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Short Communication

CHANGES IN THE ACTIVITY OF ACETYLCHOLINESTERASE AND NA,K-ATPASE IN HUMAN ERYTHROCYTES IRRADIATED WITH X-RAYS

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Abstract: The response of human erythrocytes to X-rays in the dose range from 40 Gy to 600 Gy was determined on the basis of changes in the activities of AChE and ATPase. The Na,K-ATPase activity increased above the control value at doses below 200 Gy, while at the doses higher than 200 Gy, it decreased, reaching 96% of the control value at a dose of 600 Gy. In the range of doses up to 200 Gy, the AChE activity, expressed as V_{max} , did not change. At higher doses, it fell drastically, reaching 33% of the control value at a dose of 600 Gy. Simultaneously, the enzyme substrate affinity decreased at 200 Gy, and then started to increase at lower values of V_{max} . The obtained results suggest that under appropriate conditions, low doses of radiation may have the opposite effects to high doses.

Key Words: X-rays, Na,K-ATPase, Acetylcholinesterase, Human Erythrocytes

INTRODUCTION

In radiobiology, assumptions about the effects of low doses of radiation are often based on extrapolations from results obtained with high doses. Such extrapolation may lead to results incompatible with reality. In the literature, there is data indicating that low doses of radiation may induce contrary effects to high doses. An example of such effects may be the adaptive response observed in a wide range of cellular systems including algae, protozoan cells and insect

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Abbreviations used: AChE - acetylcholinesterase; AcTCh - acetylthiocholine iodide; ATP - adenosine 5'-triphosphate; PBS - phosphate buffered saline; EDTA - ethylenediamine-tetraacetic acid, disodium salt; EGTA - ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid; PMSF - phenylmethanesulfonyl fluoride; TBARS, thiobarbituric acid-reactive substances.

cells [1, 2]. Another, found in normal and tumor cells in humans, is the hypersensitive survival response to very low doses of radiation followed by an increase in the level of resistance to higher doses [3, 4]. These observed effects were related to DNA damage and the contribution of DNA in possible repair processes or to the factor controlling the fidelity of repair of antioxidant defense systems [2, 3]. Our previous results [5, 6] revealed a decrease in the level of erythrocyte hemolysis, and a decrease in the contents of lipid peroxidation and hemoglobin oxidation products when human erythrocytes were irradiated with split doses of radiation; this contrasted with the results obtained with the same single doses. These results suggest that under various irradiation conditions, various effects may be found, and they are not necessarily directly connected with DNA.

In this study, we concentrated on the influence of the dose size of X-radiation on human peripheral erythrocytes. As they are highly specialized cells without a nucleus or any other cellular organelles, they are a good model for the study of processes taking place without the involvement of DNA. Erythrocytes are considerably more resistant to the influence of ionizing radiation than other blood cells [7]. Generally speaking, enzymatic activity is a sensitive indicator of changes caused by irradiation. Therefore, in this study, the response of erythrocytes to irradiation doses which did not cause erythrocyte hemolysis was estimated on the basis of the changes in the activity of the membrane enzymes acetylcholinesterase (AChE) and Na,K-ATPase.

MATERIALS AND METHODS

Preparation of erythrocytes

Blood samples from adult donors were provided by the Central Blood Bank in Łódź. Erythrocytes were separated from the blood plasma and leucocytes by centrifugation at 2000 x g for 10 min (4°C), washed three times with sodium phosphate buffered saline (145 mM NaCl in 10 mM sodium phosphate, pH 7.4; PBS) and the buffy coat was aspirated each time. In order to remove the residual leucocytes, the erythrocytes were passed through an α -cellulose column, washed with 0.1 M Na-phosphate buffer (pH 7.4), and resuspended in the same buffer to obtain a hematocrit of 2%.

Irradiation conditions

Human erythrocyte suspensions in an isotonic Na-phosphate buffer (pH 7.4) with a hematocrit of 2% were exposed under air to X-radiation (200 kV, 20 mA) at a dose rate of 23 Gy·min⁻¹ in a dose range of 40 to 600 Gy. The dose-rate was estimated with a Fricke dosimeter. During irradiation, the erythrocyte suspensions were stirred with a magnetic bar.

