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BALANCE BETWEEN VASOCONSTRICTION AND ENHANCED OXYGEN DELIVERY

Pedro Cabrales¹, Amy G. Tsai^{1,2}, and Marcos Intaglietta²

¹La Jolla Bioengineering Institute La Jolla, CA 92037

²Department of Bioengineering University of California, San Diego La Jolla, CA 92093

Abstract

Background—Hemoglobin (Hb) solutions are potential alternatives to blood transfusion when native oxygen (O₂) carrying capacity is lacking. Polymerized bovine Hb (PBH) solutions are characterized by its vasoactivity, low O₂ affinity, oncotic effect, prolonged shelf life and stability. Responses to facilitated O₂ transport, after exchange transfusion with PBH, were studied in the hamster window chamber model during acute extreme anemia to determine how PBH affects microvascular perfusion and tissue oxygenation.

Study Design and Methods—An anemic state was induced by hemodilution with a plasma expander (dextran 70 kDa). After hemodilution animals were randomly assigned to exchange transfusion groups based on the concentration of PBH used, namely: PBH at 13gHb/dl [**PBH13**], PBH diluted to 8 or 4 gHb/dl in albumin solution at matching colloidal osmotic pressure (COP) [**PBH8** and **PBH4**], and no PBH only albumin solution at matching COP [**PBH0**]. Measurement of systemic parameters, microvascular hemodynamics, capillary perfusion and intravascular and tissue O₂ levels were performed at 11% Hct.

Results—Restitution of O₂ carrying capacity with **PBH13**, restored higher arterial pressure, triggered vasoconstriction, low perfusion and higher peripheral resistance. **PBH4** and **PBH0** had lower arterial pressure than **PBH13**. **PBH4** and **PBH8** maintained higher perfusion and functional capillary density than **PBH13** and **PBH0**. In all groups, blood gas parameters and acid-base balance were recovered proportional to microvascular perfusion. Arterial O₂ tensions were improved for **PBH4** and **PBH8** by preventing O₂ precapillary release and increasing O₂ reserve.

Conclusion—Further studies to establish acellular Hb optimal dosage, efficacy, safety, and effects on outcome are indicated before these solutions are implemented in routine practice.

Keywords

Microcirculation; red blood cells; hemodilution; functional capillary density; hemodilution; hemoglobin oxygen affinity; tissue oxygen

INTRODUCTION

Blood transfusion is performed to augment oxygen(O₂)-carrying capacity, since allogeneic transfusion is not always available and can result in transmission of infectious and immunologic diseases, allergic, alloimmune and other adverse events. Transfusions are reserved for situations of high risk for inadequate O₂ delivery, mostly assessed by low hemoglobin (Hb)

concentrations (1). The “transfusion trigger” or minimal hematocrit (Hct) or Hb level at which transfusion is required is a subject of controversy. In some situations the concept of a set “transfusion trigger” has been replaced by a functional parameter monitoring for inadequate tissue O₂ delivery on a case-by-case basis. On the other hand, it is recommended by the American Society of Anesthesiologists that erythrocytes be administered when the Hb concentration is less than 6 g/dL in young patients (1).

The amount of O₂ released in the peripheral circulation depends on the quantity of the circulating red blood cells (RBCs), blood perfusion, arterial O₂ saturation, RBC Hb O₂ affinity and metabolic O₂ needs. Under normal physiological conditions, only about 25% of the oxygenated Hb will be deoxygenated, while 75% recirculates, returning to the lungs. Thus, a major fraction of the O₂ present in blood does not participate in the processes of O₂ metabolism.

Hb based O₂ carriers (HBOCs) blood substitutes are being developed to overcome limitations inherent to blood transfusion (2). It has been amply demonstrated that molecular solutions of natural Hb cannot be used as HBOCs due to a variety of associated toxicities, which are partially overcome by Hb polymerization. However, polymerized Hb remains vasoactive, an effect attributed to nitric oxide (NO) scavenging, extravasation and vasculature Hyperoxygenation (3,4). Hypertension is the most frequent manifestation of vasoconstriction, and appears to be the main unwanted side effect of many Hb solutions. HBOCs have been formulated to reproduce O₂ carrying capacity similar to blood. However, they tend increase O₂ release from RBCs to tissues via the process of facilitated O₂ diffusion (5).

