Comparison of erythrocyte antioxidative enzyme activities between two types of haemoglobin H disease

S PRASARTKAEW, A BUNYARATVEJ, S FUCHAROEN,* P WASI*

*From the Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, and the *Department of Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University Bangkok, Thailand*

SUMMARY The activities of erythrocyte antioxidative enzymes were measured in two groups of patients with different genotypes of haemoglobin (Hb) H disease: 21 with α -thalassaemia 1 or α -thalassaemia 2 (α -thalassaemia 1/2) and 21 with α -thalassaemia 1/Hb Constant Spring (HbCS). They were compared with 21 normal subjects. Both genotypes of Hb H disease had increased activities of erythrocyte superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase when compared with those of controls. Comparison of the two genotypes showed that subjects with α -thalassaemia 1/Hb CS, the more severe disease, had higher SOD and GSH-Px activities but lower catalase activity than those with α -thalassaemia 1/2. This indicates that there are compensatory mechanisms in Hb H erythrocytes to cope with increased generation of oxygen free radicals as a result of increased excess β chain.

Autoxidation of haemoglobin can produce superoxide free radicals, which can cause red cell damage, leading to short red cell survival. Production of the superoxide is increased in the presence of isolated α or β globin chains of the haemoglobin molecule. Haemoglobin (Hb) H disease, one of the thalassaemia syndromes, is characterised by excess β chains, which form relatively insoluble β_4 (Hb H). This should lead to generation of an increased amount of superoxide. Evidence supporting this hypothesis is an increased erythrocyte superoxide dismutase (SOD) activity in Hb H patients.¹ SOD is an enzyme that catalyses the breakdown of superoxide into the less potent oxidant H_2O_2 and oxygen to lessen the oxidative damage to the cell. The H_2O_2 , still capable of producing cell damage, is further reduced to water and oxygen by glutathione peroxidase (GSH-Px) and catalase.²

There are two common genotypes of Hb H disease: one results from double heterozygosity between α -thalassaemia 1 and α -thalassaemia 2 genes (α -thalassaemia 1/2), and the other from concomitant inheritance of α -thalassaemia 1 and Hb Constant Spring genes (HbCS). There are some differences between these two genotypes, with a greater proportion of red cells containing inclusion bodies, and higher levels of Hb Bart's and Hb H being seen in the

HbCS variety.³ These differences suggest that there might be different degrees of red cell oxidative stress and so we measured the activities of SOD, GSH-Px, and catalase in the red cells of the two types of Hb H disease.

Material and methods

Sixty three subjects were examined, 21 with genotypes α -thalassaemia 1/2, 21 with HbCS, and 21 normal controls. The patients were from the files of the division of haematology, department of medicine, faculty of medicine, Siriraj Hospital; the normal controls were laboratory staff. The patients had received no blood transfusions for at least three months before examination. All of them were in steady state. Detailed clinical and laboratory findings of the patients have been reported previously.^{4,5} Informed consent was obtained from all subjects.

HAEMATOLOGICAL STUDIES

Red cell count, white cell count, mean corpuscular volume and haematocrit were determined in a Coulter counter, model ZF6. Haemoglobin concentrations were measured on a Coulter haemoglobinometer. Haemoglobin types were identified by starch-gel electrophoresis, using tris-edetic acid-borate buffer (pH 8.6).⁶ Quantitation of HbA₂, HbH, and Hb Bart's was performed by cellulose-acetate electrophoresis.⁷

PREPARATION OF HAEMOLYSATES FOR ENZYME ASSAY

The heparinised blood was centrifuged; plasma and buffy coat were removed by aspiration. Packed red cells were washed three times with ice cold isotonic

buffer. Leucocytes and platelets were removed according to the method of Oski and Bowman.⁸ About a 20% red cell suspension was filtered through Whatman No 2 filter paper. The filtrate was collected, washed twice with 10 volumes of ice cold isotonic

Activities of erythrocyte antioxidant enzymes (a) superoxide dismutase, (b) glutathione peroxidase, and (c) catalase in normal subjects (N), patients with α -thalassaemia 1/2 (H), and those with HbCS (H + CS). Bars represent mean (SD).

buffer, and resuspended to a 50% cell suspension. The cell suspension was haemolysed and assayed for SOD,⁹ GSH-Px, and catalase.¹⁰

ENZYME ASSAYS

The method for SOD measurement is based on the ability of the enzyme to inhibit the reduction of nitroblue tetrazolium by superoxide radical, which is generated by the reaction of photoreduced riboflavin and oxygen.⁹ Measurement of erythrocyte GSH-Px activity depends on the oxidation of NADPH by the oxidised form of glutathione (GSSG), which is generated by a catalytic reaction of GSH-Px.¹¹ The method for measuring catalase activity is based on the ability of catalase to decompose hydrogen peroxide.¹² Units of the enzyme assays were reported as U/g Hb, U/ml red blood cells, and U/10¹⁰ red blood cells.

