Targeted O₂ delivery by low-P₅₀ hemoglobin: a new basis for O₂ therapeutics

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Targeted O₂ delivery by low-P₅₀ hemoglobin: a new basis for O₂ therapeutics. *Am J Physiol Heart Circ Physiol* 285: H1411–H1419, 2003. First published June 12, 2003; 10.1152/ajpheart.00307.2003.—To assess O₂ delivery to tissue by a new surface-modified, polyethylene glycol-conjugated human hemoglobin [MP4; P₅₀ at 50% saturation of hemoglobin (P₅₀; 5.4 mmHg)], we studied microcirculatory hemodynamics and O₂ release in golden Syrian hamsters hemodiluted with MP4 or polymerized bovine hemoglobin (PolyBvHb; P₅₀ 54.2 mmHg). Comparisons were made with the animals’ hemodiluted blood with a non-O₂ carrying plasma expander with similar solution properties (Dextran-70). Systemic hemodynamics (arterial blood pressure and heart rate) and acid-base parameters were not correlated with microhemodynamics (arteriolar and venular diameter, red blood cell velocity, and flow). Microscopic measurements of P₅₀ and the O₂ equilibrium curves permitted analysis of O₂ release in precapillary and capillary vessels by red blood cells and plasma hemoglobin separately. No significant differences between the groups of animals with respect to arteriolar diameter, flow, or flow velocity were observed, but the functional capillary density was significantly higher in the MP4-treated animals (67%) compared with PolvyBvHb-treated animals (37%; P < 0.05) or dextran-treated animals (53%). In the PolvyBvHb-treated animals, predominant O₂ release (both red blood cells and plasma hemoglobin) occurred in precapillary vessels, whereas in MP4 animals most of the O₂ was released from both red blood cells and plasma hemoglobin in capillaries. Base excess correlated directly with capillary O₂ release but not systemic O₂ content or total O₂ release. Higher O₂ extraction of both red blood cell and plasma hemoglobin in capillaries represents a new mechanism of action of cell-free hemoglobin. High O₂ affinity appears to be an important property for cell-free hemoglobin solutions.

ARTERIOLAR VASOCONSTRICTION limits tissue perfusion by some early-generation hemoglobin-based O₂ carriers, increasing vascular resistance, which may be manifest as systemic hypertension, offsetting potential efficacy (40). Although the mechanism of vasoactivity has been disputed, one popular explanation is that nitric oxide (NO) is scavenged by cell-free hemoglobin (9), either directly in the lumen of the vessel or in the interstitial space after extravasation. We observed, however, that derivatized hemoglobins with different hypertensive effects have nearly identical NO binding constants (26). An alternative (or additional) mechanism is the involvement of autoregulation, by which vasoconstriction results from an oversupply of O₂ to vascular walls, particularly in arterioles, which regulate the entry of blood into capillary networks (23). An O₂ oversupply would result from facilitated diffusion of O₂ as oxymoglobin in plasma. Oxymoglobin diffusion is a function of molecular size, viscosity, and O₂ affinity, and manipulation of these three parameters offers strategies for potentially overcoming autoregulatory vasoconstriction. We explored these ideas in an artificial capillary system and concluded that the O₂ delivery in small vessels is more similar to that of red blood cells when the hemoglobin molecules have a low P₅₀ at 50% saturation of hemoglobin (P₅₀) and a large molecular volume (23). As a consequence of these studies and considerations, we have developed polyethylene glycol (PEG)-modified human hemoglobin (MP4) (36), which is now in clinical trials.

In another study, we found that the hemoglobin surface modified with PEG was remarkably efficient in its ability to protect against the ill effects of a severe hemorrhage (41). The particular PEG-hemoglobin used in that study was not well characterized, but it did not cause systemic hypertension. We hypothesized that the effectiveness of this molecule arose from a lack of vasoconstriction and consequent improved tissue perfusion, although there was no direct evidence to support that conclusion.

MP4 has been studied in its ability to resuscitate hamsters in hemorrhagic shock (38), and it also does not appear to cause hypertension in exchange transfusions in rats (36). Furthermore, it is formulated at a relatively low hemoglobin concentration (4.2 g/dl) to keep its solution properties (viscosity, oncotic pressure) within a range that is acceptable for large volume use in patients. It was therefore desired to assess the ability of this solution to delivery O₂ to tissues by direct measurement. Such measurements are now available in the hamster skinfold model (30).

