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THE RELATIONSHIP BETWEEN REDUCED GLUTATHIONE LEVEL AND GLUTATHIONE S-TRANSFERASE ACTIVITY IN SHEEP ERYTHROCYTES

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Abstract

The relationship between reduced glutathione (GSH) level and glutathione S-transferase (GST) activity in erythrocytes was examined, using sheep erythrocytes, which have varying GSH concentrations, and dog erythrocytes with an inherited high concentration of GSH. There was a positive correlation (r= 0.529, p<0.001) between the GSH level and GST activity in sheep erythrocytes. In dog erythrocytes, the GST activity in high-GSH cells was significantly (p< 0.001) higher than that in normal-GSH cells. These results indicate that the activity of GST in erythrocytes is directly correlated with the intracellular GSH level.

Key words : glutathione, glutathione S-transferase, sheep erythrocyte

Introduction

Glutathione S-transferase is present in most human and animal tissues investigated so far. Through its catalytic and non-catalytic functions, this enzyme is presumed to protect the tissues from the toxic effects of xenobiotics[9,10] and endogeneous lipid hydroperoxides[11,16]. This enzyme has been found in erythrocytes of humans and some domestic animals[8,15]. Erythrocyte GST catalyzes the conjugation of electrophilic agents, such as 1–chloro–2, 4–dinitrobenzene, to form glutathione thiol ethers, and detoxifies them[4]. The physiological role of this enzyme in erythrocytes is not known, but it has been suggested that the location of the enzyme in erythrocytes is ideal for the removal of circulating xenobiotics[15]. However, the normal substrate of GST in erythrocytes has not been determined.

Recently, Beutler et al.[6] reported that a patient with a deficiency of GSH-synthetase activity of erythrocytes, showed not only GSH deficiency but also a severe

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deficiency of GST in the erythrocytes. They assumed that the deficiency of GST in the erythrocytes of the patient was due to the instability of this enzyme in the absence of adequate intracellular GSH levels. If GSH is indispensable to stabilize GST as suggested by Beutler et al., it seems likely that there is a relation between the GSH level and GST activity in erythrocytes. However, Beutler et al.[7] also found a patient with hemolytic anemia characterized by unexplained erythrocyte GST deficiency. Interestingly, the level of GSH in the erythrocytes of this patient was normal, while the GST activity was only about 15% of the normal mean value. Thus, the relationship between the GSH level and GST activity is still unclear.

To clarify this interesting problem, we examined the relationship between the GSH level and GST activity in erythrocytes, using sheep and dogs as experimental animals. Sheep erythrocytes seemed to be suitable for this experiment, because individual sheep have widely different levels of erythrocyte GSH. Furthermore, we used some dogs with an inherited high concentration of erythrocyte GSH.

MATERIALS AND METHODS

Erythrocytes were obtained from 80 adult Australian merino sheep, six mixed breed dogs with an inherited high concentration of erythrocyte GSH and six dogs with a normal level of GSH. Sheep were raised in the University of New England, Australia and the dogs were kept at Hokkaido University, Japan. Venous blood was collected into heparinized tubes from each animal. The blood was filtered through microcrystalline cellulose-α-cellulose to remove the leukocytes and platelets. Erythrocyte GSH was measured in metaphosphoric acid extracts using 5–5'–dithiobis (2–nitrobenzoic acid)[5]. Both GSH and the GST activity of erythrocytes were determined according to the method of Beutler[5].

Statistical analysis was carried out using student's t-test for paired observations. Linear regression analysis of the data in Fig. 1 (A) was performed to obtain the product-moment correlation value (r).

RESULTS

Erythrocyte GSH levels in the sheep varied from 0.5 to 16.5 μmol/g hemoglobin. The distribution of the erythrocyte GSH concentration in these sheep is shown in Fig. 1 (B). The GST activity in sheep erythrocytes varied from 2.4 to 12 IU/g hemoglobin. A significant and positive correlation was found (r=0.529, p<0.001) between the erythrocyte GSH level and GST activity (Fig. 1 (A)).

