Improved Exercise Performance After Exchange Transfusion in Subjects With Sickle Cell Anemia


Ten patients with sickle cell anemia underwent partial exchange transfusion with hemoglobin-A-containing cells using a technique that allowed hemoglobin concentration and blood volume to remain constant. The mean fraction of hemoglobin-A in these patients increased from 9% to 55%, but the mean hemoglobin concentration increased by only 1.44 g/dl. The exchange resulted in a large improvement in submaximal exercise capacity: the mean of the anaerobic threshold (the work at which lactic acid begins to accumulate in the blood) increased from 68 to 114 W. The mean work performed at a heart rate of 170/min, an estimation of maximal work capacity, increased from 128 to 187 W. Improved exercise performance after partial exchange transfusion may result from the superior flow properties of hemoglobin-A-containing red cells. Furthermore, we believe that exercise testing in sickle cell anemia has great potential utility as a means to monitor therapy and to evaluate the benefits of exchange transfusion.

Infarction in sickle cell anemia occurs because red cells containing hemoglobin-S occlude capillaries as they give up their oxygen. Therefore, a close relationship must exist between the oxygen transport properties of sickle cells and the clinical manifestations of the disease.

The oxygen affinity of blood from persons with sickle cell anemia is reduced, although the degree of reduction may vary among individuals and with the technique used for its measurement. The reduction appears to be primarily due to the effect of intracellular polymerization of hemoglobin-S molecules, but sickle cells also have increased 2,3-DPG concentration and reduced intracellular pH, which also influence oxygen affinity.

Because of the relationship between hemoglobin-S polymerization and oxygen affinity, many investigators have proposed pharmacologic manipulation to increase the oxygen affinity of hemoglobin-S and thereby inhibit polymerization. Conversely, any therapeutic maneuver that inhibits polymerization might also be expected to increase blood oxygen affinity and decrease oxygen delivery to tissues unless the agent has secondary effects on other properties of the red cell or oxygen delivery system. In fact, Rossi-Bernardi and coworkers suggested that the low oxygen affinity of sickle cell blood might compensate, in part, for the severe anemia. If this hypothesis is true, a therapeutic increase in blood oxygen affinity might lead to embarrassed oxygen delivery unless the hemoglobin concentration is simultaneously increased.

In this article, we report initial experiments designed to test whether the study of oxygen transport in sickle cell anemia patients can be used to obtain quantitative information about the benefits of therapy. Ten subjects with sickle cell anemia underwent partial isovolemic exchange transfusion with hemoglobin-A-containing red cells, and exercise performance was studied before and after the procedure. By design, hemoglobin concentration was held nearly constant, so that any physiologic changes that result could be related to the properties of the red cells, not their numbers.

Materials and Methods

All subjects were admitted to the Clinical Center of the National Institutes of Health, and after being informed about the nature and risks of the procedures, gave consent to participate. Whole blood oxygen affinity was measured as described previously. The fraction of hemoglobin-S was quantitated by densitometric scanning of cellulose acetate strips after electrophoresis at pH 8.6. 2,3-DPG determinations were performed according to the method of Nygaard and Rørth using kits supplied by Calbiochem (Behring Corp., San Diego, Calif.). Other hematologic measurements were done according to standard clinical techniques.

The exercise protocol was adapted from that described by Wasserman and Whipp. Emphasis using this test is on quantitation of submaximal performance, although maximal work can also be estimated. As the workload is progressively increased, a point is reached at which the delivery of oxygen to exercising muscle cells is no longer adequate to meet the demand. At that point, the anaerobic threshold (AT), lactic acid begins to accumulate in the blood. The AT occurs at about 60% of maximal work and is highly reproducible for an individual. Since the heart rate (HR) response to this increasing work load is linear, an individual's maximum work capacity can be estimated by extrapolation. Using the convention of Sjøstrand et al., we have chosen the work performed at a heart rate of 170/min (W170) as an estimate of maximum work. Several minutes of unloaded pedaling on a cycle ergometer (Collins—Braintree, Mass.) was performed at 60 rpm with electrocardiographic monitoring. The work was then increased in 25-W increments each minute until the subject reached 80% of maximal predicted heart rate or until the test was terminated by fatigue. In control experiments, oxygen consumption was measured at each of several work levels by collection of expired gas in a Tissot spirometer.

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and measurement of the gas volumes and compositions using a mass spectrometer (Medtron Medispect II, Waterford, Wisc.).

For each patient, measurements of heart rate versus work were carried out on several successive days to insure that no training effect could be observed and to allow the patient to become thoroughly familiar with the experimental protocol. For the complete test, a radial artery catheter was inserted and blood was sampled at each work level. The samples were cooled on ice, and within 1 hr of the test, perchloric acid extracts were made. These were either assayed directly for lactic acid or stored frozen overnight for analysis the next day using kits from Sigma (St. Louis, Mo.) Both procedures gave equivalent results.