The objective of this study was to identify the effect of concentration of low O₂ affinity Hb on vasoconstriction, blood perfusion and tissue oxygen delivery at extreme anemic conditions. Extreme isovolemic hemodilution is an experimental model where systemic Hct is lowered to the point of minimal native O₂ carrying capacity (11% Hct). This critical point is a fulcrum, where comparatively small changes in O₂ carrying capacity and other blood properties can sustain, improve, or deteriorate systemic and microvascular parameters. To achieve this objective, the hamster chamber window model was subjected to moderate hemodilution to Hct of 18% using a non-O₂ carrier (6% dextran 70 kDa, Pharmacia, Uppsala, Sweden). Hemodilution was then continued to 11% Hct using different concentrations of polymerized bovine Hb (PBH, Oxyglobin®, Biopure Corporation, Cambridge, MA; approved in the United States and European Union for veterinary use). PBH concentrations tested were 0, 4, 8 and 13 g_{Hb}/dl at matching colloidal osmotic pressure (COP). COP was adjusted with human serum albumin diluted in normal saline.

METHODS

Animal Preparation

Investigations were performed in 55 – 65 g male Golden Syrian Hamsters (Charles River Laboratories, Boston, MA) fitted with a dorsal window chamber. The hamster window chamber model is widely used for microvascular studies in the unanesthetized state, and the complete surgical technique is described in detail elsewhere (6,7). Arterial and venous catheters filled with a heparinized saline solution (30 IU/ml) were implanted in to the carotid and jugular vessels. Catheters were tunneled under the skin, exteriorized at the dorsal side of the neck, and securely attached to the window frame. Animal handling and care followed the NIH Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the local animal care committee.

Inclusion Criteria

The microvasculature was examined 3 to 4 days after the window implantation surgery. Animals were suitable for the experiments if: 1) systemic parameters were within normal range, namely, heart rate (HR) > 340 beat/min, mean arterial blood pressure (MAP) > 80 mmHg, systemic Hct > 45%, and arterial O₂ partial pressure (p_AO₂) > 50 mmHg; and 2) microscopic examination of the tissue in the chamber observed under a ×650 magnification did not reveal signs of low perfusion, inflammation, edema or bleeding. Hamsters are a fossorial species with a lower arterial pO₂, however, their microvascular pO₂ distribution in the chamber window model is similar to other rodents (8).

Test solutions

Polymerized bovine Hb (PBH) commercially available as Oxyglobin® (Oxyglobin®, Biopure Corporation, Cambridge, MA). PBH is produced by non specific polymerization with glutaraldehyde (mean molecular weight 200 kDa) in a modified Lactated Ringer's Solution. PBH (Oxyglobin®) has a concentration of 13 g_{Hb}/dl (COP: 38 mmHg, viscosity: 1.9 cp). Other PBH concentrations tested were 0, 4 and 8 g_{Hb}/dl. COP of all solution was balance using human serum albumin diluted in normal saline to 38 mmHg. PBH solution viscosities for 0, 4, and 8 g_{Hb}/dl were 1.2 cp, 1.3 cp and 1.6 cp, respectively.

Acute Isovolemic Exchange Transfusion (Hemodilution) Protocol

Acute anemia was induced by two isovolemic hemodilution steps. This protocol was described in detail in our previous reports (9–11). Briefly, the volume of each exchange-transfusion step was calculated as a percentage of the blood volume (BV), estimated as 7% of body weight (BW). The acute anemic state was induced by lowering systemic Hct to 18% by two steps of progressive isovolemic hemodilution using 6% dextran 70 kDa (Pharmacia, Uppsala, Sweden). First exchange was 40% of BV and second exchange was 35% of BV, respectively. Moderate hemodiluted animals were randomly divided into four experimental groups (12). Exchange transfusion protocol was continued by exchanging 35% of the BV with the test solution.

Blood was simultaneously withdrawn at the same rate from the carotid artery catheter according to a previously established protocol (9–11). Blood samples were withdrawn at the end of the experiment for subsequent analysis of viscosity and COP. The duration of the experiments was 4 h. Each exchange and the respective observation time point post-exchange were completed in 1 h. Systemic and microcirculation data was taken after a stabilization period of 10 min.

Experimental groups

The experimental groups were labeled **PBH0**, **PBH4**, **PBH8** and **PBH13** based in the concentration of PBH used. Group 1) **PBH13**, exchange transfusion with stock solution of PBH (Oxyglobin®); Group 2) **PBH8**, exchange transfusion with PBH diluted to 8 g_{Hb}/dl; Group 3) **PBH4**, exchange transfusion with PBH diluted to 4 g_{Hb}/dl; Group 4) **PBH0**, exchange transfusion without PBH.

Systemic Parameters

MAP and (HR) were recorded continuously (MP 150, Biopac System; Santa Barbara, CA). Hct was measured from centrifuged arterial blood samples taken in heparinized capillary tubes. Hb content was determined spectrophotometrically (B-Hemoglobin, Hemocue, Stockholm, Sweden).

