "Mayak" plant. The radioactive trace crossed territory of Kamensky rajon and caused contamination by ^{90}Sr up to 190 kBq/m^2.

Rural population of the district (more than 30 000 peoples in 1957) was chosen for analysis because its homogeneity in relation to social and environmental factors other than accidental radiation exposure. Radiation exposure of rural population directly associates with the levels of initial contamination of residential area and agricultural lands. Due to consumption of contaminated food and milk gastrointestinal tract received higher doses. This presentation describes the results of analysis of case of deaths related to cancers of gastrointestinal organs (esophagus, stomach, liver and colon) in rural population of northern part of EURT.

Material and methods

The primary source of information on causes of deaths of EURT population from Kamensky rajon was the archive of Sverdlovsk oblast civilian registry office. The death certificates issued by rural municipalities, which stored in the archive, contain the information as follow: unique identification of death certificate, sex, date of death, date of birth, place of birth, last place of residence, duration of life at last place of residence and the cause of death. Totally data on 15,685 cases of death for period 1954-2000 were obtained from the archuve. Obtained data were used to create Register of causes of deaths of rural population of Kamensky rajon (Register).

Cumulated doses absorbed due to radioactive contamination were estimated according to Guidelines "Reconstruction of doses accumulated by residents of the Techa river basin and the area affected by the accident at the "Mayak" plant in 1957" approved by Ministry of Health of Russia in 1995 (Guideline 2.6.1.024-95). The main factors contributed to radiation doses are consumption of contaminated crops, vegetables and milk, external exposure from contaminated soil and inhalation as well as external exposure during transfer of radioactive cloud. The Guideline suggests intake rates of radionuclides due to inhalation and ingestion and external exposure rates standardized per unit contamination by ^{90}Sr.

Case and control groups of the study were formed by dividing the Register due to level of initial contamination. Control group was formed from the residents of settlements with initial contamination level equal or bellow 0.1 Ci/km^2 and case group – from residents of settlements with initial contamination higher than 0.1 Ci/km^2. Thus control and case groups include residents of the same administrative territory.

Relative contribution of gastrointestinal cancers to the total mortality in the control group was estimated as ratio of number of deaths from gastrointestinal cancers (n) to total number of deaths (N). Estimated relative contribution was then applied to total number of deaths in case group (M) to assess expected number of deaths from gastrointestinal cancers in case group (E):

$$E = M\frac{n}{N}. \qquad (1)$$

Comparing the expected and observed numbers of deaths the excess number of deaths O-E and observed to expected ratio O/E were estimated. The variances of O and E were assessed accepting Poison distribution for n and O.

For each case of death at age A the years of expected life lost ($L(A)$), was accepted using data of demographic statistics for region. Then observed and expected total years of life lost (TYLL) for cases of gastrointestinal cancer deaths in case group were estimated using equations:

$$ObsTYLL = \sum_{\substack{Case\\Group}} L(A) \qquad (2)$$

$$ExpTYLL = \frac{M}{N}\sum_{\substack{Control\\Group}} L(A) \qquad (3)$$

Estimated expected and observed values were applied to assess excess TYLL of life lost $\boldsymbol{ObsTYLL - ExpTYLL}$ and relative value $\frac{\boldsymbol{ObsTYLL}}{\boldsymbol{ExpTYLL}}$.

To estimate the variance of TYLL the variance of expected life lost was accepted as follows:

$$\mathrm{Var}\left(\sum_{i=1}^{K} L_i\right) = \mathrm{Var}(L_0) + \ldots + \mathrm{Var}(L_i) + \ldots + \mathrm{Var}(L_K) \approx K \cdot \mathrm{Var}(L), \qquad (4)$$

To adjust assessments for sex and age at exposure factors the estimations were performed in subgroups formed according to categories of the factors (for age in 1957 five years categories were considered). Then the estimations were summarized to draw the final results.

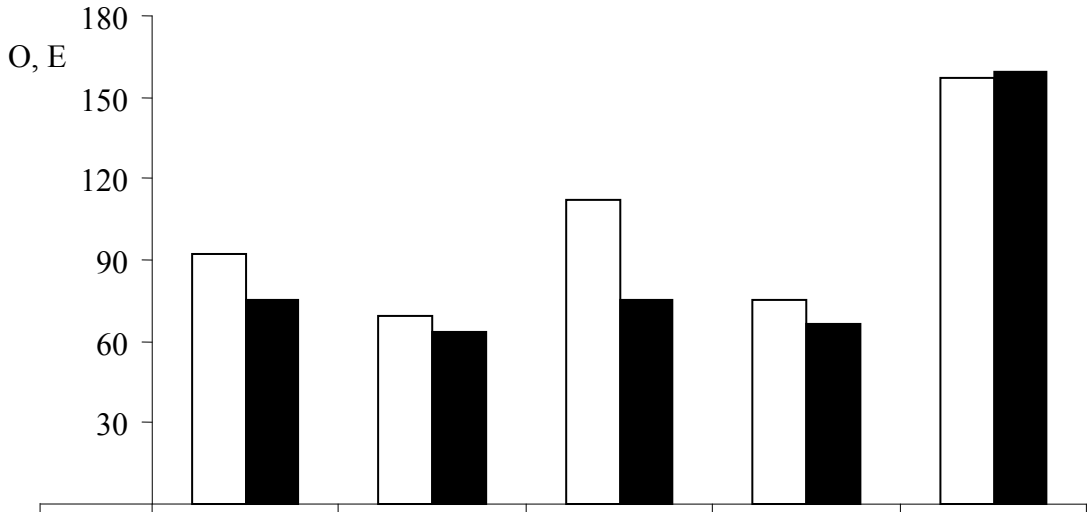
Results

General characteristics of case and control groups are presented in Table 1. Dependence of total observed and expected cases of gastrointestinal cancers in case group on calendar year is presented on Fig. 1. For periods before 1967 the difference between expected and observed values is insignificant. Significant difference ($p<0.05$) is obtained for period 1967-1977, for periods after 1977 the difference demonstrates a tendency of decreasing ($p>0.05$). Values of excess cancer deaths in case group are also presented on Fig. 1. After summarizing for period 1967-2000 estimated number of excess gastrointestinal cancer cases is equal to 57±39 (with 90% confidence interval). Estimated related excess TYLL in case group is 870±280 years, which is equivalent to about 15 years of life lost per excess case of cancer. For cancers other than gastrointestinal estimated number of excess cases (6±49) and excess TYLL (-222±410) in case group are insignificant.

Values of O-E and ObsTYLL-ExpTYLL in case group for period 1967-2000 in dependence on age in 1957 were tested (Table 2). For age at exposure interval 35-65 total value O-E = 54±39 (with 90% confidence interval). For age younger 35 in 1957 insignificant value of O-E is obtained. Significant values of excess TYLL in case group are observed for each ten years category in interval 35-65, where total value is 797±129 (with 90% confidence interval). For younger and elder ages at exposure the values of excess TYLL are insignificant.

Table 1. General characteristics of case and control groups

Characteristic	Case group	Control group
Number of entries	6 158	4 844
Male/female ratio	0.486/0.514	0.482/0.518
Total years of life after 1957	120 877	107 238
Mean colon dose, mGy	70	4.5
Number of gastrointestinal cancer deaths	440	278



Year	1954-1961	1962-1966	1967-1976	1977-1986	1987-2000
O-E	17±26	5.6±22	37±25	8.7±21	-2.3±32

Fig. 1. Observed (white boxes) and expected (black boxes) number and excess cases (O-E) of gastrointestinal cancer deaths in case group.

Table 2. Estimated number of excess of gastrointestinal cancer deaths and related excess TYLL in case group.

Age in 1957	O-E	ObsTYLL-ExpTYLL
<35	3.5±16	13±167
35-45	15±17	292±170
45-55	20±19	270±98
55-65	18±21	235±78
65-75	5.5±13	12±39
>70	-5.1±5	-

To compare risk of gastrointestinal cancers in case and control groups by age at exposure categories the relative risk parameters O/E and ObsTYLL/ExpTYLL were estimated. Two approaches yielded matching results though uncertainty of estimation by excess TYLL is in general lower than by excess cancer cases estimation. The parameter ObsTYLL/ExpTYLL demonstrates a tendency to decreasing with age at exposure from 1.5 (1.2-2.1 with 90% confidence interval) at age 35-45 to 1.0 (0.97-1.1 with 90% confidence interval) at age 65-75 in 1957.

Discussion

The crucial issue of the analysis is association of obtained excess cases of gastrointestinal cancer deaths and excess TYLL with accidental radiation exposure. In general the correlation of excess values with radiation exposure can be considered as reasonable evidence. However, the created Register is not sufficient to search such correlation. Nevertheless, we believe that the reasons such as follow support conclusion on relationship between estimated excess gastrointestinal cancer mortality in the case group of Register and the exposure. The case and control groups of studied rural population live within the same administrative and geographical territory and are homogeneous considering the social and demographic factors. It can be quite certainly supposed that the groups differ only by the factor of accidental radiation exposure. Considerable decreasing of gastrointestinal cancer mortality appeared within the period 10-20 years after accidental exposure, which can be related to the period of latency of the gastrointestinal cancers. There are no excess cases in the elderly population. Absence of excess cases deaths due to cancers other than gastrointestinal is in agreement with estimated nonuniform radiation exposure of organs and tissues.

Conclusions

Analysis of the register of causes of death of population live within northern part of EURT revealed increase of mortality due to gastrointestinal cancers, which can be associated with radiation exposure after accident at Mayak nuclear plant in 1957.

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Incidence of childhood leukaemia in the vicinity of Finnish nuclear power plants

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Abstract
Childhood leukaemia near nuclear installations has been investigated widely in the past decades, but the results have been inconclusive. Radiation exposure for the population appears negligible.

We investigated leukaemia incidence in children living near the two Finnish nuclear power plants (NPPs) using both cohort and case-control analysis.

The residential cohorts defined by censuses in 1980 and 1990 showed no increased risk of childhood leukaemia in the population residing within a 15-km zone from the NPPs compared to the 15–50-km zone.

In the case-control analysis with individual residential histories for 16 children with leukaemia and their 64 matched controls, residential distance from the NPP was not associated with leukaemia.

The results of the cohort and case-control analyses were consistent and neither of them indicated an increased risk of leukaemia. Even though the small sample size and lack of population residing within the 5-km radius limit the strength of the conclusions, the findings are reassuring from the public health perspective.

Introduction
Since 1983, childhood leukaemia near nuclear installations has been investigated widely but the results have been inconclusive so far (Laurier et al. 2008). The positive findings have been mainly limited to reprocessing sites while studies covering more than 100 nuclear power plants (NPPs) have shown little indication of increased risks. An exception was the recent German study reporting an increased risk of leukaemia among young children (aged <5 years) but not older ones living within a 5-km range around NPPs (Kaatsch et al. 2008). This finding raised public health concern and the need for information in Finland where a new NPP is being built but no published study has been available on childhood leukaemia and nuclear power.

We investigated leukaemia incidence near Finnish NPPs in children (aged <15 years) using cohort and case-control analyses which included information on residential history (Heinävaara et al. 2010).

Material and methods

Finland has four nuclear power reactors located in two nuclear sites, both having two reactors. Loviisa has been in commercial production since May 1977 and Olkiluoto since October 1979. Both sites are located at the seashore (Fig 1, large graph). The fifth nuclear power reactor is currently under construction in Olkiluoto.

Municipalities with any area within 15 km from the NPP were defined as being situated near to an NPP. According to this definition seven municipalities situate near to NPPs (Fig 1, small graphs).

In the cohort analysis, cohorts of people living near NPPs were formed based on census data at the end of 1980 and 1990. The coordinates of residence were obtained for these cohorts at the end of 1980 and 1990 from the Population Register Centre. Leukaemia cases diagnosed during the follow-up of the cohorts until the end of 2000 were obtained from the Finnish Cancer Registry. Leukaemia cases and data population counts were aggregated into 2 km * 2 km squares based on the coordinates of residence in the beginning of the follow-up. Leukaemia incidence in cohorts living within a 15-km zone around the NPPs was compared to that in reference cohorts living in the 15–50-km zone (Fig 1, small graphs). We calculated the indirectly standardized risk ratios (RRs) adjusting for age and socioeconomic status.

In the case-control analysis, the association between the residential distance from the nearest NPP and leukaemia was studied. Cases diagnosed with leukaemia between January 1, 1977 and December 31, 2004 and living in the municipalities near an NPP were identified from the Finnish Cancer Registry. Four controls were randomly chosen from the Population Registry Centre and matched to cases with respect to sex, age, and the municipality of residence at index date (i.e., date of diagnosis of the corresponding date). Residential histories of all cases and controls were obtained from the Population Register Centre. Case-control analysis included 16 children, 11 boys and 5 girls, with their 64 matched controls. All cases had acute lymphocytic leukaemia.

In the case-control analysis, residential history was taken into account from the date of production of the nearest NPP until the index date. The distances from the nearest NPP to each residence and the corresponding durations were calculated for each subject. Average distance was calculated as the sum of distances weighted by their relative durations. Categorized average distance was used as a primary measure for residential distance. Categorized distance at diagnosis and minimum distance were used as secondary measures for residential distance. Minimum distance was the shortest distance from any of the subjects' residence to the NPPs. The association between leukaemia and the residential distance measures was assessed with odds ratios (ORs).

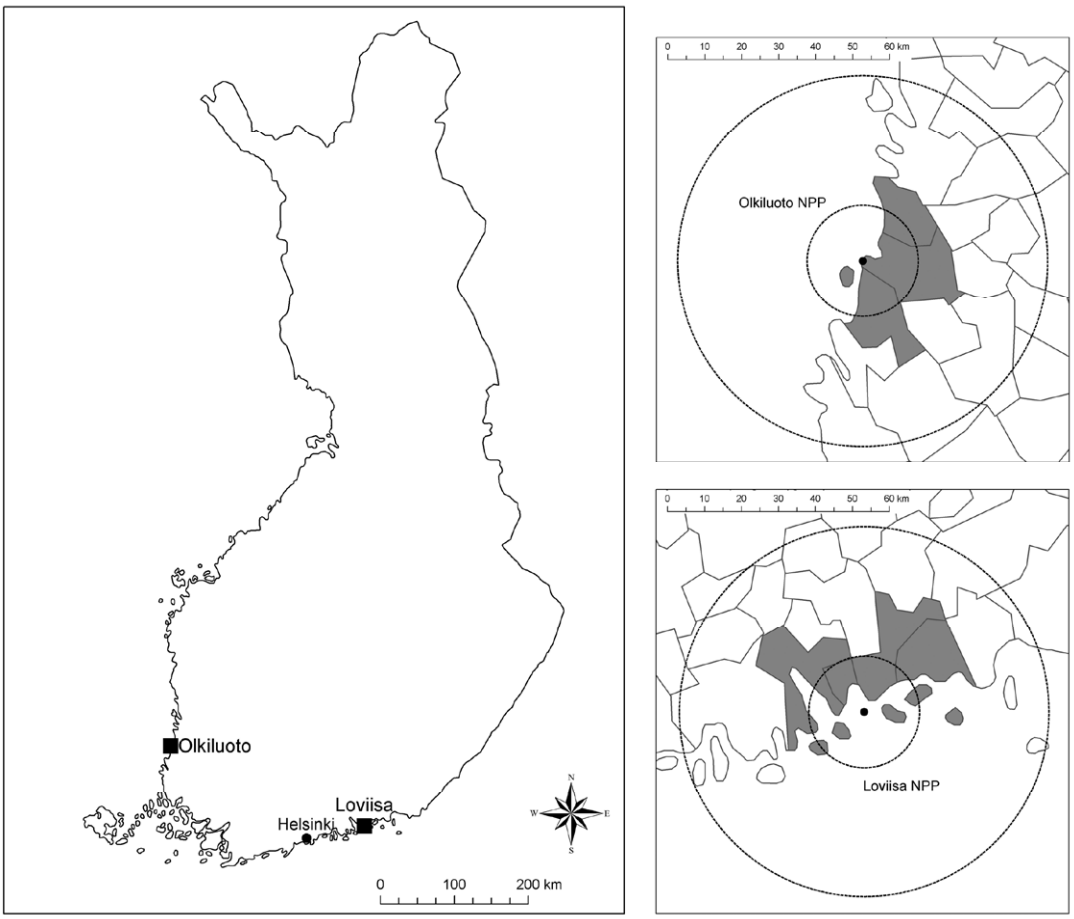

Fig. 1. Nuclear power sites Loviisa and Olkiluoto are located at seashore in the Southern and Western Finland, respectively (large graph). Municipalities near Olkiluoto site are Eurajoki, Luvia and Rauma whereas Loviisa, Ruotsinpyhtää, Pernaja and Pyhtää are near Loviisa site. Case-control analysis cover grey area. Small and large circles illustrate residential zones of 0-15 km and 15-50 km around the NPPs used in cohort analysis (small graphs). *Source:* Municipal boundaries © National Land Survey of Finland, licence 53/MML/10.

