BRIEF REPORT

Hemozygous Erythrocyte Glutathione-Peroxidase Deficiency: Clinical and Biochemical Studies

By T. F. Necheles, Norman Maldonado, Antonio Barquet-Chediak, and D. M. Allen

The enzyme glutathione peroxidase appears to play a major role in the red cell in the detoxification of potential oxidizing agents. A genetically determined partial, or presumably heterozygous, deficiency of this enzyme has been associated with a transient hemolytic anemia and hyperbilirubinemia in newborns.1* Unless subjected to oxidative stress, red cells from older children and adults with a partial deficiency of glutathione peroxidase do not appear to be especially susceptible to hemolysis. A homozygous defect in erythrocyte glutathione peroxidase, which has not been previously described, could be predicted to result in a clinical syndrome similar to that seen in some cases of severe erythrocyte glucose-6-phosphate dehydrogenase deficiency, i.e., a mild hemolytic process except in the presence of oxidizing agents whereupon a severe hemolytic episode may occur. Clinical and biochemical studies of a patient with homozygous erythrocyte glutathione peroxidase deficiency confirm this hypothesis and serve to further clarify the role of this enzyme in erythrocyte metabolism.

MATERIAL AND METHODS

Routine hematologic technics were used throughout. Red cell enzyme activities were measured using previously published technics. These included tests for glucose-6-phosphate dehydrogenase,2 6-phosphogluconate dehydrogenase,2 pyruvic kinase,3 and glutathione reductase4 activity.

Erythrocyte glutathione peroxidase levels were determined using an assay system recently published1 and quite similar to that reported by Gross et al.5 Washed hemolysate (30 to 45 mg. hemoglobin) was incubated with reduced glutathione (1.2 mg), sodium azide (1 mg.), ethylenediaminetetraacetic acid (1.75 mg.), and buffered (phosphate buffer, pH 7.4, 0.1M) isotonic saline pH 7.4 to a total volume of 5.0 ml. Hydro-

From the Departments of Pediatrics and Medicine, New England Medical Center Hospitals, and Tufts University School of Medicine, Boston, Mass. and the Department of Medicine, University of Puerto Rico, San Juan, Puerto Rico.

This work was supported in part by U.S. Public Health training grant TIAM 5302-06.
First submitted March 3, 1968; accepted for publication August 5, 1968.

Thomas F. Necheles, M.D., Ph.D.: Hematologist, New England Medical Center Hospitals; Assistant Professor of Pediatrics, Tufts University School of Medicine; Established Investigator, American Heart Association. Norman Maldonado, M.D.: Chief of Hematology, Department of Medicine, University of Puerto Rico, San Juan, Puerto Rico. Antonio Barquet-Chediak: Chief of Clinical Laboratories, Department of Medicine, University of Puerto Rico, San Juan, Puerto Rico. Donald M. Allen, M.D.: Chief Pediatric Hematologist, New England Medical Center Hospitals; Associate Professor of Pediatrics, Tufts University School of Medicine.

*A somewhat similar infant has been previously described by P. Benedetti et al. in a Symposium on Problems of Fetal Distress held in Siena, Italy, in September, 1966. No family information was available.
GLUTATHIONE-PEROXIDASE DEFICIENCY

Fig. 1.—Erythrocyte glutathione peroxidase activity in normal adults, adults heterozygous for glutathione peroxidase deficiency, and in the propositus. Enzyme activity is expressed as units/Gm. Hemoglobin.

**Erythrocyte GSH-Peroxidase Activity**

---

gen peroxide (1.8μM) was rapidly added through a blow-out pipette and samples were taken at exactly 1 and 3 minutes. The reaction was stopped by precipitation with metaphosphoric acid. Residual reduced glutathione was measured using the technic of Beutler et al.6 and the reaction rate constant K calculated. As Gross and her associates5 have pointed out, the reaction under these conditions follows apparent first order kinetics over the initial 3 minutes.

