Cardiovascular and hemorheological effects of three modified human hemoglobin solutions in hemodiluted rabbits

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Caron, Alexis, Patrick Menu, Beatrice Faivre-Fiorina, Pierre Labrude, Abdu I. Alayash, and Claude Vigneron. Cardiovascular and hemorheological effects of three modified human hemoglobin solutions in hemodiluted rabbits. J. Appl. Physiol. 86(2): 541–548, 1999.—The cardiovascular effects of human albumin (Alb) and three human hemoglobin (Hb) solutions, dextran-benzene-tetracarboxylate Hb, αααα-crosslinked Hb, and αααα-raffinose-polymerized Hb were compared in anesthetized rabbits undergoing acute isovolemic hemodilution with Hct reduction from 41.4 ± 2.7 to 28.8 ± 1.6%. The impact of the vasoconstricting properties of Hb was examined by measuring heart rate (HR), mean arterial pressure (MAP), abdominal aortic, and femoral arterial blood flow, vascular resistance (VR), and aortic distension during the first 3 h after hemodilution. The impact of the hemorheological parameters was assessed by measurements of hemodiluted blood viscosity. In contrast to Alb, the Hb solutions elicited an immediate increase in MAP (20–38%). The effects of Alb and Hb solutions on HR, as well as on aortic and femoral arterial blood flow, were similar. VR decreased with Alb (20–28%) and increased with all three Hb solutions (30–90%), but the MAP and VR rising trends were different with each Hb solution. Aortic distension decreased in Hb groups compared with the Alb group for the first 60 min. The viscosity of hemodiluted blood was similar for all groups at high shear rates but was dependent on the viscosity of the solutions at low shear rates. We conclude that the vasoconstriction elicited by the Hb solutions overrides the vasodilation associated with viscosity changes due to hemodilution and would be the major factor responsible for the cardiovascular changes.

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The accepted explanation is that free Hb (in the ferrous form Fe2+) traps nitric oxide (NO) released by the endothelium and thus impairs the vasodilating action of this relaxing factor (14, 19, 23). Nakai et al. (19) compared cellular Hb forms and free Hb derivatives in rabbit aorta strips. Nakai et al. emphasized the contribution of Hb chemical form to the NO-trapping action of the solutions. However, in a recent report, Rohlfis et al. (24) concluded that the blood pressure increases observed in rats after 50% exchange transfusion with Hb solutions could not be the result of NO scavenging by the heme and indicated that other physiological mechanisms were more likely to be involved. Gulati et al. (8) also suggested a contribution by endothelin to the pressor action of diaspirin cross-linked Hb (αααα-Hb) in hemorrhaged rats. Stimulation of αααα-adrenergic receptors has also been proposed as a reason for peripheral vasoconstriction after αααα-Hb administration in rats (7, 26). An autoregulatory control of blood pressure in response to changes in tissue oxygenation was also proposed by Intaglietta et al. (13) as a contributing mechanism to vasoactivity. Nolte et al. (20, 21) indicated that the increased blood O2-carrying capacity in the microcirculation after injection of αααα-Hb could also contribute to the pressor response of Hb as a result of alterations in vasomotion frequency and amplitude. Despite the large number of studies of Hb-based O2 carriers, the incidence of the type of modification applied to Hb on the cardiovascular function after hemodilution is poorly documented. Therefore, we examined the effects of three human Hb solutions: dextran-benzene-tetracarboxylate-conjugated Hb (Dex-BTC-Hb), αααα-Hb, and αααα-raffinose-polymerized Hb (PolyHb) after acute isovolemic hemodilution, compared with albumin (Alb), a solution used in clinical practice for intravenous volume replacement. Hemodynamic parameters and vascular resistance (VR) were examined periodically during the first 3 h. We also measured the variations in aortic distension, a mechanical parameter of conductance vessels which is likely to be affected by an increase in blood pressure. In separate experiments, measurements of blood viscosity after hemodilution were performed to determine the possible impact of this rheological parameter on hemodynamic changes.