Results

In the cohort analysis, the rate ratios (RRs) of childhood leukaemia for the cohort living within the 15-km zone from an NPP were 1.0 (95% CI 0.3, 2.6) for the 1980 residential cohort and 0.9 (95% CI 0.2, 2.7) for the cohorts of 1990. For both cohorts, incidence of childhood leukaemia within the 15-km zone from the NPPs was comparable to that in the 15-50-km zone (Table 1).

Table 1. Observed leukaemia cases (Obs) within the 15-km zone from the NPPs and rate ratios (RRs) with 95% confidence intervals (95% CI). Expected incidence within the 15-50-km zone from the NPPs was used as a reference.

	Boys			Girls		
Childhood leukaemia	**Obs**	**RR**	**95% CI**	**Obs**	**RR**	**95% CI**
Residential cohort of 1980	3	1.1	0.2, 3.3	1	0.8	0.0, 4.5
Residential cohort of 1990	2	1.2	0.2, 4.5	1	0.6	0.0, 3.3

In the case-control analysis, the mean (maximum) of average distance was 18.4 km (59.7 km) km for leukaemia cases and 19.3 km (86.4 km) for controls. None of the children lived within the 5-km zone around the NPPs (Table 2). The odds ratio (OR) of leukaemia in the closest category (5-9 km) was 0.7 (95% CI 0.1, 10.4) compared to the reference, ≥30 km zone. None of the ORs related to categorized average distance differed significantly from the unity (Table 2). The result was the same for categorized distance at diagnosis and minimum distance.

Table 2. Odd ratios (ORs) with 95% confidence intervals (95% CI) of childhood leukaemia related to categorized average distance in the municipalities near the NPPs.

Average distance	Cases	Controls	OR	95% CI
0-4 km	0	0	-	
5-9 km	1	5	0.7	0.1, 10.4
10-19 km	11	41	0.9	0.2, 4.4
20-29 km	1	9	0.3	0.0, 3.6
≥30 km	3	9	1.0	

The results showed no heterogeneity between nuclear power sites, sexes, 5-year age groups and 10-year calendar period in cohort or case-control analysis.

Discussion

We assessed the risk of childhood leukaemia in populations living near the Finnish nuclear power plants. The results of the cohort and case-control analysis were consistent and did not indicate an excess of leukaemia.

The strengths of the study include comprehensive individual residential histories for all subjects in the case-control study. The nationwide, population-based cancer registry allowed identification of the complete roster of leukaemia cases in the study population. Combining two approaches in the study allowed a thorough evaluation of the leukaemia risk.

A key limitation of the analyses was the small number of cases. Therefore more detailed analyses, such as childhood leukaemia in young children aged less than 5 years living within a 5-km zone around the NPPs (corresponding to the findings of the German KiKK study), could not be assessed. As in other studies on the issue, exposure assessment was challenging as the monitoring of exposures indicates practically zero doses from the power plant exposures. If there is any excess radiation exposure to the

population residing close to the power plants, it would have to be from internal exposure, which is more difficult to measure than external radiation. Yet, the concentrations of radionuclides are very low and calculated maximal dose contribution negligible.

Conclusions

This study showed no evidence of increased risk of leukaemia in children living close to the nuclear power plants in Finland.

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Radiation doses from global fallout and cancer incidence among reindeer herders and Sami in Northern Finland

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Abstract

People in the Arctic regions can be heavily exposed from the global radioactive fallout due to their diet rich in reindeer meat in which radionuclides accumulate. The primary aim of this study was to assess whether the estimated lifetime cumulative radiation doses for the Arctic population from atomic bomb testing have had detectable effects on the incidence of cancer. A cohort of the Arctic population in Finland (n = 34,653) was identified through the Population Register Center with grouping by reindeer herding status, ethnicity and radiation exposure. Annual average radiation doses, based on ^{137}Cs whole-body measurements, were assigned by birth-year, gender and reindeer herder status. Cancer cases of radiation-related cancer types (cancers of the bladder, female breast, colon, esophagus, liver, lung, ovary, stomach, brain and nervous system, bone and thyroid, non-melanoma of the skin, basal cell carcinoma of the skin, and leukemia) during 1971-2005 were identified from the Finnish Cancer Registry. A total of 1,580 cancer cases were observed versus 1,948 expected on the basis of incidence rates in Northern Finland [standardized incidence ratio (SIR) was 0.81 with 95% confidence interval (CI) of 0.77-0.85]. For the reindeer herders SIR was 0.72 (95% CI 0.60-0.85) and for the Sami people SIR was even lower, 0.47 (95% CI 0.37-0.60). No association between the lifetime cumulative radiation exposure from global radioactive fallout and cancer incidence in the Arctic population was found. Potential underestimation and misclassification of the radiation dose may affect the results and the findings should be interpreted with caution.

Introduction

Most of the atmospheric testing of nuclear weapons was performed during 1945-1963 and the highest global fallout from these tests occurred in the early 1960s (UNSCEAR 2000). ^{137}Cs is the most important component of human radiation exposure from the fallout. Its persistence in the environment is enhanced by a slow turnover in the northern ecosystems. After the A-bomb testing period further exposure resulted from the Chernobyl nuclear power plant accident in 1986. However, the ^{137}Cs deposition in Northern Finland was only a fraction of that received from atomic bomb testing. The route of exposure among Arctic population is mainly through the intake of food with elevated radioactivity, resulting in protracted internal exposure (Rahola and Suomela 1998). For human exposure, the lichen-reindeer-human pathway is important, because ^{137}Cs is enriched in the food chain (Golikov et al. 2004; Miettinen and Häsänen 1967). The diet of Arctic people in Lapland is rich in reindeer meat and they have several-fold higher ^{137}Cs body burdens from the global test fallout than other populations of the Nordic countries (Rahola and Muikku 2004; Rahola and Suomela 1998).

The Sami people are the indigenous population of the Northern Scandinavia and the Kola Peninsula, and are genetically distinct from other Northern European people (Lahermo et al. 1996). In all published epidemiological studies, the Sami populations have had a cancer incidence below the national and regional average (Haldorsen and Tynes 2005; Hassler et al. 2004; Hassler et al. 2005; Hassler et al. 2008; Soininen et al. 2002; Wiklund et al. 1990; Wiklund et al. 1991). The lower cancer risk of the Sami has been suggested to reflect lifestyle and/or genetic factors. However, the non-Sami population in the Arctic areas has not been investigated. Even though the radiation exposure has been the motivation for several studies on cancer risk among the Nordic indigenous populations, no information on radiation doses has been available in earlier studies.

Material and methods

The baseline cohort consisted of all 36,461 Finnish residents identifiable from the Population Register Center born in the northernmost municipalities of Finland (Figure 1). These areas were chosen because they have the highest numbers of reindeer herders and people of Sami ethnicity. Subjects in the baseline cohort were born between years 1860 and 2001.

People registered as reindeer herders in the censuses carried out in the end of 1970, 1975, 1980, 1985, 1990, or 1995 were identified from Statistics Finland. Persons resident in Finland on 1 January 1971 or later were included in the final study cohort (n = 34,653). Persons whose own mother tongue or that of both parents was any of the three Sami languages were defined as ethnic *Sami* (Table 1).

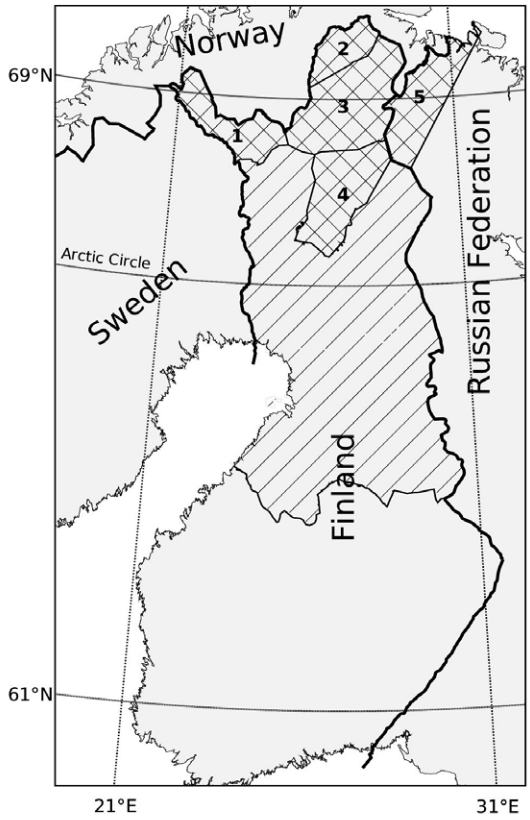

Figure 1. Birth municipalities of the study cohort members: 1 = Enontekiö; 2 = Utsjoki; 3 = Inari; 4 = Sodankylä; 5 =Petsamo (in Russia). The reference region for defining the expected numbers of cases (Cancer Care Responsibility Region of the Oulu University Hospital) is illustrated with a raster without a number.

Mean doses were assigned to groups defined by birth-year, gender, and reindeer herding status. Radiation dose assessment was primarily based on the ^{137}Cs body burden among the reindeer herders and other people in Lapland during 1961-2003. The ^{137}Cs body burden estimates from the whole-body measurements were available from surveys carried out by the Department of Radiochemistry, University of Helsinki in 1961-1977 and by STUK in 1986-2003 (Rahola and Muikku 2004). Annual doses were estimated for reindeer herders and non-reindeer herders according to gender (Figure 2). The cumulative whole-body dose was calculated as the sum of the assigned calendar-year-specific annual doses, taking into account the reindeer herding status. If a subject had a reindeer herding occupation in the earliest available census or if either parent of a child was a reindeer herder, the beginning of the reindeer-related exposure was assumed to start at three years of age. For spouses of reindeer herders, the beginning of the reindeer-related exposure was assumed to be the wedding year. Parents of a reindeer herder whose own occupation was unknown were assumed to have been reindeer herders, and calculation of reindeer-related exposure for them was assigned to start at three years of age. Subjects classified as reindeer herders remained in that exposure category regardless of subsequent changes in occupation or place of residence (assuming that their consumption of reindeer meat remained high).

Table 1. Description of the study population. Number of subjects (N) and person-years during 1971-2005.

		Total	
		N	Person-years
All		34,653	874,391
	Men	17,262	429,433
	Women	17,391	444,958
Reindeer herder		2,786	70,287
	Non-Sami	716,154	46,225
	Sami	917	24,063
Non reindeer herder		31,867	804,104
	Non-Sami	30,747	774,869
	Sami	1,120	29,236
Estimated cumulative radiation dose[a] (mGy)			
	0-0.9	13,523	172,293
	1-4.9	9,916	192,209
	5-9.9	19,665	376,65
	≥10	6,612	133,24

[a] Based on ^{137}Cs whole body measurements. A person may change exposure category during the follow-up period and therefore contribute to numbers of more than one category

The cumulative dose (D) was assigned as a function of follow-up

$$D = \sum_{t_o}^{t_1} d_{ijk}$$

where t_0 is the start of exposure and t_1 is the time of observation. The annual dose, d, depends on: gender (i), calendar year (j), and reindeer herding status (k).

Incident cancer cases occurring in 1971–2005 were identified from the Finnish Cancer Registry. Results are given for *a priori* selected radiation-related cancer sites. The category of "*radiation-related cancer sites*" is defined to consist of cancers consistently associated with radiation in previous studies, i.e., cancers of the urinary bladder, female breast, colon, esophagus, liver, lung, ovary, stomach, bone, basal cell carcinoma of the skin, brain and nervous system and thyroid, as well as leukemia.

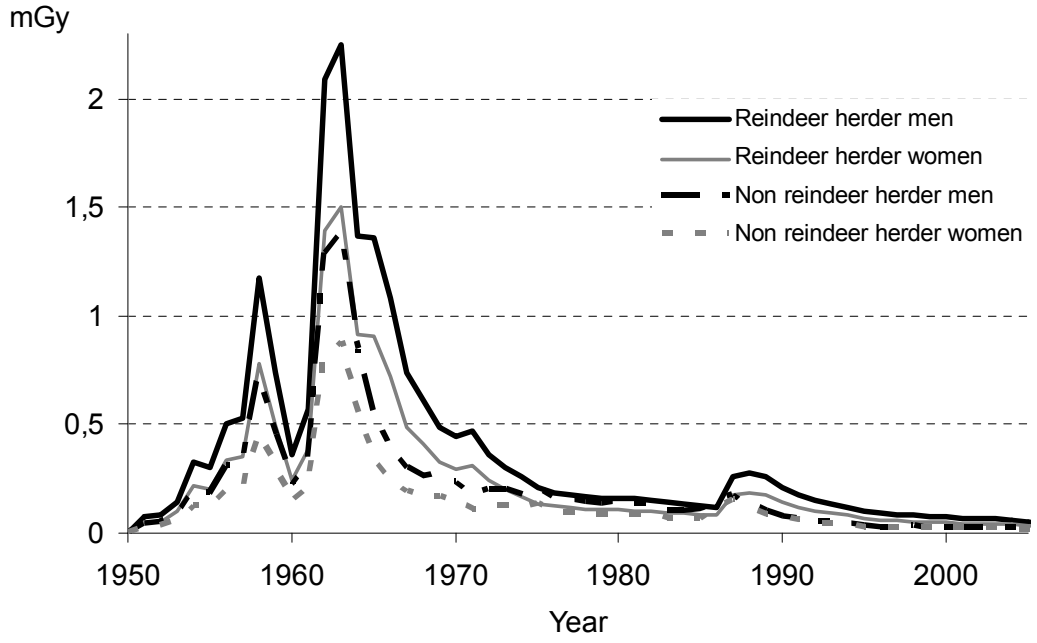

Figure 2. Average annual internal doses (mGy) from the ^{137}Cs for reindeer herders and other persons in Lapland.

The observed numbers of cancer cases and person-years at risk in the study cohort were counted according to sex and five-year age groups, separately for five calendar periods. The person-years were calculated until date of cancer diagnosis, death, emigration or the end of 2005, whichever came first. Follow-up in a given dose category was started when the cumulative dose reached the threshold level of the category with lagging by the induction period (latency). A latency of two years was allowed for leukemia, five years for thyroid cancer and ten years for other cancers. The expected numbers of cancer cases were calculated by multiplying the number of person-years in each stratum by the corresponding average cancer incidence in Northern Finland (Figure 1). The standardized incidence ratio (SIR) was defined as the ratio of observed to expected number of cases. The exact 95% confidence interval (95% CI) for each SIR was estimated on the presumption that the number of observed cases followed the Poisson distribution.

An ethical review of the study protocol was conducted by the Radiation Protection Advisory Board. The Population Register Centre gave the permission to use the population database for identifying the study population. The permission to use the census data on occupation was obtained from Statistics Finland. The National Research and Development Centre for Welfare and Health gave permission to use Finnish Cancer Registry data.

