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Effects of Ultra-Purified Polymerized Bovine Hemoglobin on Local Tissue Oxygen Tension in Striated Skin Muscle – An Efficacy Study in the Hamster

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Key Words

Hemoglobin-based oxygen carriers · Blood substitutes · Tissue oxygenation

Abstract

The development of hemoglobin-based oxygen carriers has been propagated for replacement of the oxygen carrying properties of red blood cells for almost one century. Using a Clark-type multi-wire oxygen surface electrode and the dorsal skin fold chamber model of the awake Syrian golden hamster, local tissue pO_2 was analyzed in the thin striated skin muscle before and after administration of an ultrapurified polymerized bovine hemoglobin solution (U-PBHb®, Biopure Corp., Boston, Mass., USA) under the following experimental conditions: (a) hypervolemic infusion with U-PBHb at ~10% of calculated blood volume, and (b) isovolemic exchange transfusion with U-PBHb by replacing ~50% of calculated blood volume. Control animals of group a received equivalent treatment with either isotonic saline or dextran 60, control animals of group b received dextran 60. Local tissue pO_2 was found slightly decreased after both hypervolemic infusion and isovolemic exchange transfusion with U-PBHb, while frequency distribution curves of local tissue pO_2 were found more narrow (less values <10 mm Hg and >25 mm Hg), suggesting a more homogeneous tissue pO_2 distribution. The data thus indicate that U-PBHb slightly decreases mean tissue pO_2 after both hypervolemic infusion and isovolemic exchange transfusion which is accompanied by an effective homogenization of local tissue pO_2 distribution as compared to dextran 60.

Introduction

Red cell substitutes derived from either recombinant, transgenic, or chemically modified natural hemoglobins are a promising approach to cover the enormous demand for red blood cell concentrates. Some of those have meanwhile also undergone clinical phase I and II trials in healthy volunteers and patients, respectively [1, 2].

U-PBHb® is an iso-oncotic solution of glutaraldehyde-polymerized α- and β-chains of bovine hemoglobin. This material has previously been investigated in a microcirculation model in the hamster allowing chronic visualization of the intact microcirculation of striated skin muscle in conscious animals: even under the condition of resuscitation from hemorrhagic shock, no enhancement of leukocyte/endothelial cell interaction or macromolecular leakage across the microvascular wall was observed indicating the abscence of unwanted side effects in the microcirculation during ischemia/reperfusion. U-PBHb was found a safe resuscitation fluid, superior to commonly used resuscitation fluids like Ringer's solution or dextran 60 [3].

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The aim of the present study was to prove these microcirculatory effects of U-PBHb as an oxygen-carrying red cell substitute by assessing both mean tissue pO_2 and its spatial distribution pattern. This is of particular interest due to the influence of the hemoglobin molecule on arteriolar constriction and its sequelae on local tissue oxygenation. To allow direct comparability to our microcirculation study in the striated skin muscle [3], we used the same animal model and experimental protocols for analysis of local tissue pO_2. Animals were subjected to the protocols of (a) hypervolemic infusion and (b) isovolemic exchange transfusion of ~50% of the estimated blood volume to a hematocrit of ~30%, thus providing direct correlation between the already obtained microcirculation data and tissue oxygenation properties. Mean tissue pO_2 and spatial pO_2 distribution were monitored prior to and after administration of U-PBHb or respective control fluids using the Clark-type multi-wire oxygen surface electrode [4, 5].

Table 1. Properties of U-PBHb solutions

Parameter	U-PBHb®
Hemoglobin	13 g/dl
Methemoglobin content	<10%
pO_{50}	34 mm Hg
pH	7.8
Colloid osmotic pressure	20 mm Hg
Osmolarity	295 mmol/kg
Na^+	150 mmol/l
K^+	4.0 mmol/l
Cl^-	145 mmol/l
M_r	64–500 kDa
Phospholipids	<3 nmol/ml
Endotoxin content	<0.5 EU/ml

Data provided by Biopure Corp., Boston, Mass., USA.

