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Effect of the Ionizing Radiation Dose on Histone H2AX

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ABSTRACT

In case the radiation dosimeters are not available, biological dosimetry represents an important method to estimate the absorbed dose of the exposed individuals during nuclear events. Nevertheless, the eukaryotic DNA is constantly exposed to exogenous and endogenous factors. Apart from the ionizing radiation, widescale DNA lesions are also induced by other harmful effects. DNA double-strand breaks (DSBs) are the gravest lesions. DSBs provoke an extensive reaction characterized by the expression of the H2AX molecule. The scope of this work is an assessment of a gamma radiation dose-effect on a human body in terms of expression of the H2AX in DNA.

This contribution is focused on the expression scale of the phosphorylated H2AX molecule (γ-H2AX), which highlights a DNA damage induced by the exposure to gamma radiation. The dependency between the share of γ-H2AX molecule in an irradiated sample and the radiation dose was examined. The investigated subjects consist of fourteen samples of uncoagulable blood from healthy donors. The sample of each donor was divided into four test tubes – a negative control + three levels of gamma radiation (0.5 Gray, 1 Gray, 2 Gray). The irradiation was performed on a medical caesium source "Gammacell® 1000 Elite." The evaluation was based on the method for determining γ-H2AX after chemical stimulation DNA – extracorporeal photopheresis.

The outcome of this work is the confirmation that the production of this molecule is dependent on the dose of gamma radiation. Owing to the γ-H2AX characteristic, the finding of the relation between the share of γ-H2AX molecule in a sample and a dose of radiation was statistically confirmed. Accordingly, the H2AX molecule can be considered a reliable specific marker for DNA damage. In the future, this method could find a purpose in practical events, for example, re-determination dose of radiation after nuclear events.

1 INTRODUCTION

This study with the title "Effect of the Ionizing Radiation Dose on Histone H2AX" deals with assessing the specific marker that refers to DNA damage caused by the ionizing radiation. This marker called γ-H2AX is induced in reaction to the gravest damage of DNA – double-strand breaks (DSBs).

The main aim of this study was the evaluation of gamma radiation effect on human cells. The expected goal was the determination of the dependency between the expression of the γ-H2AX molecule and the dose of gamma radiation. The part-goals were to find an appropriate methodology of samples preparation and results interpretation.

2 γ-H2AX

DNA in the form of chromatin is stored in the cell nucleus. Structure of the chromatin is based on nucleosomes, the basic building units. Each nucleosome has a structure of octamer, and it is composed of eight subunits – histones. Around this octamer, the DNA double helix is spooled. All this is a very stable complex, shown in Figure 1: Structure of nucleosomeFigure 1 [1][2].

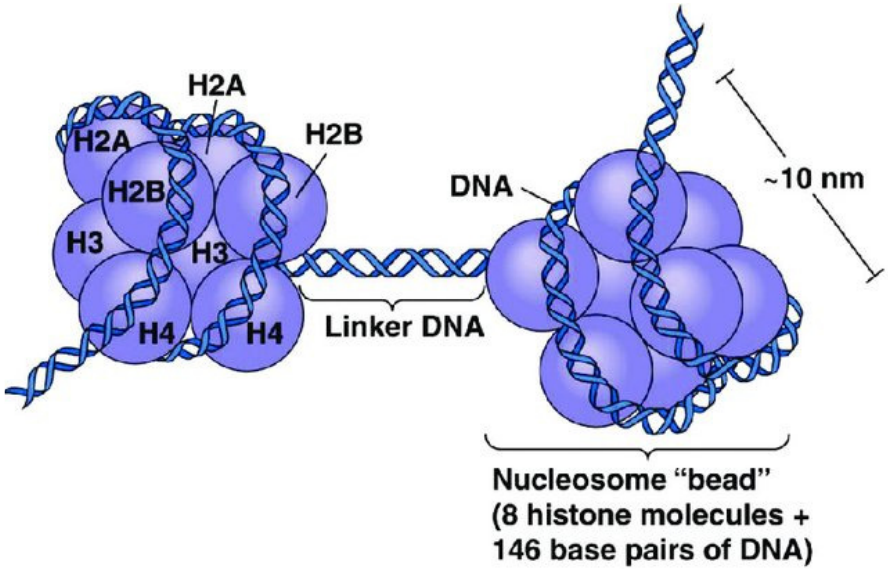

Figure 1: Structure of nucleosome [3]

The H2A histone family has several isoforms – H2A1, H2A2, H2AX, and H2AZ. The H2AX plays an essential role in repairing the DNA DSBs. It can also be classified as a tumour marker [4].