Results

A total of 1,580 cases of radiation-related cancer were observed versus 1,948 expected in the study population, indicating lower cancer risk in the study area than in the entire Northern Finland. The cancer incidence among the Sami was lower than in the rest of the study cohort, independent of the reindeer herding status. Reindeer herders had a slightly lower cancer incidence than non-reindeer herders (Table 2). The SIR for radiation-related cancers was not associated with the estimated cumulative radiation dose (Table 3).

Table 2. Observed (Obs) and expected (Exp) number of cancer cases and standardized incidence ratios (SIR) and 95% confidence intervals (95% CI) of radiation-related cancer sites by the reindeer herding status and ethnicity in the Arctic population in Finland.

	Sami		Non-Sami		All	
	Obs/Exp	SIR (95% CI)	Obs/Exp	SIR (95% CI)	Obs/Exp	SIR (95% CI)
Reindeer herder	35/67	0.52 (0.36-0.73)	102/124	0.82 (0.67-1.00)	137/191	0.72 (0.60-0.85)
Non reindeer herder	33/77	0.43 (0.29-0.60)	1,410/1,680	0.84 (0.80-0.88)	1,443/1,757	0.82 (0.78-0.86)
All	68/144	0.47 (0.37-0.60)	1,512/1,804	0.84 (0.80-0.88)	1,580/1,948	0.81 (0.77-0.85)

Table 3. Observed (Obs) and expected (Exp) number of radiation-related cancer cases and standardized incidence ratios (SIR) and 95% confidence intervals (95% CI) of the Arctic population in Finland by estimated radiation dose based on ^{137}Cs whole body measurements during 1971-2005.

Dose	Obs/Exp	SIR (95% CI)
0-0.9 mGy	137/191	0.72 (0.60-0.85)
1-4.9 mGy	33/44	0.76 (0.52-1.06)
5-9.9 mGy	811/966	0.84 (0.85-0.97)
>10mGy	307/378	0.81 (0.72-0.91)

Discussion

We evaluated cancer risks associated with estimated cumulative radiation doses, based on ^{137}Cs whole-body measurements, among the population in northernmost Finland. The cancer incidence for several primary sites was lower than among the other inhabitants of Northern Finland. No association was found between the cumulative radiation dose and radiation-related cancer sites.

The dose estimation of the current study is prone to underestimation and misclassification, mainly because reindeer meat is also rich in natural polonium, while only dose from ^{137}Cs was considered. The estimated group-level radiation doses have significant individual uncertainty. Due to uncertainties in the quantitative dose estimation quantitative risk estimation was not performed in the current paper. Northern people have a relatively high intake of smoked and salted food and a low intake of fresh vegetables and fruit, but on the other hand, a high consumption of berries. These etiologic factors constitute potential confounders that we were unable to control. Interaction between radiation and dietary risk factors is also a possibility.

Conclusions

The overall cancer incidence in Arctic population cohort was lower than in the general population in Northern Finland. Ethnic Sami people had a lower cancer incidence than the rest of the cohort. No association was observed between the lifetime cumulative radiation exposure from global radioactive fallout and radiation-related cancer risk.

Acknowledgements

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Health impairments in occupational ionizing gamma exposure

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Abstract

Work involving the preparation and assay of radiopharmaceuticals is associated with the highest occupational medical staff exposure from gamma emitters (^{99m}Tc, ^{131}I).

Our health status surveillance, a 5 years follow-up, of 16 subjects (18.11±13.76 years exposure length, 31,3 % easy-smokers) included mandatory national recommendations effect indices: clinical, haematological, cytogenetic micronuclei-MN in peripheral blood. Moreover we performed MN in sputum, oral exfoliated cells, oxidative stress indices (whole blood superoxide dismutase activity – SOD, serum lipoperoxides - Lpox).

During follow-up, 18.7% subjects over 12 years ^{131}I exposure were monitored for occupational thyroidal pathology, other 12.5% for dermic papillose, 56.3% for cardiovascular disorders or allergic rhinitis. Lymphocytes decreased level correlated inversely with exposure length in 31.2% cases. HGB, HCT modifications were associated with reticulocytes response in 12.5% subjects, but inverse correlated with years of exposure (r= -0.42). In 56.3% cases were neutrophil modifications with a 3 years tendency of normal values return at 28.1%. For 6.3% were found numerical and structural blood MN disorders due to workload or no radioprotection rules used. In 36.4% cases MN high level in oral mucous epithelial cells were indirect correlated with exposure length but direct correlated with smoking habit (r=0.58). Sputum type II cytology showed 16.7% ferruginous bodies over 20 years exposure, no correlation with smoking habit. Despite of allowable external exposure limits recorded, chronic exposure increased the SOD activity (148.8±106.6 U/ml) in 12.5% cases and Lpox level (2.4±1.9 µmol/l) at 18.7%, having no correlation with tobacco use.

We emphasized that continuously clinical and bioassay monitoring in nuclear medicine practices can reveal early changes of radiation induced effects. New proposals on national legislation for improving health surveillance of medical staff will be made.

Introduction

Nuclear medicine practices have a greater contribution to the exposure of medical staff involved as compared to other medical uses of radiation. While effective doses for radiologists in diagnostic radiology are at average of 1 mSv/year with increased values for interventional radiology, for medical personnel from diagnostic or therapeutic nuclear medicine is associated with high exposure average doses of 1-2 mSv/year from gamma emitters (^{99m}Tc, ^{131}I), low-linear-energy-transfer (LET) radiation (UNSCEAR 2000, 2006).

Occupational exposure control is usually made for both workplaces– external exposure (dose rate and radioactive contamination) and individual monitoring– individual dose estimation (dosimetry).

Internal dose assessment can be quantified by radioactive measurements (whole body counting, activity excreted in body fluids or in the breathing zone).

Health status assessment of medical staff with ionizing radiation exposure is annually performed by occupational medicine physicians with radio-pathology empowerment and based on national recommendations for effect indices in nuclear medicine exposure (clinical, haematological, cytogenetic micronuclei-MN-in peripheral blood, psychological test, thyroidal hormone determinations) (MPH 2007).

Material and methods

In a 5 years surveillance study of 16 subjects (81.2% females, 31.3% easy smokers, 18.11±13.76 years mean exposure length) we investigated effects indices, mandatory in Romanian national recommendations (MPH 2007): complete physical examination, haematological tests (blood count and blood cells` morphology: HGB, HCT, RBCs, RBC indices, WBC, platelets, reticulocytes, lymphocytes), cytogenetic test – unstable aberrations - number of cells with micronuclei (MN)/ 1000 counted cells in peripheral blood lymphocytes cultures (Popescu 2006) and specialty exams (endocrinology - T_3 triiodothyronine and T_4 thyroxine blood level, ophthalmology, psychology examinations).

Moreover we performed blood oxidative markers as whole blood superoxide dismutase activity (SOD), serum lipoperoxides (Lpox) and MN in sputum and oral exfoliated cells- by mucous cell brushing - a non invasive, not expensive method useful as screening technique.

In the final interpretation, clinical and biodosimetry results were correlated with individual exposure recorded by individual film and electronic dosimeters. Analytical methods used in this study were: t-Student, correlation and descriptive statistics by EXCEL functions.

Results

During follow-up, 18.7% subjects over 12 years ^{131}I exposure were monitored for occupational thyroidal pathology (polinodular goitre) with T_4 levels inversely correlated with exposure length.

Other clinical findings were pigmentation and skin papillose (12.5%), cardiovascular disorders (18.7%) as hypertension and respiratory arithmia, allergic rhinitis (18.7%) or musculoskeletal diseases (12.5%) and minor epileptic strokes (6.2%).

Severe anaemia (2.98*10^6 RBC, HGB 8.8 g/dl, HCT 26.8%) were found in one case with 40 years of ionizing radiation exposure.

As haematological marrow cells are the most affected even after short time of exposure, we found the lymphocytes decreased level correlated inversely with exposure length in 31.2% cases.

Trombocytes and neutrophil granulocytes modifications found in 56.3% cases. HGB, HCT and erythrocytes modifications were associated with reticulocytes response in 12.5% subjects, but indirect correlated with years of exposure (r= -0.42).

Genomic instability such as micronucleation were found for 6.3% of subjects, both numerical and structural blood MN disorders related to workload or no radioprotection rules used. In 36.4% cases MN high levels in oral cells were indirect correlated with exposure length but strongly direct correlated with smoking habit (r=0.58). The modifications trend had maintained during 5 years follow up.

Sputum type II cytology in 36.4% of subjects (Babes-Papanicolau classification) showed 16.7% ferruginous bodies over 20 years exposure, but with no correlation with smoking habit.

Despite of allowable external exposure limits recorded, chronic exposure increased the stress oxidative markers such as SOD activity (148.8±106.6) in 18.8% cases and Lpox level (2.4±1.9) at 31.3%, also in our study, having no correlation with smoking.

Discussion

Doses control in nuclear medicine practices implies radioprotection regarding inhalation or ingestion during preparation or injection the radiopharmaceuticals.

In ^{99m}Tc handling the external exposure is important, with high annual dose rates up to 500 mSv especially at hands and fingers, while the average annual effective dose to monitored medical workers averaged over five - years period can vary between 1.04 mSv and2 mSv. (UNSCEAR 2000, 2006).

In our follow-up study for effective dose estimation due to external exposure were monthly recorded the individual dose equivalent Hp (10) which did not exceeded 170 µSv, admitted value for occupational exposure for occupational ionizing radiation exposed personnel. (NCNAC 1996, BSS 1996, ICRP 2007).

There were rare cases of slight exceeding of Hp (10) limit (180µSv/month) in 5.8% of subjects. The cumulative film badge, dose for medical staff were under 24 mSv/year.

Gamma ionizing radiation-induced health effects are not specific and the targeted damages are observed as deterministic effects in hematopoetic system, lungs, thyroid, skin, eye, gonads, embrio/faetus and as stochastic effects by direct DNA damage or delayed effects – carcinogenesis (Little 2000), potential for biological effects expressed within one or two cell generation radiation risk (Morgan et al.1996, Little 2000, Ward 1999, 2002).

We focused to monitor in hematopoetic system the number of lymphocytes, with knowing tendency of decreasing immediately after exposure. In 32.1% cases the decrease of lymphocytes number (95% CI: 29.56-37.68) % were inverse correlated with exposure length (r=-0.35) (fig.1).

Platelets number had no significant changes in all years of monitoring period (fig. 2).

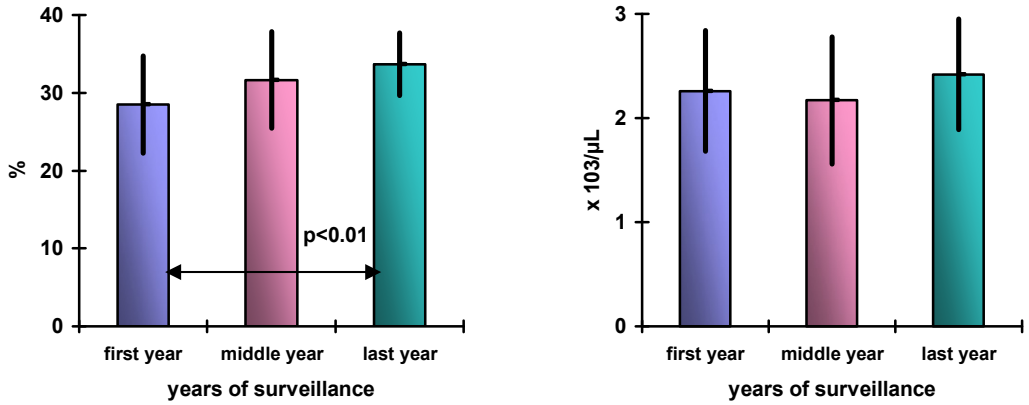

Fig. 1. Lymphocytes mean values in surveillance period.

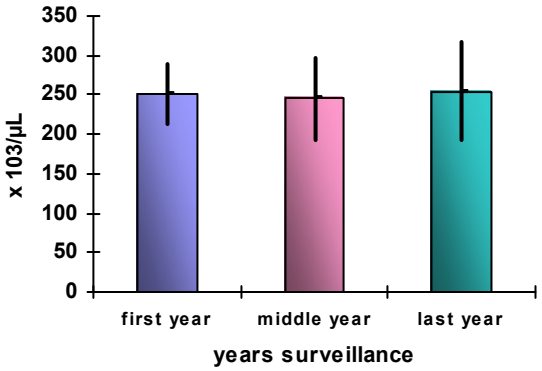

Fig. 2. Platelets mean values in surveillance period.

Numerical modifications (95%CI: 49.60-61.22) % - neutrophil granulocytes immunostimulation / immunosuppressed were found in 56.3% cases correlated inversely with occupational length (r=-0.31) and smoking habit for easy smokers: Brinkmann index under 200 (r=-0.28), statistically non-significant (p>0.05). We noticed at 28.1% subjects the trend to normal values recovery after 3 years of ionizing radiation exposure (fig. 3).

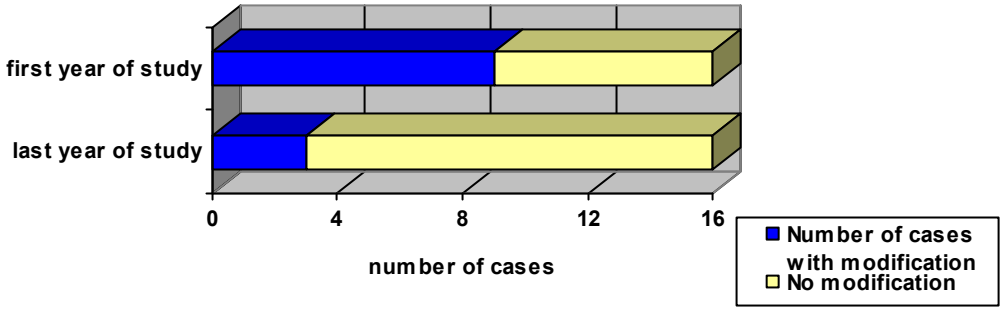

Fig. 3. Number of neutrophil granulocytes distribution in surveillance period.

Erythrocytes, which smoothly decrease after weeks of exposure (UNSCEAR 2000, 2006), in our study this trend was maintained in same values with no significant variations (fig. 4).

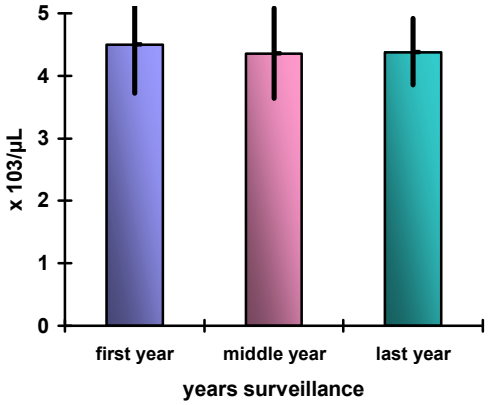

Fig. 4. Erythrocytes mean values in surveillance period.

Also HCT (95% CI: 34.42, 42.74) % correlated inversely with exposure length (r=-0.42), anaemic syndrome being observed in 12.5% of cases, one severe type. HGB (95% CI: 11.43, 14.59) g%, HCT and erythrocytes modifications were associated with reticulocytes response (95% CI: 6.06, 22.96) ‰ in 12.5% subjects, but significantly inverse correlated with years of exposure (r= -0.42).

Eosinophils granulocytes (95% CI: 0.62, 5.56) % in 12.5% of cases had a constantly numerical increasing trend over a 5 years-surveillance but had significantly positive correlation only with cigarette smoking for moderate smokers: Brinkmann index over 220 ($r=0.79$, $p<0.01$).

At subjects over 12 years ^{131}I exposure were monitored, clinical and therapeutic, an occupational multinodular goitre with hyperthyroidism, T_3 and T_4 levels trend were inverse correlated with exposure length. (r=-0.47).

