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Vasoactive hemoglobin solution improves survival in hemodilution followed by hemorrhagic shock

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Abstract

Objective—To compare survival after exchange transfusion followed by hemorrhage using: 1) The vasoactive, oxygen carrying, bovine hemoglobin based blood substitute Oxyglobin (Biopure Inc., 12.9 g Hb/dl); and 2) The hydroxyethyl starch plasma expander, Hextend (HEX, high molecular weight and low degree of substitution, 6%).

Design—Comparison between treatments.

Setting—Laboratory.

Patients/Subjects—Awake hamster chamber window model.

Interventions—50% blood volume exchange transfusion followed by a 60% hemorrhage over one hour, followed by one hour observation. Measurement of blood gases, mean arterial blood pressure (MAP), functional capillary density (FCD), arteriolar and venular diameter and microvascular oxygen (pO₂) tension distribution.

Results—Survival with Oxyglobin was 100% and only 50% for the HEX group. Vasoconstriction was evident in the microcirculation. MAP was higher in the Oxyglobin group. FCD was significantly reduced, although to a lesser extent by Oxyglobin. There was no difference in microvascular pO₂ distribution after one hour of shock between groups.

Conclusion—Higher MAP during the initial stages of hemorrhage could be due to vasoconstriction in the Oxyglobin group as compared to the HEX group. It is concluded that the pressor effect due to a vasoactive oxygen carrier may be beneficial in maintaining perfusion in conditions of severe hemodilution followed by hypovolemia.

Keywords

Shock; hemodilution; survival; molecular hemoglobin oxygen carrier; hydroxyethyl starch; microcirculation; functional capillary density

Colloids (gelatin, hydroxyethyl starch (HES) and albumin) are used for blood volume replacement therapy and perioperative fluid administration. Which colloid to use remains a point of contention since in addition to their intravascular volume expansion properties these materials also affect to a varying degree other factors such as coagulation and renal function. Efficacy and functionality of colloidal plasma expanders may be increased by using hemoglobin (Hb) to provide oxygen carrying capacity, thus postponing the need for blood transfusions. This approach, however, has been fraught with difficulties presumed to originate from the vasoactivity intrinsic to the nitric oxide (NO) scavenging properties of Hb, resulting in the rapid development of hypertension upon introduction into the organism. Hypertension related to the presence of molecular Hb in plasma has been associated with negative outcomes of clinical trials of so called Hb based blood substitutes (HBOCs).

The hypertension associated with the presence of molecular Hb in the circulation is widely accepted to be deleterious. Hypertension is clearly attributable to the presence of molecular Hb in plasma; however, it is questionable whether negative outcomes arising from the use of Hb solutions are a direct consequence of their pressor effect given that hypotensive conditions arising from hemorrhage and other forms of shock are treated with vasopressors (1). There is at least one report proposing that the Hb pressor effect could be beneficial (2), therefore the vasoconstrictive effect due to HBOCs proposed for the treatment of hemorrhage may provide immediate positive results independently of other pathologies (3).

Pressure is increased during resuscitation to insure perfusion primarily by giving fluid and secondarily using vasopressor agents if fluid alone is not sufficient. The increase in pressure caused by vasopressors does not necessarily transmit central pressure to the periphery. If the induced vasoconstriction exceeds a certain level it becomes deleterious, causing focal ischemia, tissue necrosis and eventually multi organ failure. This is a clinical experience with vasopressors if dosing becomes excessive (4). An alternative approach for pressurizing a hemodiluted circulation is increasing plasma viscosity with a viscogenic plasma expander, which redistributes viscous losses and pressurizes the capillaries maintaining FCD (5, 6).

In the present study we investigated whether a small molecular Hb based oxygen carrying solution extends survival in an extreme experimental protocol that induces a major hemodynamic challenge in the organism. This solution was evaluated by performing a 50% isovolemic exchange transfusion followed by a 60% hemorrhage with 1 hr survival as an end point. This previously developed protocol was used in an earlier study to compare the efficacy of polyethylene glycol based albumin (PEG-Albumin) and Voluven (VLV, Fresenius Kabi, Austria) a starch based resuscitation fluid prevalently used in Europe (7).