METHODS

Animals and anesthesia. Thirty male New Zealand White rabbits (La Garenne, Ville Saint-Etienne, France), which weighed 2.7 ± 0.3 kg, were anesthetized with ketamine (Ketalar 50, Parke-Davis, France; 50 mg/kg im) and pentobarbital sodium (Sanofi, France; 40 mg/kg iv, followed by infusion
at 5 mg·kg$^{-1}$·h$^{-1}$ in the right ear marginal vein). The rabbits were placed in the dorsal decubitus position on a heating table, and they were warmed to maintain a constant body temperature. The trachea was intubated, and the animal spontaneously breathed room air. At the end of the experiments, the animals were killed by excess dose of pentobarbital sodium. The study design was approved by the Animal Protection Bureau of the French Ministry for Fishing, Agriculture, and Food, and the experiments were conducted in accordance with the Guiding Principles for Research Involving Animals.

Surgery and instrumentation. The left femoral artery was exposed, and a directional, pulsed, Doppler flow probe (DBF-120A, Crystal Biotech, Holliston, MA) was placed on it. The right femoral artery was cannulated with a heparin-farin polymerized Hb (lot 95HM0404); P50, oxygen half-saturation pressure of Hb measured at 37°C; MetHb, methemoglobin. Kinematic viscosities of the Hb solutions were determined at 37°C with an automatic capillary viscometer (module V, Amtec, France).

Data acquisition. The femoral arterial catheter was connected to a pressure transducer (Viggo-Spectramed, Paris, France) to measure the pulsatile arterial pressure. The aortic and femoral arterial flow probes were connected to 20-MHz modules with pulse-repeated frequency of 125 kHz (PD-20, Crystal Biotech) to measure the blood flow, as reported by Haywood et al. (10). The crystal was connected to a 20-MHz echo-tracking module (WT-20, Crystal Biotech) to measure the aorta diameter in millimeters. The blood flow and echo-tracking modules were connected to a dedicated amplifier (CBI-8000, Crystal Biotech) to measure the blood flow, as reported by Garenne et al. (21). The pressure transducer and the echo-tracking module (WT-20, Crystal Biotech) were placed on the vessel. Saline (10 ml·kg$^{-1}$·h$^{-1}$ iv) was infused by the right ear marginal vein to maintain fluid after the laparotomy.

Data acquisition. The femoral arterial catheter was connected to a pressure transducer (Viggo-Spectramed, Paris, France) to measure the pulsatile arterial pressure. The aortic and femoral arterial flow probes were connected to 20-MHz modules with pulse-repeated frequency of 125 kHz (PD-20, Crystal Biotech) to measure the blood flow, as reported by Haywood et al. (10). The crystal was connected to a 20-MHz echo-tracking module (WT-20, Crystal Biotech) to measure the aorta diameter in millimeters. The blood flow and echo-tracking modules were connected to a dedicated amplifier (CBI-8000, Crystal Biotech). The pressure transducer and the amplifier were connected to a personal computer for on-line data acquisition at a rate of 75 Hz (Acqknowledge + MP100 hardware and software, Biopac Systems, Goleta, CA).

Acute isovolemic hemodilution. The rabbits were randomly allocated to experimental groups in which hemodilution was performed with human Alb (n = 8) or with Dex-BTC-Hb (n = 8), αα-Hb (n = 8), or PolyHb (n = 6). After instrumentation was completed, the animals were allowed to equilibrate during a 1-h baseline period. Hemodilution with one of the solutions described below was then initiated by partial-exchange transfusion to achieve a final Hct of ~28%. Blood was withdrawn at 100 ml/h with a syringe pump (Vial Médical SE 400, Saint-Martin-le-Vinoux, Grenoble, France) connected to the femoral arterial catheter. The solutions were infused at the same rate with a reciprocating syringe pump (Vial Médical SE 400) by the right ear marginal vein. The exchange transfusion was achieved in ~50 min, and the end of the infusion was considered as time point t = 0.

