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Low Oxygen Affinity Hemoglobin Solution Increases Oxygenation of Partially Ischemic Tissues during Acute Anemia

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Abstract

BACKGROUND—Maintenance of post-surgical tissue oxygenation depends on the ability of the specific tissue to recruit perfusion and oxygen (O₂) supply. However, when native O₂ carrying capacity is lacking, fluids to improve O₂ carrying capacity based in hemoglobin (Hb) could prevent partially ischemic tissue hypoxia by increasing O₂ release from the remaining red blood cells.

STUDY DESIGN—Responses to facilitated O₂ transport after exchange transfusion with polymerized bovine Hb (PBH) were studied in a chronic partially ischemic tissue model, induced by large feeding arterioles ligation during hamster window chamber model implantation. PBH affects in microvascular perfusion and tissue oxygenation were studied after exchange transfusion of 40% of animal's blood volume. Experimental groups were defined by the concentration of PBH used, namely: PBH at 13g/dl [**PBH13**], PBH at 4 g/dl in albumin solution to matching colloidal osmotic pressure (COP) [**PBH4**], and no PBH only albumin solution at matching COP [**PBH0**].

RESULTS—Restitution of O₂ carrying capacity with **PBH13** increased blood pressure and produced vasoconstriction compared to **PBH4** and **PBH0**. On the other hand, **PBH4** maintained blood pressure without significant vasoconstriction, increased tissue pO₂, arteriolar O₂ supply and extraction to the partially ischemic tissue compared to **PBH0** and **PBH13**.

CONCLUSIONS—Results suggest the existence of an optimal concentration of low O₂ affinity acellular Hb to increase oxygenation of partially ischemic tissue during anemic conditions.

Keywords

Microcirculation; hemoglobin based oxygen carriers; post-surgical tissue; hemodilution; functional capillary density; exchange transfusion; hemoglobin oxygen affinity; tissue oxygen

INTRODUCTION

Partial ischemia is defined as moderated restriction of blood supply to an organ or tissue. This ischemia induces moderated accumulation of metabolic products, and changes on inflammation and permeability, mostly because the oxygenation and energy supply are still provided by the high oxygen (O₂) caring capacity in the blood, and blood perfusion is reduced. Although, many partially ischemic conditions occur in conjunction with moderated changes

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in blood O₂ carrying capacity (surgery, bleeding, trauma, etc), impeding maintenance of homeostasis and cell function, and if untreated it will result in local tissue necrosis.¹ During surgery, the reasons for ischemia are manifold. Soft tissue trauma, burn injuries, and prolonged immobilization can result in partially ischemia-related complications. Almost any surgical dissection implies direct vascular trauma, thus compromising microvascular perfusion.² Reduction of the anatomical blood supply secondary to surgery results in areas of tissue with microhemodynamic disturbances, which are prone to ischemic complications and consequently may cause significant morbidity.

Maintenance of post-surgical tissue oxygenation during acute isovolemic anemia depends on physiologic adjustments occurring at both the systemic and the microcirculatory level. Undisturbed tissues compensate for the anemia by increasing perfusion and O₂ extraction. However, the relative contribution of these mechanisms depends on the ability of the specific tissue to recruit each of them. The tolerance to partial ischemic tissue to acute isovolemic anemia will also depend on the level of O₂ demand. The inspired O₂ fraction could also influence the tolerance to anemia, but the contribution of O₂ dissolved in the plasma is very limited, compared to lung free radical formation and subsequent tissue damage.³

Hemoglobin (Hb) based O₂ carriers (HBOCs) blood substitutes are being developed to overcome limitations inherent to blood transfusion.⁴ It has been amply demonstrated that unmodified Hb can not be used as HBOCs due to its nephrotoxicity, which is partially overcome by Hb polymerization.⁵ However, polymerized Hb remains vasoactive, an effect attributed to nitric oxide (NO) scavenging, extravasation and vasculature hyperoxygenation.⁶ Increase in blood pressure is the most frequent clinical manifestation of vasoconstriction and remains the main unwanted side effect. 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 oxygen diffusion.

