The Potential Use of Xylitol in Glucose-6-Phosphate Dehydrogenase Deficiency Anemia

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Abstract NADP-linked xylitol dehydrogenase has been found to be present in human red blood cells. This enzyme activity is normal in most glucose-6-phosphate dehydrogenase (G6PD)-deficient red cells. Xylitol was explored as a potential agent for treatment of hemolysis in patients with G6PD-deficiency. Intracellular GSH (glutathione, reduced) was first converted to its oxidized form by incubation of the erythrocytes with acetylphenylhydrazine or by pretreatment with methyl phenyl-diazene-carboxylate. The addition of 0.15 mM xylitol was shown to be more effective than 0.15 mM glucose in maintaining the levels of GSH in G6PD-deficient red cells during such oxidative challenge. Rabbit erythrocytes contain less activity of G6PD and glutathione reductase compared with the normal human adult values, but have an active xylitol dehydrogenase. The rabbit erythrocyte is sensitive to acetylphenylhydrazine and primaquine phosphate. In both in vivo and in vitro experiments, xylitol was found to partially prevent acetylphenylhydrazine induced acute hemolysis of the rabbit red cell and GSH content was found to be preserved. The intravenous injection of xylitol (0.5 g/kg body weight per 6 hr) for 6 days, seemed to be nontoxic to the animal. The results suggest that xylitol should be further investigated as an agent for the treatment of G6PD-deficient patients during acute hemolytic episodes.

Introduction

Red blood cell glucose-6-phosphate dehydrogenase (G6PD) deficiency is a widespread genetic disorder. About 3% of the world population is estimated to be affected. 10% of American Negroes have been found to carry such genetic defect. Hemolysis, resulting from G6PD-deficiency can be expressed in five ways depending on the clinical setting; (a) drug induced hemolytic anemia; (b) hemolytic anemia in infectious and other febrile illness; (c) chronic nonspherocytic hemolytic anemia; (d) favism; and (e) neonatal jaundice. Drug induced hemolysis is usually a self-limited disease, but among enzyme-deficient patients, 2% have a chronic hemolytic anemia in the absence of drug challenge (1). The disease is characterized biochemically by a low or almost absent G6PD-activity. Secondarily, the GSH is unstable on in vitro incubation of such red cells in the presence of oxidizing agents. There are many enzyme variants, and clinical expression correlates both with enzyme activity, and, directly or indirectly, with the type of enzyme. The mechanism of hemolysis is still far from clear. It has been presumed that a normal GSH concentration plays an important role in the maintenance of the integrity of the red cell. Treatment with either ACTH (adrenocorticotropin) (2) or cortisone (3, 4), appears to be of no value for the congenital nonspherocytic hemolytic anemia secondary to G6PD-deficiency. Splenectomy is much less effective than in hereditary spherocytosis. In fact, no adequate treatment of such disease has thus far been developed. Transfusion is the only method known to maintain the patient during acute hemolytic crises.

Recently, we were able to demonstrate the presence of a triphosphopyridine nucleotide (NADP)-linked xylitol dehydrogenase (XDH) in the human red blood cell. The red cell hemolysate can utilize xylitol and produce L-xylulose and NADPH (reduced form of triphosphopyridine nucleotide) (5). It thus seems possible that xylitol might be able to replace glucose-6-phosphate for the maintenance of NADPH in the G6PD-deficient state. In such a way, the intracellular GSH concentration could be restored in the deficient cells, thereby protecting red cells from oxidative injury as shown in the Scheme 1. The purpose of this report is to present evidence from both in vitro and animal experiments which...
suggest that administration of xylitol might help patients with G6PD-deficient anemia during acute hemolytic crises.