Experiments were performed using a bovine Hb-based oxygen carrying solution Oxyglobin (Biopure Inc., Cambridge, MA). This current study evaluates systemic and microcirculatory conditions, blood oxygen content and survival time with the study solution and compares results to a hydroxyethyl starch solution used clinically (Hextend, HEX, Hospira, IL), a relatively high viscosity plasma expander. The objective was to determine the relative merits of these colloids as preoperative hemodilution plasma expanders and to determine whether they affect survival time and maintenance of FCD after a severe hemorrhage.

MATERIALS AND METHODS

Animal Preparation

Studies were performed in golden Syrian male hamsters (Charles River Laboratories, Boston, MA), weight range of 51 - 67 g. The Guide for the Care and Use of Laboratory Animals (US National Research Council, 1996) was followed for animal handling. Experiments were approved by the University of California, San Diego Animal Subjects

Committee. The hamster window chamber model is a widely used model for microvascular studies in unanesthetized animals. Chamber implantation and vascular catheterization surgeries were performed under general anesthesia, 50 mg/kg IP injections as previously described (8, 9). Following chamber implantation, animals recovered for 2 days before catheterization. Animals were anesthetized for carotid artery and jugular vein catheter implantation (polyethylene-50), following microscopic chamber assessment for ruling out edema, bleeding, or signs of infection. Animals were entered into the study no earlier than two days after the last surgery.

Inclusion Criteria

Tissue preparations were inspected under high magnification (40x, LUMPFL-WIR, numerical aperture 0.8; Olympus, New Hyde Park, NY) before inclusion and only those free from edema and/or signs of bleeding were included. Additional baseline systemic parameter requirements were: Mean arterial pressure (MAP) $\times$ 80 mm Hg, heart rate (HR) $\times$ 320 beats/min, systemic hematocrit (hematocrit) $\times$ 45% and arterial oxygen tension (PaO₂) $\times$ 50 mm Hg.

Hemodynamic Parameters

MAP and HR were continuously monitored using a Biopac acquisition system (MP 150; Biopac Systems, Inc., Santa Barbara, CA) excluding periods where the arterial catheter was used for blood sampling, hemodilution, and hemorrhage.

Blood Chemistry and Hematologic Measurements

Arterial blood collected with heparinized capillary tubes was used to determine arterial blood gases, PaO₂ and carbon dioxide (PaCO₂), base excess (BE), and blood pH (Blood Chemistry Analyzer 248; Siemens, Norwood, MA). Comparatively low baseline PaO₂ and high PaCO₂ are normal for these animals due to their adaptation to a fossorial environment. Systemic hematocrit was measured from arterial blood collected in heparinized microcapillary tubes and centrifuged.

Functional Capillary Density

FCD was determined in 10-15 stepwise vertically successive microscopic fields using 40x magnification. FCD (cm⁻¹) is the total length of RBC-perfused capillaries divided by the surface area of tissue in which they are observed. Capillaries were considered functional having at least one RBC transit during a 30 sec observation period. Initial baseline fields were chosen by a distinguishing anatomical feature to allow quick recognition during repeated measurements.

Microvascular Po₂ Distribution

High resolution noninvasive microvascular pO₂ measurements were made using phosphorescence quenching microscopy (PQM) (10) PQM is based on the O₂-dependent quenching of phosphorescence emitted by albumin-bound metalloporphyrin complex after pulsed light excitation. PQM is independent of tissue dye concentration and is well suited for detecting hypoxia because the method is more precise at low pO₂s. This technique is used to measure both intravascular and extravascular pO₂ since the albumin-dye complex extravasates from the circulation. Tissue pO₂ was measured in tissue regions void of capillaries and away from large vessels

Experimental Design

The conscious, unanesthetized animal was placed in a Plexiglas tube secured to the stage of an upright transilluminated intravital microscope (BX51WI, Olympus). Animals were

randomly assigned to either group. Animals were allowed a 30 min tube adjustment period before assessing baseline parameters (MAP, HR, arterial blood gases, pH, and base excess, systemic hematocrit, vessel diameter, and FCD).