Non targeted effects, like bystander effects (Yier 2000, Belyakov 2001), genomic instability (Emerit 1994, Morgan 1996, Wright 1998, 2000), adaptive response (Wolf 1998) are specific significant at low doses and this evidence is a new model for classical theory of radiation biology (Baverstock 2005).

We performed cytogenetic tests based on the assay of unstable aberrations such as MN in peripheral lymphocytes, sputum and oral exfoliated cells, visible in cellular cytoplasm at the first post-irradiation mitosis (Muller 1996).

These methods available at present for our study seemed useful to estimate the individual effects in moderate – low LET radiation doses. For 6.3% subjects were found numerical and structural blood MN disorders due to workload, meaning an acute recent exposure. Blood MN changes had no correlation with smoking habit but had an inverse correlation with occupational exposure length (fig. 5).

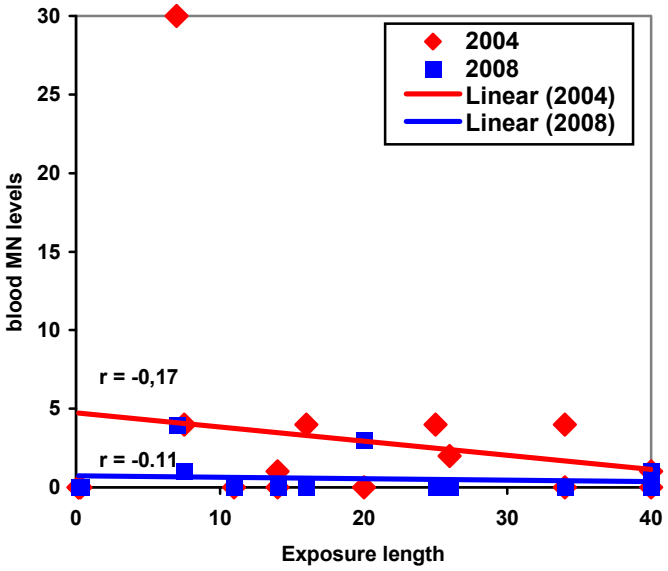

Fig. 5. Correlation between blood MN and exposure length during study surveillance.

Also, we observed a direct correlation of cases with blood/oral MN level modifications during 5 years surveillance (r=0.44). In 36.4% cases MN high numerical level in oral cells were inverse correlated with exposure length (r=-0.44) but significantly positive with smoking habit (r=0.58) (fig. 6).

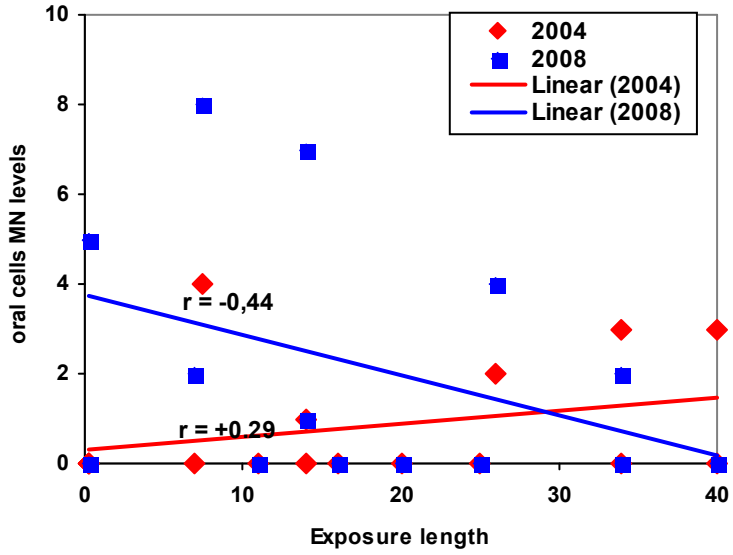

Fig. 6. Correlation between oral cells MN and exposure length during study surveillance.

Sputum type II cytology in 36.4% of subjects showed also 16.7% ferruginous bodies over 20 years exposure, but with no correlation with smoking habit. It seems that non-uniform/not constantly medical staff monthly exposure (for gamma generators) and adaptive mechanisms are responsible for the indirect correlation between length of exposure and MN alterations either in sputum or oral cells. Recently it was

demonstrated that chromosome instability in cells can develop specific cell variants more resistant to radiation exposure (Limoli 2001).

The bystander effect is cell type dependent and energy metabolism or reactive oxygen species (ROS) (Mothersill 2000) are involved in mediation of this kind of response. (Emerit 1994, Pollycove 1998, 1999, Lyng 2001). Antioxidant enzymes SOD/Lpox annihilate ROS, stress oxidative markers disturbances indicating a continuously oxidative processes and cellular stress aggression of health status caused by ionizing radiation exposure (Kłucinski et al. 2008).

For medical personnel involved in our study SOD activity (95% CI: 42.25-255.51) U/ml had increased levels in 12.5% of cases with no correlation with exposure length or tobacco use (Kłucinski et al. 2008). Lpox varied (95% CI: 0.4-4.38) µmol/l from 18.7% high levels directly with occupational exposure, to 6.2% decreased level after 7 years of occupational exposure at smoker personnel (fig. 7).

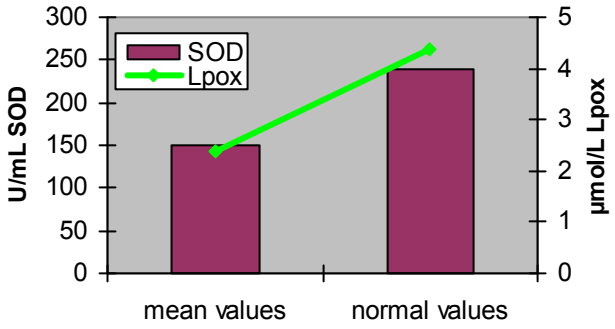

Fig. 7. Comparison trend of SOD and Lpox mean values in study surveillance.

An accurate assessment of oxidative stress in biological system is hard to perform, because a comprehensive surveillance must include measurements of all antioxidants, but that would be expensive, long time processing and technically difficult (Olisekodiaka MJ et al. 2009).

Conclusions

We emphasized that continuously clinical and bioassay monitoring in nuclear medicine practices – haematological, cytogenetic and oxidative stress status - can reveal early changes of radiation-induced effects.

Long term exposure to gamma-rays changes the antioxidant response of medical staff, having no correlation with cigarette smoking.

New proposals (such as MN in oral mucous epithelial cells) on national legislation for improving health surveillance of health care staff will be made.

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Spatial correlations of microdosimetric parameters and biological endpoints associated with radon inhalation

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Abstract
Radon and its progenies can be inhaled and deposited in the airways. Many of the decay products of the radioactive radon isotopes are short lived alpha, beta and gamma active atoms interacting with the epithelial tissue after their deposition and decay in the lung. Some of the radon and thoron decay products are short lived alpha-emitters and can transmit large amount of localized energies to the surrounding cells causing cellular and tissue damages. Since these interactions take place at a microscopic scale, the averaging of radiation dose to the whole body or the whole lung seems not to be fully appropriate for the quantification of the health effects. The aim of this study was to reveal the exact spatial correlations of different microdosimetric quantities and biological endpoints in the epithelium following the deposition and decay of short lived radon progenies. For this purpose complex numerical methods were developed. Air and particle transports in reconstructed three-dimensional airways were simulated. Particle deposition coordinates were recorded and alpha tracks generated. The cell nucleus architecture of the epithelium was reconstructed and interactions of alpha tracks with the nuclei were modelled. Our results demonstrate that in some clusters of nuclei the probabilities of single and even multiple hits are high even at low levels of macroscopic exposure. For the cells located in the deposition hot spots the probability of cell killing and transformation is more than one order of magnitude higher than the corresponding values assuming uniform surface activities.

Introduction
Radiation protection directives and regulations are based on our knowledge about health effects of radiations. Although by the accumulating experience, development of experimental and computational techniques and the emergence of new, multidisciplinary approaches a series of questions have been answered, some others are still to be clarified. One of these challenging tasks regards the biological response

triggered by low dose densely ionising radiations. Densely ionizing radiation produces a unique type of damage in which multiple lesions are encountered within close spatial proximity. The degree of damage clusterization can be further accentuated by the nonuniform radiation source distributions. Such a nonuniform surface activity distribution is produced by the inhaled radionuclides deposited within the airways. The site specificity of radioaerosol deposition in the lungs has been demonstrated both experimentally and by computational methods. However, the consequences of the inhomogeneous radionuclide deposition at cellular and tissue level are not fully explored to date. Although a comprehensive experimental study on the effect of spatial distribution of hit cells on different biological endpoints is still missing, it is clear from the existing experimental results that the spatial distribution of activity and related cellular alpha hit distributions are essential for the induced biological damage.

This study proposes to quantify the spatial distribution of the cell nucleus hits and the related biological endpoints, such as cell killing and cell transformation. Knowledge of distances between hit, killed and transformed nuclei instead of providing only their number distribution or simply assuming that they are uniformly distributed may be an important step towards a qualitatively better risk assessment. Elucidation of spatial correlations is essential especially for low doses, where neither epidemiology nor the experiments could establish a statistically convincing plausible dose-effect relationship.

Methods

Based on detailed histopathological studies (e.g. Saccomanno et al. 1996) the radon induced carcinomas arise preferentially in the upper lobes, especially in the right upper lobe. Therefore, in the present study a representative central airway segment consisting of a single bronchial bifurcation was considered (Figure 1). The 3D geometry model was constructed based on the numerical technique described by Hegedűs et al. (2004). To get the expected locations of the depositing radioactive particles it is necessary to track them within the airways. Since particle trajectories are strongly influenced by the airstreams, only a coupled (particle-air) approach can yield appropriate results. Air- and particle transport within the model airway bifurcation was simulated by the FLUENT CFD (computational fluid dynamics) code (FLUENT User's Guide 2001). Breathing parameters characteristic of light physical activity were selected. Radiation exposure conditions were characteristic of the former Czech uranium mines derived from Tomasek et al. (2008) publication. Inhaled particles were tracked until they deposited in the model airway or left the geometry. Deposition coordinates were recorded for further processing. Inhomogeneity of the deposition pattern was quantified by the help of activity maps. Activity maps were constructed by scanning the surface of the airway bifurcation by a regular triangle shaped surface element (surface area = 0.43 mm^2) and assigning to each patch a relative activity density value. Activity density is given by the sum of the activities provided by the individual radionuclides deposited on a patch divided by the area of the patch (computational cell). The ratio of the local activity density to the average activity density yields the relative activity density.

In order to model the interaction of the radiation with the radiosensitive epithelial cell nuclei, alpha tracks were generated and a three-dimensional bronchial epithelium was constructed (see also Szőke et al. 2008). Alpha-tracks were simulated as straight lines with randomly selected directions. Their lengths (ranges) were derived based on

the initial kinetic energies of alpha particles and published stopping power functions for air and tissue (ICRU 1993). Near wall and far wall alpha tracks were distinguished based on their direction to the nearest surface element. By definition, tracks entering directly into the tissue are near wall alpha tracks, whereas tracks first entering the airway lumen then penetrating into the epithelium are far wall alpha tracks. The reconstruction of the three-dimensional bronchial cell nucleus structures is based on the histological data of Mercer et al. 1991. In this work, basal, indeterminate, ciliated, preciliated, goblet and other secretory cell nuclei types were distinguished. Hit probabilities and cell killing probabilities were computed for each type of cells, including both proliferative and terminally differentiated cells. However, transformation probabilities were computed only for the radiosensitive basal and goblet cells (see Figure 1). For the simulation of cell killing and cell transformation, results of the *in vitro* cell irradiation experiments from the open literature were used.

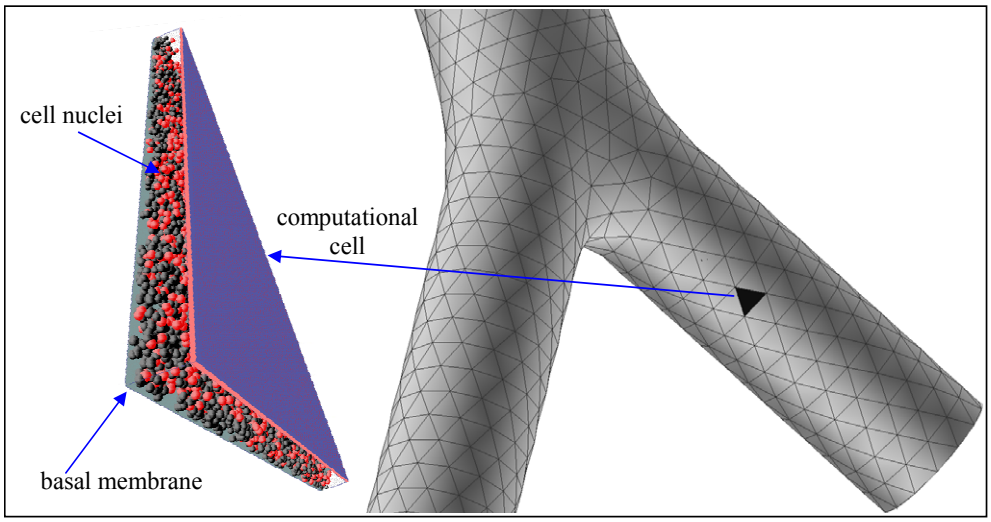

Fig. 1. Discretized (meshed) model airway bifurcation geometry and reconstructed epithelial cell nuclei.

Results and discussion

Figure 2 depicts the deposition patterns of attached (red dots) and unattached (blue dots) radon decay products corresponding to 0.01 WLM. As expected, particles deposited nonuniformly along the airway bifurcation, with the dividing spur as a preferential deposition site. The deposition efficiency, defined as the ratio of the number of deposited to the number of entering particles, was 0.46 % and 16.2% for the attached and unattached radon daughters, respectively (tracheal airflow rate was 50 l/min). Furthermore, the left panel of Fgure 2 demonstrates that the deposition of the molecular (unattached) radon progenies is less inhomogeneous than that of the attached ones. Quantification of the inhomogeneity of deposition distribution is demonstrated in the right panel. In the most exposed patch, the activity is roughly 40 times higher than the activity averaged over the whole bifurcation geometry. This suggests that cells located within this patch can be at considerable risk even if the average activity is not so high. To verify this, hit probability distributions were computed. The scanning technique presented at the description of activity maps (Methods section) was used to

count the number of hits for each computational cell, then the distribution of hit numbers per patch was plotted for the 0.25 WLM exposure level (Figure 3, left panel). To highlight the importance of considering realistic deposition distributions, the hit number per patch distribution in case of uniform activity distribution is also presented for the same exposure level (Figure 3, right panel).

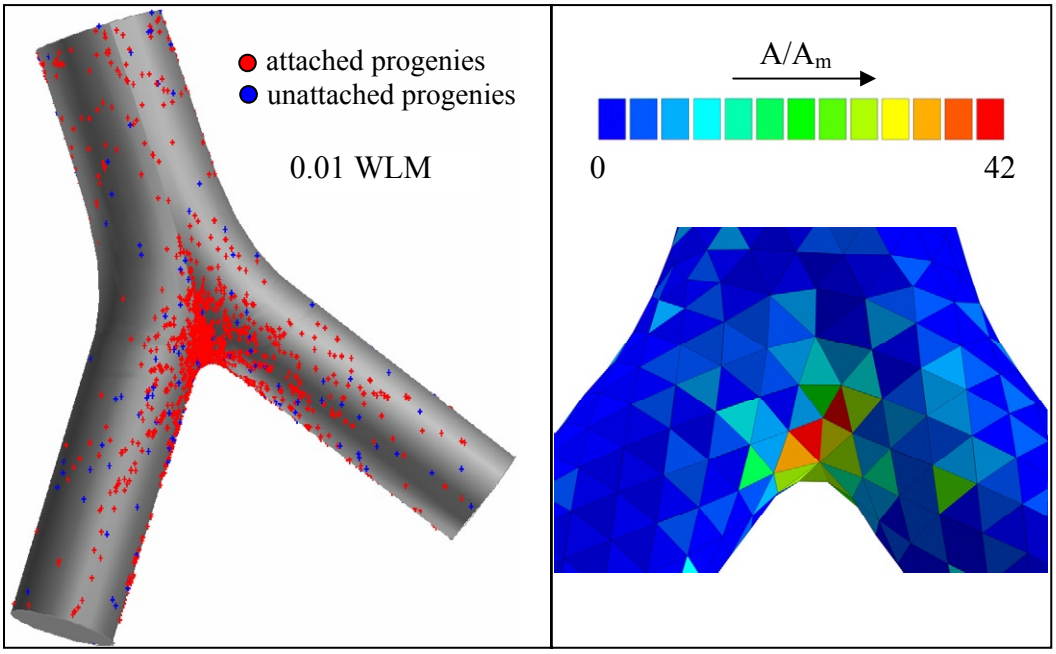

Fig. 2. Deposition pattern of attached and unattached radon progenies assuming an exposure level of 0.01 WLM (left panel) and activity density map in the vicinity of the carinal ridge normalized to the mean activity density (right panel).