Preparation of erythrocyte membranes

Erythrocyte membranes were prepared using the modified method of Dodge *et al.* [8]. The erythrocytes were hemolysed with 20 mM TRIS-HCl buffer (pH

7.4), containing 1 mM EDTA and 0.5 mM PMSF, and the resulting ghosts were washed with decreasing concentrations of TRIS-HCl buffer.

The protein content of the membrane preparations was determined via the method of Lowry *et al.* [9].

Measurement of acetylcholinesterase (AChE) activity

The activity of AChE was determined using the spectrophotometric method of Ellman *et al.* [10]. The acetylthiocholine iodide hydrolysis reaction rate was calculated as follows:

$$V = \frac{\Delta A_{412nm}(1 \text{ min}) \cdot F}{13600 M^{-1} \cdot cm^{-1}} \left[\frac{\text{mol}_{AcTCh}}{\text{min} \cdot \text{ml}_{packedER}} \right]$$

where: ΔA is the increase in absorbance in min^{-1} at 22°C ; F is the dilution factor; and $13600 \text{ M}^{-1} \text{ cm}^{-1}$ is the extinction coefficient for 5-thio-2-benzoic acid (TNB).

Measurement of Na,K-ATPase activity and total ATPase activity

Na,K-ATPase activity was determined by measuring the difference in the level of liberation of inorganic phosphate from ATP in the absence and in the presence of 0.1 mM ouabain during a 30-min. incubation of the membrane preparations at 37°C . The composition of the incubating medium was: 100 mM TRIS-HCl buffer (pH 7.4), 85 mM NaCl, 15 mM KCl, 10 mM MgCl_2 , 1 mM EGTA, 1 mM ATP and $0.5 \text{ mg}_{\text{prot}} \cdot \text{ml}^{-1}$ erythrocyte membranes. The amount of released Pi was determined spectrophotometrically at 610 nm as a complex with heptamolybdate and malachite green. Pi concentration was taken from a calibration curve made for 2 to 40 μM KH_2PO_4 as a standard. Na,K-ATPase activity was calculated by subtracting the activity assessed in the presence of ouabain from the activity in the absence of ouabain (total ATPase activity). The enzyme activity was expressed in $\text{nmol Pi mg}_{\text{prot}}^{-1} \text{ h}^{-1}$.

Osmotic fragility test

Osmotic fragility curves were drawn on the basis of the percentage of erythrocyte hemolysis in solutions of various NaCl concentrations buffered with 5 mM Na-phosphate (pH 7.4). Suspensions of erythrocytes were diluted to a hematocrit of 0.2%, and the degree of hemolysis was estimated after 10 min. The samples were centrifuged at 10000 rpm for 3 min. The absorbance of the supernatants was assessed at 570 nm. The degree of hemolysis was calculated on the assumption that the absorbance of a sample hemolysed with distilled water equaled 100%.

RESULTS AND DISCUSSION

The results presented in this paper describe the effects of the interaction of X-rays with human erythrocytes in the range of doses from 40 Gy to 600 Gy. Under the conditions used in this study, X-radiation did not induce cell hemolysis. Similarly, cell parameters such as the level of MetHb and the level of

membrane lipid peroxidation (TBARS), assessed according to the methods described earlier [5], did not change after irradiation. However, in this range of doses, changes in AChE and ATPase activity were observed. Fig. 1 shows the Lineweaver-Burk plots for erythrocyte membrane AChE activity before and after cell irradiation. Two kinetic parameters were estimated using these plots: V_{max} , which is the maximal rate of the enzymatic reaction, and K_m (the Michaelis-Menten constant), which corresponds to the substrate concentration at which the reaction rate equals half of the maximum.

