Microcirculatory effects of a hyperviscous hemoglobin-based solution administered intravenously in dogs with experimentally induced hemorrhagic shock

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Objective—To determine whether increasing the viscosity of a standard hemoglobin-based oxygen-carrying solution (HBOC) would offset its associated vasoconstrictive effects and result in improved microvascular perfusion in healthy splenectomized dogs with experimentally induced hemorrhagic shock.

Animals—12 male American Foxhounds.

Procedures—Each dog underwent anesthesia and splenectomy. Shock was induced by controlled hemorrhage until a mean arterial blood pressure of 40 mm Hg was achieved and maintained for 60 minutes. Dogs were then randomly assigned to receive either a standard or hyperviscous HBOC (6 dogs/group). Sidestream dark-field microscopy was used to assess the effects of shock and HBOC administration on the microcirculation of the buccal mucosa and the jejunal serosa. Video recordings of the microcirculation were collected before shock was induced (baseline) and at intervals up to 180 minutes following HBOC administration. Vascular analysis software was used to compute microcirculatory variables.

Results—Compared with baseline findings, hemorrhagic shock resulted in decreases in all microvascular variables in the buccal mucosa and the jejunal serosa. At all time points following HBOC administration, microvascular variables were similar to initial values and no significant differences between treatment groups were detected. At all time points following HBOC administration, blood and plasma viscosities in dogs treated with the hyperviscous solution were significantly higher than values in dogs receiving the standard solution.

Conclusions and Clinical Relevance—In splenectomized dogs with experimentally induced hemorrhagic shock, administration of a hyperviscous HBOC did not significantly affect microvascular variables, compared with effects of a standard HBOC. Microcirculatory flow returned to baseline values in both treatment groups, suggesting that marked HBOC-associated vasoconstriction did not occur. (*Am J Vet Res* 2014;75:77–84)

Hemorrhagic shock can be an unfortunate sequela of severe trauma, surgery, and coagulopathy. Survival from hemorrhagic shock depends on restoration of tissue oxygenation and blood flow. Despite numerous experimental and clinical efforts, no clear benefit has been demonstrated for IV administration of one solution over another for resuscitation from hemorrhagic shock.

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Abbreviations

CI	Cardiac index
FCD	Functional capillary density
HBOC	Hemoglobin-based oxygen-carrying solution
LRS	Lactated Ringer's solution
MAP	Mean arterial blood pressure
O_2ER	Oxygen extraction ratio
$Scvo_2$	Central venous oxygen saturation
$SVRI^2$	Systemic vascular resistance index

Hemoglobin-based oxygen carriers are modified purified hemoglobin solutions designed to restore oxygen-carrying capacity as well as to provide volume expansion in individuals with severe anemia or hemorrhage. Although effective at carrying oxygen, there are several limitations to the use of HBOCs, the most important of which is vasoconstriction secondary to scavenging of nitric oxide from the endothelial surface of blood vessels.[1] This has been linked to development of hypertension and myocardial infarction, thereby limit-

ing the use of these products.[2,3] In rodents, it is known that HBOCs also cause a significant decrease in FCD, an assessment of regional perfusion that is highly correlated with tissue survival.[1,4]

A novel treatment strategy that has been explored in rodents as well as dogs with experimentally induced hemorrhagic shock is administration of viscosity-enhanced solutions.[5–9] These solutions are created by adding an inert polymer that increases viscosity of the original solution without affecting oncotic pressure. During hemorrhage, decreased blood viscosity develops from loss of RBC mass as well as dilution of the blood through transcompartmental fluid shifting and administration of resuscitative fluid therapy. Decreased plasma viscosity leads to decreased endothelial surface shear stress and decreased endothelial nitric oxide production, subsequently resulting in vasoconstriction and maldistribution of blood flow.[6,8,10] Resuscitation with hyperviscous solutions has been performed in rodents with hemorrhagic shock, and these solutions restored microcirculatory flow (through assessment of FCD) more effectively than did their standard counterparts.[4,6,8,11]