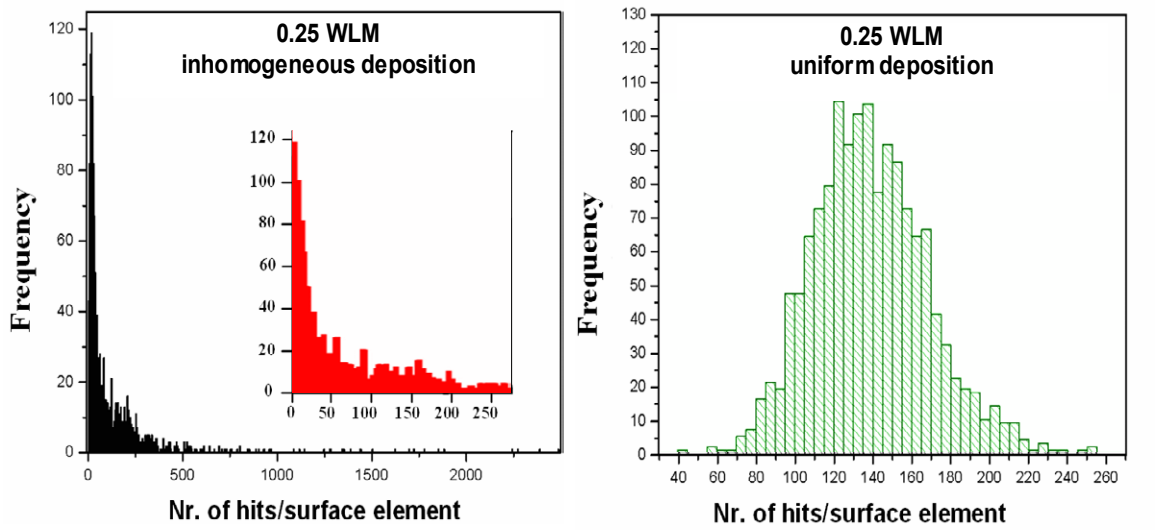

Fig. 3. Distribution of the number of hit cell nuclei located in a pre-specified surface element assuming inhomogeneous (left panel) and uniform (right panel) radioaerosol deposition distributions corresponding to 0.25 WLM radiation exposure.

Figure 3 reveals that the two hit distributions are quite different. Considering realistic deposition distribution while most of the patches receive no hit or only a small number of hits, a few patches receive hundreds and even thousands of hits. In contrast assuming uniform deposition, like most of the current models, the number of hits per patch is closely distributed around a mean value of about 130. This means that for the same exposure the degree of clusterization of the hit cells is much higher in case of inhomogeneous deposition compared to the case of uniform deposition. To check whether this observation is valid for other exposure levels, as well, the mean and maximum number of hits per patch were plotted as a function of WLM. Figure 4 demonstrates that the inhomogeneity in the distribution of hit cell nuclei per patch is present at any exposure level in the low dose range. This result suggests that although the proportion of cell nuclei receiving hits is quite low in the low dose range, there are areas, where the number of hit nuclei is very high and thus the most exposed cells are close to each other. The above fact is usually neglected by current risk models, which operate with average hit numbers.

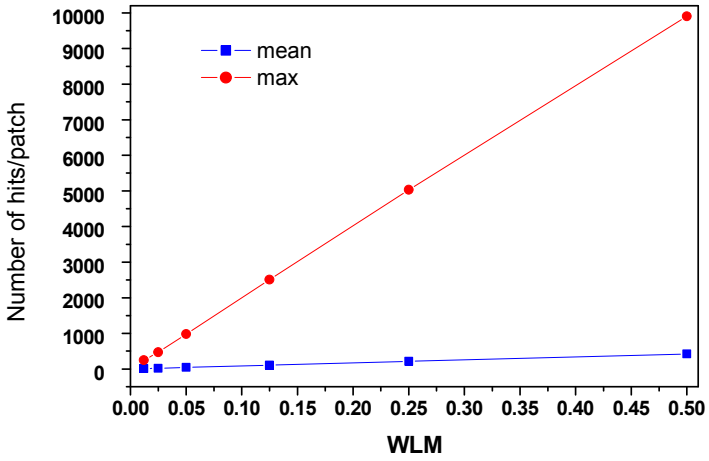

Fig. 4. Average and maximum number of cell nucleus alpha hits per patch (computational cell) as a function of working level.

It is worth mentioning that computations related to Figures 3 and 4 do not account for multiple hits. Taking into account the multiple hits the inhomogeneity becomes even more accentuated. Location of the cell nuclei receiving multiple hits are illustrated in Figure 5. The figure demonstrates that assuming realistic particle deposition patterns, the probability that a cell nucleus located in the carinal ridge of the airway bifurcation receives multiple hits is much higher than it is for a nucleus from the cylindrical parts of the bifurcation. In addition, the mean value of the distances of cell nuclei receiving multiple hits to their multiple hit neighbours (d_m) is also significantly lower than the same parameter characteristic of uniform deposition, indicating that cells at direct risk are closer to each other when assuming realistic deposition scenarios. To verify whether the finding holds for other exposure levels, as well, the probability that a cell nucleus receives at least one and at least two hits was calculated for a range of WLM values (see Figure 6). Although assuming uniform radionuclide deposition, in the considered working level interval, the probability of at least one and at least two hits increases linearly with the increase of the exposure level, there is little chance for a nucleus to

receive at least one hit below 6 WLM and the probability of more than two hits is almost zero. In contrast, in the hot spot of the inhomogeneous deposition, the curves are nonlinear and the probability of at least one and at least two hits can be high even at low exposure levels, suggesting that for these regions of the tissue the "low dose problem" is actually a" high dose problem".

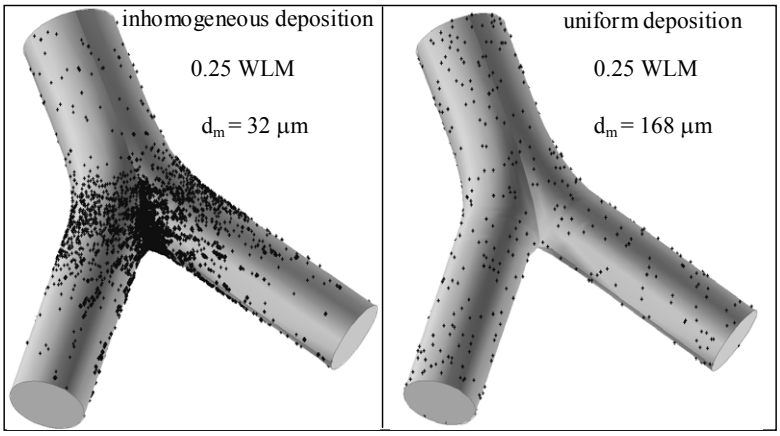

Fig. 5 Locations of cell nuclei receiving multiple hits in case of realistic nonuniform (left panel) and uniform (right panel) radionuclide surface distributions. d_m denotes the mean value of the distances between the nuclei receiving multiple hits and their nearest neighbours receiving multiple hits.

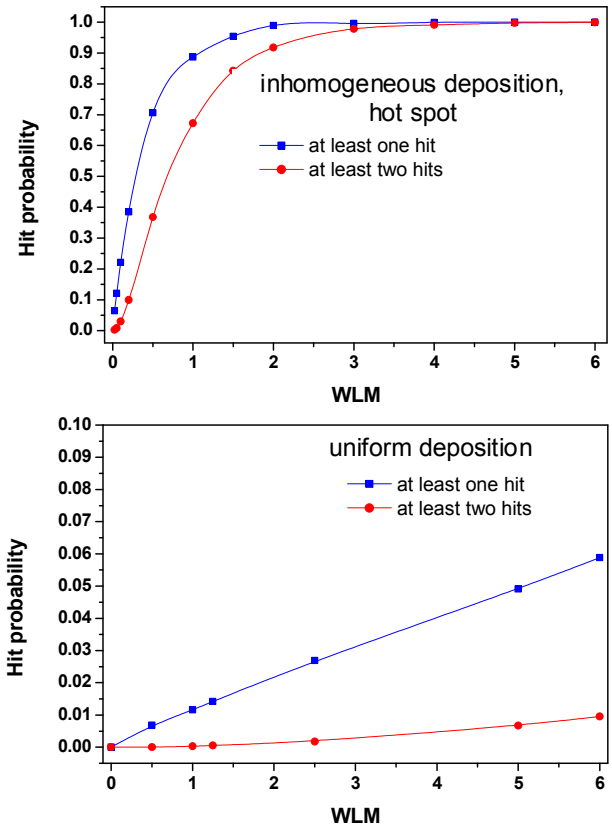

Fig. 6. Probability that a cell nucleus is hit at least once or twice as a function of WLM. The upper panel refers to nuclei in the deposition hot spot of the inhomogeneous deposition pattern, whereas the bottom panel presents the case of uniform deposition.

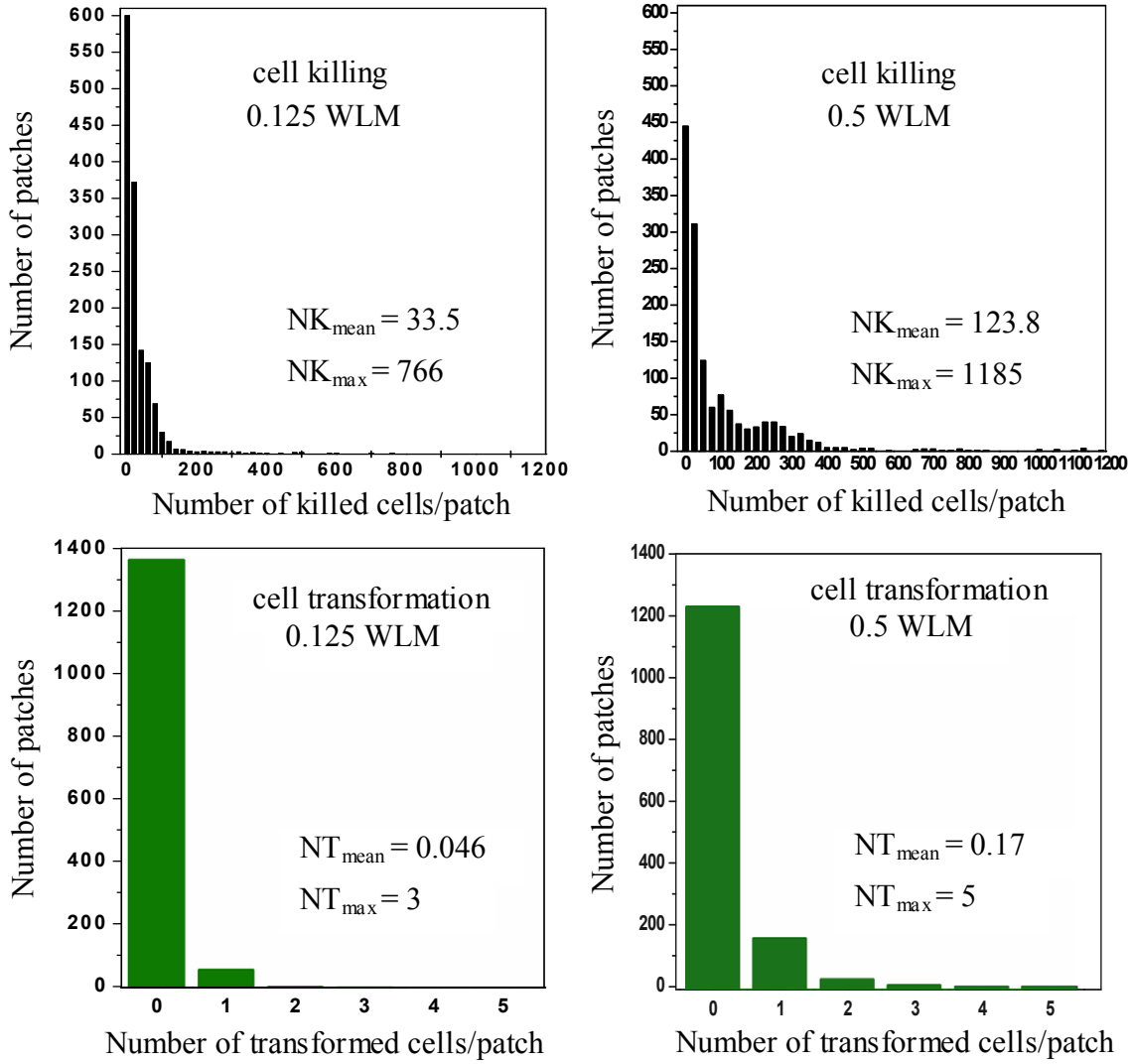

Fig. 7. Distribution of the number of killed (upper panels) and transformed (bottom panels) cells per patch at 0.125 WLM (left panels) and 0.5 WLM (right panels) exposures, assuming inhomogeneous deposition patterns. NK_{mean} – mean number of dead cells per patch; NK_{max} – maximum number of dead cells per patch; NT_{mean} – mean number of transformed cells per patch; NT_{max} – maximum number of transformed cells per patch.

To reveal the cell biological consequences of the nonuniform deposition, cell death and cell transformation distributions were computed. The results of these simulations are presented in Figure 7. The figure reveals that following the tendencies concerning the microdosimetric parameters illustrated in Figs. 3-6, spatial distribution of the biological endpoints is also heterogeneous. While the average number of inactivated cells in a patch is 33.5 at 0.125 WLM, it reaches 766 in the hot spot. The corresponding values for 0.5 WLM are 123.8 and 1185, respectively. It has been demonstrated by different experiments that the transformation frequency due to ionising radiation is extremely low in the low dose range (e.g. Sawant et al. 2001). For instance, according to Miller's microbeam irradiation experiments performed on CH310T1/2 cells (Miller et al. 1999) only one cell out of ten thousand surviving cells receiving exactly one hit will be transformed. Applying the computational schemes described in

the methods section, the average number of transformed cells on a patch is 0.046 at 0.125 WLM and 0.17 at 0.5 WLM. However, our computations demonstrated that even at low exposures and hence low average transformation probabilities, there were three transformed cells at 0.125 WLM and 5 at 0.5 WLM in the hot spots. It is worth mentioning that one patch corresponds to about 10^4 cells (or nuclei). The existence of significant number of cell death and cell transformation events in restricted areas suggests that, extended detrimental effects might occur even in the low or intermediate dose range.

