Osmotic fragility and lipid peroxidation of irradiated erythrocytes in the presence of radioprotectors[1]

Z. Helszer, Z. Jóźwiak and W. Leyko

Department of Biophysics, Institute of Biochemsitry and Biophysics, University of Łódź, PL-90237 Łódź (Poland), 27 June 1979

Summary. Gamma radiation causes a decrease in the osmotic fragility of porcine erythrocytes and induces peroxidation of membrane lipids, as reflected by an increase in malondialdehyde content. With respect to osmotic fragility all the protectors used have the radioprotective effect. Malondialdehyde content was reduced in the presence of catalase, cysteine and glutathione, whereas SOD had no protective effect.

Ionizing radiation causes disorganization of the lipoprotein structure of biological membranes[2], as reflected, for example, in the inactivation of various bound enzymes and the peroxidation of membrane lipids[3]. It has also been suggested that radiation-induced perturbations in Na^+ and K^+ transport systems are connected with the oxidation of membrane protein sulfhydryl groups[4]. There is, however, still a lack of relevant information about the molecular mechanisms of radiation-induced damage to biological membranes and especially about the role of radioprotectors in irradiated cells.

In the present paper we studied the influence of various substances on osmotic fragility and lipid peroxidation in gamma-irradiated porcine erythrocytes.

Materials and methods. 1. Determination of the osmotic fragility of porcine erythrocytes. 50-µl samples of erythrocyte cell-suspension (hematocrit 50%) were added to test tubes containing 5 ml NaCl solution of different concentrations. After 1-h incubation at 37 °C samples were centrifuged (2000 × g, 10 min) and the absorbance of the supernatant was measured at 542 nm. The degree of hemolysis was expressed as the percent total hemolysis of the same volume of erythrocytes in distilled water.

2. Determination of malondialdehyde content. Porcine erythrocyte membranes were prepared by the method of Dodge et al.[5]. The formation of malondialdehyde (MDA) was used as an index of lipid peroxidation. The content of MDA in a membrane suspension (protein concentration 3 mg/ml) was determined spectrofotometrically by the thiobarbituric acid method, as described by Fong et al.[6].

3. Irradiation conditions. Intact erythrocytes were gamma-irradiated at a dose-rate of 60 krad/h with doses in the range of 5–50 krad. Isolated erythrocyte membranes were irradiated from a ^{60}Co source at a dose-rate of 0.5 Mrad/h with doses in the range of 0.25–2 Mrad.

Results and discussion. Gamma radiation causes a decrease in the osmotic fragility of porcine erythrocytes as reflected in a shifting of the osmotic fragility curves towards higher NaCl concentrations (figure 1). In the presence of superoxide dismutase (200 µg/ml) and catalase, statistically significant protection from radiation-induced decrease in osmotic fragility was observed for higher radiation doses (table 1). A similar protective effect was found in the presence of cysteine and glutathione (table 2). Also other substances used in our study, i.e. glucose, 2,4,6-trinitrophenol and histidine, exhibit some protective effect.

Changes in osmotic fragility observed in the present study may be attributed to several different mechanisms, such as an increase in erythrocyte volume in isotonic solution, decrease in the cell membrane surface, or alterations in the mechanical properties of the erythrocyte membrane. According to Murphy[7], temperature influences the critical

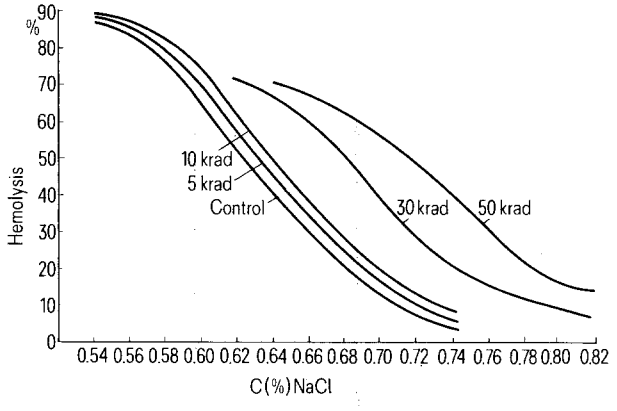

Fig. 1. Effect of gamma radiation on osmotic fragility of porcine erythrocytes.

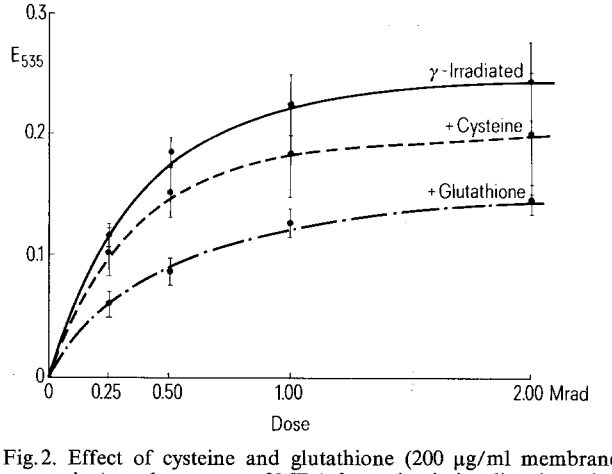

Fig. 2. Effect of cysteine and glutathione (200 µg/ml membrane suspension) on the content of MDA formation in irradiated porcine erythrocyte membrane suspension.