Material and Method

Animal Model

Effects of hypervolemic infusion and isovolemic exchange transfusion on local tissue pO_2 were analyzed in male Syrian golden hamsters (6–8 weeks old, 50–70 g body weight). All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1985). Fitted with a dorsal skin fold chamber [6, 7], the preparation allows either intravital microscopic investigation or local tissue pO_2 analysis of a thin striated skin muscle consisting of arterioles, capillaries, and postcapillary venules contained within the observation window. Chamber implantation and catheterization of the left carotid artery and jugular vein were performed under intraperitoneal pentobarbital anesthesia (60 mg/kg body weight, Nembutal®, Abbott, Wiesbaden, Germany) as previously described [3, 8]. Measurements were made after a recovery period of at least 24–48 h to eliminate the effects of surgical intervention and anesthesia. Only animals showing no alterations in sleeping, feeding, and hygiene habits, and no signs of inflammation, edema, or low flow perfusion in the microvascular bed [9] underwent the experimental protocols.

Local Tissue pO_2 Measurements

Using the Clark-type oxygen multi-wire surface electrode (MDO, Trolag, Schwabach, Germany) as introduced by Kessler and Lübbers [4, 5], both mean tissue pO_2 and its local distribution can be analyzed. The electrode consists of eight platinum wires and allows simultaneous monitoring of eight different tissue points by recording the pO_2 signals from a spheroid tissue volume of 12 mm in diameter. The employment of a micromanipulator [10] facilitates the gentle movement of the electrode in 8–10 0.5-mm steps permitting assessment of pO_2 at n = 64–80 local data points per animal and time point of investigation. The pO_2 values were registered from the striated skin muscle and classified into 5-mm ranges versus frequency of occurrence. Therefore, the method allows measurement of both spatial and temporal variations of local tissue pO_2 values which range between zero and the arterial pO_2 [5]. With use of this method, the pO_2 distribution curve from the hamster dorsal skin fold tissue has been shown to be highly reproducible [11, 12] at baseline values in the range of 19.5 ± 1 mm Hg. These values are distinct from those reported for skeletal muscle tissue [4, 5, 13–15]. To permit superfusion of the tissue with isotonic saline (Delta Pharma GmbH, Pfullingen, Germany) and placement of the electrode, the cover slip of the skin fold chamber had to be removed from the observation window of titanium chambers before performance of the measurements. This procedure has been shown not to alter the microvascular perfusion and leukocyte/endothelial cell interaction in the underlying tissue when gently performed [16]. As reported by other researchers [17], the tissue temperature was kept between 24 and 26 °C, while calibration and experimental measurements were performed.

Hemoglobin Solutions

The ultrapurified polymerized bovine hemoglobin solution (U-PBHB®, table 1) was obtained from Biopure Corp. (Boston, Mass., USA). Preparation was performed in accordance with previously described techniques [18–20] consisting of hypertonic lysis of the blood cells, filtration, chromatography, and polymerization with glutaraldehyde and storage at room temperature. In comparison to human hemoglobin, the amino acid composition of an αβ-dimer of bovine hemoglobin shows 28 substitutions, whereof two are located in the β-chain and are suggested relevant for the decreased oxygen affinity of bovine hemoglobin when compared to human [21]. Furthermore, the oxygen affinity is not regulated by 2,3-diphosphoglycerate (2,3-DPG) but by chloride ions [22]. Therefore, in a cell-free preparation of bovine hemoglobin, there is no increase in oxygen affinity due to absence of intracellular allosteric regulators. The same solution has already been reported in a microcirculation study on safety and toxicity [3].

