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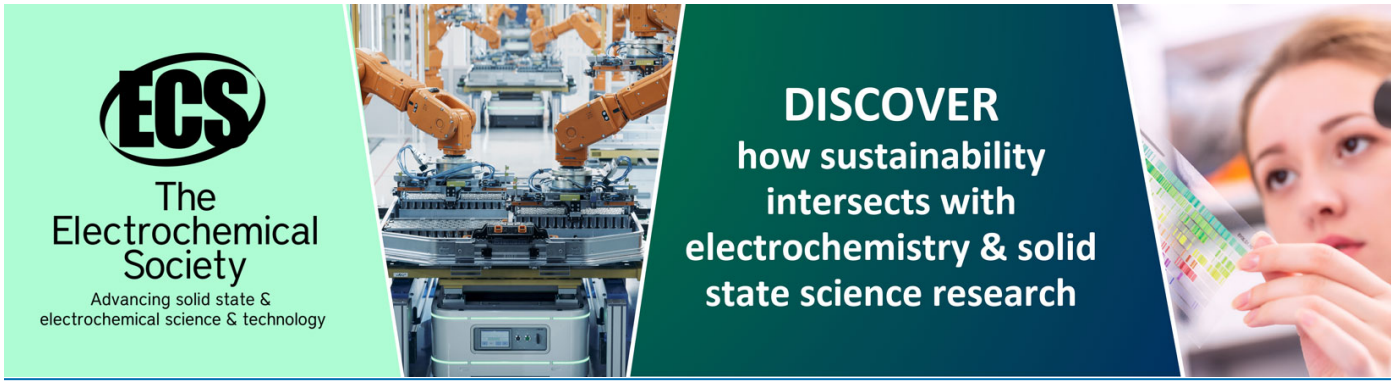

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Comparison of DNA damage level in irradiated blood cells of mouse, frog and human

I Y Mitroshina, N P Sirota and E A Kuznetsova

Institute of Theoretical and Experimental Biophysics of the Russian Academy of Science, Moscow Region, Institutskaya st. 3, 142290 Pushchino Russia

E-mail: xf2@rambler.ru

Abstract. Here, we present the comparative studies of DNA damage induced by X-ray irradiation in vitro in mouse, frog and human blood cells and assessed by the "comet test" (alkaline version). We showed that, in contrast to mouse and human cells, DNA repair processes in frog blood cells are not completed by 60 minutes of the post-radiation period. The results suggest that frogs can be used as indicators of environmental radiation pollution. In this regard, additional experiments are needed to assess the different types of external and internal exposure of animals.

1. Introduction

Studying the effects of ionizing radiation (IR) on living organisms remains important, because there is always a possibility of radiation exposure when using radioactive materials for industrial and medical purposes as well as during radiation accidents. Fast and minimally invasive methods are required for an individual retrospective assessment of the radiation response in the body. The assessment is carried out mainly using hematological and cytogenetic methods. The analysis of chromosome aberrations remains the "gold standard" for early biodosimetry in case of accidents, but it is a time-consuming procedure [1]. Therefore, the study of the processes occurring in cells and tissues of living organisms after irradiation including the evaluation of DNA damage levels and the detection of sensitive objects of radiation exposure are in demand at the present time.

To determine the levels of DNA damage in individual cells from tissues of various organisms, including human leukocytes, the method "Comet test" is used [2, 3]. The alkaline version of the method makes it possible to reveal a wide range of DNA damage: single- and double-strand breaks, alkaline-labile sites and sites with damaged bases [4].

To assess environmental changes, comparative studies of DNA damage levels in cells obtained from organisms living in contaminated and background areas are needed. Currently, the whole blood has become one of the most useful objects for assessing the level of DNA damage by different methods, including the "Comet test" method. Blood in members of different taxons differs in blood cell types, and it is not known how this difference correlates with the levels of DNA damage in blood cells.

In this work, we carried out comparative studies of DNA damage levels in blood cells mice, frogs and humans after radiation exposure in vitro. Frog blood was taken for comparison with mouse and human blood, as frogs are considered as sensitive indicators for environmental pollution [5]. The level of DNA damage in the frog's blood cells was evaluated both immediately and 1 hour after irradiation.