Blood samples and hematologic parameters. Blood samples consisting of 750 µl were collected at the end of the baseline period and at various time points (t = 5, 60, 120, and 180 min), and they were replaced by an equal volume of saline. Blood samples were used for Hct determination, pH, and analysis of blood gases (ABL2, Radiometer, Copenhagen, Denmark) as well as blood and plasma total Hb concentrations (Hbtotal, Co-oximeter 482, Instrumentation Laboratory, Lexington, MA). Because blood was collected by the femoral arterial catheter, measurements of arterial pressure were discontinued during the collection procedure.

Solutions. Human Alb (5 g/dl) dissolved in Tyrode medium (in mM: 6.7 glucose, 140 Na$^+$, 5.0 K$^+$, 2.5 Ca$^{2+}$, 1.1 Mg$^{2+}$, 115.8 Cl$^-$, 0.8 phosphates, 30.0 carbonates) was supplied by Pasteur-Mérieux Sérums & Vaccins (Marcy l’Etoile, France). The Alb solution was sterile and pyrogen free. Dex-BTC-Hb consists of 8.5 g/dl of human Hb extracted from outdated red blood cells and conjugated to a macromolecular allosteric effector, dextran-benzene-tetracarboxylate. Dex-BTC-Hb was produced in collaboration with Pasteur-Mérieux Sérums & Vaccins, according to the protocol previously described, and was suspended in Tyrode medium (pasteurized, sterile, pyrogen free) and then frozen at ~20°C without preservatives (17, 22). αα-Hb (from the US Army) consists of 8.2 g/dl of heat-treated human Hb obtained from outdated red blood cells, stabilized by cross-linking between the two αα-subunits with bis(3,5-dibromosalicyl)umarate, suspended in Ringer lactate, and frozen at ~80°C (28). PolyHb consists of a 10 g/dl pasteurized solution of human Hb extracted from outdated red blood cells, cross-linked internally with raffinose, and polymerized to form PolyHb (11, 12). PolyHb was suspended in lactated Ringer injection [US Pharmacopoeia (in mM): 123.9–137.0 Na$^+$, 3.6–4.4 K$^+$, 1.2–1.5 Ca$^{2+}$, 103.9–115.2 Cl$^-$, 25.7–29.0 lactate] and was frozen at ~80°C without preservatives. PolyHb was generously provided by Hemosol (Etobicoke, Ontario, Canada).

Each of the solutions had low endotoxin levels, and their mean physicochemical characteristics are described in Table 1. In vitro viscosity measurements. The kinematic viscosity of Alb, Dex-BTC-Hb, αα-Hb, and PolyHb was determined at 37°C with an automatic capillary viscometer (module V, Amtec, Villeneuve-Loubet, France) and expressed in centistokes (Table 1). The principle of the instrument is to measure automatically the flowing time of a solution in a capillary between two points that are optically defined. The measurement of this time is equivalent to the measurement of the kinematic viscosity.

Ex vivo viscosity measurements. In separate experiments, 25 male New Zealand White rabbits weighing 2.4–2.7 kg (La Garenne) were used. While the animals were under general anesthesia with 1% halothane (Belmont, France) mixed in 95% O$_2$-5% CO$_2$, a polyethylene tube was inserted into the right carotid artery and tunneled subcutaneously to emerge at the top of the back. The animals were treated with

