CONCISE REPORT

Enhanced Survival of Sickle Erythrocytes Upon Treatment With Glyceraldehyde

By Lennette J. Benjamin and James M. Manning

Glyceraldehyde has been demonstrated to be an antischickling agent in vitro. In the present investigation, chromium-51 red cell studies were used to investigate the life span in vivo of sickle erythrocytes after treatment with glyceraldehyde in vitro. The mean survival ($T_{1/2}$) of control cells was 5.8 ± 1.6 days, whereas cells treated with 10 mmol/L or 20 mmol/L glyceraldehyde survived 9.0 ± 1.4 (P < .05) and 11.3 ± 0.8 (P < .002) days, respectively. The extent of modification by glyceraldehyde was 0.4 to 1.0 lysine residue 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.

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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 1½ hours at room temperature. After removal of the plasma, the cells were washed twice with sterile normal saline and labeled with 100 μCi of $^{51}$Cr. 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 $T_{1/2}$ (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 1½ 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 $^{51}$Cr, 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 by treatment with glyceraldehyde but also the degree of anemia ranged from 6.5 to 8.5 g Hb. None of the 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 ($T_{1/2}$) of control cells was 5.8 ± 1.6 days, whereas cells treated with 10 mmol/L or 20 mmol/L glyceraldehyde survived 9.0 ± 1.4 (P < .05) and 11.3 ± 0.8 (P < .002) days, respectively.
SICKLE CELL SURVIVAL WITH GLYCERALDEHYDE

The mean survival time (T1/2) was increased significantly by 10 mmol/L of sickle erythrocytes after treatment with 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 T1/2 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 the erythrocytes. The pharmacologic literature on glyceraldehyde is fairly extensive in animal studies where it has been evaluated as an antileukemia agent.6-12 In one such study, it was determined that glyceraldehyde led to a decrease in the incorporation of 0.8 to 1.0 groups incorporated at the 20 mmol/L concentrations.

**Fig 1.** Reaction of glyceraldehyde with hemoglobin S.

1.6 days, whereas cells treated with 10 mmol/L and 20 mmol/L glyceraldehyde survived 9.0 ± 1.4 (P < .05) and 11.3 ± 0.8 days (P < .002), respectively. Paired t-test analysis of the T1/2 values also indicated statistically significant improvement (P < .002) in the survival of the treated cells. In a separate study in vitro, we found that the 51Cr elution profile was not affected by treatment with glyceraldehyde.

**Modification of hemoglobin.** The extent of the reaction of glyceraldehyde was determined after reducing the ketoamine adduct with sodium borohydride. Upon acid hydrolysis, 0.4 to 0.5 dihydroxypropyllysine groups per hemoglobin tetramer were found at the 10 mmol/L concentrations and 0.8 to 1.0 groups incorporated at the 20 mmol/L concentrations.

**DISCUSSION**

These studies demonstrate a prolongation of the life span of sickle erythrocytes after treatment with glyceraldehyde. The mean T1/2 was increased significantly by 10 mmol/L glyceraldehyde. 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 T1/2 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.

**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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<tr>
<td>A 1*</td>
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<tr>
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</tr>
<tr>
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<td></td>
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</tbody>
</table>

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

**Fig 2.** Chromium-51 red cell survival studies of treated and untreated erythrocytes. Cell survival is plotted as log % counts vs 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 (0) or treated with 10 mmol/L glyceraldehyde (□) as described in the text. The right panel shows the profiles of control cells (0) and cells treated with 20 mmol/L glyceraldehyde (□). The mean survival times (T1/2), as indicated by the arrows, are as follows: patient A, 4.0 days (untreated), 8.0 days (treated); patient B, 7.0 days (untreated), 10.0 days (treated); patient C, 4.7 days (untreated), 10.6 days (treated); patient D, 6.6 days (untreated), 12.2 days (treated); patient E, 7.8 days (untreated), 11.2 days (treated).
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 LD$_{50}$ 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.

ACKNOWLEDGMENT

We are grateful to Judith A. Gallea for her expert assistance in the preparation of this manuscript and to Diane Spagnoli of Dr Mary Jeanne Kreek's laboratory for her expertise in using the Prophet Computer Network (NIH Division of Research Resources). We are also indebted to the nursing staff at Rockefeller University Hospital for clinical assistance in performing these studies.

REFERENCES

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