Systemic and renal hemodynamics after moderate hemodilution with HbOCs in anesthetized rabbits

ALEXIS CARON,1 PATRICK MENU,1 BÉATRICE FAIVRE-FIORINA,1 PIERRE LABRUDA,1 ABDU ALAYASH,2 AND CLAUDE VIGNERON1

1Department of Hematology and Physiology, School of Pharmacy, University Henri Poincaré-Nancy 1, 54001 Nancy Cedex, France; and Laboratory of Plasma Derivatives, Center for Biologics Evaluation and Research, US Food and Drug Administration, Bethesda, Maryland 20892

Caron, Alexis, Patrick Menu, Béatrice Faivre-Fiorina, Pierre Labruda, Abdu Alayash, and Claude Vigneron. Systemic and renal hemodynamics after moderate hemodilution with HbOCs in anesthetized rabbits. Am J Physiol Heart Circ Physiol 278: H1974–H1983, 2000.—Hb-based O2-carrying solutions (HbOCs) have been developed as red blood cell substitutes for use in patients undergoing hemodilution. Various modified Hb with diverse solution properties have been shown to produce variable hemodynamic responses. We examined, through pulsed-Doppler velocimetry, the systemic and renal hemodynamic effects of dextran-benzene-tetracarboxylate-conjugated (Hb-Dex-BTC), bis(3,5-dibromosalicyl)fumarate cross-linked (αα-Hb), and o-raffinose-polymerized (o-raffinose-Hb) Hb perfused in rabbits after moderate hemodilution (30% hematocrit), and we compared the effects of these Hb solutions with the effects elicited by plasma volume expanders. In addition, vascular hindrance (resistance/blood viscosity at 128.5 s⁻¹) was calculated to determine whether a moderate decrease in the viscosity of blood mixed with HbOCs may impair vasconstriction as a result of autoregulation after infusion of cell-free Hb. No changes were observed in renal hemodynamics after hemodilution with reference or Hb solutions. Increase in blood pressure and vascular resistance was found with Hb-Dex-BTC and αα-Hb (for 180 min) and, to a lesser extent, with o-raffinose-Hb (for 120 min). Furthermore, Hb-Dex-BTC (high viscosity) and o-raffinose-Hb (medium viscosity) induced comparable increases in vascular hindrance (from 0.091 to 0.159 and from 0.092 to 0.162 cm²·s⁻¹, respectively) but far less than that produced by αα-Hb (low viscosity, from 0.092 to 0.200 cm²·s⁻¹). These results suggest that maintaining the viscosity of blood by infusing solutions with high viscosity makes it possible to limit vasconstriction due to autoregulation mechanisms and mainly caused by hemodilution per se.

blood substitutes; vascular resistance; blood flow; vascular hindrance; viscosity; exchange transfusion

HEMODILUTION HAS BEEN PROPOSED to reduce the use of allogeneic packed red blood cells in transfusion medicine (25). In clinical practice, colloids and/or crystalloids are used as substitute fluids to maintain normal blood volume to obtain autologous blood for subsequent transfusion (9). However, in settings where blood Hb concentration falls below a critical level, the use of blood is necessary to ensure O₂ transport to tissues (39). Hb-based O₂-carrying solutions (HbOCs) have been developed as blood substitutes to reduce the viral and immunologic risks associated with blood transfusion (15). Recent studies have shown that the use of some HbOCs could reduce significantly the need for allogeneic blood transfusion in orthopedic patients and cardiovascular surgery or cardiopulmonary bypass (15, 16).

Despite this obvious benefit, most HbOCs have been reported to increase systemic and pulmonary vascular resistance in preclinical and clinical settings, thus limiting the range of the therapeutic applications for these solutions (3, 6, 11). Some authors have proposed that the vasoconstrictive properties of HbOCs could be beneficial in the treatment of septic shock to restore hemodynamics (5, 21). This practice resulted in the correction of blood pressure but appeared to be ineffective in preventing the pulmonary vasoconstriction and O₂ debt associated with shock (14). The use of HbOCs as substitute fluids has also been proposed in the treatment of hypovolemic hemorrhage, but deleterious changes have been reported in animals and humans with DCLHb [commercial analog of bis(3,5-dibromosalicyl)fumarate-cross-linked Hb (αα-Hb)] because of vasoconstriction, which blunted the O₂ transport enhancement by Hb (18, 28). In a recent study, Winslow et al. (41) indicated that the perfusion of αα-Hb in hemodiluted hemorrhaged rats led to deleterious effects in vascular resistance compared with animals treated with polyethylene glycol-modified Hb, suggesting that the formulation and the physicochemical characteristics of the infused solutions were important properties. This phenomenon has been explained by Tsai et al. (37), who indicated that increasing blood O₂-carrying capacity and lowering blood viscosity had deleterious effects because of microvascular autoregulation processes that lead to vasoconstriction and impaired O₂ supply to tissues.

