Acute Changes in Oxyhemoglobin Affinity

EFFECTS ON OXYGEN TRANSPORT AND UTILIZATION

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ABSTRACT It has been postulated that 2,3-diphosphoglycerate (DPG)-mediated changes in oxyhemoglobin affinity play an important role in oxygen delivery; however, the effect of an acute increase in affinity without changing red cell mass has not been systematically evaluated. This study was designed to measure changes in oxygen transport and oxygen consumption produced by an acute increase in oxyhemoglobin affinity caused by an autologous exchange transfusion using DPG-depleted stored blood.

From each of 10 5-kg rhesus monkeys, 100 ml of blood was taken on the 1st and 3rd wk of the study and each stored in 25 ml of acid-citrate-dextrose storage solution. On the 5th wk, each animal underwent an exchange transfusion with 200 ml of its stored blood. Hemodynamic data were obtained before and 30 min after transfusion. The oxyhemoglobin dissociation curve shifted to the left (P_{50} changed from 33.9 to 27.2 mm Hg), as mean red cell DPG decreased from 28.6 to 12.7 μmol/g of hemoglobin. No significant change was noted in pH, P_{CO_2}, base deficit, arterial or venous percent saturation of hemoglobin, cardiac output, or oxygen consumption. However, a fall in mixed venous P_{O_2} from 35.3 to 27.9 mm Hg occurred.

Thus, an acute shift of the oxyhemoglobin curve to the left was accompanied by a significant decrease in the mixed venous P_{O_2} without evidence of acidosis, decreased oxygen consumption, or a compensatory increase in cardiac output.

INTRODUCTION

Within the past 5 yr, there has been a marked increase in interest in oxyhemoglobin affinity and factors that affect it. A decrease in oxyhemoglobin affinity has been described as an important physiological adaptive response to conditions in which oxygen delivery was impaired. Conversely, it has been suggested that increases in oxyhemoglobin affinity (as described by a shift of the oxyhemoglobin dissociation curve to the left) may actually impair tissue oxygenation. No previous studies have documented the effects of an acute increase in oxyhemoglobin affinity, mediated by a decrease in 2,3-diphosphoglycerate (DPG),[1] on oxygen transport and utilization without changing the concentration of hemoglobin. It was the purpose of this study to acutely increase oxyhemoglobin affinity in an anemic animal without changing the hemoglobin concentration and evaluate changes in oxygen delivery (cardiac output, arterial and mixed venous P_{O_2}, and hemoglobin saturation), oxygen consumption, and base excess and deficit.

It is known that red cells stored in an acid-citrate-dextrose storage solution (ACD) will lose their intracellular DPG over a 2 wk period and develop a very high oxyhemoglobin affinity (1). Consequently, if an exchange transfusion with ACD stored blood were performed, the oxyhemoglobin affinity of the subject should markedly increase. Such a situation is frequently seen in massive blood loss where large amounts of stored blood are given. Our experimental model was designed to measure the components of the oxygen delivery system as well as oxygen consumption before and 30 min after an acute increase in oxyhemoglobin affinity had been induced by an autologous exchange transfusion of DPG-depleted red cells.

METHODS

10 imported rhesus monkeys weighing approximately 5 kg each were used in the study. On the 1st and again on the 3rd wk of the study, each animal was phlebotomized 100 ml of blood that was stored in 25 ml of ACD solution (ACD,

[1] *Abbreviations used in this paper:* ACD, acid-citrate-dextrose; DPG, 2,3-diphosphoglycerate.

National Institute of Health, formula A) at 4°C. At the beginning of the 5th wk, all animals were anesthetized with sodium pentobarbital, 25 mg/kg body weight and the trachea intubated. Studies were performed during a control period. This was followed by a 200 ml exchange transfusion in 25-ml increments over a 20 min period with autologous blood that previously had been warmed to body temperature. 30 min after the transfusion repeat hemodynamic studies were obtained.

A flow-directed polyethylene (PE 90) catheter was placed in the main pulmonary artery and its position confirmed fluoroscopically and by pulse contours. A Teflon catheter was placed in the femoral artery. Cardiac output was measured during the expired gas collection by the indicator-dilution technique, as previously described (2), before the blood samples for gases and saturations were withdrawn.

