CONCISE REPORT

Enhanced Survival of Sickle Erythrocytes Upon Treatment With Glyceraldehyde

By Lennette J. Benjamin and James M. Manning

Glyceraldehyde has been demonstrated to have significant antisickling activity in vitro through its ability to modify hemoglobin S. This modification occurs as a result of the reaction between the aldehyde portion of the molecule and some of the amino groups of hemoglobin S leading to Schiff base formation and subsequent Amadori rearrangement to a stable ketoamine (Fig 1).

The major sites of the reaction were found to be Lys-16(a), Val-1(β), Lys-82(β), Lys-59(β), and Lys-120(β). The major effect of this reaction is a reduction in the ability of deoxyhemoglobin S to polymerize, due mainly to the modification at Lys-16(a). The modification has a slight effect on the oxygen affinity of hemoglobin at low concentrations of glyceraldehyde (5 mmol/L and 10 mmol/L). However, at higher concentrations of the compound (20 mmol/L), there is a measurable decrease in the P50 (Fig 2). The stability of the ketoamine adduct suggests that glyceraldehyde has a suitable half-life for therapeutic use. However, prior to performing pharmacologic and toxicity studies and subsequent therapeutic trials, such a drug should be demonstrated to be nontoxic to sickle erythrocytes. Earlier studies in vitro indicated that even though there was a discernible amount of reaction of glyceraldehyde with red cell membrane proteins, there was no adverse effect on either the osmotic fragility or the filterability of the cells or on the activity of red cell enzymes. Other indications that the red cell was not compromised were the findings that both the high viscosity and the elevated cation permeability of sickle cells were decreased.

The study reported here was designed to determine whether in vitro incubation of sickle erythrocytes with glyceraldehyde was attended by a beneficial or a deleterious effect on their survival in vivo.

MATERIALS AND METHODS

Patients. Five patients with homozygous sickle cell anemia were studied at Rockefeller University Hospital during the asymptomatic or steady-state period. The study protocols were approved by the institutional review board for human studies. The patients gave informed consent and were admitted as inpatients during the studies. Red cell survival studies. Red cell survival studies were performed using chromium-51 according to the method of Grey and Sterling. An initial control study was performed on whole blood that was incubated with sterile normal saline for 1/2 hours at room temperature. After removal of the plasma, the cells were washed twice with sterile normal saline and labeled with 100 μCi of 51Cr. Unreacted radiolabel was removed by washing, and the cells were then suspended in sterile normal saline and infused into the patient. A blood sample was obtained 24 hours later (day 0) and each day thereafter until T1/2 (50% of the initial radioactivity on day 0) was reached. After the disappearance of this initial dosage of radioactivity, the drug phase of the study was performed, using the identical procedure as in the control study except that whole blood was incubated for 1/2 hours at room temperature with 10 mmol/L or 20 mmol/L glyceraldehyde dissolved in sterile normal saline. After removal of plasma, the cells were washed, labeled with 51Cr, washed, and infused into the patient as described.

Determination of hemoglobin modification. The extent of modification of hemoglobin S was analyzed on an aliquot of the protein that was reduced with sodium borohydride at pH 6 and then subjected to acid hydrolysis. By the reduction treatment, lysine residues that have reacted with glyceraldehyde yield a dihydroxyproplysine derivative that is stable to acid hydrolysis in 6N HCl. This derivative is eluted from the 0.6 x 10 cm column of the amino acid analyzer in a position different from other amino acids in the hemoglobin tetramer. The amount of reaction with glyceraldehyde is expressed as the ratio of dihydroxyproplysine to the amount of hemoglobin tetramer.

Data analysis. Red cell survival studies were analyzed using the Prophet Computer Network (NIH Division of Research Resources). Cell survival was plotted as log % radioactive counts per hemoglobin tetramer. These studies demonstrate not only a prolongation of the life span of sickle erythrocytes by treatment with glyceraldehyde but also the absence of any deleterious effects that would be revealed by this study.

RESULTS

Patients. Five patients (three males and two females), aged 25–39, with hemoglobin S were studied (Table 1). The degree of anemia ranged from 6.5 to 8.5 g Hb. None of the patients had a palpable spleen. Red cell survival studies. The erythrocytes of all patients showed an increase in their mean survival time after treatment with glyceraldehyde compared with the mean survival time of buffer-treated autologous control cells (Fig 2). The mean survival (T1/2) of control cells was 5.8 ±
SICKLE CELL SURVIVAL WITH GLYCERALDEHYDE

The mean 1/2 was increased significantly by 10 mmol/L of sickle erythrocytes after treatment with glyceraldehyde. A 1/2 profile was not affected by treatment with glyceraldehyde.