Experiments with normal volunteers were done in the same manner, with the exception that venous blood was obtained from the forearm for the lactic acid analysis. The absolute concentration of lactic acid in venous and arterial blood may differ slightly, but the point at which the concentration increases during graded exercise can be detected satisfactorily with either.

Partial exchange transfusions were performed as described elsewhere using a Haemonetics Blood Cell Separator (Haemonetics Corp., Natick, Mass.). Five to six units of frozen deglycerolized compatible red cells were pooled before the procedure and then divided so that each unit was identical. After removal of a unit of donor blood from the patient (approximately 450 ml), the patient's plasma and a unit of donor cells were returned, and the cycle was repeated several times. The procedure was designed to maintain constant hemoglobin concentration and blood volume, while increasing the red cell 2,3-DPG concentration, the percentage of hemoglobin-S, and p50 were measured, and the exercise test was repeated 1-3 days later.

RESULTS

Subjects

The subjects were not chosen to reflect any degree of severity of sickle cell disease or any set of complications. In each case, the diagnosis of sickle cell anemia was based on the clinical history, the pattern of hemoglobin electrophoresis on cellulose acetate (pH 8.6) and citrate agar (pH 6.0) media, and the family history. The minimal requirements for participation in the study were accessibility of veins and the ability to perform the exercise test without undue discomfort.

Hemoglobin concentrations ranged from 6.2 to 10.5 g/dl before transfusion (see Table 1). Some of the subjects had detectable hemoglobin-A, the residual of prior transfusions. None of the subjects, however, was on a chronic transfusion program, and none had been transfused within the previous 6 wk.

Nine control subjects were chosen from laboratory and nursing personnel. No effort was made to match the subjects and controls in age or sex, but the two

<table>
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<th>Subject</th>
<th>Sex</th>
<th>Age</th>
<th>Hb (g/dl)</th>
<th>Retic (%)</th>
<th>DPG (M/M Hb)</th>
<th>p50 (mm Hg)</th>
<th>HbS %</th>
<th>W170 (Watts)</th>
<th>AT (Watts)</th>
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*p > .01 < .02 < .01 < .01 < .01 < .01

Controls 5  F  15.2  .93  26.7  207  131
n = 9  M  ±1.2  ±1.3  ±1.7  ±47  ±53

*Student's t test.
groups subsequently proved to be similar in these regards.

Exercise Test

In order to compare our results with those found in the literature, calibration of the cycle ergometer was carried out (Fig. 1). Values for the oxygen consumption at the anaerobic threshold have been given by Wasserman and Whipp as 0.5–1.0 liters/min for cardiac patients, 1.0–1.5 liters/min for normal sedentary subjects, and 2.0–2.5 liters/min for trained normal subjects. According to our calibration in Fig. 1, these correspond to about 50, 100, and 200 W, respectively. Since oxygen consumption was not measured in each subsequent test, the anaerobic thresholds and W170s are expressed in watts only.

The sickle cell anemia patients performed the test with little difficulty. The test was terminated if the subject reached 80% of his predicted maximal heart rate, but in some instances the test was stopped because of fatigue or leg pain. No cardiac arrhythmias were noted.

To test reproducibility of the anaerobic threshold determination, several subjects were tested repeatedly using measurements of heart rate and the ratio CO₂ produced/O₂ consumed. This method, being noninvasive, is convenient for repetitive testing of an individual. No difference could be detected either in anaerobic threshold or W170 in three successive tests. These results confirm that the 1-min incremental test does not induce a significant training effect even in sickle cell anemia subjects.

Pretransfusion Performance

The average anaerobic threshold was 131 W for our normal group and 68 W for the sickle cell group (Table I). In exercise performance, these two groups correspond to the normal sedentary and cardiac groups, respectively, described by Wasserman and Whipp. It must be noted, however, that the exercise test does not necessarily implicate cardiac dysfunction in our sickle cell anemia patients. Exercise limitation in these subjects could be the result of hematologic, pulmonary, muscle, circulatory, or cardiac disturbances either alone or in concert. To assess the influence of each factor on exercise performance, it would be necessary to hold as many constant as possible while varying only the one under consideration. Thus, according to our experimental design, only the quality of the red cells has been changed by exchange transfusion.

Postexchange Performance

In all cases, submaximal exercise capacity (anaerobic threshold) improved after exchange transfusion (Fig. 2). This is also true for W170, an approximation of maximal work when the subjects are young. On the average, hemoglobin concentration increased slightly (1.44 g/dl), but in two cases, it decreased, and in three others the increase was less than 1.5 g/dl. In a representative case (Fig. 3), the hemoglobin concentration fell slightly after exchange and the p50 returned to normal from a pretransfusion value of 37.4 mm Hg, changes which should be detrimental to oxygen delivery. Anaerobic threshold was not only increased with respect to work, but after transfusion the absolute concentrations of lactic acid were lower for each work level. In addition, the heart rate at a given work level was reduced significantly.

