Delivering Blood Transfusion in Experimental Acute Anemia with a Perfluorocarbon Emulsion

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ABSTRACT

Background: To avoid unnecessary blood transfusions, physiologic transfusion triggers, rather than exclusively hemoglobin-based transfusion triggers, have been suggested. The objective of this study was to determine systemic and microvascular effects of using a perfluorocarbon-based oxygen carrier (PFCOC) to maintain perfusion and oxygenation during extreme anemia.

Methods: The hamster (weight, 55–65 g) window chamber model was used. Two isovolemic hemodilution steps were performed using hydroxyethyl starch, 10%, at normoxic conditions to a hematocrit of 19% (hemoglobin, 5.5 g/dl), the point at which the transfusion trigger was reached. Two additional hemodilution exchanges using the PFCOC (Oxyocyte) and increasing the fraction of inspired oxygen to 1.0 were performed to reduce the hematocrit to 11% (hemoglobin, 3.8 g/dl) and 6% (hemoglobin, 2.0 g/dl), respectively. No control group was used in the study because this concentration of hemodilution is lethal with conventional plasma expanders. Systemic parameters, microvascular perfusion, functional capillary density, and oxygen tensions across the microvascular network were measured.

Results: At 6% hematocrit, the PFCOC maintained mean arterial pressure, cardiac output, systemic oxygen delivery, and oxygen consumption. As hematocrit was decreased from 11% to 6%, functional capillary density, calculated microvascular oxygen delivery, and oxygen consumption decreased; and the oxygen extraction ratio was close to 100%. Peripheral tissue oxygenation was not predicted by systemic oxygenation.

Conclusions: The PFCOC, in conjunction with hyperoxia, was able to sustain organ function and partially provide systemic oxygenation during extreme anemia during the observation period. The PFCOC can work as a bridge until erythrocytes are available for transfusion or when additional oxygen is required, despite the possible limitations in peripheral tissue oxygenation.

ALLOGENIC blood transfusions treat insufficient oxygen-carrying capacity, so-called anemia. However, although oxygen transport capacity is invariably restored by blood transfusion, it has also been associated with increased morbidity and mortality.1–3 As a result of increased awareness about the risks associated with allogenic blood transfusion, increased costs associated with this procedure,1 and controversies about the real implication of anemia in critically ill patients, several strategies that aim to reduce blood use have been proposed.1–3 Recently, the blood transfusion controversy has increased because fresh and stored erythro-
cytes do not similarly restore oxygenation. Moreover, transfusion-related adverse events, both short- and long-term, are among the costliest contributors to healthcare expenditures, including illness, future outcomes, lost wages, and impact on quality of life. To avoid unnecessary blood transfusions, the use of physiologic transfusion triggers and goal-directed, rather than exclusively hemoglobin-based, transfusion triggers have been suggested.

An oxygen-carrying fluid that sustains life in the absence of blood may have many benefits. Although an oxygen carrier is not available yet, the development of these solutions will have unprecedented medical applications. Perfluorocarbons are derived from hydrocarbons by replacing all the hydrogen atoms by fluorine atoms, available in large quantities and at a relatively low cost. Perfluorocarbons have a high solubility for gases and are chemically and biologically inert. In principle, they lead to a convenient, largely available, cost-effective, pathogen-free, and storable oxygen carrier plasma expander. However, perfluorocarbons are not soluble in water and must be emulsified using a surfactant. Perfluorocarbon-based oxygen carriers (PFCOCs) carry oxygen as a function of their oxygen solubility and the fraction of inspired oxygen (F\textsubscript{IO\textsubscript{2}}). Preclinical and phase 1, 2, and 3 clinical trials with PFCOCs have been reported with a perfluorbl emulsion (Oxygent; Alliance Pharmaceutical Corp., San Diego, CA); however, this initiative was subsequently abandoned. Currently, Oxycyte (Synthetic Blood International, Inc., Costa Mesa, CA) remains in clinical trials: a phase 2 clinical safety trial of traumatic brain injury has been completed, and a phase 2 dose-escalation study in Switzerland and Israel has been performed.

In the current study, we addressed the question of whether adjunctive therapy with PFCOCs and increased F\textsubscript{IO\textsubscript{2}} can compensate for severe decreases in native oxygen-carrying capacity. We also addressed the physiologic changes in perfusion, PO\textsubscript{2} gradients, and oxygen delivery and extraction generated by this compensation. The objective of the study was to determine systemic and microvascular changes induced by coadministration of PFCOCs and increased F\textsubscript{IO\textsubscript{2}} during lethal extreme anemia (6% hematocrit). We developed an experimental model in which systemic and microvascular hemodynamics and tissue oxygenation can be concurrently studied. To drastically reduce native oxygen-carrying capacity, our experimental hamster window chamber model was first subjected to moderate hemodilution at normoxic conditions via two isovolemic exchanges to 18% hematocrit using a plasma expander (hydroxyethyl starch, 10%). After moderate hemodilution, hematocrit was further decreased to 11% and 6% using a PFCOC (Oxycyte; Synthetic Blood International, Inc.). Based on previous results with our experimental model, 11% is the transfusion trigger value for awake hamsters because it is the minimal hematocrit supplying the tissue with sufficient oxygen necessary for organ function; a further hematocrit reduction does not adequately maintain vital functions.

