Physiologic effects of normal- or low-oxygen-affinity red cells in hypoxic baboons

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Red blood cell transfusions are given to maintain or restore cellular aerobic respiration. To be therapeutically effective, the circulating red blood cells must be able to load oxygen in the lungs and release it to the tissue. The red blood cell level of the organic phosphate compound 2,3-diphosphoglycerate (2,3-DPG), which falls during storage in acid-citrate-dextrose (ACD) or citrate-phosphate-dextrose (CPD) at 4°C, affects the red cell affinity for oxygen (3, 4, 6). Red blood cells with reduced 2,3-DPG levels have an increased affinity for oxygen (15, 16, 21-23), and they produce an increase in cardiac output and/or a decrease in mixed venous PO₂ after transfusion (15, 17-22). Red blood cells with decreased affinity for oxygen have improved oxygen delivery to tissue under conditions of low arterial PO₂ tension, but their ability to load oxygen in the lungs is impaired.

In a previous study from this laboratory (17), the transfusion of preserved red blood cells with low 2,3-DPG levels to anemic patients with traumatic injuries produced a significant increase in cardiac output during the 4-h posttransfusion period. Another of our studies (20) showed that when hyperventilated anemic baboons were given preserved red blood cells with decreased 2,3-DPG levels, there was a significant increase in cerebral blood flow during the 2-h posttransfusion period. Anemic patients in hemorrhagic or septic shock with myocardial or cerebrovascular insufficiency may fare better if the red blood cells they receive have 1½-2 times the normal 2,3-DPG levels (15).

Red blood cells can be biochemically modified after storage in the liquid state to increase the 2,3-DPG levels. This is done by incubating the red blood cells at 37°C for 1 h in vitro with a solution containing pyruvate, inosine, glucose, phosphate, and adenine (PIGPA), and the process is called "rejuvenation" (5, 11, 14, 15, 22). These substances are potentially toxic, and intravenous administration may be dangerous. The biochemically modified red cells are washed before transfusion to remove the additives, whether or not they are frozen. Biochemically modified red blood cells have excellent posttransfusion survival values and increased oxygen-releasing capacity for about 72 h after transfusion (14, 21). A method for restoring the 2,3-DPG level in vivo by intravenous administration of a solution of inosine, pyruvate, and phosphate has been reported (9, 10, 13).

We used baboons in our study because the metabolism and function of baboon red blood cells are similar to those of human red blood cells (13, 19, 20).

MATERIALS AND METHODS

Recipients. Fifteen adult male baboons (Papio cynocephalus, anubis), weighing 19-24 kg, were given 1.5 ml of phenycyclidine hydrochloride (Sernylan) and 2 ml of pentobarbital (65 mg/ml) intramuscularly for sedation. Anesthesia was maintained throughout the experiment by constant intravenous infusion of pentobarbital (0.1 mg/kg per min). Two to three mg of curare were given intravenously. Radiopaque catheters were placed in the femoral artery, the jugular vein, and the pulmonary artery. The catheters were kept open by periodic flushing with an isotonic saline solution. The femoral artery catheter was connected to a recorder via a preamplifier (Sanborn), and the arterial blood pressure was monitored throughout the experiment. The baboons were intubated and connected to a respirator (model 607,
During the baseline period the respiratory rate was 10-12 counts/min, with a tidal volume of 10-13 ml/kg. Body temperature was monitored with an esophageal thermistor probe. On completion of the base-line studies, 1 unit of whole blood (450 ml), approximately 35% of the blood volume from the arterial catheter, was drawn within 10 min. To maintain the blood pressure at nearly normal levels, the volume was immediately replaced with 500 ml of isotonic, nonbuffered saline solution.

One hour after phlebotomy, each baboon was given 350-400 ml of washed red blood cells concentrated to a hematocrit of about 55 vol % . The red blood cells were administered through a standard 170-µm filter (Fenwal Laboratories, Morton Grove, Ill.) by external pressure of 100 mmHg within 15 min. Twenty minutes after transfusion, we lowered the percentage of oxygen in the inspired air from the normal value of 21 to 15% and maintained it at this level for 2 h, after which it was restored to normal value. Using a recording spirometer in a closed respiratory circuit we measured oxygen consumption before and after phlebotomy, after transfusion, 1 and 2 h after reduction of the inspired oxygen content (hypoxic period), and 30 min after restoration of the inspired oxygen content to 21% (normoxia). A 15-min period of equilibration was allowed to pass before measurements at each stage of the study.