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H1411
In the present experiments, we directly measured the delivery of O\textsubscript{2} in the microcirculation of the hamster skinfold window by red blood cells and plasma hemoglobin separately after hemodilution to a hematocrit of 11%. MP4 was compared with polymerized bovine hemoglobin (PolyBvHb), which is very different with regard to molecular size and O\textsubscript{2} affinity, and with Dextran-70. The microcirculation parameters, combined with the known oxygenation properties of the solutions, allow for the first time, direct calculation of O\textsubscript{2} transport in the microcirculation.

**MATERIALS AND METHODS**

**Experimental solutions.** Dextran-70 was purchased from Braun Medical (Irvine, CA), and PolyBvHb (Oxygoblin) was purchased from Biopure (Boston, MA). MP4 is PEG-modified human hemoglobin manufactured by Sangart (San Diego, CA), and its preparation and properties have been described previously (36). Both fluids were formulated in physiological solutions; however, the hemoglobin concentration of the PolyBvHb (13.1 g/dl) was higher than the MP4 (4.2 g/dl).

Viscosity of the solutions was measured with a cone/plate rheometer (model DV-III, Brookfield; Middleboro, MA) with the CPE-40 cone spindle at a shear rate 200/s. Colloid oncotic pressure was measured with the use of a colloid osmometer (model 4420, Wescor; Logan, UT) (34). O\textsubscript{2} equilibrium curves of MP4 and of freshly collected hamster red blood cells were measured as described previously (37) in 100 mM phosphate buffer, pH 7.4. In the case of PolyBvHb, the published equilibrium curve (19) was digitized. All three sets of data were analyzed to determine Adair parameters with the use of MLAB software (Civilized Software; Bethesda, MD) according to previously published procedures (42). In all cases, the final saturation at the highest P\textsubscript{O2} was adjusted to minimize the sum of squared residuals.

**Animal preparations.** Animal handling and care were provided in accordance with the procedures outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All animal studies were approved by the Animal Subject Committee of the University of California, San Diego. The placement of dorsal skinfold window is described in detail elsewhere (32). Briefly, the animals were prepared for chamber implantation by an injection of pentobarbital sodium (50 mg/kg ip). After the hair was removed, the dorsal skin was lifted and mounted on the titanium frame of the window. Skin and subcutaneous tissue were removed until only a thin layer of retractor muscle and subcutaneous tissue remained. The exposed area was sealed with a glass coverslip incorporated into the chamber frame, and animals were allowed at least 2 days to recover. Animals were rejected from the study if there were significant signs of edema, bleeding, or unusual neovascularization. Those animals that were accepted for study were then reanesthetized for placement of polyethylene (PE) catheters in a femoral artery (PE-50) and jugular vein (PE-10). After recovery, and while awake, the animals were then progressively exchange-transfused with Dextran-70 until their hematocrit reached ~60% of baseline. A third exchange was then performed with Dextran-70, PolyBvHb, or MP4 to a final hematocrit of ~11%. Mean arterial pressure was recorded continuously over the experiment, and heart rate was determined from the pressure trace (Beckman Recorder; Spectramed pressure transducer).

**Microvascular hemodynamics.** At least five animals were studied in each treatment group. In each animal, at least five arterioles and venules were selected for hemodynamic measurements. To study the same microvessels throughout the experiments, we mapped the chamber vasculature as described previously (32). Before and after the hemodilution, arteriolar and venular diameter and blood flow velocity were measured online with the use of the photodiode cross-correlation technique (27). Flow (Q) in arterioles and venules was calculated as:

\[ Q = \pi V \left( \frac{D}{2} \right)^2 \]  

where \( D \) is vessel diameter and \( V \) is red blood cell velocity. Because there is variation within individual animals with respect to diameter, velocity, and flow, at least five each arterioles and venules were studied. To determine the final parameters, diameter, velocity, and flow were averaged for all vessels of all animals such that the number of determinations for each was at least 25.

**Microvascular P\textsubscript{O2}.** P\textsubscript{O2} measurements were made within vessels and in adjacent tissues by the Palladium-porphyrin decay methods as described previously (29). Animals received a slow intravenous injection of 15 mg/kg body wt at a concentration of 10.1 mg/ml of palladium-meso-tetral(4-carboxyphenyl) porphine (catalog no. T790, Frontier Scientific; Logan, UT). The dye was allowed to circulate for 10 min before measurements by the phosphorescence decay method. At concomitant points, arterial blood pressure, blood gases, and acid-base status were also determined (Blood Chemistry Analyzer model 248, Bayer Medical; Northwood, MA). Hematocrit in arterial blood was measured by microcentrifugation, and total and plasma hemoglobin were measured using the B-Hemoglobin instrument (HemoCue; Angelholm, Sweden).