Materials and Methods

Animal Preparation

Investigations were performed in male golden Syrian hamsters (Charles River Laboratories, Boston, MA; weight, 55–65 g) fitted with a dorsal window chamber. Animal handling and care followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the local animal care committee (Institutional Animal Care and Use Committee, University of California, San Diego, La Jolla, California). 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.

Catheters were tunneled under the skin, exteriorized at the dorsal side of the neck, and securely attached to the window frame.

Inclusion Criteria

Animals were suitable for the experiments if the following variables existed: (1) systemic parameters were within the normal range (i.e., heart rate more than 340 beat/min, mean arterial blood pressure more than 80 mmHg, systemic hematocrit more than 45%, and PO\textsubscript{2} more than 50 mmHg); and (2) microscopic examination results of the tissue in the chamber observed under X650 magnification did not reveal signs of edema or bleeding.

Plasma Expander and PFCOC

The non–oxygen carrier plasma expander used in the study was Pentaspan (B. Braun Medical, Irvine, CA). The PFCOC used in the study was Oxycyte (Synthetic Blood International, Inc.), a third-generation PFCOC; the PFC is 60% vol/vol F-tert-butyl cyclohexane (molecular formula, C\textsubscript{10}F\textsubscript{20}). PFCOC does not have colloidal osmotic pressure and was mixed with hydroxethyl starch solution, 20% (mean molecular mass, 200 kDa; Leopold Pharma, Graz, Austria), in a proportion of PFCOC, 80%, and colloidal hydroxethyl starch solution, 20%, according to the method of Nolte et al.

Systemic Parameters

Mean arterial pressure and heart rate were recorded continuously (MP 150; Biopac System, Santa Barbara, CA). Hematocrit was measured from centrifuged arterial blood samples taken in heparinized capillary tubes. Hemoglobin content was determined spectrophotometrically (B-Hemoglobin; Hemocue, Stockholm, Sweden). The proportion of blood volume occupied by PFCOC, fluorocrit (Fct), was measured using standard hematocrit procedures.

Cardiac Output

Cardiac output (CO) was measured by a modified thermodilution technique. Animals instrumented for CO measurements were surgically prepared and recovered identically to...
animals studied for microvascular measurements. However, the complexity of the setup for the thermodilution prohibited positioning them on the microscope. CO was measured 15–20 min after each exchange.

**Blood Chemistry and Biophysical Properties**

Arterial and venous blood were collected in heparinized glass capillaries and analyzed for PO2, PCO2, base excess, and pH (Blood Chemistry Analyzer 248; Bayer, Norwood, MA). The comparatively low arterial PO2 and high PCO2 of hamsters are indicative of the extent of capillary perfusion.14,15 Venous blood samples were only obtained after decreasing the hematocrit to 11%, which decreased blood viscosity and, therefore, the resistance of the small catheters implanted in the animal. Blood samples for viscosity and colloidal osmotic pressure measurements were quickly withdrawn into heparinized 5-ml syringes at the end of the experiment. Viscosity was measured in a cone and plate viscometer DV-II plus (Brookfield, Middleboro, MA). Colloidal osmotic pressure was measured using an osmometer (model 4420 Colloid Osmometer; Wescor, Logan, UT).22

**Hemoglobin Oxygen Saturation**

Oxygen equilibrium curves for erythrocytes were measured using an analyzer (Hemox Analyzer; TCS Scientific Corporation, New Hope, PA).23

**Functional Capillary Density**

Functional capillaries, defined as those capillary segments that have an erythrocyte transit of at least a single erythrocyte in a 45-s period in 10 successive microscopic fields were assessed, totaling a region of 0.46 mm². Each field had between two and five capillary segments with erythrocyte flow. The functional capillary density (FCD) per centimeter (i.e., the total length of erythrocyte perfused capillaries divided by the area of the microscopic field of view) was evaluated by measuring and adding the length of capillaries that had erythrocyte transit in the field of view. The relative change in FCD from baseline concentrations after each intervention is indicative of the extent of capillary perfusion.14,15

**Microhemodynamics**

A video image-shearing method was used to measure vessel diameter.24 Changes in arteriolar and venular diameter from baseline were used as indicators of a change in vascular tone. Arteriolar and venular centerline velocities were measured online by using the photodiode cross-correlation method (Photo Diode/Velocity Tracker Model 102B; Vista Electronics, San Diego, CA).25 The measured centerline velocity was corrected according to vessel size to obtain the mean erythrocyte velocity.26 Blood flow was calculated from the measured values as follows: 

\[ Q = \frac{\pi \times V \times (D/2)}{2}, \]

where \( Q \) is blood flow, \( V \), velocity; and \( D \), diameter.2 This calculation assumes a parabolic velocity profile and has been applicable for tubes with internal diameters of 15–80 μm and for hematocrits of 6–60%.26

**Microvascular Po2 Distribution**

High-resolution noninvasive microvascular Po2 measurements were made using phosphorescence quenching microscopy.27,28 Phosphorescence quenching microscopy is based on the oxygen-dependent quenching of phosphorescence emitted by an albumin-bound metalloporphyrin complex after pulsed-light excitation. Phosphorescence quenching microscopy 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 concentration, causing the method to be more precise at a low Po2 value. This technique is used to measure both intravascular and extravascular Po2 because the albumin-dye complex continuously extravasates the circulation into the interstitial fluid.27,28 Extravascular fluid Po2 (interstitial fluid) was measured in tissue regions in between functional capillaries. Phosphorescence quenching microscopy allows for precise localization of the Po2 measurements without subjecting the tissue to injury. These measurements provide a detailed understanding of microvascular oxygen distribution and indicate whether oxygen is delivered to the interstitial areas.