Blood Chemistry and Biophysical Properties

Arterial blood was collected in heparinized glass capillaries (50 µl) and immediately analyzed for pO₂, pCO₂, base excess (BE) and pH (Blood Chemistry Analyzer 248, Bayer, Norwood,

MA). Viscosity was measured in a DV-II plus (Brookfield, Middleboro, MA). Plasma COP was measured using a 4420 Colloid Osmometer (Wescor, Logan, UT).

Microvascular Experimental Setup

The unanesthetized animal was placed in a restraining tube with a longitudinal slit from which the window chamber protruded, then fixed to the microscopic stage for transillumination with the intravital microscope (BX51WI, Olympus, New Hyde Park, NY). Animals were given 20 min to adjust to the tube environment before any measurement. Tissue image was projected onto a charge-coupled device camera (COHU 4815) connected to a videocassette recorder and viewed on a monitor. Measurements were carried out using a 40X (LUMPFL-WIR, numerical aperture 0.8, Olympus) water immersion objective.

Functional Capillary Density (FCD)

Functional capillaries, defined as those capillary segments that have RBC transit of at least a single RBC in a 45s period in 10 successive microscopic fields were assessed, totaling a region of 0.46 mm². The relative change in FCD from baseline levels after each intervention is indicative of the extent of capillary perfusion (10).

Microhemodynamics

Arteriolar and venular blood flow velocities were measured online by using the photodiode cross-correlation method (Photo Diode/Velocity Tracker Model 102B, Vista Electronics, San Diego, CA). The measured centerline velocity (V) was corrected according to vessel size to obtain the mean RBC velocity. A video image-shearing method was used to measure vessel diameter (D) (13). Blood flow (Q) was calculated from the measured values as $Q = \pi \times V (D/2)^2$. This calculation assumes a parabolic velocity profile and has been found to be applicable to tubes of 15 – 80 μm internal diameters and for Hcts in the range of 6 – 60% (14).

Microvascular pO₂ distribution

High resolution non-invasive microvascular pO₂ measurements were made using phosphorescence quenching microscopy (PQM) (11,15). PQM is based on the O₂-dependent quenching of phosphorescence emitted by albumin-bound metalloporphyrin complex after pulsed light excitation. PQM is independent of the dye concentration within the tissue and is well suited for detecting hypoxia because its decay time is inversely proportional to the pO₂ level, causing the method to be more precise at low pO₂'s. This technique is used to measure both intravascular and extravascular pO₂ since the albumin-dye complex continuously extravasates from the circulation into the interstitial tissue (11,15). Tissue pO₂ was measured in tissue regions in between functional capillaries. PQM allows for precise localization of the pO₂ measurements, without subjecting the tissue to injury. These measurements provide a detailed understanding of microvascular O₂ distribution and indicate whether O₂ is delivered to the interstitial areas.

Oxygen Delivery and Extraction

The microvascular methodology used in our studies allows a detailed analysis of O₂ delivery to the tissue. Calculations are made using equation 1, O₂ delivery (DO₂) and 2, O₂ extraction (VO₂) (9).

$$DO_2 = \left[(RBC_{hb} \times Y \times S_A) + (Plasma_{hb} \times Y \times \check{S}_A) \right] \times Q \quad (\text{Eq. 1})$$

$$VO_2 = \left[(RBC_{Hb} \times Y \times S_{A-V}) + (Plasma_{Hb} \times Y \times \check{S}_{A-V}) \right] \times Q \quad (\text{Eq. 2})$$

where RBC_{Hb} is the Hb from RBCs equal to Total Hb – plasma Hb [g_{Hb}/dl_{blood}], $Plasma_{Hb}$ is the acellular Hb [g_{Hb}/dl_{blood}], Y is the O_2 carrying capacity of saturated Hb [$1.34 \text{ mlO}_2/g_{Hb}$], $S_A\%$ is the arteriolar RBC O_2 saturation and $\check{S}_A\%$ is the arteriolar PBH O_2 saturation, $A-V$ indicates the arteriolar/venular differences, and Q is the microvascular flow. Fresh hamster RBCs at pH 7.4 and 37.6°C had a P50 (50% of the Hb is saturated with oxygen) of 32 mmHg and Hill number of 2.9 measured using Hemox Analyzer (TCS Corp., PA). PBH is only 72% saturated at 150 mmHg, therefore P50 is 54 mmHg and Hill number of 1.2 (16).