**Functional capillary density.** Functional capillary density (FCD), defined as capillary segments that have red blood cell transit in a 30-s period, were assessed in a region of ~0.5 mm\(^2\). This parameter was originally described by Lindbom and Arfors (20) and has been shown to be the best single predictor of outcome in shock in the hamster model (16). Observation of the selected regions was done systematically by displacing the microscopic field of view by one field width at a time in 10–15 successive steps. The first field was chosen close to some identifiable landmarks (e.g., a vessel bifurcation) to reestablish the same fields for subsequent examination. At the magnification used, each field had 2–5 capillaries. FCD was estimated from the capillary lengths with red blood cell flow in areas composed of 10 successive defined as the total length of red blood cell-perfused capillaries divided by the area of the microscopic field of view. One value of FCD was assigned to each animal, so that the number of determinations for each group of animals was at least five.

**O\textsubscript{2} distribution.** The P\textsubscript{O2} values of systemic, arteriolar, and venular blood were used to calculate saturation from the Adair parameters and the algorithms previously described (39). The O\textsubscript{2} content of plasma hemoglobin was calculated as:

\[ \text{O}_{2}\text{plasmaHb} = \text{Hb}_{\text{plasma}} \times [1 - (0.01 \times \text{Hct})] \times 1.34 \times Y_{\text{plasmaHb}} \]  

where \( \text{Hb}_{\text{plasma}} \) is the hemoglobin concentration (in g/dl) measured in isolated plasma. Hct is the percent volume of packed red blood cells (hematocrit). \( Y_{\text{plasmaHb}} \) is the fractional saturation of the plasma hemoglobin, calculated from...
O2, as described above. The coefficient 1.34 converts grams per deciliter of hemoglobin to milliliter per deciliter of O2. The O2 content of red blood cell hemoglobin was calculated as

$$O_{2\text{RBC}} = 1.34 \times [Hb_{\text{total}} - (Hb_{\text{plasma}} \times (1 - 0.01 \times \text{Hct})] \times Y_{\text{RBC}}$$

where $Hb_{\text{total}}$ is the concentration of hemoglobin measured on an unseparated blood sample, and $Y_{\text{RBC}}$ is the O2 saturation of red blood cell hemoglobin. The amount of O2 dissolved ($O_{2\text{dis}}$) in plasma was calculated as

$$O_{2\text{dis}} = O_2 \times \left(\frac{2.3}{760}\right)$$

where 2.3 is the solubility coefficient for O2 in blood (in ml·dl⁻¹·atm⁻¹) (42).

Finally, O2 release in the precapillary and capillary vessels was calculated as

$$O_{2\text{precap}} = (C_{\text{arterial}}O_2 - C_{\text{arteriole}}O_2)$$

$$O_{2\text{cap}} = (C_{\text{arteriole}}O_2 - C_{\text{venular}}O_2)$$

where $O_{2\text{precap}}$ and $O_{2\text{cap}}$ are the quantities of O2 released in precapillary and capillary vessels, respectively, and $C_{\text{arteriole}}O_2$ and $C_{\text{venular}}O_2$ are the concentrations of O2 in precapillary and capillary vessels, respectively.

**Statistical methods.** Values are means ± SE. Differences between groups were evaluated by one-way ANOVA, with α = 0.05 and $P < 0.05$, indicating significance with the use of either Tukey’s or Bonferroni post hoc error protection. Changes in parameters were evaluated with the use of Student’s t-test.

**RESULTS**

**Experimental solutions.** The solutions are characterized in Table 1. Dextran-70 was used as a non-O2-carrying control solution because its viscosity and oncotic pressure are nearly identical to those of MP4. The viscosities of PolyBvHb and MP4 (2.0 and 2.5 cP, respectively) are similar, as is the colloid osmotic pressure (45 and 49 mmHg, respectively). The hemoglobin concentration in the stock MP4 solution is 4.2 g/dl compared with 13.1 g/dl for PolyBvHb and 14.5 g/dl for hamster blood.

The P50 of hamster blood is slightly higher than that of human blood, and the P50 of PolyBvHb is considerably higher (Fig. 1). The degree of right shift of the O2 equilibrium curves for polyethylene glycol (PEG)-conjugated human hemoglobin (MP4), hamster red blood cells, and polymerized bovine hemoglobin (PolyBvHb). Measurements were made at 37°C in 100 mM phosphate buffer, pH 7.4.

