Oxygen delivery following transfusion of stored blood. I. Normal rats

JERRY T. GUY, PHILIP A. BROMBERG, EARL N. METZ, ROBERT RINGLE, AND STANLEY P. BALCERZAK

Department of Medicine, The Ohio State University, Columbus, Ohio 43210

Blood stored in acid-citrate-dextrose (ACD) shows a progressive increase in oxygen affinity primarily as a result of a decrease in red cell 2,3-diphosphoglycerate (DPG) (5). Whether massive transfusion with such DPG-depleted blood impairs oxygen delivery is not certain. Sugerman et al. (8) reported low central venous oxygen tensions (P_{\text{cvO}}) in three patients who received large quantities of DPG-depleted blood. Valeri and Collins (9) found little change in initially normal venous oxygen tensions (P_{\text{vO}}) in seven anemic patients who were transfused with 3–5 units of ACD-stored red cells within 3 h.

An important determinant of the effect on oxygen delivery of transfusion with ACD-stored blood is the rate at which red cell DPG and oxygen affinity is returned to normal. Two studies have shown a relatively rapid restoration of donor red cell DPG levels after transfusion, but the rates of regeneration varied considerably, red cell oxygen affinity was not measured simultaneously, and no indices of tissue oxygenation were obtained (4, 10).

The present studies of exchange transfusion with ACD-stored, DPG-depleted blood were undertaken to define more precisely the effect of such transfusion on tissue oxygenation and on the rate of restoration of red cell DPG and blood oxygen affinity.

Materials and Methods

Female Sprague-Dawley rats weighing 200 g were anesthetized with pentobarbital and exchange-transfused via the femoral artery and vein (1). One group of animals received isologous blood collected in ACD on the day of the study (1 ml ACD to 4 ml blood). Mean DPG level of this fresh blood was 24.7 \( \mu \text{mole/g Hb} \) (SD \( \pm 6.0 \)). A second group was exchanged with blood collected in a similar fashion but stored for 7 days at 4°C. Mean DPG level of the stored blood was 3.4 \( \mu \text{mole/g Hb} \) (SD \( \pm 2.4 \)). Stored and fresh blood had similar ATP levels.

Exchange transfusions in both groups of animals were done in 2-ml aliquots and continued until 1.5–2.0 times the animal's calculated total blood volume was exchanged. Experiments with \(^{51}\text{Cr}\)-labeled red cells established that such an exchange was 85–90% complete. The blood volume of each rat was increased 15% by hypertransfusion in order to avoid anemia from repeated serial blood sampling.

After completion of exchange, the venous catheter was removed and the arterial catheter was pulled through a subcutaneous tunnel which surfaced on the back. This catheter was heparinized and left in place for blood sampling. Serial blood samples (0.4 ml) were obtained before and immediately after transfusion (time 0) and at 1, 4, 9, and 24 h.

Hematocrit, blood gases, red cell DPG and adenosine triphosphate (ATP), and oxygen affinity (P_{\text{so}}) were measured on donor blood and on each serial sample. Blood gases were measured with microelectrodes (Corning model 160). DPG was determined using the method of Keitt (7) with minor modification. ATP was assayed using the firefly method of Beutler and Baluda (3). Red cell oxygen affinity at 37°C was determined using a spectrophotometric technique similar to that employed by Bellingham and Huehns (2). With this method five points were obtained on the oxygen dissociation curve using about 50 \( \mu \)l of whole blood suspended in phosphate-buffered saline at pH 7.4. Skin bubble oxygen tension (P_{\text{sbo}}) was used as an index of tissue oxygenation (11). Seven days before exchange transfusion 30 ml of sulfur hexafluoride was in-
jected subcutaneously in the back. Six days later, but 24 h before the exchange, the skin bubble was inflated with 30–50 ml of air. Immediately before exchange, skin bubble P_{O_2} was measured. After the exchange, the skin bubble was reinflated with air and 24 h later P_{O_2} was again measured. In our laboratory P_{O_2} falls exponentially and reaches 90% equilibration within 6 h after refilling with room air.