Data Analysis

Tabular results are presented as mean $\pm$ standard deviation. The box-whisker plot separates the data into quartiles, top of the box defining the 75th percentile, the line within the box giving the median, and the bottom of the box showing the 25th percentile. Upper whisker defines the 95th percentile, and lower whisker the 5th percentile. Data within each group were analyzed using analysis of variance for repeated measurements (ANOVA, Kruskal-Wallis test). When appropriate, post hoc analyses were performed with the Dunns multiple comparison test. Microhemodynamic data are presented as absolute values and ratios relative to baseline values. A ratio of 1.0 signifies no change from baseline while lower and higher ratios are indicative of changes proportionally lower and higher than baseline (i.e., 1.5 would mean a 50% increase from the baseline level). The same vessels and capillary fields were followed so that direct comparisons to their baseline levels could be performed, allowing for more robust statistics for small sample populations. All statistics were calculated using GraphPad Prism 4.01 (GraphPad Software, Inc., San Diego, CA). Changes were considered statistically significant if $P < 0.05$.

RESULTS

Twenty-four animals were entered into the study; all animals tolerated the entire hemodilution protocol without visible signs of discomfort. Six animals were assigned to each experimental group. Groups were statistically similar ($P > 0.30$) in systemic and microcirculation parameters at baseline and moderate hemodilution. Systemic and microhemodynamic data sets for baseline and moderate hemodilution were obtained by combining data from all experimental groups.

Systemic Parameters

Moderate hemodilution reduced Hct and Hb. Blood chemistry at baseline, moderate hemodilution and after the exchange transfusion is presented in Table 1. Hct for all experimental groups was similar ($11.5 \pm 0.6\%$). Total Hb was increased proportional to the concentration of PBH used during the exchange transfusion. **PBH8** and **PBH13** had total Hb level higher than moderate hemodilution, more than $2 \text{ g}_{PBH}/dl$ in plasma. Hct after exchange transfusion remained stable over time until the end of the experiment for all experimental groups.

MAP was statistically decreased from baseline after moderate hemodilution. Exchange transfusion affected MAP proportional to the concentration of PBH. **PBH13** statistically recovered MAP from moderate hemodilution and was higher than **PBH4**, although no different from baseline. MAP for **PBH4** and **PBH8** were not different from moderate hemodilution, while significantly lower than baseline. **PBH0** had lower MAP than moderate hemodilution, baseline and all experimental groups with PBH (**PBH4**, **PBH8** and **PBH13**)

Systemic arterial blood gas analysis is presented in Table 1. Arterial pO₂ was statistically and significantly increased from baseline after moderate hemodilution and in all experimental groups. Arterial pCO₂ decreased significantly from baseline for all experimental groups. Arterial pO₂, pCO₂ and pH were not different among experimental groups. Blood BE was statistically significantly decreased for all experimental groups compared to baseline. **PBH8** maintained positive acid base balance and statistically improve when compared to **PBH0**.

Blood Biophysical Properties after Exchange

Blood viscosity, plasma viscosity and plasma COP after hemodilution for baseline and all experimental groups are presented in Table 2. Blood viscosities were lower than baseline for all exchange transfused groups and not different between groups. This further supports the importance of matching colloid properties in order to obtain a fair comparison of different concentrations of PBH. Plasma viscosity did not change from baseline. Plasma COP was higher from baseline for all exchange transfused groups.

Microhemodynamics

Microvascular parameter were characterized for large feeding and small arcading arterioles (range 45 – 74 µm) and small collecting and venules (range 46 – 76 µm). The changes in diameter, RBC velocity and blood flow from baseline are presented in Figure 1. Absolute values are given in the Figure 1 legend. Arterioles after moderate hemodilution were dilated, and RBC velocity and blood flow were statistically increased from baseline. Arterioles were statistically constricted for **PBH13** when compared to baseline or other experimental groups (**PBH0**, **PBH4** and **PBH8**). Arteriolar RBC velocity was statistically higher than baseline for **PBH4** and **PBH8**, they were also higher than **PBH0** and **PBH13**. Arteriolar blood flows were statistically higher for **PBH4** and **PBH8** when compared to **PBH0** and **PBH13**.

Venular microvascular tone, RBC velocity and blood flow changes are presented in Figure 1. Moderate hemodilution induced venular dilation and increased RBC velocity and blood flow from baseline. Venular RBC velocities were decrease from baseline for **PBH0**, and were also lower than venular velocities of **PBH4** and **PBH8**. **PBH13** decreased venular RBC velocities when compared to **PBH4** and **PBH8**, although no different from **PBH0**. Venular blood flows for **PBH13** were lower than baseline, and **PBH4** and **PBH8**. Venular blood flows for **PBH0** were not different from baseline but lower than **PBH4** and **PBH8**. Shear rate and shear stress for all experimental groups are presented in Table 2.