To best characterize the effects of HBOC administration and viscosity manipulation, direct visual assessment of the microcirculation is ideal. A videomicroscope[a] that incorporates sidestream dark-field technology can be used to image and assess microvascular perfusion. On the basis of previously established consensus criteria, the video recordings obtained during sidestream dark-field microscopy can be analyzed to determine several microcirculatory variables including total vessel density, perfused vessel density (a value analogous to FCD), proportion of perfused vessels, and microvascular flow index.[12] This device has been used extensively in both experimental and clinical settings to document normal microvascular anatomy, and to evaluate changes induced by sepsis and hemorrhagic shock as well as a variety of other disease states.[13–17]

The purpose of the study reported here was to determine whether increasing the viscosity of a standard HBOC would offset its associated vasoconstrictive effects and result in improved microvascular perfusion in healthy splenectomized dogs with experimentally induced hemorrhagic shock. We hypothesized that administration of the hyperviscous HBOC would result in significantly improved tissue perfusion, compared with the effects of the standard HBOC, as determined by assessments of both macrovascular and microvascular variables.

Materials and Methods

All procedures were performed with approval from The Ohio State University Institutional Animal Care and Use Committee. Twelve healthy sexually intact male American Foxhounds with a mean ± SD body weight of 24.9 ± 2.56 kg (range, 19 to 29 kg) were used in the study. Food was withheld from the dogs the night before the day of the experiment, but free access to water was allowed until the next morning.

Preparation of the test fluid—The control fluid was a standard HBOC[b] (mean dynamic viscosity, 1.37 cP [1 cP = 0.001 Pa•s]) approved for use in dogs. The hyperviscous HBOC was made by adding 0.375 g of alginate to each 125-mL bag of standard HBOC to create a 0.3% solution. The solution was thoroughly mixed by use of a heated magnetic mixer. The resulting mean ± SD viscosity of the hyperviscous HBOC was 4.96 ± 0.52 cP, which closely approximates the viscosity of whole blood (4 cP) in clinically normal dogs.

Anesthesia and instrumentation—On the day of the experiment, each dog was instrumented with an IV catheter in the left cephalic vein and given hydromorphone hydrochloride (0.1 mg/kg, IV) for premedication. Anesthesia was induced with diazepam (0.5 mg/kg, IV) and ketamine hydrochloride (5 to 10 mg/kg, IV). The dogs were orotracheally intubated, and anesthesia was maintained with sevoflurane gas administered in 100% oxygen. Mechanical ventilation was performed to maintain end-tidal carbon dioxide concentration at 35 to 45 mm Hg. The dogs received fentanyl citrate as a continuous rate infusion (6 to 10 μg/kg/h). Lactated Ringer's solution was administered IV to each dog throughout the experiment at a rate of 5 mL/kg/h. Pulse oximetry,[c] ECG, and monitoring of heart rate and end-tidal carbon dioxide concentration[c] were continuously performed. A catheter was placed in the right second dorsal metatarsal artery for direct arterial blood pressure measurement[d] and lithium dilution cardiac output monitoring.[e] A 20-cm double lumen central venous catheter was placed in the left jugular vein for blood sample collection, determination of central venous pressures,[c] and administration of lithium. The left carotid artery was surgically exposed for placement of a 10F polyethylene catheter connected to a 3-way stopcock for induction of controlled hemorrhage and collection of arterial blood samples. A splenectomy was performed through a ventral celiotomy incision to limit the variable hemodynamic effects of splenic contraction, which can occur during hemorrhagic shock.[18] The abdomen was partially closed to prevent dehydration and loss of heat; however, a loop of jejunum was exposed and packed off in gauze soaked in saline (0.9% NaCl) solution for microvascular imaging. A mesenteric vein was catheterized for blood sample collection. An 8F indwelling red rubber catheter was placed into the urinary bladder and connected to a closed collection system for urine output monitoring.