The objective of this study was to identify the role of circulating concentration of low O₂ affinity Hb in oxygenation of partially ischemic tissue during anemic conditions. Partially ischemic tissue was induced by ligation of major feeding arterioles during hamster chamber window model preparation. The partially ischemic hamster chamber window model tissue had significant reduction in blood perfusion. Acute anemic conditions were induced by isovolemic hemodilution of 40% of the blood volume, decreasing systemic Hct from 49% to 29% (RBC Hb from 14.8 g/dl to 9.7 g/dl). To achieve this objective, the window model was exchanged transfused with 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, and 13 g_{PBH}/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). 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.^{7, 8} Partial ischemia was induced by ligation of major feeding arterioles during dorsal window implantation (Figure 1A). 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, and only animals passing an established systemic and microcirculatory inclusion criteria. Animals were suitable for the experiments if 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 microscopic examination of the tissue in the chamber observed under a x650 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.⁹

Acellular Hb solution

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). Additional PBH concentrations evaluated were 0 and 4 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 and 4 g_{Hb}/dl were 1.2 cp and 1.3 cp, respectively.

Acute Isovolemic Exchange Transfusion (Hemodilution) Protocol

Prior to the exchange transfusion, animals were randomly divided into three experimental groups.¹⁰ Briefly, the volume of the exchange-transfusion was calculated as 40% of the blood volume, estimated as 7% of body weight. The acute anemic state was induced by exchanging 40% of the animal blood volume with the test solution. Simultaneously, blood was withdrawn from the carotid artery catheter, and test solution was infused using the from the jugular vein catheter at the same rate, Figure 2.

Experimental groups

The experimental groups were labeled **PBH0**, **PBH4** and **PBH13** based in the concentration of PBH used. Group 1) **PBH13**, exchange transfusion with stock solution of PBH (Oxyglobin®); Group 2) **PBH4**, exchange transfusion with PBH diluted to 4 g_{Hb}/dl; Group 3) **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). Blood samples were withdrawn at the end of the experiment for subsequent analysis of viscosity and COP.

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. Systemic and microcirculation data was taken after a stabilization period of 20 min post hemodilution.

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.¹¹

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). Arteriolar and venular blood flow velocities were measured on-line by using the photodiode cross-correlation method (Photo Diode/Velocity Tracker Model 102B, Vista Electronics, San Diego, CA). The measured centerline velocity was corrected according to vessel size to obtain the mean RBC velocity (V)¹². 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%.¹²

Microvascular pO₂ distribution

High resolution non-invasive microvascular pO₂ measurements were made using phosphorescence quenching microscopy (PQM).^{14, 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.^{14, 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 supply 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 supply (DO₂) and 2, O₂ extraction (VO₂).¹¹

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

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

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}], γ 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 $\dot{S}_A\%$ 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

Eighteen animals were entered the exchange transfusion protocol. Six animals were assigned to each experimental group. Groups were statistically similar ($P > 0.30$) in systemic and microcirculation parameters at baseline. Systemic and microhemodynamic data for baseline was obtained combining data from all experimental groups. Microhemodynamics of partial ischemic dorsal hamster windows at baseline compared, non ischemic dorsal hamster windows are shown in Figure 1B. Data for non ischemic dorsal hamster windows was obtained from six additional animals.

Systemic Parameters

Exchange transfusion reduced Hct and Hb. Blood chemistry at baseline and exchange transfusion is presented in Table 1. Hct for all experimental groups was similar. Total Hb was increased proportional to the concentration of PBH used during the exchange transfusion. Hct after exchange transfusion remained stable over time until the end of the experiment for all experimental groups.

MAP was statistically decreased from baseline for **PBH0**. **PBH13** statistically increased MAP from baseline and compared to **PBH0** and **PBH4**. HR was not statistically affected by the exchange transfusion. Systemic arterial blood gases are presented in Table 1. Arterial pO_2 was statistically increased from baseline after exchange transfusion for all groups. Arterial pCO_2 decreased from baseline after exchange transfusion. Arterial pO_2 , pCO_2 and pH were not different among experimental groups. Blood BE was decreased after exchange transfusion for

all experimental groups compared to baseline, however no difference among experimental groups was observed.