Red cell glucose utilization, CO₂ production and hexose monophosphate shunt activity were measured using technics described by Jacob and Jandl.7 Red cells, anticoagulated with heparin, were washed three times in isotonic buffered (pH 7.4) saline and the buffy coat removed. The cells were resuspended at a concentration of 35 per cent in a media containing isotonic saline (Na⁺, 145mM), bicarbonate buffer (HCO₃⁻, 25mM) (pH 7.4), potassium (K⁺, 5mM), glucose at a final concentration of 200 mg./100 ml., and 5 per cent salt-poor human albumin. Sodium cyanide (neutralized with HC₁) was added to a final concentration of 0.01 M. Glucose-1-C¹⁴ was the source of radioactive label. The cell suspension was gassed with 5 per cent CO₂ in air prior to incubation. Incubation was carried out in Warburg flasks fitted with a rubber serum cap through which material could be injected. After 2 to 4 hours, the medium was acidified with 1 N H₂SO₄ and the C¹⁴O₂ trapped in 0.2 ml. NCS† which had been injected into the center well.

The NCS was transferred into a scintillation solution (0.4 per cent 2.5 diphenyloxazole (PPO) and 0.01 per cent 1.4-bis-2-(5-Phenylloxazolyl)-benzene (POPOP) in toluene and the radioactivity counted in a Picker scintillation counter. Glucose consumption was calculated from the concentrations of glucose which were determined with the glucose oxidase method.8 All experiments were carried out at least in duplicate, and usually in triplicate, and the determinations agreed within 5 per cent.

Low steady-state concentrations of hydrogen peroxide were generated using the D-amino

---

†A quaternary ammonium base obtained from Nuclear Chicago Corp., Chicago, Ill.
HOMOZYGOUS GSH-PEROXIDASE DEFICIENCY

Fig. 2.—Family study in erythrocyte glutathione peroxidase deficiency.

CASE REPORT

The propositus is an 18 year old Puerto Rican male who was originally scheduled to undergo open heart surgery for repair of an intraventricular septal defect. Three units of his own blood were withdrawn into standard blood bank solutions, stored for 10 to 20 days, and reinfused at the time of surgery. He immediately developed a hemolytic transfusion reaction with hemoglobinemia, hemoglobinuria, and jaundice. Physical examination revealed only jaundice, and neither the spleen nor the liver was enlarged to palpation. Direct and indirect Coombs’ tests were negative and blood cultures were negative. Erythrocyte levels of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, pyruvic kinase, and glutathione reductase were all either elevated or within normal limits. Supravital staining of the patient’s erythrocytes shortly after the transfusion revealed numerous red cells containing Heinz bodies, but these disappeared within a few weeks. Incubation of the patient’s erythrocytes with acetylphenylhydrazine continued, however, to elicit increased numbers of inclusion bodies with 53 per cent of cells showing five or more inclusions. In normal individuals less than 20 per cent of cells will show such inclusions. There was a moderate increase in in vitro autohemolysis when the erythrocytes were incubated for 48 hours in saline; this was correctible with glucose (200 mg./100 ml.). Erythrocyte osmotic fragility was normal.

Hematologic studies three months after recovery from the acute hemolytic episode revealed a normal hemoglobin concentration of 15.4 Gms./100 ml. but a persistent elevation in the reticulocyte count of 2.8 to 5 per cent. The patient has received no medications during this interval and there was no history suggestive of previous hemolytic episodes.

Assay of the patient’s erythrocyte glutathione peroxidase activity revealed a markedly reduced level of 16.4 units/Gm. Hb. These results are compared, in Figure 1, with the enzyme levels found in erythrocytes from normal adults and from adults who are presumably (on the basis of family studies) heterozygous for glutathione peroxidase deficiency. There is little overlap between the three groups and the heterozygous range in the adult lies approximately half-way between the normal adult range and the level found in the present individual.