METHODS

Blood samples and enzyme determinations. Patients with G6PD-deficiency were diagnosed among the Vanderbilt Hospital Patient population by a screening test (6). The diagnosis was confirmed by spectrophotometric assay and all samples from deficient patients were checked by electrophoresis using a standard buffer and stain (7) but using cellulose acetate in place of starch. All patients appeared to have the A− variant. All patients were black. Normal blood was obtained from healthy volunteers among faculty and students. G6PD was measured by a modified method by Zinkham, Lenhard, and Childs (8). Glutathione reductase (GSSG-R) was determined by the method of Long and Carson (9). XDH was measured by the increase of NADPH at pH 8.0 as described previously (5). G6PD, GSSG-R, and XDH activities were measured in rabbit red cell hemolysates by the same methods.

In vitro restoration of GSH. Experiments to evaluate the maintenance and regeneration of intracellular GSH in normal and G6PD-deficient red cells were carried out in the following two ways. First, a modified glutathione instability test was used (10): an equal volume of an isotonic glycylglycine buffer (0.1 M glycylglycine, 0.03 M NaH2PO4, and 0.55 M NaCl at pH 7.2) was added to saline-washed packed cells. Xylitol or glucose were added as needed. The hematocrit of these suspensions varied from 35 to 40%. The final incubation volume was 2 ml. Exposure to acetylphenylhydrazine (APH) was continued throughout the incubation period. A concentration of 5 µg APH/ml of final incubation mixture was used. Control experiments without APH and/or xylitol or glucose were included.

When methyl phenyl diazenecarboxylate (CHN = NCOOC2H5) was used as oxidant, the azoester was first dissolved in the isotonic glycylglycine buffer in a final concentration 1.6 µg/ml as described by Kosower, Vanderhoff, and London (11). The cell suspension was kept at room temperature for 10 min and washed with the same buffer without azoester until the supernatant became clear. Usually, the GSH-content was reduced to approximately 10–20% of the original value. Such pretreated cells were then incubated at 37°C with glycylglycine buffer and xylitol or glucose as additives. A 0.4 ml sample was taken from each tube for GSH-determination. GSH was measured by Ellman's reagent (12), but the freezing and thawing step was omitted. The same experiments were carried out with rabbit erythrocytes. In addition to experiments with APH and the azoester, primaquine phosphate (2.5 mg/ml incubation mixture) was used.

Animal experiments. New Zealand–white male rabbits were used throughout the study. For a long-term xylitol administration experiment, 12 rabbits were divided into four groups for evaluation of the effectiveness of xylitol in vivo. The optimum dose of APH to induce a reproducible degree of acute hemolysis in the rabbit was determined. The toxicity for rabbits of a single intravenous injection of xylitol was checked. The details of each experiment are described in the legend of the appropriate figures.

Chemical determinations. Xylitol was measured as formaldehyde after periodate oxidation (13). Glucose concentration was determined using a commercial version of the glucose oxidase method (Worthington Kit) (14). Xylulose was estimated as ketopenose by the cysteine carbazole method (15) using 6% perchloric acid as deproteinizing solution. Total and conjugated serum bilirubin were determined by a manual method (16).

Materials. Methyl phenyl diazenecarboxylate was synthesized by a method described by Huang and Kosower (17). The compound was identified by infrared, ultraviolet, and nuclear magnetic-resonance spectra analysis. The use of the azoester and its mechanism of reactions have been discussed in detail by the same group (18).

Xylitol was kindly supplied by the Eisai Co., Ltd. Tokyo, Japan. No inhibition of growth of Escherichia coli could be detected. No contaminant could be detected in the xylitol infusion solution as judged by the ultraviolet absorption spectrum.

All other reagents were purchased from standard American commercial sources.

RESULTS

To evaluate the usefulness of xylitol as a potential form of rational therapy, the following questions needed to be answered: (a) is XDH present in normal and G6PD-deficient red cells in equal activity; (b) is xylitol permeable to the red cell and is it effective in restoration of intracellular GSH in oxidatively challenged red blood cell in vitro; (c) is it effective in animal models in vivo, and (d) is it justified for use in human subjects on the basis of the known toxicity of the compound and its metabolites.