Experimental design—Following instrumentation and splenectomy, each dog was placed in right lateral recumbency and a 30-minute equilibration period was allowed to elapse prior to obtaining baseline measurements. A stopcock and closed collection system containing heparin were connected to the carotid artery catheter, and the dog was allowed to bleed freely until a sustained MAP of 35 to 45 mm Hg was achieved. The shock state was maintained for 60 minutes by additional removal of blood or administration of LRS as needed. The total amount of blood removed was recorded for each dog. At the conclusion of the shock period, collection of all data was repeated.

The dogs were randomized by selection of sealed envelopes to receive either standard or hyperviscous

HBOC (6 dogs/group) as a resuscitative measure. As recommended by the manufacturer, each solution was administered as a bolus (30 mL/kg) over a 30-minute period during which the LRS infusion was discontinued. To assess efficiency of fluid resuscitation, the time and volume required to reach a MAP of 65 mm Hg (the target minimum resuscitation pressure) after initiation of HBOC infusion were recorded. Once HBOC administration was completed, the LRS infusion was restarted at the previous rate. Boluses of 5 to 10 mL of LRS/kg were administered as needed to maintain a MAP of 65 mm Hg.

For each dog, anesthesia was maintained, and all data were repeatedly collected at 30, 60, 120, and 180 minutes after completion of resuscitative fluid therapy and achieving target MAP of at least 65 mm Hg. Following the final data collection, each dog was euthanized while anesthetized with 100 mg of pentobarbital/kg administered IV.

Measurement of macrovascular, blood gas, and rheological variables—Macrovascular variables measured included heart rate, central venous pressure, systolic arterial blood pressure, diastolic arterial blood pressure, and MAP. Cardiac output was determined with a lithium dilution technique as previously described.[19,20] Data obtained from arterial, mesenteric venous, and central venous blood gas analyses[f] included blood pH, P_{O_2} and P_{CO_2}, oxygen saturation, base excess, and concentrations of lactate, bicarbonate, total hemoglobin, and sodium. Sodium concentrations were acquired for use in lithium dilution cardiac output determination.[19] Packed cell volume was determined by centrifugation, and total plasma protein concentration was measured by refractometry. Colloid osmotic pressure was measured with a colloid osmometer.[g] Whole blood viscosity and plasma viscosity were measured within 20 minutes after sample collection with a viscometer[h] at a shear rate of 150 seconds^{-1} and temperature of 37°C. By use of standard equations, values of CI, SVRI, systemic oxygen delivery, systemic oxygen consumption, and systemic O_2ER were calculated.[21]

Measurement of microvascular variables—At each time of data collection, 3 video recordings of the microcirculation of both the buccal mucosa and the jejunal serosa were obtained with a videomicroscope. According to previously established consensus criteria, it is recommended to obtain video recordings of 20 seconds' duration.[12] However, owing to technical limitations of the study design, the duration of the video recordings obtained was only 5 seconds. Specifically, peristalsis of the small intestine limited the ability to acquire stable videos of > 5 seconds' duration. This time frame was then used at both sites to maintain consistent analysis.

For the buccal site, video recordings were obtained from the mucogingival junction dorsal to a carnassial tooth. For the intestinal site in the loop of jejunum that was exteriorized and packed off with laparotomy sponges, video recordings were obtained from an area of serosa that appeared free of large vessels. In all circumstances, care was taken to minimize application pressure and risk of vessel compression. The video recordings were then stored and analyzed with special-

Table 1—Macrovascular variables determined in dogs 30 minutes following splenectomy (baseline), at 60 minutes after induction of hemorrhagic shock by means of controlled hemorrhage, and at 30, 60, 120, and 180 minutes after administration of a standard or hyperviscous HBOC (bolus [30 mL/kg] administered over a 30-minute period; 6 dogs/group).