As demonstrated by these few examples, an objective description of the effects of HbOCs on cardiovascular function is necessary but difficult to accomplish because of the complex interaction between the reduction in blood viscosity due to hemodilution and the systemic vasoreactivity of the different HbOCs under preclinical or clinical investigation. The purpose of this study is to describe the systemic and renal hemodynamic effects of...
three chemically modified human Hb solutions in a single and reproducible experimental model of hemodilution. The effects of these HbOcs were compared with those elicited by clinically used volume expanders, human albumin and low-molecular-weight hydroxyethyl starch. In addition, we investigated, by vascular hindrance measurements (resistance/blood viscosity), whether adapting viscosity of blood mixed with HbOcs could limit vasoconstriction elicited by autoregulation mechanisms after the increase of dissolved O2 into plasma.

MATERIALS AND METHODS

Test Solutions

Human albumin. Human albumin (20 g/dl) was obtained from Pasteur-Mérieux sérums & vaccins (Marcy l’Etoile, France) and dissolved in Tyrode medium (in mM: 6.7 glucose, 141.0 Na+, 5.0 K+, 2.5 Ca2+, 1.1 Mg2+, 115.8 Cl−, 0.8 phosphates, 30.0 carbonates) to obtain a final isoncotic solution at a concentration of 5 g/dl.

HES 200. HES 200 solution was obtained from Biosedra (Louviers, France) and contains 6 g/dl of low-molecular-weight hydroxyethyl starch in saline (commercial product Elohes, 6%).

Dextran-benzene-tetracarboxylate-conjugated Hb. Dextran-benzene-tetracarboxylate-conjugated Hb (Hb-Dex-BTC) solution contains 8.5 g/dl of human Hb prepared from outdated red blood cells and conjugated to a macromolecular allosteric effector, dextran-benzene-tetracarboxylate, as previously described (31). Hb-Dex-BTC was produced in collaboration with Pasteur-Mérieux sérums & vaccins. The solution was suspended in Tyrode medium, pasteurized, and frozen at −20°C without preservatives.

αα-Hb. αα-Hb solution (US Army; gift from Dr. A. Alayash, Center for Biologics Evaluation and Research, Bethesda, MD) contains 9.2 g/dl of heat-treated human Hb obtained from outdated red blood cells that were stabilized by cross-linking between the two α-subunits with bis(3,5-dibromosalicyl)fumarate, suspended in Ringer lactate (in mM: 123.9–137.0 Na+, 3.6–4.4 K+, 1.2–1.5 Ca2+, 103.9–115.2 Cl−, 25.7–29.0 lactate), and frozen at −80°C (40).

α-Raffinose-polymerized Hb. α-Raffinose-polymerized Hb solution contains 15 g/dl of pasteurized solution of human Hb prepared from outdated red blood cells, cross-linked internally with raffinose and polymerized to form α-raffinose poly-Hb (2). This Hb solution is suspended in Ringer lactate and was frozen at −80°C without preservatives. α-Raffinose-Hb was generously provided by Hemosol (Toronto, ON, Canada).

Table 1. Physicochemical data of the plasma substitutes used as reference solutions

<table>
<thead>
<tr>
<th></th>
<th>Human Albumin</th>
<th>HES 200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration, g/dl</td>
<td>5.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Mw wt</td>
<td>68,000</td>
<td>200,000*</td>
</tr>
<tr>
<td>Viscosity, cP</td>
<td>0.94</td>
<td>1.97</td>
</tr>
<tr>
<td>Oncotic pressure, Torr</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Osmolarity, mosmol/l</td>
<td>300</td>
<td>310</td>
</tr>
<tr>
<td>Endotoxin level, EU/ml</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>pH</td>
<td>7.5</td>
<td>4.0–7.0</td>
</tr>
</tbody>
</table>

*Weighted average molecular weight. †Number averaged molecular weight. Kinematic viscosity determined at 37°C with an automatic viscosimeter (Viscomatic VCD, Amtec).