Microhemodynamics

The changes in diameter and blood flow after exchange transfusion relative to baseline are presented in Figure 3. Absolute values are given in the Figure 2 legend. Arteriolar diameter of the partially ischemic tissue was statistically constricted for exchange transfusion with **PBH13** compared to **PBH4** and **PBH0**. Venular diameters of the partially ischemic tissue were not different after exchange transfusion. Arteriolar and venular blood flows were not different after exchange transfusion among groups.

Functional Capillary Density

FCD of the partially ischemic tissue after exchange transfusion with different PBH concentration are presented in Figure 4. FCD was reduced after exchange transfusion for all groups. FCD after exchange transfusion with **PBH13** was statistically decreased compared to **PBH0**.

Microvascular Oxygen Tensions

Intravascular and tissue pO₂s of the partially ischemic tissue after exchange transfusion are shown in Figure 5. **PBH4** yielded high intravascular (arteriolar and venular) pO₂s when compared to **PBH0** and **PBH13**. Additionally, exchange transfusion with **PBH4** produced higher interstitial tissue pO₂ than **PBH0** and **PBH13**.

Oxygen Supply and Extraction

Figure 6 shows analysis of systemic and microvascular O₂ supply and extraction. Systemic O₂ supply after exchange transfusion was not different among groups. Arteriolar O₂ supply to the partially ischemic tissue was higher for **PBH4** compared to **PBH0**, and **PBH13**. Microcirculation O₂ extraction was statistically reduced for **PBH0** when compared to **PBH4** and **PBH13**. Microcirculation O₂ extraction ratio (O₂ supplied/O₂ extracted) was lower for **PBH4** when compared to **PBH0** and **PBH13**. Consequentially venular O₂ reserve was statistically increased for **PBH4** compared to **PBH0** and **PBH13**.

DISCUSSION

The principal finding of the study is that low O₂ affinity Hb solutions can be used to increase microvascular O₂ transport to partially ischemic tissues during anemic conditions. Moderate concentrations of PBH (**PBH4**) appear to be more effective than stock concentration of PBH (**PBH13**) to enhance oxygenation of the partially ischemic tissue. The importance of increasing plasma O₂ carrying capacity to prevent anoxia of partially ischemic tissue during acute anemic conditions is shown by the higher interstitial tissue pO₂ and calculated microvascular O₂ extraction compared to without acellular Hb in plasma (**PBH0**). Colloidal osmotic properties were not a factor during the study, because solution COP was matched before infusion, and 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 the PBH.

Partially ischemic tissue induced by major arterial ligation, are noticeably sensitive to changes in native O₂ carrying capacity. Previous experiments performed in the hamster window model, without the arteriole ligation, produced changes in hemodynamic parameters to compensate for the reduction in O₂ carrying capacity.¹⁴ Normal tissues maintained O₂ delivery during moderate hemodilution by increasing local perfusion, facilitated by the decrease in blood

viscosity and the systemic increases in and cardiac output.¹⁷ A decreased of 40% of the Hct in hamster window model without arterial ligation does not produce significant changes in interstitial tissue pO_2 .¹⁴ However, when partial ischemia is induced, by feeding arterial ligation, a 40% decrease in Hct significantly compromised interstitial tissue pO_2 , as presented by the current study. Exchange transfusion with different concentrations of PBH produced MAP and vascular tone responses proportional to plasma PBH concentration. PBH plasma concentration did not increase central O_2 arterial supply, while it significantly affected arteriolar O_2 supply to the partially ischemic tissue. However, the arteriolar O_2 supply to the partially ischemic tissue was not proportional to the PBH plasma concentration, as only the moderate concentrations of PBH sustaining perfusion and took advantage of the increase in O_2 carrying capacity. This result suggests the potential of moderate amounts of acellular low O_2 affinity HBOC to ensure oxygenation of partially ischemic tissues.