Experimental Design

The macrohemodynamic parameters mean arterial pressure (MAP) and heart rate (HR) were continuously monitored during the

Table 2. Mean arterial pressure (MAP) before and after hypervolemic infusion and isovolemic exchange transfusion

	MAP, mm Hg					
	baseline	5 min	25 min	40 min	55 min	70 min
Hypervolemic infusion						
NaCl 0.9 %	100 ± 7	105 ± 7	100 ± 7	95 ± 8	100 ± 6	105 ± 6
Dextran 60	100 ± 7	100 ± 6	95 ± 8	105 ± 6	102 ± 5	100 ± 7
U-PBHb	100 ± 7	115 ± 6	113 ± 7	117 ± 4	109 ± 10	111 ± 8
Isovolemic exchange transfusion						
Dextran 60	100 ± 7	102 ± 6	98 ± 4	105 ± 4	102 ± 6	98 ± 4
U-PBHb	100 ± 7	120 ± 6	115 ± 6	118 ± 4	110 ± 5	115 ± 4

Table 3. Heart rate (HR) before and after hypervolemic infusion and isovolemic exchange transfusion

	HR, min^{-1}					
	baseline	5 min	25 min	40 min	55 min	70 min
Hypervolemic infusion						
NaCl 0.9 %	352 ± 25	335 ± 23	352 ± 25	371 ± 30	351 ± 23	335 ± 21
Dextran 60	352 ± 25	351 ± 23	371 ± 30	335 ± 21	345 ± 16	352 ± 25
U-PBHb	352 ± 25	305 ± 17	310 ± 20	300 ± 10	323 ± 29	318 ± 26
Isovolemic exchange transfusion						
Dextran 60	352 ± 25	345 ± 19	356 ± 13	334 ± 13	345 ± 19	356 ± 13
U-PBHb	352 ± 25	292 ± 14	305 ± 15	298 ± 9	319 ± 14	305 ± 11

experiments using a Statham element (PD 23, Gould Statham, Calif., USA) connected to the indwelling carotid catheter and to a micropressure transducer and cardiovascular analyzer (Type Brush 2400, Gould-Allco, Ballainvilliers, France). In consistence with a previous microcirculatory study [3], the experimental protocol was as follows.

Hypervolemic Infusion

The influence of a low-dose administration of U-PBHb on the microcirculation of the striated skin muscle was determined by infusing the animals with 5 ml per kg body weight (~ 10% total blood volume; 500 mg/kg) of either isotonic saline (Delta Pharma GmbH, Pfullingen, Germany), 6% dextran 60 (Dx-60, Schiwa GmbH, Glandorf, Germany) or bovine hemoglobin solution (U-PBHb). Measurements of pO_2 values were performed prior to hypervolemic infusion (baseline) and 10, 30, and 60 min thereafter.

Isovolemic Exchange Transfusion

The effect of a 5.5-fold higher dose of U-PBHb was assessed by exchange transfusion of the animals with either 6% dextran 60 or hemoglobin solution at a rate of 0.3 ml/min i.v. to a final hematocrit of 30 ± 3%. The exchange volume amounted to ~ 50% of total blood volume which was calculated from 7% of total body weight [23]. Measurements of pO_2 values were made prior to exchange transfusion (baseline) and 10, 30, and 60 min thereafter.

Experimental Design

Thirty animals fulfilling the entry criteria [6] were involved in the study: 18 of them underwent hypervolemic infusion grouped into isotonic saline-treated animals (n = 6), dextran 60-treated animals (n = 6), and those who received U-PBHb (n = 6). Another 12 animals were subjected to the isovolemic exchange transfusion protocol and grouped into dextran 60 (n = 6) and U-PBHb-treated animals (n = 6).

Statistics

All data are given as mean ± standard deviation (SD). For all statistical tests the minimum level of significance considered was 5% ($p < 0.05$). For differences between groups, time-related changes were assessed with Kruskal-Wallis ANOVA (Sigma Stat® Version 1.0, 1992–1994, Jandel Corp., USA), for differences within groups (repeated measurements), Student-Newman-Keuls test was applied.

Results

Hypervolemic Infusion

MAP and HR. Under baseline conditions, MAP and HR ranged between 95 and 105 mm Hg and between 300 and 400 beats per minute, respectively. Hypervolemic infusion of isotonic saline or dextran 60 caused no changes of these parameters, whereas application of U-PBHb initiated an immediate increase in MAP by ~ 15% which remained elevated during the entire observation time of 60 min and a HR response in reciprocal manner (table 2, 3).

