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Radiation damage to human erythrocytes. Relative contribution of hydroxyl and chloride radicals in N_2O-saturated buffers

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Abstract

The erythrocyte suspensions in Na-phosphate buffered isotonic NaCl solution (PBS) or Na-phosphate isotonic buffer (PB) (hematocrit 1%) were irradiated with the dose of 400 Gy under N_2O. Erythrocytes were incubated in the medium in which the cells were irradiated or in fresh PBS. The level of damage to cells was estimated on the basis of the course of post-radiation hemolysis and hemoglobin (Hb) oxidation. The medium in which the cells were irradiated and incubated influenced the course of the post-radiation hemolysis and Hb oxidation as well as some other parameters. We discussed the contribution of hydroxyl and chloride radicals in the initiation of erythrocyte damage and oxygen modification of these processes.

Keywords: Ionizing radiation; Human erythrocytes; Nitrous oxide; Chloride-derived radicals; Hydroxyl radicals

1. Introduction

In our previous paper (Komorowska et al., 2007), we presented the results concerning the influence of Na-phosphate buffered saline (PBS) and phosphate buffer (PB) medium on radiation-induced damage to human erythrocytes under aerobic conditions. The level of damage in both environments was assessed on the basis of the course of the post-radiation hemolysis of erythrocytes, which was faster in cells suspended and irradiated in PBS than in PB. On the basis of the differences in chemical composition of both media and the data in literature (Saran and Bors, 1997) concerning PBS radiolysis, it might be suggested that the secondary chloride-derived radicals generated during irradiation in PBS could contribute to the damage to erythrocytes. These radicals could cause a greater damage to the plasma membrane than $^{\bullet}OH$ radicals that could lead to a faster post-radiation hemolysis.

It should be also stressed that the erythrocytes suspended in either of both media were irradiated in the atmosphere of air. Molecular oxygen present in the medium is an additional factor reacting with secondary radicals generated in biological target molecules and thus catalyzing the further oxidation steps. In this way, oxygen modifies the reactions initiated by primary radicals. It is well known that oxygen is a factor enhancing the radical damage in biological systems (Greenstock, 1984; Petkau, 1986). Therefore, the efficacy of radicals generated in both systems for cell damage should be rather evaluated in the absence of oxygen.

In order to eliminate the influence of oxygen, in this work the suspension of erythrocytes in PBS or PB were irradiated under N_2O. During irradiation, N_2O present in the system reacts very rapidly with e^-_{aq}, which are further transformed into $^{\bullet}OH$ radicals. In this way, the radiation yield of $^{\bullet}OH$ radicals under N_2O is doubled ($G_{OH} = 5.3$) in comparison to air conditions (von Sontag, 1987). If Cl^- ions are present in the medium in physiological concentration (145 mM), there is a high probability that $^{\bullet}OH$ radicals will be transformed into the short-living radicals derived from chloride (Saran et al., 1993).

The aim of the work was to assess the efficiency of both radiation-generated primary $^{\bullet}OH$ radicals and secondary radicals derived from chloride in damage to human erythrocytes under anaerobic conditions.

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2. Materials and methods

2.1. Chemicals

α-Cellulose, acethylthiocholine iodide, 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), gentamycine, 2,2,6,6 tetrametyl-4 maleimidopiperidine-*N*-oxyl (MSL) (maleimido-TEMPO), sodium dodecyl sulfate (SDS), TRIS and phenyl-methylsulfonyl fluoride (PMSF) were purchased from Sigma Chemical Co., Poole Dorset, UK. Other chemicals were obtained from POCh (Gliwice, Poland) and all of them were of analytical grade. All solutions were made with water purified by the Millipore Q system.

2.2. Preparation of erythrocyte suspensions

Erythrocytes were prepared from blood obtained from healthy adult donors using ACD (0.023 M citric acid, 0.045 M sodium citrate, 0.045 M glucose) as an anticoagulant. Erythrocytes were separated from blood plasma and leucocytes by centrifugation and washed three times with (PBS—145 mM NaCl in 10 mM Na-phosphate buffer, pH 7.4) or 0.1 M phosphate buffer, pH 7.4 (PB). The buffy coat was aspirated each time. In order to remove the residual leucocytes, erythrocytes were passed through an α-microcrystalline cellulose column, washed with PBS or PB and resuspended in PBS or PB to obtain a hematocrit of 1%.