Conclusions

In this study, spatio-temporal distributions of microdosimetric parameters and related biological endpoints were determined in the central bronchial airways by complex computational methods. As expected, the deposition patterns of the inhaled attached and unattached radon progenies are nonuniform within an airway bifurcation with primary activity hot spots located at the dividing spur (carinal ridge). Cell nucleus hits of radon progeny alpha particles were also heterogeneous in space, although to a slightly lower extent than the distribution of the deposited radionuclides. The probabilities of single and multiple hits were quite high in the deposition hot spot even at low doses and increased in a nonlinear manner with increasing exposure level. The mean distances between hit nuclei were significantly reduced for hot spots compared to the corresponding mean distances in case of uniform deposition. Our computations revealed high cell killing rates in the deposition hot spots at low macroscopic doses. Oncogenic transformation probabilities are also significantly higher at the carinal ridges where transformed cells are closer to each other. Present results may help in the elucidation of different aspects concerning the biological consequences of radio-aerosol inhalation and may serve as inputs for future risk assessment models.

Acknowledgements

This work has been supported by the European Commission under contract FI6R-CT-2003-508842 (RISC-RAD) and by the Hungarian OTKA K61193 and ETT 317-08 projects.

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Cardiovascular risk after low-dose radiation exposure

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Abstract

Introduction: There are considerable uncertainties concerning health effects of low doses of ionising radiation on heart. The Life Span Study of the Japanese atomic bomb survivors shows excess radiation-associated risk for cardiac disease even at doses of 0.5 Gy.

Aim: We have used both a human endothelial cell line and a mouse model to study proteomic alterations after low-dose irradiation.

Methods: The human endothelial cell line Ea.hy926 was irradiated with 0.2 Gy gamma rays with two different dose rates and the cells were harvested 4h and 24h after the irradiation. The proteome changes were analysed using 2 DE-DIGE techniques. Using a C57/Bl6 mouse model functional and proteomic alterations in mitochondria isolated from irradiated and sham-irradiated murine hearts 4 weeks after heart-focussed irradiation (0.2 Gy, 2 Gy X ray) were analysed.

Findings: In the endothelial cell line, out of more than fifty protein spots that showed significant alterations in their expression 22 proteins were identified. Among the pathways affected by the low-dose ionising radiation are Ran and Rho/Rock pathways, stress response and glycolysis. In the mouse model, no differences between sham- and irradiated cardiac mitochondria were found in swelling, respiratory coupling and production of ATP. However, we could identify significantly increased ROS formation in cardiac mitochondria 4 weeks after the exposure to 2 Gy ionising radiation.

Conclusions: The immediate response to low-dose ionising radiation in the human endothelial cell line includes alterations in the expression of small GTPases (or their regulators) such as Ran and RhoA, the expression of which is known to be regulated by the production of reactive oxygen species (ROS). Similarly, we find in the long-term response of irradiated murine heart mitochondria an increased production of endogenous ROS. We conclude that changes in the oxidative stress may be an important factor in the development of radiation-induced cardiac disease.

Modelling and experimental studies on adaptive response

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Abstract

The ability of a low "priming" radiation dose to decrease the cell response to a subsequent higher "challenging" dose is termed adaptive response (AR). The main proposed mechanisms to explain AR are: increased efficiency of DNA repair, induction of anti-oxidant enzymes, alteration of cell cycle progression, changes in chromatin conformation. The model proposed by Curtis in 1986 that considers a modulation of the efficiency of DNA repair activity and of the level of anti-oxidant enzymes, starting from the framework of lethal-potentially lethal (LPL) model, was extended with the inclusion of the dynamical variables representing the efficiency of repair, the levels of radiation induced radicals and of anti-oxidant enzymes. In the simulation code particular attention was devoted to the induction of anti-oxidant enzymes as adaptive response mechanism, even if the more relevant mechanism remains the modulation of the repair efficiency, by which the cells processes the initial radiation damage. Furthermore, in order to describe different radiation qualities, the weight of the two factors (i) production of direct damage and (ii) production of free radicals have been made variable in the model. Our model is able to describe the protective effect of a priming dose. Moreover, in agreement with the literature data, the simulations show that the AR happens in a given priming dose and priming dose rate ranges only, and requires at least 4 hours to develop. In order to get more insights on the role of cell-cell communication as factors affecting the AR, experimental studies have been performed using sparse or confluent AG1522 cell monolayer. The results obtained after gamma-irradiation suggest that cell density is a crucial factor for observing an AR. The possibility of an AR induced as bystander effects and as a function of radiation quality is under study.

The α-particle irradiator at the ISS: a useful tool for low dose/dose rate studies

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Abstract

An α-particle irradiator has been developed for radiobiological studies at the Istituto Superiore di Sanità (ISS), Rome, Italy. It consists in a 200 mm diameter stainless steel chamber that can be equipped with alpha sources of different activities, allowing modulation of dose and dose-rate.

In the present configuration the irradiator, equipped with ^{244}Cm or ^{241}Am sources, provides a useful facility for irradiation of cultured mammalian cells with α-particles at dose rate in the range of 1-100 mGy/min, with spatial variations of less then ± 7%. Moreover, for both sources the photon doses calculated at the cell entrance are negligible compared to the α–particle dose. A further important feature of the irradiator is that the seal necessary to keep the helium gas inside the chamber is provided by the Mylar® base of the sample holder. This solution decreases energy degradation and eliminates the problems related to the estimation of the air layer between the exit window and the sample holder. Mylar®-based Petri dishes with different irradiation area were designed that can house permeable membrane inserts, mimicking the same geometry of commercial cell culture insert companion plates. Due to the limited residual range of α-particles, this feature is particularly valuable for co-culture experiments aimed at investigating bystander effects.

The small size allows the irradiator to be easily positioned into a standard CO2 incubator, avoiding use of "*ad hoc*" and separate devices for temperature control and for keeping the cells in the proper air/CO_2 mixture. This important feature makes it possible to carry out long term irradiation and/or post-irradiation incubation of cells in physiological conditions.

The irradiator has been already successfully used to continuously irradiate confluent primary human fibroblasts for up to 14 days as well as to investigate cellular and molecular end points in directly hit and in bystander primary human fibroblasts.

Using of radioprotectors and antidotes during the external radiation exposure and radionuclide incorporation

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Abstract

We have studied experimentally on mice the compatibility of radioportectors (indralin and riboxin) and antidote drugs (potassium iodide and ferrocin) during the external gamma ray exposure and incorporation of ^{131}I and ^{137}Cs. We have shown that during combined action of both external and internal radiation factor radioprotectors and antidotes do not affect the efficiency of each other. In the absence of antidotes the radioprotectors alternates the exchange kinetics of the radionucildes. Application of the indralin alone increases the concentration of ^{131}I in the thyroid and kidneys and ^{137}Cs in the liver. Application of riboxin alone decreases the concentration of ^{137}Cs in organs and tissues at early times. The accumulation rate and concentration values for radioniclides in different organs depends on the time of radioprotector application.

Low-dose irradiation delays neuronal differentiation during early embryonic brain development

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Abstract

Recent interest into the effects of low-dose ionizing irradiation on the human brain has been rather limited partly due to the fact that the adult brain (postmitotic neurons) is very insensitive to radiation damage. However, epidemiological studies on individuals who were exposed to ionizing radiation *in utero* after the atomic bombings in Hiroshima and Nagasaki, showed an increased incidence of mental retardation and behavioural defects. These defects were most outspoken when the irradiation occurred between weeks 8 and 15 of gestation, which is a critical time for human embryonic neuronal development. This suggests a higher sensitivity of neurons during their early differentiation.

Initial experiments in our laboratory showed that young adult mice that had been irradiated with doses (1 Gy) *in utero* at day 12 of gestation (E12) and at lesser extent with lower doses (0.2 Gy) at day 11 of gestation (E11) suffered from memory and learning defects as assessed by Morris Water Maze testing. In order to identify possible pathways involved in neurogenesis that could be affected by low-dose irradiation during early neuronal development, we irradiated pregnant mice at day 10 (E10) and 11 of gestation at 0, 0.1 or 0.2 Gy. Three hours after irradiation, embryonic brains were isolated for total RNA extraction and subsequent microarray analysis using the MoGene 1.0 ST Arrays (Affymetrix, USA).

In line with earlier observations, our analysis revealed upregulation of several genes involved in p53-responsive pathways in a dose-dependent way in both E10 and E11 irradiated embryos, indicating that these pathways are important for the response of the embryonic brain to low doses of irradiation. Furthermore, we observed that low doses of irradiation attenuate many functions of the normal physiological changes in gene expression, and in particular functions related to neurogenesis and synaptogenesis. This suggests that normal neuronal development may be delayed by low-dose irradiation. A significant amount of the affected genes involved in neurogenesis were predicted targets of the neuronal gene silencer RE-1 silencing transcription factor (REST) of which the expression in the embryonic brain decreases during differentiation. Interestingly, expression of REST was increased when E11 brains were

irradiated both at 0.1 and 0.2 Gy, while the expression of the REST target genes dose-dependently declined.

Together, our data indicate that the cognitive defects observed in young adult mice that had been irradiated *in utero* may be related to delayed neuronal differentiation due to a repression of neuronal genes following increased expression of the neuronal silencer REST. The exact mechanism behind the radiation-induced stimulation of REST remains to be elucidated.

This work is financially supported by the NOTE project EC contract: FI6R-036465.

EU GENRISK-T project: Thyroid cancer risk in the Balb/c mouse strain after exposure to low doses of X-rays

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Abstract

Thyroid cancer is one of the malignancies that are induced by radiation exposure as supported by epidemiological studies of different radiation-exposed groups such as: the survivors of atomic bombing in Japan, Marshall islanders exposed to nuclear test fall out and children undergoing head or neck radiotherapy or accidentally exposed to radiation like in Chernobyl accident. In these two last groups, the age was an important determinant of the radiation-exposure outcome.

Besides the age at the time of exposure, genetic susceptibility is one of the factors that can interfere in the development of thyroid cancer. The aim of the FP6 EU GENRISK-T project is to define the genetic component influencing the risk of developing thyroid cancer after exposures to low dose radiation as there is currently no accurate model of the dose response curve for thyroid cancer at low doses. To shed light on the cause-effect relationship between low radiation exposure, thyroid cancer and genetic background we used a mouse model with low sporadic rate of developing thyroid cancers. Such studies of genetic susceptibility would be impossible to conduct in humans due to the significant incidence of sporadic thyroid cancers and to the presence of "confounding" non-radiation induced tumours.

Balb/c mice were irradiated at 12 weeks of age with either low (25cGy, 50cGy, 100cGy) or high (4Gy) X-ray doses and sacrificed 4 hours, 9 or 18 months post-irradiation. Thyroid cancer was assessed via histopathology examinations to detect any morphological alteration in the thyroid tissue structure. In addition, we used immunohistochemistry to detect the level of some proliferation markers (PCNA and Cyclin-D1) that would be indicative of the proliferation status of the thyroid tissue.

Based on histopathology examinations of thyroid tissue sections from the 9 month mouse group, we found that no mice had developed thyroid tumors even at the highest doses. However, at a dose of 1 Gy, we found two specific cases, one of microfollicular hyperplasia and the other one of acute infiltrate with granulocyte. Regarding the immunolabeling with Cyclin-D1 and PCNA, we found a dose-dependent increase of these molecules significant from 25cGy onwards compared to the controls. Examination of thyroid tissue sections from the remaining mouse groups (4 hours and 18 months) is currently under progress. In parallel, high-throughput microarray technology is currently performed.

This work is financially supported by the EU Euratom programme (GENRISK-T : contract FP6-36495).

Identification of health risks in workers staying and working on the terrains contaminated with depleted uranium

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Abbreviations:

L-ALP	Leukocyte alkaline phosphatise
MCV	Mean corpuscular volume
IU	international units
DU	depleted uranium
NU	natural uranium
TLD	thermo luminescent dosimeters
DOE	duration of occupational exposure
NATO	North Atlantic Treaty Organization
FRY	Federal Republic of Yugoslavia

Abstract

Objectives: This study investigated health risks in workers permanently or occasionally residing in the contaminated terrains by low ionizing radiation doses originated from ammunition containing depleted uranium (DU).

Methods: The studied population had been composed of two test groups (T-I, T-II) occasionally .exposed to DU, and two referent (R-I, R-II) groups not exposed at any time to DU. All of them had been evaluated for the following: complete clinical examination and blood count, presence of immature forms and blasts, leukocyte alkaline phosphatase activity and cytogenetic tests (lymphocyte karyotype and chromosomal aberrations). The probability of onset of the characteristic complete biomarkers – chromosomal aberrations, had been specially analyzed using logarithmic function of the Poisson regression.

Results: The difference between the groups was found in potential exposure to DU, which was not found in the reference group R-I and R-II being probable in the test group T-I and T-II. Estimated function of density of probabilities of Poisson distribution of the chromosomal aberrations in the test group T-II was drastically different from the corresponding distribution of the referent group R-I and to the somewhat lesser extent from the group R-II; Wilcoxon test exactly confirms presence of significant difference between the reference group R-II and test group T-II, $p<0.05$.

Conclusion: Damages of chromosomes and cells, had been used for the relative radiation risk assessment, were highest in the test group T-II of workers additionally occupatioanlly exposed to DU. Group of workers T-I, had been exposed to DU working on contaminated terrain, have had certain risks of cell and chromosome damages, and that risk was not greater than the risk to the referent group R-II of workers occupationally exposed to ionizing radiation.

Introduction

The research studies directly related to depleted uranium (DU) and its utilisation for military purposes are relatively rare in comparison to the studies related to the natural uranium.[1] Natural uranium is normal component of the lithosphere (in the region of Serbia, averagely ranging between 0.5–5g/1 ton of the soil) and it is composed of 3 mutually balanced isotopes: 234, 235 and 238 (depleted).[2 3]

Utilization of ammunition containing depleted uranium (DU) has resulted in contamination of the terrains after the bombing and continuous exposure of the living world to small ionizing radiation doses,[4 - 6] in addition to the already existing natural (e.g., uranium) and artificial (e.g., radio cesium) radionuclides. Local human population living and working in the contaminated environment has been also exposed to continuously increased radioactivity to DU.[7 8]

Two ways of DU transfer from the contaminated environment to humans are possible. Ingestion is the predominant form of DU contamination transfer from the environment to the human bodies in the post-conflict period. DU containing in the soil finds its different ways to be included in the food chain. Through contamination of underground and ground water, radionuclide comes into the plants and animals, to be finally consumed by human population.

The mechanism of internal contamination through inhalation is also possible. The aerosol forms are deposited in the soil, to be thereafter returned to the aerosol forms under the influence of wind or human activities, thus coming into the human body through inhalation.[3]

The study is aimed at identification of health risks in workers permanently or occasionally residing in the contaminated territory associated with contamination of the terrain by low ionizing radiation doses resulting from utilization of ammunition containing DU.

Methods

According to the health consequences monitoring program on the contaminated terrain proposed by the World Health Organization in 1996,[9] the studied population was evaluated for the following:

- Complete clinical examination;
- Complete blood count;
- Leukocyte formula and leukocyte/lymphocyte ratio;
- Microscopically observed morphological changes; presence of immature forms and blasts;
- Leukocyte alkaline phosphatase (L-ALP) activity;
- Cytogenetic tests (lymphocyte karyotype and chromosomal aberrations).

The method had been limited to analysis of the peripheral blood cells and cytogenetic analysis of lymphocyte karyotype as the most sensitive parameters of low dose influence, due to short time period (5 years after initial contamination) for clinical presentation of the possible diseases. Nevertheless this is sufficiently long time of period to expect positive findings of DU blood and urine values, regardless of the previous presence of absence of contamination.

Identification of environmental radionuclide presence effects on the body was performed indirectly, using analysis of blood count parameters: total count of red blood cells, haemoglobin concentration, mean corpuscular volume (MCV), platelets, white blood cells, leukocyte formula. Particular attention had been given to lymphocyte/leukocyte ratio, leukocyte alkaline phosphatase (L-ALP) activity, chromosomal aberration and lesion frequency, probability of onset of chromosomal aberrations.[8 10 - 12]

The venous blood was used for the blood cells count. An automatic counter counted erythrocytes, reticulocytes, platelets and leukocytes.

Blood smears stained with May-Grunewald-Gyms were studied through the optical microscope with immersion for the differences the white cell components, presence of morphologically altered blood cells, young precursors, immature forms and blasts.

