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Short Communication

**THE EFFECT OF HYPOCHLORITE ON HUMAN ERYTHROCYTES
PRETREATED WITH X-RADIATION**

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Abstract: Both hypochlorite and ionizing radiation induce oxidation processes of biomolecules. The effects are dependent to a large degree on the dose of the oxidizing agent. Previously we observed that split doses of gamma radiation caused lower hemolysis than the same but single doses. The critical factors influencing the occurrence of this effect were: the value of the first dose and the time between the doses.

In this work we examined the effect of gamma radiation (40-400 Gy) on hemolysis of human erythrocytes induced by hypochlorite.

Erythrocytes in PBS, hematocrit 2 %, were irradiated with doses of 40, 200 or 400 Gy. The dose-rate was 23.8 Gy/min. Cell suspensions were stirred during irradiation. After irradiation the erythrocytes were incubated for 1, 3 or 4 hours at room temperature and then hypochlorite was added to a 250 microM concentration. Control samples were erythrocytes treated only with NaOCl.

The level of hemolysis was determined after NaOCl addition. Hemolysis of erythrocytes preirradiated with the dose of 400 Gy was lower than hemolysis of erythrocytes treated only with NaOCl. The effect was dependent on the time between the end of irradiation and the addition of NaOCl.

In contrast, slightly higher hemolysis was observed for erythrocytes preirradiated with lower (40 or 200 Gy) doses of radiation.

The observed effect is similar to that obtained for radiation-induced hemolysis.

It suggests that ionizing radiation may induce structural and/or functional changes in erythrocytes, which make the cell more resistant to further oxidative damage.

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Abbreviations used: PBS - phosphate-buffered saline, pH 7.4; TBA-RS - thiobarbituric acid-reactive substances; NaOCl - mixture of hypochlorite, sodium salt and hypochlorous acid present at pH 7.4; Hb - hemoglobin; MetHb - oxidized form of Hb.

Key Words: X-Radiation, Human Erythrocyte, Hypochlorite

INTRODUCTION

There exists considerable literature concerning the influence of ionizing radiation and hypochlorite on the structure and function of mammalian erythrocytes [1-4]. Some of these papers contain proofs of repair of radiation damage in mammalian erythrocytes. The effects of both factors are dependent on the dose and time of the treatment. Previous works from our laboratory demonstrated that the hemolysis of erythrocytes irradiated with a dose given in two fractions with a break between the following expositions was lower in comparison with the hemolysis of erythrocytes irradiated with a single dose [5-7]. This effect was observed in aerobic conditions and under N₂O [5]. The most pronounced effect was observed for the first dose equal to 0.4 kGy and the time interval 3-3.5 hours.

It may be suggested that relatively low doses of ionizing radiation induce structural and/or functional changes in enucleated human erythrocytes. These changes make erythrocytes more resistant to the next portion of radiation.

In this work the second dose of radiation which induced hemolysis was exchanged for hypochlorite at a concentration of 250 μ M.

We studied the influence of X-radiation in a dose range of 40-400 Gy on hypochlorite-induced hemolysis.

MATERIALS AND METHODS

Human erythrocytes were obtained from the blood of healthy donors. Erythrocytes were separated from blood plasma and leukocytes by centrifugation for 10 minutes at 2000 rpm, and were washed with phosphate-buffered saline (145 mM NaCl in 10 mM sodium phosphate, pH 7.4). In order to remove residual leukocytes, erythrocytes were passed through a α -cellulose (Sigma) column. Purified erythrocytes were washed twice with PBS and resuspended at a hematocrit of 2 %.

Erythrocytes were X-irradiated under air with the doses of 20, 200 or 400 Gy. The source of X-radiation was a Roentgen lamp (Stabilipan, 200 kV, 20 mA). The dose-rate was 23.8 Gy \cdot min⁻¹. During irradiation the erythrocyte suspensions were stirred with a magnetic bar. Irradiated erythrocytes were incubated at room temperature for 1, 3 or 4 hours. After this time hypochlorite at a concentration of 250 μ M was added to preirradiated erythrocytes. Control cells were unirradiated erythrocytes treated only with hypochlorite at a concentration of 250 μ M.

The level of hemolysis of erythrocytes was determined by measurement of hemoglobin (Hb) released from the cells, relative to the total cellular Hb content. The amount of Hb was estimated on the basis of absorbance at 540 nm. Absorbance of the supernatant after complete hemolysis with distilled water was

taken as 100 % hemolysis. Hemolysis was determined as a function of time after hypochlorite addition.

The level of MetHb within the erythrocytes was determined spectrophotometrically at 630 nm with the correction at 700 nm after release of Hb from the erythrocytes.

Lipid peroxidation was quantified spectrophotometrically by measuring the formation of thiobarbituric acid reactive substances (TBA-RS) at 532 nm [8].

RESULTS AND DISCUSSION

Figure 1 shows the hypochlorite-induced hemolysis of erythrocytes preirradiated with the dose of 400 Gy. The interval between irradiation and hypochlorite addition was 3 hours. Hemolysis of preirradiated erythrocytes was lower compared to that of unirradiated ones. After 90 minutes from the addition of hypochlorite the hemolysis of unirradiated erythrocytes was 22 %, while for the preirradiated erythrocytes only 13 %.