Results

Figs a–c show the results of SOD, GSH-Px, and catalase activities. Erythrocytes from both genotypes of Hb H disease had higher SOD activities than the normal erythrocytes in all units determined. The Hb CS erythrocytes also had higher SOD activities than the α -thalassaemia 1/2 erythrocytes, but only on the basis of U/g Hb and U/10¹⁰ red blood cells not U/ml red blood cells.

Erythrocyte GSH-Px activity was higher in both genotypes when compared with normal values. GSH-Px activity was also higher in Hb CS erythrocytes compared with the values for α -thalassaemia 1/2 genotype in all respects.

Red cell catalase activity of α -thalassaemia 1/2 erythrocytes was higher than normal erythrocytes

Mean (SD) GSH-Px, catalase, and SOD, activities and concentrations of red cells in top and bottom layers

Cell population	Reticulocyte count (%)	SOD (U/gHb)	GSH-Px (IU/gHb)	Catalase ($\times 10^4$ IU/gHb)
Top layer (n = 6)	5.0 (0.4)	7214 (826)	65.09 (7.77)	31.52 (0.81)
Bottom layer (n = 6)	1.8 (0.3)	6897 (1120)	63.72 (4.77)	30.96 (3.06)
p value	< 0.001	NS	NS	NS

only when expressed as $\bar{U}/g$ Hb and U/ml red blood cells, and catalase activity of Hb CS erythrocytes was statistically higher than normal only in U/g Hb. The activity of catalase was higher in α -thalassaemia 1/2 than in Hb CS, expressed both as U/g Hb and U/ml red blood cells. No statistical differences were found between the three groups when catalase activity was expressed as U/10¹⁰ red blood cells.

To exclude the effect of excess young cells on enzyme activities whole blood from six patients was centrifuged and separated into top and bottom layers. Reticulocyte counts and the three enzyme activities were measured in both fractions (table). No significant differences in activities of all the studied enzymes were found between the top and the bottom layers.

Discussion

Red cells of patients with Hb H disease contain excess β -globin chains that are unstable and polymerise to form β_4 inclusion bodies. Concentrations of Hb H and the number of red cells containing inclusion bodies were greater in patients with the genotype of Hb CS compared with those with the genotype α -thalassaemia 1/2.³ In addition, the distribution pattern of inclusion bodies was also different between the two genotypes.¹³ Our findings indicate that patients with the more severe disease, Hb CS, having a greater excess of β -globin chain, also have higher activities of SOD and GSH-Px but lower catalase activity than the milder genotype, α -thalassaemia 1/2.

Increased red cell SOD values in thalassaemic patients have previously been explained as a reaction to, or compensation for, the increased production of superoxide radicals, the amount of which is related to excess globin chain.¹ Our results show that the greater increase in SOD activity is seen in Hb CS erythrocytes which have the highest numbers of red cell inclusions, the highest Hb H concentrations, and the greatest clinical severity of the two genotypes.

The generally increased red cell GSH-Px activity in patients with Hb H disease we describe has previously been reported.¹⁴ We found also that the values of GSH-Px are higher in the genotype Hb CS than the α -thalassaemia 1/2. The greater SOD activity in Hb CS erythrocytes probably generates a greater amount of H₂O₂, which in turn induces a compensatory rise in GSH-Px activity. An increase in red cell GSH-Px activity has been shown in vitro by the addition of acetylphenylhydrazine, an agent promoting release of superoxide radicals from haemoglobin.¹⁵

The Hb CS erythrocytes produce higher concentrations of H₂O₂ than α -thalassaemia 1/2 erythrocytes. This study shows significantly lower red cell catalase activity (although higher than normal sub-

jects), in patients with the more severe form of the disease expressed both as per g Hb and as per ml red blood cells. A possible explanation for lower red cell catalase activity found in the more severe genotype of Hb H disease is that the greater amount of H₂O₂ might produce direct toxic damage to catalase.¹⁶ Although such damage can be prevented by NADPH,^{16,17} the concentration of this is considerably reduced in conditions of high oxidative stress. In Hb CS erythrocytes H₂O₂ is mainly detoxified to water and oxygen through the catalytic reaction of GSH-Px in which the GSH is simultaneously converted to GSSG. The GSSG is then reduced back to GSH by glutathione reductase, which requires NADPH. Decreased amounts of NADPH could result in insufficient protection of the toxic effect of H₂O₂ to catalase, leading to the lower activity of this enzyme.

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Requests for reprints to: Dr A Bunyaratvej, Department of Pathology, Ramathibodi Hospital, Rama Road 6, Bangkok 10400, Thailand.