Percentages of the white cell components of the blood (specific leukocyte shapes) were used to determine the absolute number of lymphocytes.

The capillary blood smears were stained for alkaline phosphatase using a modified Kaplow's method.[8] The colourless cytoplasm was marked 0, the mildly stained 1, the clearly stained 2, that with numerous granules 3, the intensely stained 4. Every hundred of counted cells was multiplied by the stain intensity index. The sum of these products makes the L-ALP score, i.e. the value of enzyme activity. Score of enzyme activity was presented as the international units (IU).

Chromosomes were observed in peripheral blood lymphocytes. Moorhead's method and conventional cytogenetic techniques were used for preparation of lymphocytes.[10 12] The cells in metaphase were microscopically examined in stained (2% M.G. Gyms) smears under immersion (magnification 100x16). The karyotypes of 200 prepared metaphase lymphocytes were analyzed, when the chromosomes were arranged in equatorial plane. The most characteristic aberrations were dicentric chromosomes. Ring chromosomes and acentric fragments were considered the equivalent to dicentric (chromosome aberrations – ca). Chromatid and chromosomal breaks and chromatid exchanges were designated as chromosomal lesions – cl. Lymphocytes having karyotype damages were marked as damaged cells – dc.[12]

The final studies were performed during 2004, five years after the initial contamination caused by DU radioactive ammunition used in 1999, and the whole five-year period had been taken into account.

Environmental follow-up measurements were performed on several occasions (2001, 2002 and 2004) applying the well known methods, according to which, the initial contamination of the terrain was determined.[5 6] The above evidenced chronically increased population exposure due to DU transfer into the biosphere.[8]

Measurement of the total drinking water alpha-activity resulting from both and DU (DU) from different sources (the total of 18 points) in the contaminated region revealed the values below 1 Bq/l, i.e., averagely 10mBq/l.

The specific DU activity in water samples ranges between 0.03 and 0.21Bq/l.

The total alpha-activity in the samples that are indicators of biosphere contamination (animals – rabbit; and plants – vegetation moss and lichen) ranges between 1340± 200 and 1740 ±260 (rabbit); moss 1370±210 and lichen 1860±280 Becquerel at the average.

The specific activity of the DU in meat (rabbit) was 2.3Bq/kg., while in plants it was 16-48 Bq/kg, averagely: 23± 5Bq/kg.

Cesium 137gamma activity as the indicator of radioactive contamination (even before the conflict) was lower, 0.16 ±0.05Bq/kg (rabbit) and 2.0± 0.1(vegetation).

Statistical analysis included two groups of subjects (table 1): reference group (without potential risk of exposure to DU) and test group (with potential risk of exposure to DU).

Reference group was designed to include two characteristic samples. The first reference sample was obtained from the population considered to be exposed one to natural phone without occupational exposure to ionizing radiation, physical or chemical mutagens. The group comprised young workers employed at the Federal Customs Administration, averagely aged 36.6 years, exclusively males with average 7.6 years of service and zero duration of exposure (group R-I).

As opposed of the above group, another reference group (R-II) was introduced composed of workers employed at the Institute of Oncology in Belgrade, who had been occupationally exposed to ionizing radiation effects. They were constantly subjected to dosimetric and medical control. Dosimetric control included monitoring doses by personnel dosimeters. The absorbed external doses of ionizing radiation to the bodies were measured by personnel thermo luminescent dosimeters (TLD) for the duration of occupational exposure (DOE). The TLD measurements were expressed in mSv, as equivalent doses. Average annual doses for the observed five-year period (1999-2004) have been presented (table 1).

Medical control included periodic check-ups based on the program proposed by the Ionizing radiation protection law,[11] incorporating ICRP recommendations, in order to get the insight into general health status, symptoms and clinical signs (complete clinical evaluation).

The group was averagely aged 42.7 years, with 28.6% of males, 15.6 years of service at the average and 23.8 years of occupational exposure at the average.

Test group comprised two specific samples. The first sample (T-I) included the workers employed at the Radio Television of Serbia from Belgrade, who had stayed, during the North Atlantic Treaty Organization (NATO) bombing of Federal Republic of Yugoslavia (FRY), on several locations including Pljačkovica hill near Vranje, the town in south Serbia ,in order to repair malfunctions on the TV antenna hit by DU ammunition. The group was not occupationally exposed to ionizing radiation before that. However, due to the nature of their occupation, the test group was exposed to increased electromagnetic radiation present on TV antenna. Their average age was 53.7 years, 95% of them were males with 28.7 years of service and 0 years of occupational exposure.

The second test group (T-II) included health care workers from Vranje, who were occupationally exposed to ionizing radiation since they have lived and worked in vicinity of the terrain contaminated with DU.

The workers have been constantly subjected to dosimetric and medical check-ups, similarly to R-II group.

The average age of the second test group (T-II) was 43.3 years, 59.6 % of them were males with 14.8 years of service and 11.1 years of occupational exposure.

Statistical methods

The samples were compared based on the observed parameters using the estimated Poisson distribution and the significance of the difference was tested using Wilcoxon rank sum test with 95% confidence interval and significance threshold at the level of 0.05. The probability of onset of the characteristic complete biomarkers was specially analyzed using logarithmic function of the Poisson regression and it was quantified by lambda parameter (λ).

Linear regression correlation analysis and Student's t-test comparison at the probability level of 0.05 were used.

Results

Measurements performed at the working sites confirmed that the exposure doses absorbed by the occupationally exposed medical professionals in Belgrade (group R-II) and Vranje (group T-II) were admissible and low. The readings were performed in the reference group II using thermo luminescent dosimeters (TLD) and the equivalent doses ranged between 1.00 mSv and 2.04 mSv (1.34mSv/year, at the average). As for the test group II, the mean annual dose was 2.08 mSv, with minimal and maximal values being 0.9 mSv and 4.98 mSv, respectively (Table 1).

The obtained results were tabularly presented in the context of the comparative pair analysis (table 1), both with negative clinical finding of the diseases associated with low ionizing dose radiation effects:

1. Referent group I (R-I) and test group I (T-I);
2. Referent group II (R-II) and test group II (T-II).

Table 1. Statistical parameters of reference and test groups with average annual dose.

Statistical parameters		Groups			
		Reference (R)		Test (T)	
		R-I	R-II	T-I	T-II
N° (persons)		25	42	20	52
Equivalent dose (TLD)		*< 1 mSv*	*1.34 mSv*	*< 1 mSv*	*2.08 mSv*
Age (year) α_ci = 0.05	μ	36.6	42.7	53.7	43.3
	σ	5.8	9.5	12.1	9.7
	ci_lower	34.2	39.8	48	40.6
	ci_upper	39	45.7	59.3	46
Sex masculinum –M, versus femininum-F	M	25	12	19	31
	%	100	28.6	95	59.6
	F	0	30	1	21
	%	0	71.4	5	40.4
Years of services (year) α_ci = 0.05	μ	7.6	15.6	28.7	14.8
	σ	5.9	9.3	12	9.6
	ci_lower	5.2	12.7	23	12.1
	ci_upper	10.1	18.5	34.3	17.5
DOE (year) α_ci = 0.05	μ	0	13.8	0	11.1
	σ	0	9.3	0	9.2
	ci_lower	0	10.9	0	8.6
	ci_upper	0	16.7	0	13.7

TLD - term luminescence dosimeter
DOE - duration of occupationally exposure
μ – mean value – central tendency measure
σ – standard deviation
α_ci = 0.05; ci_ lower, and ci_ upper = confidence interval and deviation below and above it

The R-I/T-I pair differs with respect to average age and average years of service, in absence of any differences related to sex and years of exposure which has had zero value in both groups. The difference in sample size may be considered negligible. The difference was found in potential exposure to ionizing radiation – it was not found in the group R-I, being probable in group T-I because of exposure to DU.

As for the pair R-II/T-II, i.e., health professionals employed in the ionizing radiation zones in two cities (Belgrade and Vranje) in Serbia, no differences were found related to age, years of service, years of exposure, occupation (approximately the same level of qualification). However, the difference was found with respect to their age, mean value (1.34mSv/2.08mSv) and range of annual equivalent ionizing radiation dose over the observed five-year period (1.0-2.04 mSv/0.9-4.98mSv). The equivalent dose of ionizing radiation in T-II was by 55% higher from the dose in R-II as a consequence of the working site conditions. Workers from the test group II have head higher work load (greater number of radiological procedures per single shift). The difference between the groups was also found in potential exposure to DU, which was not found in R-II being probable in the T-II.

The analysis included blood count elements (Tables 2 and 3).

Table 2. Statistical parameters of reference and test groups of blood count.

Blood count		Groups			
		Reference (R)		Test (T)	
		R-I	R-II	T-I	T-II
N° (persons)		25	42	20	47
Hemoglobin (Hb)	Standardized interval		120 – 160g/l		
	Mean value of standardized interval		140		
α_ci = 0.05	μ	**139.1**	**133.1**	**132**	**133.2**
	σ	±3.8	±12.9	±7.1	±17.4
	ci_lower	137.5	129.1	128.6	128.1
	ci_upper	140.6	137.1	135.3	138.3
	Below limit	0	4 (9.5%)	0	10 (18.9%)
	Above limit	0	2 (4.8%)	0	2 (3.8%)
	Without limit	**0**	**6 (14.3%)**	**0**	**12 (22.7%)**
Eerythrocytes (Er)	Standardized interval		$4.0 – 5.5x10^{12}/l$		
	Mean value of standardized interval		4.75		
α_ci = 0.05	μ	**4.71**	**4.418**	**4.61**	**4.64**
	σ	±0.350	±0.475	±0.363	±0.445
	ci_lower	4.56	4.27	4.44	4.51
	ci_upper	4.85	4.56	4.78	4.77
	Below limit	0	3 (7.1%)	0	2 (3.8%)
	Above limit	0	2 (4.8%)	0	3 (5.7%)
	Without limit	**0**	**5 (11.9%)**	**0**	**5 (9.5%)**
Mean corpuscular volume	Standardized interval		80.0 – 94.0		
	Mean value of standardized interval		87.0		
α_ci = 0.05	μ	**91.9**	**82.0**	**92.9**	**87.9**
	σ	±4.0	±23.9	±6.3	±7.5
	ci_lower	90.2	74.5	90.0	85.6
	ci_upper	93.5	89.4	95.8	90.3
	Below limit	0 (0%)	9 (21.4%)	0 (0%)	4 (7.6%)
	Above limit	7 (28%)	6 (14.3%)	10 (50%)	5 (9.4%)
	Without limit	**7 (28%)**	**15 (35.7%)**	**10 (50%)**	**9 (17%)**
Retikulocytes (Ret)	Standardized interval		0.5 – 1.5 %		
	Mean value of standardized interval		1.0		
α_ci = 0.05	μ	**0.80**	**1.11**	**1.11**	**1.06**
	σ	±0.316	± 0.803	±0.174	±0.440
	ci_lower	0.674	0.864	1.028	0.817
	ci_upper	0.934	1.365	1.192	1.305
	Below limit	0 (0%)	5 (11.9%)	0	1 (1.9%)
	Above limit	2 (8 %)	11 (26.2%)	0	1 (1.9%)
	Without limit	**2 (8 %)**	**16 (38.1%)**	**0**	**2 (3.8%)**
Platelets (Plt)	Standardized interval		$150 – 350x10^{9}/l$		
	Mean value of standardized interval		250		
α_ci = 0.05	μ	**286**	**250**	**303**	**270**
	σ	±22.0	±69.1	±50.8	±65.3
	ci_lower	277.3	228.5	278.7	249.3
	ci_upper	295.5	271.5	326.3	291.0
	Below limit	0	2 (4.8%)	0	2 (3.8%)
	Above limit	0	2 (4.8%)	0	2 (3.8%)
	Without limit	**0**	**4 (9.6%)**	**0**	**4 (7.6%)**

α_ci = 0.05 Confidence 95%; probability p at 0.05
MCV – mean corpuscular volumeof erythrocytes
Standardized interval – adopted from the Ionizing radiation protection law and based on ICRP 2005 and WHO 1996 recommendations (7, 15)
μ– mean value – central tendency measure
σ – standard deviation
α_ci = 0.05; ci_ lower; ci_ upper = confidence interval and deviation below and above it
Below limit – below standardized interval borderline value
Above limit – above standardized interval borderline value
Without limit – below or/and above (without) standardized interval borderline value

Table 3. Statistical parameters of reference and test samples for total leukocytes, leukocyte formula and alkaline phosphatase.

Leukocytes, leukocyte formula, and alkaline phosphatase		Groups			
		Reference (R)		Test (T)	
		R-I	R-II	T-I	T-II
	N° (persons)	25	42	20	47
Leukocyte	Standardized interval		4.0 – 9.0 x10^9/l		
Le	Mean value of standardized interval		6.5x10^9/l		
α_ci = 0.05	μ	**6.44**	**6.54**	**6.35**	**6.95**
	σ	1.41	2.18	1.69	2.27
	ci_lower	5.86	5.87	5.57	6.28
	ci_upper	7.03	7.22	7.14	7.62
	Below limit	0	2(4.8%)	0	1(1.9%)
	Above limit	2 (8%)	5(11.9%)	2 (10%)	8(15.1%)
	Without limit	**2 (8%)**	**7(16.7%)**	**2 (10%)**	**9(17%)**
Granulocyte	Standardized interval		0.51– 0.61		
G	Mean value of standardized interval		0.56		
α_ci = 0.05	μ	0.598	0.577	0.630	0.597
	σ	0.060	0.113	0.152	0.087
	ci_lower	0.573	0.542	0.558	0.571
	ci_upper	0.622	0.612	0.701	0.622
	Below limit	1(4%)	10(23.8%)	1(5%)	3(5.7%)
	Above limit	0	1(2.4%)	0%	2(3.8%)
	Without limit	4%	11(26.2%)	5%	5(9.5%)
Lymphocyte	Standardized interval		0.21– 0.35		
Ly	Mean value of standardized interval		0.280		
α_ci = 0.05	μ	0.319	0.326	0.288	0.357
	σ	0.0805	0.0815	0.0363	0.0829
	ci_lower	0.2860	0.3006	0.2705	0.3322
	ci_upper	0.3524	0.3513	0.3045	0.3809
	Below limit	2 (8%)	6(14.3%)	8 40%)	4(7.6%)
	Above limit	1 (4%)	5(11.9%)	1(5%)	11(20.8%)
	Without limit	3 (12%)	11(26.2%)	9(45%)	15(28.4%)
Monocyte	Standardized interval		0.04 – 0.08		
Mo	Mean value of standardized interval		0.060		
α_ci = 0.05	μ	0.0500	0.0657	0.0425	0.0430
	σ	0.0144	0.1008	0.0234	0.0203
	ci_lower	0.0440	0.0343	0.0316	0.0370
	ci_upper	0.0560	0.0971	0.0534	0.0489
	Below limit	3(12%)	12(28.6%)	6(30%)	12(22.6%)
	Above limit	1(4%)	3(7.1%)	0	1(1.9%)
	Without limit	4(16%)	15(35.7%)	6(30%)	13(24.5%)
Leukocyte alkaline phosphatase L-ALP	Standardized interval		20.0 – 80.0 IU		
	Mean value of standardized interval		50.0		
	μ	**64.9**	**57.1**	**74.9**	**67.1**
	σ	8.8	15.3	9.8	16.3
α_ci = 0.05	ci_lower	60.3	51.8	70.3	61.8
	ci_upper	69.5	62.3	79.5	72.3
	Below limit	0	0	0	0
	Above limit	0	0	7 (35%)	4(7.6%)
	Without limit	0	0	**7(35%)**	**4 (7.6%)**

α_ci = 0.05 – Confidence 95%; probability (p) at 0.05
Standardized interval – adopted from the Ionizing radiation protection law and based on ICRP 2005 and WHO 1996 recommendations (7, 15)
μ – mean value – central tendency measure
σ – standard deviation
α_ci = 0.05; ci_ lower, and ci_ upper; = confidence interval and deviation below and above it
Below limit – below standardized interval borderline value
Above limit - above standardized interval borderline value
Without limit – below or/and above (without) standardized interval borderline value

Mean erythrocyte values were not statistically significant for probability threshold condition α=0.05 for the subject pair R-I/T-I (table 2). The subjects from the both groups were within the standard range for the erythrocytes. Mean haemoglobin values were also within standard range in the both groups. The difference was not found with respect to the criterion of falling of the measured haemoglobin values out of the standard interval. However, mean haemoglobin values were statistically different with respect to the probability threshold condition α=0.05.