In a second example in which two tests were carried out before exchange transfusion (Fig. 4), the hemoglobin concentration increased from 8.5 to 10.0 g/dl. The anaerobic threshold increased from 100 to 140 W and the W170 from 150 to 225 W. This case illustrates that the variation in heart rate versus work in consecutive tests (i.e., training effect) is small compared to the effect of exchange transfusion.

Platelet counts were unaffected by the exchange
procedure. On the average, reticulocyte counts decreased, in about the same proportion as the HbS (Table 1). The individual variation was large, however, and did not correspond to impairment in exercise.

**Blood Oxygen Affinity**

In all cases in which the measurement was made, whole blood p50 decreased to nearly normal values. In a representative case (subject J.T.), calculation of the theoretical arterial-venous oxygen content difference, assuming arterial and venous oxygen tensions of 100 and 40 mm Hg, respectively, yields 6.70 ml/dl before and 5.66 ml/dl after transfusion. This suggests that higher cardiac output might be required after transfusion for a given amount of work. In fact, the anaerobic threshold for this subject more than doubled (68–154 W after transfusion), and the W170 increased (115–161 W). Thus, we must conclude that in the sickle anemia patients we have studied, reduced blood oxygen affinity, irrespective of the actual degree of reduction, is not a significant compensation for anemia.

**DISCUSSION**

We have found that replacement of about 50% of the subjects’ sickle cells with normal cells leads to increased exercise performance in each of 10 cases. That is, the point at which tissues switch to anaerobic metabolism occurs at higher work levels. Since oxygen consumption is a constant function of work for an individual in the 1-min incremental exercise test (Fig. 1), we conclude that exchange transfusion in our patients led to more efficient delivery of oxygen to tissues. Moreover, more work can be performed (i.e., more oxygen delivered) at a given heart rate after the exchange than before (cf., Fig. 3, and the increased W170, Table 1).

Many possible mechanisms for the improved exercise performance could be involved, and our studies do not distinguish among them. Increased hemoglobin concentration, however, would seem very unlikely, since the mean increase in our subjects was only 1.44 g/dl, and in 2 patients the concentration actually decreased. In a previous study of normal subjects, Woodson et al. found that for each increase of 1 g/dl in hemoglobin concentration, maximal work increase in W170 of 57 W or 45% of the pretransfusion value could not be explained by an increase of 1.44 g/dl in hemoglobin concentration. Moreover, although quantitative data are lacking, the effect of hemoglobin concentration on submaximal exercise capacity seems to be even less than on maximal exercise capacity.

Decreased blood viscosity in our subjects after exchange transfusions could explain at least a part of
the results. It is known that at equivalent hematocrit, SS blood is more viscous than AA blood\textsuperscript{17} and that mixtures of AA and SS have better flow properties than SS cells alone.\textsuperscript{18} Thus, decreased viscosity after exchange transfusion could lower peripheral resistance with a resultant increase in cardiac output, independent of the oxygen delivery properties of red cells.\textsuperscript{19} However, the increased cardiac output of anemia is primarily due to increased heart rate\textsuperscript{20} and therefore would not seem comparable with our findings that heart rate is decreased at all work levels after transfusion.

We have previously demonstrated that the oxygen affinity of sickle cell blood depends somewhat on the method used for its measurement.\textsuperscript{2} Although the method used in the present studies gives a maximal value of p50, and in vivo p50 in sickle cell anemia patients is increased with respect to normal.\textsuperscript{2} A rightward shift of the oxygen equilibrium curve, due to increased 2,3-DPG concentration, is usually thought to be beneficial to oxygen delivery in other types of anemia, and Rossi-Bernardi and coworkers suggested that the rightward shift in sickle cell anemia could serve a similar compensatory function.\textsuperscript{4} While our finding of improved exercise performance after a decrease in p50 does not refute the latter concept, we believe that blood oxygen affinity in sickle cell anemia is less important than red cell rheology as a determinant of oxygen delivery.

The major difficulty in evaluation of therapy in sickle cell anemia is that a clearly defined indicator of success is lacking. Various authors have used decreased reticulocytes or irreversibly sickled cells, increased hemoglobin concentration, or decreased crisis frequency as indicators of benefit. These measurements, however, often require that large numbers of subjects be studied for long periods of time, usually with detailed and expensive double-blind crossover protocols before conclusive results can be obtained. Therefore, an objective, quantitative, and reproducible in vivo measure of oxygen delivery, such as the exercise test we have described, would prove to be of immense value. Such a measure could be used to follow the clinical course of individuals who receive experimental therapy. Drugs that have no effect in an individual or in a small group of patients could be eliminated from further consideration.

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Improved exercise performance after exchange transfusion in subjects with sickle cell anemia

DM Miller, RM Winslow, HG Klein, KC Wilson, FL Brown and NJ Statham