The study presents a significant increase in partially ischemic tissue O_2 supply when a moderate amount of PBH (1.4 g/dl) was present in the anemic blood, while 89% of the O_2 supplied was supplied by the RBCs and only 11% by the PBH. Without PBH in plasma, RBCs in the anemic blood arrived to the partially ischemic tissue with only 60% of the O_2 presented in the blood when 1.4 g/dl of PBH is present in the plasma. The presence of 3.7 g/dl of PBH in plasma did not increase the O_2 provided by the RBC to the partially ischemic tissue, however it presented similar O_2 supplied that moderate concentration PBH by increasing the amount of O_2 provided by the plasma Hb. The low O_2 affinity acellular HBOC brings more O_2 into the partially ischemic area by increasing the fraction of O_2 supplied by the RBCs and enhances O_2 offload increasing arteriolar-venular O_2 extraction and tissue pO_2 . These results show that moderated supplementation of plasma O_2 carrying capacity is more effective than large increases, since a fraction of the O_2 is preserved, to be released at the microcirculation, sustaining precapillary O_2 released without triggering vascular O_2 regulatory mechanisms. After a large reduction in the native O_2 carrying capacity, maintenance of perfusion and O_2 supply are advantageous. If only a plasma expander is used in optimal conditions only perfusion is sustained. When using an HBOC, perfusion and oxygenation can be affected by the simple strategy of HBOC dose titration as shown in the current study.

A possible mechanism for our demonstrated improvement in oxygenation with the low circulating acellular Hb concentration was the O_2 supply to the partially ischemic tissue created by the low O_2 affinity Hb. While the MAP was sustained for all groups that received PBH, only animals exchanged transfused with 13 g/dl of PBH showed vasoconstriction. The higher perfusion pressure as a consequence of the higher MAP alone can not explain higher arteriolar pO_2 s, however combined with Hb-bound O_2 diffusion (facilitated diffusion), HBOC can offload O_2 more efficiently in arteriolar-sized vessels than blood, because they overcome transport barriers associated with increased distance for O_2 diffusion and low plasma O_2 solubility. Moderated increases in plasma O_2 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 by the facilitated O_2 diffusion and the improved perfusions pressure. Therefore, the circulatory effects of PBH after exchange transfusion appear to represent a balance of positive (e.g., increased O_2 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 microvascular function and FCD.

PBH is a non specific glutaraldehyde polymerized Hb, with low O_2 affinity (P_{50} of 54 mmHg), drastically reduced cooperativity and a broad distribution of molecular weights which significantly facilitates O_2 offloading.¹⁸ To some extent low O_2 affinity and reduced

cooperativity has been interpreted as negative properties to replace or reinstate blood O₂ carrying capacity. Although, during partial ischemic conditions a small acellular Hb diffuse fast and augment O₂ transport by carrying Hb-bound O₂ to the partially ischemic tissue. In this condition PBH has a larger Biot number as a consequence of its reduced O₂ affinity and O₂ dissociation kinetics. The O₂ delivery in the present of HBOC becomes a specific problem for each Hb preparation, depending on O₂ affinity, molecular size, and amount infused. Nevertheless, the optimal point to use a HBOC should (target) be defined by the O₂ metabolic needs.

This study demonstrated that the administration of a low concentration of acellular HBOC with low O₂ affinity, prevents hypoxic areas at plasma concentration of 1.4 g_{PBH}/dl, and it appears that higher doses are not necessary because vasoconstriction induced by a larger dose counteracts the benefits in O₂ transport. In recent studies, the toxicities of Hb solutions has been linked to oxidative stress, vasoconstriction and inflammation.^{5, 19, 20} Oxidative stress can be induced by Hb autooxidation and leads to the formation of reactive oxygen species.^{20, 21} There are many published experimental studies where the objective was to substitute the native O₂ carrying capacity with HBOCs, without understanding the different nature of the materials.²² Over-expectation has been a major limiting factor in the development and approval of effective HBOC blood substitutes. Future investigations would be directed to potential side effects of this product with regard to partially ischemic tissue oxygenation.