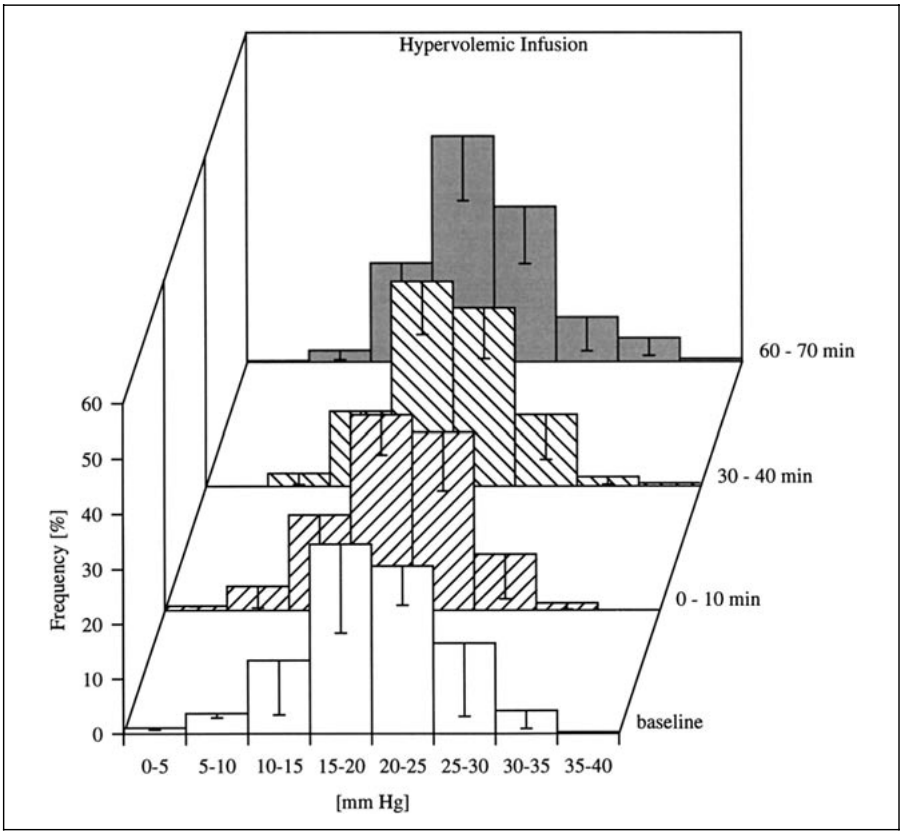

Fig. 1. Spatial distribution of tissue pO_2 before and after hypervolemic infusion of isotonic saline (n = 6, mean ± SD).