Table 1 shows the GSH concentration and GST activity in high-and normal-GSH dog erythrocytes. The level of erythrocyte GSH in high-GSH dogs was about six times the normal level. The activity of GST in high-GSH erythrocytes was significantly (p<0.001) higher than that in normal-GSH erythrocytes.
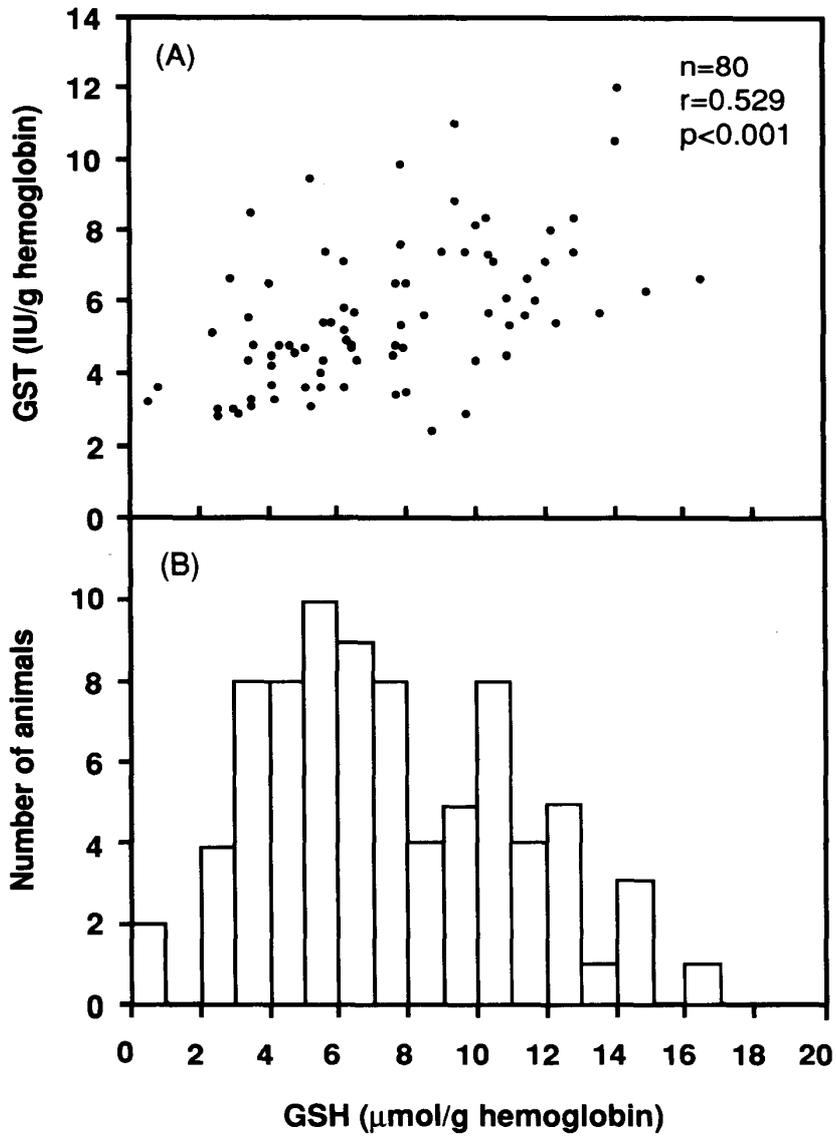
Fig. 1. The correlation between GSH content and GST activity (A), and the distribution of the GSH concentrations in sheep erythrocytes (B).

Table 1. Concentrations of GSH and GST activities in high-and normal-GSH dog
         erythrocytes (mean ± SD, n= number of animals)

Cell type	GSH (μmol/g hemoglobin)	GST (IU/g hemoglobin)
High-GSH erythrocyte (n=6)	52. 38±9. 09*	5. 87±0. 75*
Normal-GSH erythrocyte (n=6)	9. 46±0. 97	3. 35±0. 53

* Significantly different from the value for normal-GSH erythrocytes (P<0.001).

DISCUSSION

The present study demonstrated that the activity of GST of erythrocytes is correlated with the intracellular GSH level.   That is, erythrocytes with high intracellular GSH levels exhibited high GST activity in both sheep and dogs.   In regard to human erythrocyte GST, Beutler et al.[7] demonstrated that there was a significant positive correlation between GST and hexokinase activity, and suggested that GST is affected by erythrocyte age, because hexokinase is a well-known enzyme exhibiting a rapid loss of activity with increasing erythrocyte age.   In general, the concentration of GSH in young erythrocytes is higher than that in mature cells and it decreases with aging of erythrocytes[17].   As reported previously, dogs having inherited high-GSH erythrocytes also show high activity of hexokinase[14].   The high level of erythrocyte GSH in these dogs is due to increased glutamate transport in erythrocytes that is accelerated by the function of Na,K-ATPase in these cells[12].   Since Na,K-ATPase was found in dog reticulocytes[13], but not in dog mature erythrocytes, it was assumed that the high-GSH cells may have remained at an immature stage of their development.   From this, the high activity of GST observed in high-GSH dog erythrocytes in the present study may also reflect the immaturity of these cells, and may support the opinion of Beutler et al. mentioned above.   However, Scott and Wright[18] reported that the level of GST in human erythrocytes was varied by at least sixfold, and that the variation of the GST level was not due to a difference in the ratio of immature to old cells.   Furthermore, the level of erythrocyte GSH in the sheep used in the present study was not dependent on the cell age but on the activity of γ-glutamylcystein synthetase, and there were no differences in biochemical and physiological properties between low-and normal-GSH cells except for the level of GSH[1-3,19].   On the basis of the results obtained from sheep erythrocytes, we conclude that the activity of GST in erythrocytes may not be dependent on erythroyte age but directly correlated with the intracellular GSH level.   The present study also suggests that GSH may be indispensable to stabilize GST.

