Oxygen release from low and normal P50 Hb vesicles in transiently occluded arterioles of the hamster window model

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Sakai, Hiromi, Pedro Cabrales, Amy G. Tsai, Eishun Tsuchida, and Marcos Intaglietta. Oxygen release from low and normal P50 Hb vesicles in transiently occluded arterioles of the hamster window model. Am J Physiol Heart Circ Physiol 288: H2897–H2903, 2005. First published January 28, 2005; doi:10.1152/ajpheart.01184.2004.—A phospholipid vesicle encapsulating Hb [Hb vesicle (HbV)] has been developed as a transfusion alternative. One characteristic of HbV is that the O2 affinity [P50 at which Hb is 50% saturated (P50)] of Hb can be easily regulated by the amount of the coencapsulated allosteric effector pyridoxal 5’-phosphate. In this study, we prepared two HbVs with different P50s (8 and 29 mmHg, termed HbV8 and HbV29, respectively) and observed their O2-releasing behavior from an occluded arteriole in a hamster skinfold window model. Conscious hamsters received HbV8 or HbV29 at a dose rate of 7 ml/kg. In the microscopic view, an arteriole (diameter: 53.0 ± 6.6 μm) was occluded transectaneously by a glass pipette on a manipulator, and the reduction of the intra-arteriolar Po2 100 μm down from the occlusion was measured by the phosphorescence quenching of preinfused Pd-porphyrin. The baseline arteriolar Po2 (50–52 mmHg) decreased to about 5 mmHg for all the groups. Occlusion after HbV8 infusion showed a slightly slower rate of Po2 reduction compared with that after HbV29 infusion. The arteriolar O2 content was calculated at each reducing Po2 in combination with the O2 equilibrium curves of HbVs, and it was clarified that HbV8 showed a significantly slower rate of O2 release compared with HbV29 and was a primary source of O2 (maximum fraction, 0.55) overwhelming red blood cells when the Po2 was reduced (e.g., <10 mmHg) despite a small dosage of HbV. This result supports the possible utilization of Hb-based O2 carriers with lower P50 for oxygenation of ischemic tissues.

blood substitutes; artificial red blood cells; occlusion; microhemodynamics; liposome

PHOSPHOLIPID VESICLES encapsulating concentrated human Hb [Hb vesicles (HbV)] or liposome-encapsulated Hb can serve as a transfusion alternative whose O2 carrying capacity can be formulated to be comparable to that of blood (1, 5, 8, 16, 24, 30). The capsular structure of HbV (particle diameter ~250 nm) has characteristics similar to those of natural red blood cells (RBCs), because both have membranes that prevent direct contact of Hb with the components of blood and the endothelial lining, mitigating cellular injury due to Hb-mediated prooxidative species (4, 38). Furthermore, Hb encapsulation in vesicles prevents a hypertensive response induced by free Hbs that scavenge the endogenous vasorelaxation factors nitric oxide (NO) and carbon monoxide (12, 18, 26). The safety of HbV has been confirmed in rodent models in terms of the prompt metabolism of the components of HbV in the reticuloendothelial system, which was demonstrated by histopathological analysis and plasma biochemical analysis (28, 29).

One of the characteristics of the capsular HbV is that its physicochemical characteristics such as O2 affinity [O2 tension at which Hb is half-saturated with O2 (P50)] can be easily regulated by manipulating the amount of an allosteric effector coencapsulated in HbV. This property provides additional flexibility in formulating the O2 transport properties of HbV by comparison with the chemically modified Hbs whose P50 is modified and fixed by chemical reactions such as cross-linking or polymer conjugation (34). We use pyridoxal 5’-phosphate (PLP) as the allosteric effector (33, 45). For example, coencapsulation of PLP at the molar ratio of PLP to Hb of 2.5:1 yields a P50 of about 29 mmHg. On the other hand, HbVs without PLP have a P50 of 8 mmHg. Historically, P50 was set similar to that of RBCs or about 25–30 mmHg, which theoretically allows sufficient O2 unloading as blood transits the microcirculation. Decreasing O2 affinity (increasing P50) increases O2 unloading in the peripheral blood circulation as shown by the enhanced O2 release and improved exercise capacity in mutant mice that carry high P50 RBCs (36). Hemoglobin-based O2 carriers (HBOCs) of molecular dimensions as well as HbV could be effective for the targeted oxygenation of ischemic tissues (6, 43) because the small particle dimension would allow their passage through constricted or partially occluded vessels that do not allow the passage of RBCs (19). Blood flow in these vessels and in collateral vessels is usually slow, thus increasing RBC transit times (7, 11). As a result, tissue Po2 is low and RBCs release most of their O2 before reaching the capillary circulation. As an example, if tissue Po2 is below 5 mmHg, O2 saturation (SaO2) of RBCs would be around 5%, and RBCs will have released most of their O2 before they reach the ischemic tissue. Thus an HBOC with a normal P50 similar to RBCs (36).