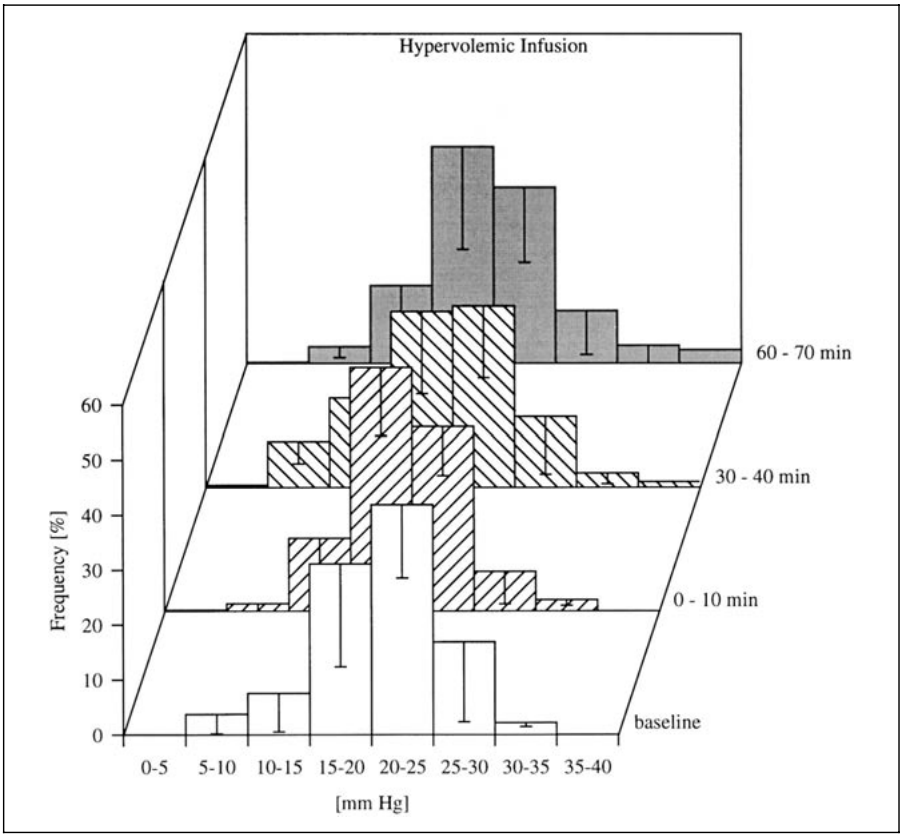

Fig. 2. Spatial distribution of tissue pO_2 before and after hypervolemic infusion of dextran 60 (n = 6, mean ± SD).

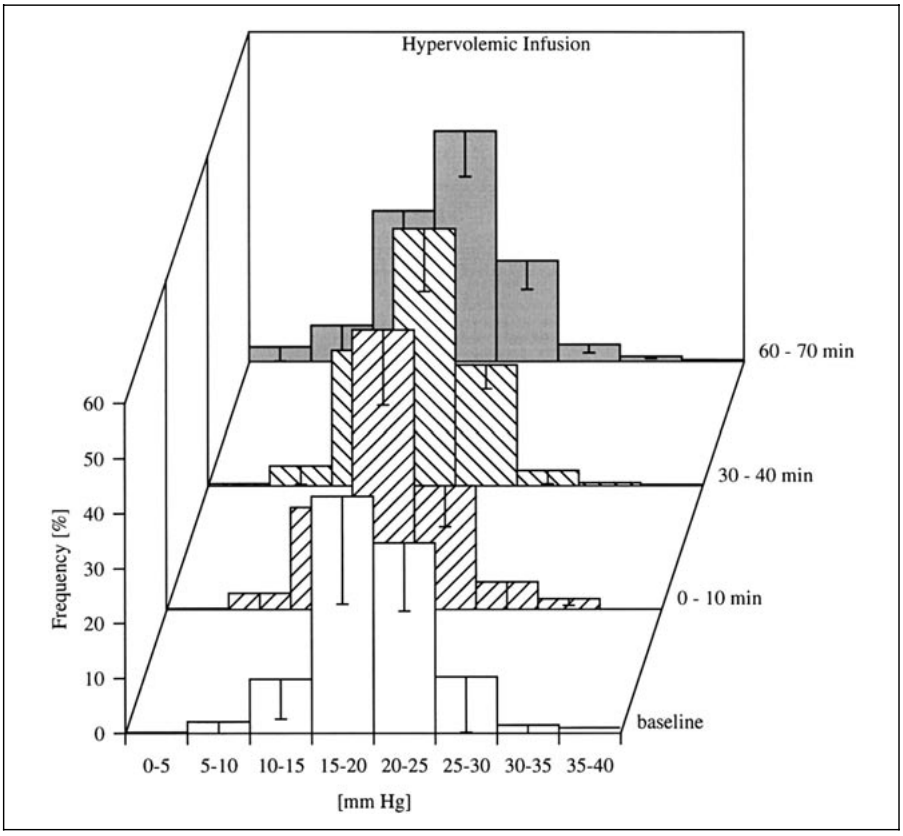

Fig. 3. Spatial distribution of tissue pO_2 before and after hypervolemic infusion of U-PBHb (n = 6, mean ± SD).