Table 2. Physico-chemical data of Hb-based O2 carriers

<table>
<thead>
<tr>
<th></th>
<th>Hb-Dex-BTC</th>
<th>αα-Hb</th>
<th>α-Raffinose-Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb concn, g/dl</td>
<td>8.5</td>
<td>8.2</td>
<td>10.0</td>
</tr>
<tr>
<td>Free Hb, %</td>
<td>4.9</td>
<td>&lt;5</td>
<td>4.2</td>
</tr>
<tr>
<td>HbO2, %</td>
<td>93</td>
<td>90</td>
<td>78</td>
</tr>
<tr>
<td>MetHb, %</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Mw wt</td>
<td>32,000</td>
<td>&lt;5%</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>64,000</td>
<td>&lt;5%</td>
<td>&gt;95%</td>
<td>32 ± 6%</td>
</tr>
<tr>
<td>&gt;64,000</td>
<td>&gt;90%</td>
<td>64 ± 7%</td>
<td></td>
</tr>
<tr>
<td>&gt;500,000</td>
<td>&lt;5%</td>
<td>&lt;1%</td>
<td></td>
</tr>
<tr>
<td>P50, Torr</td>
<td>23</td>
<td>29.5</td>
<td>34</td>
</tr>
<tr>
<td>Viscosity, cP</td>
<td>2.15</td>
<td>0.99</td>
<td>1.26</td>
</tr>
<tr>
<td>Oncotic pressure, Torr</td>
<td>40</td>
<td>34.5</td>
<td>26</td>
</tr>
<tr>
<td>Osmolarity, mosmol/l</td>
<td>280</td>
<td>280</td>
<td>280–300</td>
</tr>
<tr>
<td>Endotoxin level, EU/ml</td>
<td>&lt;0.5</td>
<td>0.25</td>
<td>&lt;0.06</td>
</tr>
<tr>
<td>pH</td>
<td>7.24</td>
<td>7.52</td>
<td>7.30</td>
</tr>
</tbody>
</table>
Hemodynamic Monitoring and Calculation

The catheter was connected to a pressure transducer (Viggo-Spectramed) to measure the pulsatile arterial pressure. The aortic blood flow transducer was connected to a 20-MHz module (model PD-20, Crystal Biotech), and the renal arterial blood flow transducer was connected to a high-velocity 20-MHz module (model HVDP-20, Crystal Biotech) to measure the blood flow velocity in centimeters per second. Both velocity modules were used with a pulse repeated frequency of 125 kHz. Although we did not measure absolute blood flow in aorta and renal artery, blood flow velocity is proportional to volume flow, because we used hard-epoxy Doppler probes for which the vessel diameter is constant throughout the experiments. The crystal was connected to a 20-MHz echo-tracking module (model WT-20, Crystal Biotech) to measure the aortic diameter in millimeters (7). The blood flow and echo-tracking modules were connected to a dedicated amplifier (model CBI-8000, Crystal Biotech). The pressure transducer and the amplifier were connected to a personal computer for continuous data acquisition at 100 samples/s (Acqknowledge software and MP100 hardware, Biopac Systems).

All hemodynamic parameters were calculated with software developed in collaboration with the Centre Interuniversitaire des Ressources Informatiques de Lorraine (Vandoeuvre-lès-Nancy, France). Mean values of arterial pressure (MAP) and aortic and renal blood flow were calculated directly from the digital signals. Heart rate (HR) was calculated from the aortic blood flow signal as the reciprocal between two consecutive systolic peaks. Vascular resistance (VR) was calculated as MAP/aortic blood flow velocity, as previously described (6). Aortic distensibility coefficient (DC) was calculated according to the following formula: $DC = \frac{2\Delta d}{d} - \frac{\Delta P}{D_P}$, where $\Delta d$ is the difference between systolic and diastolic aortic diameter, d is the mean aortic diameter, and $\Delta P$ is the pulse pressure (22).

Blood Gas and Hematologic Values

Arterial pH, $P_{O_2}$, and $P_{C_2}$ were measured in a blood-gas analyzer (model ABL 330, Radiometer, Copenhagen, Denmark) with use of 100-µl samples of heparinized blood. Arterial blood and plasma total Hb ($Hb_{tot}$) concentration, blood methemoglobin ($MeTHb$), and blood O$_2$ content ($C_{aO_2}$) were measured with a CO-oximeter (model 682, Instrumentation Laboratory) with use of 65-µl samples. Hematocrit (Hct) was measured in duplicate with use of 75-µl samples of arterial blood by microcentrifugation (Celлокrit 2, Lars Ljungberg, Stockholm, Sweden). For each sample, the collected blood was replaced by an equal volume of saline. Because blood was collected by the femoral arterial catheter, measurements of arterial pressure were discontinued during the collection.

Hemodilution Protocol

Hemodilution was performed by exchange transfusion, as previously described (6). Briefly, 20% of total blood volume (estimated as 6.5% of total body weight) or 13 ml/kg was exchanged with one of the test solutions in three consecutive steps. Blood was collected from the arterial catheter at 100 ml/h with a syringe pump (Vial médical SE 400), and the solutions were infused at the same rate with a reciprocating syringe pump through the right ear marginal vein.

