Systemic and microvascular responses to hemorrhagic shock and resuscitation with Hb vesicles

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Sakai, Hiromi, Shinti Takeoka, Reto Wettstein, Amy G. Tsai, Marcos Intaglietta, and Eishun Tsuchida. Systemic and microvascular responses to hemorrhagic shock and resuscitation with Hb vesicles. Am J Physiol Heart Circ Physiol 283: H1191–H1199, 2002. First published May 16, 2002; 10.1152/ajpheart.00080.2002.—A phospholipid vesicle encapsulating hemoglobin (Hb vesicle, HbV) has been developed to provide O2-carrying capacity to plasma expanders. Its ability to restore systemic and microcirculatory conditions after hemorrhagic shock was evaluated in the dorsal skinfold window preparation of conscious hamsters. The HbV was suspended in 8% human serum albumin (HSA) at Hb concentrations of 3.8 g/dl [HbV(3.8)/HSA] and 7.6 g/dl [HbV(7.6)/HSA]. Shock was induced by 50% blood withdrawal, and mean arterial pressure (MAP) at 40 mmHg was maintained for 1 h by the additional blood withdrawal. The hamsters receiving either HbV(3.8)/HSA or HbV(7.6)/HSA suspensions restored MAP to 93 ± 14 and 93 ± 10 mmHg, respectively, similar with those receiving the shed blood (98 ± 13 mmHg), which were significantly higher by comparison with resuscitation with HSA alone (62 ± 12 mmHg). Only the HSA group tended to maintain hyperventilation and negative base excess after the resuscitation. Subcutaneous microvascular blood flow reduced to ~10–20% of baseline during shock, and reinfusion of shed blood restored blood flow to ~60–80% of baseline, an effect primarily due to the sustained constriction of small arteries A0 (diameter 143 ± 29 μm). The HbV(3.8)/HSA group had significantly better microvascular blood flow recovery and nonsignificantly better tissue oxygenation than of the HSA group. The recovery of base excess and improved tissue oxygenation appears to be primarily due to the increased oxygen-carrying capacity of HbV fluid resuscitation.

PHOSPHOLIPID VESICLES encapsulating concentrated human hemoglobin (Hb) (Hb vesicles, HbV) can serve as blood substitutes of which their O2-carrying capacity can be formulated to be comparable to that of blood (2, 4, 10, 23, 40). They are void of blood-type antigens and infectious viruses and are stable and suitable for long-term storage (26). The cellular structure of HbV (particle diameter ca. 280 nm) has characteristics similar to those of natural red blood cells (RBCs), because both have cell membranes that prevent direct contact of Hb with the components of blood and the endothelial lining. Furthermore, Hb encapsulation in vesicles suppresses hypertension induced by vasoconstriction due to scavenging of the endogenous vasorelaxation factors nitric oxide (NO) and carbon monoxide (5, 14, 26) consequent to their high affinity with Hb. Once in the circulation, HbV particles are captured by the phagocytes in the reticuloendothelial system (mainly the spleen and liver), and they are metabolized completely within 14 days, with no deposition of iron or lipids (27).

O2-carrying blood replacement fluids using molecular or encapsulated Hb as an O2 carrier have been proposed for volume restoration in hemorrhagic shock (16, 18, 43) and hemodilution being generally assumed that low O2 affinity (high P50) and high Hb concentration should be effective for O2 delivery. However, in previous studies (31) of extreme hemodilution in the hamster dorsal skinfold preparation, we found that the optimal O2 dissociation curve of HbVs is shifted to the left. In this report, we analyze systemic and microvascular responses after resuscitation from hemorrhagic shock by using HbVs with different Hb concentrations to determine the optimal oxygen-carrying capacity, focusing on the responses of small resistance arteries, which were found to be the critical vessels in regulating microvascular blood flow (24–26, 32).