XDH in G6PD-deficient patients. G6PD and XDH were measured in groups of normal and G6PD-deficient subjects. Table I reports the activities of the two enzymes in the red cell hemolysates. It is clear that the G6PD-deficient patients have a normal XDH activity averaging 7.3 ± 1.2 nM NADPH/min per g Hb.

| Table I Glucose-6-Phosphate Dehydrogenase (G6PD)- and NADP-Linked Xylitol Dehydrogenase (XDH) Activities in Normal and G6PD-Deficient Erythrocytes |
|-------------|--------|-------------|
| G6PD        | XDH    |
| IU/100 ml   | nM NADPH/min per g Hb |
| Normal erythrocytes* | 153.5 ± 15.3 | 7.0 ± 0.8 |
| G6PD-deficient erythrocytes* | 12.9 ± 8.0 | 7.3 ± 1.2 |

* Average of eight subjects ± 1 SD.
Maintenance and restoration of intracellular GSH by xylitol. Xylitol is actively metabolized by red cells and has been shown to stimulate lactate formation (19) and methemoglobin reduction (20, 21). Xylitol can restore GSH in sodium nitrite-treated normal erythrocytes (22). The permeability of xylitol to the red cell was evaluated by Asakura et al. (22), and a simple diffusion mechanism has been suggested. Xylitol passes through the red cell membrane easily and equilibrates in less than 1 min. Long-term storage with xylitol as an additive to red cells has been shown in our laboratory to help the maintenance of the 2, 3 DPG-level. A direct demonstration of the effectiveness of xylitol in restoring GSH in both normal and G6PD-deficient cells has been made by using APH and methyl phenyl diazenecarboxylate.

Fig. 1 gives the results of a representative experiment with APH. Fig. 1A shows that glucose could maintain the GSH level better than xylitol in normal red cells. However, Figure 1B demonstrates that xylitol preserved more intracellular GSH in the G6PD-deficient cells. In the normal cells, glucose maintains 90% GSH and xylitol 55% after 4 hr incubation. In the deficient cell, glucose preserved only 26% and xylitol 35%. This result was reproducible on multiple experiments and the difference is significant (P < 0.05). Fig. 2 shows similar results of a representative experiment using the azoester. Fig. 2A illustrates that glucose is far better than xylitol in GSH-recovery in the normal blood cells, in which, glucose restores 85% GSH and xylitol 56%. In the G6PD-deficient cell xylitol shows better ability to regenerate GSH as shown in Fig. 2B. Xylitol restores 65% of GSH and glucose only 48%. The difference is reproducible on multiple trials and is significant (P < 0.05). A 100% recovery is not likely to be reached, since the GSSG has been reported to leak out of the red cells (23). Even without an oxidant challenge, xylitol maintained GSH better than glucose in the G6PD-deficient cells. This is shown in the top curve of Figs. 1B and 2B. In red cells from persons heterozygous for G6PD-deficiency (around 55 IU per 100 ml RBC) the benefit of xylitol was much less marked.

Glucose and xylitol at final concentrations of 0.015 M, which is 10 times lower than that used in the experiments described, gave similar results to glucose and xylitol at 0.15 M.

The effect of xylitol on rabbit erythrocytes in vitro. There is no suitable animal model for G6PD-deficient studies. The rabbit was chosen because it is known to be drug sensitive. In a preliminary experiment the levels of the pertinent enzymes were measured. Table II gives the G6PD, GSSG-R, and XDH activities in the rabbit red cell hemolysates. With the average of 4.3%...
Table II
Comparison of G6PD-, Glutathione Reductase (GSSG-R)-, and XDH-Activities and Reticulocyte Percentage in Human and Rabbit Erythrocytes

<table>
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<tr>
<th></th>
<th>Human</th>
<th>Rabbit</th>
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<tbody>
<tr>
<td>G6PD IU/100 ml RBC</td>
<td>153.5 ± 15.3 (8)*†</td>
<td>166.0 ± 15.2 (5)</td>
</tr>
<tr>
<td>GSSG-R IU/100 ml RBC</td>
<td>95.8 ± 15.4 (5)</td>
<td>54.9 ± 9.1 (5)</td>
</tr>
<tr>
<td>XDH xNADPH/min per Hb</td>
<td>7.0 ± 0.8 (8)</td>
<td>8.6 ± 1.3 (5)</td>
</tr>
<tr>
<td>Average reticulo-</td>
<td>1</td>
<td>4.3</td>
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<tr>
<td>cytes, %</td>
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* Mean ±1 sd.
† Figure in parenthesis indicates the number of subjects.

added, the same results were obtained. Fig. 3 shows a representative experiment.