Local Tissue pO_2. Mean local tissue pO_2 in striated skin muscle after hypervolemic infusion of normal saline and dextran 60 disclosed no changes when compared with preinfusion values, while a 17% decrease was observed 1 h after infusion of U-PBHb (fig. 6). Analysis of the underlying local tissue pO_2 distribution curves demonstrates that mean local tissue pO_2 under baseline conditions and after treatment with either normal saline or dextran 60 mainly results from values in the ranges of 15–20 and 20–25 mm Hg (fig. 1–3), while application of U-PBHb led to a left shift of this distribution pattern by 5 mm Hg (fig. 3).

Isovolemic Exchange Transfusion

MAP and HR. Exchange transfusion with U-PBHb resulted in a 20% increase of MAP which was seen during the entire observation period and – similar to the results of hypervolemic infusion – in a HR response in reciprocal manner. These changes were absent after exchange transfusion with dextran 60, where only a slight decrease in MAP was noted (table 2, 3).

Local Tissue pO_2. Mean local tissue pO_2 in striated skin muscle decreased by 7% 1 h after isovolemic exchange transfusion with dextran 60, and by 27% after use of U-PBHb, respectively (fig. 7). While isovolemic exchange transfusion with dextran 60 did not alter tissue pO_2 distribution pattern (fig. 4), the treatment with U-PBHb resulted in a left shift by 5 mm Hg and a narrowing of this pattern (fig. 5), meeting the demand of adequate tissue oxygenation and avoiding tissue hypoxia (values in the range of 0–5 mm Hg).

Discussion

The present study suggests the effective homogenization of local tissue pO_2 by U-PBHb by providing a more homogeneous distribution of local tissue oxygen tensions under the condition of isovolemic exchange tranfusion as assessed in the striated skin muscle of conscious Syrian golden hamsters.

Similar to findings by others with U-PBHb [24, 25], further compositions of bovine [26, 27] and other mam-

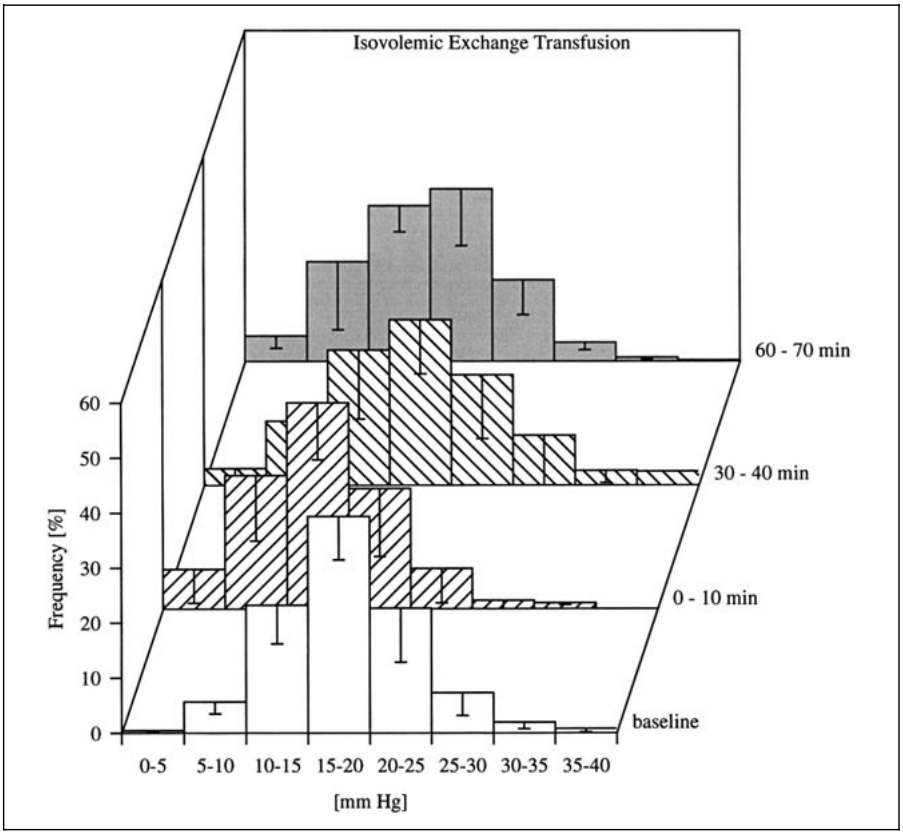

Fig. 4. Spatial distribution of tissue pO_2 before and after isovolemic exchange transfusion of dextran 60 (n = 6, mean ± SD).