Blood samples were drawn from the femoral and pul-

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**Fig. 1.** Measurements of pH, Po2, Pco2, and O2 content in blood collected anaerobically from femoral artery, pulmonary artery, and jugular vein. Blood samples were collected before and after phlebotomy, after transfusion, during 2-h period of arterial hypoxemia, and 30 min and 24 h after restoration of arterial oxygen tension to normal. Eight baboons were given red blood cells with decreased affinity for oxygen, and 7 baboons were given red blood cells with normal affinity for oxygen. Statistical significance between the 2 groups is noted with asterisks. Number in parentheses indicates number of baboons in each group that was studied for that sampling period. If no number is present, then n signifies 7 baboons in normal 2,3-DPG group and n signifies 8 baboons in 1½ times normal 2,3-DPG group.
NORMAL- OR LOW-AFFINITY RED CELLS IN HYPOXIC BABOONS

Pulmonary arteries and from the jugular vein in heparinized, gas-tight glass syringes before and after phlebotomy, after transfusion, and 1 and 2 h after the onset of hypoxia, and 30 min after return to normoxia. The samples were kept on ice, and within 30 min of collection measurements were made of pH, PO₂, PCO₂, oxygen content, and oxygen-carrying capacity (19). The oxygen saturation of hemoglobin was measured in the blood obtained from the femoral artery, pulmonary artery, and jugular vein. In the blood obtained from the pulmonary artery, the in vivo P₅₀ value was determined as previously described (19). After red cell washing, the in vitro P₅₀ values were measured at 37°C at pH of 7.2 and PCO₂ of 0 mmHg by the Bellingham and Huehns procedure (2).

Blood samples were collected in heparin for measurement of red blood cell 2,3-DPG, ATP, and inorganic phosphorus levels (18). Blood lactate (µmol/ml) was measured spectrophotometrically (7). The cardiac output was calculated from the measured oxygen consumption and the difference in oxygen content between the femoral and pulmonary arterial blood.

Transfused blood. On the morning of the experiment, about 700 ml of blood was collected in CPD from each blood group-compatible baboon. Some of the units were incubated for 1 h at 37°C in a 50-ml solution of pyruvate, inosine, glucose, phosphate, and adenine (solution A) (14, 21); these red cells (rejuvenated) had 1/5 times the normal 2,3-DPG levels and reduced affinity for oxygen. The units were washed in the IBM blood processor using 2 liters of an isotonic sodium chloride solution with a pH of 7.2 that contained 200 mg/100 ml glucose and 200 mg/100 ml disodium phosphate. Red cell washing removes the inosine and adenine (16, 21, 22). Other units were incubated at 37°C for 1 h without a rejuvenation solution and were washed as described above (nonrejuvenated); these units had normal 2,3-DPG levels and normal affinity for oxygen. Both the rejuvenated and the nonrejuvenated washed red blood cells had hematocrit values of about 55 vol % at the time of transfusion.

RESULTS

Seven baboons received red blood cells with normal 2,3-DPG levels (averaging 16.3 µmol/g Hb), and eight baboons received red blood cells with increased 2,3-DPG levels (averaging 26.6 µmol/g Hb). Figure 1 shows the pH, PO₂, PCO₂, and oxygen content. The transfusion of washed biochemically modified red blood cells caused the 2,3-DPG levels to rise from 17.5 to 21.6 µmol/g Hb; this represented an increase of 125% of normal (Fig. 2). The transfusion of washed nonrejuvenated red blood cells produced no significant change in the 2,3-DPG level.

Fifteen minutes after phlebotomy, the hemoglobin concentration was reduced by about 27%, and the oxygen content and capacity by about 28% in both groups (Figs. 1 and 2), but measurements of pH, PO₂, PCO₂, and oxygen saturation were similar to prephlebotomy values (Figs. 1 and 3). After transfusion the hemoglobin concentration and oxygen-carrying capacity were similar to the prephlebotomy values in both groups, but oxygen content and saturation values were slightly lower in the high 2,3-DPG group; the in vivo P₅₀ value was 6.0 mmHg higher in this group (Fig. 4). Oxygen consumption remained constant throughout the study and was not significantly different between the two groups. Neither the phlebotomy nor the subsequent transfusion had any significant effect on cardiac output (Fig. 5).

During hypoxia there was a greater fall in oxygen saturation in the baboons transfused with high 2,3-DPG red cells than in those with normal 2,3-DPG red cells, with a significant difference (P < 0.02) after 2 h of hypoxia (Fig. 3). Throughout the hypoxic period, the in vivo P₅₀ value was 3-5 mmHg higher in the high 2,3-DPG group, and a small but consistent decrease in cardiac output was seen throughout the hypoxic period (Figs. 4 and 5). Thirty minutes after restoration of the arterial PO₂ tension to normal, there was a significant decrease in cardiac output (P < 0.02) in the high 2,3-DPG group compared with the control group (Fig. 5).