MATERIALS AND METHODS

Preparation of HbVs. HbVs were prepared under sterile conditions as previously reported (26, 28, 40). Hb was purified from outdated donated blood provided by the Hokkaido Red Cross Blood Center, Sapporo, Japan. The encapsulated Hb (38 g/dl) contained 5.9 mM of pyridoxal 5'-phosphate (PLP, Merck; Darmstadt, Germany) as an allosteric effector at a molar ratio of PLP/Hb = 2.5. The lipid bilayer was composed of Presome PPG-I (a mixture of 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine, cholesterol, and 1,5-dipalmitoyl-glycerol-3-phosphatidyl-N-succinimide acid at a molar ratio of 5:5:1;...
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Nippon Fine Chemical, Osaka, Japan). The surface of the HbV was modified with polyethylene glycol (mol mass: 5 kDa, 0.3 mol% of the lipids in the outer surface of vesicles) by using 1,2-distearoyl-sn-glycero-3-phosphatidylethanolamine-N-polyethylene glycol (Sunbright DSPE-50H, H-form, NOF; Tokyo, Japan). Carbonylhemoglobin was converted to oxyhemoglobin by exposure to visible light in an O2 atmosphere. HbVs were suspended in a physiological salt solution and filtered through sterilizable filters (pore size: 0.45 μm, Dismic, Toyo Roshi; Tokyo, Japan) and deoxygenated with N2 bubbling for storage (29). Physicochemical parameters of the HbVs are listed in Table 1.

Before use, the HbV suspension ([Hb] = 10 g/dl) was mixed with a human albumin solution (HSA, 25%, Bayer; Leverkusen, Germany) and saline to regulate the colloid osmotic pressure of the suspension to ~40 mmHg (20, 24, 31). Two suspensions with different Hb concentrations, 3.8 g/dl and 7.6 g/dl, but the same HSA concentration in the suspending medium, and the resulting colloid osmotic pressure (40 mmHg) were prepared. The two samples are abbreviated as HbV(3.8)/HSA and HbV(7.6)/HSA, respectively. The viscosities of HbV(3.8)/HSA, HbV(7.6)/HSA, and HSA alone measured with a cone-plate viscometer (PVII+, Brookfield Engineering; Middleboro, MA) at 37°C were 1.8, 3.0, and 1.0 cP, respectively (150 s⁻¹).

Animal model and preparation. Experiments were carried out in 26 male Syrian golden hamsters (64 ± 7 g body wt, Charles River; Worcester, MA). All animals were housed in cages and provided with food and water ad libitum in a temperature-controlled room with a 12:12 h dark-light cycle. The muscular supply to the exposed tissue allowing intravital observation of the microvasculature in the subcutaneous tissue was obtained by removing the entire layer of epidermis and subcutaneous fat overlying the target area, leaving the superficial dermis intact. Skin muscle was separated from the subcutaneous tissue and removed until a thin monolayer of muscle including the skin muscle were observed by transillumination with an incandescent light source. The patency of the catheters was monitored throughout the experiment.

Measurements of systemic and microhemodynamic parameters. Systemic and microhemodynamic parameters and blood gases were evaluated before hemorrhage (baseline), after 50% hemorrhage, before resuscitation, just after resuscitation, and 0.5, and 1.0 h after resuscitation. The in situ microcirculation of the skinfold chamber was observed using a video microscope system. After 1 h from resuscitation, palladium-porphyrin bound to bovine albumin solution (7.6 wt%, 0.1 ml) was injected intravenously to measure the PO2 in the vessels and interstitium (11, 36).

Blood samples were collected in heparinized microtubes (<100 μl, Curtin Matheson Scientific; Norcross, GA) for hematocrit (Hct) and blood gas analyses. A pH/blood gas analyzer (Blood Chemistry Analyzer 248, Bayer Medical; Northwood, MA) was used for analysis of arterial blood O2 tension (PaO2), arterial blood CO2 tension (Pa CO2), pH, and base excess (BE). A recording system (MP 150, Biopac System; Santa Barbara, CA) was used for continuous monitoring of MAP and heart rate (HR).