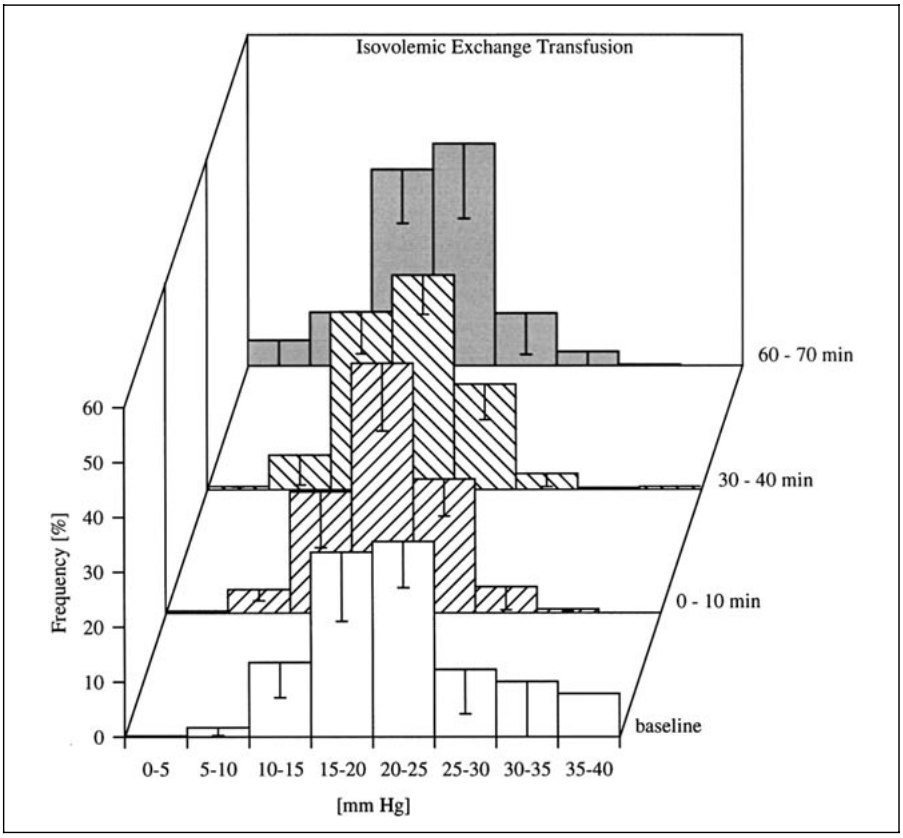

Fig. 5. Spatial distribution of tissue pO_2 before and after isovolemic exchange transfusion of U-PBHb (n = 6, mean ± SD).

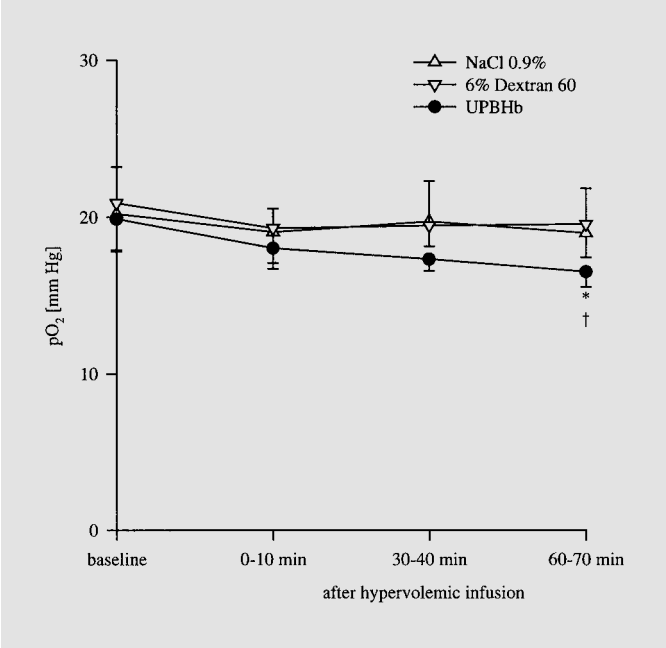

Fig. 6. Mean tissue pO_2 before and after hypervolemic infusion (n = 6, mean ± SD). * $p < 0.05$ vs. baseline (Student-Newman-Keuls-test), † $p < 0.05$ vs. 6% dextran 60 (Kruskal-Wallis-test).