PolyBvHb may be controversial; however, assignment of a P50 value to very right-shifted curves is a problem with the use of commercial measurement techniques. In these methods, the saturation, usually with room air (~150 mmHg), is set to 100%. If hemoglobin is not fully saturated at this PO2, then the P50, as measured, will be falsely low. The problem can be reduced somewhat by equilibration of the hemoglobin solution with gas with increased PO2, but this is not according to the recommendations of manufacturers of such instruments. Another way to deal with this problem is to include the final saturation as a parameter in the fit of the data to the Adair equation, as we have done in the present case. Note that the P50 of MP4 is much lower (5.4 mmHg) than either of hamster red blood cells or PolyBvHb. Note also the reduced cooperativity (N) of all three O2 carriers.

**Exchange transfusions.** Five animals (55–65 g body wt) in each experimental group entered into the hemodilution and exchange-transfusion protocol, and all of them tolerated the experiment without any visible discomfort. The hematological changes are shown in Table 2. The MP4 exchange resulted in a plasma hemoglobin level of 1.12 ± 0.03 g/dl, which increased the total hemoglobin in blood (RBCs + MP4) to 4.80 ± 0.12 g/dl. Because the stock PolyBvHb concentration is

**Table 1. Solution and O2 binding parameters**

<table>
<thead>
<tr>
<th></th>
<th>Hamster Blood</th>
<th>PolyBvHb</th>
<th>MP4</th>
<th>Dextran</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygenation parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$a_1$</td>
<td>1.756 × 10⁻²</td>
<td>4.365 × 10⁻²</td>
<td>5.791 × 10⁻¹</td>
<td></td>
</tr>
<tr>
<td>$a_2$</td>
<td>0.000</td>
<td>1.299 × 10⁻³</td>
<td>1.591 × 10⁻¹</td>
<td></td>
</tr>
<tr>
<td>$a_3$</td>
<td>4.688 × 10⁻⁶</td>
<td>1.637 × 10⁻⁵</td>
<td>1.325 × 10⁻²</td>
<td></td>
</tr>
<tr>
<td>$a_4$</td>
<td>9.073 × 10⁻⁷</td>
<td>1.032 × 10⁻⁷</td>
<td>1.869 × 10⁻³</td>
<td></td>
</tr>
<tr>
<td>$P_{50}$</td>
<td>35.90</td>
<td>54.20</td>
<td>5.35</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>1.51</td>
<td>1.17</td>
<td>1.25</td>
<td></td>
</tr>
<tr>
<td>Viscosity, cPs</td>
<td>4.5</td>
<td>2.0</td>
<td>2.2</td>
<td>2.8</td>
</tr>
<tr>
<td>COP, mmHg</td>
<td>45.0</td>
<td>49.0</td>
<td>49.9</td>
<td></td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>14.5</td>
<td>13.1</td>
<td>4.2</td>
<td></td>
</tr>
</tbody>
</table>

$a_1$–$a_4$, Parameters for the Adair equation used to calculate saturation from PO2; PolyBvHb, polymerized bovine hemoglobin; MP4, polyethylene glycol-conjugated human hemoglobin; COP, colloid oncotic pressure; Hb, hemoglobin; N, cooperativity; $P_{50}$, PO2 at 50% saturation of Hb. See Ref. 42 for a complete description of the parameters for the Adair equation.
Table 2. Hematologic parameters after hemodilution

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Total Hb, g/dl</th>
<th>Plasma Hb, g/dl</th>
<th>Hct, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP4</td>
<td>6</td>
<td>15.3 ± 0.02</td>
<td>0.00</td>
<td>48.6 ± 0.79</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemodilution</td>
<td>5</td>
<td>4.80 ± 0.12a</td>
<td>1.12 ± 0.03</td>
<td>11.2 ± 0.16</td>
</tr>
<tr>
<td>PolyBvHb</td>
<td>5</td>
<td>15.30 ± 0.035</td>
<td>0.00</td>
<td>47.3 ± 0.46</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemodilution</td>
<td>5</td>
<td>6.70 ± 0.25</td>
<td>3.74 ± 0.29c</td>
<td>11.2 ± 0.35</td>
</tr>
<tr>
<td>Dextran</td>
<td>7</td>
<td>13.90 ± 0.039b</td>
<td>0.00</td>
<td>46.0 ± 0.52</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemodilution</td>
<td>5</td>
<td>3.44 ± 0.11d</td>
<td>0.00</td>
<td>11.2 ± 0.46</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. Hct, hematocrit. *P < 0.02, MP4 > dextran; †P < 0.01, dextran < MP4 and PolyBvHb; ‡P < 0.001, PolyBvHb > MP4; §P < 0.01, dextran < MP4 and PolyBvHb; *P < 0.01, MP4 < PolyBvHb.