Expired gases were collected in a recording underwater seal spirometer to permit measurement of oxygen consumption. Simultaneous arterial and mixed venous (pulmonary arterial) blood samples were obtained. The expired gases and blood were analyzed with an Instrumentation Laboratory model 113 blood gas analyzer and pH electrode (Instrumentation Laboratory, Inc., Lexington, Mass.). Hemoglobin concentration and oxygen saturation were measured with an Instrumentation Laboratory model 182 Cooximeter. Base excess was determined using the Severinghaus blood gas calculator (3). Hematocrits and mean corpuscular hemoglobin concentrations were not measured.

Hemoglobin's affinity for oxygen, expressed as P_{50} (the P_{O_2} at which hemoglobin is 50% saturated), was measured by reproducing the oxyhemoglobin dissociation curve, using the mixing technique as previously described (4). Each oxyhemoglobin dissociation curve was determined in duplicate, however, and four points were used to plot each curve (at 35, 45, 55, and 65% saturation of hemoglobin). Hill's number was used to describe the slope of the oxyhemoglobin dissociation curve (5). The effect of pH on the dissociation curve was corrected for by using the formula $\Delta \log P_{O_2} = 0.52 \ \Delta$ pH, as described for rhesus monkeys by Lenfant and Aucutt (5). An increase in oxyhemoglobin affinity was measured by a leftward shift of the oxyhemoglobin dissociation curve and a decrease in the P_{50}. DPG was measured by a modification of the technique of Krimsky (1). The standard deviation of repeated determinations on single blood samples was 0.4 mm Hg for P_{50} and 0.1 μmol/g hemoglobin for DPG.

Body temperature of the animals was not monitored during the study. Previous work with rhesus monkeys in our laboratory has shown less than ±1°C variation during a 2 h study, with no consistent change. This could produce variation in P_{50} of as much as 1.9 mm Hg at this level of P_{50}, but would be expected to vary at random.

Statistical analysis of all the data was performed using the Student "t" test for paired comparisons, each animal serving as its own control.

RESULTS

The results are listed in Table I.

Oxyhemoglobin dissociation curve. With the exchange transfusion the mean P_{50} fell from 33.9 to 27.4 mm Hg as the mean red cell DPG decreased from 28.6 μmol/g of hemoglobin to 12.7 μmol/g of hemoglobin. Thus, an acute

increase in oxyhemoglobin affinity had been induced. A statistically significant change in the slope of the curve, as represented by Hill's number, also occurred; but this was of such a small magnitude that it had little effect on hemoglobin binding of oxygen.

Cardiac output. The mean cardiac output decreased 5 ml/min/kg after transfusion. (The method is consistently reproducible to within ±10% [2 SD]). Therefore, this represented no significant change in cardiac output. The base-line cardiac output per kilogram was approximately 20% greater than other control groups of rhesus monkeys in our laboratory (2, 6). This difference may have been due to the anemia induced in these animals by the phlebotomies.

Hemoglobin and hemoglobin saturation. Because of the previous phlebotomies, the animals began with a hemoglobin of only 7.4 g/100 ml that did not change with the exchange transfusion. No significant change was recorded in either the arterial or venous percent saturation. Consequently, the amount of oxygen being released by hemoglobin (the arterial-venous difference) remained constant.

TABLE I

Results of the Statistical Analysis of all Data

	Control ±1 SD	Posttransfusion ±1 SD
P_{50} (in vivo pH), mm Hg	33.9±1.1	27.2±0.7*
DPG, μmol/g, hemoglobin	28.6±8.2	12.7±3.4*
Hill's number	2.91±0.16	2.40±0.13*
Cardiac output, ml/min/kg	211.0±48.0	206.0±56.0‡
Hemoglobin, g/100 ml	7.4±1.4	7.4±1.1‡
Art-Ven sat of hemoglobin, %	37.9±8.9	39.4±6.7‡
Oxygen consumption, ml/min/kg	6.8±1.1	7.3±3.0‡
Arterial pH	7.40±0.03	7.41±0.03‡
Arterial P_{CO_2}, mm Hg	39.6±4.5	38.0±4.8‡
Base deficit, meq/liter	−0.39±1.74	−0.76±2.03‡
Arterial P_{O_2}, mm Hg	76.2±11.9	73.7±20.4‡
Mixed venous P_{O_2}, mm Hg	35.3±6.9	27.9±5.8*

* $P < 0.01$.
‡ No significant change.