Functional Capillary Density

FCD after moderated hemodilution and exchange transfusion with different PBH concentration are presented in Figure 2. Moderated hemodilution was lower than baseline. FCD was further reduced after exchange transfusion with different concentration PBH. FCD after exchange with **PBH4** and **PBH8** were higher than **PBH0**. Exchange with **PBH13** produced the lowest FCD.

Microvascular Oxygen Tensions

Microvascular and tissue pO₂s are shown in Figure 3. **PBH4** and **PBH8** yielded high arteriolar pO₂ when compared to **PBH0** and **PBH13**. Venular pO₂ were lower for **PBH13** when compared to **PBH4** and **PBH8**. Exchange with **PBH4** produced higher interstitial tissue pO₂ than **PBH0** and **PBH13**. Interstitial tissue pO₂ in all experimental groups was lower from normal tissue pO₂ at full Hct, 21.7 ± 3.5 mmHg (17).

Microvascular Oxygen Delivery and Extraction

Figure 4 shows analysis of systemic and microvascular O₂ delivery and extraction. Systemic O₂ delivery was higher for **PBH8** than **PBH0**, **PBH4** and **PBH13**. Premicrocirculation O₂

extraction was statistically higher for **PBH0** when compared to **PBH4**, **PBH8** and **PBH13**. Microcirculation O₂ delivery was statistically higher for **PBH8** when compared to **PBH0**, **PBH4** and **PBH13**. Microcirculation O₂ extraction was also higher for **PBH8** when compared to other groups. **PBH0** had lower microvascular O₂ delivery and extraction when compared to **PBH4**, **PBH8** and **PBH13**.

Systemic RBC O₂ extraction ratio was higher after exchange with **PBH0** and **PBH13** when compared to **PBH4** and **PBH8**. Systemic plasma Hb extraction ratio was higher after exchange with **PBH13** when compared to **PBH4** and **PBH8**. Microvascular RBC and plasma Hb extraction ratios presented trends that were similar to those found in systemic extraction ratios. Systemic and microvascular RBC O₂ reserve from RBC was higher for **PBH4** when compared to **PBH0** and **PBH13**. Systemic plasma Hb O₂ reserve was lower for **PBH13** when compared to **PBH4** and **PBH8**. Microcirculation plasma Hb O₂ reserve was higher than the systemic plasma Hb reserve and lower for **PBH13** when compared to **PBH4** and **PBH8**.

DISCUSSION

The principal finding of the study is that moderate concentrations of PBH (**PBH4** and **PBH8**) can be used to enhance microvascular O₂ transport and maintain systemic and microhemodynamic parameters at extreme anemia when compared to stock concentration of PBH (**PBH13**) or without PBH (**PBH0**). The importance of increasing plasma O₂ carrying capacity during extreme acute anemic conditions is shown by maintained microhemodynamics as PBH is added to the circulation at different concentrations (**PBH4**, **PBH8** and **PBH13**). Colloidal osmotic properties were not a factor during the study, because solution COP was matched before infusion, verified by the lack of differences in Hct between groups. Since there were no differences in native O₂ transport capacity (Hct), the differences found between groups are mostly due to changes in plasma O₂ carrying capacity by PBH.

Moderate hemodilution produced changes in systemic and hemodynamic parameters to compensate for the reduction in native O₂ carrying capacity. Exchange transfusion with different concentrations of PBH produced MAP and vascular tone responses proportional to plasma PBH concentration. Microvascular hemodynamic parameters did not correlate with PBH plasma concentration. Responses to plasma O₂ carrying capacity, affected tissues O₂ delivery by sustaining perfusion, carrying capacity and facilitating O₂ release from RBCs (18,19). Our results suggest the potential importance of using moderate amounts of PBH to ensure maintenance of hemodynamic conditions and effectively exploit its plasma O₂ carrying capacity.

The study results show a significant increase in microvascular oxygenation when moderate amounts of PBH were present in the anemic blood. Without PBH in plasma RBCs released similar amounts of O₂ prior arrival to the microcirculation as in the microcirculation (43% before arriving to the microcirculation and 57% in the microcirculation). This balance was drastically affected by the presence of 1.1 g_{PBH}/dl in plasma (**PBH4**), which caused RBCs to release of 19% before and 64% in the microcirculation of the total O₂ extracted. The remaining 16% of the total O₂ was offloaded from plasma PBH, divided in 5% before and 11% at the microcirculation. The presence of 2.1 g_{PBH}/dl in plasma (**PBH8**) favored the release of O₂ from RBC prior to the microcirculation, dividing the total O₂ extracted in 19% before and 53% in the microcirculation. The remaining 29% of the total O₂ was offloaded from plasma PBH, divided in 10% before and 19% in the microcirculation. Calculation of improved systemic O₂ release for **PBH8**, correlates with the maintenance of systemic conditions observed. However, when 3.5 g_{PBH}/dl were presented in the plasma, O₂ release from RBC decreased, dividing the RBC O₂ extracted in 23% before and 38% in the microcirculation. The remaining

39% of the total O₂ was offloaded from plasma PBH, divided in 15% before and 24% in the microcirculation.