Table 3. Blood gases and acid-base balance after hemodilution

<table>
<thead>
<tr>
<th></th>
<th>PO2, mmHg</th>
<th>PCO2, mmHg</th>
<th>pH</th>
<th>ΔBase Excess, mEq/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP4</td>
<td>Systemic</td>
<td>91.43 ± 3.18</td>
<td>46.42 ± 3.22</td>
<td>7.40 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Arteriolar</td>
<td>39.35 ± 4.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Venular</td>
<td>2.71 ± 0.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tissue</td>
<td>1.74 ± 0.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PolyBvHb</td>
<td>Systemic</td>
<td>93.53 ± 4.43†</td>
<td>45.78 ± 2.52</td>
<td>7.37 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Arteriolar</td>
<td>23.27 ± 1.51†</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Venular</td>
<td>0.74 ± 0.24‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tissue</td>
<td>0.27 ± 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextran</td>
<td>Systemic</td>
<td>103.87 ± 5.36</td>
<td>39.69 ± 8.35</td>
<td>7.30 ± 0.02‖</td>
</tr>
<tr>
<td></td>
<td>Arteriolar</td>
<td>35.23 ± 4.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Venular</td>
<td>13.34 ± 4.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tissue</td>
<td>2.12 ± 0.46</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. *Change from baseline. †P < 0.05 vs. MP4. ‡P < 0.05 vs. PolyBvHb.

Significant reductions in PO2 are observed in arterioles compared with systemic arteries in all animals. However, the arteriolar, venular, and tissue PO2 values in the PolyBvHb animals are all significantly less than those in the MP4-treated animals (P < 0.05). The arterial pH is significantly lower in dextran-treated animals compared with MP4-treated animals after hemodilution (P < 0.05). Finally, although animal variability in the baseline base excess values does not establish statistical significance, the fall in base excess in the dextran-treated animals (12.62 meq/l) is significantly greater than the fall in either MP4- (1.48 meq/l) or PolyBvHb (3.00 meq/l)-treated animals (P < 0.05).

Tissue PO2 values are all very low, as expected in these severely hemodiluted animals. However, the higher tissue PO2 in the MP4 compared with PolyBvHb animals is statistically significant (P < 0.05).

Systemic and microvascular hemodynamics. Both the MP4 and PolyBvHb animals maintained systemic mean arterial pressure; however, in the dextran-treated animals, the mean arterial pressure fell to 65% of baseline (Fig. 2). Likewise, the heart rate in both MP4 and PolyBvHb-treated animals was not different from baseline, but there was a relatively small but significant fall in heart rate in the dextran-treated animals.

Arteriolar diameter diminished slightly but significantly only in the PolyBvHb-treated animals. Arteriolar flow and red blood cell flow velocity fell significantly in all groups, but there were no differences between groups. In contrast, venular diameter was significantly less in the dextran-treated animals compared with both MP4- and PolyBvHb-treated animals, but venular red blood cell velocity and flow were increased in the dextran-treated animals. Venular red blood cell velocity and flow were reduced significantly only in the PolyBvHb-treated animals.

Finally, all FCD fell in all groups, but FCD was significantly less in the PolyBvHb (36.7% of baseline) compared with MP4 (66.5% of baseline) (P < 0.05). FCD in the dextran-treated animals was intermediate.
(53.2%), which was significantly less than baseline, but not different from the other two groups of animals.

O₂ distribution. The amount of O₂ in the three spaces, red blood cells, plasma hemoglobin, and dissolved in plasma, can be calculated using the distribution of hemoglobin (Table 1), the measured Po₂ (Table 3), and the hemoglobin saturation (Fig. 1). The O₂ contents of these various vessel type are presented in Table 4, and the O₂ release values are given in Table 5 and Fig. 3.

