Hemodynamic response and oxygen transport in pigs resuscitated with maleimide-polyethylene glycol-modified hemoglobin (MP4)

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Hemodynamic response and oxygen transport in pigs resuscitated with maleimide-polyethylene glycol-modified hemoglobin (MP4). J Appl Physiol 96: 1843–1853, 2004. First published January 16, 2004; 10.1152/japplphysiol.00530.2003.—Cell-free Hb increases systemic and pulmonary pressure and resistance and reduces cardiac output and heart rate in animals and humans, effects that have limited their clinical development as “blood substitutes.” The primary aim of this study was to evaluate the hemodynamic response to infusion of several formulations of a new polyethylene glycol (PEG)-modified human Hb [maleimide PEG Hb (MalPEG-Hb)] in swine, an animal known to be sensitive to Hb-induced vasoconstriction. Anesthetized animals underwent controlled hemorrhage (50% of blood volume), followed by resuscitation (70% of shed volume) with 10% pentastarch (PS), 4% MalPEG-Hb in lactated Ringer (MP4), 4% MalPEG-Hb in pentastarch (HS4), 2% MalPEG-Hb in pentastarch (HS2), or 4% stroma-free Hb in lactated Ringer solution (SFH). Compared with baseline, restoration of blood volume after resuscitation was similar and not significantly different for the PS (103%), HS2 (99%), HS4 (106%), and MP4 (87%) animals but significantly less for the SFH animals (66%) (P < 0.05). All solutions that contained MalPEG-Hb restored mean arterial and pulmonary pressure and cardiac output. Systemic vascular resistance was unchanged, and pulmonary arterial pressure and resistance were increased slightly. Both systemic and pulmonary vascular resistance increased significantly in animals that received SFH, despite less adequate blood volume restoration. Oxygen consumption was maintained in all animals that received MalPEG-Hb, but not PS. Base excess improved only with MalPEG-Hb and PS, but not SFH. Red blood cell O2 extraction was significantly increased in animals that received Hb, regardless of formulation. These data demonstrate resuscitation with MalPEG-human Hb without increasing systemic vascular resistance and support our previous observations in animals suggesting that the efficacy of low concentrations of PEG-Hb in the plasma results from reduced vasoconstriction.

Nitric oxide (NO) scavenging by cell-free Hb would appear to be an obvious explanation for the vasoactive effects; however, several modified Hb with different hypertensive effects have nearly identical NO-binding properties (24). We have proposed an alternative hypothesis. In an in vitro system, McCarthy et al. (16) found that, by decreasing the diffusivity of cell-free Hb by conjugation with polyethylene glycol (PEG), the diffusive transfer of oxygen in a low-PO2 environment could be reduced or eliminated, compared with HbA0 or α-Hb. We further suggested that this modified Hb could preserve oxygen delivery (DO2) to capillaries in vivo and limit vasoconstriction in the microcirculation, resulting from premature unloading of oxygen at the level of small arterioles. Consistent with this hypothesis, we found that PEG-modified Hb did not lead to elevated systemic blood pressure and resistance in rats, compared with α-Hb or polymerized Hb, and further demonstrated that PEG-Hb could preserve DO2, oxygen consumption (VO2), and acid-base status (39).

In view of these findings, a new surface-modified human Hb [maleimide PEG Hb (MalPEG-Hb)] was designed by using novel attachment of six strands of maleimide-activated PEG (5 kDa) to human Hb (32). Based on the previous demonstration that the pig circulation was very sensitive to the hypertensive effects of cell-free Hb (11), the present experiments were performed to determine whether MalPEG-Hb solutions caused the characteristic hemodynamic effects observed previously with other Hb solutions. In addition, we examined DO2 and VO2 in response to hemorrhage and resuscitation to determine whether the concentrations of plasma Hb tested support oxygen requirements to offset the effects of hemodilution after resuscitation. To accomplish these goals, three different formulations of MalPEG-Hb were compared with 10% pentastarch.
(PS) and 4% stroma-free Hb (SFH) as resuscitation solutions in anesthetized pigs following controlled hemorrhage of 50% of estimated blood volume. The results confirm the lack of hemodynamic effects previously reported and further demonstrate that MalPEG-Hb preserves VO2 after severe hemorrhage and resuscitation in pigs.

**METHODS**

**Test solutions.** Hb solutions were prepared and characterized as described previously (32). Briefly, outdated human packed red blood cells, obtained from the San Diego Blood Center, were washed with saline, lysed with distilled water, and dialyzed to achieve a SFH concentration of 4.3 g/dl in lactated Ringer solution. MalPEG-Hb was produced by the reaction of human Hb with iminohistidine to introduce sulfhydryl groups at surface ε-amino groups (lysine), which were then conjugated to six strands per tetramer of maleimide-activated PEG, 5-kDa molecular mass (NOF, Tokyo, Japan) (1, 32). One formulation of pentastarch, three formulations of MalPEG-Hb, and one formulation of SFH were prepared before infusion: 1) PS, 10 g/dl pentastarch (Pentaspan, a gift from B. Braun Medical, Irvine, CA); 2) MP4, containing 4.2 g/dl MalPEG-Hb in lactated Ringer solution; 3) HS4, containing 4.2 g/dl MalPEG-Hb and 5 g/dl pentastarch; 4) HS2, containing 2 g/dl MalPEG-Hb in 5 g/dl pentastarch; and 5) SFH, containing 4.3 g/dl SFH in lactated Ringer solution. Although the molecular mass of MalPEG-Hb is ~95 kDa, compared with 64 kDa for unmodified Hb, concentrations are expressed on hemolytic basis. That is, concentrations are estimated from optical density and extinction coefficients for Hb, so that, gram for gram, MalPEG-Hb has the same O2 capacity as native Hb. The physical properties of the solutions are summarized in Table 1. Oxygen equilibrium curves were measured by using the method described previously (33), and other physical characterizations were as previously reported (32).