These results show that moderated supplementation of plasma O₂ carrying capacity is more effective than large increases, since a fraction of the O₂ is preserved to be released at the microcirculation, sustaining precapillary O₂ released without triggering vascular O₂ regulatory mechanisms. After a large reduction in the native O₂ carrying capacity, maintenance of perfusion and O₂ supply are advantageous. If only a plasma expander is used, even in optimal conditions, only perfusion can be influenced. When using an HBOC, perfusion and oxygenation can be affected by the simple strategy of HBOC dose titration, as shown in the current study.

Detailed analysis of the O₂ extraction ratios (O₂ extracted divided O₂ supplied) shows that in the absence of PBH 95% of the total O₂ was extracted. When 1.1 g_{PBH}/dl was presented in the plasma, 90% of total O₂ was extracted with a similar result obtained, when the concentration in plasma was 2.1 g_{PBH}/dl (92% of total O₂ extracted). Although a plasma concentration of 3.5 g_{PBH}/dl increased O₂ extraction ratio to 97%, total oxygen delivery and extraction in the microcirculation was similar or lower to that with 1.1 g_{PBH}/dl due to vasoconstriction and hypoperfusion, which indicates that there is a limit to the increase plasma O₂ carrying capacity than can be attained with PBH. It is that the improvement of O₂ transport at moderate concentrations of PBH (**PBH4** and **PBH8**) is not proportional to the concentration of PBH.

Moderated increases in plasma O₂ carrying capacity with PBH provided better and prolonged maintenance of homeostasis when compared to stock concentration of PBH (**PBH13**) or non PBH (**PBH0**). Although PBH is broad mix of different molecular sizes, it appears that at low concentration the vasoconstrictive properties of the smaller Hb polymers are compensated for by facilitated O₂ diffusion and an improved perfusion pressure. Therefore, the circulatory effects of PBH after exchange transfusion, appear to represent a balance of positive (e.g., increased O₂ carrying capacity and perfusion pressure) and negative (e.g. vasoconstriction) stimuli. In this context, a moderate vasoactivity may have the beneficial effect of increasing perfusion pressure without compromising FCD.

PBH is a non-specific glutaraldehyde polymerized Hb, with low O₂ affinity (P50 of 54 mmHg) and drastically reduced cooperativity, which significantly facilitates O₂ offloading (20,21). To some extent, low O₂ affinity and reduced cooperativity have been interpreted as negative properties to replace or reinstate blood O₂ carrying capacity (22). Adding this to PBH's inherent vasoactivity complicates the specifying of conditions in which this product could be optimally employed. To the present it has been proven impossible to fully replace RBCs O₂ transport with HBOC; however, enhancement of O₂ transport and offloading from the remaining RBCs by small additions of acellular Hb solution could be an alternative application for using HBOCs, specifically PBH (23,24). Additionally, since HBOC are primarily extracellular, chemically modified and/or recombinant when administered in large volumes, it is likely that removal of this rather heterogeneous class of proteins from the circulation occurs, in part, via pathways common to those for extracellular Hb. Buehler et al. have found that chemical modification involving increased molecular size has an important influence on CD163 monocyte / macrophage mediated uptake of polymerized Hb-based blood substitutes (25).

A large number of experimental studies have demonstrated that resuscitation with different HBOCs has the capacity to restore blood pressure, and improve tissue blood flow, FCD, and tissue O₂ tension (26–28). These positive effects reversed the base deficit, reduced anerobic metabolisms and lactate production, and decreased acute mortality. Improved outcomes in these experimental studies were principally attributed to blood volume and pressure restoration, and secondarily, to the increase in O₂ carrying capacity (28). The current extreme hemodilution

results suggest that O₂ delivery from PBH does not equate to O₂ delivery from native RBCs. They work in different ways, and the optimal amount of Hb from PBH needed to restore O₂ delivery from hemorrhagic shock (if volume is restored) is much less than the Hb needed in the form of RBCs. This concept could of great benefit during resuscitation from hemorrhagic shock, since at this condition, no more Hb is needed, rather, better O₂ transport through a combination of O₂ offloading and local perfusion. In fact, vasoconstriction due to arterial (premicrocirculation) hyperoxygenation could be detrimental to a successful resuscitation.