The eucaryotic DNA is constantly exposed to the effects of the environment that cause its damages. The damage may arise by the effects of exogenous factors, for example, various types of radiation. An example of the endogenous factors represents faults in DNA formed during DNA replication. These events can generate a wide scale of the DNA lesions. Of all the DNA damages, DSBs are the gravest. The DSBs represent a significant intervention in the DNA integrity because the DNA "spinal" is fragmented [5][6].

After a series of cascading cells reactions, the histone H2AX becomes a phosphorylated form (γ-H2AX). Phosphorylation of molecule means the connection of a phosphoryl group. Therefore, the cells developed several repairing mechanisms. The repair mechanisms respond to the formed γ-H2AX fields [7].

3 METHODOLOGY

From the uncoagulable blood samples of 14 healthy donors, the part of blood cells (mononuclear cell fraction) was separated. The mononuclear cell fractions were separated by density gradient centrifugation. Subsequently, the prepared cells were left in the thermostat for

24 hours at 37 °C for their cultivation and growth. After that time, the cells of each sample were divided into four tubes and prepared for irradiation.

Three tubes were exposed to gamma radiation of intensity of 0.5 Gray, 1 Gray, and 2 Gray. The last tube was used as a negative control. The irradiation was performed on a medical caesium source "Gammacell® 1000 Elite." This apparatus is regularly controlled and calibrated by a technician.

These irradiated samples were left in the thermostat for 24 hours at 37 °C again. Then, the cells were marked by the appropriate intracellular antibodies. The antibodies bind the formed γ-H2AX molecules. The final suspension was analysed by flow cytometry (FC).

Before the final analysis, the test measuring the γ-H2AX molecules expression 24, 48, and 72 hours after the irradiation was carried out. The results confirmed the highest γ-H2AX molecule expression 24 hours after irradiation, subsequently used for all the samples. The reduction of the expression after a more extended time could be caused by repairing ways of the DNA or by the death of the cells after exposure to gamma radiation.

4 EVALUATION

The raw data as an output of the measurement of the FC was analysed in the programme "Kaluza Analysis 2.1." Needed data were obtained by using a "gating strategy," see Figure 2 and Figure 3. This step was an essential part of the methodology for the data gaining and evaluating the expression of the γ-H2AX molecule.

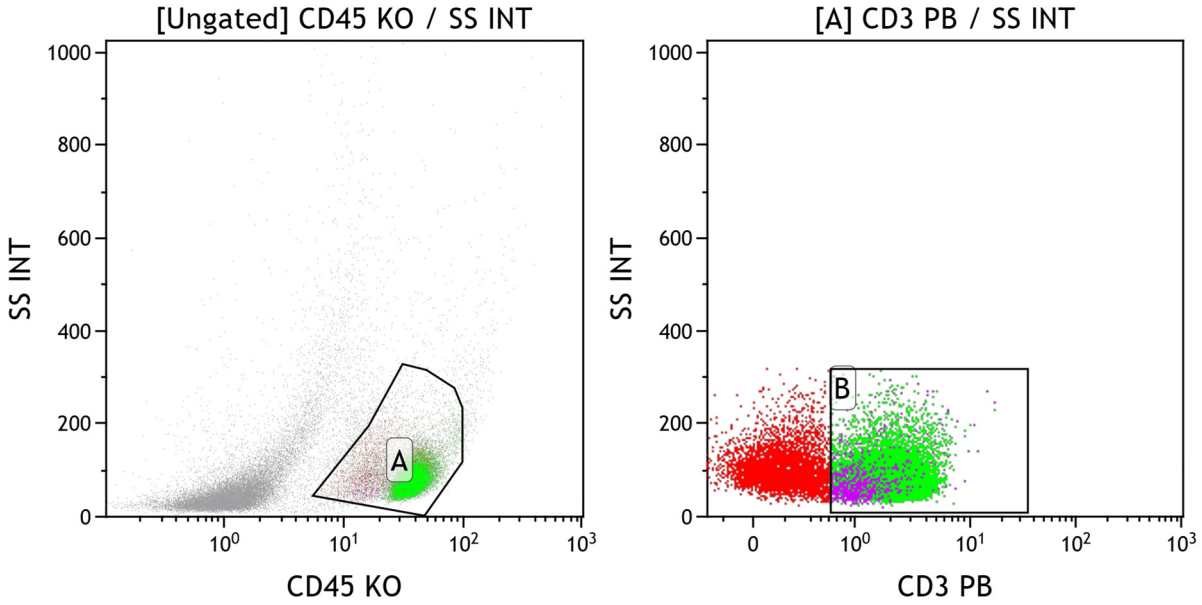

Figure 2: Gating strategy from FC analysis

Figure 2: Cells have on their surface protein markers that can be detected by specific antibodies conjugated with fluorophores. Each coloured dot represents a single cell. Gate A shows a CD45-positive lymphocytes' marker. Gate B describes a CD3-positive T-lymphocytes' marker. Other white blood cell types like monocytes and NK cells were not measured.