Blood Viscosity and Vascular Hindrance

Arterial whole blood was obtained from separate anesthetized rabbits ($n = 3$), because the amount of blood required for viscosity determinations would have altered hemodynamics in the hemodilution experiments. Blood was collected in sterile tubes containing 5% EDTA (wt/vol), and blood viscosity was measured in vitro at Hct levels of 40 and 30% to mimic pre- and posthemodilution conditions, respectively, in accordance with Hct values shown in Table 3. Hemodilution conditions were achieved by mixing whole blood with each test solution to reach a final Hct of 30%. The viscosity of whole or diluted blood was determined at 37°C with a viscometer (Low Shear 30, Coutette, Contraves, Switzerland) for shear rates of 0.2–128.5 s$^{-1}$ and expressed in millipascals times seconds. Vascular hindrance (vascular resistance/blood viscosity) was calculated according to Chien (8) before and after hemodilution for each group. Vascular hindrance was calculated for a relevant shear rate value, namely, 128.5 s$^{-1}$, representing shear rate in large arteries (20).

Statistical Analysis

The animals were randomly allocated to one of the five following experimental groups: albumin ($n = 7$), HES 200 ($n = 7$), Hb-Dex-BTC ($n = 7$), $\alpha_{0}$-Hb ($n = 5$), and $\alpha_{0}$-raffinose-Hb ($n = 5$). Values are means ± SE. Statistical comparisons were made before hemodilution and at various posthemodilution time points (5, 30, 60, 120, and 180 min) for each group by one-factor ANOVA for repeated measures (Statview, Abacus Concepts). Comparisons between groups were made for each time point by use of one-factor ANOVA for repeated measures with Bonferroni-Dunn correction. $P < 0.05$ was considered significant.

RESULTS

Blood Gases and Hematologic Values

Hct decreased significantly immediately as a result of the hemodilution in each group, and no differences between the groups were found during the 180 min of subsequent observation (Table 3). As expected, the fall in $Hb_{tot}$ and $C_{aO_2}$ followed closely that of Hct in all groups (Table 3). In all HbOC groups, blood MetHb concentration increased immediately after exchange transfusion. This increase was dependent on the MetHb concentration in the HbOC solution and, therefore, appeared to be smaller and more transient in Hb-Dex-BTC and $\alpha_{0}$-Hb animals than in $\alpha_{0}$-raffinose-Hb animals (Table 3). MetHb concentration returned to preinfusion values after 120 and 180 min with Hb-Dex-BTC and $\alpha_{0}$-Hb, respectively, whereas it was still increased at the end of the experiments in the $\alpha_{0}$-raffinose-Hb group. Measurements performed in HbOC-treated animals showed an immediate rise in plasma $Hb_{tot}$ after the hemodilution that appeared to be dose dependent (Fig. 1). The plasma $Hb_{tot}$ was unchanged after hemodilution in albumin and HES 200 groups. The plasma half-life for each HbOC was estimated by extrapolation of the plasma $Hb_{tot}$ values from the initial 3 h and gave the following data: 7 h for $\alpha_{0}$-raffinose-Hb, 6 h for Hb-Dex-BTC, and 4 h for $\alpha_{0}$-Hb. The pH and blood gas values are shown in Fig. 2. A slight fall in pH was observed 120 min after hemodilution in albumin and HES 200 animals. A transient increase in pH was observed in the $\alpha_{0}$-raffinose-Hb group 60 min after hemodilution. $P_{O_2}$ increased in HES 200 animals after 120 min and remained unchanged from baseline values.
found. Exchange transfusion with albumin or HES 200. Hemodilution remained unchanged after exchange transfusion compared with baseline with albumin or HES 200. No significant changes in PCO₂ were found.

### Hemodynamic Parameters

Blood pressure. Systolic, diastolic, and mean blood pressures (BP) are shown in Fig. 3. BP was not statistically changed from baseline during the follow-up period with albumin or HES 200. Exchange transfusion with Hb-Dex-BTC produced a rise over the 180 min from 67.3 ± 5.3 to 76.6 ± 3.8 mmHg (P < 0.0001), from 80.4 ± 5.46 to 90.5 ± 4.6 mmHg (P < 0.0001), and from 72.9 ± 5.1 to 82.5 ± 3.7 mmHg (P < 0.0001) in diastolic, systolic, and mean BP, respectively. A rise from 66.7 ± 2.7 to 81.8 ± 2.6 mmHg (P < 0.0001) in diastolic BP, from 80.1 ± 7.3 to 93. ± 2.5 mmHg (P < 0.0001) in systolic BP, and from 72.7 ± 4.1 to 85.7 ± 3.1 mmHg (P < 0.0001) in mean BP was observed in the α-Hb group over the 180 min. Exchange transfusion with o-raffinose-Hb induced a rise over the 180 min from 65.8 ± 4.9 to 74.4 ± 4.9 mmHg (P = 0.0009), from 75.1 ± 4.8 to 83.0 ± 5.1 mmHg (P = 0.0003), and from 68.0 ± 4.5 to 77.2 ± 5.2 mmHg (P = 0.0009) in diastolic, systolic, and mean BP, respectively. However, no significant differences were found when the effects of the various HbOcs on BP were compared.

