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EXPRESSION OF CELLULAR ISOFORM OF PRION PROTEIN ON THE SURFACE OF PERIPHERAL BLOOD LYMPHOCYTES AMONG WOMEN EXPOSED TO LOW DOSES OF IONIZING RADIATION

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Abstract: Ionizing radiation affects the expression of adhesive and co-stimulatory molecules in lymphocytes. The physiological function of cellular isoform of prion protein (PrP^c) is little known. Evidences indicate a link between lymphocytes activation and PrPc expression on their surface; however, no direct effect of radiation on PrP^c level in these cells was investigated. The objective of this study was to determinate the effect of low doses of ionizing radiation on the expression of PrP^c on the surface peripheral blood lymphocytes in the women operating X-ray equipment. In 36 female workers and 30 persons of the control group the PrP^c expression on CD3 (T lymphocytes), CD4 (T helper), CD8 (T cytotoxic) and CD19 (B lymphocytes), as well as the percentage of lymphocytes with PrP^c on their surface, were tested. Subgroups with respect to age and length of employment were selected. A significant increase was observed in PrP^c expression on CD3 and CD4 with lowered PrP^c level on CD8 and percentage of CD8 cells with PrP^c in workers compared to control. The PrP^c level did not show significant changes in subgroups in relation to age (below and over 40 years old) both in the investigated and control groups, whereas a lower percentage of PrP^c expressing CD19 cells showed in employed women below 40 years of age. A significant decrease was found in PrP^c expression on the surface of CD3, CD4 and CD8 cells in the subgroup employed for over 10 years than in the subgroup with less than 10 years of employment.

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INTRODUCTION

The normal prion protein (PrP^c) and its confirmatory changed isoform PrP^{Sc} is chiefly involved in the transmissible spongiform encephalopaties [23]. The physiological PrP^c is expressed at a high level in nervous tissues, but it is also detectable at a lower level in most other tissues and cells, including peripheral blood cells. PrP^c is detected on the surface of human T and B lymphocytes, monocytes/macrophages, dendritic cells, natural killers and on CD34 bone marrow stem cells [3, 4, 5]. The virtual absence or its very low expression on the granulocyte surface was revealed [4, 9]. The observations indicate that the level of PrP^c expression on lymphocytes increases after polyclonal stimulation with lecithin or mitogenic antibodies. On the other hand, proliferation of human T cells stimulated

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with an anti-CD3 antibody or concanavalin A was inhibited by anti-PrP^c antibodies, suggesting a role of PrP^c in signal transduction in these cells [3, 17]. It was also suggested that PrP^c is a component of the multicellular-signaling complex in the T cell activation associated with phosphorylation protein ZAP-70 [1, 12, 20]. The physiological function PrP^c is little known. It has been reported that PrP^c possesses super oxide dismutase-like activity and may be associated with cellular stress response [2, 14, 16].

Technicians operating X-ray machinery are occupationally more sensitively exposured to long-term low level of ionizing radiation, which may cause alternations in the function of the immune system. Among white blood cells the highest sensitivity to X-ray irradiation was shown by lymphocytes [10]. The destructive influence of irradiation on technicians in X-ray departments revealed an increase of chromosomal aberrations in lymphocytes that were associated with cumulative occupational doses [8]. Additionally, ionizing radiation caused abnormalities in composition and function of lymphocytes as well as changes in the lysosomal membrane of lymphocytes, functional TCR (T cell receptor), lymphocyte kinase activity among others [13, 15, 21, 26, 27]. Knowledge concerning the influence of X-ray radiation on PrP^c expression on lymphocytes is scanty.

Therefore, the objective of our study was to assess the possible influence of low doses of ionizing radiation on the cellular prion expression on the surface of peripheral blood lymphocytes in women operating X-ray machinery.

MATERIAL AND METHODS

The study group covered 36 women aged 25-54 years (average 42.8) operating X-ray equipment. The period of employment ranged 2-33 years (average length 16.4). Eight women aged 25-54 years (mean 42) smoked cigarettes (19.1 pack-years). An annual effective dose of X-radiation was below 1 mSv. The control group, similar to the exposed group, included 30 clinically healthy women aged 26-52 years (mean 38.7), not employed in radiology and never occupationally exposured to ionizing radiation. Ten women aged 28-50 years (mean 40) smoked cigarettes (17.2 pack-years). All participants were subjected to medical examination and passed the basic haematological and biochemical parameters to assess their state of health. No deviations in the basic laboratory test, no infections during the month before the study, nor any acute or chronic diseases were found. Their economic and social state and place of residence was similar.