**Animal preparation.** All animal experiments were performed at the Department of Experimental Traumatology, Defense Research Agency (FOI), Stockholm, Sweden, at the Söder Hospital. The local institutional review committee approved the protocols. Animals (n = 38) were female or castrated male Swedish landrace pigs, weighing an average of 20.8 kg (range, 17.0–27.5 kg). The PS, HS2, HS4, and MP4 animals were randomized as to test article on the day of experiment without identification of the animal or knowledge of its weight, condition, or any other parameters. The SFH studies were performed as a separate cohort and were not randomized to as treatment group.

Before anesthesia, pigs were given 10–15 ml of ketamine-HCl (50 mg/ml) by intramuscular injection in the neck, followed by intrave-

**Hemodynamic measurements.** Arterial pressure, Ppa, and CVP signals were recorded continuously and sampled at 100 Hz by the BIOPAC system. Mean pressures were calculated as diastolic pressure peaks. Minute averages for mean arterial pressure (MAP), mean pulmonary arterial pressure (MPAP), CVP, and heart rate were calculated from the continuously recorded data. Cardiac output was measured by using an Edwards 9420A Laboratory Cardiac Output computer (Edwards Laboratories, Santa Ana, CA) and the thermodilution catheter, by injection of 5 ml of saline at ambient temperature into the pulmonary artery catheter. The average of two measurements that agreed to within 10% of each other was used. 

**Experimental groups and hemorrhage protocol.** After anesthesia and surgical instrumentation, animals were assigned to one of five groups: PS (n = 8), HS2 (n = 8), HS4 (n = 8), MP4 (n = 8), and SFH (n = 6). After a 30-min stabilization period, a 50% hemorrhage (50% of blood volume) was begun (blood volume calculated as 65 ml/kg) at constant rate (1.08 ml/kg·min−1) by using the arterial catheter. Because all animals were of comparable weight, the duration of hemorrhage was predetermined to be 30 min in all pigs. Fifteen minutes after completion of the blood withdrawal, resuscitation fluid was initiated at a rate of 1.14 ml/kg·min−1, the entire volume to be infused over the course of 20 min. The replacement volume, arbitrarily 70% of the shed volume, was chosen because the solutions are hyperonotic (see Table 1) and thus would lead to overexpansion of the blood volume if volumes equal to the shed volume were replaced. Consequently, ~450 ml of each product were infused into each animal, corresponding to 0.9, 900, 450, and 900 mg/kg of Hb in the PS, MP4, HS4, HS2, and SFH groups, respectively.

**Systemic vascular resistance index (SVRI) was calculated as**

\[
SVRI = \frac{80(MAP - CVP)}{Q}
\]

where MAP is in mmHg, CVP is in mmHg, and Q is cardiac index (ml·min−1·kg body wt−1) (units for SVRI are dyn·kg−1·s·cm−5). An approximation of pulmonary vascular resistance (PVRI*) was calculated, without the measurement of pulmonary capillary wedge pressure, by

\[
PVRI* = \frac{80(MPAP)}{Q}
\]

where MPAP is in mmHg (units for PVRI* are dyn·kg−1·s·cm−5). Cardiac output, stroke volume, systemic vascular resistance, and pulmonary vascular resistance are expressed as “index” values, normalized to body weight.

**Table 1. Test solutions and their compositions**

<table>
<thead>
<tr>
<th>Solution</th>
<th>Pentastarch, g/dl</th>
<th>Hemoglobin, g/dl</th>
<th>Viscosity, cP</th>
<th>COP, mmHg</th>
<th>Pso, mmHg</th>
<th>nso</th>
<th>Pso, mmHg</th>
<th>nso</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>10.0</td>
<td>4.0</td>
<td>4.0</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MP4</td>
<td>4.2</td>
<td>2.2</td>
<td>2.2</td>
<td>49</td>
<td>5.9</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS2</td>
<td>5.0</td>
<td>2.0</td>
<td>3.1</td>
<td>64</td>
<td>6.2</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS4</td>
<td>5.0</td>
<td>2.0</td>
<td>3.1</td>
<td>64</td>
<td>6.2</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFH</td>
<td>4.3</td>
<td>1.5</td>
<td>1.5</td>
<td>12.3</td>
<td>2.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

