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Does occupational exposure to low-dose ionizing radiation induce cell membrane damage?

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ABSTRACT

BACKGROUND: *Chronic exposure to low-dose radiation doses could be much more harmful than high, short-term doses because of lipid peroxidation initiated by free radicals. The cell membranes and cellular organelles are the main targets for free radicals attack. Peroxidation of cell membrane increases with decreasing dose rate (Petkau effect). The aim of this study was to establish if chronic occupational exposure to low-dose ionizing radiation could induce cell membrane damage.*

METHODS: *Our investigation comprised 77 medical workers: 44 occupationally exposed to ionizing radiation (E), divided in two subgroups-exposed to x-rays (Ex) or gamma rays (En), and 33 controls (C). Informed consent and questionnaire containing dietary, habits, medical factors and exposure history were taken. Groups were matched in gender, age, dietary habits, alcohol consumption, smoking habit, and specific exposure time. Radiation dose accumulated by occupationally exposed over years was calculated on the basis of individual TL-dose records. Besides regular biochemical and cytogenetic tests, lipid peroxidation index, expressed as malondyaldehyde production was performed.*

RESULTS: *Significantly higher lipid peroxidation index was found in workers occupationally exposed to low-dose of ionizing radiation ($p>0.000028$), which is correlated with age, smoking habit, and significantly correlated with doses. After blood samples in vitro irradiation by 2 Gy of gamma-radiation malondyaldehyde production significantly increased in each group, but were not significantly different between groups.*

CONCLUSION: *Lipid peroxidation index could be considered as triage parameter for further cytogenetic studies in workers chronically exposed to low-dose radiation.*

KEY WORDS: *Occupational Exposure; Radiation, Ionizing; Lipid Peroxidation; Malondialdehyde*

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INTRODUCTION

Reactive oxygen species (ROS) formation and oxidative stress that follows are very important in injuries induced by low-dose (LD) radiation (1-3).

DNA and cell membranes are the main targets of induced free radicals. Considering DNA as the most sensitive part of the cell and oxidative DNA damage as the main lesion in radiation injury, the most common early diagnostic and biodosimetric tests in accidental and occupational situations are cytogenetic tests (4-5). They are very useful, but long lasting, expensive, and above all not sufficiently sensitive to low-dose radiation. That is the reason why health risk assessments for LD exposure based on cytogenetic tests data are often not highly correlated with epidemiological studies results. Thus, new or more diagnostic tests are needed for harmful effects of LD assessment. Some of them could be based on oxidative injury of cell membrane (6-10).

At high doses ionizing radiation has a plasma membrane-direct effects such as increase of membrane intrinsic viscosity, rigidity of lipid bilayer, dielectric constant, and lamellar structure. Additionally, radiation indirectly affects the plasma membrane through radiolysis, resulting in production of free radicals. The membranes of the cells and cellular organelles are the main targets for free radicals attack. They could initiate lipid peroxidation (LP), and destruction of cell surface. The final product of LP is malondyaldehyde (MDA), which is mutagenic and carcinogenic (2,3,11,12).

Petkau discovered that chronic low doses could be much more harmful than high, short-term doses. According to Petkau's results peroxidation of cell membrane, expressed as production of MDA, increases with decreasing dose rate (Petkau effect). Once initiated in the membrane, the damaging chain reactions propagate by themselves (13,14).

The aim of this study was to establish if chronic occupational exposure to low-dose ionizing radiation could induce cell membrane damage.

MATERIAL AND METHODS

Our investigation comprised 77 healthy medical workers: 25 (32.47%) women and 52 (67.53%) men. Controls (K) were 10 (30.30%) women and 23 (69.70%) men - medical workers from Preventive Medicine Department occupationally not exposed to ionizing radiation. Medical staff from Radiology (exposed to x-rays - Ex) and Nuclear Medicine (exposed to gamma rays - En) was in occupationally exposed group (E): 15 (34.10%) women and 29 (65.90%) men. Mean age of our examinees was 43.3(6.01) years (range: 32-55 years). Group K and subgroups Ex and En were matched in gender ($p= 0.73$) and age ($p= 0.77$). Informed consent and questionnaire containing dietary, habits, medical factors and exposure history were taken. There were no statistically significant differences between groups in dietary style, alcohol consumption ($p=0.16$), and smoking habits

(p=0.74) considering smoking years (p=0.99) and daily cigarette consumption (p=0.53).

Accumulated radiation dose was calculated on the basis of individual TL-dose records and multiplied with exposure time. All measurements were performed by calibrated TLD typeCaF_2: Mn.