Investigation of family members. (Fig. 2) confirmed the previous suggestion that glutathione peroxidase deficiency is inherited as an autosomal recessive characteristic. Both parents proved to be heterozygous with enzyme levels of 32.5 and 35.7 units/Gm. Hb. One sibling was also heterozygous while two siblings had normal enzyme levels. All

*Obtained from Sigma Chemical Co., St. Louis, Mo.
GLUTATHIONE-PEROXIDASE DEFICIENCY

Table 1.—Biochemical Studies

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose consumption pM/hr./ml. red cells</th>
<th>CO₂ production pM/hr./ml. red cells</th>
<th>% via the hexose monophosphate shunt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (10)</td>
<td>2.72±0.67</td>
<td>0.103±0.39</td>
<td>3.88±1.22</td>
</tr>
<tr>
<td>&quot; &amp; H₂O₂ (4)</td>
<td>3.67±0.17</td>
<td>0.730±0.220</td>
<td>19.59±5.23</td>
</tr>
<tr>
<td>G-6-PD deficient</td>
<td>6.40</td>
<td>0.069</td>
<td>1.08</td>
</tr>
<tr>
<td>&quot; H₂O₂</td>
<td>6.88</td>
<td>0.070</td>
<td>1.01</td>
</tr>
<tr>
<td>GSH-peroxidase deficient</td>
<td>2.84</td>
<td>0.101</td>
<td>3.56</td>
</tr>
<tr>
<td>&quot; &quot; &amp; H₂O₂</td>
<td>3.68</td>
<td>0.112</td>
<td>3.04</td>
</tr>
</tbody>
</table>

family members were completely asymptomatic and had no previous history suggestive of hemolytic episodes. The results of routine hematologic studies were within normal limits.

Biochemical Studies

Glycolysis and hexose monophosphate shunt activity were measured in erythrocytes from normal individuals, from a patient with severe red cell G-6-PD deficiency (p.10 IU/10⁶ rbc) associated with a mild compensated hemolytic anemia, and from the patient with homozygous GSH-peroxidase deficiency. Total glucose consumption was found to correlate relatively closely with the degree of reticulocytosis (Table 1). In each of several experiments, addition of a peroxide-generating system to normal erythrocytes led to a four-fold or greater rise in C¹⁴O₂ production. Even in the absence of peroxide, C¹⁴O₂ production was significantly depressed in the erythrocytes from a patient with severe G-6-PD deficiency. Addition of a peroxide-generating system to these erythrocytes resulted in no significant change in C¹⁴O₂ production. In the red cells from the individual with homozygous GSH-peroxidase deficiency, C¹⁴O₂ production in the absence of peroxide was normal, but these cells failed to respond to the presence of oxidizing agents.

Discussion

Glutathione peroxidase is the last step in the chain of reactions which link the "detoxification" of hydrogen peroxide to glucose metabolism in the mature erythrocyte. Cohen and Hochstein originally suggested that detoxification of hydrogen peroxide was important in the protection of erythrocytes from drug-induced damage. These authors demonstrated that in G-6-PD-deficient erythrocytes, low steady-state concentrations of H₂O₂ led to a progressive loss in intracellular concentrations of GSH followed by the appearance of methemoglobin and increased osmotic fragility. Similarly, in the presence of low concentrations of H₂O₂ depletion of glucose in catalase-rich erythrocytes was followed by loss of reduced glutathione, progressive increase in osmotic fragility, and eventual lysis. If catalase was inhibited with azide, or in erythrocytes from acatalasic individuals, loss of reduced glutathione and subsequent oxidative damage could be prevented by the presence of adequate concentrations of glucose. These studies suggested that glutathione peroxidase is the primary agent for the elimination of hydrogen peroxide in erythrocytes while catalase appears to play a secondary role.

Although erythrocyte glutathione peroxidase activity in the homozygous
patient was 30 per cent of the normal value by enzyme assay, the metabolic studies suggest a much lower level of activity in the intact red cell. Whether this discrepancy is due to limitations of the assay method in detecting low levels of activity or to specific conditions within the cell influencing activity in the intact cell cannot be determined. However, in this patient a marked reduction in enzyme activity as assayed in an in vitro system is associated with Heinz body formation in the intact cell, suggesting that this enzyme, glutathione peroxidase, plays an important role in the protection of the erythrocyte from oxidative stress. Furthermore, this enzyme appears to play an important role in mediating the response of the hexose monophosphate shunt to oxidative agents. Previous studies have shown that this response is dependent upon the presence of reduced glutathione. These observations indicate that the presence of adequate levels of the enzyme glutathione peroxidase are also essential.

