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ADAPTATION OF THE BLOOD ANTIOXIDANT DEFENCE MECHANISMS OF SHEEP WITH A GENETIC LESION RESULTING IN LOW RED CELL GLUTATHIONE CONCENTRATIONS

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Finnish Landrace sheep with a genetic lesion which results in restricted cysteine transport across the red cell membrane have total glutathione concentrations in their red blood cells that are approximately 40% of those in normal sheep of the same breed. However, dimethyldisulphide-challenged red blood cells from both phenotypes produce an ESR-spin adduct at similar rates. The resistance of the low glutathione phenotype red cells to oxidant challenge is reflected by increases in the activities of antioxidant enzymes. Sheep with a genotypic disorder in cysteine transport may be a suitable model for studying the genetic expression of antioxidant enzymes in response to oxidant loads.

KEY WORDS: Glutathione, antioxidants, ESR, sheep.

INTRODUCTION

Approximately twenty percent of Finnish Landrace sheep have abnormally low intracellular glutathione (GSH) concentrations arising from a phenotypic expression of a genotypic effect which interferes with the transport of cysteine across the red blood cell membrane.[1] Red blood cells from these individuals may provide a system to study the influence of low glutathione status on cellular antioxidant defence mechanisms and their responses to oxidant-challenge. This can be achieved by treatment of the cells with dimethyldisulphide (DMDS), which reacts with GSH via a disulphide exchange mechanism[2] thereby decreasing the availability of thiol substrate for glutathione peroxidase and leading to an increased pool of cellular peroxides which may act as a source of hydroxyl radicals via the Fenton reaction.[3,4]

Using spin trapping and electron spin resonance (ESR) spectroscopy, the present study has compared the response of the red cells of low glutathione and normal phenotypes to DMDS-challenge. The production of free radical adducts was then related to the red cell glutathione concentrations and to the activities of the enzymes involved in the antioxidant defence system.

MATERIALS AND METHODS

Blood (8 ml) was removed via jugular venipuncture (Heparinised Vacutainers: Beckton Dickinson, Cowley, Oxford, U.K.) from 6 adult Finnish Landrace ewes of the low-glutathione phenotype (LGP) and 6 controls of the same breed (weight range 40–45 kg). Blood samples were placed on ice for the immediate determination

in whole blood of reduced and oxidised glutathione concentrations[5] and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) activity with a test-kit (Sigma Chemical Co., Poole, Dorset). The blood was then centrifuged (1500 × g, 4°C, 15 min) and the plasma was stored at -70°C prior to the measurement of concentrations of conjugated dienes[6], thiobarbituric acid reactive substances (TBARS)[7] and vitamin E[8]; the activities of pyruvate kinase (EC 2.7.1.40) and creatine kinase (EC 2.7.3.2) in plasma were determined with test kits (Boehringer Mannheim UK, Lewes, Sussex and Sigma Chemical Co., Poole, Dorset). The red cells were washed twice and resuspended to the original blood volume in iso-osmotic phosphate-buffered saline (pH 7.4). Red cell glutathione peroxidase (EC 1.11.1.9), superoxide dismutase (EC 1.15.1.1), catalase (EC 1.11.1.6) and glutathione reductase (EC 1.6.4.2) activities were determined as described previously.[9]

Free radical generation in red cells exposed to DMDS was measured by an ESR spin trapping procedure.[3] In brief, 2 ml of the reconstituted red cell suspension and an equal volume of the spin trap α-(4-pyridyl 1-oxide)-N-*tert*-butylnitrone (4-POBN; 200 mM in iso-osmotic phosphate-buffered saline, pH 7.4; Aldrich, Gillingham, UK) were added to a conical-bottomed glass vial ("Reactivial", Pierce, Rockford, Illinois, USA) open to the atmosphere, maintained at 37°C with constant stirring. Following the addition of 0.6 μl DMDS, aliquots (0.6 ml) were transferred at regular intervals (see Figure 1) to a Bruker ECS 106 spectrometer operating at 9.5 GHz (X-band) frequency with a cylindrical (TM mode) cavity, microwave power level of 10 mW, field modulation of 100 kHz and amplitude of 0.2 mT.

All values are expressed as mean ± SEM and the Student 2-tailed t-test was used to compare mean values between the two groups (LGP vs controls).

RESULTS

The mean total glutathione concentration in the whole blood of LGP sheep was 40% of that in the control animals. In addition, there was a significantly higher concentration of oxidised glutathione in the LGP sheep compared with the normal phenotype (Table I).

Despite these differences in glutathione concentration, the rate of production of a 4-POBN-spin adduct by DMDS-challenged red cells was similar in both groups of sheep (Figure 1). The adduct had an isotropic g-value of 2.0060 ± 0.0005, $A(^{14}N) = 1.57 \pm 0.01$ mT and $A(^{1}H) = 0.26 \pm 0.01$ mT where $A(^{14}N)$ refers to the isotropic hyperfine coupling costant arising from the interaction of the unpaired

TABLE I

Total, reduced and oxidised glutathione concentrations on whole blood of sheep with a genetic lesion which interferes with cysteine transport and results in low glutathione concentrations (low phenotype) and controls of the same breed (normal phenotype).