REFERENCES

1) AGAR, N. S. & SMITH, J. E. (1973): Glutathione regeneration related to enzyme activities in erythrocytes of sheep. Enzyme, 14, 82–86

2) AGAR, N. S. (1975): Glutathione polymorphism in the sheep red blood cells. Int. J. Biochem., 6, 843–852

3) AGAR, N. S., ROBERTS, J. & SHEEDY, J. (1975): Certain features of erythrocytes of normal and glutathione-deficient sheep. Am. J. Vet. Res., 36, 949–951

4) AWASTHI, Y. C., MISRA, G., RASSIN, D. K. & SRIVASTAVA, S. K. (1983): Detoxification of xenobiotics by glutathione S-transferases in erythrocytes: the transport of the conjugate of glutathione and 1–chloro–2, 4–dinitrobenzene. Br. J. Haematol., 55, 419–425

5) BEUTLER, E. (1984): Red Cell Metabolism. A manual of biochemical methods, 3 ed. 122–157, Orlando: Grune and Stratton

6) BEUTLER, E., GELBART, T. & PEGELOW, C. (1986): Erythrocyte glutatione synthetase deficiency leads not only to glutathione but also to glutathione-S-transferase deficiency. J. Clin. Invest., 77, 38–41

7) BEUTLER, E., DUNNING, D., DABE, I. B. & FORMAN, L. (1988): Erythrocyte glutathione S-transferase deficiency and hemolytic anemia. Blood, 72, 73–77

8) BOARD, P. G. & AGAR, N. S. (1983): Glutathione metabolism in erythrocytes. In: Red blood cells of domestic mammals Ed. Agar N. S. & Board P. G. 253–290, Amsterdam: Elisvier

9) CHASSEUAD, L. F. (1979): The role of glutathione and glutathione S-transferases in the metabolism of chemical carcinogens and other electrophilic agents. Adv. Cancer Res., 29, 175–274

10) JAKOBY, W. B. (1978): The glutathion S-transferases: A group of multifunctional detoxification proteins. Adv. Enzymol. Relate. Areas Mol. Biol., 46, 383–414

11) LAWRENCE, R. A. & BURK, R. F. (1976): Glutathione peroxidase activity in selenium-deficient rat liver. Biochem. Biophys. Res. Commun., 71, 952–958

12) MAEDE, Y., KASAI, N. & TANIGUCHI, N. (1982): Hereditary high concentration of glutathione in canine erythrocytes associated with high accumulation of glutamate, glutamine, and aspartate. Blood., 59, 883–889

13) MAEDE, Y. & INABA, M. (1985): (Na,K)-ATPase and ouabain binding in reticulocytes from dogs with high K and low K erythrocytes and their changes during maturation. J. Biol. Chem., 260, 3337–3343

14) MAEDE, Y. & INABA, M. (1987): Energy metabolism in canine erythrocytes associated with inherited high Na^+-and K^+-stimulated adenosine triphosphatase activity. Am. J. Vet. Res., 48, 114–118

15) MARCUS, C. J., HABIG, W. H. & JAKOBY, W. B. (1978): Glutathione transferase from human erythrocytes. Arch. Biochem. Biophys., 188, 287–293

16) PROHASKA, J. R. & GANTHER, H. E. (1977): Glutathione peroxidase activity of glutathione-S-transferases purified from rat liver. Biochem. Biophys. Res. Commun., **76**, 437–445

17) SASS, M. D., CARUSO, C. J. & O'CONNELL, D. J. (1965) Decreased glutathione in aging red cells. Clin. Chim. Acta, **11**, 334–340

18) SCOTT, E. M. & WRIGHT, R. C. (1980): Variability of glutathione S-transferase of human erythrocytes. Am. J. Hum. Genet., **32**, 115–117

19) SUZUKI, T. & AGAR, N. S. (1983): Glutathione peroxidase, superoxide disumutase and catalase in the red blood cell of GSH-normal and GSH-deficient sheep. Experientia, **39**, 103–104