In conclusion, this study shows that the criteria for specifying the optimal amount of acellular Hb O₂ carrier is not unique, and requires in part, selecting between the enhancement of blood pressure, blood perfusion, microvascular function and O₂ delivery. In this study, microvascular function showed a tendency to be optimal at concentrations between 1 and 2 g_{PBH}/dl in plasma, a result only valid for 11% Hct. However, if tissue pO₂ is the criterion for the most advantageous condition, this could be obtained if the concentration PBH in plasma is equal to 46% of the remaining Hb in native RBCs. There was no O₂ delivery and extraction limitation in the anemic state tested in the presence of PBH in plasma; however results were limited by its low affinity and uncompleted O₂ saturation at normal air. A notable finding was high O₂ delivery and extraction found with moderate concentrations of PBH corresponding to the higher tissue pO₂s. These results were obtained at a specific anemic state, and it is likely that the relationship between pre-microcirculation and at the microcirculation O₂ delivery, O₂ reserve and tissue pO₂ will change for different anemic conditions. Moderate amounts of PBH during anaemic conditions increased microvascular pO₂, O₂ delivery and extraction and facilitated microvascular O₂ offload from RBCs. These effects are negated at plasma concentrations of 3.5 g_{PBH}/dl, producing increases MAP, peripheral vascular resistance and diminished O₂ transport benefits relative to those observed at lower concentrations. In summary, these results suggest the existence of an upper limit of concentration for increasing O₂ carrying capacity with acellular Hb.

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Figure 1. Relative changes to baseline in arteriolar and venular hemodynamics

#, $P < 0.05$ compared to baseline; †, $P < 0.05$ compared to **PBH0**; ‡, $P < 0.05$ compared to **PBH4**; and §, $P < 0.05$ compared to **PBH8**. Baseline diameters (μm , mean $\pm$ SD) for each animal group were as follows: **PBH0** (arterioles (A): 61.7 ± 9.0 , $n = 28$; venules (V): 68.2 ± 9.1 , $n = 32$); **PBH4** (A: 67.6 ± 7.9 , $n = 30$; V: 64.1 ± 8.6 , $n = 34$); **PBH8** (A: 65.0 ± 8.6 , $n = 29$, V: 69.2 ± 8.3 , $n = 34$); **PBH13** (A: 64.2 ± 8.2 , $n = 30$, V: 63.4 ± 8.4 , $n = 32$). n = number of vessels studied. Calculated baseline blood flows (nl/s, mean $\pm$ SD) for each animal group were as follows: **PBH0** (A: 13.4 ± 7.5 ; V: 8.1 ± 5.0); **PBH4** (A: 14.2 ± 8.2 ; V: 9.0 ± 5.4); **PBH8** (A: 13.9 ± 6.2 ; V: 8.7 ± 4.7); **PBH13** (A: 13.6 ± 6.2 ; 8.4, V: 7.9 ± 5.2).

§, $P < 0.05$ compared to baseline; ★, $P < 0.05$ compared to shock; †, $P < 0.05$ compared to **PBH4**; and ‡, $P < 0.05$ compared to **PBH0**.

Figure 2. Capillary perfusion during hemodilution

#, $P < 0.05$ compared to baseline; ¶, $P < 0.05$ compared to **MH**; †, $P < 0.05$ compared to **PBH0**; ‡, $P < 0.05$ compared to **PBH4**; and §, $P < 0.05$ compared to **PBH8**. FCD (cm-1) at baseline was as follows: **PBH0** (116 ± 10); **PBH4** (110 ± 8); **PBH8** (A: 106 ± 8); **PBH13** (A: 117 ± 9).

Figure 3. Intravascular and tissue pO₂ after exchange transfusion

†, P<0.05 compared to **PBH0**; ‡, P<0.05 compared to **PBH4**; and §, P<0.05 compared to **PBH8**.

Figure 4. Microvascular oxygen delivery and extraction

Oxygen transport by red blood cells (RBCs) and plasma Hb. †, $P < 0.05$ compared to **PBH0**; ‡, $P < 0.05$ compared to **PBH4**; and §, $P < 0.05$ compared to **PBH8**. **A)** Systemic oxygen delivery; **B)** Pre-microvascular oxygen extraction; and **C)** Microvascular oxygen extraction and delivery.