COP, colloid oncotic pressure; Pso, O2 half-saturation pressure measured at 37°C; pH 7.4, nso, Hill coefficient at Pso; PS, pentastarch; MP4, 4% maleimide polyethylene glycol-Hb (MalPEG-Hb); HS2, 2% MalPEG-Hb in 5% PS; HS4, 5% MalPEG-Hb in lactated Ringer solution; SFH, stroma-free Hb.
Hematology. Arterial and venous blood samples were drawn into plastic syringes and immediately capped and measured by using a GEM Premier Plus blood-gas analyzer (Instrumentation Laboratories, Lexington, MA) for PO\textsubscript{2}, PCO\textsubscript{2}, pH, and base excess (BE). Hct was measured by using centrifuged arterial blood samples in microhematocrit tubes. Total Hb was measured by using a HemoCue B-Hemoglobin (HemoCue, Angelholm, Sweden), and plasma Hb was measured, after centrifugation of whole blood, by using a HemoCue Plasma/Low Hb system, also from HemoCue. Plasma methemoglobin was not measured routinely. However, it was shown in control experiments that met-MalPEG-Hb does not increase over the period of these experiments (data not shown).

Although red cell Hb is confined to the red cell space, it is measured in whole blood, and the value is used as if Hb were evenly distributed in blood. Plasma Hb, however, is measured only in plasma. Therefore, to compare the two Hb compartments, it is necessary to correct plasma Hb to the same volume of distribution as total Hb. This quantity, blood-free Hb (BFH) is

\[ \text{BFH} = (\text{Plasma Hb}) \times (1 - \text{Hct}) \]

where Hct is expressed as a fraction. Red blood cell Hb concentration is then calculated as the difference between total Hb and BFH. All values are expressed as grams per deciliter of blood.

Red cell Hb saturation was calculated by using the Rovida formulas for pig blood (25) using a 2,3-diphospho-D-glycerate-to-Hb molar ratio of 1.9 measured in one of our animals (unpublished observation). The saturation of the plasma Hb was calculated by using the PO\textsubscript{2} as ratio of 1.9 measured in one of our animals (unpublished observation).

...
The degree of hemodilution in each of the experimental groups was given by the ratio TBV₂/TBV₁ (Table 2). The degree of plasma expansion is related to the oncotic pressure of the test solutions (Table 1). In particular, it should be noted that the animals that received SFH were underresuscitated relative to the other groups. The degree of blood volume expansion for SFH is significantly less than that for the PS group immediately postresuscitation and 60 min later.

**Blood-gas and acid-base status.** Arterial Po₂ fell slightly after resuscitation in the MP4, HS2, and HS4 animals (Table 3), and this was significant in the HS4 group (P < 0.05 from baseline). These values did not reach statistical significance compared with those of the PS group. The mixed-venous Po₂ fell in all groups after hemorrhage and remained below baseline in each of the groups administered Hb solutions. Mixed-venous Po₂ was significantly lower in the SFH animals compared with the PS animals immediately postresuscitation, but there were no other statistically significant differences between groups. Arterial pH values fell significantly from baseline in all groups after resuscitation, and these recovered in all groups except the SFH-treated animals. Arterial pH was significantly less in SFH animals compared with PS-treated animals at 120 min postresuscitation. Finally, arterial BE fell in all groups but failed to recover only in the SFH animals. At 120 min postresuscitation, BE was significantly lower in this group (−1.43 ± 2.09 meq/l) compared with the PS animals (6.76 ± 1.78 meq/l).

**Systemic hemodynamic response.** Baseline values for hemodynamic parameters are reported in Table 4. There were no differences in the baseline values for any of these variables, and there were no differences immediately after hemorrhage in any of the groups. After resuscitation, cardiac index rose above baseline in the PS, HS2, and HS4 animals. Cardiac index increased less in the MP4 and SFH animals, and the cardiac index was significantly lower in MP4 animals (resuscitation to 60 min) and in SFH animals (resuscitation to 120 min).
HEMODYNAMIC RESPONSE TO MALPEG-HB