In this study, we evaluate the rate of O2 release from HbVs with high and low P50s from arterioles immediately after their occlusion. We selected arterioles with diameters of about 50 μm because this size of arterioles contributes significantly to tissue oxygenation in normal conditions (13). This model was selected to determine the ability of HbVs to retain or release O2 in hypoxic conditions and establish their suitability for oxygenating ischemic tissues.
Preparation of HbVs. HbVs with different P₅₀s were prepared under sterile conditions as previously reported (32, 34, 37). Hb was purified from outdated donated human blood provided by the Japanese Red Cross Society (Tokyo, Japan). HbVs with a P₅₀ = 29 mmHg (HbV₂₉) was prepared by adding the allosteric effector pyridoxal 5'-phosphate (PLP; 14.7 mM, Sigma Chemical; St. Louis, MO) to Hb (38 g/dl) at a molar ratio of PLP to Hb = 2.5. HbVs with a P₅₀ = 8 mmHg (HbV₈) were prepared by adding no allosteric effector to the Hb solution. The Hb solution was encapsulated within vesicles composed of Presome PPG-I [a mixture of 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine, cholesterol, and 1,5-di-O-octadecyl-N-succinyl-1-glutamate at a molar ratio of 5:5:1 (Nippon Fine Chemicals; Osaka, Japan)], and the particle size of HbVs was regulated by an extrusion method. The surface of the HbVs was modified with polyethylene glycol (molecular mass: 5 kDa, 0.3 mol% of the lipids in the outer surface of vesicles) using 1,2-distearoyl-sn-glycero-3-phosphatidylethanolamine-N-polyethylene glycol (Sunbright DSPE-50H, H-form, NOF; Tokyo, Japan). HbVs were suspended in a physiological salt solution and sterilized with filters (Dismic, Toyo Roshi; Tokyo, Japan; pore size: 0.45 μm) and deoxygenated with N₂ bubbling for storage. The endotoxin content was measured with a modified Limulus amebocyte lysate assay, and the level was less than 0.2 EU/ml.

Physicochemical properties of HbV₈ and HbV₂₉ compared with hamster blood

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HbV₈</th>
<th>HbV₂₉</th>
<th>Hamster Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb concentration, g/dl</td>
<td>10</td>
<td>10</td>
<td>14.8 ± 0.5</td>
</tr>
<tr>
<td>Particle diameter, nm</td>
<td>250 ± 64</td>
<td>247 ± 44</td>
<td>5,000–7,000*</td>
</tr>
<tr>
<td>P₅₀, mmHg</td>
<td>8</td>
<td>29</td>
<td>28</td>
</tr>
<tr>
<td>Molar ratio of PLP to Hb</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>MetHb, %</td>
<td>&lt;3</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>HbCO, %</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td></td>
</tr>
</tbody>
</table>

HU/ml. Microvascular observations of the awake and unanesthetized hamsters were performed 5 days after chamber implantation to mitigate the effects of surgery. The hamster was placed in a perforated plastic tube from which the window chamber protruded to minimize animal movement without impeding respiration. All animal studies were approved by the Animal Care and Use Committee of University of California-San Diego and performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Washington, DC: National Academy Press, 1996).