In conclusion, anemic conditions drastically affect oxygenation of partially ischemic tissue, which can progress from hypoxia to more severe necrotizing form, if untreated. The arterial ligation produced tissue microcirculation disturbances, and as a consequence, decreased tissue oxygenation. At this point, specific therapy must be directed towards microcirculation perfusion and tissue oxygenation, as their preservation will prevent further progression. In essence, treatment of experimental partially ischemic tissue after exchange transfusion with HBOC like low O₂ affinity acellular Hb can preserve microcirculation and improve tissue oxygenation, compared to hemodilution alone. These results were obtained at a specific anemic state, and it is likely that the relationship between central arterial supply and the ischemic tissue arteriolar O₂ supply, microcirculation O₂ extraction and tissue pO₂ will change for different anemic conditions. A decrease of 40% of the native O₂ carrying capacity, in the current partially ischemic model drastically affects its tissue pO₂ in the absence of PBH, while a plasma concentration of 1.4 g_{PBH}/dl increased tissue pO₂ and arteriolar supply, without vasoconstriction or hypertension. These effects were negated at plasma concentrations of 3.5 g_{PBH}/dl, which produced an increase in MAP, peripheral vascular resistance and diminishing O₂ transport benefits of PBH, relative to those observed at lower concentrations. In summary, the results suggest the existence of an optimal concentration of low O₂ affinity Hb to increase oxygenation of partially ischemic tissue during anemic conditions.

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References

1. Sarani B, Strong M, Pascual J, Schwab CW. Necrotizing fasciitis: current concepts and review of the literature. *J Am Coll Surg* 2009;208:279–288. [PubMed: 19228540]
2. Nicholls TP, Shoemaker WC, Wo CC, et al. Survival, hemodynamics, and tissue oxygenation after head trauma. *J Am Coll Surg* 2006;202:120–130. [PubMed: 16377505]

3. Freeman BA, Crapo JD. Hyperoxia increases oxygen radical production in rat lungs and lung mitochondria. *J Biol Chem* 1981;256:10986–10992. [PubMed: 7287745]
4. Winslow RM. Blood substitutes. *Curr Opin Hematol* 2002;9:146–151. [PubMed: 11844999]
5. Alayash AI. Oxygen therapeutics: Can we tame haemoglobin? *Nature Reviews Drug Discovery* 2004;3:152–159.
6. Winslow RM. Oxygen transport agents: a new approach to red blood cell alternatives. *TATM* 2004;5:498–504.
7. Colantuoni A, Bertuglia S, Intaglietta M. Quantitation of rhythmic diameter changes in arterial microcirculation. *Am J Physiol* 1984;246:H508–H517. [PubMed: 6720909]
8. Endrich B, Asaishi K, Götz A, Messmer K. Technical report: A new chamber technique for microvascular studies in unanaesthetized hamsters. *Res Exp Med* 1980;177:125–134.
9. Cabrales P, Tsai AG, Frangos JA, Intaglietta M. Role of endothelial nitric oxide in microvascular oxygen delivery and consumption. *Free Radic Biol Med* 2005;39:1229–1237. [PubMed: 16214038]
10. Altman DG, Bland JM. Statistics notes: How to randomise. *BMJ* 1999;319:703–704. [PubMed: 10480833]
11. Cabrales P, Tsai AG, Intaglietta M. Microvascular pressure and functional capillary density in extreme hemodilution with low and high plasma viscosity expanders. *Am J Physiol* 2004;287:H363–H373.
12. Lipowsky HH, Zweifach BW. Application of the “two-slit” photometric technique to the measurement of microvascular volumetric flow rates. *Microvasc Res* 1978;15:93–101. [PubMed: 634160]
13. Intaglietta M, Tompkins WR. Microvascular measurements by video image shearing and splitting. *Microvasc Res* 1973;5:309–312. [PubMed: 4709728]
14. Tsai AG, Friesenecker B, McCarthy M, et al. Plasma viscosity regulates capillary perfusion during extreme hemodilution in hamster skin fold model. *Am J Physiol* 1998;275:H2170–H2180. [PubMed: 9843817]
15. Kerger H, Groth G, Kalenka A, et al. pO₂ measurements by phosphorescence quenching: characteristics and applications of an automated system. *Microvasc Res* 2003;65:32–38. [PubMed: 12535869]
16. Cabrales P, Tsai AG, Intaglietta M. Increased tissue PO₂ and decreased O₂ delivery and consumption after 80% exchange transfusion with polymerized hemoglobin. *Am J Physiol* 2004;287:H2825–2833.
17. Cabrales P, Tsai AG. Plasma viscosity regulates systemic and microvascular perfusion during acute extreme anemic conditions. *Am J Physiol Heart Circ Physiol* 2006;291:H2445–2452. [PubMed: 16731641]
18. Tsai AG, Vandegriff KD, Intaglietta M, Winslow RM. Targeted O₂ delivery by low-P50 hemoglobin: a new basis for O₂ therapeutics. *Am J Physiol Heart Circ Physiol* 2003;285:H1411–H1419. [PubMed: 12805024]
19. D’Agnillo F, Alayash AI. Redox cycling of diaspirin crosslinked hemoglobin induces G2/M arrest and apoptosis in cultured endothelial cells. *Blood* 2001;98:3315–3323. [PubMed: 11719369]
20. Goldman DW, Breyer RJ 3rd, Yeh D, et al. Acellular hemoglobin-mediated oxidative stress toward endothelium: a role for ferryl iron. *Am J Physiol* 1998;275:H1046–1053. [PubMed: 9724312]
21. Giulivi C, Cadenas E. Heme protein radicals: formation, fate, and biological consequences. *Free Radic Biol Med* 1998;24:269–279. [PubMed: 9433902]
22. Fitzpatrick CM, Savage SA, Kerby JD, et al. Resuscitation with a blood substitute causes vasoconstriction without nitric oxide scavenging in a model of arterial hemorrhage. *J Am Coll Surg* 2004;199:693–701. [PubMed: 15501108]