The list of the evaluated laboratory parameters included expression of PrP^c on the surface of the following cells: T lymphocytes (CD3), T helper (CD4), T cytotoxic (CD8) and B lymphocytes (CD19) and percentage of lymphocytes (CD3, CD4, CD8, CD19) expressing PrP^c was calculated.

Blood samples from the antecubital vein were collected in test tubes with EDTA; later, peripheral blood lymphocytes were isolated from whole blood using Ficoll Paque (Pharmacia, Sweden). The resulting layer was polled, washed 3 times with phosphate buffered saline (PBS) with 2% calf fetal serum (CFS). Cells were counted and prepared to the final level of 5,000/mm^3 in 0.3 ml PBS. To block non-specific binding the cells were incubated for 30 minutes at 4ºC in PBS with 10% rabbit serum, and washed 3 times. Thereafter, mouse monoclonal anti-prion 3F4 antibody (0.00005 mg per tube) was added. After incubation (30 minutes at 4ºC) and washed 3 times with 3F4 antibody, followed by R-Phycoerythrin-Cy5 (RPE-Cy5)-conjugated $F(ab')_2$ fragment of rabbit anti-mouse immunoglobulin (0.0027 mg per tube). After incubation (30 minutes at 4ºC) cells were washed 3 times in PBS with 2% CFS. Phenotypes of lymphocytes were stained with specific antibodies for CD3 (FITC), CD4 (RPE), CD8 (FITC), and CD19 (RPE) (FITC – fluorescein, RPE- R-phycoerythrin). The prion expression was measured as median immunofluorescence RPE-Cy5 intensity in lymphocyte populations. Cells were analysed by multiparametr flow cytometry with 3 color analyses. The negative control mouse FITC/RPE was used. All antibodies were purchased from DakoCytomation (Denmark). After staining, analyses were performed on a FACScan (Becton Dickinson, San Jose, CA, USA) equipped with an Argon laser tuned to 480 nm. Samples were first analysed conventionally, and using the same material, life gates were then set at appropriate fluorescence vs SSC correlations and gated events acquired to 10,000 events. All data were analysed using Cell Quest software (Becton Dickinson).

The results were compared in the following manner:

A. Between the total group of employed women (36 persons) and the control group (30 persons).

B. Between the subgroups of women exposed to radiation <40 years old (16 persons) and >40 years old (20 persons).

C. Between the subgroup of 18 women employed <10 years and the subgroup of 18 women employed >10 years.

D. Between the subgroup of control <40 years old (16 women) and the subgroup of control >40 years old (14 women).

The Gaussian composition of results to normal distribution analysed by using Shapiro-Wilk tests and then the arithmetic means ($\bar{x}$) and the standard deviations (SD) were calculated. The data were estimated by Kruskal-Wallis test. P values of < 0.05 were taken as significant. The study was approved by Ethics Commission of the Medical University of Silesia.

RESULTS

The cellular prion expression was significantly higher on the surface of CD3 and CD4 cells in female workers, whereas CD8 lymphocytes showed a decrease of this parameter. The comparison of PrP^c level on examined lymphocytes with respect to age (<40> years old) in both the

Table 1. Expression of prion on the surface of lymphocyte subpopulations.

Investigated group/subgroup	CD3 $\bar{x}$ ± SD	CD4 $\bar{x}$ ± SD	CD8 $\bar{x}$ ± SD	CD19 $\bar{x}$±SD
Female workers (n=36)	123.3 ± 87.8[a]	95.6 ± 35.0[a]	128.2 ± 65.2[a]	88.8 ± 77.7
Control group (n=30)	107.0 ± 34.3	85.7 ± 62.7	163.9 ± 51.4	103.2 ± 48.8
Female workers < 40 years old (n=16)	130.4 ± 90.0	104.1 ± 67.1	145.9 ± 69.9	107.0 ± 76.0
Female workers > 40 years old (n=20)	91.4 ± 86.4	71.1 ± 56.4	113.9 ± 59.2	73.4 ± 84.3
Control < 40 years old (n=16)	100.2 ± 38.5	86.2 ± 35.5	153.6 ± 54.5	103.1 ± 54.0
Control > 40 years old (n=14)	116.8 ± 27.6	106.5 ± 32.3	175.7 ± 46.7	103.4 ± 44.0
Women employed < 10 years (n=18)	121.8 ± 74.4[a]	105.4 ± 45.4[a]	158.3 ± 51.8[a]	93.7 ± 43.7
Women employed > 10 years (n=18)	76.3 ± 72.5	59.8 ± 47.5	102.5 ± 52.0	73.0 ± 85.1

[a] – statistically significant

Table 2. Percentage of lymphocytes expressing prion protein.