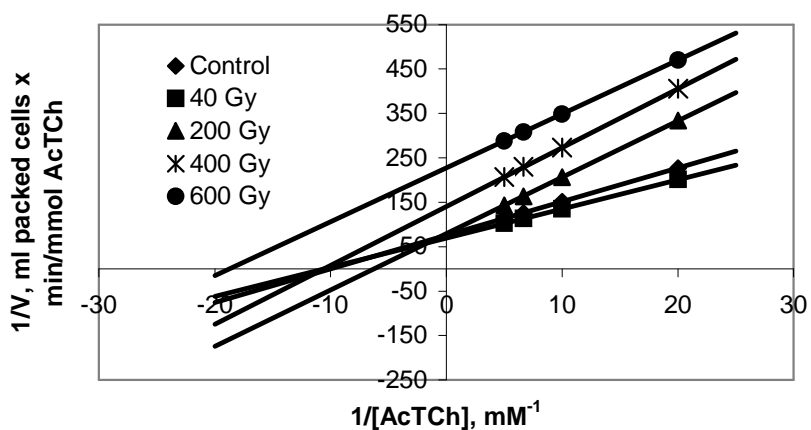


Fig. 1. The Lineweaver-Burke plots for erythrocyte membrane AChE activity before and after the X-irradiation of the cells: (-♦-) control, (-■-) 40 Gy, (-▲-) 200 Gy, (-*) 400 Gy, (-●-) 600 Gy.

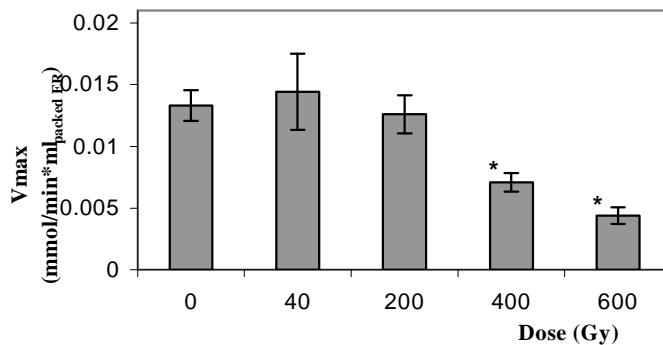


Fig. 2. Acetylcholinesterase activity in human erythrocytes expressed as V_{max} . Each bar represents the average value \pm SD of 3-11 experiments. Values are statistically significant (*) with respect to the control at $p < 0.05$.

Fig. 2 shows the maximal rate of the enzymatic reaction (V_{\max}) for AChE at different radiation doses. Doses up to 200 Gy did not cause any significant changes in the V_{\max} in comparison with the control value. V_{\max} visibly decreased at 400 Gy and 600 Gy, respectively to 53% and 33% of the control value. However, the Michaelis-Menten constant (K_m) underwent irregular changes, reaching its maximum level at 200 Gy (Fig. 3). This increase amounts to 58% of the control value. Doses of 40 Gy and 400 Gy did not change the K_m value significantly. Increasing the irradiation dose to 600 Gy caused a decrease in K_m to 53% of the control value.

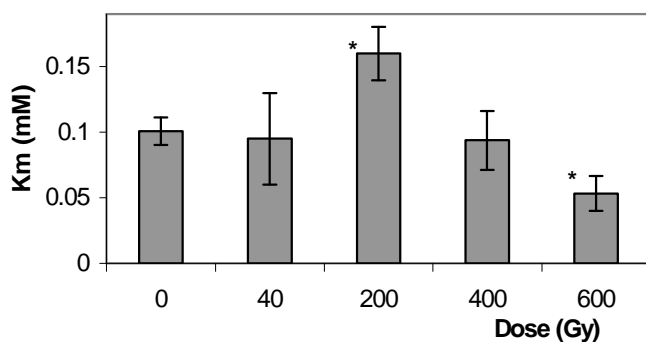


Fig. 3. AChE substrate affinity in human erythrocytes after X-irradiation, expressed as the Michaelis-Menten constant (K_m). Each bar represents the average value \pm SD of 3-11 experiments. Values are statistically significant (*) with respect to the control at $p < 0.05$.