Infusion of HbV₈ and HbV₂₉ and occlusion of an arteriole. The unanesthetized animal was placed in a perforated plastic tube and stabilized under the microscope. Animals were suitable for the experiments if systemic variables were within normal range, namely, heart rate >340 beats/min, mean arterial pressure >80 mmHg, systemic hematocrit >45%, and arterial PO₂ >50 mmHg, and microscopic examination of the tissue in the chamber did not reveal signs of edema or bleeding. Baseline measurements of microvascular parameters and PO₂ (see below) were performed before the infusion of HbV₈ or HbV₂₉ suspended in physiological saline solution into the venous line at 7 ml/kg. Systemic blood volume was estimated as 70 ml/kg. In our previous reports of resuscitation from hemorrhagic shock or hemorrhage, HbVs were suspended in an albumin solution to regulate colloid osmotic pressure (30, 33). However, in the present study, we did not use albumin to minimize the hypervolemic effect. For the same reason, the infusion amount was minimized to equal 10% blood volume (7 ml/kg).

After we stabilized the condition and measured the systemic parameters for 20 min, diameter and blood flow of the selected arterioles were measured. Large feeding arterioles or small arcading arterioles (diameter 53.0 ± 6.6 μm) were selected for observation. The arterioles were occluded by means of a glass micropipette whose end was drawn into a long fiber by a pipette puller (Fig. 2). The fiber was bent over a flame, and the knee of the bend was used to press on the intact skin of the preparation mounted in an inverted microscope that allowed observation of the opposite side, i.e., the intact microcirculation. Once an arteriole was selected for measurement, the microoccluder was moved to the skin side, between the intact skin and the optics of the substage illumination. The tip of the occluder was placed near the center of the optical field of view of the microscope, and the vessel was similarly placed using the stage micrometric position control. This arrangement allowed for direct microscopic observation of the occluded vessel and the stopped flow as shown in Fig. 2. The duration of occlusion was 30 s.

Measurement of microhemodynamic parameters. Microvessels were observed by transillumination with an inverted microscope (IMT-2, Olympus; Tokyo, Japan). Microscopic images were video recorded (Cohu 4815-2000; San Diego, CA) and transferred to a television videotape recorder (Sony Trinitron PVM-1271Q monitor; Tokyo, Japan) and Panasonic AG-7355 video recorder (Tokyo, Japan). Arterioles were classified according to their position within the microvascular network according to the previously reported scheme (33). Microvascular diameter and RBC velocity before occlu-

Fig. 1. Oxygen equilibrium curves (OECs) of Hb vesicles (HbVs) at a PO₂ where Hb is half-saturated (P₅₀) of 8 mmHg (HbV₈) and 29 mmHg (HbV₂₉) measured with a Hemox Analyzer (TCS Medical Products) at 37°C compared with hamster blood, RBC, red blood cells.
Light was gathered from an optical window of 20 × 5 μm placed longitudinally along the blood vessels. Measurements in the blood compartment were made every second using a single flash.

The PO2 decay curves induced by the occlusion were obtained before the infusion of HbVs and 20 min after the infusion of HbVs. The SaO2 of HbVs at every PO2 were obtained from the OECs (Fig. 1), and the total O2 content in blood (ml O2 in 1 dl blood) can be estimated using the following equation:

\[
\text{O2 content} = \frac{23.6 \times [\text{SaO2(RBC)} + 0.0667 \times \text{SaO2(HbV)}]}{100} + 2.42 \times \frac{\text{PO2}}{713}
\]

In this calculation, we used 15 g/dl as the average Hb concentration in arterial blood (14.8 ± 0.5 g/dl, heme concentration 9.3 mM), which was measured with a handheld photometer (B-Hemoglobin Photometer, Hemocue). One hundred milliliters of blood contain 23.6 ml O2 bound to Hb when SaO2 is 100% (volume of an ideal gas at 37°C) according to Boyle-Charle's gas law, \( PV = nRT \), where \( P \) (in atm) is atmospheric pressure, \( V \) (in liters) is gas volume, \( n \) is mole number, \( R \) is the gas constant (0.082 atm·l·K⁻¹·mol⁻¹), and \( T \) is absolute temperature [23.6 (ml) = 9.3 × 10⁻³ (mol) × 0.082 × (273 + 37) × 1,000]. The physically dissolved O2 content at 1 atm O2 (713 mmHg) after subtracting the vapor pressure of water = 47 mmHg) at 37°C was calculated to be 2.42 ml in 100 ml water. SaO2(RBC) and SaO2(HbV) are SaO2 of RBCs and HbVs, respectively, at each arteriolar PO2 during the experiments.