**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. The tissue image was projected onto a charge-coupled device camera (COHU 4815; Cohn Electronics, San Diego, CA) connected to a video cassette recorder and viewed on a monitor. Measurements were performed using a ×40 water-immersion objective (LUMPFL-WIR; numerical aperture, 0.8; Olympus). The same sites of study were followed throughout the experiment so that comparisons could be made directly to baseline concentrations.

**Experimental Group**

Animals included in the study were divided into two groups. One group of animals was used to measure CO, and another group was used to characterize microvascular hemodynamics and oxygenation.

**Moderate Isovolumic Hemodilution**

Both groups of animals were prepared identically and underwent an identical progressive hemodilution scheme. Progressive hemodilution was accomplished by two isovolemic exchange steps using hydroxyethyl starch at a normoxic condition (FiO2, 0.21). This protocol has been previously described in detail.14,15 Blood volume was estimated as 7% of body weight. The first exchange was 40% of blood volume (29% hematocrit [level 1]). The second exchange was 35% of blood volume and decreased the hematocrit to 18% (hemoglobin, 5.5 g/dl level).
for microvascular oxygen delivery (DmicO2) and equation 2 for tissue oxygen supply. Calculations were made using equation 1

\[ D_{micO2} = [(\text{erythrocyte hemoglobin} \times \gamma \times S_H) + (1 - \text{hematocrit} - \text{Fct}) \times (\alpha_{\text{plasma}} + \text{Fct} \times \alpha_{\text{PFC}} \times P_{\text{Fct}})] \times Q \] (equation 1)

\[ V_{micO2} = [(\text{erythrocyte hemoglobin} \times \gamma \times S_{\text{A-V}}) + (1 - \text{hematocrit} - \text{Fct}) \times (\alpha_{\text{plasma}} + \text{Fct} \times \alpha_{\text{PFC}} \times P_{\text{A-V}O2})] \times Q \] (equation 2)

where erythrocyte hemoglobin is the hemoglobin in erythrocytes (measured in g/dl), \( \gamma \) is the oxygen-carrying capacity of saturated hemoglobin (1.34 ml O2/g), \( S_H \) is the arteriolar oxygen saturation, (1 - hematocrit - Fct) is the fractional plasma volume (measured in dlplasma/dl), \( \alpha_{\text{plasma}} \) is the solubility of oxygen in plasma (3.14 \times 10^{-3} \text{ ml O2/dl plasma} \times \text{mmHg}), \( \alpha_{\text{PFC}} \) is the solubility of oxygen in PFCOC (2.4 \times 10^{-2} \text{ ml O2/dlOxycyte} \times \text{mmHg}), A-V is the arteriolar–venular difference, and Q is the microvascular flow.

Detailed analysis of systemic oxygen delivery (DO2) and systemic oxygen consumption (VO2) was established using the following equations:

\[ \text{DO2} = [(\text{erythrocyte hemoglobin} \times \gamma \times S_H) + (1 - \text{hematocrit} - \text{Fct}) \times (\alpha_{\text{plasma}} + \text{Fct} \times \alpha_{\text{PFC}} \times P_{\text{Fct}})] \times \text{CO} \] (equation 3)

\[ \text{VO2} = [(\text{erythrocyte hemoglobin} \times \gamma \times S_{\text{A-V}}) + (1 - \text{hematocrit} - \text{Fct}) \times (\alpha_{\text{plasma}} + \text{Fct} \times \alpha_{\text{PFC}} \times P_{\text{A-V}O2})] \times \text{CO} \] (equation 4)

where all parameters are as previously described. In addition, the fractional contribution to oxygen delivery of each phase and the fractional contribution of each phase to oxygen consumption were calculated.

**Statistical Analysis**

Results are given as the mean ± SD. In the box–whisker plot, the top of the box, the line within the box, and the bottom of the box indicate the 75th percentile, the median, and the 25th percentile, respectively. The upper and lower whiskers define the 95th and 5th percentiles, respectively.

After the moderate hemodilution, the protocol was continued by exchanging 35% of blood volume to 11% hematocrit (hemoglobin, 3.8 g/dl [level 3]); last, there was a final exchange of 35% of blood volume to 6% hematocrit (hemoglobin, 2.0 g/dl [level 4]) using the PFCOC mixture previously described. Animals were changed to hyperoxia (FIO2, 1.0) as the third exchange was started. Each exchange and the respective observation time point after exchanges were fully completed in 1 h; after the last exchange, animals were observed for 90 min. Thus, the total duration of the experiments was 5 h. Systemic and microcirculation data were obtained 10 min after a stabilization period after the hemodilution step. Figure 1 shows the experimental scheme, volume exchanges and time line of the protocol.