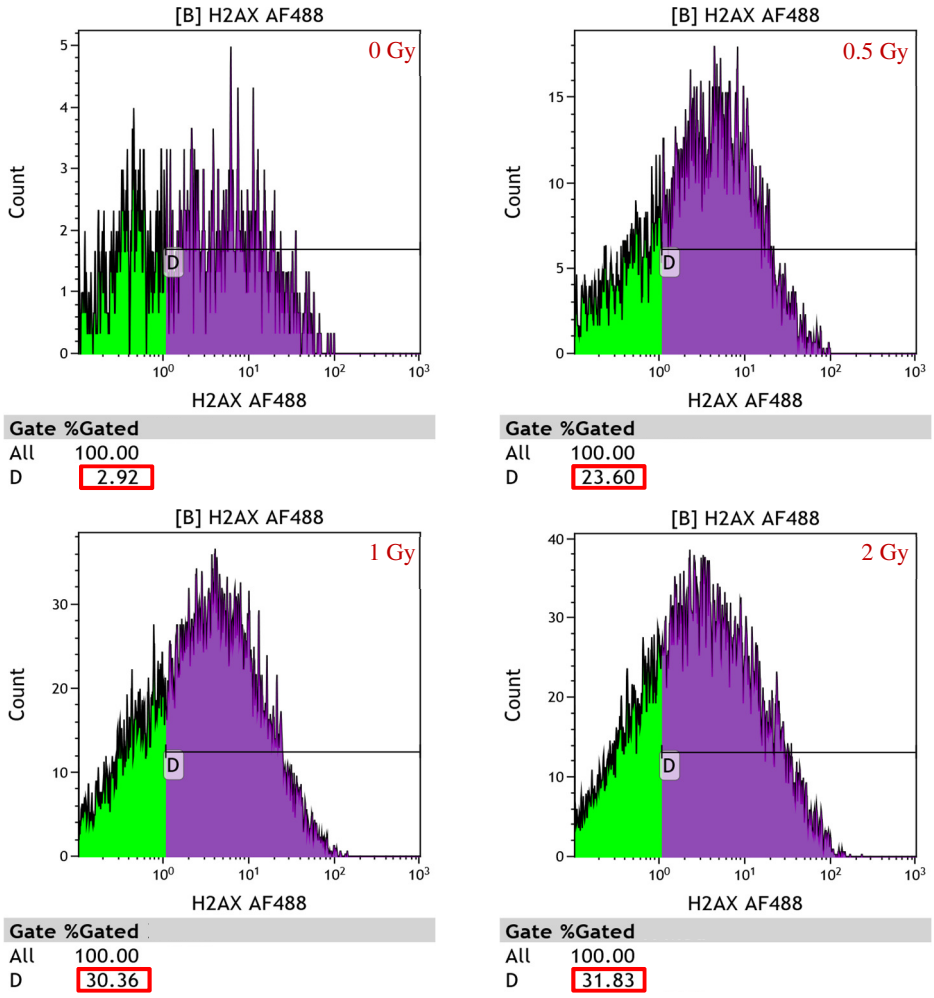

Figure 3: Expression of the γ-H2AX analysis

Figure 3: Line D describes a percentage of DNA damaged T-cells. Histograms represent an increasing expression of the γ-H2AX with an increasing dose of the gamma radiation.

5 RESULTS

For analysis, there were 14 blood samples from which the mononuclear cell fractions were separated. Each fraction was divided into four samples (a negative control, 0.5 Gray, 1 Gray, 2 Gray); thus, the 56 final results were obtained. The results represent the percentage expression of the γ-H2AX molecules from all the cells in the test tube. Because of very different numbers of each blood sample, caused by various donor's age, sex, and lifestyle, the medians were determined for the evaluation of the results, as shown in Table 1.

Table 1: Medians of the percentage expression of γ-H2AX

	0 Gray [%]	0.5 Gray [%]	1 Gray [%]	2 Gray [%]
Median	6.315	27.875	31.795	31.850

These results were statistically evaluated in the programme "GraphPad Prism 9". After this evaluation, the Wilcoxon test, which monitors differences between medians, was applied.

Figure 4 shows a range of the results and the medians. A significant statistical difference was between the median of the negative control (0 Gray) and the sample irradiated with 0.5 Gray. A value of probability, calculated from the Wilcoxon test, confirms this statistical significance.