Microvessels in the subcutaneous tissue and the skeletal muscle 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 TV-VCR (Sony Trinitron PVM-1271Q monitor; Tokyo, Japan) and Panasonic AG-7355 video recorder (Tokyo, Japan).

Table 1. Physicochemical properties of HbV/HSA in comparison with hamster blood

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HbV(3.8)/HSA</th>
<th>HbV(7.6)/HSA</th>
<th>Hamster Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb concentration, g/dl</td>
<td>3.8</td>
<td>7.6</td>
<td>ca. 14*</td>
</tr>
<tr>
<td>Particle diameter, nm</td>
<td>281 ± 11</td>
<td>281 ± 11</td>
<td>5,000–7,000†</td>
</tr>
<tr>
<td>P50, mmHg</td>
<td>33</td>
<td>33</td>
<td>28</td>
</tr>
<tr>
<td>metHb, %</td>
<td>2.5</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Viscosity at 150 s⁻¹, cP</td>
<td>1.8</td>
<td>3.0</td>
<td>4.47 ± 0.50*</td>
</tr>
<tr>
<td>Oncotic pressure, mmHg</td>
<td>40</td>
<td>40</td>
<td>17.5 ± 1.7†</td>
</tr>
</tbody>
</table>

* Ref. 39; † Ref. 35. Hb, hemoglobin; HbV (3.8)/HSA, Hb vesicles/human serum albumin with 3.8 g Hb/dl; HbV (7.6)/HSA, HbV/HSA with 7.6 g Hb/dl; met Hb.
Microvessels were classified according to their position within the microvascular network according to the previously reported scheme (24). Arteriolar microvessels were grouped into small artery (A0, diameter 143 ± 29 μm), large feeding arterioles (A1, 60 ± 12 μm), small arcading arterioles (A2, 27 ± 6 μm), and transverse arterioles (A3, 11 ± 3 μm). Venules were classified as small collecting venules (Vc, 31 ± 8 μm), large venules (VL, 89 ± 18 μm), and small veins (Vo, 376 ± 95 μm). These microvessels and capillaries were sketched in advance to plan the sequence of measurements.

Microvascular diameter and RBC velocity were analyzed online in arterioles and venules (8, 9). Vessel diameter was measured with an image-shearing system (Digital Video Image Shearing Monitor 908, I.P.M.; San Diego, CA), whereas RBC velocity was analyzed by photodiodes and the cross-correlation technique (Velocity Tracker Mod-102 B, I.P.M.). Blood flow rates (Q) were calculated using the equation

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

where $R_c$ is the ratio of the centerline velocity to average blood velocity according to data from glass tubes, $R_c = 1.3$ being used for $A_3$ and 1.6 for the other vessels (15). The blood flow rates were summarized as arteriolar blood flow rates ($A_0, A_1, A_2$, and $A_3$) and venular blood flow rates ($V_c, V_l$, and $V_o$).

Functional capillary density (FCD) was analyzed online by counting the number of capillaries with RBC flow stemming from one $A_3$ arteriole, usually 40–80 capillaries, and expressed as a percentage of the basal value.

Subcutaneous microvascular and interstitial $P_O_2$ was determined with the $O_2$-dependent quenching of phosphorescence emitted by bovine serum albumin-bound metalloporphyrin complexes after pulsed light excitation (11, 36). The method allows noninvasive assessment of intravascular $P_O_2$ and determination of interstitial oxygenation because intravascularly injected porphyrin complexes bound to albumin extravasate into the interstitium over time. The relationship between phosphorescence lifetime and $P_O_2$ is given by the Stern-Volmer equation. The baseline $P_O_2$ values were obtained separately with six hamsters without hemorrhage.

**Data analysis.** Data are given as means ± SD for the indicated number of animals. Data were analyzed by using analysis of variance, followed by Fisher’s protected least-significant difference test between the groups according to the previous studies (11, 12, 24). Student’s t-test was used for the comparisons within each group. The level of confidence was placed at 95% for all the experiments.