**Tissue and Systemic Oxygen Delivery and Consumption**

The method used in our studies allows a detailed analysis of tissue oxygen supply. Calculations were made using equation 1 for microvascular oxygen delivery (DmicO2) and equation 2 for microvascular oxygen consumption (VmicO2), as follows14:

**Fig. 1.** Hemodilution was attained by a progressive, stepwise, isovolemic, blood exchange–transfusion protocol in which the erythrocyte (RBC) volume (dark bar) is continuously decreased and the plasma volume (clear bar) is increased while maintaining the total blood volume constant (dotted line). The volume of each exchange–transfusion step was calculated as a percentage of the blood volume, estimated as 7% of the body weight. The third and fourth hemodilution steps were achieved (35% of blood volume) using a mixture of perflurocarbon oxygen carrier (PFCOC) and hydroxyethyl starch (HES), and the fraction of inspired oxygen (FIO2) was increased. PE = plasma expander (HES).

Extreme Isovolemic Hemodilution

After the moderate hemodilution, the protocol was continued by exchanging 35% of blood volume to 11% hematocrit (hemoglobin, 3.8 g/dl [level 3]); last, there was a final exchange of 35% of blood volume to 6% hematocrit (hemoglobin, 2.0 g/dl [level 4]) using the PFCOC mixture previously described. Animals were changed to hyperoxia (FIO2, 1.0) as the third exchange was started. Each exchange and the respective observation time point after exchanges were fully completed in 1 h; after the last exchange, animals were observed for 90 min. Thus, the total duration of the experiments was 5 h. Systemic and microcirculation data were obtained 10 min after a stabilization period after the hemodilution step. Figure 1 shows the experimental scheme, volume exchanges and time line of the protocol.

**Exchange Protocol**

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**Results**

The number of animals used in the current study was 25% (SD, 12%) after hemodilution with PFCOC. Thereafter, the box–whisker plot, the top of the box, the line within the box, and the bottom of the box indicate the 75th percentile, the median, and the 25th percentile, respectively. The upper and lower whiskers define the 95th and 5th percentiles, respectively. Because animals instrumented for CO measurements were an independent group, from the animals used for tissue PO2, as the complexity of the setup for the thermodilution prohibited positioning them on the microscope, two sample sizes (CO and tissue PO2) were independently calculated. An a priori calculation of the sample size for testing the changes in CO and tissue PO2 after hemodilution with PFCOC in hamsters was based on previous results15; during hemodilution, CO changed by 25% (SD, 12%) after hemodilution with PFCOC. Therefore, the number of animals used in the current study was based on a power analysis of CO using an \( \alpha = 0.05 \) and a \( 1 - \beta = 0.9 \) (an estimated nine animals were required to identify differences in CO). In addition, tissue PO2 decreased by 17% (SD, 8%) after hemodilution with PFCOC; therefore, the number of animals was based on a power analysis of PO2 using an \( \alpha = 0.05 \) and a \( 1 - \beta = 0.9 \) (an estimated six animals were required). As the data were collected, interim analyses were implemented; by following animal care regulations at our institution, no more animals were included as statistical significance was reached. No attempts were made to adjust the significance level for the interim analyses. Data within the group were analyzed using an ANOVA for non-parametric repeated measurements, Friedman test. When appropriate, post hoc analyses were performed with the
Dunns multiple comparison test. Changes in Po2, V\textsubscript{mc}O\textsubscript{2}, VO\textsubscript{2}, and V\textsubscript{mc}O\textsubscript{2} were evaluated using a two-tailed paired t test to compare 11% hematocrit (concentration 3) against 6% hematocrit (concentration 4). Closeness within a gaussian population for all measured values at baseline for each animal was quantified with the Grubbs test. Oxygen distributions were analyzed using the Kurtosis test and the Skewness test. Microhemodynamic data are presented as absolute values and ratios relative to baseline values. The same vessels and functional capillary fields were followed so that direct comparisons to their baseline concentrations could be performed. All statistics were calculated using computer software (GraphPad Prism 4.01; GraphPad Software, Inc., San Diego, CA). Changes were considered statistically significant if $P < 0.05$.

Results

Eleven animals were enrolled into this study, and all animals tolerated and survived the entire protocol without visible signs of discomfort. Blood gases, mean arterial pressure, and heart rate were measured in all animals. Six animals were used to study microvascular changes, and five animals were used to study CO changes. Blood gases, mean arterial pressure, and heart rate were no different between the animals used for microhemodynamics and CO ($P > 0.30$). All animals included in the study passed the Grubbs test, ensuring that all the measured parameter values at baseline were within a similar population ($P < 0.05$).