Figure 1.

(A) Partially ischemic hamster window chamber. Window implantation surgery includes ligation of large feeding arterioles. Ligations are marked with triple line. (B) Microhemodynamic changes induced by feeding arterioles ligation. Significant decrease in blood flow all microvessel diameters were measured between conventional hamster window (red, control, without ligation) and partially ischemic hamster window (blue, partially ischemic). †, $p < 0.05$. Functional capillary density was higher in partially ischemic windows ($122 \pm 11 \text{ cm}^{-1}$) compared to control windows ($111 \pm 10 \text{ cm}^{-1}$), $p < 0.10$. Intravascular and tissue O_2 tensions are affected by arterioles ligation compared to non-ligated preparation. Arteriolar, venular and tissue pO_2 for partially ischemic window are 33.5 ± 3.3 , 16.4 ± 2.8 and $11.8 \pm 2.0 \text{ mmHg}$, respectively. Non-ischemic hamster window pO_2 was statistically higher ($p < 0.05$) for arteriolar, venular and tissue pO_2 , 46.2 ± 4.8 , 27.0 ± 3.6 and $20.9 \pm 2.9 \text{ mmHg}$, respectively.

Figure 2.

Hemodilution protocol. Hemodilution was attained by means of isovolemic blood exchange-transfusion protocol in which red blood cell (RBC) volume (red bar) is continuously decreased and plasma volume is increased (open bar) while total blood volume remains constant (dashed line). The volume of the exchange-transfusion was calculated as 40% of blood volume, estimated as 7% of body weight. The acute anemic state was induced by exchange transfusion with polymerized bovine Hb (PBH, Oxyglobin®, Biopure Corporation, Cambridge, MA; approved in the United States and European Union for veterinary use). PBH concentrations evaluated were 0, 4, and 13 g_{PBH}/dl at matching colloidal osmotic pressure (COP). COP was adjusted with human serum albumin diluted in normal saline. Experimental groups were defined by the concentration of PBH used, namely: PBH at 13g/dl (PBH13), PBH at 4 g/dl in albumin solution to matching colloidal osmotic pressure (COP) (PBH4), and no PBH only albumin solution at matching COP (PBH0).

Figure 3.