HbVs were suspended in physiological saline solution ([Hb] = 10 g/dl); therefore, their infusion lowered colloid osmotic pressure, causing the extravasation of plasma fluid. To account for this, we carried out our measurements 20 min after HbV infusion and assumed that this interval was sufficient for normalizing blood volume through the release of extra fluid to the interstitium, thus increasing plasma Hb concentration by 6.7%.

**Data analysis.** Data are given as means ± SD for the indicated number of animals. Data were analyzed using ANOVA followed by Fisher’s protected least-significant difference test between groups according to the previous studies. Student’s \( t \)-test was used for comparisons within each group. All statistics were calculated using GraphPad Prism 4.01 (Graph Pad Software; San Diego, CA). Changes were considered statistically significant if \( P < 0.05 \).

**RESULTS**

**Hemodynamic properties of arterioles.** The profiles of the selected arterioles, diameters, centerline RBC velocities, blood flow rates, and intra-arteriolar PO2 values before and after infusion of HbVs are listed in Table 2. There was no significant difference between the groups. The O2 content in blood attributed to hamster RBCs and physically dissolved O2 at the observed arteriolar PO2 was estimated as 18.61 ± 1.23 ml O2/dl blood according to Eq. 3. After the infusion of HbVs and HbV29, the O2 content increased to 20.30 ± 1.18 and 20.17 ± 1.54 ml O2/dl blood, respectively, due to the O2 bound to HbVs. The contributions of HbV8 and HbV29 to whole O2 content were 1.51 ± 0.01 and 1.25 ± 0.07 ml O2/dl blood, respectively. The HbV8 group showed higher O2 content than the HbV29 group due to the higher SaO2(HbV8), which was 95.9 ± 0.6% compared with the SaO2(HbV29) of 79.6 ± 4.7%.

**Changes in PO2 in arterioles after occlusion in the presence of HbVs.** Arteriolar PO2 before occlusion was about 10 ± 5 mmHg and decreased significantly immediately after occlusion, as shown in Fig. 3. In all groups, PO2 fell to about 10 and 5 mmHg after 10- and 20-min occlusion, respectively. The HbV8 group showed higher PO2 than the HbV29 group due to the higher SaO2(HbV8), which was 95.9 ± 0.6% compared with the SaO2(HbV29) of 79.6 ± 4.7%.

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**Fig. 2.** A: microscopic image of an occluded arteriole in the hamster window chamber. The glass fiber lies across the arteriole. Scale bar = 100 μm. B: schematic representation of occlusion of A showing the different tissue layers of the skin (not to scale).

**Table 2:** Contributions of hemoglobin (Hb) vesicles (HbVs) to whole O2 content in blood.

<table>
<thead>
<tr>
<th>Group</th>
<th>HbVs (ml O2/dl)</th>
<th>Contribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbV8</td>
<td>1.51 ± 0.01</td>
<td>8.1 ± 0.4</td>
</tr>
<tr>
<td>HbV29</td>
<td>1.25 ± 0.07</td>
<td>6.7 ± 0.3</td>
</tr>
<tr>
<td>Total</td>
<td>2.76 ± 0.08</td>
<td>14.8 ± 0.7</td>
</tr>
</tbody>
</table>

---

**Appendix:**

**Eq. 1:**

\[ Q = \frac{(\text{RBC velocity}/R_c) \times \text{diameter}^2}{2} \]

**Eq. 2:**

\[ \tau / \tau_o = 1 + k_o / \tau_o \times \text{PO2} \]

**Eq. 3:**

\[ \text{O2 content} = \frac{23.6 \times [\text{SaO2(RBC)} + 0.0667 \times \text{SaO2(HbV)}]}{100} + 2.42 \times \frac{\text{PO2}}{713} \]