With regard to red blood cell O₂ release, there is no statistically significant difference between the amount in precapillary vessels between MP4-, PolyBvHb-, and dextran-treated animals, although there is a trend toward a larger amount in the PolyBvHb-treated group. However, a striking feature of the data is that a large amount of cell-free hemoglobin bound O₂ is released in precapillary vessels compared with the MP4 animals (P < 0.001). Essentially none of the MP4-bound O₂ is released in precapillary vessels, owing to the high O₂ affinity of this hemoglobin and the relatively high Po₂ in these vessels.

Significantly more O₂ is released in capillaries compared with precapillary vessels in the MP4 animals, and significantly less O₂ is released in capillaries compared with precapillary vessels in the PolyBvHb animals, respectively, and both differences are statistically significant (P < 0.001). In the dextran-treated animals, O₂ release is evenly distributed between precapillary and capillary vessels.

Table 5 also presents the fractional O₂ extraction values for the red blood cell, plasma hemoglobin, and plasma dissolved compartments. The values are presented for both precapillary and capillary vessels. Most interestingly, the calculations show that the O₂ extraction ratio (i.e., percent of arterial O₂ that is delivered) for red blood cells in the MP4 animals is 28% in precapillary vessels and 47% in capillaries. In contrast, the red blood cells of animals treated with PolyBvHb release 43% of the bound O₂ in precapillary vessels, and only 14% in capillaries. In terms of overall O₂ release (red cell + plasma Hb + dissolved), MP4 animals release 62% of the arterial content of O₂ into capillaries, compared with 31% in precapillary vessels.

Table 4. Oxygen distribution after hemodilution

<table>
<thead>
<tr>
<th></th>
<th>Red Blood Cell O₂, ml/dl</th>
<th>Plasma Hb O₂, ml/dl</th>
<th>Dissolved O₂, ml/dl</th>
<th>Total O₂, ml/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MP4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>4.87 ± 0.12</td>
<td>1.30 ± 0.04*</td>
<td>0.28 ± 0.01</td>
<td>6.44 ± 0.14†</td>
</tr>
<tr>
<td>Arteriolar</td>
<td>3.09 ± 0.54*</td>
<td>1.27 ± 0.04</td>
<td>0.12 ± 0.01*</td>
<td>4.47 ± 0.58*</td>
</tr>
<tr>
<td>Venular</td>
<td>0.06 ± 0.01*</td>
<td>0.41 ± 0.07*</td>
<td>0.01 ± 0.00*</td>
<td>0.48 ± 0.08*</td>
</tr>
<tr>
<td>PolyBvHb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>4.44 ± 0.19</td>
<td>2.96 ± 0.30*</td>
<td>0.28 ± 0.01</td>
<td>7.68 ± 0.22†</td>
</tr>
<tr>
<td>Arteriolar</td>
<td>1.13 ± 0.15*</td>
<td>1.21 ± 0.15</td>
<td>0.07 ± 0.00*</td>
<td>2.41 ± 0.28*</td>
</tr>
<tr>
<td>Venular</td>
<td>0.01 ± 0.00*</td>
<td>0.04 ± 0.02*</td>
<td>0.00 ± 0.00*</td>
<td>0.06 ± 0.02*</td>
</tr>
<tr>
<td>Dextran</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>4.53 ± 0.14</td>
<td>0.00 ± 0.00†</td>
<td>0.31 ± 0.02</td>
<td>4.85 ± 0.14</td>
</tr>
<tr>
<td>Arteriolar</td>
<td>2.57 ± 0.48</td>
<td>0.00 ± 0.00†</td>
<td>0.11 ± 0.01</td>
<td>2.69 ± 0.49</td>
</tr>
<tr>
<td>Venular</td>
<td>0.58 ± 0.27</td>
<td>0.00 ± 0.00†</td>
<td>0.04 ± 0.01</td>
<td>0.63 ± 0.28</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05, MP4 vs. PolyBvHb; †P < 0.05, MP4 vs. dextran; ‡P < 0.05, PolyBvHb vs. dextran.
In contrast, the PolyBvHb animals release 69% of their total arterial O\textsubscript{2} content into precapillary vessels and only 31% into capillaries. In the dextran-treated animals, O\textsubscript{2} release is about evenly distributed between precapillary and capillary vessels for red blood cells, and the contribution from dissolved O\textsubscript{2} is negligible.