**RESULTS**

Systemic responses to the hemorrhagic shock and resuscitation. MAP of the hamsters before hemorrhage was 105 ± 13 mmHg and declined to ~40 mmHg during shock, a level maintained for 60 min (Fig. 1). Immediately after resuscitation, the MAP of the SAB group recovered to 98 ± 13 mmHg, which was maintained for 1 h. MAP of the HbV(7.6)/HSA and HbV(3.8)/HSA groups recovered on retransfusion to 93 ± 10 and 93 ± 14 mmHg, respectively, values that were maintained for 1 h. The HbV/HSA groups were statistically not different from the SAB group and showed significantly higher MAP at all time points than the HSA.

![Fig. 1. Changes in mean arterial pressure (MAP), heart rate (HR), and hematocrit (Hct) during hemorrhagic shock and resuscitation with infusion of hemoglobin vesicles (HbV)/human serum albumin (HSA) with 3.8 g Hb/dl [HbV(3.8)/HSA], HbV/HSA with 7.6 g Hb/dl [HbV(7.6)/HSA], HSA, and shed autologous blood (SAB). *Significantly different from baseline ($P < 0.05$); #significantly different from the SAB group ($P < 0.05$); †significantly different from “before infusion” ($P < 0.05$).](Image)
group, of which its MAP was 62 ± 12 mmHg after resuscitation and remained at this level for 1 h.

Average HR before hemorrhage was 440 ± 67 beats/min and fell to 362 ± 64 beats/min during hemorrhagic shock (Fig. 1). Although recovery of HR was not immediate after infusion, it tended to return to the original level after 0.5 h for the SAB, HSA(7.6)/HSA, and HbV(3.8)/HSA groups, whereas the HSA group tended to remain lower and was significantly lower than the SAB group after 1.0 h.

Hct before hemorrhage was 47 ± 3% and was reduced to 35 ± 4% after bleeding and to 29 ± 4% before resuscitation (Fig. 1). This gradual Hct reduction is due to the compensatory increase in plasma volume. After resuscitation, Hct in the SAB group increased to 40 ± 4%. Hct in the HbV(7.6)/HSA, HbV(3.8)/HSA, and HSA groups were significantly reduced to 15 ± 2%, 16 ± 1%, and 13 ± 2%, respectively, because of the dilution of blood with the different solutions. These three values were not significantly different from each other. The HbV particles remained dispersed in the plasma phase in the glass capillaries used for Hct measurements.

Normal hamsters at baseline conditions had relatively lower PaO₂ (60 ± 6 mmHg) and higher PaCO₂ (54 ± 6 mmHg) values because of alveolar hypoventilation, a result from their adaptation to a fossorial environment (21) (Fig. 2). Hemorrhagic shock induced hyperventilation, which significantly increased PaO₂ to 106 ± 14 mmHg and decreased PaCO₂ to 38 ± 11 mmHg. There was significant metabolic acidosis shown by the decrease in pH from 7.38 ± 0.03 to 7.30 ± 0.08 and the decrease in BE from 5.3 ± 2.2 to −7.6 ± 6.6 mM before infusion. The SAB, HSA(7.6)/HSA, and HbV(3.8)/HSA groups tended to recover immediately from the hyperventilation after resuscitation, and there was no significant differences between the HbV/ HSA and SAB groups. Only the HSA group had significantly higher PaO₂ values than the SAB groups at all the time points. However, all of the groups showed significantly higher PaO₂ values than basal values at all the time points. All of the groups increased pH and BE after 0.5 h. The HSA group had the lowest BE values at 0.5 and 1 h after resuscitation.

Microhemodynamic responses to hemorrhage and resuscitation. Hemorrhagic shock induced significant constrictions of Aₒ arterioles to 60 ± 11% of the basal values (P < 0.0001) (Fig. 3). As seen in previous studies (24), other vessels did not show such significant changes (data not shown). All groups tended to recover from Aₒ constriction after resuscitation to ~80% of the basal values; however, diameters remained significantly constricted with no significant difference between groups.