### Table 1. Physicochemical characteristics of solutions used for hemodilution

<table>
<thead>
<tr>
<th>Solution</th>
<th>Protein Concentration, g/dl</th>
<th>Molecular Mass, kDa</th>
<th>Pso, mmHg</th>
<th>Kinematic Viscosity, cSt</th>
<th>Oncotic Pressure, mmHg</th>
<th>Osmolality, mosmol/l</th>
<th>MetHb Content, %</th>
<th>Endotoxin Level, EU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>5.0</td>
<td>68</td>
<td>0.92</td>
<td>25</td>
<td>300</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Dex-BTC-Hb</td>
<td>8.5</td>
<td>64–500</td>
<td>23</td>
<td>2.11</td>
<td>40</td>
<td>280</td>
<td>&lt;5</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>αα-Hb</td>
<td>8.2</td>
<td>64</td>
<td>29.5</td>
<td>0.97</td>
<td>34.5</td>
<td>280</td>
<td>&lt;5</td>
<td>0.25</td>
</tr>
<tr>
<td>PolyHb</td>
<td>10.0</td>
<td>64–500</td>
<td>34</td>
<td>1.24</td>
<td>26</td>
<td>280–300</td>
<td>&lt;10</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Dex-BTC-Hb, dextran-benzene-tetracarboxylate hemoglobin (lot 94PS235); αα-Hb, αα-cross-linked Hb (lot 950404-00-X); PolyHb, o-raffinose polymerized Hb (lot 95HM0404); Pso, oxygen half-saturation pressure of Hb measured at 37°C; MetHb, methemoglobin. Kinematic viscosity was determined at 37°C with an automatic capillary viscometer (module V, Amtec, France).
penicillin (200,000 U/kg im) and were allowed a recovery period of 1 day before experiments. On the day of experiments, the animals received heparin (150 U/kg iv); 5 min later, an exchange transfusion was performed to decrease Hct to 28%. In these experiments, Alb, Dex-BTC-Hb, αα-Hb, and PolyHb (n = 5, each) were used for hemodilution. The whole blood and the blood + solution mixtures were collected with 5% EDTA (wt/vol). The viscosity was determined 5 min after the end of exchange transfusion, at 37°C, by using a Couette viscometer (Low Shear 30, Contraves, Zurich, Switzerland) for shear rates ranging from 0.5 to 128 s⁻¹ and expressed in millipascals per second. For determination of control blood viscosity, the Hct was adjusted to 40 and 30% (n = 5 each) by addition or subtraction of autologous plasma.

Data analysis. Mean arterial pressure (MAP) was calculated as \[\frac{1}{3} (\text{systolic pressure} - \text{diastolic pressure}) + \text{diastolic pressure}\]. Heart rate (HR) was calculated from the aortic blood flow signal as the reciprocal between two consecutive systolic peaks. VR was calculated as MAP/aortic blood flow. Aortic distension was expressed as the difference between the aortic systolic and diastolic diameters for each beat. MAP, HR, aortic blood flow, aortic distension, VR, and femoral blood flow are expressed as means ± SE. Statistical comparisons were made before hemodilution and at posthemodilution time points (t = 5, 30, 60, 120, and 180 min) for each group by using an analysis of variance for repeated measures. Comparisons between groups were made for each time point by using an analysis of variance for repeated measures with Dunn-Bonferroni correction. A value of \(P < 0.05\) was considered significant.

RESULTS

Hematological results. Hct decreased similarly in each group after exchange transfusion (from 41.4 ± 2.7 to 28.8 ± 1.6%; \(P < 0.05\)) and was stable for the 3 h of experiments (Fig. 1A).

Hemodilution led to a decrease in blood Hb tot which was significant in the Alb group (Fig. 1B). Hb appeared in plasma immediately after exchange transfusion with the three Hb solutions, and plasma Hb tot decreased with time (Fig. 1C).

The order of the kinematic viscosity of the solutions measured in vitro was Dex-BTC-Hb > PolyHb = αα-Hb > Alb (Table 1). At 5 min after exchange transfusion, marked differences were found in blood viscosity at low shear rates; blood hemodiluted with Dex-BTC-Hb exhibited a higher viscosity than blood hemodiluted with αα-Hb, PolyHb, and Alb, respectively (Fig. 2). In contrast, at high shear rates, the viscosity values were similar between the groups.

No major changes in arterial blood gases and pH were found after exchange transfusion; this indicates that the acid-base status and oxygenation were well maintained all through the experiments (Table 2).

Effects of hemodilution with Alb. Hemodilution with Alb caused a progressive decrease in MAP of 12–25% and had no effect on HR (Fig. 3). Aortic blood flow decreased by 26% at \(t = 180\) min, and femoral arterial blood flow increased by 33% after 120 min (Fig. 4). VR decreased (20%) after 30 min (Fig. 5). Aortic distension significantly increased by 20% in the first 30 min after exchange transfusion (Fig. 5).