Systemic and blood laboratory parameters are shown in table 1. The first and second hemodilution exchanges with non–oxygen-carrying plasma expander statistically reduced hematocrit and hemoglobin concentrations. The third and fourth hemodilution exchanges with PFCCOC further decreased hematocrit and hemoglobin concentrations. Arterial blood gas analysis showed an increase in arterial Po2 at hemodilution exchanges with non–oxygen-carrying plasma expander at an FiO\textsubscript{2} of 0.21; however, this value was not statistically significant. Only after hemodilution exchange to 11% hematocrit with PFCCOC and an increased FiO\textsubscript{2} to 1.00, arterial Po2 statistically increased compared with baseline and 18% hematocrit. A further decrease in hematocrit to 6% with PFCCOC increased arterial Po2 compared with 11% hematocrit. During the entire hemodilution protocol, arterial Paco2 decreased; however, no statistical difference was measured. At 11% and 6% hematocrit, PFCCOC and an increased FiO\textsubscript{2} to 1.00 prevented hyperventilation and maintained venular Paco2 within the specified physiologic range, respectively. In addition, the benefits of PFCCOC and increased FiO\textsubscript{2} to 1.00 were evident on the central venous Paco2, which decreased after hemodilution from 11% to 6% hematocrit; however, central venous oxygen saturation remained greater than 50%. Moreover, arterial pH did not present a significant change through the extreme hemodilution protocol, indicating the absence of acidosis; in addition, because the arterial PCO2 did not show major deviations, this suggested metabolic maintenance. The benefits of PFCCOC and an increased FiO\textsubscript{2} to 1.00 at 11% and 6% hematocrit are confirmed by the maintenance of acid–base balance, as judged by arterial Paco2 and pH; these variables were not significantly altered at this degree of hemodilution.

Systemic hemodynamic changes are provided in figure 2. The arterial pressure decreased progressively from 103.5 ± 9.8 mmHg at baseline to 95.0 ± 6.9 mmHg at 29% hematocrit, to 87.5 ± 5.8 mmHg at 19% hematocrit, to 81.0 ± 4.5 mmHg at 11% hematocrit, and to 70.5 ± 5.6 mmHg at 6% hematocrit (fig. 2A). The heart rate remained unchanged during hemodilution with the plasma expander until 18% hematocrit; continuing the hemodilution with PFCCOC statistically increased CO at 11% and 6% hematocrit compared with baseline and previous hemodilution concentrations, respectively (fig. 2B). The CO statistically increased after initial reduction of hematocrit to 29% and 18%. Continuing the hemodilution to 11% hematocrit with PFCCOC maintained CO statistically increased compared with baseline.
The final hemodilution to reach 6% hematocrit using PF-COC statistically reduced CO compared with previous hemodilution concentrations. However, CO throughout the entire progressive hemodilution remained greater than baseline concentrations (fig. 2C).

Changes in microcirculation diameters and flows and statistical significance are presented in figure 3. Arteriolar diameter statistically increased from baseline after hemodilution to 29% hematocrit and was not different from baseline after continuing the hemodilution to 11% and 6% hematocrit with PFCOC. Venular diameter statistically decreased from baseline after hemodilution to 29% hematocrit. This venular constriction was statistically reverted after hemodilution to 11% hematocrit with PFCOC (fig. 3A). Arteriolar microvascular flows were statistically increased after hemodilution to 29% hematocrit. Continuing the hemodilution to 11% hematocrit with PFCOC statistically decreased microvascular flows (arterioles and venules) at 11% and 6% hematocrit, respectively (fig. 3B). Microvascular flows at 11% and 6% hematocrit were statistically lower than previous hemodilution concentrations. The FCD decreased to 0.92 ± 0.06 of baseline after hemodilution to 29% hematocrit; when the hemodilution was continued to 18% hematocrit, the FCD was statistically decreased to 0.79 ± 0.12 of baseline (fig. 3C). Hemodilution to 11% hematocrit with PFCOC statistically decreased the FCD to 0.69 ± 0.10 of baseline; when the hemodilution was continued to 6% hematocrit, the FCD further decreased to 0.37 ± 0.08 of baseline.

Intravascular oxygen tension values and distribution curves are presented in figure 4A. Arteriolar PO2 was 42.0 ± 6.8 mmHg at 11% hematocrit and statistically decreased to 29.0 ± 6.6 mmHg at 6% hematocrit. Venular oxygen tension was 7.6 ± 2.5 mmHg at 11% hematocrit and statistically decreased to 6.2 ± 2.0 mmHg at 6% hematocrit. Tissue PO2 and distribution curves are illustrated in figure 4B. Tissue PO2 was 3.8 ± 1.1 mmHg at 11% hematocrit and statistically decreased to 2.8 ± 1.4 mmHg at 6% hematocrit. Arteriolar oxygen distributions at 11% and 6% hematocrit (skewness: 11% hematocrit, 0.94; 6% hematocrit, 1.01) were not symmetrical and skewed to higher values; both distributions did not closely match a gaussian distribution (kurtosis: 11% hematocrit, −1.79; and 6% hematocrit, −0.92). Venular oxygen distributions at 11% and 6% hematocrit were highly symmetrical (skewness: 11% hematocrit, 0.35; and 6% hematocrit, 0.20) and closely match a gaussian distribution (kurtosis: 11% hematocrit, −0.36; and 6% hematocrit, −0.46).
6% hematocrit, −0.04). Tissue oxygen distributions at 11% and 6% hematocrit were both highly symmetrical (skewness: 11% hematocrit, 0.71; and 6% hematocrit, 0.78); however, only 6% hematocrit closely matched a gaussian distribution (kurtosis: 11% hematocrit, 1.52; and 6% hematocrit, 0.20).

