Original Article

Effects of Oxidative Agents on the Erythrocytes from High GSH and Low GSH Sheep

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Abstract. The responses of sheep erythrocytes with high and low reduced glutathione (GSH) content were investigated under extracellular oxidative stress induced by 2,2'-azobis(amidinopropane) dihydrochloride (AAPH) and ferric ion. In terms of GSH depletion, haemolysis, lipid peroxidation and methaemoglobin formation, the extent of oxidant damage to high and low GSH sheep erythrocytes was remarkably similar. These results suggest that red cell GSH content per se is relatively unimportant in susceptibility of sheep erythrocytes to extracellular oxidative insults.

Keywords: AAPH; Erythrocyte; Ferric ion; Glutathione; Haemolysis; Lipid peroxidation; Methaemoglobin; Sheep

INTRODUCTION

Erythrocyte glutathione (GSH) is considered essential in protecting haemoglobin, some enzymes and membrane proteins and lipids from oxidative insults. GSH eliminates hydrogen peroxide and other peroxides through its oxidation to GSSG, and is regenerated by NADPH (Strivastava and Beutler 1970). GSH is maintained at appropriate levels by metabolic synthesis and regeneration. Deficiencies of the enzymes that are required for GSH synthesis, or those associated with the pentose pathway, lead to increased susceptibility of red cells to oxidative haemolysis (Beutler 1975).

Certain breeds of sheep have an inherited deficiency of GSH in erythrocytes (Board and Agar 1983). Merino-type deficiency is associated with a diminished glutamylcysteine synthetase and Finn-type deficiency is due to defective amino acid transport across the membrane. GSH-deficient cells of Finn sheep have a shortened life span and are more susceptible to oxidative stress than normal cells. GSH-deficient and normal GSH cells from Merino sheep not only have similar activities of catalase, superoxide dismutase and glutathione peroxidase (Suzuki and Agar 1983) but are also equally susceptible to oxidation caused by enzymatically generated superoxide/hydrogen peroxide (Eaton et al. 1989). These results provided evidence that red cell GSH concentration is a rela-
tively unimportant variable in oxidant susceptibility. However, Goto et al. (1993) reported that GSH-deficient erythrocytes of Merino sheep produced higher Heinz body formation than normal cells when the cells were incubated with acetylphenylhydrazine (APH). Their findings are contradictory to those of Eaton et al. (1989).

Whether GSH level per se is critical in oxidant susceptibility remains to be solved. To clarify this issue, we have examined the responses of high GSH and low GSH sheep erythrocytes to oxidant challenge by 2,2'-azobis(amidinopropane) dihydrochloride (AAPH) and ferric ion. These oxidative agents were chosen because they generate reactive oxygen species outside the cells or during interaction with the cell membrane without using haemoglobin.

MATERIALS AND METHODS

Venous blood was taken from five high GSH and five low GSH Merino sheep and collected into heparinised tubes. After centrifugation at 750 g for 10 min the plasma andbuffy coat were discarded and erythrocytes were washed three times with ice-cold 0.9% NaCl.

Incubation of Erythrocytes with AAPH

The washed erythrocytes were suspended to 10% haematocrit in medium consisting of 137 mM NaCl, 5 mM KCl, 1.2 mM MgCl₂, 5 mM glucose, 20 mM sodium phosphate buffer (pH 7.4). The erythrocyte suspension was incubated with 50 mM AAPH at 37°C. Aliquots of the suspension were withdrawn at 1, 3 and 6 h. GSH concentration was determined by the method of Beutler et al. (1963) and methaemoglobin was assayed according to the procedure of Hegesh et al. (1970). The degree of lipid peroxidation was measured by estimation of malondialdehyde (MDA) as described by Jain (1984). For the measurement of haemolysis, red cell suspension was centrifuged at 750 g for 5 min and the concentration of haemoglobin in the supernatant was determined spectrophotometrically at 540 nm. The value of 100% haemolysis was determined from the supernatant of 1 volume of erythrocytes mixed with 9 volumes of water incubated for 10 min at 37°C.

Incubation of Erythrocytes with Ferric Ion

The washed erythrocytes were suspended to 1% haematocrit in 0.9% NaCl and incubated with 1 mM ferric nitrate at 37°C. The incubation of erythrocytes was also conducted in the presence of 1 mM 8-hydroxyquinoline (oxine) which helps ferric ion to penetrate the cell membrane (Bella et al. 1990). Aliquots of the suspension were withdrawn for analysis at the indicated times. GSH, methaemoglobin concentration, haemolysis and lipid peroxidation were assayed as described above.

RESULTS

Response of Erythrocytes to AAPH

Initial erythrocyte GSH concentrations were 8.67 ± 0.12 μmol/g Hb in high-GSH sheep and 2.85 ± 0.20 μmol/g Hb in low-GSH sheep. In the presence of AAPH, GSH concentrations decreased rapidly to 50% of initial levels at 1 h whereas haemolysis and methaemoglobin formation were not yet detected (Fig. 1). At 3 h, haemolysis and methaemoglobin formation were only slight but lipid peroxidation was remarkably elevated in both high-GSH and low-GSH sheep (Table 1). At 6 h, methaemoglobin formation rose steeply to 100% and haemolysis to 40% whereas lipid peroxidation increased only a little from the levels at 3 h.

Despite a large difference in GSH concentrations, the degrees of oxidative insults produced on erythrocytes were not significantly different between high-GSH and low-GSH sheep.

Response of Erythrocytes to Ferric Ion

As shown in Fig. 2, ferric ion alone produced a 30% decrease in GSH concentrations and 25% haemolysis at 2 h in the erythrocytes of both high-GSH and low-GSH sheep. There was very little methaemoglobin formation and lipid peroxidation (not shown). When 8-hydroxyquinoline (oxine) was added, GSH depletion was greatly accelerated, and methaemoglobin formation increased to 40% at 2 h (Fig. 2a and c). However, haemolysis was virtually unaffected by oxine (Fig. 2b), and lipid peroxidation remained very little