Relative changes to baseline in arteriolar and venular hemodynamics. †, $p < 0.05$. Baseline diameters (μm , mean $\pm$ SD) for each animal group were as follows: PBH0 (arterioles (A): 66.9 ± 12.8 , $n = 22$; venules (V): 65.4 ± 14.2 , $n = 32$); PBH4 (A: 65.4 ± 14.5 , $n = 30$; V: 64.5 ± 14.2 , $n = 32$); PBH13 (A: 63.2 ± 13.5 , $n = 26$, V: 64.1 ± 15.2 , $n = 31$). n = number of vessels studied. Calculated baseline blood flows (nl/s , mean $\pm$ SD) for each animal group were as follows: PBH0 (arterioles (A): 7.1 ± 3.1 , $n = 22$; venules (V): 4.5 ± 2.2 , $n = 32$); PBH4 (A: 6.7 ± 3.4 , $n = 30$; V: 4.7 ± 2.5 , $n = 32$); **PBH13** (A: 7.2 ± 2.8 , $n = 26$, V: 4.7 ± 1.9 , $n = 31$).

Figure 4.

Capillary perfusion during hemodilution. †, $p < 0.05$. FCD (cm-1) at baseline was as follows: PBH0 (124 ± 11); PBH4 (119 ± 9); PBH13 (A: 120 ± 12).

Figure 5.

Intravascular and tissue pO₂ after exchange transfusion. †, p<0.05. Exchange transfusion with PBH4 produced higher intravascular (arteriolar and venular) pO₂s compared to exchange transfusion with PBH0 and PBH13, respectively. Interstitial tissue pO₂ was also higher for exchange transfusion with PBH4 compared to exchange transfusion with PBH0 and PBH13.

Figure 6.

Microvascular oxygen delivery and extraction. Oxygen transport by red blood cells (RBCs) and plasma Hb. †, $p < 0.05$. Systemic O₂ supply after exchange transfusion was not different among groups. Arteriolar O₂ supply to the partially ischemic tissue was higher for exchange transfusion with PBH4 compare to exchange transfusion with PBH0, and PBH13, respectively. Microcirculation O₂ extraction was statistically reduced for exchange transfusion with PBH0 when compared to exchange transfusion with PBH4 and PBH13, respectively. Microcirculation O₂ extraction ratio (O₂ supplied / O₂ extracted) was lower for exchange transfusion with PBH4 when compared to exchange transfusion with PBH0 and PBH13, respectively.

Table 1**Blood Gas Parameters**

		Exchange transfusion, PBH solution		
	Baseline	PBH0	PBH4	PBH13
n	24	6	6	6
Hct, %	48.8±0.9	28.8±0.8 [*]	29.1±0.7 [*]	29.2±0.7 [*]
Total Hb, g/dL	14.7±0.5	9.4±0.4 ^{*†‡}	10.7±0.5 ^{*‡}	13.2±0.5 ^{*‡}
Plasma Hb, g/dL			1.4±0.3	3.7±0.4
MAP, mmHg	112±7	92±7 ^{*†‡}	110±8 ^{*‡}	126±10 ^{*‡}
HR, bpm	431±27	452±36	433±34	409±30
P _a O ₂ , mmHG	57.9±6.2	72.7±8.9 [*]	68.7±6.8 [*]	74.7±8.1 [*]
P _a CO ₂ , mmHg	54.0±5.2	48.6±6.5	50.2±6.0	44.8±6.4
PH _a	7.343±0.021	7.337±0.020	7.337±0.019	7.353±0.026
BE _a , mmol	3.2±1.4	0.8±1.6	0.9±1.2	0.2±1.8 [*]

Values are means ± SD. Baseline includes all the animals.

^{*} p<0.05 compared to baseline.

[†] p<0.05 compared to PBH.

[‡] p<0.05 compared to PBH13.

Hct, systemic hematocrit; Hb, hemoglobin content of blood; MAP, mean arterial blood pressure; HR, Heart rate; PaO₂, arterial partial O₂ pressure; PaCO₂, arterial partial pressure of CO₂; BE_a, arterial base excess.