Variable	HBOC treatment group	Baseline	After shock period	Time after HBOC administration (min)			
				30	60	120	180
Heart rate (beats/min)	Standard	72 ± 15	210 ± 9*	91.2 ± 18	104 ± 22	95.5 ± 19	101 ± 21
	Hyperviscous	54 ± 5	200 ± 6*	96 ± 21	98 ± 12	115 ± 15	125 ± 19
SAP (mm Hg)	Standard	136.8 ± 9.7	43.0 ± 1.9*	144.5 ± 10.9	151.0 ± 4.6	144.0 ±12.2	142.0 ± 8.8
	Hyperviscous	147.5 ± 8.4	46.2 ± 2.1*	142.3 ± 15.2	147.0 ± 7.9	150.3 ± 9.8	158.3 ± 15.3
MAP (mm Hg)	Standard	91.33 ± 8.1	37.67 ± 1.3*	92.23 ± 9.1	86.79 ± 2.4	90.50 ± 8.8	89.83 ± 5.0†
	Hyperviscous	96.83 ± 7.6	39.00 ± 0.6*	93.48 ± 11.2	92.00 ± 4.4	100.50 ± 6.4	110.80 ± 10.0†
DAP (mm Hg)	Standard	78.83 ± 8.0	32.67 ± 1.1*	81.23 ± 5.8	79.36 ± 7.2	73.33 ± 6.3	85.17 ± 16.7
	Hyperviscous	81.17 ± 6.5	33.67 ± 0.6*	83.46 ± 6.7	81.87 ± 10.2	86.33 ± 5.9	93.17 ± 10.1
CI (L/min/m2)	Standard	3.53 ± 0.37	1.51 ± 0.17*	3.33 ± 0.21†	3.38 ± 0.12†	3.04 ± 0.19†	3.39 ± 0.15†
	Hyperviscous	2.96 ± 0.28	1.41 ± 0.34*	2.24 ± 0.18†	2.34 ± 0.31†	2.21 ± 0.27†	2.56 ± 0.20†
SVRI (dyne•s/cm^5/m^2)	Standard	2,143 ± 421	2,175 ± 257	2,100 ± 302	2,439 ± 238	2,338 ± 261†	2,067 ± 129†
	Hyperviscous	2,608 ± 334	2,797 ± 501	2,068 ± 459	3,441 ± 573	3,361 ± 293	3,402 ± 598
Oxygen delivery (mL/min)	Standard	705 ± 192	233 ± 62*	624 ± 64	482 ± 75	415 ± 62	443 ± 54
	Hyperviscous	548 ± 218	220 ± 146*	450 ± 164	374 ± 75	293 ± 96	340 ± 94
Oxygen consumption (mL/min)	Standard	51.04 ± 42.7	153.69 ± 48.1*	54.28 ± 16.7	90.20 ± 35.7	94.92 ± 49.4*	92.90 ± 26.9*
	Hyperviscous	26.60 ± 16.5	144.29 ± 86.1*	62.70 ± 22.4	85.75 ± 25.9	80.69 ± 18.1*	105.87 ± 32.1*
Central venous pressure (mm Hg)	Standard	4.83 ± 2.6	−1.17 ± 1.2*	2.17 ± 1.2*	2.50 ± 2.5*	2.50 ± 2.6*	2.50 ± 2.6*
	Hyperviscous	4.83 ± 2.4	−1.17 ± 1.2*	2.33 ± 1.0*	2.17 ± 1.8*	1.83 ± 1.2*	0.83 ± 1.2*
Colloid osmotic pressure (mm Hg)	Standard	16.1 ± 2.0	11.2 ± 1.2	18.1 ± 1.1	16.9 ± 0.8	15.9 ± 0.8	14.8 ± 0.8
	Hyperviscous	16.1 ± 0.8	11.5 ± 1.4	18.9 ± 1.5	17.4 ± 1.4	15.8 ± 1.6	14.6 ± 2.2
Total protein (g/dL)	Standard	5.7 ± 0.6	4.6 ± 0.5	UD	UD	UD	UD
	Hyperviscous	5.7 ± 0.4	4.7 ± 0.5	UD	UD	UD	UD
Hemoglobin (mg/dL)	Standard	16.1 ± 1.1	12.0 ± 1.5	11.5 ± 0.6	10.9 ± 0.5	10.7 ± 0.6	10.4 ± 0.7
	Hyperviscous	16.1 ± 1.0	12.4 ± 0. 7	11.0 ± 0.7	10.6 ± 0.8	10.5 ± 0.7	10.3 ± 0.5
PCV (%)	Standard	46.5 ± 3.2	36.0 ± 5.0	20.0 ± 2.6	19.2 ± 2.9	19.0 ± 1.0	19.5 ± 2.6
	Hyperviscous	47.7 ± 2.5	37.2 ± 1.8	22.0 ± 2.5	20.0 ± 2.7	20.8 ± 3.1	20.1 ± 1.7

