The Effect of Sodium Cyanate on Globin Synthesis

By Michael Habib, Veronica Watson, and Elias Schwartz

The incubation of peripheral blood with sodium cyanate produced dose-related suppression of hemoglobin synthesis. Addition of 5 mM sodium cyanate reduced globin synthesis in the peripheral blood of a patient with sickle cell disease by 34%, while 25 mM cyanate reduced synthesis by 92%. The relative synthesis of α- and β-chains remained constant despite the reduction of total synthesis. The effects were also found in peripheral blood and bone marrow of patients without hemoglobinopathies. The results suggest that further studies of the effect of cyanate on protein synthesis are necessary before widespread use of the drug in sickle cell disease.

SICKLE CELL DISEASE is a hemoglobinopathy with hemolytic anemia and vaso-occlusive crises. Cyanate salt have been used in an attempt to correct the anemia and to prevent the thrombotic complications that occur as a result of the sickling process. Cyanate irreversibly carbamylates the N-terminal amino groups in α- and β-chains of hemoglobin. Although beneficial effects of cyanate have been demonstrated on red cell survival and oxyhemoglobin levels in sickle cell disease, studies of red cell enzymes and endocrine and neurologic function indicate that there may also be adverse effects.

We have studied the action of cyanate on hemoglobin production and found significant dose-related suppression of globin synthesis.

MATERIALS AND METHODS

Heparinized whole blood was obtained from patients with sickle cell disease, sickle-beta thalassemia, and homozygous beta thalassemia. The reticulocytes in each patient were 6% or higher.

In the experiments determining the effect of sodium cyanate on total protein synthesis in the red cells, aliquots of whole blood or bone marrow were preincubated with sodium cyanate (NaOCN) at concentrations from 5 mM to 50 mM and with dextrose (2 mg/ml) at 37°C for 15 min in a metabolic shaker. After preincubation of 1 ml samples, 1.0 μCi of 14C-leucine was added to each sample, and incubation was continued for 2 hr at 37°C.

The red cells were then washed three times with cold saline and were hemolyzed with 4 volumes of 1.0 mM magnesium chloride. After 90 sec 1 volume of 1.5 M potassium chloride was added, and the hemolysates were centrifuged at 15,000 g for 20 min. Duplicate aliquots of each hemolysate were used to prepare globin. Globin was precipitated with cold acid acetone twice and once with cold acetone, in order to remove heme.


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The precipitated globin was dried overnight and dissolved in 8 M urea; the radioactivity of the solution was determined in a liquid scintillation spectrometer. The hemoglobin concentration of each hemolysate was determined, and the final results were expressed as cpm per mg of hemoglobin.

Further experiments were performed to determine the effect of sodium cyanate and of an equivalent concentration of sodium chloride used as a control on synthesis of individual globin chains. After preincubation of 4.0 ml of peripheral blood with 10 or 20 mM sodium cyanate and dextrose, 10 μCi of 14C-leucine were added, and incubation at 37°C was continued for 2 hr. The red cells were then washed, hemolyzed, and centrifuged to remove the cell membranes. Globin chains were separated by chromatography on carboxymethyl cellulose in 8 M urea at pH 6.7 using a sodium phosphate gradient. Radioactivity was determined in a liquid scintillation spectrometer, and absorption was measured in a spectrophotometer at 280 nm. The total radioactivity of each peak was calculated by summation of the radioactivities of the tubes containing the peak.

In one experiment on blood from a patient with sickle-beta thalassemia that had been incubated with 20 mM sodium cyanate, the identity of the globin peaks was confirmed by urea starch gel electrophoresis and by fingerprinting of the peptides obtained by tryptic digestion.

RESULTS

The effects of sodium cyanate on total protein synthesis in red cells from patients with sickle cell anemia, sickle-beta thalassemia, and homozygous beta thalassemia were similar. A representative response occurred in a patient with sickle cell disease where there was a 34% reduction in total reticulocyte protein synthesis with 5 mM sodium cyanate and more than 90% suppression with concentrations of 25 mM or higher (Fig. 1). A similar response was noted with bone marrow from a patient without a hemoglobinopathy, where concentrations of 10 mM, 20 mM, and 30 mM sodium cyanate caused 60%, 92%, and 96% suppression of protein synthesis, respectively. The synthesis of separate globin chains in peripheral blood was similarly suppressed by cyanate. In a patient with sickle cell disease, 10 mM sodium cyanate produced a 35% reduction in the synthesis of alpha chain and a 40% reduction in beta chain, the effect on both being approximately the same. Similar suppression was noted in a patient with sickle-beta thalassemia (Fig. 2). In each of the patients in whom synthesis of individual globin
GLOBIN SYNTHESIS

Fig. 2. Comparison of chromatograms from patient (S.S.) with sickle-beta thalassemia (transfused 2 mo previously) whose red cells were incubated with 10 mM NaCl(A) or 10 mM NaOCN(B). Suppression of synthesis and appearance of new peaks are apparent in cells incubated with cyanate.

chains was studied, the $\beta/\alpha$ ratio of radioactivities was not altered by the action of cyanate (Table 1). The suppression of $\alpha$-chain synthesis with 10 mM cyanate ranged from 35% to 65%.