Table 3. Blood-gas and acid-base status

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Posthemorrhage</th>
<th>Postresuscitation</th>
<th>60 Min</th>
<th>120 Min</th>
</tr>
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<tbody>
<tr>
<td>PAO2, Torr</td>
<td></td>
<td></td>
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<tr>
<td>PS</td>
<td>88.1 ± 2.5</td>
<td>87.0 ± 2.8</td>
<td>84.2 ± 3.9</td>
<td>91.5 ± 4.4</td>
<td>98.7 ± 4.0</td>
</tr>
<tr>
<td>MP4</td>
<td>83.2 ± 2.9</td>
<td>78.7 ± 2.2</td>
<td>72.0 ± 4.6</td>
<td>83.1 ± 4.7</td>
<td>80.5 ± 3.8</td>
</tr>
<tr>
<td>HS2</td>
<td>84.2 ± 4.1</td>
<td>82.6 ± 4.5</td>
<td>78.6 ± 4.1</td>
<td>87.6 ± 6.0</td>
<td>83.7 ± 7.8</td>
</tr>
<tr>
<td>HS4</td>
<td>85.3 ± 2.9</td>
<td>87.2 ± 4.5</td>
<td>72.0 ± 4.6†</td>
<td>83.4 ± 3.9</td>
<td>85.4 ± 4.2</td>
</tr>
<tr>
<td>SFH</td>
<td>91.0 ± 2.5</td>
<td>89.7 ± 3.1</td>
<td>89.5 ± 3.9</td>
<td>98.3 ± 4.9</td>
<td>98.7 ± 4.0</td>
</tr>
<tr>
<td>PVO2, Torr</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>PS</td>
<td>35.7 ± 2.0</td>
<td>25.8 ± 2.2†</td>
<td>37.1 ± 1.9</td>
<td>33.9 ± 1.9</td>
<td>32.4 ± 2.0</td>
</tr>
<tr>
<td>MP4</td>
<td>38.6 ± 0.9</td>
<td>21.8 ± 1.6†</td>
<td>31.3 ± 1.9†</td>
<td>27.8 ± 1.2</td>
<td>27.5 ± 1.3†</td>
</tr>
<tr>
<td>HS2</td>
<td>39.0 ± 1.5</td>
<td>25.0 ± 1.9†</td>
<td>35.7 ± 1.6</td>
<td>30.4 ± 2.2</td>
<td>29.7 ± 2.4†</td>
</tr>
<tr>
<td>HS4</td>
<td>37.4 ± 2.0</td>
<td>26.4 ± 4.0†</td>
<td>32.2 ± 2.3</td>
<td>29.2 ± 1.3</td>
<td>27.4 ± 1.7</td>
</tr>
<tr>
<td>SFH</td>
<td>33.7 ± 2.0</td>
<td>21.7 ± 0.8†</td>
<td>28.7 ± 1.9*</td>
<td>24.6 ± 1.5</td>
<td>25.0 ± 2.0</td>
</tr>
<tr>
<td>pHb units</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS</td>
<td>7.514 ± 0.032</td>
<td>7.482 ± 0.071</td>
<td>7.365 ± 0.089†</td>
<td>7.472 ± 0.073</td>
<td>7.491 ± 0.057</td>
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<tr>
<td>MP4</td>
<td>7.474 ± 0.050</td>
<td>7.448 ± 0.067</td>
<td>7.366 ± 0.069†</td>
<td>7.424 ± 0.063</td>
<td>7.432 ± 0.059</td>
</tr>
<tr>
<td>HS2</td>
<td>7.495 ± 0.032</td>
<td>7.452 ± 0.062</td>
<td>7.366 ± 0.061†</td>
<td>7.452 ± 0.057</td>
<td>7.453 ± 0.050</td>
</tr>
<tr>
<td>HS4</td>
<td>7.490 ± 0.036</td>
<td>7.469 ± 0.033</td>
<td>7.349 ± 0.097†</td>
<td>7.435 ± 0.053</td>
<td>7.464 ± 0.043</td>
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<tr>
<td>SFH</td>
<td>7.502 ± 0.027</td>
<td>7.445 ± 0.024</td>
<td>7.353 ± 0.071†</td>
<td>7.432 ± 0.073</td>
<td>7.402 ± 0.098†</td>
</tr>
<tr>
<td>BE, meq/l</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>PS</td>
<td>6.86 ± 0.072</td>
<td>1.80 ± 1.90†</td>
<td>0.67 ± 1.85*</td>
<td>4.43 ± 1.98</td>
<td>6.76 ± 1.78</td>
</tr>
<tr>
<td>MP4</td>
<td>6.06 ± 0.076</td>
<td>1.92 ± 0.98†</td>
<td>1.51 ± 0.96†</td>
<td>4.06 ± 1.22</td>
<td>4.51 ± 1.12</td>
</tr>
<tr>
<td>HS2</td>
<td>6.47 ± 0.83</td>
<td>0.56 ± 1.38†</td>
<td>1.53 ± 1.32†</td>
<td>4.80 ± 1.58</td>
<td>4.45 ± 1.51</td>
</tr>
<tr>
<td>HS4</td>
<td>6.09 ± 0.73</td>
<td>0.14 ± 1.59†</td>
<td>0.64 ± 1.64†</td>
<td>4.21 ± 0.98</td>
<td>5.32 ± 0.66</td>
</tr>
<tr>
<td>SFH</td>
<td>5.92 ± 0.72</td>
<td>-0.25 ± 1.33†</td>
<td>-1.43 ± 1.51†</td>
<td>0.38 ± 1.61†</td>
<td>-1.43 ± 2.09†</td>
</tr>
</tbody>
</table>

Values are means ± SE. PAO2, arterial PO2; PVO2, venous PO2; pHb, arterial pH; BE, base excess. *P < 0.05 compared with PS at the same time point. †P < 0.05 compared with corresponding baseline value.

compared with PS (Fig. 2). Cardiac index was maintained slightly above baseline in MP4 animals and actually fell below baseline in SFH-treated animals, reflecting the difference in COP.

MAP rose to values greater than baseline in all groups that received Hb-containing solutions, regardless of their formulation, but not in PS animals (Fig. 3A). MAP was significantly greater in all groups, compared with PS, from immediately after resuscitation to 75 min postresuscitation, but had returned to baseline in all groups at 120 min. Similar results were found for CVP. CVP was significantly lower after infusion of SFH, compared with PS, from resuscitation to 30 min postresuscitation, consistent with its lower COP.