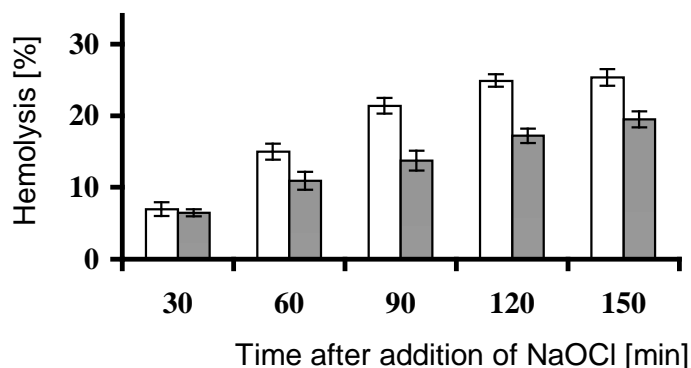


Fig. 1. Hypochlorite-induced hemolysis of erythrocytes. Cells were treated with NaOCl at a concentration of 250 μ M (open bars) or preirradiated with a dose of 400 Gy, incubated for 3 hours at room temperature, and then NaOCl was added to a concentration of 250 μ M (closed bars). Each bar represents mean \pm SD for 3-5 experiments.

The attenuation of hemolysis was time-dependent (Fig. 2). The most pronounced effect was observed for a 3-hour incubation between the end of irradiation and the addition of hypochlorite. The relative hemolysis of the preirradiated erythrocytes decreased to 59 % of that of the unirradiated ones. For 1-hour and 4-hour incubations the relative hemolysis was 85 and 80 %, respectively. These results suggest that decrease in hemolysis is dependent on biochemical changes occurring during the time after irradiation. It should be mentioned that the doses in the range of 40-400 Gy did not induce hemolysis themselves. The oxidation of hemoglobin was not detected either after the irradiation or after the addition

of hypochlorite, or after the joint action of these two oxidants. A minute increase in TBA-reactive substances was observed (from 0.030 to 0.044 in absorbance units) in the irradiated erythrocytes. This increase was not dependent on the dose and on the time after the irradiation.

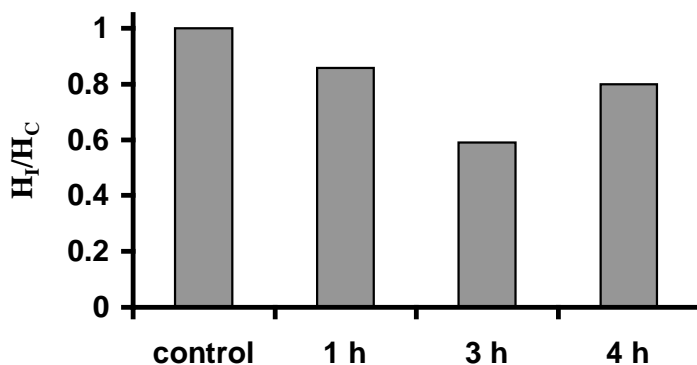


Fig. 2. Relative hemolysis of erythrocytes irradiated with a dose of 400 Gy is dependent on the time between irradiation and NaOCl addition. Control - only NaOCl, 250 μ M, H_I/H_C - the ratio of hemolysis of preirradiated erythrocytes induced by NaOCl to hemolysis of erythrocytes treated only with NaOCl, determined 90 minutes after NaOCl addition.

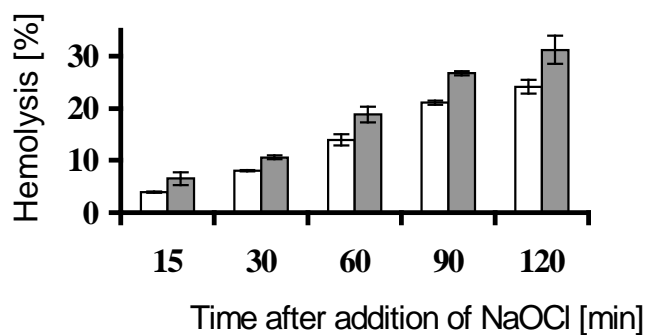


Fig. 3. Hypochlorite-induced hemolysis of erythrocytes. Cells were treated with NaOCl at a concentration of 250 μ M (open bars) or preirradiated with a dose of 40 Gy, incubated for 3 hours at room temperature, and then NaOCl was added to a concentration of 250 μ M (closed bars). Each bar represents mean \pm SD for 3-5 experiments.

Erythrocytes irradiated with the dose of 40 Gy hemolysed more intensively than control erythrocytes. After 90 minutes from hypochlorite addition hemolysis was 21.1 and 26.8 %. Similar data were obtained for the dose of 200 Gy (not shown). These results may confirm our previous findings that properly matched doses of ionizing radiation may induce resistance in human erythrocytes to the next dose of

an oxidant [5-7]. The oxidant can be not only ionizing radiation, but also a chemical oxidant.

The occurrence of resistance might be connected with conformational changes in membrane proteins and lipid fluidity, and with interactions between these two components of the plasma membrane. Previously we observed a non-linear dependence between a few parameters e.g. lipid fluidity, membrane proteins conformation, Na,K-ATPase activity and the dose of radiation [9]. It could be supposed that the resistance of human erythrocytes to hypochlorite-induced hemolysis is due to the unique conformational state of the membrane after a specific dose of ionizing radiation. The observed resistance of erythrocytes was not permanent. It was also strictly correlated with the time of addition of the next portion of the oxidant.

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