Oxygen extraction was similar in the two groups throughout the experiment, and the pulmonary artery and jugular venous PO₂ tensions were slightly lower in the high 2,3-DPG group than in the normal 2,3-DPG group (Fig. 1). Although the baboons in the high 2,3-DPG group showed a slight increase in extraction during the first hour of hypoxia, only a modest increase prevailed throughout the study period (Fig. 4).

An increase in red blood cell ATP paralleled an in-
PIGPA (solution A) at 37°C, the in vitro $P_{50}$ value does not rise above 44 mmHg even in normal red blood cells, notwithstanding that there is a 25% increase in the 2,3-

crease in the 2,3-DPG level (Fig. 2). There were no changes in blood lactate levels after phlebotomy, after transfusion, during hypoxia, or after restoration of the arterial oxygen tension to normal. There were no significant differences in plasma creatinine or uric acid levels, or blood calcium or phosphorus levels between the two groups.

DISCUSSION

When one-third the volume of red blood cells was replaced with type-specific, washed homologous red blood cells with 1.12 times the normal 2,3-DPG levels, the circulating red blood cells of the baboons showed an increase in 2,3-DPG to 125% of normal, and a slight, insignificant increase in the in vivo $P_{50}$ value. Apparently the red blood cells with 160% of normal 2,3-DPG levels produced only a slight increase in the in vivo $P_{50}$ value after transfusion because the value had already increased after the phlebotomy. We have noted that between the first and second hour after incubation with
DPG level during this period. Our findings indicate that after an increase in red blood cell 2,3-DPG beyond a certain level, there is little effect on the in vivo $P_{50}$ value when pH, $PCO_2$, and temperature are constant.

By reducing the arterial $P_O_2$ levels to 55 and 60 mmHg, we created a situation in the baboon similar to that usually seen in patients with pulmonary and cardiac disease who require red blood cell transfusions. Under these circumstances, high 2,3-DPG red blood cells caused a greater reduction in the femoral artery oxygen saturation than did red blood cells with normal 2,3-DPG levels (Fig. 3). The systemic oxygen extraction was not significantly different between the two groups, suggesting that the decrease in oxygen loading in the lungs produced by the low-affinity red blood cells was compensated for by greater delivery of oxygen to the tissues (Fig. 4). The modest decrease in cardiac output during hypoxia in the high 2,3-DPG group and the significantly lower cardiac output after restoration of normal arterial oxygen tension suggest that red blood cells with increased 2,3-DPG levels facilitated oxygen delivery to the tissues. The slightly increased oxygen extraction in baboons transfused with red blood cells with an increased 2,3-DPG level was associated with a decrease in the red cell affinity for oxygen in vivo and a slight but insignificant reduction in pulmonary artery and jugular venous $P_O_2$ tensions (Figs. 1 and 4).

There have been indications that red blood cells with a decreased 2,3-DPG level and increased affinity for oxygen in vivo may be detrimental in certain clinical settings. Riggs et al. (12) reported that exchange transfusions of autologous red blood cells with low 2,3-DPG levels to rhesus monkeys produced a significant decrease in mixed venous $P_O_2$. Red blood cells with low 2,3-DPG levels and an increased affinity for oxygen have also been shown to produce ischemia of the canine myocardium when coronary perfusion is kept constant (8).

The transfusion of red blood cells with normal or 1½-2 times normal 2,3-DPG levels can minimize the requirement for increased blood flow and ensure adequate tissue oxygenation in patients in hemorrhagic shock and those subjected to extracorporeal circulation (19, 20). Agostoni and co-workers (1) found that when arterial $P_O_2$ values are greater than 45 mmHg, a decrease in red cell affinity for oxygen in vivo produces an increase in oxygen delivery to tissue in the presence of acute acidosis. Despite a decreased oxygen-loading content and decreased oxygen saturation, the transfusion of red blood cells with 1½ times normal 2,3-DPG levels to hypoxic baboons resulted in oxygen extraction similar to that in control baboons. After restoration of the arterial oxygen tension to normal, the oxygen extraction was similar in the two groups but cardiac output was significantly reduced in the baboons transfused with high 2,3-DPG red cells.

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The animals used in this study were handled in accordance with the provisions of Public Law 89-44 as amended by Public Law 91-579, the Animal Welfare Act of 1970, and the principles outlined in the Guide for the Care and Use of Laboratory Animals, Department of Health, Education and Welfare Publication (NIH)72-23.

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