SVRI, calculated according to Eq. 1, is best examined as differences between measured value and baseline (Fig. 3B) because of individual differences in baseline values among animals. SVRI was unchanged immediately after hemorrhage in all groups, but fell significantly below the baseline value after administration of PS and for 90 min thereafter. SVRI fell immediately after resuscitation with HS2, but was unchanged at all other time points after administration of MALPEG-HB solutions. With SFH, SVRI rose significantly above baseline at 30–60 min postresuscitation and was significantly greater than PS at all time points (resuscitation to 120 min).

Pulmonary hemodynamic response. MPAP fell during hemorrhage and increased above baseline on resuscitation in all groups (Fig. 4A). The pattern of response of the MPAP was identical to the response in systemic pressure, except that the sensitivity in the pulmonary circulation appears to have been greater in all groups. Compared with infusion of PS, MPAP values were significantly higher in all groups immediately after resuscitation through 45 min postresuscitation. MPAP recovered toward baseline in each of the groups but remained significantly elevated from baseline values in the MP4 and

Table 4. Baseline hemodynamic values

<table>
<thead>
<tr>
<th></th>
<th>HS2</th>
<th>HS4</th>
<th>MP4</th>
<th>PS</th>
<th>SFH</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>CI, ml-min⁻¹kg⁻¹</td>
<td>137 ± 14.5</td>
<td>113 ± 8.7</td>
<td>113 ± 9.2</td>
<td>112 ± 6.6</td>
<td>123 ± 7.3</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>104 ± 6</td>
<td>96.2 ± 6</td>
<td>105 ± 4</td>
<td>100 ± 6</td>
<td>103 ± 4</td>
</tr>
<tr>
<td>VO₂, ml-min⁻¹kg⁻¹</td>
<td>4.3 ± 0.24</td>
<td>4.3 ± 0.18</td>
<td>3.9 ± 0.36</td>
<td>4.8 ± 0.39</td>
<td>4.6 ± 0.46</td>
</tr>
<tr>
<td>DO₂, ml-min⁻¹kg⁻¹</td>
<td>17.2 ± 1.88</td>
<td>15.4 ± 1.77</td>
<td>14.5 ± 1.17</td>
<td>13.3 ± 0.60</td>
<td>12.5 ± 0.31</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>83.7 ± 4.1</td>
<td>74.8 ± 3.8</td>
<td>76.8 ± 4.2</td>
<td>75.5 ± 5.1</td>
<td>78.1 ± 7.0</td>
</tr>
<tr>
<td>CVP, mmHg</td>
<td>2.9 ± 0.6</td>
<td>1.5 ± 0.7</td>
<td>2.8 ± 0.9</td>
<td>5.5 ± 2.3</td>
<td>2.9 ± 0.4</td>
</tr>
<tr>
<td>MPAP, mmHg</td>
<td>17.5 ± 1.7</td>
<td>14.0 ± 1.8</td>
<td>14.4 ± 1.6</td>
<td>17.3 ± 1.5</td>
<td>14.0 ± 0.5</td>
</tr>
<tr>
<td>SVRI, dyn·s⁻¹·cm⁻³·m⁻³</td>
<td>50.5 ± 5.3</td>
<td>54.4 ± 5.9</td>
<td>56.0 ± 5.9</td>
<td>51.5 ± 3.1</td>
<td>48.7 ± 3.8</td>
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<td>PVRI*, dyn·s⁻¹·cm⁻³·m⁻³</td>
<td>11.2 ± 1.6</td>
<td>10.0 ± 1.3</td>
<td>11.0 ± 1.5</td>
<td>12.3 ± 1.3</td>
<td>9.3 ± 0.8</td>
</tr>
<tr>
<td>SVI, ml/kg</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. CI, cardiac index; HR, heart rate; VO₂, O₂ consumption; DO₂, O₂ delivery; MAP, mean arterial pressure; CVP, central venous pressure; MPAP, mean pulmonary arterial pressure; SVRI, systemic vascular resistance index; PVRI*, approximation of pulmonary vascular resistance index; SVI, stroke volume index.

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SFH groups at the completion of the protocol (120 min post-resuscitation).

We did not measure left arterial pressure or pulmonary capillary wedge pressure in these experiments, so calculation of PVRI* according to Eq. 2 is an approximation and, therefore, an overestimation. As with SVRI, because of variability between animals, the PVRI* is best appreciated by examining the differences at each time point relative to baseline (Fig. 4B). This analysis shows that SFH produces the greatest degree of pulmonary vascular resistance but that some increase is also seen for MP4 and, to a still lesser degree, HS4. PVRI* was elevated significantly above baseline in SFH (resuscitation through 120 min) and MP4 (resuscitation through 60 min). The formulations HS2 and HS4 did not result in elevated resistance from baseline. Thus, with the caution that absolute resistance cannot be calculated from our data, the analysis of PVRI* suggests that the effect on the pulmonary circulation is in the order SFH > MP4 > HS4 > HS2, and that PS causes a slight, but not significant, reduction in pulmonary resistance from baseline.