The parameter of the erythrocyte volume (mean corpuscular volume -MCV) indicating haemoglobin content in the erythrocytes and cellular size and shape relevant for its function, was not statistically significant, likewise reticulocyte number. No significant difference was found between the subject pair R-I/T-I with respect to red blood cell line parameters (table 2).

Mean platelet values were not statistically different. All the subjects from both groups were within standard range for platelets (table 2).

As for the subject pair R-II/T-II (table 2) mean red blood cell values ranged within standard limits and the difference found between them cannot be considered statistically significant with respect to the probability threshold condition α=0.05. The difference was found with respect to the criterion of falling of the measured values out of the standard interval for erythrocytes. However, it cannot be considered statistically significant.

As for the test group (T-II) the total number of subjects found to be out of the standard range was 5 (11,9%) being in the reference group (R-II) 5 or 9,5%, with their deviation ratio of 1,2. Mean haemoglobin values were within standard limits and the difference between them cannot be considered to be statistically significant for the condition of probability threshold α=0.05. The difference was found with respect to the criterion of falling out of the measured values out of the standard interval for haemoglobin, MCV and reticulocytes and it must be considered statistically significant.

As for the test group T-II, there was significantly higher number of subjects who were out of the standard interval for haemoglobin when compared to the reference group, while the number of subjects out of the standard interval for MCV was significantly lower when compared to the reference group R-II (table 2). Therefore, red blood cell line generally cannot be considered significant in this pair as well with respect to the ionizing radiation influence. As it may be seen in table 2, no differences in platelet line were found for the test pair R-II/T-II. The mean values do not differ significantly while deviations from the standard interval are approximately the same in the both groups.

As for the pair comprising reference and test group R-I/T-I (table 3) mean values of white blood cells were within standard limits and they were not statistically different for α=0.05 (confidence 95%). Additionally, no difference was found with respect to falling out of the borderline values of the standard while blood cell interval, while deviation above the upper limits was almost the same in the both groups. No significant difference was found in number of lymphocytes. The average distribution in the leukocyte formula was 0.32% vs. 0.29% (table 3), while mean values of the absolute number of lymphocytes were 2.1± 0.5 vs.1.8±0.5. The difference was found with respect to the criterion of deviation of the measures values from the standard interval

for lymphocytes. As for the reference group I, the number of subject falling out of the standard interval was significantly lower (12%) in comparison to the test group I (45%).

As for the R-II/T-II pair, no difference was found in white blood cell count, being 16.7% for the reference group and 17% for the test group, meaning that this difference in deviation was not significant. Significant difference was not found with respect to other elements of the leukocyte formula. Basophiles were not evidenced. Moreover, no immature cells or blasts were observed.

Although the comparison between the groups revealed no statistical difference, deviation from the standard intervals for white blood cells and lymphocytes was still present, however it was approximately the same in the both groups (reference II and test II), which included health care professionals.

In comparison to the reference group I (free of ionizing radiation risk) the deviations are prominent (tables 2 and 3).

It has been known that, in addition to alteration of white blood count, ionizing radiation **also** influences leukocyte alkaline phosphatase activity changes as well as the changes of the natural proportions of the leukocytes formula (table 3). As for the L-ALP activity, the changes were characterized by increased activity and they cannot be considered statistically significant, particularly owing to the fact that they follow the increase in white blood count, which is normal phenomenon.

Due to specific lymphocyte sensitivity, lymphocyte-related changes are also particularly characteristic with respect to their number and correlation with white blood cells as well as with respect to chromosomal aberrations in their nuclei.

The correlation analysis of leukocyte and lymphocyte relation was performed for all subjects from the reference and test groups. The analysis of linear regression of the relation between leukocytes and lymphocytes gave regression coefficient for the reference and test groups R-I, R-II, T-I and T-II: 0.76, 0.83, 0.90 and 0.78, respectively. The results of comparison of the linear regression parameters using parametric Student's t-test with significance threshold of 0.05 did not show any difference. In none of the cases statistical difference of white blood cells and lymphocyte proportions was identified for the probability level of 0.05.

Presence of chromosomal aberrations was analyzed in the reference and test groups (Tables 4 and 5).

Table 4. Poison distribution of chromosomal aberrations, chromosomal lesions and damaged cells in the reference and test groups.

Chromosomal aberration, lesion, and damaged cells		Groups			
		Reference (R)		Test (T)	
		R-I	R-II	T-I	T-II
N° (persons)		25	42	19	41
n° (cells)		5000	8050	3800	7920
Chromosomal aberrations –ca	N° persons with ca	4	7	6	20
	%	16.0	16.7	31.6	48.8
	Total ca	4	14	8	32
	% - frequenci ca	**0.08**	**0.17**	**0.21**	**0.48**
	Poison λ	**0.1600**	**0.3333**	**0.4211**	**0.9268**
	distribution ci_ lower	0.0269	0.1483	0.1353	0.5853
	α_ci = 0.05 ci_ upper	0.5038	0.6390	0.9778	1.3892
Chromatid lesions - cl	N°persons with cl	1	16	0	17
	%	4.0	38.1	0.0	41.5
	Total cl	1	29	0	24
	%	0.02	0.36	0.00	0.30
	Poison λ	0.0400	0.6905	0	0.5854
	distribution ci_ lower	0.0002	0.4049	NaN	0.3233
	α_ci = 0.05 ci_ upper	0.2972	1.0947	0.2789	0.9694
Damaged cells - dc	N°persons with dc	5	18	6	26
	%	20.0	42.9	31.6	63.4
	Total dc	5	27	8	47
	%	0.10	0.34	0.21	0.60
	Poisson λ	0.2000	0.6429	0.4211	1.1463
	distribution ci_lower	0.0431	0.3688	0.1353	0.7614
	α_ci = 0.05 ci_upper	0.5660	1.0356	0.9778	1.6516

λ - lambdą parameter of density of probabilities of Poisson distribution
α_ci = 0.05 Confidence 95%; probability (p) at 0.05
α_ci = 0.05; ci_ lower, and ci_ upper; = confidence interval and deviation below and above it

Table 5. Significance of difference in chromosomal aberrations and lesions between the reference and test groups.

Reference groups	Chromosomal aberration, lesion, and damaged cells	Test group 1		Test group 2	
		Confidence 95% alfa=0.05	p	Confidence 95% alfa=0.05	P
Referent group 1	Chromosomal aberration	No	0.2103	**Significance *<0.01***	**p=0.0041**
	Chromatid lesion	No	0.4089	*Significance*	9.86e-004
	Damaged cells	No	0.3531	*Significance*	1.29e-004
Referent group 2	Chromosomal aberration	No	0.3449	**Significance *<0.01***	**p=0.0045**
	Chromatid lesion	*Significance*	0.0022	No	0.7582
	Damaged cells	No	0.3513	*Significance <0.05*	p=0.0203

alfa=0.05 - Confidence 95%; probability (p) at 0.05

Observation of the first pair from the groups R-I and T-I evidenced presence of chromosomal aberrations (table 4.) in the both group. Poisson distribution parameter λ differs, however the corresponding confidence intervals are crossed over for the significance threshold α=0.05, which indicates that the difference is not statistically significant. The former is confirmed by Wilcoxon test of matching of the estimated parameters of the Poisson distribution. The former indicates the presence of tendency toward chromosomal aberrations (higher risk of their onset) in the group T-I in comparison to the comparative reference group R-I. However, the difference in incidence of chromosomal aberrations is not significant, at the average (tables 4 and 5),

although the frequency of chromosomal aberrations (table 4) is higher in the group T-I (0.21% vs. 0.08%) in comparison to the reference one.

The equivalent analysis for the test pair R-II and T-II shows similar tendencies, however they are more prominent and exceed the limits of statistic tolerance in testing of differences (do not belong to the same set - tables 4 and 5). Estimated function of density of probabilities of Poisson distribution of the chromosomal aberrations in the test group T-II is drastically different from the corresponding distribution of the reference group R-I and to the somewhat lesser extent from the group R-II; Wilcoxon test exactly confirms presence of significant difference between the reference group R-II and test group T-II, $p<0.05$ (tables 4 and 5).

The difference in chromosomal aberrations is observed between the reference groups R-I and R-II which is expected since the groups are mutually different with respect to the occupational exposure to low ionizing radiation doses (table 4). Reference group II has significantly higher incidence of the unspecific chromosomal lesions ($p=0.0012$) in comparison to R-I group, as well as significantly higher number of the damaged lymphocytes for that reason (damaged cells – dc, $p=0,0054$ Frequency of chromosomal aberrations characteristic for radiation is increased (0.17% vs. 0.08%) however the increase is non-significant ($p=0.13$, Wilcoxon test). Poisson distribution parameter λ is higher (0.33 vs. 0.16) in the group R-II. This indicates that the probability for onset of chromosomal aberrations is higher in R-II in comparison to R-I group (tables 4).

The results of the chromosomal aberration analysis indicate that the test group I, which has been temporarily occupationally exposed to the effects of low environmental ionizing radiation doses as well as to non-ionizing radiation, had been different from the groups R-I and T- II being at the same time the most similar with the results of the health-related risk analyzes to the referent group R-II, which was chronically occupationally exposed to ionizing radiation.

Group T-I has been significance low chromatid lesions than R-II ($p=0.0022$, table 5). Frequency of chromosomal aberrations characteristic for radiation is increased (0.21%) and Poisson distribution parameter λ is higher than in referent groups ($\lambda=0,42$), but they are no significant.

Test group II, despite test group I, had been significantly different (confidence 95%; table 5) from the both of referent groups (R-I and R-II) taking into consideration chromosomal aberrations in lymphocytes ($p<0.01$) and damaged cells ($p<0.05$).

Accordance to logarithmic function Poisson regression, complete biomarkers (chromosomal aberration – ca) showed that relative risk (RR) in group T-II was 4; in group T-I is 2 and in group R-II it was 3, while in group R-I exposed only nature radiation, relative risk had been 1.

Discussion

Identification of field contamination resulting from application of radioactive ammunition risks on the health on individuals living and working on the contaminated territory is significant for studying of the admissible levels of absorbed radiation of different population groups, particularly individuals occupationally exposed to the ionizing radiation as well as their patients and general population.

The obtained results indicate the presence of the objective risk if exposure of workers living and working on the known contaminated terrain on the south of Serbia as a result of DU originating from the military conflicts that took place on the terrain in 1999.

DU ammunition has become a source of environmental contamination.[4 5 6] On one hand, it is superimposed to the natural phone, while on the other, it penetrates to the soil and ground water to come thereby into the biosphere. Thus, DU becomes a residual radiological risk for human life in the course of their life on the affected terrain.[13] As for the non-occupationally exposed population, each dose above 1mSv/1year is conspired to be a significant dose. Low environmental doses are cumulated during the stay in such regions and within five-year period, if they are above 1 mSv per year, they may reach 6mSv, which represents the upper limit for the occupationally exposed individuals in the category B zone.[11] For this reason, the individuals working and living on the contaminated territories are subjected to health monitoring.[3 6 9]

The analysis included blood count elements, as the most sensitive parameters associated with the reaction of the organism to ionizing radiation effects, which may be monitored and point out to the early signs of the diseases using simple techniques. Blood element differences were observed between the studies groups, however they were insufficiently specific or statistically significant, and thus the analyses were not sufficient for risk assessment.

Lack of specificity of the blood count elements as health indicators for assessment of ionizing radiation risk points out to the need of analysis of chromosomal aberration presence.[12 - 14]

As for the workers living in a wider region with verified contaminated zones who are occupationally exposed to low doses of ionizing radiation, statistical differences were identified upon their comparison with the referent group of the occupationally equivalent subjects, particularly with respect to onset of the chromosomal aberrations. For that reason, the number of damages lymphocytes is higher, which is essential for their organism defence role.

No statistically significant difference was found with respect to unspecific, single-stranded chromatid changes (lesions), which do not form chromosomal figures (dicentrics). Most probably, they are also the result of other causes, in addition to radiation (chemical metal toxicity, habits, smoking etc.) and they may influence karyotype instability.[12]

Additionally, it has also been evidenced that onset of these lesions on DNA is accompanied by the same number of the stable aberrations (deletion, inversion and translocation), which indirectly suggests the probability of onset of mutations (due to stable aberrations fixed in the cell division).

Test group II (workers in the radiation zone in the contaminated region in the southern Serbia) had been composed of two groups of subjects. The first one was composed of individuals at risk of uniform low-dose irradiation on their working sites, while the second one comprised the individuals at risk of contamination with DU. For this reason, the radiation risk was the highest in this group and it has been quantified by the highest probability of onset of chromosomal aberrations and the highest frequency of chromosomal aberrations and damaged lymphocytes. Damaged lymphocytes lost

function because since 50% of them tend to disappear after the initial divisions, and before division ten, all the remaining damages ones also disappear.[8 10 12 14]

Only referent group I was free of any of the 2 above mentioned risks – radiation at the working site or radiation at the contaminated terrain. Therefore, it is clear why the referent groups are also mutually different, since the referent group II had been composed with the individuals chronically exposed to low ionizing radiation doses at their work sites. Nevertheless, the difference was found between the reference groups I and II with respect to chromosomal lesions, i.e., higher risk expressed as probability of onset of lesions was found in the referent group II composed of the occupationally exposed health care professionals from Belgrade. The group was also proved to have higher frequency of unspecific lesions in comparison to the test group I, in which occupational exposure was not continuous but only occasional during repair works on the TV antenna on the contaminated terrain. This group was not exposed to increased radiation and did not have relative risk due to exposure to DU. It was not significantly different from other referent groups with respect to other parameters, however if was different from the test group II composed of subjects continuously staying in the vicinity of the contaminated region.

Accordingly, statistically significant differences related in comparison with the referent group had not been observed, although changes values of certain parameters were evidenced in workers who had lived out of the contaminated regions but who were occupationally exposed for some time due to their stay in the contaminated zone performing their professional tasks.

Therefore, life in the contaminated region is associated with higher radiological risk, particularly for those who work in the ionizing radiation zone and probability of potential occupational diseases may be higher and thus workers rights related to occupational diseases, treatment and assessment of their working abilities should be considered. Additionally, the criteria for recognition of radiation-induced occupational diseases must be also reconsidered in the new circumstances associated with increased risk of environmental contamination as well.

The above fact points out need for further investigations of the association between occupational exposure to the ionizing radiation effects and effects of the underlying environmental contaminants, particularly within the context of the increased relative risk that way quantitatively verified in this study. Accordingly, the aspect of legal regulations in the fields of occupational protection should be also reconsidered, since the situation is new and uncovered by the current regulations. This is particularly important for the health risks of medical professionals in the ionizing radiation zone in southern Serbia, having in mind the fact that their work load is higher due to higher morbidity rate among the population in the underdeveloped region.[9 15]

Conclusions

For all the above, the latent period before onset of the disease is prolonged, and thus the consequences of the DU effects are yet to be expected. The former points out to the need of reassessment of the admissible level of radiation in case of occupational exposure. Decrease of occupational exposure (admissible dose level) may suppress the influence of the environmental doses, since natural phon is increase by superimposed contamination with DU.

Increased health risk of the workers exposed to ionizing radiation caused by profession and additionally by contamination from the contaminated environment by DU, has become because of cumulative radiobiology effects of small doses over the continual exposition and it depends to the time of period of exposure duration.

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