Isovolemic Hemodilution

Isovolemic hemodilution of 50% blood volume (BV, estimated as 7% of body weight) was performed by simultaneous withdrawal of blood from the arterial catheter and infusion of the study solution into the venous catheter at a rate of 0.1 mL/min (Harvard Apparatus 33 pump, Hollister, MA) (7).

Sixty Percent Blood Volume Exponential Hemorrhage and Shock

Hemorrhage (60% of BV) was completed during a 1 hr period, starting 10 min post hemodilution. Arterial blood was removed by connecting the arterial catheter to a dual syringe pump (model 33 syringe pump; Harvard Apparatus; Holliston, MA). Pumping was initiated at the start of each 10 min interval and continued for the predetermined time needed to achieve a 60% blood volume loss within 1 hr. The total blood volume (TBV) at the end of each 10 min period was calculated as follows:

$$Tbv = Tbv_0 \times E^{0.015t}$$

where TBV_0 is the baseline blood volume (assumed to be 70 mL/kg) and t is time (min). The amount of blood removed each time was calculated according to this equation (11) at a rate of 0.3 mL/min. The amount of blood withdrawn progressively diminished, simulating a severe surgical blood loss. Animals were monitored for an additional hour following the end of the 60 min hemorrhage phase before being euthanized.

Systemic and microvascular parameters were measured at baseline (BL) and after hemodilution (HD), hemorrhage (H) and shock (S). MAP, HR and FCD were measured during the hemorrhage period after each blood withdrawal. Microvascular vessel diameter and blood flow were measured at 30 min intervals after the first hemorrhage (H30, H60) and in the shock phase (S30, S60). Arterial blood gases and hematocrit were measured at BL, HD, and the end of the shock period. Systemic blood chemistry and microcirculatory parameters were assessed at baseline and after HD, hemorrhage, and shock. MAP, HR, and FCD were determined during hemorrhage, after blood withdrawal, through the end of the shock phase. Oxygen was measured at the end of the shock period.

Study Material

Physical Properties of Oxyglobin and Hextend Are Summarized in Table 1.

Statistical Methods

Data are presented in terms of mean $\pm$ STD. Kruskal Wallis test was performed between time points of interest within a treatment group with Dunns post hoc analysis when differences were found. Mann-Whitney test was used to compare the two treatment groups. The product limit method (Kaplan-Meier) was used to produce survival curves and analysis of survival was made using the Log-rank test (Mantel-Cox). Statistical analyses were performed with Prism 4 software (Graphpad, San Diego, CA). Results were considered statistically significantly different when $p < .05$.

RESULTS

Survival

Eleven animals were entered into the study and divided randomly into 2 treatment groups before the experiment: **Oxyglobin** (n = 5) and **HEX** (n = 6). Baseline systemic data for the two groups were combined since there were no differences between groups.

Figure 2 shows the percent survival during the course of the experiment. All animals in the Oxyglobin group survived the protocol while there were some nonsurvivors in the HEX treatment group. Survival was significantly different between groups.

Hemodynamic Parameters

MAP was recorded at baseline, after the start of HD at time zero immediately following the first largest bleed H0, and 30 (H30) and 60 mins (H60) after, and during the shock period at 30 (S30) and 60 (S60) minutes after start of shock are given in Table 2. MAP and HR baseline values did not differ between Oxyglobin and HEX animal groups and exchange transfusion did not significantly influence MAP in either group. MAP tended to increase relative to baseline during hemodilution with Oxyglobin and to decrease with HEX causing MAP to be significantly different ($p < .02$) between groups at H60.

Hemorrhage significantly reduced MAP and HR in all treatments and Oxyglobin maintained a statistically higher MAP and HR relative to HEX.