Oxygen consumption. No significant change was measured in oxygen consumption; considerable posttransfusion variability was seen and may have reflected the level of anesthesia.

Blood gases. By 30 min after transfusion, the pH and P_{CO_2} had both returned to control values; there was no evidence of a significant base deficit. The arterial P_{O_2} had not significantly changed, but the mixed venous P_{O_2} taken from the pulmonary artery had decreased 7.4 mm Hg.

DISCUSSION

Many clinical conditions are associated with an increase in oxyhemoglobin affinity, i.e., respiratory or metabolic alkalosis, hypothermia, carbon monoxide inhalation, hexokinase deficiency, and transfusions of large amounts of stored blood. Such an increase in affinity would require a lower tissue oxygen tension for the same amount of oxygen to be released from hemoglobin. Consequently, it has been postulated that each of these conditions may be detrimental by ultimately causing tissue hypoxia. To avert such tissue hypoxia, however, one might predict compensatory changes in oxygen delivery, or if these are inadequate, evidence of anaerobic metabolism.

In this study we were able to acutely induce an increase in oxyhemoglobin affinity, without changing hemoglobin concentration. The increase in affinity was accompanied by a fall in the mixed venous P_{O_2} which must reflect a decrease in oxygen tension, at least in some tissues. The precise relationship between mixed venous P_{O_2} and tissue oxygen tension is unknown, as there are obviously large differences in regional tissue perfusion and oxygen utilization. With the fall in mixed venous P_{O_2} there was no significant change in cardiac output, oxyhemoglobin saturations, or oxygen consumption. Furthermore, there was no evidence of a change in base deficit, suggesting that there was no increase in lactic acid production. Thus, in this group of animals, large increases in oxyhemoglobin affinity occurred without embarrassing oxygen delivery or consumption. On the other hand, it is possible that certain tissues may have undergone critical oxygen lack not reflected in these "total body" measurements. Certainly these methods of assessing aerobic metabolism (oxygen consumption and base deficit) have limited sensitivity in spite of their usefulness in severe hypoxic states.

It is noteworthy, that in a similar experiment involving human subjects, Valeri and Collins infused 3–5 U of ACD stored blood in eight anemic patients and noted no change in cardiac index or oxygen consumption as the oxyhemoglobin dissociation curve shifted from a mean

P_{50} of 30.7–27.5 mm Hg (7). However, in their study there was a significant increase in red cell mass and oxygen-carrying capacity with transfusion, that may have modified the effects of the acute change in oxyhemoglobin affinity. An additional study involving exchange transfusion in infants (8) did not evaluate the effects of an acute change in oxyhemoglobin affinity on other aspects of the oxygen transport system.

Oski, Marshall, Cohen, Sugerman, and Miller (9) attempted to compare the hemodynamic differences of a subject with increased oxyhemoglobin affinity and a subject with decreased affinity; both were anemic. At high exercise levels, the subject with increased affinity did appear to require a higher cardiac output. However, the subjects were not matched for age, sex, or previous physical training.

Direct application of these findings in the monkeys, to humans, should be made with caution, because of the difference in their base-line oxyhemoglobin affinity (34.6 and 26.6 mm Hg). Furthermore, the possibility that regional vascular disease may modify the significance of the changes in oxyhemoglobin affinity should be considered. The significance of acute increases in oxyhemoglobin affinity may well go unanswered until improved techniques are available for evaluating the critical level of oxygen tension in specific tissues, and its effects on cellular metabolism. In this experimental model, however, a large increase in oxyhemoglobin affinity was induced without changing the hemoglobin concentration and this was accompanied by a decrease in the mixed venous P_{O_2}. There was no evidence of anaerobic metabolism, decreased oxygen consumption or compensatory changes in cardiac output.

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