In a separate study in vitro, we found that the 5'Cr elution improvement (P < .002) was determined after reducing the concentrations and 0.8 to 1.0 groups incorporated at the 20 mmol/L hemoglobin tetramer were found at the 10 mmol/L concentrations.

Table 1. Baseline Hematologic Data on Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Hb (g/dL)</th>
<th>Hematocrit (%)</th>
<th>Red Cells (x 10^6 cells/mm^3)</th>
<th>Final Concentration of Glyceraldehyde (mmol/L)</th>
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<tbody>
<tr>
<td>A 1*</td>
<td>25</td>
<td>M</td>
<td>7.7</td>
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<td>2.96</td>
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<td>8.2</td>
<td>23.8</td>
<td>3.10</td>
<td>10</td>
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<tr>
<td>B 1</td>
<td>39</td>
<td>M</td>
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<tr>
<td>B 2</td>
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<td></td>
<td>20.2</td>
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<td></td>
<td></td>
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<td>C 1</td>
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<td>F</td>
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</tbody>
</table>

*1, initial control study; 2, glyceraldehyde-treated red cells.

DISCUSSION

These studies demonstrate a prolongation of the life span of sickle erythrocytes after treatment with glyceraldehyde. The mean T_{1/2} was increased significantly by 10 mmol/L and 20 mmol/L glyceraldehyde. The same conclusion was reached in comparing the results for each patient by a paired t test. In three of the five patients studied, the T_{1/2} was increased at least twofold. These studies demonstrate not only a beneficial effect on sickle erythrocytes but also that glyceraldehyde in the concentrations used is not toxic to sickle erythrocytes.

Glyceraldehyde has been shown to have the properties that a compound should possess before performing toxicity and pharmacology studies and prior to consideration for clinical trials: (1) It has been shown to have significant antisickling effects in vitro by several criteria in different laboratories, (2) its mechanism of action is established, (3) it has been shown to have no severe adverse effects on red blood cell function and metabolism, and (4) as demonstrated in this study, it has a beneficial effect on erythrocyte survival time. Thus the slight degree of crosslinking of membrane proteins observed at high concentrations of glyceraldehyde in vitro is apparently of little consequence in vivo, at least as ascertained by these initial cell survival studies.

The pharmacologic literature on glyceraldehyde is fairly extensive in animal studies where it has been evaluated as an antileukemia agent. In one such study, it was determined that glyceraldehyde led to a decrease in the incorporation of acids, suggesting a biochemical basis for its therapeutic property. In another study, glyceraldehyde was found to inhibit the growth of some human tumor cell lines in vitro. In a third study, glyceraldehyde was shown to inhibit the proliferation of human lymphocytes in vitro. These studies demonstrate the potential of glyceraldehyde as a therapeutic agent in the treatment of sickle cell anemia.

Fig 1. Reaction of glyceraldehyde with hemoglobin S.

Fig 2. Chromium-51 red cell survival studies of treated and untreated erythrocytes. Cell survival is plotted as log % counts v days using the straight line of the best fit (see text). The left panel shows the survival profiles of the red cells from two patients whose erythrocytes were untreated (O) or treated with 10 mmol/L glyceraldehyde (□) as described in the text. The right panel shows the profiles of control cells (O) and cells treated with 20 mmol/L glyceraldehyde (□).
labeled amino acids into proteins. This result might have implications regarding future toxicity. However, of the hemoglobin-modifying agents presently being considered for treatment of sickle cell anemia, glyceraldehyde is estimated by toxic-to-therapeutic ratios to be one of the least toxic. It has an LD₉₀ of approximately 3 g/kg when injected intraperitoneally into mice.

Although these findings are encouraging, extensive evaluation needs to be performed regarding the toxicity of glyceraldehyde before clinical trials can be carried out via the oral or parenteral route. The results described here and in previous studies, which show significant antisickling activity, suggest the feasibility of limited clinical testing of glyceraldehyde via the extracorporeal route. This approach would permit the testing of a larger population of cells and thereby address the question of whether there is a selective sparing of deoxygenation of a small aliquot of cells such as used in the present study. Investigations to extend these studies using a double isotope cohort label and to define the optimal reaction conditions are in progress. This information will permit an evaluation not only of the efficacy of this agent but also of potential problems regarding antigenicity and any adventitious reactions with macromolecules other than hemoglobin S.

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REFERENCES

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