HR. HR values are shown in Fig. 4A. HR values remained unchanged after exchange transfusion compared with baseline with albumin or HES 200. Hemodilution led to a fall in HR from 242 ± 26 to 210 ± 21 beats/min (P = 0.0028) and from 249 ± 22 to 226 ± 22 beats/min (P = 0.0039) over the 180 min with Hb-Dex-BTC and α-Hb, respectively. A statistically nonsignificant drop in HR from 224 ± 6 to 220 ± 10 beats/min (P = 0.3835) was observed in the o-raffinose-Hb group over the 180 min. The comparison of the HR values revealed no significant differences between the HbOc groups.

VR. VR values are shown in Fig. 4B. Exchange transfusion induced a rise in VR values over the 180 min from 3.0 ± 0.7 to 3.8 ± 0.8 mmHg·s·cm⁻¹ (P = 0.0557) and from 2.6 ± 0.1 to 3.7 ± 0.4 mmHg·s·cm⁻¹ (P = 0.0013) with albumin and HES 200, respectively. VR rose from 2.8 ± 0.7 to 4.9 ± 1.1 mmHg·s·cm⁻¹ (P <
Exchanger transfusion with $\alpha$-Hb produced an increase in VR from $2.8 \pm 0.6$ to $4.6 \pm 0.7$ mmHg·s·cm$^{-1}$ ($P = 0.0001$) over the 180 min. Hemodilution with $\alpha$-raffinose-Hb led to a rise in VR from $2.8 \pm 0.4$ to $4.1 \pm 0.9$ mmHg·s·cm$^{-1}$ ($P = 0.0019$) over the 180 min. However, no significant differences were found when the effects of the various HbOCs on vascular resistance were compared.

Renal blood flow. Renal blood flow values are shown in Fig. 5B. The statistical analysis indicated that renal blood flow remained unchanged after exchange transfusion regardless of the solution and the posthemodilution time point.

Fig. 2. Variations of arterial blood pH (A), $P_{O_2}$ (B), and $P_{CO_2}$ (C) after acute normovolemic hemodilution in rabbits with albumin ($n = 7$), low-molecular-weight hydroxyethyl starch (HES 200; $n = 7$), Hb-Dex-BTC ($n = 7$), $\alpha$-Hb ($n = 5$), or $\alpha$-raffinose-Hb ($n = 5$).

Aortic blood flow. Aortic blood flow values are shown in Fig. 5A. Aortic blood flow returned to baseline values in all groups immediately after hemodilution. Exchange transfusion induced, however, a fall over the 180 min from $24.2 \pm 2.1$ to $18.4 \pm 2.9$ cm/s ($P = 0.0066$) with albumin and from $27.0 \pm 3.9$ to $19.2 \pm 4.7$ cm/s (P = 0.0004) with HES 200. A similar drop in aortic blood flow was also observed after hemodilution with Hb-Dex-BTC, $\alpha$-Hb, and $\alpha$-raffinose-Hb from $26.5 \pm 5.0$ to $17.2 \pm 3.8$ cm/s ($P < 0.0001$), from $26.2 \pm 3.0$ to $18.9 \pm 2.4$ cm/s ($P < 0.0001$), and from $24.3 \pm 3.1$ to $18.9 \pm 3.6$ cm/s ($P = 0.0030$), respectively. The comparison of the aortic blood flow values revealed no significant differences between the groups.
Aortic distensibility. DC values are shown in Fig. 6. Posthemodilution DC values in albumin, Hb-Dex-BTC, αα-Hb, and o-raffinose-Hb groups were not significantly different from baseline values. Exchange transfusion with HES 200 induced a mean rise in DC from 4.2 ± 0.7 to 6.5 ± 1.7 × 10⁻³ Torr⁻¹ (P < 0.0001). The comparison of the aortic distensibility values revealed no significant differences between the HbOC groups.

Blood Viscosity and Vascular Hindrance Measurements

Viscosity. Viscosity values are presented in Fig. 7A. For the lowest shear rate values, the viscosity of diluted blood is greatly affected by the viscosity of the solution used for hemodilution (Tables 1 and 2). Hence, the viscosity of blood after dilution with HES 200 or Hb-Dex-BTC is higher than the viscosity of blood diluted with autologous plasma (blood at Hct 30%). Conversely, the viscosity of blood diluted with albumin, αα-Hb, or o-raffinose-Hb is lower than the viscosity of blood diluted with autologous plasma (blood at Hct 30%). As expected, the differences in the viscosity of diluted blood decrease progressively when shear rate increases.