Optimal dose of APH-induction of in vivo hemolysis. Fig. 4 shows the percentage hemolysis after different doses of APH were injected intraperitoneally. 40% hemolysis was observed consistently after 72 hr when more than 20 mg APH per kg body weight was injected into rabbits. Some animals died with a dosage exceeding 25 mg APH per kg body weight. There was a drastic decrease of the GSH content along with a severe drop of PCV and hemoglobin concentration. The reticulocyte count rose proportionately. A 70% reticulocyte count was observed in one rabbit before death. Serum hemoglobin was found increased occasionally. 10 mg APH per kg body weight was chosen to be a reasonable dose and is therefore, the one used in the following long-term animal experiments.

Administration of xylitol to rabbits with APH-induced hemolysis. Fig. 5 gives the result in a long-term xylitol treatment of animals anemic from an APH-injection. PCV, hemoglobin concentration, reticulocyte counts, and GSH content of single animals are shown. Rabbits in each group followed a similar pattern in their hematological values. While there are no observable changes in control animals, animals to which only xylitol was given intracellular GSH content increased slightly. The value returned to normal at the end of experimental period. In animals to which only APH was administered, the PCV dropped from 36 to 24%, the hemoglobin concentration fell from 12.8 to 8.6 g per 100 ml in 120 hr. GSH content showed an 18% decrease. With the injection of xylitol and the administration of APH, the animal showed only an absolute drop in PCV of 6%, while hemoglobin fell only from 12.2 to 10.3 g per 100 ml blood. GSH measured within normal range. An increase of reticulocyte count was seen after the 5th day. In the animals of the control and xylitol treated group, the reticulocyte counts increase slightly, which is likely due to the continued withdrawal of 2-3 ml blood from the animals daily.

Fig. 6 summarizes the average differences of PCV- and GSH-content in per cent of initial value among the four groups. The baseline value is set at 100%. Fig. 6A indicates the differences in PCV among groups. Fig. 6B demonstrates that GSH has been conserved by the treatment of xylitol in the APH-treated animals. It seems clear that the production of NADPH by xylitol maintained the intracellular GSH content.

Toxicity of xylitol to the rabbit. Xylitol and glucose levels in three animals after a single xylitol intravenous injection (0.5 g xylitol per kg body weight in a 20% solution) are shown in Fig. 7. The values are expressed in mg per 100 ml. The plasma xylitol declines rapidly five min after intravenous infusion. The mean half disap-
pearance rate, ti, of xylitol is estimated to be about 20 min. The decay constant of xylitol in plasma is computed to be 3.3% per min. The results are in agreement with the results of the elimination study in human blood (24).

The xylitol content in the red cell has been found elevated after xylitol injection. Fig. 7A points out that xylitol is higher in whole blood than plasma after 6 hr. Red cell xylitol reached about 13 mg per 100 ml. The glucose concentration in both whole blood or plasma was not significantly altered by the injection of xylitol. The parallel curve between plasma and whole blood glucose indicates that red cell glucose was maintained unchanged. The fall of glucose at 60 min might be caused by an increase of insulin in the plasma (25). Fig. 8 shows the time course of the plasma xylulose after an intravenous xylitol injection. The ketopentose formation reached a peak around 5 min after the injection. The total conversion to xylulose was estimated to be only 4% of the xylitol injected. It is likely that the ketopentose is L-xylulose. However, the plasma ketopentose was not characterized.

Serum bilirubin has been determined in two animals who received xylitol only. There was no elevation of serum bilirubin in 24 or 48 hr during chronic xylitol administration.

No deterioration has been observed in the group which received only xylitol and all animals survived throughout the study.