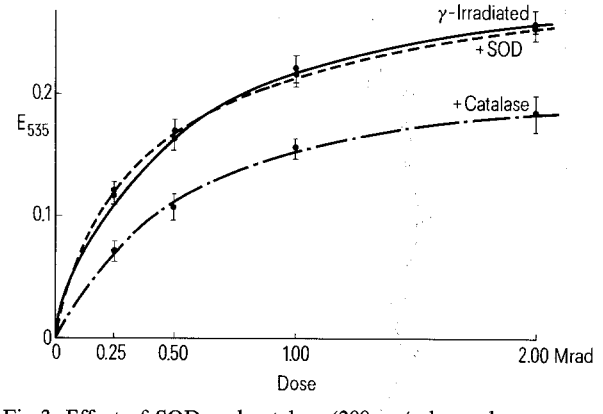

Fig. 3. Effect of SOD and catalase (200 µg/ml membrane suspension) on MDA formation.

hemolytic volume. Aloni et al.[8] observed temperature-induced changes in osmotic fragility of rabbit, rat, guinea-pig and human red blood cells. Erythrocytes studied by these authors exhibited a considerably higher stability at 37 °C than at 0 °C.

The decrease in osmotic fragility observed in the present study after erythrocyte irradiation results mainly from the damage to the cell membrane caused by exogenous superoxide anion radicals generated during the water radiolysis process. The destructive action of hydrogen peroxide formed in the dismutation reaction and singlet oxygen may also be of considerable importance.

Gamma radiation causes also peroxidation of membrane lipids. After irradiation of porcine erythrocyte membranes with doses in the range of 0.25–2 Mrad the amount of MDA increases gradually, and above a dose of 2 Mrad it saturates, indicating that at this dose all possible substrates involved in MDA formation have already been utilized.

When membranes were irradiated in the presence of radioprotectors the MDA content was reduced. In the presence of glutathione, catalase and cysteine it was reduced by averages of about 50%, 30% and 20% respectively (figures 2 and 3). In contrast, superoxide dismutase did not protect membrane lipids from MDA formation (figure 3). These results suggest that the main role in radiation-induced damage to membrane lipids should be attributed to hydroxyl radicals and not to superoxide radicals.

Ching-San and Lawrence[9] have shown that OH· radicals generated in liver microsomes initiate NADPH-dependent lipid peroxidation. These radicals are produced mainly according to the classical Fenton reaction. Peroxidative degradation of arachidonic acid proceeds very easily in the presence of OH· radicals and may be stopped by thiourea – a potential scavenger of hydroxyl radicals. Similarly, trapping of OH· radicals by 5,5-dimethyl-1-pyrroline-1-oxide prevents lipid peroxidation. Raleigh et al.[10] have proved that hydroxyl radicals initiate autooxidation of linoleic acid subjected to X-rays.

The results obtained indicate that superoxide radicals and hydroxyl radicals play the main role in radiation-induced damage to porcine erythrocyte membranes.

Table 1. Protective effect of superoxide dismutase and catalase on osmotic fragility of irradiated porcine erythrocytes

Dose	$C_{50\%}$	p
30 krad	0.68±0.03	
+SOD	0.66±0.02	0.01<p<0.02
+Catalase	0.66±0.02	0.01<p<0.02
50 krad	0.72±0.02	
+SOD	0.70±0.01	0.005<p<0.01
+Catalase	0.69±0.02	0.005<p<0.01

Concentration of SOD and catalase, 200 µg/ml erythrocyte suspension; $C_{50\%}$, NaCl concentration at which 50% hemolysis was observed.

Table 2. Protective effect of cysteine and glutathione on osmotic fragility of irradiated porcine erythrocytes. Concentration of cysteine and glutathione: 200 µg/ml

Dose	$C_{50\%}$	p
30 krad	0.70±0.01	
+Cysteine	0.68±0.01	0.01<p<0.02
+Glutathione	0.68±0.01	0.01<p<0.02
50 krad	0.73±0.01	
+Cysteine	0.70±0.02	0.01<p<0.02
+Glutathione	0.71±0.01	0.01<p<0.02

1 This work was performed under the contract R.III.13.

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^{13}C NMR-study of osmoregulatory metabolites in the marine mollusc *Tapes watlingi*[1]

R.S. Norton[2] and P. de Rome

Roche Research Institute of Marine Pharmacology, Dee Why (N.S.W. 2099, Australia), 26 July 1979

Summary. Examination of homogenates of tissue from the marine bivalve mollusc *Tapes watlingi* by natural-abundance ^{13}C NMR indicates that taurine, betaine, and some free amino acids play a significant role in osmoregulation.

Recently we reported that natural-abundance ^{13}C NMR-spectroscopy provides a convenient means of identifying and quantitating the major organic metabolites in tissue from marine molluscs[3]. As it is likely that the metabolites observed in that study are involved in osmoregulation in those organisms, we decided to investigate this possibility in a representative species. In the work described here, ^{13}C NMR is used to monitor the levels of the major organic ions in tissue from specimens of the marine bivalve mollusc *Tapes watlingi* exposed to a range of external salinities. The results yield information about the osmoregulatory process in *T. watlingi*, while at the same time illustrating the advantages of ^{13}C NMR as a tool with which to study osmoregulation in marine organisms.

Materials and methods. Specimens of *T. watlingi* were collected in Sydney Harbour between March and July 1978, and kept in aerated natural sea water (collected at Dee Why headland) at room temperature (about 22 °C). The animals used in the 2-day experiments (see below) were all from a single collection which was held in natural sea water for 3 weeks before use. Reduced salinities were generated by dilution of natural sea water with deionized water,