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**Notes:**

*Volmer equation:* molecular O2 and at a given PO2, respectively, and phosphorescence lifetime and PO2 is given by the following Stern-Volmer equation:

\[ \tau / \tau_o = 1 + k_o / \tau_o \times \text{PO2} \]

where \( \tau_o \) and \( \tau \) are the phosphorescence lifetimes in the absence of molecular O2 and at a given PO2, respectively, and \( k_o \) is the quenching constant, with both factors being pH and temperature dependent.
30-s occlusion, respectively. When the \( P_{O_2} \) values were expressed as relative to the baseline values (before occlusion), infusion of HbV8 tended to show a slower rate of reduction of \( P_{O_2} \) compared with the infusion of HbV29 and without infusion (Fig. 4). There was a significant difference between the HbV8 infusion and before infusion groups only at 7 s (\( P = 0.035 \)).

\[
\text{Sa}_{O_2}(RBC) \text{ and } \text{Sa}_{O_2}(HbV) \text{ at every arteriolar } P_{O_2} \text{ value can be estimated using the OECs in Fig. 1 assuming that the conditions in the arteriole (such as temperature and pH) do not change significantly from the normal condition (37°C, pH 7.4).}
\]

![Fig. 3](image_url)

**Fig. 3.** Time course of \( P_{O_2} \) in the blood of an occluded arteriole (diameter, \( 53.0 \pm 6.6 \) \( \mu \)m) before and after infusion of 7 ml/kg HbV8 or HbV29 into hamsters. Measurements were made in blood at a distance of 50 \( \mu \)m from the point of occlusion. Most vessels equilibrate to intravascular partial pressure in the range of 4–6 mmHg about 15–20 s after occlusion.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before Infusion</th>
<th>( \text{HbV}_8 )</th>
<th>( \text{HbV}_{29} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arteriolar diameter, ( \mu )m</td>
<td>53.0 ± 6.6</td>
<td>56.2 ± 6.8</td>
<td>55.8 ± 6.9</td>
</tr>
<tr>
<td>Centerline flow velocity, mm/s</td>
<td>3.1 ± 0.5</td>
<td>3.4 ± 0.7</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>Blood flow rate, nl/s</td>
<td>6.8 ± 1.6</td>
<td>8.7 ± 3.1</td>
<td>8.5 ± 2.1</td>
</tr>
<tr>
<td>Arteriolar ( P_{O_2} ), mmHg</td>
<td>50.7 ± 4.7</td>
<td>51.4 ± 4.8</td>
<td>52.1 ± 5.3</td>
</tr>
<tr>
<td>( \text{Sa}_{O_2}(RBC) ), %</td>
<td>78.1 ± 5.1</td>
<td>76.0 ± 7.7</td>
<td>77.9 ± 6.5</td>
</tr>
<tr>
<td>( \text{Sa}_{O_2}(HbV) ), %</td>
<td>95.9 ± 0.6†</td>
<td>79.6 ± 4.7</td>
<td></td>
</tr>
<tr>
<td>( P_{O_2} ) content in whole blood, ml O2/dl blood</td>
<td>18.61 ± 1.23</td>
<td>20.30 ± 1.18*</td>
<td>20.17 ± 1.54*</td>
</tr>
<tr>
<td>( O_2 ) content in HbV, ml O2/dl blood</td>
<td>1.51 ± 0.01</td>
<td>1.25 ± 0.07*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. Arteriolar \( P_{O_2} \), \( O_2 \) saturation (\( \text{Sa}_{O_2} \)) and \( O_2 \) contents were obtained during 6 s before occlusion. *\( P < 0.05 \) vs. before infusion; †\( P < 0.05 \) vs. RBCs and HbV29.

\( \text{Sa}_{O_2}(RBC) \) and \( \text{Sa}_{O_2}(HbV) \) at every arteriolar \( P_{O_2} \) value can be estimated using the OECs in Fig. 1 assuming that the conditions in the arteriole (such as temperature and pH) do not change significantly from the normal condition (37°C, pH 7.4).

**Fig. 4.** Changes in \( P_{O_2} \) relative to before occlusion. The data in Fig. 3 were averaged. Baseline values before occlusion were obtained as the average of 6 values before occlusion and fixed as 1.0. There was a significant difference between the \( \text{HbV}_8 \) infusion and before infusion groups only at 7 s (\( P = 0.035 \)).
Although the O₂ supply was significantly reduced, diffusion and continued to release O₂ until 30 s.