Heart rate and stroke volume index. The heart rate response to hemorrhage was quite variable (Fig. 5A), and there were no statistically significant differences among the groups at any point in the experiment. However, as significant differences did occur in the cardiac output (Fig. 2), the computed stroke volume index (SVI = cardiac index/heart rate) is different for
the various groups (Fig. 5B). The SVI fell equally in all groups during hemorrhage. After resuscitation, SVI increased significantly above baseline in PS, HS2, and HS4 groups but did not change in the MP4 and SFH groups. SVI actually tended to fall below baseline after resuscitation with SFH, although the decline did not reach statistical significance from baseline.

Compared with the PS group, SVI was significantly lower in SFH (resuscitation through 120 min) and in the MP4 group (resuscitation through 45 min).

Blood-gas and oxygen transport. Arterial O$_2$ content fell with hemorrhage and resuscitation due to loss of red cell mass and subsequent hemodilution after infusion of the resuscitation fluids. However, perfusion and oxygenation of tissue appeared to be adequate in all of the groups of animals, except those that received SFH, as indicated by BE (Fig. 6A) and lactic acid (Fig. 6B) measurements. BE fell significantly from baseline in all groups during hemorrhage, resuscitation, and 15 min postresuscitation. This began to normalize in all groups at 30 min after resuscitation, but continued to deteriorate in SFH animals, and BE was significantly different from baseline and compared with the PS group ($P < 0.05$) between 75 and 120 postresuscitation. Although lactic acid did not normalize completely in any of the groups, the trend was in the direction of normalization, except in the SFH animals, in which the trend reversed and lactic acid appeared to be rising by the end of the observation period.

D$_2$O was not significantly different among the various groups, as shown by a comparison of relative changes (Fig. 7A). V$_O_2$ was maintained in all groups except the PS animals, in which V$_O_2$ fell significantly from baseline values and did not recover (Fig. 7B). Statistical significance was achieved for differences between Hb-resuscitated and PS-treated animals from 45 min to the end of the experiment at 120 min postresuscitation.

The amount of O$_2$ delivered by plasma Hb is small in all cases because of the low-plasma Hb concentration (Table 2). Thus the maintenance of V$_O_2$ in these severely anemic animals after resuscitation and hemodilution is a result, in part, of elevated OER both from red blood cells and from plasma Hb (Fig. 8). Oxygen extraction from red blood cells (Fig. 8A) increased significantly during hemorrhage in all groups, normalized after resuscitation, and increased subsequently in all groups except PS ($P < 0.05$ at 45–120 min). Interestingly, the
OER from red blood cells was significantly lower in the PS animals compared with animals that received Hb, regardless of the formulation, suggesting that plasma Hb facilitates \( \dot{D}O_2 \) from red blood cells. Oxygen extraction from plasma Hb was greatest in SFH animals and intermediate in animals resuscitated with MalPEG-Hb solutions (Fig. 8B), reflecting the lower oxygen affinity of SFH compared with MalPEG-Hb.

**DISCUSSION**

Our working hypothesis in the development of Hb-based O\(_2\) carriers (“blood substitutes”) has been that vasoconstriction is the major obstacle preventing successful clinical application. This hypothesis has been presented in detail and is based on the published literature generated by \( \alpha\alpha\)-cross-linked Hb (38). The present study was carried out to determine whether a new Hb derivative, based on our current understanding of the mechanism of vasoconstriction (16), would demonstrate vasoconstriction in swine, considered to be a sensitive animal model. Because \( \alpha\alpha\)-cross-linked Hb is no longer available, due to the shutdown of the US Army’s pilot plant, we used SFH as a reference material.

The primary result of these studies demonstrates that formulations of MalPEG-Hb did not promote an increase in systemic vascular resistance, a direct measure of vasoconstriction, in contrast to numerous reports with other formulations of cell-free Hb (4, 6, 19, 35). SFH, used as a control solution, produced a greater rise in systemic and pulmonary vascular resistance and was, therefore, less effective in satisfying tissue O\(_2\) demands as demonstrated by significantly less correction in BE and lactic acid after resuscitation. These studies with PEG-modified human Hb extend, in a large animal model, our previous observations with PEG-modified bovine Hb in rats (39) and underscore the importance of eliminating vasoconstriction in achieving adequate resuscitation from hemorrhagic shock.