2.3. Conditions of irradiation

Human erythrocyte suspensions in an isotonic Na-phosphate buffer (pH 7.4, $[H_2PO_4^-]$-23.5 mM, $[HPO_4^{2-}]$-76.5 mM, $[Na^+]$-176.5 mM) (PB) or Na-phosphate buffered saline ($[Cl^-]$-146.7 mM, $[H_2PO_4^-]-1.1$ mM, $[HPO_4^{2-}]$ -3.65 mM, $[Na^+]$ -155 mM) (PBS) with a hematocrit of 1% were exposed to X-ray radiation (200 kV, 20 mA, 0.1 mm Cu filter) at a room temperature under N_2O, at the dose rate of 16.4 Gy min^{-1}. Irradiation of erythrocyte suspensions was performed in the special tonometer through which N_2O had been passed for 60 min before irradiation. During irradiation the erythrocyte suspensions were stirred with a magnetic bar.

2.4. Measurement of post-radiation hemolysis of erythrocytes

The percentage of hemolysis was determined on the basis of the amount of hemoglobin (Hb) released from the cells, in relation to the total cellular Hb content. After irradiation, the erythrocyte suspensions were incubated at 37 °C in the presence of gentamycine (0.4 mg/ml of erythrocyte suspension). In a given time, the samples were taken for hemolysis measurement. Hemolysis was determined as previously described (Komorowska et al., 2007).

2.5. Methemoglobin (MetHb) measurement

The percentage of MetHb content was measured in the residual erythrocytes after their hemolysis. The absorbance of the solutions was measured at 630 nm before oxidation of iron, and after its total oxidation with $K_3[Fe(CN)_6]$. The content of MetHb was calculated as described earlier (Puchała et al., 2004).

2.6. Glutathione (GSH) measurement

GSH was determined by Ellman's method with some modifications (Ellman, 1959; Pawelski, 1990). Control and irradiated red cell suspensions of 0.15 ml (hematocrit 5%) were mixed with 1 ml of 40 mM sulfuric acid. After incubation at 25 °C for 10 min, 0.15 ml of 300 mM sodium tungstate was added and the samples were shaken for 5 min and centrifuged. To 0.6 ml of supernatant, 0.75 ml of Tris–HCl buffer (pH 8.0) and 0.06 ml DTNB (1 mM) were added. The concentration of GSH was determined spectrophotometrically at 412 nm using the absorption coefficient 13.6 mM^{-1} cm^{-1}.

2.7. Potassium efflux

The erythrocyte suspensions were centrifuged at 2000*g* for 10 min in order to obtain supernatant samples for the measurement of concentration of extracellular potassium ions. Extracellular K^+ was measured by flame emission photometry using a SpectrAA-300 apparatus (Varian).

2.8. Acetycholinesterase (AChE) activity

The activity of AChE was determined using spectrophotometric method of Ellman et al. (1961). Erythrocytes were suspended in 0.145 M NaCl solution containing 5 mM potassium phosphate (pH 8.0) at 22 °C (0.05% hematocrit). Acethylthiocholine iodide concentrations were 10–50 μM, and the final concentration of Ellman's reagent in the samples was 100 μM.

2.9. Preparation of erythrocyte membranes

Erythrocyte membranes were prepared using the modified method of Dodge et al. (1963) with some modifications. The erythrocytes were hemolyzed with 20 volumes of 10 mM Na-phosphate buffer (pH 7.4), containing 1 mM EDTA and 0.5 mM PMSF and the resulting ghosts were washed with decreasing concentrations of phosphate buffer.

The protein content in the plasma membrane preparations was determined by the method of Lowry et al. (1951).

2.10. Determination of SH groups in membrane proteins

The concentration of SH groups was determined spectrophotometrically according to the method of Ellman