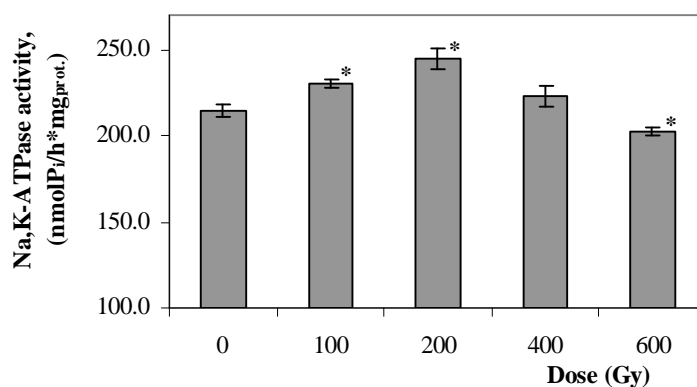


Fig. 4. Na,K-ATPase activity in X-irradiated erythrocyte membranes. Each bar represents the average value \pm SD of 3-5 experiments. Values are statistically significant (*) with respect to the control at $p < 0.05$.

The changes in the total ATPase activity in the membranes of the control and irradiated erythrocytes were statistically insignificant (data not shown). Na,K-ATPase activity (Fig. 4) increased with the dose up to 200 Gy, where it reached its maximum (114% of the control value). Then it decreased with increasing doses, reaching 94% of the control value at a dose of 600 Gy. Taking into consideration the lack of changes in the total ATP-ase activity, it may be suggested that the observed increase in Na,K-ATPase activity was accompanied by a decrease in the activity of other ATPases. These results show that doses below or equal to 200 Gy induced effects that were opposite to those observed at higher doses (400-600 Gy). The course of radiation-induced AChE inactivation was different, which suggested that AChE has a higher radioresistance than Na,K-ATPase. However, at higher doses a drastic decrease in AChE activity was observed; it reached 33% of the control value at a dose of 600 Gy, while at the same dose, the Na,K-ATPase activity decreased by 6%.

It is known that the radiolytic species generated in aerated cellular systems are forms of activated oxygen: $\cdot\text{OH}$, $\text{O}_2\cdot^-$ radicals and H_2O_2 . Taking into account the low reactivity of $\text{O}_2\cdot^-$, $\cdot\text{OH}$ radicals are the principal oxidizing species causing cell damage in the presence of air [11, 12]. The molecular oxygen present in the system reacts with the secondary free radicals of biological target molecules, catalysing further oxidation steps. It is clear that under the conditions of irradiation applied in this study, the observed changes in the activity of the studied enzymes were caused by structural modifications to the proteins initiated by $\cdot\text{OH}$ radicals, the concentration of which changed with the change in dose. Differences in the response of the two proteins to the action of low and high doses of radiation may result from differences in protein structure and location in the membrane. Moreover, Na,K-ATPase activity greatly depends on the condition of its thiol groups, whereas AChE is a non-thiol-dependent enzyme [13, 14]. On the other hand, it is known that AChE activity is modulated by the hydrophobic environment of the membrane and depends on the membrane fluidity and surface charge [15]. In the range of doses studied, no products reacting with TBA were found. However, in the area of the lipid bilayer, structural changes that could cause a decrease in the activity of this enzyme cannot be excluded.

The obtained results indicated that the two enzymes had different radiosensitivities in the range of doses defined as low and high. However, the changes in ATPase activity took place in the range of lower doses, which suggested that these proteins have a higher radiosensitivity than AChE.

The increase in Na,K-ATPase activity should also be considered in terms of changes in osmotic resistance. From the osmotic resistance plots, the C_{50} parameters were calculated (i.e. the concentration of NaCl at which 50% hemolysis occurred) and the following values were obtained: 59 mM for the control erythrocytes, 61 mM for those irradiated with 400 Gy, and 64 mM for those irradiated with 600 Gy. A decrease in the osmotic resistance was observed at the maximum dose of 600 Gy, at which Na,K-ATPase activity showed a slight drop.

The obtained results do not allow for suggestions concerning the nature of the structural or functional changes taking place in the studied proteins, but do stand as further proof showing that various doses of radiation may induce completely different effects.

By contrast to our results, the results of experiments carried out by other authors indicate Na,K-ATPase inactivation caused by ionizing and UV radiation [16, 17]. However, in the case of AChE, there are both reports on its radiation-induced inactivation [18] and on increases in its activity under the influence of ionizing and laser radiation [19, 20].

Taking into consideration the fact that the observed effects were mainly initiated by \cdot OH radicals, it may be suggested that at certain concentrations, they may be the factors modulating protein structure and function.

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