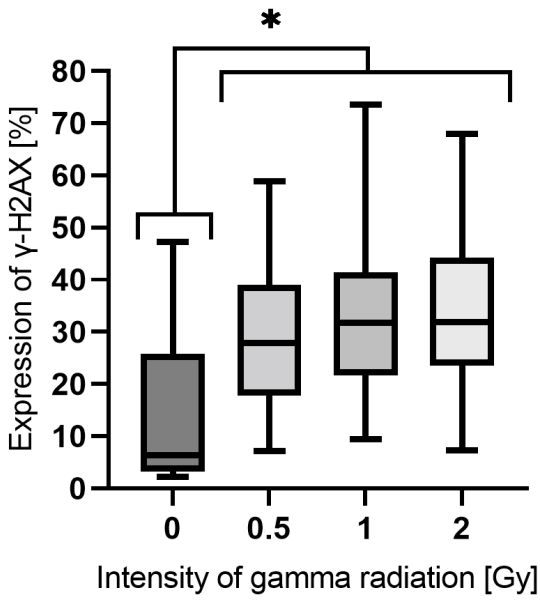

Figure 4: Boxplot of γ-H2AX expression

Figure 4: Boxplot describes γ-H2AX expressions for each group (n = 14). A significant difference in expression values was calculated with the Wilcoxon test between the median of the control sample and all irradiated samples (p-value < 0.05). A significant difference between irradiated samples was not detected.*

There are also noticeable statistical differences between the rest of the samples with higher doses of irradiation, but these differences are not statistically significant due to a low total count of the samples. If more than 14 blood samples were available and analysed, the differences could already be more significant.

Figure 5 shows a relation between the γ-H2AX expression medians and the doses of irradiation. It can be seen that with the higher dose of gamma radiation, the median increases. For the higher irradiation doses, the increase is becoming very slow (statistically irrelevant), and the line is nearly invariable. This is primarily important for future research, which will be focused on the lower dose rates between 0 Gray and 0.5 Gray.

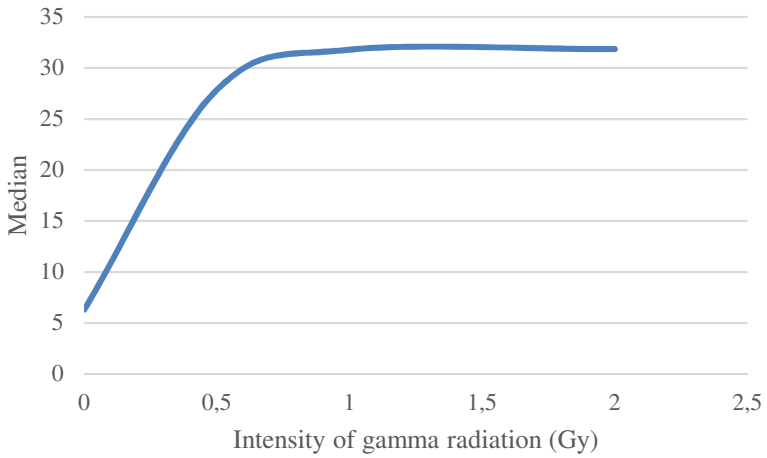

Figure 5: Dependency of the γ-H2AX expression on the dose level

6 PREVIOUS H2AX RESEARCH

Some reports introduced that the peak of the γ-H2AX formation can be seen at one hour after X-ray exposure. Then the number of foci decreases within 24 h after the induction of DNA damage. γ-H2AX kinetics is changing; therefore, it is hard to detect the original number of γ-H2AX foci [8]. To avoid this underestimation, the phosphatase inhibitor calyculin A can be used. Calyculin A reportedly inhibited the decrease in γ-H2AX foci in irradiated cells [9].

Data from 2010 suggest that the cellular response to DSBs is substantially different for low vs. high radiation doses. The study found that the cellular response might depend on the concentration of H_2O_2 [10].

7 CONCLUSION

The phosphorylated form of the H2AX molecule is easily detectable with the specific antibodies and by the flow cytometry. The γ-H2AX molecule, therefore, is a reliable specific marker DSBs. Thanks to its nature, the investigated dependency between the percentage expression of γ-H2AX and the irradiation dose was statistically confirmed. The initial method, "extracorporeal photopheresis," seems appropriate.

In further studies, research might focus on the measuring of the expression of γ-H2AX during the lower dose range exposure (below 0.5 Gray). It might also be appropriate to analyse data in a shorter time after the exposure samples to gamma radiation (in the order of tens of minutes). This could help to determine the expression of the γ-H2AX time dynamics.

In the future, this method could find a purpose in practical events, for example, re-determination dose of radiation after nuclear events.

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