Although absolute O\textsubscript{2} uptake ($V\dot{O}_{2}$) across capillary networks cannot be calculated without volume flow, the microhemodynamic data (see Fig. 2) do not support the quantitative conclusion that the increased O\textsubscript{2} extraction in MP4 animals is a result of increased capillary perfusion alone. The FCD is significantly greater in the MP4 animals compared with the PolyBvHb-treated animals ($P < 0.05$), but arteriolar and venular flow, although greater in the MP4-treated animals, is not statistically different from the values for PolyBvHb-treated animals.

**Global O\textsubscript{2} delivery.** Base excess calculated in arterial blood was used to assess whether the microcirculatory O\textsubscript{2} distribution is indicative of global (whole body) O\textsubscript{2} supply. Only weak correlations were found between arterial base excess and arterial O\textsubscript{2} content ($r^2 = 0.479$), hematocrit ($r^2 = -0.275$), total hemoglobin ($r^2 = 0.324$), and plasma hemoglobin concentration ($r^2 = 0.196$) (see Fig. 4). In contrast, the correlation between base excess and capillary O\textsubscript{2} release is 0.976, suggesting that this parameter is indicative of total body O\textsubscript{2} supply (see Fig. 5).

**DISCUSSION**

In animals that were hemodiluted with MP4, 62% of the total arterial O\textsubscript{2} content is released in capillaries. This is in distinct contrast to animals that were hemodiluted to the same degree with polymerized bovine hemoglobin, in which only 31% of the arterial O\textsubscript{2} content is released in capillaries, and to animals that were hemodiluted with Dextran-70, in which 40% of the arterial O\textsubscript{2} is released in capillaries.

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**Table 5. Oxygen extraction after hemodilution**

<table>
<thead>
<tr>
<th></th>
<th>Red Blood Cell</th>
<th>Plasma</th>
<th>Dissolved</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O\textsubscript{2} delivery, ml/dl</td>
<td>OER</td>
<td>O\textsubscript{2} delivery, ml/dl</td>
<td>OER</td>
</tr>
<tr>
<td>MP4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precapillary</td>
<td>1.78</td>
<td>0.28</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Capillary</td>
<td>3.03</td>
<td>0.47</td>
<td>0.85</td>
<td>0.13</td>
</tr>
<tr>
<td>PolyBvHb</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Precapillary</td>
<td>3.31</td>
<td>0.43</td>
<td>1.75</td>
<td>0.23</td>
</tr>
<tr>
<td>Capillary</td>
<td>1.11</td>
<td>0.14</td>
<td>1.17</td>
<td>0.15</td>
</tr>
<tr>
<td>Dextran</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precapillary</td>
<td>2.05</td>
<td>0.42</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Capillary</td>
<td>1.89</td>
<td>0.39</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

OER, O\textsubscript{2} extraction ratio.

---

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High precapillary O$_2$ loss was observed with the use of microelectrode measurements in rats and hamsters (7) and has been confirmed by more recent studies (15, 31, 33) using sophisticated microscopic PO$_2$ measurements. Precapillary PO$_2$ in the range of 20–40 mmHg (present study) suggested that intravascular PO$_2$ could be a sensitive effector of local vasoconstriction, as has been suggested by Guyton (10) for precapillary sphincters, and which would apply to arterioles as well. Studies (20, 21) in the rabbit tenuissimus microcirculation showed clearly that FCD is inversely related to PO$_2$.

The participation of cell-free hemoglobin in facilitated diffusive O$_2$ delivery has been studied by many investigators. As early as 1960, Scholander (28) showed that the rate of diffusion of O$_2$ through a solution of hemoglobin could be increased as much as eightfold compared with plasma and that this rate could be modulated by hemoglobin concentration and O$_2$ affinity. More recent measurements have confirmed these findings (3, 4). Hemoglobin, free in the plasma space, can greatly increase the supply of O$_2$ to vessel walls (8, 13), and some measurements have been made with modified hemoglobin solutions and encapsulated hemoglobin (5, 24). Most of these measurements have been made in static solutions, a very simple system compared with flow through small vessels, which is made complex because of mixing, the dependency of O$_2$ release on flow rate, and the presence of red blood cells in the solution. However, direct measurements in vitro in artificial capillaries show that cell-free hemoglobin is more efficient than red blood cells in the uptake and release of O$_2$ (2, 17, 23, 25), and PolyBvHb increases the lung-diffusing capacity in humans, presumably by a similar mechanism (14). However, despite this body of evidence, experiments designed to show more efficient oxygenation of muscle tissue after replacement of red blood cells with cell-free hemoglobin have failed to do so (1, 12). Our data suggest that in these cases the bulk of O$_2$ delivery occurs before the capillaries, thus