Vascular hindrance. Vascular hindrance is presented in Fig. 7B. Because vascular hindrance was calculated as a group variable, i.e., VR divided by the viscosity of appropriate Hct blood, no standard error is indicated. Hemodilution is followed by an increase in vascular hindrance. The slighter increase over the 180 min was found with HES 200 (59%), and the higher increase was observed in the αα-Hb group (117%). Hemodilution with albumin, o-raffinose-Hb, or Hb-Dex-BTC led to increases in vascular hindrance in the first 60 min after exchange transfusion of 49, 69, and 63%, respectively; after 120 min, vascular hindrance still increased in the albumin group (25%), whereas it appeared stable in the
DISCUSSION

We compared the systemic and renal hemodynamics after exchange transfusion to an Hct of 30% with plasma substitutes or modified human Hb solutions in anesthetized rabbits. The model of moderate hemodilution we have chosen aimed at simulating a clinical setting in which several HbOCs are under investigation (28, 35). This model affords the ability to compare the effects of different HbOCs in a single protocol and in a large animal that permits various hemodynamic measurements (7). In addition to the good reproducibility of the measurements, assessment of blood flow by the pulsed-Doppler method has the major advantage, compared with the radiolabeled microsphere technique (19), to reduce the number of animals required for the experiments, especially when numerous time points must be monitored. In this model of hemodilution, the animals have similar blood Hbtot and Ca O2, and no perturbation of acid-base regulation is observed (Table 3, Fig. 2). This indicates that mechanisms involving acid-base regulatory elements are not likely to be involved in the vascular responses after moderate hemodilution by exchange transfusion (4).

One limitation of our model is the lack of information about blood volume after exchange transfusion; we may nevertheless hypothesize that, despite differences in oncotic properties, the blood volume changes may have been limited, since the exchange transfusion led to moderate hemodilution. This was confirmed by the Hct values, which were similar in the five groups, thus suggesting the lack of large blood volume disturbances. Another limitation is the absence of measurements of cardiac output, which does not allow assessment of total peripheral resistance; thus measurements of aortic blood flow only provide an estimation of cardiac output. In addition, after hemodilution, we did not observe any increase in aortic blood flow, which may have been expected as a response to decreased viscosity and O2 content (34). This may be due to the surgical procedure, and especially laparotomy, which has led to a progressive fall in aortic blood flow throughout the follow-up period, as previously reported in the same experimental model (7).

We tested three HbOCs prepared by different chemical modifications: internal cross-linking in αα-Hb (commercial product DCLHb, clinical trials stopped in phase III), conjugation to macromolecules in Hb-Dex-BTC (in preclinical evaluation), and oligomerization in α-raffinose-Hb (commercial product Hemolink in phase III clinical trial). Accordingly, each solution has specific physicochemical properties (Table 2) that may affect hemodynamics and hematologic parameters to different extents. Moreover, because of differences in the physicochemical characteristics, such as Hb concentration, viscosity, oncotic pressure, molecular weight, or size, the choice of a reference solution must be considered thoroughly. For this reason, we have chosen two clinically used plasma volume expanders, each having some properties similar to those of the tested HbOCs (Table 1). Nevertheless, in such a study, it is not possible to control variables independently from the others.

Hematologic Parameters

The main difference in hematologic parameters was the plasma Hb tot level (Fig. 1), which appeared to be dependent on the Hb tot concentration of the infused solution and on the vascular persistence of the HbOC previously estimated to 4, 6, and 7 h for αα-Hb, Hb-Dex-BTC, and α-raffinose-Hb, respectively (6). The plasma Hb tot level is important, since it may influence the vasoconstriction produced by HbOCs, by virtue of interactions of cell-free Hb with factors involved in the regulation of the vascular tone, such as nitric oxide (NO), endothelin-1, or prostacyclin (18, 28). The plasma Hb tot level is also of great importance at the microcirculatory level, since the plasma cell-free Hb concentration determines the oxygenation capacity of plasma (29, 33, 37). Although we did not measure tissue oxygenation in this study, we may hypothesize that the O2-carrying capacity of blood mixed to cell-free Hb is dependent on the plasma Hb tot level and would, therefore, be higher in the case of α-raffinose-Hb, which, in addition, possesses the higher O2 half-saturation pressure of Hb. This putative high oxygenation capacity may be unbalanced by microvascular constriction as a result of...
increased O₂ supply, in accordance with the theory of autoregulation (37). However, to what extent the O₂-carrying properties of the HbOCs may account for changes in vascular tone and, consequently, hemodynamics at the macroscopic level has not been established in this study and requires further investigation to understand the cardiovascular effects of Hb solutions.

Another substantial difference is seen in blood MetHb levels: the amplitude and the duration of the rise in blood MetHb are dependent on the MetHb level in the infused solution and have the following order in the present experiments: α-rafﬁnose-Hb > αα-Hb > Hb-Dex-BTC (Table 3). Extraerythrocytic MetHb is, however, rapidly reduced in vivo, since the level decreased twofold between 5 and 180 min, indicating the efﬁcacy of reducing processes in rabbits. As reviewed by Faiivre-Fiorina et al. (12), the reduction of MetHb involves plasma and erythrocytic enzymes, namely, superoxide dismutase and catalase, and other factors such as ascorbic acid and glutathione.