HbV₈, and not RBCs, was the main source of the O₂ carrier significantly and was about 0.55 after 10 s. This indicated that the maximum of 0.08 ml O₂/dl blood after 10 s of occlusion HbVs clearly, only the O₂ content of HbVs is shown in Fig. 5.

FIG. 5. Time course of the fraction of O₂ loss from HbVs obtained by the differentiation of the graphs in Fig. 5B. HbV₂₉ showed the fastest O₂ loss with the maximum of 0.18 ml O₂/dl blood sec after only 2 s of occlusion and did not supply O₂ after 17 s. On the other hand, HbV₈ showed a moderate O₂ loss and showed the maximum of 0.08 ml O₂/dl blood after 10 s of occlusion and continued to release O₂ until 30 s.

FIG. 6. Time course of the fraction of O₂ content from HbVs in whole blood. The extended time of occlusion induced hypoxic conditions and the fraction of O₂ content from HbV₈ increased significantly compared with HbV₂₉.

A limitation of our experimental method is that SaO₂ is estimated under the assumption that conditions in the target arteriole are identical to that of the OEC measurement; however, the O₂ affinity of Hb changes as a function of temperature, pH, electrolyte concentration, and CO₂ content. Local ischemic conditions caused by the occlusion could affect pH and increase CO₂ tension, resulting in a slight decrease in the O₂ affinity (increased P₅₀); however, it is unlikely that this would introduce a significant error in the measurement of O₂ release considering the short duration of the occlusion (30 s).

We have previously demonstrated using an artificial narrow polymer tube (inner diameter: 28 µm) surrounded by a sodium dithionate solution to consume O₂ that a Hb solution under continuous flow conditions (1 mm/s) facilitates O₂ release when mixed with RBCs. Conversely, HbV did not show this phenomenon (31). This difference is due to the small size of O₂-bound acellular Hb molecules, which diffuse and therefore contribute to the facilitated O₂ transport (21, 31), whereas HbVs (diameter, about 250 nm) are too large to show sufficient diffusion for the facilitated O₂ transport. In these conditions, O₂ affinity (P₅₀) becomes the determining factor for the rate of O₂ release and transport to the vessels wall. Thus, in our present results, the presence of HbVs did not facilitate the reduction of PO₂ or O₂ content but retarded the reduction of PO₂ and O₂ content.

Our experimental model is designed to characterize the O₂ release behavior of blood from an occluded microvessel and does not directly related to clinical ischemic conditions because the occlusion of the small arteriole for 30 s does not induce tissue ischemia other than the transient event in the proximity of the microvessel. However, our data suggest that HbV₈ could be a significant source of O₂ in an ischemic condition with significantly lowered tissue PO₂. Because of the small dosage of HbV₈ (7 ml/kg), the O₂ content in the blood after occlusion (5 ml O₂/dl blood at 5 s) is significantly smaller than the baseline value (20 ml O₂/dl blood at 0 s). To enhance the contribution of HbVs, a larger dosage and sustained blood flow would be required. Contaldo et al. (7) recently demonstrated that inducing hemodilution using up to 50% blood exchange with HbV (P₅₀ = 15 mmHg) suspended in dextran effectively oxygenated ischemic collateralized tissue in skin flaps. This phenomenon could be explained by low P₅₀ HbVs retaining O₂ in the upstream vessels and delivering it to the ischemic tissue via collateral arterioles, even when these may have significantly slower blood flow. It has been proposed that small-sized HBOCs oxygenate ischemic tissue by being able to pass through constricted or partially occluded vessels that do not allow the passage of RBCs; however, the results from Contaldo et al. (17) as well as those from our experimental model do not serve to support this concept, because arterioles were completely ligated or occluded. It should be noted, however, that an advantage of small HBOCs, including HbVs,
it is that they are homogeneously dispersed in the plasma phase and therefore can deliver O₂ more homogeneously to the periphery than RBCs because microvascular hematocrit is heterogeneous particularly in pathological states. In such conditions, HbVs with a higher O₂ affinity should show a slower O₂ unloading that would be effective for oxygenating ischemic tissues.

In conclusion, HbVs provide the unique feature of allowing for the regulation of P₅₀ by modulating the amount of coenzyme A and therefore can deliver O₂ more homogeneously to the tissues.

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