The Effect of Carbon Monoxide on Red Cell Life Span in Sickle Cell Disease

By Ernest Beutler

Carbon monoxide at a concentration of 1000–2000 ppm was administered to sickle cell disease patients. In each of two patients, one 51Cr red cell survival study was carried out before CO administration, and a second study was initiated a few days before CO administration was started. In both, significant prolongation of red cell survival was observed, suggesting that the rheologic properties of sickle cells were favorably influenced in vivo. The administration of carbon monoxide is not recommended as a treatment for sickle cell disease. However, further trials would seem to be justified if conducted under carefully controlled conditions.

The aggregation of hemoglobin S molecules which leads to the deformation of sickle cells depends on the concentration of hemoglobin S in the deoxy conformation. The conformation of the hemoglobin S is a function not only of oxygenation, but also depends upon the binding of other ligands. Some therapeutic attempts directed at changing the conformation of sickle hemoglobin have been made. The induction of methemoglobinemia represents the earliest of these. Methemoglobin is in the oxy conformation, and the induction of methemoglobinemia by the experimental administration of sodium nitrite and p-aminopropiophenone has resulted in some degree of normalization of red cell survival in sickle cell patients. However, maintenance of stable, elevated methemoglobin levels has made the induction of methemoglobinemia an impractical therapeutic maneuver.

Liganding with carbon monoxide also very strongly favors the oxy conformation of the hemoglobin molecule. In 1963, Sirs reported that the administration of carbon monoxide exerted a favorable influence on the proportion of sickle cells in a patient with sickle cell anemia. Subsequently, however, Purugganan and McElfresh induced levels of 11.2%–17% carboxyhemoglobin twice daily in two boys with sickle cell disease and failed to demonstrate any effect on red cell survival. We are not aware of any additional therapeutic trials with carbon monoxide and now report an evaluation of the therapeutic potential of this easily administered form of therapy in three patients with sickle cell disease.

Patients

Patient BA is an 18-yr-old black female who has been known to have sickle cell disease since she was 4 yr old. Painful crises have been the main clinical manifestation of her disease; the development of back pain and pain in the lower extremities has required hospitalization several times a year. Her hemoglobin level has generally fluctuated between 7.6 and 10 g/100 ml, and there have been no leg ulcers or neurologic complications. She is a nonsmoker.
DT is a 30-yr-old black female known to have sickle cell disease since she was 8 yr old. Her hemoglobin level has ranged from 7.0 to 8.4 g/100 ml over the past few years. The principal clinical manifestation of sickle cell disease in this patient has also been painful crises, occurring approximately four times per year. Four years ago she developed a transient hemiparesis, and there has been no recurrence of neurologic symptoms. There have been no ankle ulcers. This patient is also a nonsmoker.

LJ is a 38-yr-old black female, first diagnosed as having sickle cell disease when she was 13 yr of age. Since this time she has been hospitalized some 70 times for sickle crises. These generally subsided after 1 or 2 days of conservative therapy with fluids and analgesics. Her hemoglobin level has ranged from 5.9 to 8.2 g/100 ml in recent years. She is a nonsmoker.

**MATERIALS AND METHODS**

$^{51}$Chromium studies were carried out by adding 20-100 μCi of sodium $^{51}$chromate to approximately 20 cc of blood collected in special ACD solution (Abbott Laboratories). After 20 min at room temperature, 50 mg of ascorbic acid were added, and the blood was injected intravenously. The first sample was collected 24 hr after injection and was considered as representing 100% activity. One $^{51}$Cr study was carried out on each of two patients prior to the initiation of experimental therapy with carbon monoxide. When blood radioactivity had declined to negligible levels, another blood sample was labeled with $^{51}$Cr, and after baseline $^{51}$Cr activity determinations had been obtained for a few days, the patients were admitted to the hospital. A scalp vein needle with a reseal injection site was inserted into a peripheral vein and kept filled with heparin-saline. Two- to three-milliliter samples were drawn, and carboxyhemoglobin estimations were carried out at the bedside using a co-oximeter (Instrumentation Laboratories).