Effects of hemodilution with Dex-BTC-Hb. Dex-BTC-Hb induced an increase in MAP (37% maximum) in the 3 h after exchange transfusion; MAP values were significantly higher than in the Alb group (Fig. 3). A 12% decrease in HR was observed 5 min after exchange transfusion, and HR was not different from baseline value after this time (Fig. 3). Aortic blood flow increased by 35% 5 min after hemodilution, thereafter the values were not different from those of the other groups (Fig. 4). Femoral arterial blood flow decreased between 30 and 120 min (25–40%) after exchange transfusion (Fig.
transfusion and was higher than in the Alb group all through the experiments (Fig. 5). Aortic distension decreased by 25% in the first 60 min after hemodilution and was lower than in the Alb group all through the experiments (Fig. 5).

Effects of hemodilution with αα-Hb. MAP increased by 35% for the 3 h after hemodilution with αα-Hb compared with baseline value (Fig. 3) and was higher than in the Alb group. HR and femoral arterial blood flow remained unchanged after exchange transfusion (Fig. 3 and 4). A significant decrease (35%) in aortic blood flow was observed at t = 180 min (Fig. 4). VR progressively increased (30–90%) after exchange transfusion and was higher than in the Alb group all through the experiments (Fig. 5). Aortic distension slightly decreased compared with baseline (Fig. 5).

Effects of hemodilution with PolyHb. MAP increased after hemodilution with PolyHb compared with the Alb-exchanged group, and the values were significantly different from baseline value until 180 min (Fig. 3). HR was not altered after exchange transfusion (Fig. 3). Aortic blood flow was reduced after 120 min, and femoral arterial blood flow decreased by 30% between 60 and 120 min after hemodilution (Fig. 4). VR progressively increased (25–38%) after 30 min and was higher than in the Alb group all through the experiments (Fig. 5). Aortic distension slightly decreased compared with baseline (Fig. 5).

Comparative effects of the three Hb-based solutions. The increase in MAP was immediate in Dex-BTC-Hb and PolyHb groups, and MAP decreased with time, whereas it was stable in the αα-Hb group (Fig. 3). Aortic blood flow was significantly higher in Dex-BTC-Hb group compared with αα-Hb and PolyHb at t = 5 min, but the flow was not different after this time (Fig. 4). No statistical differences in HR, femoral arterial blood flow, and aortic distension were found between the three Hb groups at the various time points.
Although no significant differences were found in VR, the increments were different in the groups (Fig. 5). There was a progressively rising trend in the Dex-BTC-Hb and \( \alpha\alpha\)-Hb groups at each time point, whereas VR increased until 60 min and was stable thereafter in the PolyHb group.

**DISCUSSION**

We examined the cardiovascular effects of three human Hb solutions that were undergoing preclinical or clinical evaluation: Dex-BTC-Hb (preclinical evaluation), and \( \alpha\alpha\)-Hb and PolyHb (clinical evaluation). The possible influence of the type of chemical modification applied to Hb was assessed in an exchange-transfusion protocol that led to a reduction in Hct from 41 to 28\%.

The model we chose did not aim at being a clinical model but was intended to permit a comparative study of cardiovascular parameters after hemodilution. An important point to consider was to have a reduction in Hct that was similar among the experimental groups to allow valid comparisons of blood flow measurements. Blood flow is indeed directly proportional to blood viscosity, and, therefore, to Hct (2) as indicated by Poiseuille-Hagen equation

\[
\dot{Q} = \Delta P \cdot \pi \cdot r^4 / 8 \eta \cdot L
\]

where \( \dot{Q} \) is blood flow, \( \Delta P \) is the pressure difference, \( \eta \) is blood viscosity, and \( r \) and \( L \) are vessel radius and length, respectively.

The contribution of \( O_2 \)-transport parameters and acid-base status to hemodynamics was comparable in the experimental groups, because no changes in arterial blood gases and pH were observed after hemodilution (Table 2). Blood Hb\( \alpha \) content was above 10 g/dl (Fig. 1), the commonly accepted threshold at which oxygenation is correctly maintained. Moreover, the low endotoxin levels of the infused solutions make it possible to assume that the animals did not react to this parameter. Thus we can assume the cardiovascular changes observed with Hb solutions are related to their vasoactive and/or hemorheological effects.

Comparative effects of Alb and Hb solutions. Human Alb was used as a control solution because it has a viscosity similar to that of plasma and most Hb solutions presently undergoing clinical tests. The cardiovascular responses to hemodilution with non-\( O_2 \)-carrying and Hb solutions have been extensively characterized. Unlike the case with Hb solutions, hemodilution with volume-replacement solutions devoid of \( O_2 \)-carrying capacity increases cardiac output by a compensatory...
mechanism that aims at maintenance of O2 delivery at prehemodilution values (27). In clinical practice, such as cardiopulmonary bypass, hemodilution is conducted to reach a final Hct of <30% (3, 5, 27). In our protocol, we did not measure absolute cardiac output, but it was estimated by aortic blood flow. We did not find major differences in aortic blood flow between the Alb and Hb groups, although Hct was reduced to ~28%, nor did we find any major difference in HR.

Blood viscosity reduction has been proposed as the major factor responsible for the increase in cardiac output with non-O2-carrying solutions rather than decreased O2 delivery (27). In a recent study, Dietz et al. (6) compared the effects of Alb and αα-Hb in a partial-exchange transfusion model in which blood viscosity changes were similar with both solutions. They concluded that the vasoconstricting properties of the Hb solution had a greater effect on vasculature than blood viscosity. Our viscosity measurements are consistent with these findings: at high shear rates, reflecting the rheological behavior of blood in the conductance vessels, such as the abdominal aorta, the viscosity of hemodiluted blood was similar for Alb and Hb, despite differences in the proper viscosity of the solutions (Fig. 2, Table 1). In contrast, at low shear rate values (0.5–5 s⁻¹), reflecting the hemorheological behavior in the small vessels, the viscosity of hemodiluted blood is highly dependent on the viscosity of the solutions (Fig. 2). The beneficial effect of the low viscosity of Hb solutions in the microcirculation is of great interest, because these solutions could thus improve oxygenation more efficiently than whole blood in ischemic tissues (3, 20, 21). But the improvement of oxygenation due to viscosity reduction could also induce vasoactive responses by autoregulation mechanisms, as proposed by Intaglietta et al. (13).

We observed major differences between Alb and Hb groups in MAP, VR, and aortic distension; these differences are related to the vasoactive properties of the solutions. MAP and VR decreased after exchange transfusion with Alb, whereas Hb solutions increased both MAP and VR. The VR, calculated in our experiments as the ratio of MAP to aortic blood flow, describes the vascular bed distal to the location of the flowmeter (i.e., the abdominal aorta). The rise in VR after Hb infusion in the three groups confirms the vasoconstricting effect of these solutions, which led to increased pressure (1, 6–8, 14, 20, 27). In contrast, the decrease in VR and MAP in the Alb-hemodiluted group indicates that hemodilution with a solution without vasoconstrictor properties (Alb) evokes vasodilation, as previously reported (6). Aortic distension was expressed as the difference between systolic and diastolic aorta diameter as measured with an echo-tracking device. In our experimental model, aortic distension was reduced in the three groups of animals hemodiluted with Hb compared with Alb-hemodiluted animals for the first 60 min after exchange transfusion (Fig. 5). In contrast, the transient increase in aortic distension after hemodilution with Alb may be due to decreased blood pressure. Although we did not measure the absolute distensibility of the property of conductance vessels which contribute to propagate the pressure pulse and to buffer stroke volume [expressed as Δdiameter/diameter × Δpressure)] (16), we can assume that the changes in aortic distension after Hb infusion are caused by increased wall stress elicited by increased blood pressure.

As discussed above, the impact of the viscosity of Alb and Hb solutions was similar at the macrohemodynamic level; thus the alterations in MAP, VR, and aortic distension can basically be attributed to the Hb-induced vasoconstriction. Many mechanisms have been proposed to explain the vasoconstrictor action of Hb: 1) inhibition of the NO-dependent vascular smooth muscle relaxation by NO-scavenging action of free Hb (4, 9, 14, 15, 23), 2) sensitization of α-adrenergic receptors (7, 26), 3) involvement of endothelin (8), and 4) increase in microcirculation vascular tone as a response to increased blood O2-carrying capacity (13, 20, 21).

Comparison of Dex-BTC-Hb, αα-Hb, and PolyHb effects. The three solutions were prepared from human Hb that was extracted from outdated banked red blood cells according to specific protocols previously described (11, 12, 22, 28). The three Hb solutions are isosmolar to plasma, but each solution has specific physicochemical properties, the contribution of which to the hemodynamic effects cannot be absolutely defined (Table 1). There is a clear difference in oncolytic pressure between Dex-BTC-Hb and αα-Hb vs. PolyHb that would differentially affect the plasma volume expansion ability of the solutions (18). This could explain at least in part the large increase in aortic blood flow after administration of Dex-BTC-Hb, as a result of an increase in preload. However, the colloidal oncotic pressure measured in vitro is only an approximation of the oncolytic behavior of the macromolecules in vivo, and the exact quantitative effect is unknown.

The Hb concentration was similar for Dex-BTC-Hb and αα-Hb, (administered dose, 3.1 g) and was higher for PolyHb (administered dose, 3.7 g). The concentration of plasma Hb was dose dependent, because it was higher in PolyHb group vs. Dex-BTC-Hb and αα-Hb (Fig. 1C). The plasma retention times, estimated from the data shown in Fig. 1C, indicated that αα-Hb had a shorter half-life (~4 h) than Dex-BTC-Hb (~6 h) and PolyHb (~7 h) in this experimental model. Despite these differences, we could have expected that the impact of the dose administered on the blood pressure was similar, because only a small dose of free Hb is necessary to achieve maximal pressor effect immediately (plateau effect). The three Hb solutions induced a significant increase in MAP, with specific increments for each of the solutions (Fig. 3). Dex-BTC-Hb and αα-Hb induced a significant rise in MAP compared with preexchange values for 3 h, whereas the increase with PolyHb was no longer significant after 2 h. There was also a progressive rising trend in the VR in Dex-BTC-Hb and αα-Hb groups, whereas VR increased until 60 min and was stable thereafter in the PolyHb group (Fig. 5). In contrast, statistical comparisons indicated that Dex-BTC-Hb, αα-Hb, and PolyHb had similar acute effects on HR, aortic blood flow, femoral blood flow, and aortic distension (Figs. 3–5).
As discussed previously, despite large differences in viscosity between Dex-BTC-Hb vs. αα-Hb and PolyHb (Table 1), the rheological properties of the Hb solutions had limited impact on the hemodynamic changes (Fig. 2). The differences in the pressor action elicited by the solutions could instead be attributed to the specific permeability characteristics of the modified Hbs. The NO-scavenging action and the role it plays in inhibiting vasodilator mechanisms are indeed thought to be related to the leakage of Hb across the endothelial barrier into the space directly surrounding the smooth muscle cells (1, 19). The polymerization of Hb, aiming to increase its molecular size, would hence result in an impaired penetration into the vascular wall (1, 19) and could explain the limited vasocostricting action of PolyHb compared with αα-Hb and Dex-BTC-Hb. The structural modification of the heme pocket induced by the chemical modifications applied to Hb, and thereby the changes in affinity of the heme moiety for NO, is another factor likely to be involved in the different cardiovascular effects of the solutions. The accessibility to the β93 cysteine residue of the globin, known to react with NO and thus to contribute to its transport by Hb (14), may also be different from one solution to another, thus resulting in different vasoactive effects. The interaction of Hb with other regulatory elements involved at the vascular wall level (endothelin, α-adrenergic receptors) could also be specific to the type of modification applied to Hb and requires further investigation.

In summary, Dex-BTC-Hb, αα-Hb, and PolyHb have specific vasoactive effects, but in regard to the multiple parameters possibly involved, the proper action of the chemical modification is difficult to establish in vivo. The contribution of viscosity to the macrohemodynamic changes induced seems, however, to be blunted by the vasocostricting properties of Hb solutions.

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