Table 1

Systemic parameters during the exchange protocol

	Baseline	Moderated Hemodilution <i>MH</i>		Extreme Hemodilution, concentration of Hb solution			
				PBH0	PBH4	PBH8	PBH13
n	24	24	6	6	6	6	6
Hct, %	48.7 ± 1.0	18.4 ± 0.7 [†]	11.3 ± 0.9 ^{†‡}	11.4 ± 0.6 ^{†‡}	11.6 ± 0.7 ^{†‡}	11.2 ± 0.5 ^{†‡}	11.2 ± 0.5 ^{†‡}
Total Hb, g/dl	14.6 ± 0.6	6.0 ± 0.5 [†]	3.7 ± 0.5 ^{†‡}	5.0 ± 0.4 ^{†‡}	6.3 ± 0.5 ^{†‡}	7.1 ± 0.4 ^{†‡}	7.1 ± 0.4 ^{†‡}
Plasma Hb, g/dl				1.1 ± 0.2	2.1 ± 0.4	3.5 ± 0.6	
MAP, mmHg	108 ± 6	92 ± 7 [†]	66 ± 7 ^{†‡}	87 ± 8 ^{†§}	92 ± 8 ^{†§}	101 ± 9 [§]	
HR, bpm	426 ± 28	448 ± 26	418 ± 41	452 ± 24	438 ± 36	409 ± 29	
pO₂, mmHg	56.1 ± 6.1	80.4 ± 8.2 [†]	105.3 ± 16.7 ^{†‡}	95.4 ± 7.1 [†]	92.4 ± 6.2 [†]	94.2 ± 6.6 [†]	
P_aCO₂, mmHg	55.8 ± 5.1	48.1 ± 5.5	39.1 ± 7.8 [†]	42.6 ± 7.2 [†]	44.3 ± 5.9 [†]	42.4 ± 5.3 [†]	
pH_a	7.343 ± 0.018	7.351 ± 0.023	7.323 ± 0.022	7.342 ± 0.033	7.351 ± 0.026	7.359 ± 0.024	
BE_a, mmol	4.1 ± 1.9	0.8 ± 2.3 [†]	-2.6 ± 2.0 [†]	-0.8 ± 1.6 [†]	0.2 ± 1.2 [†]	-0.4 ± 1.5 [†]	

Values are means ± SD. Baseline and moderated hemodilution (*MH*) included all the animals. No significant differences were detected between baseline or before exchange with test solutions (moderated hemodilution) values between groups. Hct, systemic hematocrit; Hb, hemoglobin content of blood; MAP, mean arterial blood pressure; PaO₂, arterial partial O₂ pressure; PaCO₂, arterial partial pressure of CO₂; BE_a, arterial base excess.

[†]p<0.05 compared to baseline

[‡]p<0.05 compared to *MH*

[§]p<0.05 compared to *PBH0*.

Table 2

Blood rheological properties, WSR and WSS.

	Baseline	Moderated Hemodilution <i>MH</i>	Extreme Hemodilution, concentration of Hb solution			
			0	4	8	12
n, (Het)	6, (48%)		6, (11.3)	6, (11.4)	6, (11.6)	6, (11.2)
Blood Viscosity, cP	4.2 ± 0.4		1.6 ± 0.6 [†]	1.7 ± 0.5 [†]	1.8 ± 0.5 [†]	1.8 ± 0.5 [†]
Plasma Viscosity, cP	1.2 ± 0.3		1.2 ± 0.3	1.3 ± 0.3	1.3 ± 0.4	1.4 ± 0.3
Plasma COP, mmHg	17.6 ± 0.7		20.7 ± 0.9 [†]	19.1 ± 1.1 [†]	18.9 ± 1.0 [†]	19.6 ± 0.9 [†]
Arteriolar						
WSR, s-1	635 ± 79	803 ± 95 [†]	592 ± 81	778 ± 94	839 ± 96 [§]	739 ± 102
WSS, dyn. cm-2	26.2 ± 3.3		9.5 ± 1.4 [†]	13.2 ± 1.6 [†]	15.1 ± 1.8 [†]	13.3 ± 1.8 [†]
Venular						
WSR, s-1	359 ± 62	434 ± 83 [†]	297 ± 71	406 ± 76	430 ± 88 [§]	336 ± 93
WSS, dyn. cm-2	15.1 ± 2.6		4.8 ± 1.3 [†]	6.9 ± 1.3 ^{†§}	7.8 ± 1.4 ^{†§}	6.1 ± 1.4 [†]

Shear rate of 150 s⁻¹ at 37°C; COP, colloid osmotic pressure at 27°C. Values are means ± SD; n, no. of animals studied. Hematocrits are presented in Table 2. WSR, wall shear rate; WSS, wall shear stress.

[†] P<0.05 compared to baseline (non hemodiluted animals)
[§] P<0.05 compared to *PBH0*.