Mixtures containing carbon monoxide were prepared by mixing the gas flow from a tank containing air with that from a tank containing 20% carbon monoxide and 80% nitrogen. The rate of gas flow was accurately monitored with gas-flow meters of appropriate range. The air tank was fitted with an alarm which sounded if the tank was empty, so as to preclude the possibility of unwittingly administering 20% carbon monoxide to the patient. A physician was at the bedside at all times during carbon monoxide administration, and 100% oxygen was immediately available to aid in the treatment of any carbon monoxide toxicity which might occur. The procedure was discussed in detail with the patients prior to the initiation of the studies, and informed consent was obtained. Total flow rates of 4-8 liters/min were used with a carbon monoxide level ranging from 1000 to 2000 ppm. The percentage of carbon monoxide used was adjusted so that the blood carboxyhemoglobin level rose at a rate of approximately 1%/min. Carbon monoxide was administered at intervals of 3-4 hr starting at approximately 8:00 a.m. and continuing until 9:00 or 10:00 p.m.

**RESULTS**

Control of blood carboxyhemoglobin levels proved to be quite predictable, and it was therefore a relatively simple matter to achieve the desired level of blood carboxyhemoglobin during each course of carbon monoxide administration. The baseline carboxyhemoglobin was surprisingly high in all three patients even though they were nonsmokers.* After elevation of carboxyhemoglobin...
globin levels had been achieved by the administration of carbon monoxide, the carboxyhemoglobin level gradually fell over a period of several hours while the patient respired room air. Typically, at the end of 3 or 4 hr, blood carboxyhemoglobin levels were still well above the baseline concentration when administration of the gas was resumed. Even after a 10- or 11-hr night-time gap in carbon monoxide administration, carboxyhemoglobin levels were 2% or 3% above baseline. The administration of carbon monoxide was well tolerated. Even though co-oximeter readings as high as 20.3% were achieved in patient BA, and of 23.6% in patient DT (representing approximately 12% and 15% carboxyhemoglobin, respectively), no symptoms were reported. A mild transient headache in patient BA did not appear temporally related to the administration of carbon monoxide, and no dyspnea, palpitations, or any other discomforts were reported by either subject.

During the control period, the $^{51}$Cr red cell survival curve obtained on subject BA consisted of a single exponential component with a $t_{1/2}$ of 9.2 days over the entire 14 days of the control study. This subject was given two 5-day courses of carbon monoxide. The results of this study are summarized in Fig. 1. The $^{51}$Cr red cell survival data obtained during carbon monoxide administration were calculated by the method of least squares as five components. A significant change in the slope of the $^{51}$Cr survival curve during carbon monoxide administration was observed, both when compared with the interval immediately before and the interval immediately after each period of carbon monoxide administration and when compared with the control $^{51}$Cr survival curve carried out prior to carbon monoxide therapy. The decreased rate of hemolysis documented with the $^{51}$Cr survival data was not, however, reflected in a significant decrease in the plasma bilirubin level. Although there was an increase in the hemoglobin concentration of the blood, this may have been due, at least in part, to the increased stimulus to erythropoiesis imposed by the carbon monoxide treatment. Indeed, a significant degree of reticulocytosis was noted. A sample of blood taken towards the end of the first course of carbon monoxide therapy showed no significant alteration in red cell 2,3-DPG, or intracellular pH. Three months after conclusion of the studies depicted in Fig. 1, the patient was seen in the emergency room with severe back pain of only 2-hr duration. Carbon monoxide was administered, achieving a blood carboxyhemoglobin level of 19.1% within 5 min. Although her pain disappeared rapidly and the patient was able to return home, no conclusions can be drawn regarding the effect of CO on sickle crisis (see patient LJ below).

The control $^{51}$Cr red cell survival curve obtained on patient DT could be resolved into two components: the first, lasting for 9 days with a $t_{1/2}$ of 10.5 days and the second with a $t_{1/2}$ of 18.7 days. For this reason, the patient was given

In a study of 17 nonsmoking patients with sickle cell disease, T. Bensinger (personal communication), using gas chromatography, found an average carboxyhemoglobin level of 3.5% with a standard deviation of 1.4%. The range of values obtained was 1.4%–6.4% carboxyhemoglobin. These values are probably more representative of the true carboxyhemoglobin levels of patients with sickle cell disease. They are elevated because of the increased rate of heme catabolism in these patients.
only one course of carbon monoxide therapy. Because many of this patient's veins were sclerosed, the course of treatment was limited to only 3 days. The results of this study, calculated in the same manner as those of patient BA, are shown in Fig. 2. A highly significant prolongation of red cell survival was again observed during the administration of carbon monoxide.