![Figure 5](image-url) **Figure 5** The hematological alterations in representative animals during a long-term animal experiment. The following symbols are used: (●) control group, (○) xylitol group, (▲) APH-group, (△) experimental group to which both APH and xylitol were given. APH at dose of 10 mg per kg body weight was injected intraperitoneally at zero time. Xylitol, in a 20% solution, was injected four times a day for 6 days. 0.5 g xylitol per kg body weight was injected over 4 min each time through an ear vein.

![Figure 6](image-url) **Figure 6** Hematological changes in rabbits expressed as per cent of initial values. The same symbols are used as described in Fig. 5. Baseline values were used as 100%. Each point represents an average of three animals ±1 sd.

![Figure 7](image-url) **Figure 7** A) Xylitol; B) glucose concentration in whole blood and plasma after a single xylitol injection (a 20% solution at 0.5 g xylitol per kg body wt). (○) refers to whole blood; and (△) to plasma. Each point indicates an average of three animals ±1 sd.
of xylitol may be a general one in other cells besides the erythrocyte. In patients with G6PD-deficiency this enzyme may also be decreased in liver (27), platelets (28), leucocytes (29), skin (30), and in lens tissue (31, 32). The NADP-linked xylitol dehydrogenase has been found in almost all the organs of mammals (33). Therefore, the potential usefulness of xylitol may not be limited to the erythrocyte.

Xylitol has been introduced for clinical application 10 yr ago as an adjuvant in parenteral alimentation. It has been reported that xylitol is independent of insulin for transport (34). It is also an adequate calorie source (35). Xylitol has been used in the human in Germany (36), Japan (37), Russia (38, 39), Italy (40), and South Africa (25). Besides normal subjects, it has been used to treat patients with diabetes (41), bile duct and liver disease (38), renal disease (25), ketonemia (42), pulmonary tuberculosis (43), and for parenteral nutrition during and after surgery (36). No harmful effect has been observed in these published series. Recently, however, Donahoe and Powers (44) reported that the hyperuricemia, hyperuricosuria and hyperbilirubinemia have occasionally been observed in normal subject with xylitol doses of 1.22–3.13 g per kg body weight. The infusion time was not indicated in this note. Similar adverse effects were also reported by an Australian group (45). Once again, the xylitol dose and the infusion time were not mentioned. At a lower dose, 1.5 g xylitol per kg body weight Forster, Meyer, Ziege (46) reported transient rise in serum uric acid and serum bilirubin though remaining within the normal range, in normal subjects after rapid xylitol infusion. SGOT- and SGPT-levels were not influenced by the xylitol administration. Our experience with xylitol in rabbits showed no clinical toxicity, and no serum bilirubin elevation, though the literature did mention xylitol toxicity in rabbits (47).

The plasma and whole blood glucose was found unchanged for 6 hr after a single xylitol injection. Similar results were found in dogs (48), rabbits (49), and humans (50) with different xylitol dosage. Therefore, the maintenance of the hematocrit was not due to increased serum glucose, but likely from the effect of xylitol per se. The xylitol concentration was estimated to be 13 mg per 100 ml red blood cell after a single injection of xylitol. The NADP-linked xylitol dehydrogenase has a low K_m for xylitol (5). Although the xylitol content of the red cell increased after repeated injection, it is unlikely that the intracellular xylitol concentration reached saturating levels for the enzyme. Even so, saturation is not necessary if the NADPH formed is rapidly utilized to reduce GSSG.

The liver is the major organ which metabolizes xylitol. The major products, except L-xylulose are similar
to that of glucose. The excess L-xylulose will be excreted through the urine, and is likewise a harmless substance, as shown by pentosurics who are asymptomatic in spite of the excretion of gram quantities of that sugar (51). A mild increase of lactate was reported (25, 42, 50) in the blood after intravenous xylitol administration. The serum concentration of xylulose was very low in our experiments.

For the time being human experiments are excluded. The Australian groups reported multiple cases of death from xylitol infusion although the presence of a contaminant in the xylitol solution was strongly suspected (52). Since these observations are at present not published but only informally reported, a decision must await more details.

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