MAP for Oxyglobin Was Significantly Increased Relative to HEX At HD At All Time Points.

Hemodilution did not significantly affect HR relative to baseline and both animal groups presented reduced HR at all time points after H0.

Blood Gas and Acid-base Response

Blood gases and chemical changes are shown in Table 3. No significant differences were found between Oxyglobin and HEX PaO₂ levels. Hemorrhage and shock caused a decreasing trend in Paco₂, pH, and base excess levels. Oxyglobin had a significantly increased blood pH at the end of shock vs. HEX ($p < .05$).

Tissue Perfusion

The effect of hemorrhage on FCD preceded by hemodilution with Oxyglobin is presented in Figure 3. FCD at S30 and S60 were also evaluated in addition to assessments taken at HD, H0, H10, H30 and H60. Oxyglobin maintained greater FCD until the end of the hemorrhage.

Microvascular Measurements

Microvascular diameters are shown in Figure 4. Arterioles were dilated for Hextend and constricted for Oxyglobin (A). Venular diameters tended to be unchanged for Oxyglobin and were constricted for Hextend (B).

Oxygen Distribution

There was no significant difference in the distribution of the partial pressure of oxygen between groups measured in arterioles, venules and the tissue. Pooled data are shown in Figure 5.

DISCUSSION

The principle finding of this study was that Oxyglobin had 100% survival after 1 hr shock when used in a moderate exchange transfusion followed by a severe bleed model, which simulates a major surgical bleed, a result not attained when using HEX. Statistically significant higher systemic blood pressure was also associated with better microvascular perfusion (FCD) in the Oxyglobin group when compared to HEX. This result is identical to that obtained using a plasma expander based on PEG-Albumin previously reported (7) which also yielded 100% survival, and the same final blood pressure and pH.

There was no significant difference in microhemodynamic parameters measured in the window chamber at the end of the experiment with the exception of FCD. Oxygen distribution was the same in both groups, showing that the tissue was hypoxic. Thus as in previous studies of extreme hemorrhage conditions (13) outcome appears to be primarily determined by differences in FCD rather than tissue oxygenation in subcutaneous tissue and muscle of the skin fold.

Mechanistically, oxygen delivery by constricted vessels not able to be transited by RBCs may be accomplished by an oxygen carrying molecular Hb solution, yielding a favorable and synergistic effect between increased oxygen carrying capacity and increased perfusion pressure, enhancing tissue oxygenation. This effect, not evident in the window chamber tissue, may be present in vital organs considering the more favorable base excess shown by the Oxyglobin group.

Low viscosity of the Hb solution cannot account for the survival benefit in this group; low viscosity plasma expansion in the same experimental protocol caused animals to succumb before the end of the hemorrhage period, (7) due to a significant drop in FCD. The remaining critical difference between treatments is, therefore, the increase in blood pressure due to vasoconstriction by the Hb solution that keeps up capillary perfusion and may, therefore, account for the observed survival.

Mechanistically both Hextend and Oxyglobin increase capillary pressure, and therefore FCD, however Oxyglobin appears to be more effective in these extreme conditions. This should be due to the significant difference in viscosity between solutions, which affects the heart function and its overall ability to pressurize the circulation. This suggests that there is a benefit (in these extreme conditions) in combining low viscosity perfusion with vasoconstriction.

Our objective was to determine which plasma expander improves survival under extreme conditions even though these might be rare and usually will not develop in a clinical setting. The experimental protocol chosen was sufficiently severe to immediately evidence how differences in blood composition and blood volume restitution fluid affect FCD. This protocol would rarely represent clinical conditions since an oxygen carrying transfusion would never be implemented before the use of a plasma expander. However, it may represent a situation in which a hemodiluted patient starts bleeding.

It is evident that the microcirculation of the skin is not representative of internal organs such as the viscera, the liver or the heart. Furthermore, it is known that during shock vasoconstriction of the vessels of the skin favors the shifting of blood toward more crucial organs such as the heart, the brain or the liver. Nevertheless, Kerger et al (13) demonstrated that maintenance of FCD was the only critical microvascular parameter correlated with survival in a model of severe hemorrhagic shock in the hamster window chamber. In view of this finding it seems justifiable to draw conclusions from data acquired in the hamster window chamber model.