Patient LJ was admitted to the hospital with a 6-hr history of severe back and leg pain. She was given intravenous fluids and 100 mg of Demerol intramuscularly with some relief of pain. Three hours later severe back pain, bilateral shoulder pain, and pain below the left breast required another injection of demerol. One hour following demerol injection, she was considerably improved symptomatically, but had some residual pain. She was given carbon monoxide, 1000 ppm, raising her carboxyhemoglobin concentration by 4.5% of the total pigment. This therapy produced no symptomatic improvement. Four hours later, when the effects of the demerol had been dissipated, she again had severe pain. The carboxyhemoglobin level was 1% above baseline, and this was raised by 6% by the administration of carbon monoxide. No relief of pain was experienced at the time of carbon monoxide administration, and the pain dis-
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Fig. 2. The effect of carbon monoxide administration on ⁵¹Cr survival hemoglobin, hematocrit and reticulocyte count of subject DT. The bars indicate the standard error of the 1/2.

appeared gradually over the next 2 days, in a manner not unlike the patient's previous crises.

DISCUSSION

The clinical consequences of sickle cell disease are chiefly related to the alterations of the rheologic properties of sickle red cells, rather than to the anemia. Thus, therapeutic strategies which influence the equilibrium between the oxy and deoxy conformation in favor of the oxy conformation may be successful in the treatment of sickle cell disease even if they result in some compromise in the oxygen-delivering capacity of the blood.

In our early attempts to treat sickle cell disease by inducing methemoglobinemia with nitrite or p-aminopropriophenone, prolongation of red cell survival when methemoglobinemia was induced was presumably due to the fact that methemoglobin is in the oxy conformation. However, the treatment was impractical because control of methemoglobinemia was very difficult. This was a handicap particularly in patients with sickle cell disease because of their young red cells with the highly developed capacity to reduce methemoglobin rapidly.
Carbon monoxide is another agent which has a profound effect upon the function of hemoglobin. It produces a marked increase of the oxygen affinity of hemoglobin, presumably by strongly favoring the oxy conformation of the hemoglobin molecule. In an earlier study, we were able to demonstrate that, at a fixed level of oxygenation, carbon monoxide did inhibit the aggregation of sickle hemoglobin as measured by its effect on the viscosity of concentrated hemoglobin S solutions.\textsuperscript{5} We now find that induction and maintenance of elevated blood carbon monoxide levels were technically easily achieved and were unaccompanied by any overt toxicity.

As one might anticipate from these considerations, carbon monoxide did exert a favorable effect on red cell survival of patients with sickle cell disease. It is difficult to evaluate the negative results briefly reported by Purugganan and McElfresh,\textsuperscript{3} but in our studies higher levels of carboxyhemoglobinemia were maintained for longer periods of time. Although carboxyhemoglobinemia is much more easily maintained than methemoglobinemia, the present system of administration is not suitable for long-term therapy. While the prolongation of red cell survival which we observed is an encouraging indication that we have improved the rheologic properties of sickle cell erythrocytes by treatment with carbon monoxide, the clinical value of such a treatment would have to rest upon its ability to either prevent or to terminate sickle crises. Indeed, it is possible that long-term treatment with carbon monoxide might prove to be deleterious. First of all, the CO-induced increased rate of erythropoiesis and decreased rate of destruction should result in an increase in the red cell mass and hematocrit. This alteration poses a potential for increased viscosity should sickling occur, either because of discontinuation of CO administration or because of other factors. Secondly, it has been proposed by Astrup\textsuperscript{6} that long-term CO intoxication may lead to vascular changes.

It is unlikely that crises of long duration will be favorably affected by any therapeutic maneuver directed toward the unsickling of sickled red cells, since secondary tissue changes including edema and necrosis probably play a dominant role in the etiology of pain after prolonged occlusion of blood vessels by sickled red cells. The effect of induction of modest carboxyhemoglobinemia in the early stage of crisis, however, may well warrant investigation under properly controlled conditions. Our uncontrolled experience in the treatment of two crises, one of short duration and one of longer duration, does not provide much insight into the potential value of this type of therapy. We do not recommend the use of carbon monoxide as a treatment for sickle cell disease. While its further investigation may well be warranted, it should be carried out only under conditions which permit close monitoring of the patient.

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REFERENCES

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