Data are reported as mean ± SD.

*For a given variable at this time point, value differs significantly ($P < 0.05$) from the baseline value for the respective treatment group. †Within a variable, value at this time point is significantly ($P < 0.05$) different between treatment groups.

DAP = Diastolic arterial blood pressure. SAP = Systolic arterial blood pressure. UD = Unable to be determined (owing to plasma discoloration).

Table 2—Central venous blood gas data obtained for dogs 30 minutes following splenectomy (baseline), at 60 minutes after induction of hemorrhagic shock by means of controlled hemorrhage, and at 30, 60, 120, and 180 minutes after administration of a standard or hyperviscous HBOC (bolus [30 mL/kg] administered over a 30-minute period; 6 dogs/group).

Variable	HBOC treatment group	Baseline	After shock period	Time after HBOC administration (min)			
				30	60	120	180
Blood pH	Standard	7.33 ± 0.01	7.11 ± 0.02*	7.26 ± 0.02	7.27 ± 0.01	7.28 ± 0.01	7.27 ± 0.01
	Hyperviscous	7.33 ± 0.01	7.10 ± 0.02*	7.25 ± 0.01	7.25 ± 0.02	7.25 ± 0.03	7.26 ± 0.02
Po_2 of central venous blood	Standard	83.6 ± 26.6	39.2 ± 8.1*	130.8 ± 14.6†	88.3 ± 19.3†	80.4 ± 19.4†	80.8 ± 19.1†
	Hyperviscous	83.5 ± 17.8	36.2 ± 4.9*	90.1 ± 18.8†	65.5 ± 17.5†	56.7 ± 12.1†	52.9 ± 17.1†
Pco_2 of central venous blood	Standard	45.55 ± 0.97	68.02 ± 1.17*	71.17 ± 2.34	53.09 ± 1.79	52.63 ± 1.59†	52.43 ± 1.34†
	Hyperviscous	45.47 ± 0.91	74.27 ± 2.13*	74.02 ± 1.78	53.67 ± 3.08	56.08 ± 3.61†	54.60 ± 2.90†
$Scvo_2$ (%)	Standard	95.33 ± 1.64	35.25 ± 2.56*	79.45 ± 3.25†	80.20 ± 3.76†	77.53 ± 3.76†	78.65 ± 2.95†
	Hyperviscous	94.37 ± 1.34	33.70 ± 3.24*	72.04 ± 5.40†	70.61 ± 1.75†	69.43 ± 4.85†	67.05 ± 5.10†
Base excess (mmol/L)	Standard	−1.92 ± 0.54	−7.33 ± 0.93*	−3.34 ± 0.39	−2.29 ± 0.23	−2.03 ± 0.26	−2.57 ± 0.47
	Hyperviscous	−1.63 ± 0.74	−6.38 ± 0.83*	−3.65 ± 0.56	−2.31 ± 1.12	−2.37 ± 1.95	−2.75 ± 2.00
HCO_3 (mmol/L)	Standard	22.34 ± 0.56	16.03 ± 0.68*	17.14 ± 0.48	20.98 ± 0.21	21.77 ± 0.32	21.35 ± 0.43
	Hyperviscous	22.55 ± 0.65	16.32 ± 0.65*	17.09 ± 0.78	19.78 ± 1.06	21.27 ± 1.08	21.03 ± 1.03
Lactate (mmol/L)	Standard	1.57 ± 0.13	3.57 ± 0.36*	3.01 ± 0.45*	2.56 ± 0.21	2.10 ± 0.32	1.66 ± 0.34
	Hyperviscous	1.63 ± 0.14	4.18 ± 0.46*	2.78 ± 0.19*	2.05 ± 0.43	1.32 ± 0.17	2.60 ± 0.42

