SHORT COMMUNICATION

GLUTATHIONE PEROXIDASE DEFICIENCY WITH INCREASED SUSCEPTIBILITY TO ERYTHROCYTE HEINZ BODY FORMATION

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SUMMARY

1. The production of Heinz bodies, after incubation of erythrocytes in the presence of hydrogen peroxide vapour, was significantly greater in erythrocytes with abnormally low glutathione peroxidase (GSH-Px) activity than in cells with normal enzyme activity.

2. The addition of the catalase inhibitor, sodium azide, to the incubation mixture, increased the formation of Heinz bodies after exposure to hydrogen peroxide vapour; the percentage of Heinz bodies was significantly greater in erythrocytes with low GSH-Px activity than in normal erythrocytes.

3. Erythrocyte GSH-Px deficiency thus predisposes the cell to Heinz body formation when exposed to oxidant stress.

Key words: erythrocyte enzymes, glutathione peroxidase, Heinz bodies.

Glutathione peroxidase (GSH-Px) (glutathione : hydrogen peroxide oxidoreductase, EC 1.11.1.9) is an enzyme which detoxifies peroxides, including hydrogen peroxide, via the oxidation of reduced glutathione (GSH):

\[ 2\text{GSH} + \text{H}_2\text{O}_2 \xrightarrow{\text{GSH-Px}} \text{GSSG} + 2\text{H}_2\text{O} \]

This enzyme is of importance in preventing oxidative changes in the human erythrocyte (Cohen & Hochstein, 1963; Hill, Haut, Cartwright & Wintrobe, 1964). Low activities of GSH-Px occur in most infants, particularly premature infants (Gross, Bracci, Rudolph, Schroeder & Kochen, 1967; Necheles, Boles & Allen, 1968; Bracci, Corvaglia, Princi, Bettini & Pindinelli, 1969; Emerson, Mason & Cuthbert, 1972); however, a normal activity of GSH-Px is usually attained by the age of 6 months (Necheles, Steinberg & Cameron, 1970). Reports...
have appeared of a moderate to severe deficiency of GSH-Px occurring as a genetically deter-
minded enzymopathy. In infants the expression of this has included haemolysis (Gross et al., 1967; Necheles et al., 1968; Bracci et al., 1969), and in adults drug-induced haemolysis (Boivin, Galand, Hakim & Blery, 1970; Steinberg, Brauer & Necheles, 1970; Steinberg & Necheles, 1971) and chronic haemolytic anaemia (Boivin, Galand, Hakim, Roge & Gueroult, 1969; Necheles et al., 1970) have been reported. In a study of GSH-Px in human erythrocytes in health and disease, we previously reported low activities of the enzyme in a number of cases of chronic disease (Hopkins & Tudhope, 1973). In several cases, this deficiency was as severe as the reported cases of genetic deficiency associated with haemolysis and drug-induced haemolytic anaemia. In a study of the relationship of plasma tocopherol concentrations to Heinz body susceptibility we referred to one case of GSH-Px deficiency where increased Heinz body formation occurred on exposure of erythrocytes to hydrogen peroxide (Tudhope & Hopkins, 1974a). We now report further studies of the susceptibility to Heinz body forma-
tion in erythrocytes with GSH-Px deficiency.

SUBJECTS AND METHODS

Blood was obtained by venepuncture from normal subjects (medical and laboratory personnel) and from patients who had been admitted to the acute general medical wards at Maryfield Hospital, Dundee. The blood samples were collected in tubes containing lithium heparin.

Erythrocyte glutathione peroxidase (GSH-Px) was assayed within a few hours of venepunc-
ture as described by Hopkins & Tudhope (1973), an assay based, with minor modifications, on the method of Paglia & Valentine (1967). This method depends upon a reduced nicotin-
amide-adenine dinucleotide phosphate (NADPH)-coupled reaction, in which the oxidized glutathione (GSSG) resulting from the activity of GSH-Px is converted into reduced gluta-
thione (GSH) by added glutathione reductase (GSSG-R) and NADPH. The rate of change in NADPH concentration was measured at 25°C with a Pye–Unicam SP.800 recording spectrophotometer. To 0.1 ml of the mixture of lysate and Drabkin's solution was added 2.58 ml of 0.15 mol/l phosphate buffer (pH 7.0) containing 5 mmol/l EDTA. The following solutions were then added: 0.1 ml of 8.4 mmol/l NADPH, 0.005 ml of GSSG-R (Sigma London Chemical Co. Ltd), 0.01 ml of 1.25 mol/l sodium azide and 0.1 ml of 0.15 mol/l GSH. The reagents were allowed to equilibrate for 5 min and the reaction was then initiated by the addition of 0.1 ml of 2.2 mmol/l H$_2$O$_2$. The activity of the enzyme was defined as μmol of NADPH oxidized/ min under the conditions used and was expressed as the activity of GSH-Px per 10$^{10}$ erythro-
cytes (units/10$^{10}$ erythrocytes).

Erythrocyte catalase (hydrogen peroxide: hydrogen peroxide oxidoreductase, EC 1.11.1.6) was assayed as described by Tudhope (1967). Erythrocyte glucose 6-phosphate dehydrogenase (d-glucose 6-phosphate : NADP$^+$ oxidoreductase, EC 1.1.1.49; G6PD) was measured as described by Tarlov & Kellermeyer (1961). Erythrocyte GSH was measured by the method of Beutler, Duron & Kelly (1963) and plasma tocopherol by the method of Bieri & Prival (1965).