Parameter	Low phenotype	Normal phenotype	P
Total glutathione (mg/gHb)	0.94 ± 0.09	2.34 ± 0.36	0.003
Reduced glutathione (mg/gHb)	0.71 ± 0.09	2.27 ± 0.36	0.002
Oxidised glutathione (mg/gHb)	0.08 ± 0.016	0.03 ± 0.004	0.02
Reduced/oxidised glutathione	14 ± 4.8	78 ± 16	0.003

Data as mean ± SEM: 6 sheep per group.

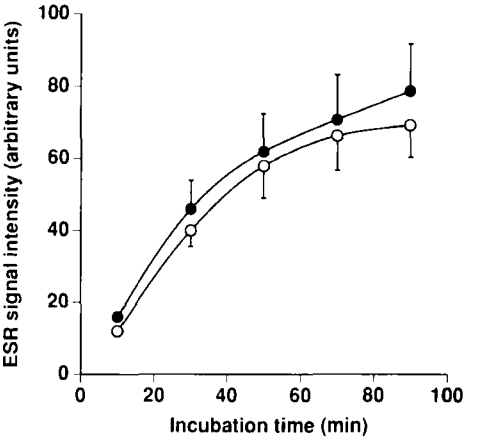

FIGURE 1 Rate of formation of 4-POBN spin adduct intensity by red blood cell suspensions from sheep with a genetic lesion which interferes with cysteine transport and results in low glutathione concentrations (low phenotype (○)) and controls of the same breed (normal phenotype (●)) following challenge with DMDS. Data as mean ± SEM: 6 sheep per group.

electron with the nuclear spin (I = 1) of the nitrogen moiety on the trap and A(^{1}H) is the coupling constant arising from the interaction with the nuclear spin (I = 1/2) of the hydrogen at the β-position to the radical centre on the trap.

Activities of erythrocyte superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase were significantly greater in the LGP sheep than in controls whereas vitamin E concentrations and the activity of glucose-6-phosphate dehydrogenase were similar in both groups (Table II). Plasma pyruvate kinase and creatine kinase activities and concentrations of conjugated dienes and thiobarbituric acid reactive substances (TBARS) were not significantly different in the two groups (Table III), indicating that there was no increase in tissue damage or lipid peroxidation in the LGP sheep.

DISCUSSION

The 4-POBN ESR spin adduct detected in the DMDS-challenged sheep red blood cell suspensions was identical to that previously reported[3] and is consistent with a carbon-centred species arising from the breakdown of linoleic acid.[10] Similar

TABLE II

Concentration of vitamin E and activities of antioxidant enzymes in red cells of sheep with a genetic lesion which interferes with cysteine transport and results in low glutathione concentrations (low phenotype) and controls of the same breed

Parameter	Low phenotype	Normal phenotype	P
Vitamin E (μg/gHb)	2.45 ± 0.38	2.45 ± 0.55	NS
Glucose-6-phosphate dehydrogenase (U/gHb)	1.55 ± 0.11	1.29 ± 0.11	NS
Superoxide dismutase (mg/gHb)	0.72 ± 0.14	0.26 ± 0.08	0.016
Catalase (K/gHb)	219 ± 19	155 ± 17	0.030
Glutathione peroxidase (U/gHb)	373 ● 9	257 ± 37	0.012
Glutathione reductase (U/gHb)	0.076 ± 0.006	0.051 ± 0.008	0.030

Data as mean ± SEM: 6 sheep per group: NS, not significantly different.

TABLE III
Indices of muscle damage and lipid peroxidation in plasma of sheep with phenotypically low red cell glutathione concentrations (low phenotype) and controls of the same breed

Parameter	Low phenotype	Normal phenotype	P
Pyruvate kinase (U/l)	73 ± 12	82 ± 9	NS
Creatine kinase (U/l)	30 ± 3	37 ± 4	NS
TBARS (nmol MDA/ml)	1.70 ± 0.17	1.63 ± 0.23	NS
Conjugated dienes (RU/ml)	66 ± 5	56 ± 11	NS

Data as mean ±SEM: 6 sheep per group: TBARS, thiobarbituric acid reactive substances: MDA, malonaldehyde equivalents: NS, not significantly different.

adducts have been observed following iron-initiated oxidation of tissue homogenates and liver microsomal preparations[11,12] which suggests that DMDS is a useful alternative probe for challenging the overall antioxidant capacity of biological preparations.

The rate of increase of the 4-POBN adduct by the red cells of the LGP sheep was similar to that in the controls despite their markedly lower glutathione concentrations. However, the increased activities of several antioxidant enzymes in the glutathione-deficient red cells suggest that there was compensatory adaptation in their red cell antioxidant defence system. Such adaptations occur in farm and experimental animals and humans experiencing prolonged oxidant loads arising from genetic disorders,[9] nutritional antioxidant deficiency,[13] exercise[14] and smoking[15]. Therefore, LGP sheep may be a useful model for the study of the control of the genetic expression of antioxidant enzymes in response to increases in oxidant metabolites. Exposure to a sustained oxidant stress is also consistent with an increased turnover of glutathione as indicated by the significant increase in the oxidised glutathione concentrations in blood from the LGP sheep.

The ESR data indicate that the red cells from LGP sheep were no more susceptible to DMDS-challenge than were the controls. Moreover, plasma indices of muscle damage and lipid peroxidation were similar in the two groups of sheep, suggesting that the low glutathione status did not result in an additional free radical burden which exceeded the protective capacity of the antioxidant defence system. This does not exclude the possibility that the imposition of a chronic, as opposed to acute, free radical stress could be more deleterious to LGP sheep compared with controls given that other antioxidants are already showing a compensatory response under benign management conditions.

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