The DO$_2$ and VO$_2$ and the fractional contribution of each phase are illustrated in figure 5. At 11% hematocrit, the Fct was 9.5% and the plasma volume represented the remaining 79.4%; at 6% hematocrit, the Fct was 19.0% and the plasma volume represented the remaining 75.2%. The DO$_2$ was 1.67 ± 0.20 ml O$_2$/min at 11% hematocrit and statistically decreased to 1.37 ± 0.14 ml O$_2$/min at 6% hematocrit. As shown in figure 5B, the fractional contribution of each phase to delivery was 65% (erythrocyte), 17% (PFCOC), and 18% (plasma) at 11% hematocrit and changed to 37% (erythrocyte), 42% (PFCOC), and 22% (plasma) at 6% hematocrit. In contrast to DO$_2$, the VO$_2$ statistically increased from 0.80 ± 0.08 ml O$_2$/min at 11% hematocrit to 1.00 ± 0.10 ml O$_2$/min at 6% hematocrit (unpaired $t$ test). The fractional contribution to consumption was 32% (erythrocyte), 32% (PFCOC), and 35% (plasma) at 11% hematocrit; and 18% (erythrocyte), 54% (PFCOC), and 28% (plasma) at 6% hematocrit. Figure 6 presents the changes in D$_{mic}$O$_2$ and V$_{mic}$O$_2$. Between 11% and 6% hematocrit, there was a significant decrease in D$_{mic}$O$_2$ and V$_{mic}$O$_2$. The reduction in hematocrit from 11% to 6% using the PFCOC did not maintain delivery or consumption of oxygen in the microcirculation.

### Discussion

The principal finding of this study is that by using a PFCOC emulsion combined with hyperoxia, hemodilution can be continued to one-third of the transfusion trigger hematocrit (6% hematocrit: hemoglobin 2.0 g/dl), partially sustaining DO$_2$ and VO$_2$. However, local microvascular tissue Po$_2$ values were physiologically low; oxygen delivery and extraction remained partially dependent on the oxygen transported by the remaining erythrocytes. The PFCOC, combined with hyperoxia, increases arterial Po$_2$, directly affecting the amount of oxygen dissolved in the blood; therefore, the blood oxygen content is affected and the longitudinal and circumferential arterial oxygen gradients are directly changed. Because oxygen diffusivity cannot be affected, the changes in blood oxygen solubility produced by PFCOC affected the local oxygen gradients; this is the only mecha-
nism that drives oxygen to leave the intravascular compartment. After moderate hemodilution, the increase of CO and microvascular blood flow, as the result of reduced blood viscosity, maintained oxygen supply to the tissues. Sufficient oxygenation of the tissues is limited by the functional reserve of the heart; at a given hematocrit, an increase in CO is needed to deliver oxygen needs.\textsuperscript{30,31} However, when the progressive hemodilution is continued, regulatory mechanisms tend to fail, even with PFCOC emulsion combined with hyperoxia. Important physiologic parameters, such as tachycardia, hypotension, and a CO decrease, that indicate the need for a blood transfusion were observed as hemodilution progressed beyond the transfusion trigger. During the initial experimental design, we planned to include a control group that underwent hemodilution with a colloid plasma expander in combination with hyperoxia. In the pilot study, the awake hamsters that tolerated hemodilution to 8% hematocrit showed signs of stress and hypotension (mean arterial pressure, lower than 40 mmHg) and no animal survived hemodilution to 6% hematocrit without PFCOC.

The avoidance of allogeneic blood transfusions has become an important issue as patients demand treatment without allogeneic blood transfusions. Thus, in recent years, a variety of methods and procedures have been developed to minimize allogeneic blood transfusions. Therefore, the current study defines a potential application for PFCOC emulsions and hyperoxia for clinical scenarios in which bleeding is not completely controlled and blood for transfusion is not available. This possible application would be limited to a few patients in such conditions, and it is far from the universal blood substitute once erroneously envisioned. The metabolic acidosis developed during progressive hemodilution may represent lactic acidosis as the result of tissue hypoxia or a loss of buffering capacity of the blood, although lactate concentrations were not measured. The progressive hemodilution with PFCOC with an increased \textit{FiO2} of 1.0 prevented metabolic acidosis and maintained arterial PaCO\textsubscript{2} unaltered (Table 1). This indicates improved perfusion and oxygen delivery to tissue during extreme hemodilution, which was partially verified by the microvascular measurements. Therefore, as found in this study, changes in pH, base excess, and HCO\textsubscript{3}\textsuperscript{−} do not correlate with the decrease in hemoglobin concentration because PFCOC was capable of sustaining oxygen delivery greater than oxygen demand. Similar clinical observation has been reported with perflubron emulsion, delaying blood transfusions during orthopedic surgery.\textsuperscript{7}