In two separate groups of 10 animals each, blood for P_{O_2} was sampled via an indwelling catheter advanced through the femoral vein into the inferior vena cava. The catheter tip lay between the entry of the renal veins and the diaphragm. P_{O_2} was measured before and serially after exchange with either stored or fresh blood. In these experiments blood was collected in ACD and stored as previously, but following storage the cells were resuspended in fresh heparinized plasma to a hematocrit of 45%. This group of animals was not hypertransfused.

Significance testing within groups with respect to pre-exchange values utilized the t-test for paired data. Where appropriate, the difference between groups was tested for significance by the two-sided t test for independent samples.

RESULTS

Ten rats in each of the two groups were matched in regard to preexchange hematocrit values. Figure 1 shows not only similar hematocrit values for each group but also the absence of significant anemia during study. Values increased initially secondary to hypertransfusion but returned to pretransfusion levels by 24 h. Reticulocyte counts, used as an index of dilution of the transfused blood by newly released endogenous red cells, were 3.5% for animals receiving fresh blood and 2.2% for those receiving stored blood at 48 h.

Red cell DPG values fell markedly immediately after exchange with stored blood (22.7 ± 3.9 to 7.9 ± 4.2 μmole/g Hb) as depicted in Fig. 2. Restoration was partial by 4 h (11.9 ± 4.4) and complete by 24 h (22.7 ± 3.8). DPG values immediately after exchange with fresh blood in ACD fell slightly and at 4 h were significantly less than preexchange levels (25.6 ± 5.5 to 21.2 ± 4.0). By 24 h DPG levels in this group were higher (33.4 ± 10.8; P < 0.02) than preexchange values. When the stored and fresh blood groups were compared, DPG levels were significantly lower at all time periods after stored blood transfusion. The P values were < 0.001 except for P < 0.02 at 24 h.

Arterial blood pH was also altered in both groups of rats (Fig. 3). Exchange transfusion with either stored (pH 6.88 ± 0.06) or fresh blood (pH 6.90 ± 0.04) was followed immediately by a fall in arterial pH. In the group receiving stored blood this was significant (7.39 ± 0.03 pretransfusion to 7.30 ± 0.03 at time 0). In both groups of animals pH values returned to pretransfusion levels by 1 h and by 9 h were significantly higher than prior to transfusion. Values at 9 h for animals receiving fresh blood were 7.45 ± 0.03 and for those receiving stored blood were 7.46 ± 0.05. This mild alkalosis was associated with a significant fall in P_{CO_2} in both groups of animals at 9 and 24 h (Fig. 4).

Red cell oxygen affinity was markedly increased immediately following exchange transfusion with stored blood (Fig. 5). P_{50} values fell from 30.4 ± 3.1 to 17.8 ± 2.6 mmHg, a decrease of 40% compared to the simultaneous 65% decrease in DPG values. Return toward normal oxygen affinity occurred promptly. At 4 h P_{50} levels were 87%
of pretransfusion values, and by 24 h they had returned to preexchange levels. \( P_{50} \) values in animals receiving fresh blood fell slightly but significantly immediately after exchange (31.0 ± 1.3 to 29.5 ± 1.7 mmHg). By 1 h post-exchange, however, \( P_{50} \) levels had been restored. Oxygen affinity in animals who had received fresh blood continued to decrease gradually and by 24 h \( P_{50} \) values were 33.9 ± 4.0 mmHg, significantly greater than preexchange levels \((P < 0.02)\). These changes paralleled those observed in DPG levels. When the stored and fresh blood groups were compared, \( P_{50} \) levels were significantly lower \((P < 0.01)\) for at least 4 h after stored blood transfusion. Mean values for Hill's \( n \) ranged from 2.25 to 2.62 and did not differ significantly between fresh and stored blood-transfused animals either before or after transfusion.