Hemodynamic Parameters

The first interesting finding in our hemodynamic investigations is the absence of signiﬁcant change in renal blood ﬂow after hemodilution with reference or Hb solutions. This suggests that renal VR changes were proportional to changes in MAP and that renal plasma ﬂow increased because of the fall of Hct. This conﬁrms recent results from Lieberthal et al. (23), who indicated that α-rafﬁnose-Hb infused in similar conditions in rats (20% exchange transfusion) had no deleterious effects on renal hemodynamics. In their study, the authors also demonstrated the potency of α-rafﬁnose cross-linking to decrease the vasoconstrictive action of unmodiﬁed cell-free Hb. In our study we did not compare the effects of the three HbOCs tested with those of unmodiﬁed Hb, because this ﬂuid has a very short circulating persistence because of rapid renal elimination and extravasation, and this would greatly affect the hemodynamic changes. However, our results indicate that α-rafﬁnose-Hb induced lesser changes in BP, HR, and VR than Hb-Dex-BTC or αα-Hb (Figs. 3 and 4). The effects of the three HbOCs (and albumin) on aortic distensibility were nevertheless similar, indicating that these solutions have no signiﬁcant direct action on the aortic vascular tone in vivo. Conversely, we found an immediate, large, and sustained increase in aortic distensibility in HES 200-treated animals that was not due to changes in BP (Figs. 3 and 6). To our knowledge, this is a novel ﬁnding of a pharmacological effect of HES 200, and it requires further investigation. Among the possible hypotheses for this effect is a positive inotropic effect that would affect the mechanical properties of conductance vessels. An action on cardiac contractile function has been described with other plasma volume-expanding ﬂuids such as dextrans, but little is known about the effects of starches (36). In other respects, an immediate expansion of plasma volume may be proposed to account for the increase in distensibility. Although this hypothesis cannot be ruled out in the absence of plasma volume measurements, the lack of changes in Hct values after infusion of HES 200 in comparison to albumin suggests that large alterations of blood volume did not occur in these experiments. Although the mechanism(s) accounting for this effect is unclear, the implications are obvious. The increased distensibility allows the accommodation of a given stroke volume within the aorta and its contiguous major branches with a lesser increment in the systolic pressure. Although we have no direct measurements of stroke volume, of all groups the HES 200-exchanged rabbits exhibited the smallest changes in aortic systolic BP (Fig. 3A). Nevertheless, in the absence of further experimental data, we cannot exclude the hypothesis that the increased distensibility may be independent of a pharmacological action of HES 200 and would, rather, be due to changes in ultrasound propagation after infusion of starch, which could alter the measurement of aortic diameter.

Many mechanisms can be proposed to explain the differences in the pressor effect of the three HbOCs, but, as discussed above, most of these variables cannot be controlled when ﬂuids prepared by the various proprietary processes are used. Among the pharmacological mechanisms, the afﬁnity of Hb for NO has long been suggested as the main mechanism, but there is growing evidence for the involvement of other mediators (reviewed with particular reference to DCLHb in Ref. 17). Moreover, the possible correlation between the afﬁnity of Hb for NO and the pressor effect of the solution has not been clearly established (11, 32).

The physicochemical properties of the solutions can also inﬂuence the cardiovascular responses to HbOCs. Migita et al. (27) showed that, in contrast to αα-Hb, bovine Hb conjugated to polyethylene glycol signiﬁcantly increased blood volume and cardiac index after 50% exchange transfusion and that these differences were due to the high colloid osmotic pressure of the conjugated HbOC. In our model, hemodilution was less aggressive; thus colloid osmotic pressures and consequent major changes in blood volume were more closely matched. Consequentially, the effect on cardiovascular homeostasis may have been of smaller extent.

The impact of the size and molecular weight of the modiﬁed Hb molecules is commonly presented as a key factor in the vasoconstrictive effect of HbOCs. Thus, Gould et al. (17) proposed that the absence of vasoconstriction after infusion of glutaraldehyde-polymerized human Hb (mol wt > 120,000) in trauma patients is due to the large size of the molecules. If this were indeed the principal determinant, the polymerization of Hb would result in an impaired penetration into the vascular wall and could explain the limited vasoconstricting action of α-rafﬁnose-Hb compared with αα-Hb and Hb-Dex-BTC found in our model. However, this statement is becoming controversial in view of several recent results. Abassi et al. (1) indicated that polymerization of diaspirin cross-linked human Hb (mol wt > 320,000) had no beneﬁcial effects on hemodynamics after exchange transfusion in rats compared with the nonpolymerized form (mol wt 64,500) (1). Moreover, Faiivre-Fiorina et
al. (13) demonstrated the ability of Hb-Dex-BTC (64,500 > mol wt > 500,000) to penetrate into and through aortic endothelial cells after exchange transfusion in guinea pigs. The latter suggests that the vasoconstrictive action of HbOCs with high molecular weight would not be restricted to the vascular lumen but could also occur into the endothelium. Further investigation is required to fully understand the role of the molecular weight and/or size and other factors in the pressor effect of HbOCs.