The mean and sd for erythrocyte GSH-Px for sixty-two normal subjects was 6.23 ± 1.35 units/10$^{10}$ erythrocytes (Hopkins & Tudhope, 1973). Erythrocyte GSH-Px deficiency was defined as an enzyme activity less than 2 sd below the normal mean, i.e. < 3.53 units/10$^{10}$ erythrocytes. Erythrocyte GSH-Px activities in the six patients studied were: 2.21, 2.33, 2.40, 2.53, 2.70 and 3.17 units/10$^{10}$ erythrocytes. In these patients the diagnoses were: haemo-
Susceptibility to erythrocyte Heinz body formation

chromatosis, chronic renal failure, advanced carcinoma of the breast, rheumatoid arthritis, chronic lymphatic leukaemia and phenacetin-induced nephropathy respectively.

In all patients with GSH-Px deficiency normal activities of erythrocyte catalase (1·01–1·53 x 10^{-8} units/g of haemoglobin), glucose 6-phosphate dehydrogenase (8·1–16·8 units/g of haemoglobin) and GSH (2·19–3·56 mmol/l of erythrocytes; 67·5–109·5 mg/100 ml of erythrocytes) were found. The plasma tocopherol concentration was normal (>11·6 μmol/l; >0·5 mg/100 ml) in all except one case in which the low value of 8·6 μmol/l (0·37 mg/100 ml) was found.

The experimental procedure has been described previously (Tudhope & Hopkins, 1974b) and consists of exposing erythrocyte suspensions in the outer well of a Conway unit to oxidant stress in the form of H_2O_2 vapour. Hydrogen peroxide (1·8 ml, 8·8 mol/l) was present in the inner well of the unit; in some experiments the catalase inhibitor sodium azide (0·1 mmol/l) was included in the erythrocyte suspension in the outer well. After incubation in an oven for 3 h at 37°C, the erythrocyte suspension was removed by Pasteur pipette and stained for Heinz bodies with Crystal Violet (Tudhope & Hopkins, 1974b).

RESULTS

Susceptibility to Heinz body formation was studied in six patients with erythrocyte GSH-Px deficiency, by exposing erythrocyte suspensions with and without the addition of sodium azide to H_2O_2 vapour. Similar experiments were performed on suspensions of erythrocytes from thirty-two normal (control) subjects. The results are shown in Fig. 1.

In thirty-two experiments, when normal erythrocytes were exposed to H_2O_2 vapour in the

![Fig. 1. Erythrocyte Heinz body formation after peroxidation of normal and GSH-Px-deficient cells (a) with hydrogen peroxide and (b) with hydrogen peroxide plus sodium azide.](attachment:image.png)
absence of sodium azide, the mean (±SD) percentage of cells showing Heinz bodies was 4.8±2.3. With GSH-Px-deficient cells the corresponding values were 11.5±6.3%; the difference between these means is significant (P<0.01).

With both normal and GSH-Px-deficient erythrocytes, the addition of sodium azide alone resulted in Heinz body formation in less than 1.5% of cells. When erythrocytes were exposed to H₂O₂ after the addition of sodium azide, the formation of Heinz bodies was 13.6±5.5% in six experiments with normal cells, and was significantly higher at 36.6±14.9% in GSH-Px-deficient cells (P<0.005).

DISCUSSION

Since the discovery of glutathione peroxidase by Mills (1957) it has become apparent that this enzyme plays an important role in the detoxification of H₂O₂ in the erythrocyte. Hydrogen peroxide may be generated in the erythrocyte as the result of therapy with certain oxidant drugs such as anti-malarials of the 8-aminoquinoline group (Cohen & Hochstein, 1964, 1965; Dausset & Contu, 1969). The potentially haemolytic effect of the therapeutic use of such drugs in subjects with deficiency of glucose 6-phosphate dehydrogenase (G6PD) is well known. G6PD is closely associated with glutathione reductase and GSH-Px in the detoxification of erythrocyte H₂O₂ (Scheme 1).

\[
\begin{align*}
\text{H}_2\text{O}_2 & \quad \text{GSH} \quad \text{NADP}^+ \quad \text{G6P} \\
\text{GSH-Px} & \quad \text{GSSG-R} \\
\text{H}_2\text{O} & \quad \text{GSSG} \quad \text{NADPH} \\
\end{align*}
\]

Scheme 1

A deficiency of any of these enzymes may therefore seriously affect the ability of the cell to deal with peroxides. In previously reported cases of GSH-Px deficiency this was shown by haemolytic anaemia and drug-induced haemolysis. Results in the present study have shown that deficiency of GSH-Px may also predispose the cell to Heinz body formation in a situation where there is oxidant stress. Certain patients with chronic debilitating disease, including carcinoma, may have low activities of erythrocyte GSH-Px (Hopkins & Tudhope, 1973) and of erythrocyte catalase (Tudhope, 1967); these enzyme deficiencies may reflect a general impairment of protein metabolism. As GSH-Px and catalase are both involved in the protection of the erythrocyte against oxidant stress, the possibility arises that drug therapy in such patients may contribute to some degree of drug-induced haemolysis, which may be unrecognized in the presence of the other haematological changes associated with the chronic disease.

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REFERENCES


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