2. Materials and Methods

2.1 Cells

In the course of the work, 10–15 μl of capillary blood of healthy donors aged 25–65 years (12 men, 8 women) were used. At the time of blood sampling, donors did not exacerbate infectious or chronic diseases. The human blood was selected for evacuators containing K-EDTA. Informed consent was obtained from each participant before the blood draw.

The blood of the frog was kindly presented by V.K. Uteshev. Whole blood samples of 15 male *Rana temporaria* aged 2–3 years used. The blood from decapitated frogs, 50 μl from each, was collected in the tube with 0.3 ml of cold PBS, 1 mM EDTA.

Mice blood was obtained by incising the tip of the tail of 20 male animals with a mass of 22–25 g. The noninbred SHK strain mice were obtained from the vivarium of the ITEB RAS. Blood samples were collected in tubes with phosphate buffered saline (PBS), pH 7.2, containing 1 mmol/L EDTA (PBS-EDTA). Splenocytes from mice were obtained according to [6]. The experiments were carried out according to the requirements set by the Institute Committee on Control for the use of animals in biomedical experiments.

Whole blood of mice and human was used, diluted with PBS-EDTA 6 times; frog blood and splenocyte suspensions were diluted with PBS-EDTA to 10^6 cells/ml.

2.2 Irradiation

Cells / nucleoids were exposed to X-ray radiation within agarose slides in RTD-250-15-1 X-ray unit (Russia) operated at 200 kV and of 20 mA. Dose rate was 1.12 Gy/min. Radiation was filtered through 1 mm Al and 1 mm Cu. The focal length was 37 cm. Immediately after irradiation, the slides were placed in a cooled lysis solution.

Part of the slides containing the frog erythrocytes after irradiation was placed in PBS or medium 199 (Gibco Lab). Next, the slides were incubated for 60 minutes at 37 °C.

2.3 Comet Assay

First, slides were placed in a solution of 1% agarose and dried. A 1% agarose layer was applied to these glasses and incubated in the refrigerator until it solidified (5–7 minutes). 1% low- melting agarose (Sigma Chem Co, USA) was melted at 70 °C in PBS-EDTA and incubated to 20–22 °C. The blood or cell suspension was mixed with an equal volume of the 1% low-melting agarose. 15 μl of this mixture was applied to the prepared agarose layer. The agarose layer with the cells was cooled until it solidified. A new layer of 0.5% low-melting agarose was applied over the cooled layer with the cells.
The slides were genotoxically exposed and placed in a lysis solution (2.5 mol / L NaCl, 0.1 mol / l EDTA, 0.01 mol/L Tris-HCl, pH 10, 1% Triton X-100) at 4 °C for 1 hour.Then the slides were exposed for 20 minutes in alkaline solution "A" (0.3 mol/L NaOH, 0.001 mol / l EDTA, pH> 13). After alkaline denaturation, the slides were moved to an electrophoretic chamber SE-1 / S-1N (OOO Helikon, Russia) and electrophoresis was carry out in a fresh portion of solution “A” for 20 minutes at 4 °C. The buffer volume was 250 ml, voltage 27 V, amperage 260-270 mA (electric field strength 2 V / cm). After electrophoresis, the slides were washed with distilled water and stained with ethidium bromide (2.0 μg / ml) for 1 hour. The preparations were analyzed using a fluorescent microscope LUMAM I-3 (LOMO, St. Petersburg, Russia). The image capturing was carried out by a photocamera Nikon CoolPix 995 (Japan). Received images transferred to the computer. The analysis of images was made by means of special software developed to register and analyze the images, where algorithms of computation of the standard comet parameters have been realized [7].

The level of DNA lesions was judged from the fraction of DNA in comet tails in percent − % TDNA. For each experimental point, at least 5 individual animals were taken, 3 slides for each one, 50–75 comets were scored per slide, according to recommendations [8]. The statistical analysis was performed by Student’s t-test ($p < 0.05$).

3. Results and Discussion

At the initial stage of the study, preliminary experiments were carried out in which the levels of DNA damage in irradiated cells and in nucleoids obtained from these cells were compared. Agarose slides containing mouse splenocytes were prepared, some of which underwent a lysis procedure for the preparation of nucleoids. After that, slides containing both whole cells and nucleoids were irradiated at 2, 4, 8 Gy, and the levels of DNA damage were measured. Figure 1 shows correlation between the level of DNA damage and the dose of X-ray irradiation in splenocyte nucleoids and whole splenocytes from a mouse irradiated at room temperature.