The current results indicate that CO and microvascular blood flow are the major determinants of systemic and D\textsubscript{micO2}, respectively. The CO increased after the initial hemodilution and started to decrease near the transfusion trigger, and the use of the PFCOC and hyperoxia maintained CO higher than baseline even when blood’s oxygen-carrying capacity was only 12% of baseline. Changes in CO were produced by changes in heart rate and stroke volume, specifically during the initial hemodilution phase; decreasing hematocrit to 29% and 18% increased stroke volume by 50% and 61% from baseline, respectively. Fluid shifts from the interstitial space as a result of hydroxyethyl starch hyperoncotic properties could be responsible for the initial elevation in stroke volume as the hydrostatic pressure of capillaries decreases; this corresponds to the observed reduction in FCD, which is mostly dependent on capillary hydrostatic pressure and plasma viscosity.\textsuperscript{14} The maintenance of normovolemia is of primordial importance during extreme hemodilution to support the compensatory mechanisms, such as the increase in CO and the decrease in mean arterial pressure. It also prevents relevant tachycardia, with potential consecutive myocardial ischemia. As hematocrit decreased from 18% to 11%, the CO and stroke volume were maintained above baseline; however, they decreased in similar proportion than the systemic oxygen delivery. The cardiac response depends on the maintenance of cardiac filling and adequate myocardial oxygenation, which itself is almost exclusively dependent on coronary blood flow.\textsuperscript{30} The tachycardia measured at a hematocrit of 11% and 6% corresponds to a 25% increase in heart rate, compared with baseline; shortening of the diastolic phase; and induction of myocardial ischemia by decreasing coronary filling and perfusion. Adequate oxygenation of the myocardium is beyond the scope of this study. However, further research to particularly identify the sensitivity of the myocardium to blood oxygen content at a reduced hematocrit is needed because no extra myocardial work is necessary to maintain a higher CO and oxygen delivery if blood is hemodiluted as the result of reduced viscosity. In addition, PFCOC combined with hyperoxia at extreme anemic states may require supplementary support to maintain perfusion pressure with catecholamines, to allow the effects of the increased physically dissolved oxygen to arrive at peripheral tissues.

The D\textsubscript{micO2} was critical at 11% and 6% hematocrit, when the microvascular oxygen extraction ratio (V\textsubscript{mic}O\textsubscript{2}/D\textsubscript{micO2}) increased from 38% at baseline\textsuperscript{32} to 92% at 11% hematocrit and to 95% at 6% hematocrit, respectively. Microvascular blood flow decreases after extreme hemodilution (11% hematocrit) as a response to the drastic decrease in oxygen-carrying capacity and blood viscosity.\textsuperscript{14,16} This is characterized by selective vasoconstriction and a decrease in FCD, as observed in this study. In previous studies,\textsuperscript{16} when extremely low hematocrit concentrations are achieved by hemodilution with low-viscosity plasma expanders, vascular hindrance increased, relative to baseline, indicating probable vasoconstriction accompanied by a redistribution of blood flow to vital organs. In light of the microvascular vasoconstriction and reduced blood flow, hydroxyethyl starch, in conjunction with hyperoxia, did not maintain CO or vascular perfusion greater than baseline at 11% hematocrit.\textsuperscript{15}

Previous studies\textsuperscript{33} have shown that VO\textsubscript{2} remained relatively constant and independent of DO\textsubscript{2} (DO\textsubscript{2} was sustained above VO\textsubscript{2}). The increase on \textit{FiO2} with the PFCOC emulsion significantly increases oxygen delivery to the tissue; in-
terestingly, most of the oxygen is the result of hemoglobin in the erythrocytes. The explanation may be related to the increase in oxygen solubility in blood that allows hemoglobin to remain partially saturated until it arrives to the hypoxic tissues. The mechanism by which the PFCOC was able to sustain life and even increase the oxygen extraction ratio after the last hemodilution step was by increasing oxygen extraction from 48% at 11% hematocrit to 73% at 6% hematocrit. From 11% to 6% hematocrit, there was a reduction in DO2 of 0.30 ml O2/min. Despite this reduction, oxygen consumption increased by 0.20 ml O2/min because of a reduction in oxygen content in venous blood of 0.50 ml O2/min.

Despite apparently sufficient DO2, local signs of hypoxia persisted. The localized measurements of tissue PO2 highlight the importance of microvascular information compared with systemic measurements, which showed that oxygen extraction was satisfied by oxygen delivery. Microvascular results show that the tissue was extracting most of the oxygen delivered, indicating that, during extreme anemia, the tissues consumed as much oxygen as is available. Under conditions in which oxygen supply becomes limited but microvascular regulation is intact, such as may occur during dysxia (local hypoxia), corrections of global hemodynamic and oxygen-derived variables may be erroneously expected to maintain tissue oxygenation. As reported by Ott and Cooley,34 there are clinically relevant situations when major surgical procedures need to be performed safely in individuals who refuse blood transfusion and preoperative and intraoperative techniques that decrease surgical blood loss, decrease oxygen consumption, and when increased oxygen delivery are required. Therefore, to clinically increase oxygen delivery after severe normovolemic hemodilution, techniques to augment oxygen content based on increased Fio2 and drugs to increase CO and to cause peripheral vasodilation are combined with sedation and paralysis to decrease oxygen consumption.

In addition, the clinical relevance of applying PFCOC in situations of extreme anemia, in conjunction with the currently used techniques, is supported by the metabolic benefit observed with PFCOC and Fio2 of 1.0, although oxygen-carrying capacity was decreased from 11% to 6% hematocrit.