We have shown experimentally that survival from hemorrhagic shock depends on the maintenance/reestablishment of an adequate FCD (5, 14). This depends directly on sustaining capillary pressure at normal levels (5, 15), a direct consequence of the transmission of central blood pressure to the periphery.

Vasopressors are used for restoring central blood pressure in the circulation; however vasoconstriction, increasing hydraulic resistance between the central and the peripheral circulation, increases pressure upstream from the constricting vessels (i.e., the arterioles) and lowers pressure downstream. The net effect being that FCD tends to decrease as shown by studies using noradrenaline or arginine-vasopressin (1, 16). These studies also showed significantly lowered tissue oxygenation due to increased arteriolar wall oxygen consumption (16), an effect also found using Oxyglobin (17).

Microvascular studies show that hypertension as a result of arteriolar vasoconstriction may lead to hypoperfusion and local tissue hypoxia/ischemia with lethal consequences on organ function when prolonged. Hypertension associated with the use of cross linked Hb solutions was related to focal necrosis (18) from vasoconstriction due to NO-scavenging (19). Notably there is no evidence that using vasopressors in the treatment of hemorrhagic shock leads to micronecrosis. Therefore, this effect may be specific to NO scavenging after use of Hb based oxygen carriers, which may aggravate hypoxia by increasing oxygen consumption of the vascular wall (20, 21), or to simply Hb-related toxicity (3).

We have shown that even if a Hb based oxygen carrier is vasoactive, it provides improved survival when used in small dosages by comparison to a non oxygen carrying colloidal resuscitation fluid such as albumin (22, 23). The actual balance between vasoconstriction and increased oxygen carrying capacity is also shown to have an optimal value in extreme hemodilution, providing improved FCD at a specific Hb plasma concentration, FCD being lower at higher and lower dosages (24).

In conclusion, Oxyglobin provides 100% survival in an extreme bleeding experimental model, while an identical intervention using a conventional starch based plasma expander leads to 50% mortality. We realize that even though the severity of the model leads to major differences in outcome, results may not be relevant in conventional medical practice. However, the eventual use of a material like Oxyglobin, even though it is vasoactive may be the determinant factor in extreme conditions when standard resuscitation procedures are unavailable. In these conditions the vasoactivity associated with this type of oxygen carrying plasma expander may be a positive factor, maintaining blood pressure and insuring perfusion. Therefore, the many functions of Oxyglobin could be beneficial in extreme scenarios with limited therapeutic options. In conventional settings a deficit in volume, oxygen delivery, and hypotension are treated in proportion of the magnitude and mechanism of each deficit, transferring control to the clinicians on how to best implement the therapeutic strategy.

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Figure 1.
Timeline of procedures and measurements.

Figure 2.
Survival following exchange transfusion and hemorrhage.

Figure 3.

Functional capillary density relative to baseline. HD: hemodilution; H0 – H60 hemorrhage phase; S30 – S60 shock phase, starting at H60. Numbers are time in minutes. $p < .05$ relative to BL (*), HD (†), H30 (‡); analysis between treatments at the same time point: $p < .05$ (§).

Figure 4.

Microvascular diameters relative to baseline. Arteriole (A) diameters: 35 - 50 μm and venules (B) 40 - 70 μm at baseline. HD: hemodilution; H30 - H60 hemorrhage phase; S30 - S60 shock phase, starting at H60. Analysis within the same treatment group: $p < .05$ relative to BL (*), HD (†), H30 (‡). Analysis between treatments at the same time point: $p < .05$ (§).

Figure 5. Distribution of pO₂ at the end of the shock phase of blood vessels and tissue treated with Oxyglobin and Hextend. The data are pooled since there was no statistical difference between groups.