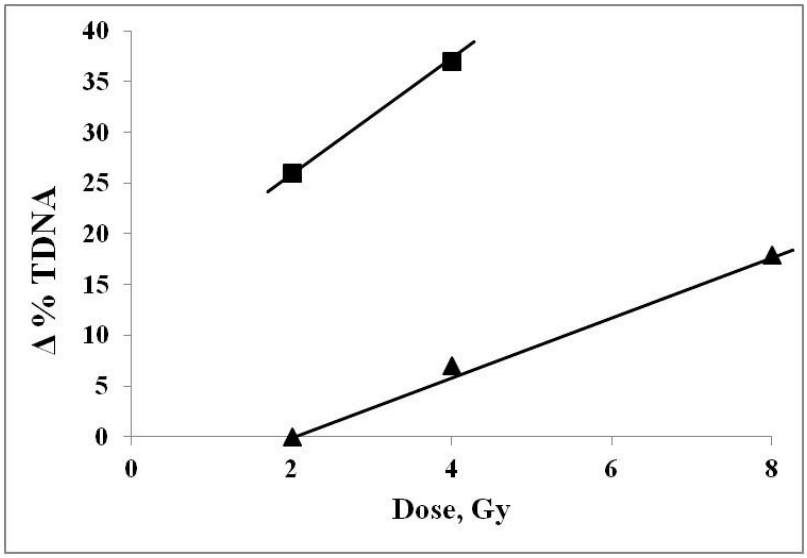

Figure 1. Dependence of the DNA damage level of mouse splenocyte nucleoids (■) and whole splenocytes (▲) on the irradiation dose.

The damage level is shown as the difference in % TDNA values between exposed and control samples. This figure shows that at the same dose, % TDNA in nucleoids is higher compared to splenocytes, what indicates active DNA repair in splenocytes. The possibility of DNA repair in the blood cells within the agarose slides was evaluated by incubating the slides in the PBS for 1 hour at 37 °C.

Figure 2 shows the change in % TDNA in human, mouse and frog blood cells after irradiation of these cells at a dose of 4 Gy at room temperature and subsequent incubation in the PBS at 37 °C for 60 minutes.

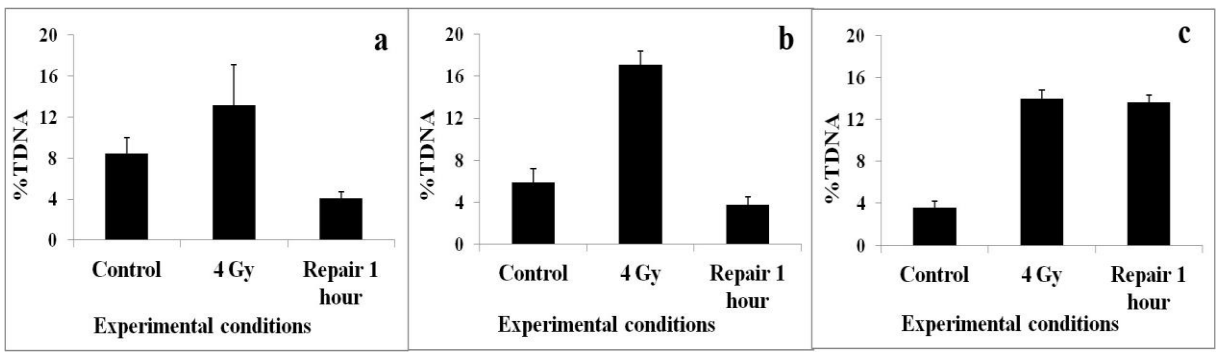

Figure 2. The level of DNA damages of nucleated cells of peripheral blood of a human (a), mouse (b) and frog (c) after irradiation at a dose of 4 Gy and incubation for 60 minutes (M ± SEM)

It can be seen that the level of DNA damage in control cells of a human, mouse and frog does not exceed 10%. This basic level is due to both: the functioning / metabolism of DNA and the continuous emergence of DNA lesions, including oxidative modifications, and their repair [9]. After exposure to X-rays at a dose of 4 Gy %TDNA increased in the cells of all organisms and it differed from the basic level significantly. After 60 minutes incubation, it significantly decreased only in mouse and human leukocytes, reaching a control level, what is consistent with the literature data [10, 11]. In frog blood cells 60 minutes after irradiation,% TDNA was comparable to that one measured immediately after irradiation, what indicates a very slow DNA repair.