Index of lipid peroxidation (ILP) was determined according to the method of Andreeva et al. (15) after stimulation of lipid peroxidation *in vitro* with Fe-salts. Thiobarbituric acid reacted with MDA, which originated from polyunsaturated fatty acids in the process of lipid peroxidation and formed a colored complex. Concentration of generated MDA was measured by spectrophotometer at 533 nm. Half of each sample were put in a sterile plastic test-tube placed in a Plexiglas container 15x15 cm, and irradiated by ^{60}Co source of γ-ray at room temperature. Employed radiation dose was 2 Gy, dose-rate 0.45 Gy/min, and distance from the source 74 cm. All blood samples were frozen at -70˚C and kept till analyses, which were performed at the same time.

All the values were presented as the mean value ± standard deviation. Mann-Whitney, U, and Kruskal Wallis tests were used nonparametric tests, with $p<0.05$ considered statistically significant. The correlations between dependent values (before and after irradiation) were evaluated by Wilcoxon test. Correlations between MDA values and radiation doses, age and smoking habit were evaluated by regression analysis.

RESULTS

Personal dosimetry results

Three subsequent years personal dosimetry results were analyzed and used to estimate mean doses. Mean annual radiation doses are presented in Table 1.

Table 1. Mean annual radiation doses

Dose	Ex($\bar{X}$±SD)	Range	En($\bar{X}$±SD)	Range	p
D1	3.31±3.27	0.100-11.200	0.75±0.66	0.100-2.360	p=0.001
D2	3.14±3.25	0.060-10.700	0.72±0.59	0.200-2.230	p=0.030
D3	2.15±2.17	0.020-7.560	0.65±0.49	0.140-1.910	p=0.025
Dmean	2.87±2.78	0.150-9.203	0.71±0.56	0.147-2.167	p=0.030

Mean occupational exposure time in subgroup Ex was 15.00(5.97) years (range: 5-27 years), and in subgroup En was 14.92(5.21) years (range: 5-23 years), which is not significant (p=0.94). The mean cumulative doses of subgroups Ex and En were significantly different (p=0.0028).

MDA production

The values of MDA production are presented in Figure 1. Observed values were 2.32-16.92 nMol MDA/ml, with mean value of 8.08(3.36) nMol MDA/ml.

The mean values before and after irradiation are presented in Figure 2. In our groups K and E, and subgroups Ex and En mean values before irradiation were 6.21(2.67), 9.49(3.16), 9.67(3.10), and 9.01(3.41) nMol MDA/ml, respectively.

Our results confirmed significantly higher lipid peroxidation index (higher MDA production) in workers occupationally exposed to LD of ionizing radiation ($p<0.0001$). The difference between controls (K) and subgroups was statistically significant too (p=0.0001 for Ex and p=0.018 for En), while it was not significant between subgroups (p=0.54).

The values of MDA_0 production after irradiation are presented in Figure 3.

Observed values were between 5.33 and 61.36 nMol MDA/ml, with mean value of 23.63(14.80) nMol MDA/ml. Our results showed the lack of statistically significant difference in MDAo production between occupationally exposed workers and controls (p=0.41), as well as between controls (K) and subgroups (p=0.41 for Ex and p=0.96 for En), and between subgroups Ex and En (p=0.68).