Table 1

Properties of hamster blood, Oxyglobin and Hextend

	Hamster blood	Oxyglobin	Hextend
Source	Hamster	Bovine	-
Concentration (g/dl)	(Hb) 15	(Hb) 13	(HES) 6
COP (mm Hg)	16	42	36
Viscosity (cP)	4.2	1.8	3.9
p50 (mm Hg)	28	34	-
Average molecular weight (kDa)	-	65 -- 130	670 (450 -- 800)
Suspending fluid	-	Modified lactated Ringer's	Lactated Ringer

Abbreviations: COP, colloid osmotic pressure. Data from (12).

Table 2

Mean arterial pressure and heart rate

	Oxyglobin		Hextend	
	MAP	HR	MAP	HR
HD	113.8 ± 12.7 ^b	406.6 ± 42.5 ^b	97.2 ± 9.0	469.7 ± 14.7
H0	101.9 ± 14.2 ^b	411.7 ± 97.3 ^b	39.5 ± 4.6 ^{*†}	295.7 ± 24.0 ^{*†}
H30	65.4 ± 19.1 ^{*†}	313.5 ± 85.8 [*]	40.7 ± 5.7	326.5 ± 50.0
H60	45.5 ± 7.2 ^{*†}	239.9 ± 59.2 ^{*b}	30.7 ± 10.4 ^{*†}	313.0 ± 32.3 [†]
S30	65.33 ± 20.4 ^{†b}	307.3 ± 89.55 [*]	40.7 ± 5.7	326.5 ± 50.0
S60	70.6 ± 11.0 ^b	316.2 ± 80.2 [*]	41.0 ± 8.8	302.5 ± 26.4 [†]

Baseline: MAP (mm Hg) = 108.1 ± 8.0; HR (bpm) = 461.3 ± 33.4

Oxyglobin (n = 5); Hextend HD/H0/H30 (n = 6), H60 (n = 4), S30/S60 (n = 3)

^{*} Analysis within the same treatment group: $p < .05$ relative to BL and HD[†] Analysis between treatments at the same time point: $p < .05$ ^b Oxyglobin vs. Hextend

Table 3

Hematocrit and Arterial Blood Gas

	Baseline	Hemodilution		Hemorrhage 60		Shock 60	
		Oxyglobin	HEX	Oxyglobin	HEX	Oxyglobin	HEX
Hct, %	43.7 ± 1.8	25.9 ± 4.7 ^{*,†}	28.6 ± 1.4 [*]	16.7 ± 1.4 ^{*,b}	19.3 ± 0.5 ^{*,†}	17.1 ± 2.0 ^{*,b}	19.4 ± 0.5 [*]
PaO ₂ , mm Hg	60 ± 7	62 ± 5 ^b	65 ± 9	128 ± 18 ^{*,†,b}	105 ± 10 [*]	125 ± 21 ^{*,†}	120 ± 23 [*]
Pa[scap]co[1] ₂ , mm Hg	54 ± 3	54 ± 2 ^b	59 ± 6	43 ± 7 [†]	48 ± 10 [†]	46 ± 7 ^{*,†}	45 ± 12 [†]
pH arterial	7.38 ± 0.02	7.38 ± 0.02	7.36 ± 0.03	7.32 ± 0.07 ^b	7.19 ± 0.05 [*]	7.23 ± 0.08 ^{*,†,b}	7.09 ± 0.10 ^{*,†}
BE, mmol/l	5.0 ± 1.3	4.8 ± 1.2 ^b	7.2 ± 1.9	-5.3 ± 2.6 ^b	-10.2 ± 5.6 [†]	-8.5 ± 5.5 ^{*,†,b}	-13.8 ± 9.1 ^{*,†}

Oxyglobin (n = 5); Hextend: Hemodilution (n = 6), Hemorrhage 60 (n = 4), Shock 60 (n = 3)

* Analysis within the same treatment group: $p < .05$ relative to BL, HD[†] Analysis between treatments at the same time point: $p < .05$ ^b Oxyglobin vs. HEX group