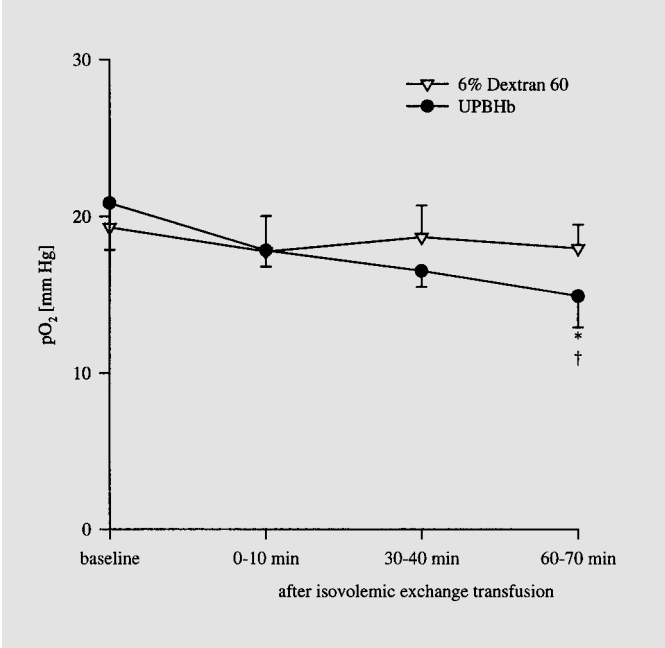

Fig. 7. Mean tissue pO_2 before and after isovolemic exchange transfusion (n = 6, mean ± SD). * $p < 0.05$ vs. baseline (Student-Newman-Keuls-test), † $p < 0.05$ vs. 6% dextran 60 (Kruskal-Wallis-test).

malian hemoglobins [8, 28] and similar to our own findings with U-PBHb [3], a significant elevation of mean arterial pressure has been found accompanied by reflex bradycardia following infusion of these solutions. The effect may be explained by the scavenge of $NO^{\bullet}$ by hemoglobin via formation of S-nitrosohemoglobin within the vascular bed [26, 29–31]. This mechanism is of particular interest in this context, since vasoconstriction – and as a consequence flowmotion – is known to influence local tissue pO_2 [32–34].

We assume hemoglobin to cause a modulation of vasomotion, since the application of U-PBHb is accompanied by a homogenization of the distribution pattern of local tissue pO_2, assessed with the Clark-type multi-wire surface electrode. After isovolemic exchange transfusion, the effect was even more pronounced than after hypervolemic infusion and the procedure simultaneously resulted in a decrease of mean local tissue pO_2. At the end of the observation period, no increase of hypoxic values (0–5 mm Hg) was registered suggesting that this phenomenon may be interpreted not to be harmful to the tissue. Other researchers even found tissue oxygenation improved after isovolemic hemodilution to a hematocrit of ~ 10% using U-PBHb as compared to autologous stored red cells [35].

Due to the demonstration of a more homogeneous pO_2 distribution pattern, we claim U-PBHb to economize oxygen distibution by lessening areas of inadequately high or low tissue oxygen tension. This is consistent with findings of raised tissue oxygenation yielding improved organ function and morphology, when U-PBHb was compared to an iso-oncotic perfusion fluid in rat kidneys [36].

In summary, this study provides in vivo evidence that U-PBHb significantly improves the homogeneity of local tissue oxygenation. To clarify whether these effects hold true under the conditions of severe ischemia and reperfusion, further trials with U-PBHb, especially under the conditions of severe hemorrhagic shock, have to be undertaken.

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