Increased Survival of Sickle-Cell Erythrocytes after Treatment In Vitro with Sodium Cyanate
(chromium-51/hemoglobin S/sickle-cell disease)

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ABSTRACT Cyanate reacts with the amino-terminal valine residues of hemoglobin S and prevents the sickling in vitro of 50-80% of the erythrocytes from patients with sickle-cell disease. The purpose of the studies reported here was to determine whether this anti-sickling effect would increase the survival of cyanate-treated cells that were returned to the patient. In seven subjects with sickle-cell disease, the mean 50% survival of 51Cr-labeled sickle erythrocytes was increased from 9.9 to 20.7 days (normal 25-35 days) after treatment of the cells in vitro with sodium cyanate. These results provide evidence that the anti-sickling effect of cyanate observed in vitro is retained in vivo, and strengthen the rationale for further investigation of cyanate as a possible therapeutic agent in sickle-cell disease.

The anemia and the other pathological manifestations associated with sickle-cell disease are presumed to be due to the abnormal morphology of the deoxygenated erythrocyte (1). Cyanate has recently been shown to inhibit in vitro the sickling of erythrocytes from patients with sickle-cell disease by specifically carbamylating amino-terminal valine residues of hemoglobin S (2). This observation has led to the initiation of clinical studies designed to evaluate the possible use of cyanate in the treatment of this disease. In the present communication, the anti-sickling effect of cyanate was assessed in a group of patients by the use of 51Cr-labeling to compare the survival in vivo of untreated erythrocytes with that of cells treated in vitro with cyanate.

METHODS

Patients. Seven patients with sickle-cell disease and two normal subjects were studied. The patients were of both sexes, ages 17-36, and they exhibited a spectrum of the illness from mild to severe. The investigative procedures were reviewed and approved by The Rockefeller University Hospital Committee and the Food and Drug Administration, and informed consent was obtained from the patients or their parents. Documentation of the diagnosis of sickle-cell disease and other clinical information are listed in Table 1. Clinical chemistry was done by the staff of the Rockefeller Hospital laboratory. The two normal volunteers had no history of serious illness or blood disorders, and no abnormalities were observed after routine hematologic testing.

Labeling with Chromium-51. Erythrocyte survival studies were done by the modified procedure of Gray and Sterling, which is in general use in clinical laboratories (3-5), except that in the present studies the unbound chromate was removed by washing the cells with sterile saline (6). 20 ml of venous blood was drawn and transferred to a 50-ml vial containing 4 ml of (Squibb) Acid-Citrate-Dextrose Solution Modified (ACD). The pH after mixing was 6.8. After the blood was oxygenated by gentle rotation, the vial was centrifuged for 3 min at 170 × g at room temperature (22°C); the plasma was withdrawn, discarded, and replaced with saline. 40-100 μCi of Na64CrO4 (Chromitope, Squibb; specific activity 22-95 Ci/g) was added, and the vial was incubated for 60-90 min at 37°C with occasional swirling. The cells were then washed three times with 25 ml of saline and centrifuged each time, as described above, in order to remove unbound 64Cr. The cells, resuspended in saline to the original volume, were infused into the patient over a period of 1-2 min.

Estimation of Apparent 50% Survival of the Cells. For determination of cell survival, blood samples were drawn at 30 ± 5 min after infusion of the labeled cells, and at intervals of several days thereafter, until 95% of the counts at 30 min had disappeared. The amount of radioactivity in each sample, which contained 1.5-2.0 ml of erythrocytes, was determined in a Nuclear-Chicago gamma counter (background less than 10 cpm) for periods of time adequate to assure a standard deviation below 5%. To avoid correction for radioactive decay, the samples were counted simultaneously at the end of each survival study, at which time the 30-min sample had 1000-2500 cpm/ml of erythrocytes.

The cpm/ml of erythrocytes were computed as a percentage of the counts at day 0; the sample drawn 30 min after infusion of the labeled cells was taken at 100%. The data were plotted on linear coordinates, and the apparent 50% survival time (half-time) was defined for these studies as the time elapsed from day 0 to the day at which 50% of the radioactivity had disappeared.