Blood flow decreased significantly in arterioles to 11 ± 10% of the basal value (Fig. 3). The HbV(3.8)/ HSA, HbV(7.6)/HSA, and SAB groups immediately showed significant increases in blood flow rate after

![Fig. 2. Changes in blood gas parameters during hemorrhagic shock and resuscitation with infusion of HbV(3.8)/ HSA, HbV(7.6)/HSA, HSA, and SAB. PaO₂, arterial PO₂; PaCO₂, arterial PCO₂; BE, base excess. *Significantly different from baseline (P < 0.05); #significantly different between the indicated groups (P < 0.05); †significantly different from “before infusion” (P < 0.05).](http://ajpheart.physiology.org/resolve/doi/10.1152/ajpheart.00483.2002)
resuscitation (51 ± 47, 37 ± 35, and 48 ± 46% of basal value, respectively) with the exception of the HSA group (15 ± 20%). Only the HbV(3.8)/HSA and SAB groups had significantly higher blood flow rates than the HSA group after 30 min. After 1 h, there was no significant difference between the HbV/HSAs and the HSA groups. HbV(3.8)/HSA tended to show higher blood flow rates than the HbV(7.6)/HSA group.

Blood flow in venules was significantly decreased to 17 ± 22% of baseline during the shock period (Fig. 3). Immediately after infusion of the resuscitation fluids, only the HSA groups remained at a lower value (13 ± 16%), whereas the other groups increased significantly where HbV(3.6)/HSA showed the highest value (84 ± 57%). The HbV(7.6)/HSA and the HSA group remained at lower levels at 0.5 and 1 h after infusion. The HbV(3.6)/HSA group showed significantly better flow than the HSA group at all the time points and better than the SAB group at 0 and 1 h after resuscitation. None of the groups showed a complete recovery of flow to baseline levels throughout the experiment.

FCD of all groups diminished during the hemorrhagic shock period to 22 ± 20% of basal values (Fig. 3). The SAB group showed an immediate recovery to 82 ± 17% just after resuscitation, whereas the other three groups showed significantly lower values than the SAB group at all the time points. The infusion of HbV(3.8)/HSA tended to show better FCD (43 ± 28% at 1 h) than the infusion of HbV(7.6)/HSA (34 ± 25%) and the HSA (28 ± 34%).

Intravascular PO$_2$ values in A$_0$ 1 h after the resuscitation for the HbV(3.8)/HSA, HbV(7.6)/HSA, and SAB are 57 ± 3, 54 ± 9, and 54 ± 3 mmHg, respectively, and almost the same level with the baseline values (54 ± 3 mmHg) (Fig. 4). However, intravascular total O$_2$ content of A$_0$ of the HSA group (42 ± 26 mmHg) was lower than that of the SAB group due to the significantly lower Hct (16%), consequently the reduction of downstream PO$_2$ was significant. There was no significant difference between the SAB group and baseline. The PO$_2$ values in both HbV/HSA treatments were consistently lower in vessels downstream from A$_2$. The HbV(3.8)/HSA and HbV(7.6)/HSA groups showed interstitial PO$_2$ of 10 ± 10 and 9 ± 11 mmHg, respectively, which were lower than the SAB group (26 ± 14 mmHg) but tended to show nonsignificantly better PO$_2$ than the HSA group (4 ± 5 mmHg).

**DISCUSSION**

Our principal findings are that infusion of HbVs suspended in HSA restores blood pressure and blood gas parameters, including BE, after hemorrhagic shock independently of the difference in Hb concentra-
tions (7.6 g/dl vs. 3.8 g/dl) in the HbV suspensions, and that microvascular PO2 were improved in the presence of HbV by comparison with HSA alone. Furthermore, the levels of recovery of blood pressure and blood gas parameters attained with the HbV/HSA suspensions are comparable with that of shed blood and are significantly higher than those attained by treatment with HSA alone.

It has been reported that resuscitation from hemorrhagic shock with acellular Hb modifications such as polymerized or intramolecularly cross-linked Hb causes the elevation of MAP beyond the baseline values (3, 6, 16, 18, 34), presumably because of NO scavenging due to the high affinity for NO of acellular Hbs and their smaller size, which enable NO trapping in the proximity of the endothelium (19, 26); however, MAP did not exceed the baseline values after resuscitation with HbV.