Cyanate attaches to the N-terminal amino groups of globin chains irreversibly, changing the chromatographic and electrophoretic behavior of the chains. The carbamylation peaks elute earlier than the normal globin peaks. The new absorption peaks in our studies contained radioactive globin, indicating carbamylation of newly formed globin chains as well as previously formed hemoglobin. Fingerprinting of tryptic digests of the individual peaks confirmed their identity. The degree of carbamylation, as indicated by the shift of absorption peaks, did not correlate with the amount of suppression of globin synthesis in individual experiments. This finding indicates that the

<table>
<thead>
<tr>
<th>Cyanate Concentration (mmole/liter)</th>
<th>Suppression of $\alpha$-Chain Production</th>
<th>$\beta/\alpha$ Ratio (Radioactivity) NaCl</th>
<th>$\beta/\alpha$ Ratio (Radioactivity) NaOCN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sickle cell anemia</td>
<td>35.4</td>
<td>1.10</td>
<td>1.02</td>
</tr>
<tr>
<td>Sickle $\beta$-thalassemia (S.S.)</td>
<td>42.6</td>
<td>0.52</td>
<td>0.47</td>
</tr>
<tr>
<td>Sickle $\beta$-thalassemia (E.W.)</td>
<td>84.3</td>
<td>0.74</td>
<td>0.78</td>
</tr>
<tr>
<td>Thalassemia major</td>
<td>65.1</td>
<td>0.24</td>
<td>0.23</td>
</tr>
</tbody>
</table>
mechanism of suppression of globin production may be independent of the carbamylation of globin chains, as has recently also been suggested on the basis of studies of initiation of protein synthesis in the presence of cyanate. 

DISCUSSION

Sodium cyanate has a number of different effects on the red cell. The in vivo survival of sickle erythrocytes after in vitro incubation with 50 mM cyanate is prolonged. The hematocrit rose in patients with sickle cell disease who received cyanate salt by mouth, possibly because of prolongation of red cell lifespan. These effects of sodium cyanate may be due to the increased oxygen affinity and higher proportion of oxyhemoglobin that occurs with carbamylation, rather than to a major direct inhibition of sickling. In addition, cyanate alters the mobility of hemoglobin and globin chains by conferring additional negative charges to the molecules. The mobility and activity of red cell enzyme are also affected. After incubation with 10 mM sodium cyanate for 1 hr, there is a decrease in the activity of the hexose monophosphate shunt and glucose-6-phosphate dehydrogenase.

Our studies indicate that, in addition to the effects outlined above, there is a major degree of suppression of globin synthesis in reticulocytes and nucleated red cells at concentrations of cyanate that have been used for previous studies. The effects on globin synthesis found in the present studies are unexpected in view of the rise in hematocrit noted in patients who were treated with cyanate. It is possible that the beneficial effect on red cell survival may more than counterbalance the impressive effect on hemoglobin synthesis, resulting in a small net rise in packed cell volume, or that the in vivo concentration of cyanate is less than those used in our study. Further studies are needed to evaluate the relative magnitude of these effects in persons treated with cyanate and also the effect of cyanate on protein synthesis in red cell precursors.

The observed suppression of globin synthesis might in part be due to an indirect effect by interfering with amino acid transport and utilization. Studies by Alter et al. have demonstrated that in the presence of 50 mM cyanate transport of amino acids into the cell was normal and that amino acylation of tRNA was not affected. The free amino acids were not carbamylated in 50 mM cyanate. The suppression of globin synthesis, therefore, is most likely a direct intracellular effect.

Hemolysis and ineffective erythropoiesis in the beta thalassemia syndromes are in large part due to precipitation of excess a-chain and resultant membrane damage. An agent that would selectively suppress a-chain synthesis in patients with beta thalassemia might cause clinical improvement in these syndromes. Although cyanate did not selectively suppress the relative synthesis of a- and b-chains in blood from patients with thalassemia in our studies, the absolute suppression of a-chain synthesis was greater than that of b-chain. Further studies are needed to determine if this effect on a-chain synthesis would be of help in prolonging survival of the thalassemic red cell.
Considerable enthusiasm has arisen about the possible use of cyanate salts in the treatment of patients with sickle cell anemia and in the prevention of sickle cell crises. If the drug is shown to be effective in reducing the incidence of vasoocclusive crises, the major need for its use will be in childhood where the effects of the disease are frequently very severe. It is of importance to evaluate the effects of this drug on nonhematopoietic tissues. There is a significant reduction in maze learning in mice as the result of carbamylation of brain proteins. Mice that have been fed cyanate for long periods of time have reduced fertility on the basis of inactivation of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). There is a marked reduction in activity of bovine thyroid-stimulating hormone (TSH) with 10 mM cyanate. The diversity of effects due to cyanate that have been previously reported and the effects on protein synthesis that have been demonstrated in the present study should alert investigators to be cautious in the clinical use of this agent, especially in children. Although cyanate may have a beneficial effect on the sickling process, its effects on growth and other body processes must be carefully evaluated before the drug can be recommended for general use in patients with sickle cell disease.

REFERENCES

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