Conventional procedures to restore oxygen-carrying capacity at extreme anemic conditions are based on the correction of DO2. The current work shows that such correction can be inadequate because regional hypoxia was observed in the peripheral tissues. Lack of knowledge about the basic mechanisms controlling oxygen transport in the microcirculation and the insufficiency of clinical techniques for assessment of the adequacy of tissue oxygenation were the principal causes of failure during the early stages of the development of blood substitutes. Regional assessment of vital organ oxygen delivery at 11% hematocrit has been previously studied; all vital organs were underoxygenated, receiving 50% of the basal oxygen supply.16 The assessment of adequate tissue oxygenation lacks universal consensus because all approaches measuring blood flow and arterial oxygen content are for determining oxygen supply and extraction. An assessment of regional dysxia has been performed mainly by lactate, gastric tonometry, and oxygen electrodes. Interstitial tissue pH measurements are considered indicators of the activity of anaerobic metabolism associated with tissue dysxia. However, to what extent measured concentrations are influenced by the balance between lactate production and clearance and by lactate produced from sources other than anaerobic metabolism is difficult to determine.23

The limitations of the study will be described. Despite the use of microvascular and global oxygen transport variables to address end points achieved with PFCOC–hyperoxia, the current study lacks the demonstration of improvement in organ function. Understanding the function of the brain in relation to PFCOC, combined with hyperoxia, will be an important step toward physiologic transfusion triggers. Anemia-sensitive neurologic transfusion triggers in animals requires further development. Analyses of evoked potentials are sensitive to cerebral ischemia and critical oxygenation concentrations. Further studies have to show their usefulness in anemia treated with perfluorocarbon emulsions. In addition to reduced oxygen transport, other factors may play a major role in producing cell and organ dysfunction; the fact that global oxygen transport variables are increased does not necessarily correlate with the outcome, and future studies addressing these points are needed. For the assessment of the progression of tissue dysfunction, oxygen delivery and extraction at the local concentration (microcirculation) rather than at the systemic concentration could be more important. Because of the resistance of the small catheters required in this experimental model, central venous blood gases were only collected after an 11% hematocrit. Therefore, in conscious hamsters, total oxygen consumption was stable at 11% and 6% hematocrit; hemodilution with PFCOC with an Fio2 of 1.0 did not improve or further compromise oxygen transport capacity. Paradoxically, oxygen delivery progressively decreased during the hemodilution with PFCOC with an Fio2 of 1.0, a consequence of the reduction of CO. In addition, local microvascular tissue blood flows and oxygen delivery were progressively reduced. These results suggest that the effects of hemodilution with PFCOC with an Fio2 of 1.0 are counteracted and/or masked by local and remote control mechanisms because of the simultaneously reduced arterial oxygen content. The effects of reduced arterial oxygen content without alterations in viscosity could shed some light on the problem of the influence of viscosity during hemodilution. This is indicated by experimental results in which reductions in arterial oxygen content are tolerated if produced by hemodilution, rather than if they are produced by carboxyhemoglobinemia.35 Although this study has restricted direct clinical significance, it is a comprehensive experimental study, with the objective of defining the mechanistic principles for translational developments. Translational research of PFCOC sets the cornerstone for transfusion medicine.
signing appropriate clinical studies depends on good, robust, and reproducible preclinical data to support the investment in prospective studies large enough to be adequately powered to reveal an effect that can change clinical practice. The results obtained in our conscious animals can be used to design future studies in the anesthetized condition to specifically address the role of PFCOC in conjunction with increased FiO₂ in a clinically relevant anemic condition. There are several elements that can also be analyzed in future studies, including longer survival time, neurologic markers, and colloid controls. Applying all the currently experimental technology to the analysis of hemodynamics, oxygenation, and metabolism will likely provide information to define suitable clinical indications and precise potential analytical challenges associated with PFCOC use.

The objective of this study was to observe systemic and microcirculatory effects of PFCOC–hyperoxia used to sustain oxygen delivery during extreme anemic conditions. This study demonstrates that infusion of an 8.0-g/kg dose of PFCOC, combined with increased FiO₂, was well tolerated and resulted in hemodynamic changes. Our investigation was performed using an extreme hemodilution protocol aimed to determine the efficacy of oxygen-carrying plasma expanders at hematocrits below the critical hematocrit (18%) where further hemodilution limits metabolic oxygen supply. A critical parameter that determines microvascular function is FCD, which remained greater than 40%, a value that is low, but not pathologic, according to the findings of Kerger et al. 36 This value was able to provide sufficient oxygen delivery to maintain systemic metabolic needs, although localized analysis of oxygenation showed that the oxygen extraction rate of peripheral tissues was close to 100% of the oxygen supplied. The degree of hemodilution achieved in the current study cannot be attained with a plasma expander, as previously determined. 15 Preservation or even temporary upholding of DO₂ and CO after drastic hemodilution with PFCOC–hyperoxia defines a potential application for PFCOC emulsions as a bridge until erythrocytes are available for transfusion; however, clear signs of microvascular dysxia were observed. In addition, in conditions in which metabolic and immunologic complications increased oxygen extraction, 37 PFCOC emulsion plus hyperoxia may provide the additional oxygen required until the problem is controlled. Even during prehospital asanguineous fluid resuscitation, PFCOC–hyperoxia could aim to restore macrocirculation oxygenation. Once the trauma victim is hospitalized, the necessary increase in oxygen-carrying capacity can be achieved by blood transfusion; however, this is often not yet available in the prehospital setting. In the absence of erythrocytes, hyperoxic ventilation in conjunction with PFCOC can help augment arterial oxygen content. These results suggest the possibility of maintaining oxygenation lower than the transfusion trigger until allogeneic blood transfusions can be accomplished.

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