Because Ao vessels constricted to the same extent in all the groups, the changes in peripheral resistance and therefore recovery of MAP, could be expected to follow a similar pattern. However, peripheral resistance is also a function of the viscosity of the circulating blood. The viscosity of the HSA solution was significantly lower than that of the HbVs solutions. Because the volume taken out and replaced was 50% of the total blood volume, we may assume that the replaced volume was 50% diluted with the remaining blood before volume restitution, which had an approximately uniform Hct of 25% in all groups. Baseline blood and plasma viscosity of hamsters are 4.47 ± 0.59 and 1.20 ± 0.04 cP, respectively, at Hct 45% and 37°C. Volume restitution with HSA reduced this Hct to 12%, and the viscosity achieved in restituting blood volume with HSA whose viscosity is 1 cP should be slightly lower than the viscosity of blood diluted to 12% with plasma, which is ~1.2 cP (shear rate, 250 s⁻¹). Conversely, restitution of volume with blood or HbVs, of which their minimum viscosity was 1.8 cP, probably restored viscosity of the circulating blood to ~1.5 cP, a 25% higher viscosity than HSA and therefore correspondingly higher peripheral vascular resistance, presumably causing the observed higher MAP. An additional contributing factor may be the slightly lower HR found for HSA resuscitation suggesting a slightly lower cardiac output.

Hb-based O2-carrying resuscitation fluids tend to be formulated with a Hb concentration close to that of normal blood. However, normal blood has a surplus O2-carrying capacity, and hemodilution up to 30% may improve O2 delivery and maintain the O2 consumption. Moreover, molecular O2 carriers and HbV, being much smaller than RBCs, release O2 in closer proximity to the arteriolar wall (17, 31), significantly augmenting the flux O2 to the arteriolar walls inducing vascular autoregulatory responses aimed at maintaining tissue oxygenation constant through vasoconstriction and the reduction of blood flow (7, 41). When these two factors are taken into consideration, Hb concentration can be adjusted to be ~5 g/dl provided that the level of blood exchange does not exceed 80%, in which case the tissue can become hypoxic (30). Lower Hb concentrations such as 3.8 g/dl may still be useful but only applicable to a 50–60% level of blood exchange.

Another parameter that regulates the O2 release is O2 affinity. A right-shifted O2 equilibrium curve for RBCs has been reported to be effective for tissue oxygenation; however, contradictory results are reported for the Hb-based O2 carriers (1, 42). In our previous report (31), reducing P50 from 30 to 16 mmHg resulted in increased FCD. Thus conventional concepts for RBCs may not be applicable to Hb-based O2 carriers, and lower Hb concentration and lower P50 may be advantageous when using Hb-based O2 carriers. However, further study is necessary to confirm this concept with systemic O2 consumption, peripheral resistance, etc.

Microvascular blood flow dropped significantly during hemorrhagic shock to nearly 10% of the baseline values as a consequence of the lowered blood pressure and the significant vasoconstriction of the resistance artery Ao as previously reported (24). Other vessels did not show such significant changes. In terms of Poiseuille’s law, blood flow in a tube is proportional to the fourth power of the radius, the pressure gradient, and inversely proportional to fluid viscosity. If we assume that the Ao vessels are the primary determinants of microvascular blood flow, application of Poiseuille’s
law for a diameter of 80% of baseline value and a reduced blood viscosity due to hemodilution (due to the reduction of Hct from ca. 50% to ca.40% for the SAB group) and the reduced MAP affecting the regional arteriovenous pressure difference (ca. 90%) shows that blood flow rate is reduced to (0.8)$^4 \times 50/40 \times 0.9 \times 100 = 46\%$ of the basal value. This calculation is speculative but corresponds to our finding on the incomplete recovery of blood flow rates of the SAB group and suggests that the reactivity of the A$_0$ small arteries is crucial in determining microvascular flow.