An additional factor that promotes O$_2$ release in capillaries is FCD. In the MP4-treated animals, FCD is 67% of baseline, whereas in the PolyBvHb-treated animals, FCD is reduced to 37% ($P < 0.05$). The FCD value for the dextran-treated animals (53%) is intermediate between the other two groups and is not significantly different from either. We propose that high precapillary O$_2$ loss stimulates microvascular arteriolar vasoconstriction, elevating systemic pressure proximal to the constriction but reducing pressure distal to it. This would result in depressurization of capillaries, leading to reduced red blood cell flow (i.e., loss of FCD), lowered hematocrit, and consequent reduced red blood cell O$_2$ delivery to the tissues served by the capillary network. The data from our experiments do not prove this mechanism, but the reduction in vessel width required to have this effect would be too small to detect by current methods.

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engaging autoregulatory vasoconstriction, with the concomitant increase of O₂ consumption by the constricting vessel wall (43). This interpretation is also consistent with the observation that when α-crosslinked hemoglobin was administered to animals and humans (11, 40) the increased O₂ capacity was offset by vasoconstriction to the detriment of tissue oxygenation.

Given the proposed sequence of events that follow introduction of hemoglobin into the plasma space, the critical parameters of a successful cell-free hemoglobin-based O₂ therapeutic become immediately apparent: 1) increase hemoglobin affinity for O₂, and 2) reduce the diffusivity of the molecule by increasing its size and viscosity, according to the Stokes-Einstein law

\[ D_{HbO_2} = \frac{K T}{6 \pi \eta r} \] (7)

where \(D_{HbO_2}\) is the diffusion coefficient for cell-free hemoglobin, \(K\) is Boltzman’s constant, \(T\) is absolute temperature, \(\eta\) is viscosity, and \(r\) is molecular radius. MP4 has increased molecular radius and viscosity compared with early generation products, reduced \(D_{HbO_2}\), and the data in the present experiments indicate that it permits high FCD, reduced vasoactivity, and effective oxygenation of capillaries.

On the basis of ex vivo measurements of aortic ring constriction by hemoglobin, it has been tempting to explain hemoglobin-induced vasoconstriction on scavenging of NO, and hemoglobin mutants with decreased NO affinity are less hypertensive than native hemoglobin (6, 18). However, whereas constriction of either large (arterial) or small (arteriolar) vessels may raise systemic blood pressure, it is arteriolar vasoconstriction that limits capillary perfusion.

Regulation of capillary blood flow, the protection of hemoglobin within red blood cells, and the low solubility of O₂ in plasma are biological, chemical, and physical features that are integrated into a system of O₂ delivery, which is the product of millions of years of evolution. The movement of O₂ out of the vessel necessitates overcoming barriers (35), which include limited diffusion within the red blood cell, diffusion through unstirred plasma surrounding the red blood cell, and diffusion through a layer of plasma with a variable thickness that depends on the size of the vessel and its flow characteristics. Ultimately, the release of O₂ from the vessel wall is a function of the diffusion distance through the plasma layer at the vessel wall (22), the diffusion constant for O₂, its solubility, and the gradient of O₂ concentration at the vessel wall. These factors combine to keep O₂ within precapillary vessels. The essential problem for complex organisms, such as mammals, is to conduct O₂ from the lung to tissue with minimal losses, so that the bulk of the O₂ can be released in capillaries, rather than in tissues that already have sufficient O₂ supply. The introduction of highly diffusible cell-free hemoglobin defeats these mechanisms and leads to accelerated release of O₂ before entering the capillaries, triggering autoregulatory protective mechanisms aimed at decreasing the supply of O₂ to highly metabolic tissues. Property selections of cell-free hemoglobin that retard O₂ release before entry into capillaries can potentially solve this problem.

Finally, it should be noted that arterial O₂ content, which in the case of red blood cell transfusions is represented by simple measurement of total hemoglobin concentration or hematocrit, does not correlate with tissue oxygenation, at least as base excess is an indicator (see Fig. 4). This has the important clinical implication that hemoglobin and hematocrit cannot reliably be used as surrogates for O₂ delivery to tissues as has been the practice for centuries. Instead, when patients are treated with cell-free O₂ carriers with properties such as those of MP4, other, and possibly novel, methods of assessing O₂ transport status will be required.

DISCLOSURES

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12. Hogan M, Kurdk S, Richardson R, and Wagner P. Partial substitution of red blood cells with free hemoglobin does not