Investigated group/subgroup	CD3 $\bar{x}$ ± SD	CD4 $\bar{x}$ ± SD	CD8 $\bar{x}$ ± SD	CD19 $\bar{x}$ ± SD
Female workers (n=36)	81.7 ± 14.4	75.6 ± 19.1	81.4 ± 12.9[a]	74.2 ± 13.1
Control group (n=30)	83.5 ± 9.2	77.4 ± 12.2	88.4 ± 10.8	79.4 ± 14.6
Female workers < 40 years old (n=16)	83.0 ± 14.3	74.5 ± 21.9	83.6 ± 13.1	71.2 ± 13.5[a]
Female workers > 40 years old (n=20)	82.9 ± 15.1	80.1 ± 17.3	85.0 ± 11.7	78.2 ± 13.6
Control < 40 years old (n=16)	86.7 ± 7.1	78.3 ± 7.6	89.9 ± 8.4	80.0 ± 15.6
Control > 40 years old (n=14)	81.6 ± 10.6	72.5 ± 16.4	85.8 ± 13.7	77.9 ± 18.2
Women employed < 10 years (n=18)	86.5 ± 14.1	77.1 ± 23.3	86.5 ± 14.2	73.2 ± 15.2
Women employed < 10 years (n=18)	81.3 ± 14.8	76.8 ± 17.5	83.8 ± 11.0	77.4 ± 13.3

[a] – statistically significant

group exposed to radiation and the control group did not show significant differences. However, significant lower PrP^c expression was found in women employed >10 years than in those employed <10 years (Tab. 1). Percent of lymphocytes with PrP^c expression was significantly lower with regard to CD8 lymphocytes in the radiology institute workers as compared to control and the employed subgroup <40 years with respect to the >40 years exposed women in relation to CD19 cells (Tab. 2).

DISCUSSION

The study showed a statistically significant higher expression of PrP^c on the surface of CD3 and CD4 with additionally lower expression on CD8 cells, as well as percentage of lymphocytes expressing PrP^c in workers compared to the control group. It has been reported that low doses of ionizing radiation enhance or diminish expression of adhesion, cluster of differentiation and co-stimulatory molecules, as well as intracellular signalling pathways in lymphocytes, stromal and endothelial cells [6, 11, 18, 24]. The higher level of PrP^c on CD3 and CD4 lymphocytes may be a consequence of protective function against oxidative stress [12, 14, 20] or activation of these cells [11, 24]. Low radiation in mouse T cells and thymocytes also up-regulated TCR, CD3, CD4, CD8 and CD28 molecules [11, 18, 19]. The lower PrP^c expression on CD8 cells and their percentage is probably associated with a higher sensitivity to radiation because CD8 population is more radiosensitive than CD4 [7, 25] and CD8 possesses a higher PrP^c level than CD4 or CD19 cells [5]. This may indicate an association with exposure to low doses of radiation and differences in sensitivity to radiation.

The PrP^c level on lymphocytes and their percentages expressing PrP^c in the subgroups <40 and >40 years aged in the workers, as well as in the control group, show significant changes only in relation to a lowered percentage of B cells with PrP^c expression in the subgroup of female workers <40 years old. This seems to indicate that these tested parameters, with exception of B cells, are not connected with age, neither in the women operating X-ray machinery nor in the control subgroups. Politopoulou *et al.* [22] showed an age-related increase of PrP^c expression in the total lymphocyte population; however a significantly higher PrP^c level was observed when a group of children (mean age 6 years) and adults (mean age 33 years) were compared to the older population (mean age 66 years). A relationship between lower PrP^c expression on the surface of CD3, CD4 and CD8 cells and the length of period of employment was revealed. Moreover, workers >40 years old demonstrated a slightly lower PrP^c expression in comparison to workers <40 years of age, whereas respective

control subgroups revealed slightly higher PrP^c levels. The above data may indicate that the length of employment period affects expression of PrP^c on these cells. It is difficult to assess if age related with the decreased PrP^c level may influence the function of investigated cells in women exposured to low levels of ionizing radiation, but it seems that a reduced response to oxidative stress should be taken into consideration due to possible protective mechanisms of PrP^c against stressful conditions [12, 14].

CONCLUSIONS

We conclude that in women operating X-ray equipment the relationship between low doses of ionizing radiation and expression of PrP^c on lymphocytes does exist concerning CD3, CD4 and CD8 lymphocytes. Moreover, the length of employment period reduced PrP^c expression on the surface of these cells. The meaning of these findings is unknown and requires further studies.

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