Cardiac output is reported to fall as much as 50% during hemorrhage (13, 22, 33), and although not measured in the present study because of the small size of the hamsters (60 g), it is unlikely that it would be reduced to nearly 10% of baseline, which is the level of skin microvascular blood flow during hemorrhagic shock. The decrease in flow seen in our experiments is probably due to a significant redistribution of vascular resistance concomitant with the “centralization” of blood flow in hemorrhage, controlled by these resistance vessels. Because the sympathetically driven A$_0$ constriction is a normal physiological response required for blood centralization, the early reversal of this phenomenon in resuscitation may not be beneficial; however, the constriction should eventually be reverted to restore normal tissue conditions. It should be noted, however, that the changes in microvascular flow were not consistent.

In the normal tissue, intravascular P$_{O_2}$ decreases from 54 ± 3 mmHg in the A$_0$ vessels to 40 ± 8 mmHg in the A$_3$. This reduction is due to O$_2$ diffusion from arterioles and consumption by the vascular wall (7, 38). In the present experiments normal interstitial P$_{O_2}$ was 22 ± 6 mmHg, and blood P$_{O_2}$ increased after passing through the capillaries being 27 ± 10 mmHg in V$_C$ and 30 ± 7 mmHg in V$_D$ due to the presence of diffusive and convective shunts between arterioles and venules (7). The two HbV/HSA groups and the SAB group tended to show similar or slightly higher P$_{O_2}$ in A$_0$ compared with the baseline value probably because of the higher central P$_{O_2}$ after resuscitation due to hyperventilation. However, intravascular P$_{O_2}$ in A$_1$, A$_2$, and A$_3$ and interstitial P$_{O_2}$ for the HbV/HSA groups were significantly lower than the baseline and higher than the HSA group. Because the recovery of FCD was similar for all groups (but significantly lower than for SAB), the higher tissue P$_{O_2}$ values are due to the increased O$_2$-carrying capacity by the addition of HbV to HSA.

The improved microvascular recovery found for SAB by comparison with HbV/HSAs may be due to the different viscosities of the fluids. The viscosities of HbV(3.8)/HSA and HbV(7.6)/HSA at 150 s$^{-1}$ are 1.8 and 3.0 cP, respectively, being lower than the viscosity of blood (4.5 cP) and that of previously reported HbV(10)/HSA (4 cP) (30). Higher viscosities should lead to higher shear stress, the consequent release of vasorelaxation factors, and higher FCD, even though we did not find a related response of microvascular diameters. Lowered blood viscosity and blood flow does not transmit adequate pressure to the capillaries, causing the decrease of FCD (37, 39). The significant decrease in Hct could also be a contributing factor to decrease FCD because the reduction of Hct increases the plasma layer and the resulting plasma skimming, leading to an underestimation of the number of perfused capillaries (30). Semitransparent elements presumed to be HbV particles were visible in the capillaries of the HbV/HSA groups, and because FCD was estimated on the basis of number of capillaries through which RBCs were flowing, FCD values might have underestimated the total number of functioning capillaries for the HbV/HSA groups.

In summary, this study shows that resuscitation from hemorrhage with HbVs suspended in HSA restore systemic parameters to the same level as shed blood, whereas subcutaneous microvascular function and tissue oxygenation return to a level that is intermediate between that attained with whole blood and HSA. The degree of systemic restoration does not appear to be dependant on Hb concentration within the range of 3.8–7.6 g/dl, indicating that low concentrations of Hb are effective and that there may be a plateau in effectiveness that can be achieved with HbVs in this model of resuscitation from hemorrhagic shock. Our results indicate that HbVs are not vasoactive (26) and that the sustained constriction of resistance arteries during the resuscitation period is a physiological response probably related to the maintenance of blood pressure and blood flow to vital organs. Thus complete microvascular recovery in our model during the observation period of this study may be related to the time needed for the relaxation of the resistance arteries of this tissue and not dependent on the specific formulation of the HbVs within the range of Hb concentrations tested.

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REFERENCES


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