The hemodynamic consequences of administration of unmodified cell-free Hb have been known for many years, and the studies demonstrating hypertension, vasoconstriction, reduced cardiac output, and bradycardia in animal models and humans have been reviewed recently (38). Hess and coworkers (10) reported sustained (>3 h) increases in MAP and MPAP in pigs after hemorrhage and resuscitation with unmodified Hb.
and αα-Hb. They also showed that cardiac output failed to rise after resuscitation, and systemic vascular and pulmonary resistance paralleled the increased pressure. Moreover, despite the increased arterial oxygen content contributed from the plasma Hb, mixed-venous oxygen content was also elevated, and oxygen utilization was not improved in the animals receiving Hb compared with those receiving human serum albumin or lactated Ringer solution. The authors concluded that the potential benefit from improved oxygen-carrying capacity was offset by the systemic vasoconstriction. This conclusion was supported by a recent study in which exchange transfusion with Hb solutions was followed by a 60% hemorrhage in conscious rats (39). In that study (39), exchange transfusion with αα-Hb initially increased MAP and systemic vascular resistance and decreased heart rate. The vasoconstriction in rats transfused with αα-Hb was exacerbated during subsequent hemorrhage to such an extent that Do2 and Vo2 were limited, acid-base disturbances were evident, and survival was compromised. That report concluded that the pressor effect of αα-Hb did not correlate positively with survival but, instead, caused the greater mortality seen in those animals. In the same study, animals transfused with PEG-modified bovine Hb exhibited no vasoconstriction, no acid-base disturbance, no decline in cardiac output, and demonstrated the greatest survival. Thus, in addition to the obvious requirement for oxygen-carrying capacity, the need for the lack of vasopressor activity in Hb-based oxygen carriers is clear from the foregoing studies.

Whereas a number of modifications to the Hb molecule have attempted to reduce the hypertensive actions of cell-free Hb, the studies published to date have not demonstrated the elimination of these effects (4, 6, 19, 35). Data from clinical studies of polymerized human Hb report efficacy without hypertension in cases of acute trauma (8, 17), but published data from preclinical studies are lacking. In contrast, the cumulative evidence with PEG-modified Hb (2, 3, 36, 39) demonstrates the lack of hemodynamic actions in a number of animal models. The primary observation in the present study, i.e., lack of hypertension or rise in systemic vascular resistance, represents the first publication, to our knowledge, of this finding with PEG-modified Hb in a large-animal model of hemorrhage and resuscitation. This is an important fundamental observation regarding Hb-based oxygen carriers, especially in view of the current lack of consensus on several issues. First, the mechanism of vasoconstriction and hypertension observed with Hb-based solutions is unclear, and potential candidates include NO-scavenging (5, 20) endothelin release (14), potentiation of catecholamines (9), and alteration of autoregulation (16). Second, the number of different Hb formulations and animal models has hampered comparison between laboratories and published observations. Third, despite the fact that vasoconstriction with most Hb-based solutions in preclinical models is well recognized, this agreement does not extend to clinical development, and programs are still in place with a number of different solutions shown to induce hypertension and vasoconstriction in animals (7, 31). Finally, depending on the specific clinical indication being considered, differing arguments exist as to whether the hypertension is, in fact, an undesirable property of Hb-based oxygen carriers (22). Whereas the current results cannot address most of the above issues, the demonstrated lack of vasoactivity in an animal model known to be sensitive to the vascular actions of Hb provides the first evidence that MalPEG-Hb eliminates the dramatic rise in systemic vascular resistance observed previously with resuscitation using Hb solutions after hemorrhage in pigs (4, 11, 15) or sheep (34).

A second observation from the present study relates to the metabolic consequences of resuscitation with the different solutions. Each of the MalPEG-Hb solutions maintained Vo2 for the duration of the resuscitation period and induced near-complete recovery of both the BE and the lactate concentrations. In contrast, BE and lactate concentrations began to deteriorate in SFH-treated animals 90 min after resuscitation, despite the fact that calculated Vo2 was similar to that in MalPEG-Hb-treated animals. This difference suggests that Vo2 during resuscitation is not uniform with different Hb solutions and that maintenance of Vo2 in SFH-treated animals does not necessarily signal an improvement in regional metabolic status as reflected by the surrogate markers of tissue oxygenation, i.e., BE and lactate. The deterioration in BE and lactate in SFH-treated animals indicates that significant portions of tissues remained ischemic in those animals, most likely as a consequence of regional vasoconstriction and increased peripheral resistance. The poor metabolic recovery in SFH-treated animals, despite equivalent Vo2 (Fig. 7) and enhanced oxygen extraction (Fig. 8), documents, in our model, the inefficiency of oxygen transport by unmodified Hb (10) and αα-cross-linked Hb (11, 39) demonstrated previously. Animals resuscitated with PS exhibited a different profile, i.e., BE and lactate recovered similar to that in MalPEG-Hb animals, despite the fact that Vo2 was significantly lower during the 120-min recovery period. The recovery of BE and lactate suggests the absence of any regional hypoperfusion, an observation supported by the increased cardiac output and decreased peripheral vascular resistance with PS. The observation of reduced Vo2 after resuscitation with PS is interesting and might reflect a less efficient repayment of the O2 debt incurred during the brief shock period, compared with that observed in animals resuscitated with Hb solutions. Other studies have demonstrated that resuscitation with crystalloid or non-Hb colloidal solutions provides adequate resuscitation from shock, reflected by improvement in hemodynamic and acid-base status (4, 10, 15), and the present results support this view. Whereas our results do not allow clear distinction of the metabolic effects of MalPEG-Hb from those of PS, an improvement in Vo2 with MalPEG-Hb has been demonstrated previously in the microcirculation of the hamster skinfold preparation (30). That study compared hemodilution with dextran, MalPEG-Hb, and polymerized bovine Hb (Oxyglobin) and demonstrated improvement in perfused capillary density, local tissue Do2 and Vo2, and systemic acid-base status with MalPEG-Hb. The observed improvement with MalPEG-Hb in the hamster model was demonstrated to result from enhanced utilization of oxygen transported by red blood cells (30) to the capillary bed. The present study results suggest that similar effects might occur in the swine hemorrhage model, although the relationship between Do2, VO2, and markers of metabolic status with MalPEG-Hb solutions warrants further experimentation.