Figure 6 shows a significant drop from 34 ± 5.9 to 27 ± 7.5 mmHg \((P < 0.001)\) in \( P_{bo} \) 24 h after the infusion of stored blood. The 3 mmHg increase in mean \( P_{bo} \) (from 33 ± 4.2 to 30 ± 3.0 at 24 h) observed in the animals receiving fresh blood was significant at the 5% level at 24 h but occurred in association with a mean 24-h \( P_{ao} \) value which was significantly lower than the preexchange mean (Fig. 7). \( P_{bo} \) in normal rats, neither transfused nor operated, was not statistically different when sampled on two occasions 24 h apart.

A separate group of animals had serial measurements of \( P_{co} \) after infusion of either fresh or stored blood. Hematocrit values did not change significantly after transfusion in either subgroup. Serial mean \( P_{co} \) after transfusion is shown in Fig. 8. No significant change in \( P_{co} \) was observed for 24 h after infusion of fresh blood. In contrast, animals exchanged with stored blood had an immediate drop in \( P_{co} \) and values remained significantly decreased for at least 9 h. When the stored and fresh blood groups were compared, \( P_{co} \) levels were significantly lower \((P < 0.02)\) for at least 4 h after stored blood transfusion.

**Discussion**

Transfusion is an effective means of restoring red cell mass and blood volume. With the discovery of the influence of DPG on blood oxygen affinity, the deleterious effects of blood storage on DPG levels, and the significant impact of altered blood oxygen affinity on tissue oxygenation, the promptness of restoration of tissue oxygenation by transfusion of stored blood has been questioned.

Several studies of the effect of stored blood transfusion on oxygen delivery have involved administration of blood to anemic patients. Valeri and Collins (9) infused relatively small amounts of blood and evaluated tissue oxygenation by “venous” \( P_{bo} \). Despite a significant rise in hematocrit, venous \( P_{bo} \) remained unaltered over a 24-h period. These investigators were unable to define precisely the physiologic effects of the transfusion of preserved red cells with high affinity for oxygen into patients with anemic hypoxia. Sugerman et al. (8) reported five massively transfused patients and two appeared to have a fall in \( P_{co} \) related to decreased \( P_{50} \). The clinical exigencies, however, made complete control of the variables impossible.

In separate studies of exchange transfusion with DPG-depleted adult blood in newborn infants with hyperbili-
rubinemia, Delivoria-Papadopoulos et al. (6) and Versmold et al. (12) showed that significant in vivo regeneration of red cell DPG occurred within the first day. No direct measurements of venous oxygen tension were performed by either group of investigators although the implications for tissue oxygen release of altered blood oxygen affinity were discussed.

Although we did not measure cardiac output or oxygen consumption, our experiments assessed other major variables known to affect tissue oxygen delivery. These include blood oxygen affinity, red cell DPG, hematocrit, arterial PO2, and pH. We evaluated tissue oxygenation both by sequential measurements of PO2 and in an “integrated” manner by Psbo2 measurement. By performing complete exchange transfusion, we avoided the problem of having two red cell populations—native cells with elevated DPG and P50 and transfused cells with low DPG and P50.

Under the conditions of our experiments in rats, the restoration of stored red cell DPG following transfusion followed a time course similar to that described by Beutler and Wood (4) and by Valeri and Hirsch (10) following transfusion of anemic humans. The expected correlation between DPG and P50 was observed and the restoration half-time of in vitro P50 to preexchange values was approximately 4 h.

The PO2 measurements indicate that tissue oxygen delivery was not normalized for at least 9 h following transfusion as compared to the preexchange value. Similarly, Psbo2, which may be regarded as a more slowly responding integrated measure of tissue PO2, was significantly decreased even at 24 h posttransfusion.

The relevance of these studies to man remains to be established but suggests that massive transfusion with ACD-stored, DPG-depleted blood is less effective than fresh blood in early delivery of oxygen to tissue.

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REFERENCES

7. Kratt, A. S. Reduced nicotinamide adenine dinucleotide-linked analysis of 2,3 diphosphoglyceric acid: spectropho-