Cyanate Treatment in Vitro. Sodium cyanate was obtained from K&K Laboratories and was recrystallized from ethanol (7). The microcrystals obtained were dissolved in sterile saline at a concentration of 0.2 M and filtered through a 0.45-μm Millipore filter. The cyanate solution was then assayed and found to be free of pyrogens.

When the level of radioactivity from the determination of the intrinsic erythrocyte survival of the patient had become negligible, a second 20-ml sample of blood from the patient was placed in 4 ml of ACD. The blood was oxygenated, centrifuged, and the plasma was removed as described above. Sodium cyanate in saline was added, to a final concentration of 0.05 M. The 51Cr was added, and the procedures described above for incubation and washing were followed.
For determination of the amount of carbamylation by the cyanate, an aliquot (1.0 ml) was removed at the start of the incubation and added to [¹⁴C]cyanate (New England Nuclear Corp.). The incubation with labeled cyanate was performed in parallel with the main sample. A portion of the radioactive aliquot was then precipitated with 5% trichloracetic acid, and the extent of carbamylation was measured (2).

**RESULTS**

Treatment of sickle-cell erythrocytes in vitro with cyanate leads to an increased survival of the cells when they are returned to the patient. The survival of cells labeled with ⁵¹Cr in three subjects is illustrated in Fig. 1. The upper graph depicts the survival of untreated cells and of cells treated in vitro with cyanate from one of the patients with sickle-cell disease. Half of the radioactivity of the untreated cells had disappeared by 4.5 days; this half-time was the shortest apparent 50% survival time observed. Treatment of this patient’s cells in vitro with cyanate extended the 50% survival time to 18 days. The center graph presents the data obtained from another patient with sickle-cell disease. This patient’s cells had the highest intrinsic half-time (17 days) of the patients studied. After cyanate treatment, the half-time of the cells increased to 27 days. The lower graph shows the survival of the cells of a normal subject; the apparent 50% survival time of 34 days falls within the normal range for this technique (25–35 days) reported by other investigators (8, 9).

The half-times of untreated and cyanate-treated cells from seven patients with sickle-cell disease, and the intrinsic values for two normal subjects are listed in Table 2. The range of intrinsic ⁵¹Cr half-survival-time of cells from patients with sickle-cell disease obtained in this study is in agreement with published data (5, 10). In each case the half-times of the cyanate-treated cells (18–27 days, mean 20.7 days) was longer than that of the untreated cells (4.5–17 days, mean 9.9 days).

**DISCUSSION**

The time-dependent disappearance of radioactivity from the blood of patients whose cells are labeled with chromium is the result both of removal of the cells from the circulation and the elution of the isotope from the cell (8, 9). If carbamylation of the sickle hemoglobin decreased the rate of chromium elution, the label would persist longer in the circulation, and a
spuriously enhanced survival would be observed. At present, an altered rate of elution in vivo has not been excluded.

In the present studies, experimental conditions were selected that produced a high extent of carbamylation (2-4 mol of cyanate incorporated per mol of hemoglobin tetramer) in order to ensure an observable in vivo effect. A lower extent of carbamylation might have been equally as effective in prolonging the apparent life span of the cells, since a maximum of 50-80% of the erythrocytes from the patients studied were prevented from sickling in vitro after incorporation of 0.1-1.0 carbamyl group per hemoglobin molecule (2). This variable responsiveness to cyanate is not understood. In addition, it was not expected that the apparent survivals of the aliquots of cyanate-treated cells would be completely in the normal range, because when tested in vitro, samples from all patients had one portion of the cyanate-treated cells that remained irreversibly sickled and another portion that was not prevented from sickling by cyanate treatment (2).

Erythrocytes from normal individuals have a normal life span when transfused into patients with sickle-cell disease (11), so the increased life span of cyanate-treated cells inferred from the results of these studies probably reflects those cells that do not sickle in the circulation. Therefore, the anti-sickling effect of cyanate observed in vitro appears to be retained in the patient.

These results strengthen the rationale for further investigation of cyanate as a therapeutic agent in sickle-cell disease. Studies in progress include evaluation of possible toxicity of cyanate in humans and determination of clinical efficacy.

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<th>Patients with sickle-cell disease</th>
<th>Untreated cells (days)</th>
<th>Cyanate-treated cells (days)</th>
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<tbody>
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<tr>
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