The animal model and hemorrhage protocol in the present study were chosen due to prior experience of reproducible hemodynamic and metabolic changes in our laboratory (41) and to facilitate comparison with previously tested Hb-based
solutions (4, 15). Three animals (~10%) died immediately before or during the resuscitation, confirming the severity of the hemorrhage protocol. In the remaining animals, hemorrhage elicited a reproducible fall in MAP between 35 and 57% and in MPAP between 33 and 43% in the five groups of animals. In addition, the fall in BE (−4.4 to −6.0 meq/l) and rise in lactate concentration (+2.4 to +4.5 meq/l) were similar in each of the five groups of animals, indicating that the hemodynamic and metabolic responses to hemorrhage were similar across the groups. In response to resuscitation, MAP and cardiac output returned to values higher than baseline, yet systemic vascular resistance rose above baseline only in the SFH animals. Pulmonary vascular resistance increased in each of the groups receiving Hb solutions, whereas pulmonary resistance fell in PS-treated animals. These data are difficult to interpret adequately in the absence of left arterial or pulmonary capillary wedge pressure because estimation of resistance requires both pressure and volume data. In the case of the systemic circulation, CVP is available, but, in the case of the pulmonary circulation, we can only speculate about the volume status. If, as seems reasonable, left atrial pressure increased with increased cardiac return (elevated CVP), then the pulmonary resistances that we calculate would be an overestimation. Resolution of this issue must await subsequent measurements of left atrial and/or pulmonary capillary wedge pressures. This shortcoming notwithstanding, the results of this study agree with the observations using unmodified Hb and αHb reported by Hess et al. (10). In that study, systemic vascular resistance doubled immediately after resuscitation and did not decline with either solution for 4 h. Pulmonary vascular resistance increased two- to threefold over baseline and remained elevated for the same duration. Heart rate declined immediately on resuscitation and remained depressed for the duration of monitoring. In contrast, our present observations with MalPEG-Hb solutions more closely resemble the results with human serum albumin and Ringer lactate in the study by Hess et al. (10), where systemic and pulmonary vascular resistance were unchanged after resuscitation.

The overall comparison of these Hb solutions is confounded somewhat by their differing COP. Thus we estimate that, after restoration of 70% of the shed blood volume, the animals that received SFH were incompletely resuscitated, with blood volumes estimated to be ~65% of baseline, compared with ~100% for the MalPEG-Hb and PS solutions. This may explain, in part, the less satisfactory response in the SFH animals in regard to acid-base restoration. However, as the primary purpose of the study was to evaluate systemic and pulmonary hemodynamics, we elected to match the dose of Hb, on heme basis, rather than their volume effects. Hence the solutions were matched for volume administered and Hb concentration. The optimal onotic pressure of a Hb-based O2 carrier has been somewhat controversial (37). One fear has been that onotic pressure could oppose hydrostatic pressure, which is necessary for glomerular filtration (26). However, recent studies indicate that reduced endothelial swelling and improved microvascular perfusion more than offset any detrim ent due to increased onotic pressure in patients undergoing major abdominal surgery (21). Our results support the concept that hypertonic and/or hyperoncotic solutions are beneficial in resuscitation from hemorrhagic shock (6, 23, 29, 36).

The present results indicate that hemodynamic and metabolic resuscitation after hemorrhage can be effectively accomplished with MalPEG-Hb solutions. We did not observe a difference in the hemodynamic actions with the four resuscitation solutions (excluding SFH). In fact, it appeared that the high onotic pressures of PS, MP4, HS2, and HS4 enabled normalization of hemodynamic parameters before complete transfusion with 70% of shed volume, and adequate resuscitation might have been realized with smaller volumes than those chosen for the present study. This is an important finding in view of the need for resuscitation fluid volumes to be minimized for military use (18, 21). The fact that we observed similar improvement in VO2 in the three groups of animals receiving MalPEG-Hb suggests that their ability to oxygenate tissue is not linear with total Hb concentration. The development of an ideal resuscitation solution will ultimately take into consideration the O2 transport characteristics and concentration of modified Hb and the onotic pressure and viscosity of the solution. However, there appears to be good agreement that vasoconstriction and pressor activity are undesirable, and probably unsafe, qualities for Hb-based resuscitation fluids (7, 17, 22, 27, 28, 38), especially when the mechanism responsible remains poorly understood. The present study lends further evidence to the concept that MalPEG-Hb solutions can be administered without the adverse effects of vasoconstriction and elevated vascular resistance observed by basic and clinical researchers for more than 50 years.

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DISCLOSURES

R. M. Winslow, K. D. Vandegrift, M. A. Young, J. Lohman, and A. Malavalli are employees of Sangart, Inc. and hold stock options in the company. R. M. Winslow is President, CEO, and Board Chairman of Sangart, Inc.

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