In other series of experiments, the frog blood cells was irradiated at a dose of 8 Gy and incubated for 60 minutes at 37 °C in both PBS and medium 199 (figure 3).

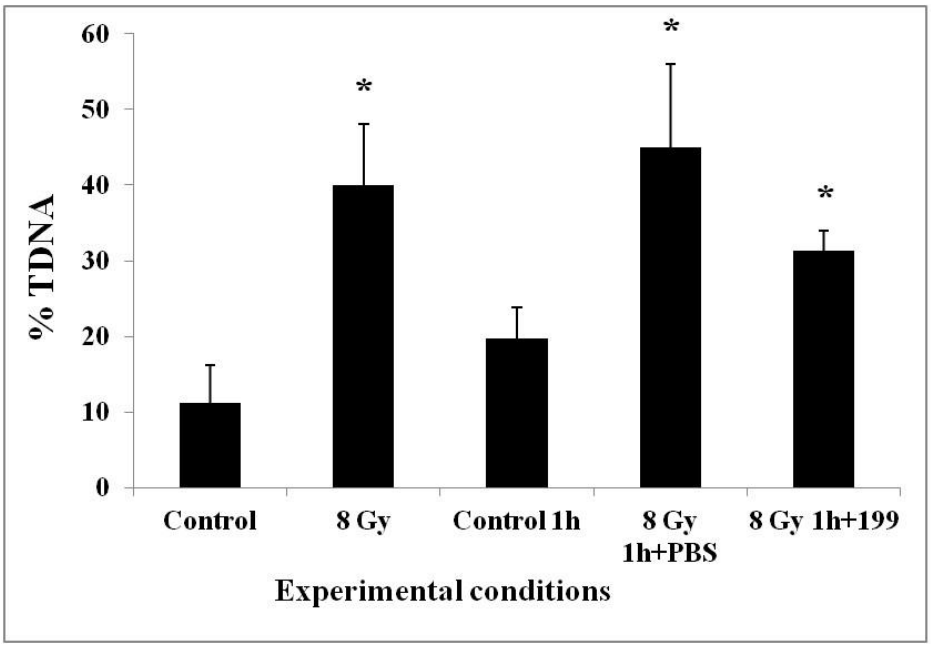

Figure 3. The level of DNA damage of the frog's blood cells after irradiating at a dose of 8 Gy at room temperature (M ± SD). 1h – 1 hour. 199 – medium 199. * − significant difference from t-test ($p < 0.05$)

% TDNA in blood cells irradiated at a dose of 8 Gy was significantly higher than at 4 Gy and after 60 minutes incubation in the PBS did not differ from level measured immediately after irradiation. After incubation in the medium 199, % TDNA decreased compared to that one in PBS, but no significant difference was found.

Note that the blood of a mouse, a human and a frog differs both in blood cell types and in their ratio. Lymphocytes form the largest group among the white blood cells in mice and neutrophils – in humans. Among the frog blood cells, nucleated red blood cells predominate. Moreover, metabolic processes in amphibians are not as active as in mammals, and it can be expected that the processes of DNA repair also proceed more slowly than in mammals. Thus, the repair of radiation-induced DNA sites sensitive to formamidopyrimidine-glycosylase was more rapid in rodent cells than in mammalian cells. It suggested the higher level of DNA polymerase β activity [10].

Unlike mammals, the nucleated erythrocytes of amphibians and birds additionally contain histone H5, which appears to play an important role in maintaining the repressed state of chromatin [12, 13]. For example, it was shown that in the nucleated erythrocytes of chickens there was no repair of single-stranded DNA breaks induced by ionizing radiation [14].

4. Conclusions

We compared the levels of DNA damage in blood cells in members of different taxons.

It can be assumed that the level of DNA damage depends on the blood cells composition and their metabolism but not their radiosensitivity.

DNA repair processes in frog blood cells are not completed by 60 minutes of the post-radiation period and the level of DNA damage remains high. Thus, frogs can be used as indicators of radiation pollution of the environment both in the background and in emergency situations.

Further studies should be performed to estimate of different types of external and internal irradiation of animals.

Acknowledgments

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