Another essential physicochemical variable in hemodilution settings is the viscosity of the circulating fluids (37). We observed differences in blood viscosity with the different solutions used to dilute blood to equal Hct (Fig. 7A). The differences may be due to rheological parameters such as plasma viscosity and, possibly at low shear rates, to differences in red blood cell aggregation, as evidenced by the high viscosities of Hb-Dex-BTC and HES 200 and as reported by Menu et al. (26). We demonstrated previously that the vasoconstrictive properties of HbOCs override the benefit of decreased viscosity and of reduced Hct after hemodilution (6). However, because VR and blood viscosity are dependent variables (in regard to the Poiseuille-Hagen formula), understanding to what extent the effect of decreased blood viscosity due to hemodilution may be compensated for by changes in vascular tone would be helpful. In this study, we therefore estimated vascular hindrance (resistance/viscosity) to determine whether a moderate decrease in the viscosity of blood mixed with HbOCs may impair vasoconstriction due to auto-regulation after infusion of cell-free Hb. Determination of vascular hindrance was performed through measurements of blood viscosity at 128.5 s⁻¹ and was carried out in vitro. The main limitation of this method is that measurements in vitro do not take into account possible changes in fluid balance that are likely to affect Hct and, consequently, blood viscosity; however, as previously discussed, the extent of such changes in our experiments may be small, since we performed moderate hemodilution. Moreover, at elevated shear rate values, viscosity measured in vitro correlates with viscosity measured in vivo and thus provides an accurate view of rheological phenomena occurring in vivo (26).

Early increase of vascular hindrance has been proven to occur after hemodilution and is aimed at maintaining intravascular resistance in the face of a decreased viscosity (24). As expected, a progressively rising trend in vascular hindrance was observed in all groups throughout the posthemodilution period; the slight increase in Hct (1–2%) is, however, probably not sufficient to account for the large increases in vascular hindrance, and other parameters such as vasoconstrictive properties are likely to be involved. Our results indicate similar increases in vascular hindrance with Hb-Dex-BTC and o-raffinose-Hb, although Hb-Dex-BTC produced a greater increase in VR. In the same conditions, αα-Hb and Hb-Dex-BTC exhibited a similar action on VR, but the lower viscosity of αα-Hb did not make it possible to reduce vascular hindrance (Figs. 4 and 7). These results suggest that infusion of HbOCs with elevated viscosity to maintain blood viscosity may limit the vasoconstriction due to hemodilution per se; as a result, vasoconstriction would be restricted to mechanisms involving the pharmacological properties of cell-free Hb, since vasoconstriction elicited by auto-regulation processes would be impaired. Similar conclusions have been drawn by Winslow and Chapmann (40), who compared the relationships between the VR changes and viscosity of αα-Hb and polyethylene glycol-conjugated Hb in an experimental model of 50% hemodilution followed by severe hemorrhage in rats. In the same respects, Tsai et al. (36) demonstrated that high plasma viscosity was needed to maintain capillary perfusion during hemodilution, and de Witt et al. (10) proposed that increased mixed blood viscosity and, therefore, increased wall shear stress lead to arteriolar dilation through an NO-dependent mechanism. Our results also indicate that elevation of mixed blood viscosity would be required not only in hypovolemic conditions but also after intentional hemodilution in order to limit their oxygenation capacity of HbOCs.

Taken together, these results confirm the need to adapt the design of HbOCs to their clinical applications. Thus high colloid osmotic pressure may be required in the treatment of hypovolemic shock, a predominantly powerful pressor effect may be useful in septic shock, and, as emphasized by this study, a balance between moderately increased VR and decreased viscosity appears to be necessary when HbOCs are used for moderate hemodilution.

The authors are grateful to Dr. G. Biro (Hemosol, Toronto, ON, Canada) for supplying o-raffinose-oligomerized Hb and for suggestions on the manuscript. The authors thank Prof. J.-F. Stoltz (Laboratoire Angéologie et Hémorhéologie, Faculté de Médecine, Université Henri Poincare–Nancy 1) for advice and M. Gentils and G. Gauchois for technical assistance.

This study was supported in part by Association Recherche et Transfusion (Paris, France) Contract 02-1995 and by the Fondation pour la Recherche Médicale (Paris, France).

Address for reprint requests and other correspondence: A. Caron, Laboratoire d’Hématologie et de Physiologie, Faculté de Pharmacie, 5 rue Albert Lebrun, 54001 Nancy cedex, France (E-mail: caron@pharma.u-nancy.fr).

Received 14 September 1999; accepted in final form 13 December 1999.

REFERENCES


HEMODYNAMIC EFFECTS OF MODERATE HEMODILUTION WITH HbOCs

H1983