The enzymes, glucose-6-phosphate dehydrogenase, glutathione reductase, and glutathione peroxidase, plus the tripeptide glutathione comprise the pathway linking the hexose monophosphate pathway to the detoxification of peroxides. A genetically determined deficiency of three of these, G-6-PD, GSSG-R, and glutathione has previously been described. The clinical picture has been quite similar; a compensated hemolytic process interspaced by acute hemolytic episodes on exposure to various oxidizing agents. Glutathione peroxidase deficiency appears also to be associated with a similar clinical picture. In the presence of reduced levels of this enzyme, the red cell becomes increasingly sensitive to the hemolytic effects of oxidizing agents. Indeed, increased hemolysis has been observed in individuals with a partial (heterozygous) deficiency while taking therapeutic doses of sulfisoxazole (Gantrisin®). The biochemical insult which precipitated the hemolytic episode in the present patient is still unclear. Whether in vitro storage of blood for periods of 10 to 21 days would result, in the presence of this enzyme deficiency, in sufficient red cell damage to lead to massive hemolysis remains problematic. Results from in vitro autohemolysis tests suggest that this indeed may be the case.

**Summary**

An adult Puerto Rican male is described who developed an acute hemolytic episode following the infusion of three units of stored autologous red cells. The acute episode was associated with the presence of numerous red cells containing Heinz bodies. Investigation of his red cells revealed a markedly decreased level of the enzyme glutathione peroxidase. Examination of family members revealed moderately decreased levels of red cell glutathione peroxidase activity in both parents and in a sibling suggesting an autosomal recessive mode of inheritance. Metabolic studies on the erythrocytes from this patient revealed a normal level of hexose monophosphate shunt activity under basal conditions but a lack of normal activation of this pathway in the presence of hydrogen peroxide. The results of these studies support the concept that this enzyme, glutathione peroxidase, plays a major role in mediating the normal red cell response to the presence of peroxides.
GLUTATHIONE-PEROXIDASE DEFICIENCY

SUMMARIO IN INTERLINGUA

Un adulte masculo portorican es describite qui disveboppava un episodio de hemolyse acute post le infusion de tres unitates de thesaurisate erythrocytos autologe. Le episodio esseva associate con be presentia de numerose erythrocytos continente corpores de Heinz. Investigationes de su erythrocytos revelava un marcatemente reducite mvelbo del enzyma peroxydase de glutathiona. Le examine de membros del familia revelava moderamente reducite nivellos de activitate de peroxydase de glutathiona in le erythrocytos de ambe parentes del patiente e in un confratemo de ille, factos suggestionante un modo recessive autosomal de hereditate. Studios metabolic del erythrocytos del probando revelava un nivello normal de activitate de shunting de monophasphato de hexosa sub conditiones basal sed un activation subnormal de iste circuito in le presentia de peroxydo de hydrogeno. Le resultatos de iste studios supporta le conception que le enzyma peroxydase de glutathiona ha un rolo major in le mediation del normal responsa erythrocytic al presentia de peroxydos.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Francisco Raffucci, Professor and Chairman of the Department of Surgery, University of Puerto Rico School of Medicine, for referring and allowing us to study the proband. We wish to thank also Mrs. T. Boles, Mrs. P. Saltman, and Mrs. J. Fradera for valuable technical assistance. Dr. T. F. Necheles is an Established Investigator of the American Heart Association.

REFERENCES

Brief Report: Hemozygous Erythrocyte Glutathione-Peroxidase Deficiency: Clinical and Biochemical Studies

T. F. NECHELES, NORMAN MALDONADO, ANTONIO BARQUET-CHEDIAK and D. M. ALLEN

Updated information and services can be found at:
http://www.bloodjournal.org/content/33/2/164.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml