Hemoglobin-based oxygen carrier HBOC-201 provides higher and faster increase in oxygen tension in skeletal muscle of anemic dogs than do stored red blood cells

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Background: **Increasing need for and potential shortage of blood products have intensified the search for alternative oxygen carriers. A solution to this problem could be use of the bovine hemoglobin-based oxygen carrier HBOC-201. While hemodynamic reactions to cell-free hemoglobin have been studied, little knowledge exists about tissue oxygenation properties of hemoglobin solutions, especially in comparison with red blood cells (RBCs).**

Study design and methods: **Tissue oxygenation in skeletal muscle of 12 anesthetized dogs was examined after decrease of hemoglobin concentrations by means of hemodilution to hematocrit 10% and subsequent transfusion with either HBOC-201 or autologous banked RBCs. In addition to hemodynamic parameters, blood gas concentrations and oxygen content in arterial and muscular venous blood, tissue oxygen tension (tPo_2) were measured in the gastrocnemius muscle with a polarographic needle probe.**

Results: **Hemodilution increased muscular blood flow and oxygen extraction and decreased tPo_2. Transfusion decreased muscular oxygen extraction in the RBC group but not in the HBOC-201 group ($P < .01$). The 10th percentile of tPo_2 increased by 400% after the first dose of HBOC-201 ($P < .001$ vs posthemodilution) but only by 33% after equivalent RBC transfusion ($P < .01$ vs HBOC-201). Increases in the 50th (120%, $P < .05$) and 90th (31%) percentiles and all percentiles of tPo_2 after the second and third HBOC-201 dose were less pronounced but higher than in the RBC group.**

Conclusion: **Compared with RBC transfusion, infusion of low doses of HBOC-201 maintain enhanced oxygen extraction after extended hemodilution and provide faster and higher increase in muscular tissue Po_2. (J Vasc Surg 2003;37: 859-65.)**

A remarkable shortage of allogeneic blood may occur in the industrialized countries within the next two or three decades.[1] Against this background and because of the remaining risks associated with the transfusion of blood components,[2-5] demand for artificial oxygen carriers could increase.[6,7] Modern cell-free hemoglobin-based oxygen carriers (HBOCs) have been used to overcome earlier problems related to impurities and red blood cell (RBC) debris by ultrapurification.[8,9] Clinical studies have documented the safety and tolerability of the ultrapurified polymerized bovine hemoglobin HBOC-201 in volunteers and patients.[10,11] Furthermore, there is evidence that HBOC-201 may diminish or eliminate the need for banked blood in patients undergoing major vascular surgery.[12,13] Indeed, South African authorities, faced with significant viral safety problems in the blood supply, in 2000 approved HBOC-201 for treatment of acute anemia. A unique feature of HBOC-201, room temperature stability for more than 2 years, offers the opportunity for widespread use of this material in clinical settings. Unlike unmodified human cell-free hemoglobin, which has a high oxygen affinity (p50 = 13 mm Hg), bovine hemoglobin has a low oxygen affinity (p50 = 36 mm Hg), which is regulated by plasma chloride concentration rather than 2,3-diphosphoglycerate.[14] Stable hemodynamic conditions during resuscitation from hemorrhagic shock[15,16] or during nearly complete blood exchange with HBOC-201 have been demonstrated,[17,18] as has increased tissue oxygen tension (tPo_2) during blood exchange[17] and in comparison with transfusion of different RBCs.[19] In a model of artificial arterial stenosis, HBOC-201 restored substantially decreased poststenotic tissue oxygen tension in skeletal muscle by increasing oxygen extraction,[20] suggesting that this material might have beneficial indications in vascular surgery, acute myocardial infarction, cerebrovascular accident (stroke), and chronic and acute vascular diseases. We investigated the clinically relevant question of equivalence or difference between cell-free and cellular oxygen carriers with respect to their tissue oxygenation potential.

METHODS

The study included 12 foxhounds and was approved by the local Animal Care Committee. On the day of the experiment the dogs (mean age, 20 ± 5 mo; mean weight, 29 ± 4 kg) were anesthetized with intravenous infusions of

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Competition of interest: none.

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fentanyl at 0.025 mg/kg/h, midazolam at 0.4 mg/kg/h, and vecuronium at 0.2 mg/kg/h, and mechanical ventilation was provided with 30% oxygen in air. Minute ventilation was adjusted to maintain end-expiratory partial pressure of carbon dioxide (Pco_2) constant (36-40 mm Hg; Normocap; Datex, Helsinki, Finland).

Measurements. An arterial line was inserted into the aorta to measure mean arterial pressure (MAP) and for blood sampling to measure arterial blood gas levels and arterial oxygen content (Cao_2). A 6F minitip catheter (Sensodyn FPO; B. Braun, Melsungen, Germany) was inserted into the left ventricle for continuous measurement of left ventricular end-diastolic pressure (LVEDP) and maintainance of intravascular volume over time (T). The left hind limb of the animals was prepared for measurements of the left gastrocnemius muscle. An electromagnetic flow probe (Cliniflow FK 701 D; Carolina Medical Electronics, Wilmington, NC) was placed at the left popliteal artery, which is the main vessel in the gastrocnemius muscle for measuring mean arterial blood flow to the muscle. A small catheter was inserted into the left popliteal vein for sampling venous blood from the left gastrocnemius muscle. Peripheral venous blood gases, venous lactate concentrations (lactate-specific test kit; Boehringer, Mannheim, Germany), and venous oxygen content ($Cvpo_2$)were measured. Oxygen concentrations were measured with an oxygen-specific fuel cell (Lex-O_2-Con, Lexington Instruments, Waltham, Mass).

tPO_2 was measured in the left gastrocnemius muscle with a microprocessor-controlled fast responding polarographic needle probe with 12.5 mm diameter (Helzel Medical Systems, Kaltenkirchen, Germany), yielding 200 single tPo_2 values within 5 minutes in a 2 to 3 cm^3 conical muscular tissue area. At each measurement 1200 single tPo_2 values were collected.

Plasma viscosity was measured with a viscosimeter (Rheomat; Fresenius, Bad Homburg, Germany) and colloid osmotic pressure (COP) with an oncometer (BMT 851; Thomae, Biberach, Germany).

Calculated variables. Variables were calculated with the following equations: muscular arteriovenous oxygen difference, $avDo_2m = Cao_2 - Cvpo_2$; muscular oxygen delivery, $Do_2m = Flow \times Cao_2$; muscular oxygen consumption, $Vo_2m = Flow \times avDo_2m$; and muscular oxygen extraction ratio, $ERo_2m = Vo_2m \times Do_2m^{-1}$.

Study protocol. Because previous experiments demonstrated that extreme isovolemic hemodilution decreases muscular tPo_2 significantly to less than 50% of baseline,[17] LVEDP-controlled hemodilution with Ringer solution was performed until Hct 10% was reached. The removed blood volume was replaced with a fourfold volume of Ringer solution, and control of Hct values was performed repetitively.

When Hct 10% was reached, the second measurement was performed after stabilization (T1) and before animals were infused with either HBOC-201 or canine RBCs, to obtain stepwise augmentation of total arterial hemoglobin concentration of approximately 1 g/dL (T2), 2 g/dL (T3), and 3 g/dL (T4).

In group 1, six animals received HBOC-201 (Biopure, Cambridge, Mass)[17] with a mean hemoglobin concentration of 13 ± 1 g/dL. In group 2, six dogs received 3-week-old banked autologous RBCs, which were stored at 4° C in 200 mL PAGGS-mannitol additive solution.

Statistics. Data are reported as mean ± SEM unless stated otherwise. Skeletal muscle tPo_2 values are expressed as median and plotted as 10th, 50th, and 90th percentiles. All single 1200 tPo_2 values were tested with the Mann Whitney U test. For all other parameters, differences within groups were tested with one-way analysis of variance and post hoc comparison with the paired Student t test. Differences between groups were tested with the unpaired Student t test. The slope ratio assay method was used to calculate estimates of relative potency between groups. Relative potency was estimated as the ratio of two slopes (HBOC-201/reference = RBCs) taken from the multiple linear regression of the posthemodilution time points. Ninety-five percent confidence limits around relative potency were calculated with the Fieller theorem. All differences were considered significant at $P < .05$.

The authors conceived and designed the study; had full access to all of the data from the study; and take responsibility for integrity of the data, accuracy of data analysis and interpretation, and for writing the manuscript and submitting it for publication.

RESULTS

Hemodynamic and rheologic parameters are given in Table I. Arterial and popliteal venous blood gas parameters, and core and skeletal muscle temperatures are shown in Table II.

Oxygen transport parameters. The target posthemodilution Hct of 10%± 1% was reached in both groups ($P < .001$) and remained unchanged during infusion of HBOC-201, but it was continuously increased during RBC transfusion in group 2 ($P < .001$ vs group 1; Table III). Total arterial hemoglobin concentration (cellular plus plasma) increased in both groups. Arterial and popliteal venous oxygen content was decreased during hemodilution ($P < .001$) and reversibly increased during application of the respective oxygen carrier in both groups, but popliteal venous oxygen content was lower during infusion of HBOC-201 when compared with group 2 during RBC transfusion ($P < .01$). Muscle oxygen delivery was decreased in both groups compared with baseline ($P < .05$), and it was higher for the RBC transfusion group compared with the HBOC-201 group at the end of infusion. Oxygen consumption was higher after the second and third infusions of HBOC-201 in comparison with group 2 ($P < .05$). The ERo_2 was increased after hemodilution in both groups ($P < .05$; Fig 1). In the RBC group ERo_2m returned to baseline during transfusion, but it remained elevated during infusion of HBOC-201 ($P < .01$) and was higher in group 1 than in group 2 ($P < .01$ vs baseline).

Table I. Hemodynamic and rheologic parameters before and after hemodilution and during decrease and increase in global hemoglobin concentration with HBOC-201 or RBCs

	MAP (mm Hg)	*LVEDP (mm Hg)*	*Flow (mL/min)*	*COP (mm Hg)*	*Vis (cp)*	*V.p.Lactate (mg/dL)*
HBOC-201						
Baseline	149 ± 16*	14 ± 2	50 ± 10	15.8 ± 0.7	1.28 ± 0.03	12.5 ± 3.2
Time 1	105 ± 5†	13 ± 2	123 ± 19†	12.4 ± 0.5†	1.18 ± 0.02†	8.7 ± 0.5
Time 2	112 ± 7	10 ± 2	114 ± 18†	11.1 ± 0.5†	1.17 ± 0.01†	9.6 ± 0.6
Time 3	110 ± 8	9 ± 2†	112 ± 16†	10.8 ± 0.5†	1.19 ± 0.01†	9.9 ± 0.8
Time 4	110 ± 7	9 ± 2†	100 ± 18†	10.9 ± 0.5†	1.24 ± 0.01*	9.9 ± 0.8
RBC						
Baseline	118 ± 7	11 ± 1	62 ± 9	14.3 ± 1.8	1.26 ± 0.03	11.1 ± 4.6
Time 1	98 ± 8	10 ± 2	149 ± 25†	12.4 ± 0.8†	1.14 ± 0.03†	8.2 ± 1.0
Time 2	103 ± 7	8 ± 2	121 ± 15†	11.5 ± 0.6†	1.16 ± 0.02†	8.6 ± 0.5
Time 3	104 ± 8	8 ± 2	110 ± 16†	10.4 ± 0.6†	1.17 ± 0.03†	9.6 ± 0.5
Time 4	107 ± 8	10 ± 2	112 ± 16†	9.2 ± 0.9†	1.17 ± 0.03†	9.6 ± 0.5

Values are expressed as mean ± SEM.
MAP, Mean arterial pressure; *LVEDP,* left ventricular end-diastolic pressure; *Flow,* popliteal artery blood flow; *COP,* colloid oncotic pressure; *Vis,* plasma viscosity; *V.p.Lactate,* popliteal venous lactate concentration.
*$P < .05$ compared with RBC; †$P < .05$ compared with baseline.

Table II. Arterial and popliteal vein blood gas level and temperature before and after hemodilution and during decrease and increase in global hemoglobin concentration with HBOC-201 or RBCs

	Pa_{O_2} (mm Hg)	*Arterial pH*	*Arterial base excess (mmol/L)*	*V.p.Po_2 (mm Hg)*	*V.p.pH*	*V.p.BE (mmol/L)*	*Core temp (° C)*	*Muscle temp (° C)*
HBOC-201								
Baseline	137 ± 4	7.39 ± 0.02	−1.5 ± 0.9*	52 ± 4	7.35 ± 0.02	−0.7 ± 0.7	36.2 ± 0.5	33.4 ± 0.7
Time 1	135 ± 7	7.24 ± 0.01†	−8.8 ± 0.4*†	46 ± 3	7.18 ± 0.02†	−9.9 ± 0.4†	37.0 ± 0.3	33.7 ± 0.5
Time 2	139 ± 6	7.27 ± 0.01†	−8.4 ± 0.2†	43 ± 5	7.18 ± 0.01*†	−9.3 ± 0.4†	36.8 ± 0.3	33.8 ± 0.5
Time 3	139 ± 4	7.26 ± 0.01*†	−8.5 ± 0.3*†	51 ± 4	7.18 ± 0.01†	−9.5 ± 0.5†	37.0 ± 0.3	33.9 ± 0.5
Time 4	136 ± 11	7.24 ± 0.01†	−9.2 ± 0.3†	57 ± 5	7.19 ± 0.01†	−8.6 ± 1.1*†	37.1 ± 0.3	34.1 ± 0.4
RBC								
Baseline	137 ± 5	7.36 ± 0.02	−3.8 ± 0.5	58 ± 2	7.31 ± 0.01	−3.1 ± 0.6	36.9 ± 0.4	34.4 ± 0.5
Time 1	135 ± 8	7.23 ± 0.01†	−10.1 ± 0.4†	50 ± 3	7.16 ± 0.01†	−10.5 ± 0.3†	37.6 ± 0.5	34.9 ± 0.6
Time 2	136 ± 5	7.26 ± 0.04†	−9.4 ± 0.4†	52 ± 8	7.16 ± 0.01†	−10.4 ± 0.4†	37.6 ± 0.4	34.7 ± 0.6
Time 3	141 ± 6	7.23 ± 0.01†	−10.1 ± 0.6†	65 ± 4	7.17 ± 0.01†	−10.6 ± 0.3†	37.2 ± 0.4	34.5 ± 0.5
Time 4	145 ± 7	7.24 ± 0.01†	−10.3 ± 0.8†	63 ± 3	7.18 ± 0.01†	−10.9 ± 0.5†	37.3 ± 0.4	34.3 ± 0.4

Values are expressed as mean ± SEM.
V.p.Po_2, Popliteal vein partial pressure of oxygen; *V.p.pH,* popliteal vein pH; *V.p.BE,* popliteal vein base excess; Core temp, cava superior; Muscle temp, skeletal muscle temperature (left gastrocnemius muscle).
*$P < .05$ compared with RBC.
†$P < .05$ compared with baseline.

Skeletal muscle oxygen tension. In both groups a significant decrease in muscular tPo_2 values was seen after hemodilution to Hct 10% ($P < .001$; Fig 2). The first hemoglobin augmentation (T2) provided a steep increase of all tPo_2 percentiles in the HBOC-201 group, but it was more pronounced for the 10th percentile, which showed an increase of 400% compared with posthemodilution measurements ($P < .001$, T2 vs T1; Fig 2). The respective increase in the 50th percentile was 120% ($P < .001$, T2 vs T1), and in the 90th percentile it was 31% ($P < .05$, T2 vs T1) compared with posthemodilution values.

The second hemoglobin augmentation with HBOC-201 (T3) continued to increase at all tPo_2 percentiles, but the enhancement was not as high as after the first application. Again, the highest increase noted was for the 10th percentile (525%) compared with posthemodilution values ($P < .001$, T3 vs T1). For the 50th percentile the increase was 186% ($P < .001$, T3 vs T1), and for the 90th percentile it was 64% ($P < .05$, T3 vs T1).

The third infusion of HBOC-201 (T4) provided additional but less pronounced increase at all percentiles when compared with the first two infusions. The greatest enhancement was again registered for the 10th percentile (625% over posthemodilution values; $P < .001$, T4 vs T1). The respective increase for the 50th percentile was 253% ($P < .001$, T4 vs T1), and for the 90th percentile it was 100% ($P < .001$, T4 vs T1).

In group 2, RBC transfusion provided a less accentuated increase in tPo_2 percentiles compared with HBOC-201 (Fig 2). The 10th percentile increased by 33% after the first transfusion (T2), by 50% after the second transfusion (T3), and by 180% after the third transfusion ($P < .05$, T4

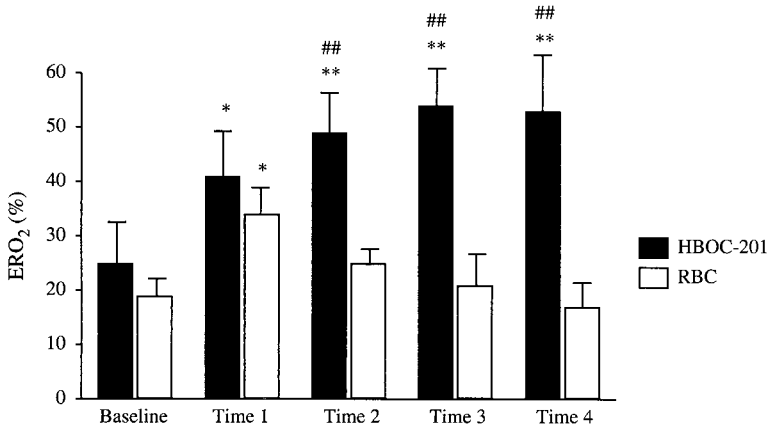

Fig 1. Changes in muscular oxygen extraction ratio (ERO_2) during decrease and increase in global hemoglobin concentration with HBOC-201 or RBCs. Values are expressed as mean ± SEM. $*P < .05$; $**P < .01$; $***P < .001$ compared with baseline; $\#P < .05$; $\#\#P < .01$ compared with RBC group.

Table III. Muscular oxygen transport parameters before and after hemodilution and during decrease and increase in global hemoglobin concentration with HBOC-201 or RBCs

	Hct (Vol%)	*Hb (g/dL)*	*C_aO_2 (mL/dL)*	*$C_{vp}O_2$ (mL/dL)*	*$avDO_2m$ (mL/dL)*	*DO_{2m} (mL/min)*	*VO_{2m} (mL/min)*
HBOC-201							
Baseline	48.0 ± 1.6	15.9 ± 0.6	20.8 ± 1.0	15.9 ± 1.9	4.9 ± 1.1	10.1 ± 1.7	2.6 ± 0.9
Time 1	9.3 ± 0.4†	2.8 ± 0.2†	4.2 ± 0.3†	2.4 ± 0.3†	1.8 ± 0.5†	5.1 ± 0.9†	2.0 ± 0.3
Time 2	9.4 ± 0.6*†	3.5 ± 0.3†	4.9 ± 0.6†	2.4 ± 0.3*†	2.5 ± 0.6	5.1 ± 0.6†	2.4 ± 0.3
Time 3	10.0 ± 1.1*†	4.4 ± 0.3†	5.5 ± 0.6†	2.6 ± 0.5*†	2.9 ± 0.5*	6.0 ± 0.8†	3.0 ± 0.2*
Time 4	10.3 ± 1.5*†	5.7 ± 0.5†	6.9 ± 0.6*†	3.2 ± 0.6*†	3.7 ± 0.8*	6.7 ± 0.9*†	3.5 ± 0.7*
RBC							
Baseline	44.5 ± 2.0	14.5 ± 0.6	19.3 ± 0.8	15.6 ± 0.6	3.7 ± 0.5	11.7 ± 1.5	2.2 ± 0.3
Time 1	9.5 ± 0.3†	2.8 ± 0.2†	4.1 ± 0.3†	2.7 ± 0.2†	1.4 ± 0.1†	6.2 ± 1.0†	2.0 ± 0.2
Time 2	12.8 ± 0.5†	4.0 ± 0.3†	5.6 ± 0.4†	4.2 ± 0.3†	1.4 ± 0.2†	7.0 ± 1.2†	1.7 ± 0.3
Time 3	16.3 ± 0.7†	5.2 ± 0.3†	7.1 ± 0.4†	5.6 ± 0.3†	1.5 ± 0.3†	7.8 ± 1.2†	1.5 ± 0.3
Time 4	20.2 ± 1.0†	6.4 ± 0.3†	8.7 ± 0.4†	7.2 ± 0.4†	1.5 ± 0.2†	10.0 ± 1.8	1.7 ± 0.3

Values are expressed as mean ± SEM.

Hct, Hematocrit; *Hb,* total hemoglobin concentration; C_aO_2, arterial oxygen content; $C_{vp}O_2$, popliteal venous oxygen content; $avDO_{2m}$, muscular arteriovenous oxygen difference; DO_{2m}, muscular oxygen delivery; VO_{2m}, muscular oxygen consumption.

$*P < .05$ compared with RBC.

$^{\dagger}P < .05$ compared with baseline.

vs T1) compared with the posthemodilution measurement (T1), and it reached baseline values only after the second RBC transfusion (Fig 2).

The 50th percentile increased by 30%, 50%, and 105% ($P < .001$, T4 vs T1) over posthemodilution measurements after the respective transfusions, and it reached baseline values between transfusions 2 and 3.

The 90th percentile showed similar increases of 45%, 65%, and 84% ($P < .001$, T4 vs T1) over posthemodilution values. However, baseline values could not be restored even after the third RBC transfusion.

Differences in muscular tPO_2 enhancement between the two treatment groups are demonstrated in Figure 2 by the different slopes of predicted tPO_2 values. The ratio of the respective slopes at the respective percentiles provides the relative potency of HBOC-201 in comparison with banked RBCs. These results are given in Table 4 and show a relative potency of HBOC-201 in the range of 2.3 to 3.0 compared with RBC. Nonlinearity of the 10th percentile (Table 4) means that the response of the first treatment with HBOC-201 may be greater than the response of the last treatment.

DISCUSSION

Because of limited clinical experience with HBOC-201,[11,12] experimental data that enable comparative assessment of the efficacy of HBOC-201 and commonly transfused RBCs could be helpful in terms of dosing and indications for clinical use of HBOC-201.

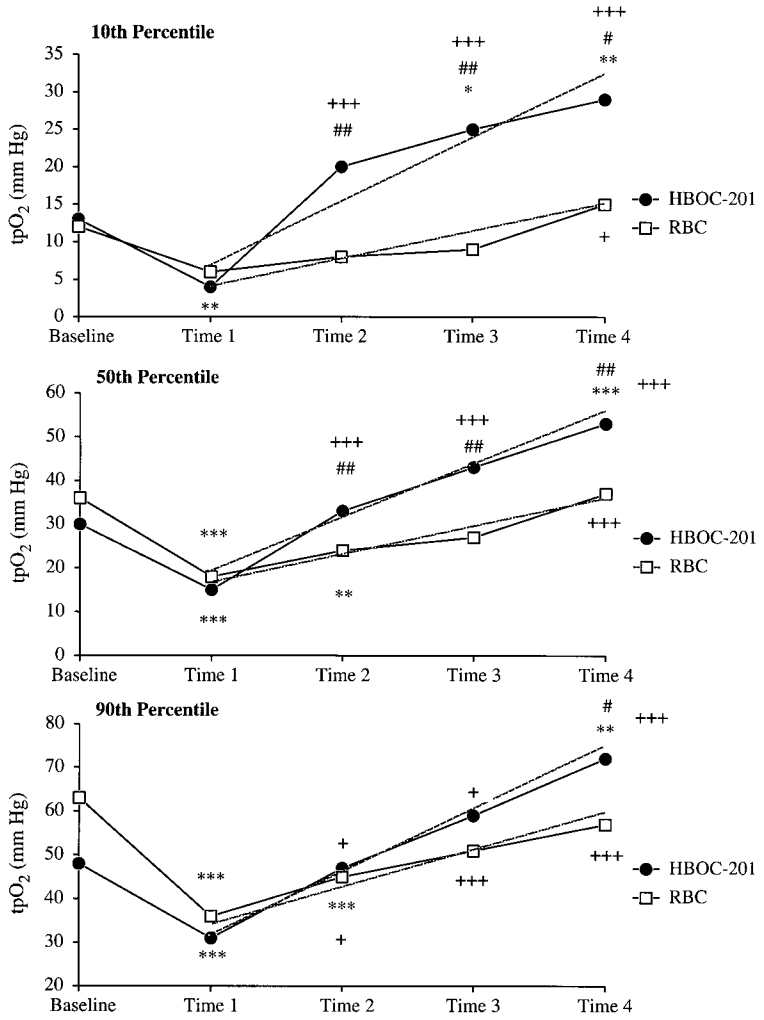

Fig 2. Observed and predicted values (dotted lines) of skeletal muscle tPO2 at the 10th, 50th , and 90th percentiles during decrease and increase in global hemoglobin concentration with HBOC-201 or RBCs. **P* < .05; ***P* < .01; *** *P* < .001 compared with baseline; # *P* < .05; ## *P* < .01 compared with RBC group; + *P* < .05; ++ *P* < .01; +++ *P* < .001 compared with T1.

Table IV. Summary of slope ratio assay method for HBOC-201 and RBCs

	Slopes (P)		*Relative potency HBOC-201/ RBC*	P	
tPo_2	*HBOC-201*	*RBC*		*Heterogeneity of intercepts*	*Nonlinearity*
10th percentile	**9.2 (.00)**	**3.0 (.00)**	3.03	0.64	**.04**
50th percentile	**14.5 (.00)**	**6.2 (.00)**	2.34	0.76	.25
90th percentile	**15.1 (.00)**	**5.8 (.00)**	2.59	0.72	.47

Note: Statistically significant *P* values ($\alpha < .05$) from testing for heterogeneity of intercepts or nonlinearity and slopes that are significantly different from zero ($\alpha < .05$) are in bold type.

For this reason, we investigated tissue oxygenation properties during infusion with HBOC-201 compared with transfusion of banked RBCs after extended isovolemic hemodilution in a model of an in vivo skeletal muscle. This situation is common in the operating room when there is major intraoperative bleeding, eg, during vascular surgery.

To decrease muscular tPo_2 to an acceptable level, we performed LVEDP-controlled isovolemic hemodilution,

inasmuch as tPo_2 is significantly decreased by reducing Hct to 10%.[17,19,21]

Hemodynamics and oxygen transport parameters. Our data show comparable hemodynamic changes during isovolemic hemodilution in both groups. Acidosis was also seen in both groups after initiating hemodilution, and it continued throughout the study, although hemodynamic and temperature stability could be achieved. Considering the observed stable muscular venous lactate concentrations, this metabolic acidosis can be attributed to loss of buffers, eg, bicarbonate, from continuous blood exchange with bicarbonate-free Ringer solution and to increased diuresis during hemodilution rather than anemia-related hypoxia.

Reduction of RBCs leading to lower blood viscosity causes at least partial increase in blood flow, which might be necessary to maintain oxygen delivery. Oxygen delivery decreased and oxygen consumption was constant after extended hemodilution. Despite increased muscle oxygen extraction in both groups, the increase in blood flow to the muscular compartment alone could not compensate for the reduced oxygen delivery and resulted in significantly decreased muscular tPo_2 values at the end of hemodilution. When the hemodiluted animals were given RBC transfusions, ERo_2m decreased toward baseline values. In contrast, in the HBOC-201 group, ERo_2m remained higher compared with baseline measurements and with the RBC group. This higher ERo_2m was paralleled by increased tPo_2 values in the HBOC-201 group. The sustained higher ERo_2m was not caused by decreased flow due to vasoconstriction after HBOC-201 infusion, because the flow and Do_2m did not differ between groups over time and the muscle was in steady state (temperature and venous lactate concentration constant, muscle paralysis by relaxation). There are several explanations for the enhanced oxygen extraction in the HBOC-201 group. First, HBOC-201 has a low oxygen affinity compared with human or canine RBC hemoglobin, which facilitates oxygen off-loading to the tissues.[14] In addition, HBOC-201 increases the oxygen off-load from remaining RBCs. In an in vitro model with an artificial capillary, HBOC-201, when mixed with RBCs, increased oxygen release from RBCs compared with RBC oxygen release alone.[22]

The high ERo_2 with HBOC-201 is therefore neither dependent on nor caused by reduced oxygen delivery, which has been noted in animal experiments and clinical studies after application of different cell-free hemoglobin solutions.[23,24] In some clinical trials with cell-free hemoglobin, systemic vasoconstriction, possibly due to nitric oxide scavenging, was associated with increased systemic vascular resistance and mean arterial pressure and with consecutive decrease in cardiac output. Because of the low dose of cell-free hemoglobin administered in the current study, the small increase in oxygen content did not compensate for the decrease in cardiac output, resulting in a demonstrated reduction in calculated oxygen delivery. However, oxygen consumption was well maintained and was associated with increased ERo_2. Preclinical animal studies have shown unaffected or even improved organ perfusion and microcirculatory blood flow in the presence of cell-free hemoglobin solutions, although systemic vascular resistance and mean arterial pressure were increased.[25,26] However, colloid osmotic pressure and plasma viscosity were finally higher in the HBOC-201 group because of the colloidal properties of the hemoglobin solution. Thus it remains unclear whether there was also a contribution from the changed colloid osmotic pressure and the blood or plasma viscosity to changes in muscular tPo_2.

Tissue oxygenation and relative potency of HBOC-201. The exact mathematical calculation of the relative oxygenation potential of both acellular and cellular oxygen carriers means that smaller doses of HBOC-201 will provide the same oxygenation effect in comparison with 3-week-old stored RBCs, which may suffer from aging, storage, and preparation processes, although rejuvenation occurs in banked RBCs within the first hours after transfusion.

The result that tissue areas with significantly reduced tPo_2 profit most by infusion of acellular hemoglobin might be explained in that HBOC-201, in contrast to RBCs, is not prioritized on the microcirculatory site. This makes an increase in tPo_2 faster and more pronounced in poorly oxygenated tissues, represented by the 10th percentile of the tPo_2 values, which are less supplied by RBCs at this time. However, the lower increase in tPo_2 in the 50th and 90th percentiles indicates that HBOC-201 does not have the same high oxygenation potential for tPo_2 enhancement in areas where high oxygen off-loading and tissue oxygenation are already provided by high RBC perfusion and oxygen off-loading, thus possibly preventing adequately supplied tissues from hyperoxygenation.

Another aspect of our results is the declining effect of HBOC-201 increasing muscular tissue oxygenation to the same extent by further application of the drug. This makes a certain first-pass effect of HBOC-201 likely, in which all tissues are reached by HBOC-201 with the first plasma stream and probably the tissues with low tPo_2 extract the highest amount of oxygen and are approximately saturated when the next dose of HBOC-201 is given. This effect equilibrates the distribution between the oxygen in plasma and tissue and may also prevent the tissues from becoming overloaded with oxygen.

The early first-pass effect of a low dose of HBOC-201 offers opportunities in experimental and clinical settings such as profound acute anemia or ischemia and reperfusion. These indications, where fast increase in tissue oxygenation is crucial, include tissue hypoxia due to arterial stenosis, myocardial infarction, stroke, and arterial clamping during vascular surgery.

In conclusion, HBOC-201 has a 2.3-fold to 3-fold higher first- pass oxygenation potential compared with banked RBCs, providing faster and higher increase in tPo_2 in muscular tissue with reduced oxygenation after acute anemia.

Limitations of the study. We did not use an isolated muscle model, because we wanted to control clinically

relevant hemodynamic parameters such as blood flow and to maintain isovolemic conditions. Since the popliteal artery and vein are the main vessels of the gastrocnemius muscle, the values obtained should reflect representative data for this muscle but certainly not for other organs or for an entire organism. However, although less important than vital organs, the skeletal muscle mass represents a major part of mammal and human tissue, is easily accessible, and can be used to measure tPo_2 even in patients, as shown by Boekstegers et al[27] in patients undergoing cardiac surgery. Under steady-state conditions (temperature, paralysis), muscular tPo_2 measurements are highly reproducible.[17,19,21]

Unlike in group 2, autologous blood was not available for animals receiving HBOC-201. As a consequence, these animals demonstrated a trend to lower baseline Hct compared with the RBC group. However this difference was not significant. The more important Hct for the evaluation of oxygenation potency is the posthemodilution value (T2), which figures as a second baseline value before initiation of therapy with the respective oxygen carrier. These Hct values were nearly identical: 9.3 ± 0.4 for the HBOC-201 group versus 9.5 ± 0.3 for the RBC group.

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REFERENCES

1. Sullivan MT, Wallace EL, Umana WO. Trends in the collection and transfusion of blood in the United States, 1987-1997. Tansfusion 1999;39(suppl):1S.
2. Blumberg N, Agarwal MM, Chuang C. Relation between recurrence of cancer of the colon and blood transfusion. BMJ 1985;290:1037-9.
3. Goodnough LT, Brecher ME, Kanter MH, AuBuchon JP. Transfusion medicine. Part I: Blood transfusion. N Engl J Med 1999;340:438-47.
4. Hébert PC, Wells G, Blajchman MA, Marschall J, Martin C, Pagliarello G, et al. A multicenter, randomized, controlled clinical trial of transfusion requirements in critical care. Transfusion Requirements in Critical Care Investigators, Canadian Critical Care Trials Group. N Engl J Med 1999;340:409-17.
5. Lackritz EM, Satten GA, Aberle-Grasse J, Dodd RY, Raimondi VP, Janssen RS, et al. Estimated risk of transmission of the human immunodeficiency virus by screened blood in the United States. N Engl J Med 1995;333:1721-5.
6. Cohn SM. The current status of haemoglobin-based blood substitutes. Ann Med 1997;29:371-6.
7. Nucci ML, Abuchowski A. The search for blood substitutes. Sci Am 1998;278:72-7.
8. Lee R, Atsumi N, Jacobs EE, Austen WG, Vlahakes, GJ. Ultrapure, stroma-free, polymerized bovine hemoglobin solution: Evaluation of renal toxicity. J Surg Res 1989;47:407-11.
9. Lipfert B, Standl T, Dullmann J, Helmchen U, Schulte am Esch J, Lorke DE. Histology and ultrastructure of liver and kidney following blood exchange with ultrapurified, polymerised bovine hemoglobin in comparison with hydroxyethyl starch. Lab Invest 1999;79:573-82.
10. Hughes GS, Antal EJ, Locker PK, Francom SF, Adams WJ, Jacobs EE. Physiology and pharmacokinetics of a novel hemoglobin-based oxygen carrier in humans. Crit Care Med 1996;24:756-64.
11. Standl T, Burmeister MA, Horn EP, Wilhelm S, Knoefel W, Schulte am Esch J. Bovine haemoglobin-based oxygen carrier for patients undergoing haemodilution before liver resection. Br J Anaesth 1998;80:189-94.
12. LaMuraglia GM, O'Hara PJ, Baker WH, Naslund TC, Norris EJ, Li J, et al. The reduction of the allogenic transfusion requirement in aortic surgery with a hemoglobin-based solution. J Vasc Surg 2000;31:299-308.
13. Sprung J, Popp H, O'Hara P, Woletz J. The successful use of hemoglobin-based oxygen carrier as a primary blood substitute during abdominal aneurysm repair with large blood loss. Anesth Analg 2001;92: 1413-5.
14. Fronticelli C, Bucci E, Orth C. Solvent regulation of oxygen affinity in hemoglobin. Sensitivity of bovine hemoglobin to chloride ions. J Biol Chem 1984;259:10841-4.
15. Bosman RJ, Minten J, Lu HR, Van Aken H, Flameng W. Free polymerized hemoglobin versus hydroxyethyl starch in resuscitation of hypovolemic dogs. Anesth Analg 1992;75:811-7.
16. Harringer W, Hodakowski GT, Svizzero T, Jacobs EE, Vlahakes GJ. Acute effects of massive transfusion of a bovine hemoglobin blood substitute in a canine model of hemorrhagic shock. Eur J Cardiothorac Surg 1992;6:649-54.
17. Standl TG, Reeker W, Redmann G, Kochs E, Werner C, Schulte am Esch J. Haemodynamic changes and skeletal muscle oxygen tension during complete blood exchange with ultrapurified polymerized bovine haemoglobin. Intensive Care Med 1997;23:865-72.
18. Vlahakes GJ, Lee R, Jacobs EE Jr, LaRaia PJ, Austen WG. Hemodynamic effects and oxygen transport properties of a new blood substitute in a model of massive blood replacement. J Thorac Cardiovasc Surg 1990;100:379-88.
19. Standl T, Horn P, Wilhelm S, Greim C, Freitag M, Freitag U, et al. Bovine haemoglobin is more potent than autologous red blood cells in restoring muscular tissue oxygenation after profound isovolaemic haemodilution in dogs. Can J Anaesth 1996;43:714-23.
20. Horn EP, Standl T, Wilhelm S, Jacobs EE, Freitag U, Freitag M, et al. Bovine hemoglobin increases skeletal muscle oxygenation during 95% artificial arterial stenosis. Surgery 1997;121:411-8.
21. Freitag M, Standl T, Horn E, Wilhelm S, Schulte am Esch J. Acute normovolaemic haemodilution beyond Hct 25%: The ratio of skeletal muscle tissue oxygen tension and cardiac index is not maintained in healthy dogs. Eur J Anaesth 2002;19:487-94.
22. Page TC, Light WR, McKay CB, Hellums JD. Oxygen transport by erythrocyte/hemoglobin solution mixtures in an in vitro capillary as a model of hemoglobin-based oxygen carrier performance. Microvasc Res 1998;55:54-64.
23. De Figueiredo LF, Mathru M, Solanki D, Macdonald VW, Hess J, Kramer GC. Pulmonary hypertension and systemic vasoconstriction may offset the benefits of acellular hemoglobin blood substitutes. J Trauma 1997;42:847-56.
24. Kasper SM, Walter M, Grune F, Bischoff A, Erasmi H, Buzello W. Effects of a hemoglobin-based oxygen carrier (HBOC-201) on hemodynamics and oxygen transport in patients undergoing preoperative hemodilution for elective abdominal aortic surgery. Anesth Analg 1996;83:921-7.
25. Gulati A, Sharma AC, Burhop KE. Effect of stroma-free hemoglobin and diaspirin cross-linked hemoglobin on the regional circulation and systemic hemodynamics. Life Sci 1994;55:827-37.
26. Sherman IA, Dlugosz JA, Perelman V, Hsia CJ, Wong LT, Condie RM. Systemic hemodynamic and hepatic microvascular responses to a 33% blood volume exchange with whole blood, stroma-free hemoglobin, and oxypolyhemoglobin solutions. Biomater Artif Cells Immobilization Biotechnol 1993;21:537-51.
27. Boekstegers P, Riessen R, Seyde W. Oxygen partial pressure distribution within skeletal muscle: Indicator of whole body oxygen delivery in patients? Adv Exp Med Biol 1990;277:507-14.

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