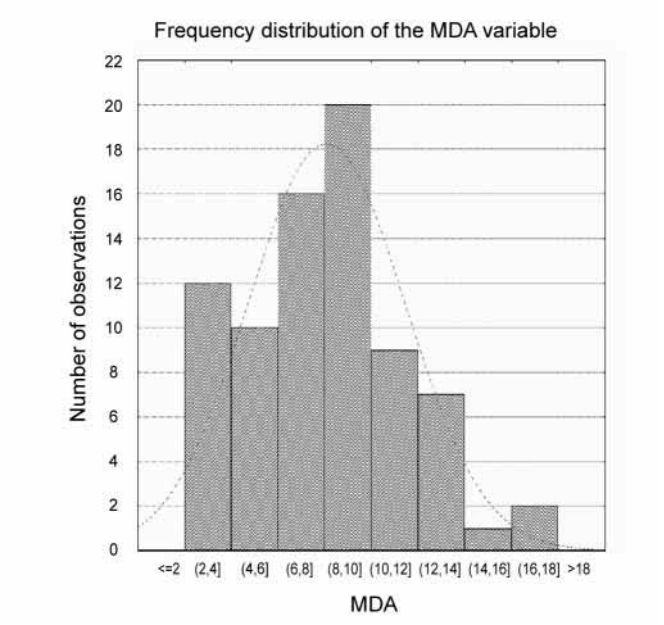

Figure 1. Comparison of mean values in variable MDA/MDA_0

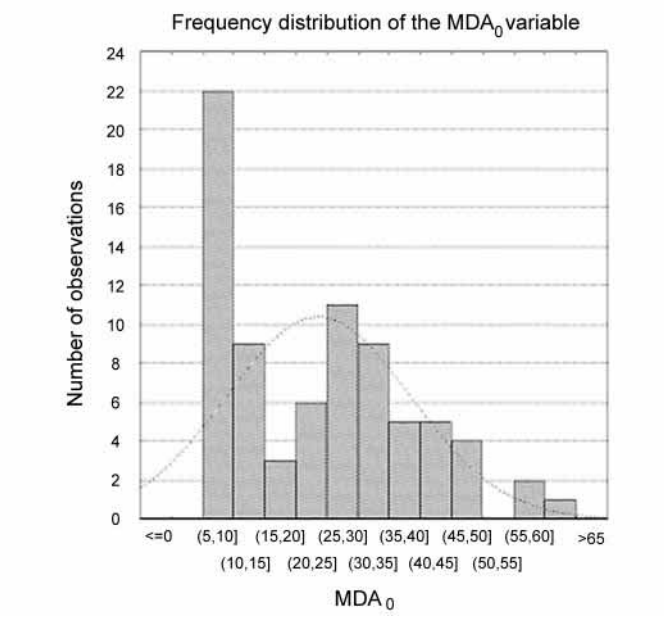

Figure 2. Frequency distribution of the MDA_0 variable

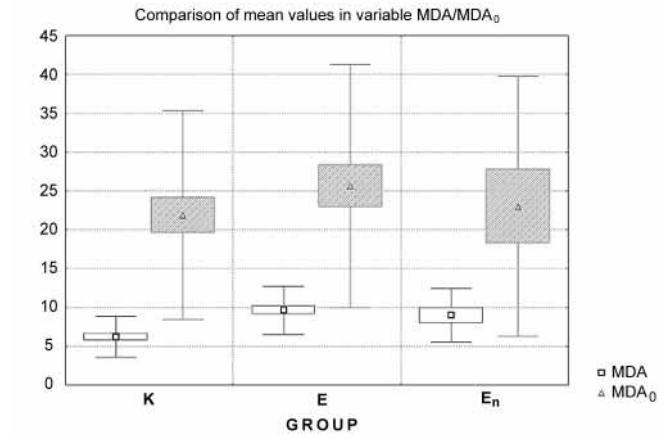

Figure 3. Frequency distribution of the MDA variable

The difference between dependent values (MDA and MDA_0) was statistically significant for the whole group ($p<0.0001$), for the controls ($p<0.0001$), exposed group ($p<0.0001$), and subgroup Ex ($p<0.0001$), but not significant for subgroup En ($p=0.099$).

DISCUSSION

Oxygen free radicals can damage many cellular structures and affect human health under ordinary conditions (1,14,15). Expose to ionizing radiation results in overproduction of oxygen derived free radicals (12,13,17).

Petkau (13) showed that production of oxygen free radicals is negatively correlated with dose rate. Considering the DNA as the most important and by DNA-repair mechanisms highly protected molecule of the cell, Petkau concluded that health effects of LD of ionizing radiation are the result of cell membrane damage, as initiating factor in cell damage. The damage of cell membrane disturbs the first DNA-repair mechanism, i.e., mitotic delay. In his experiments, Petkau showed that lipid peroxidation is produced even by normal background radiation (dose rate of 0.18 μGy/h) and after less then 100 h (total dose of 19 μGy) an increase in lipid peroxidation could be measured (6,13,14). Our results confirmed significantly higher MDA production in workers occupationally exposed to LD of ionizing radiation versus unexposed. At the same time, MDA production was not correlated with low-dose range and the type of ionizing radiation (X ray or gamma ray).

Production of MDA was highly correlated with age and smoking habit of our examinees. Considering the fact that our groups were matched by many different criteria (age, smoking habit, etc.) we concluded that further differences could be correlated with exposure to LD of ionizing radiation.

After irradiation, MDA production significantly increased in all groups, which could be a consequence of direct effect and/or insufficiency of antioxidative defense (2,12).

Although MDA is a naturally occurring product of LP, it appears to be both mutagenic and carcinogenic (18). LD of ionizing radiation could also initiate biochemical reactions at the cell surface, resulting in the production of the second messengers as ceramide, which triggers apoptosis, or play a role in cell adaptive response to irradiation (2,3,17). Finally, cell membrane damage induced by LP is the molecular basis for disturbance of signal transduction, gene expression and regulation of cell functions involved in apoptosis, adaptation and/or genomic instability which are closely correlated to cancerogenesis (17,19).

Our results suggest that occupational exposure to ionizing radiation could induce severe cell membrane damage, which is not inversely correlated with dose. Decomposition products of lipid peroxidation, such as MDA, possess carcinogenic properties. According to present knowledge, the aging process and cancerogenesis are coupled to lipid peroxidation process, too. DNA adducts, induced by MDA are correlated with malignancy, but still uninvestigated how. Cell membrane damage could be the first step in explanation the link between low-level radiation and cancer.

We can assume that lipid peroxidation index, as easy, quick and not expensive test should be considered as a marker of radiation exposure and triage parameter before further cytogenetic studies in workers chronically exposed to low-dose radiation.

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