Data are reported as mean ± SD. *See* Table 1 for key.

ized vascular analysis software[i] by a single investigator (AMP) who was blinded to the treatment group as well as the study time point at which the video recordings were obtained. All video recordings were assessed for overall quality. Video recordings that contained poor image resolution, excessive motion, or pressure artifact were withdrawn from analysis. The analysis software was then used to determine the microvascular variables previously established by consensus criteria.[12] The microvascular variables of interest were total vessel density, perfused vessel density, proportion of perfused vessels, and microvascular flow index. For each image, total vessel density was derived by dividing number of vessels crossing a superimposed grid of 3 equidistant horizontal and 3 vertical lines by the length of the lines. The proportion of perfused vessels is a value based on the assignment of a flow category to each vessel (continuous, intermittent, or no flow). The proportion of perfused vessels was calculated as follows:

Proportion of perfused vessels = 100 × (total number of vessels − [number of vessels with no flow + intermittent flow])/ total number of vessels

The perfused vessel density was calculated by multiplying the total vessel density by the proportion (decimal fraction) of perfused vessels. The analysis software was also used to determine the microvascular flow index. This value is obtained by dividing the visual field into quadrants and characterizing the flow as absent (0), intermittent (1), sluggish (2), or normal (3).[31] The mean value from each quadrant is then calculated to determine the microvascular flow index.

To assess measurement reliability, intraobserver variability was determined for all microvascular variables. Video recordings from 8 dogs were randomly selected and analyzed in triplicate by the same blinded investigator who performed the study analysis.

Statistical analysis—The data were analyzed for normality with the Kolmogorov-Smirnov test. To evaluate differences between groups and among time points, a 2-way ANOVA for repeated measures was used. When significant differences were found, a Holm-Sidak post test was used to further characterize the differences between groups. For single-measure variables (eg, cumulative urine output), a Student *t* test was performed to determine differences between control and experimental groups. All statistical analyses were performed with commercially available statistics software.[j] For all tests, a value of $P < 0.05$ was considered significant.

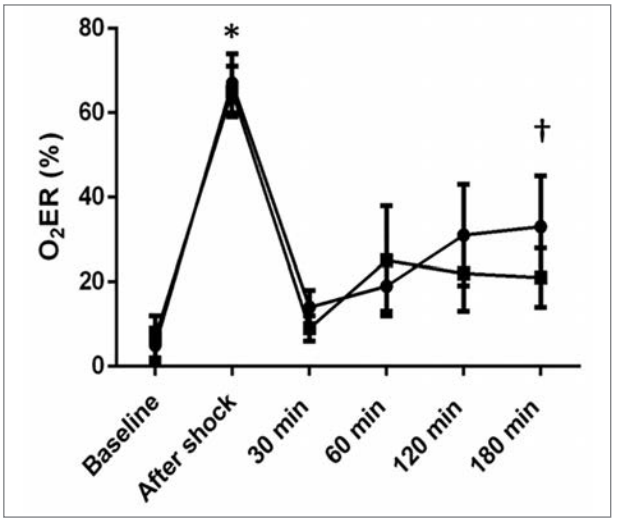

Figure 1—Mean ± SD O_2ER in splenectomized dogs in which hemorrhagic shock was experimentally induced by means of controlled hemorrhage followed by IV administration of a standard (squares) or hyperviscous (circles) HBOC (bolus [30 mL/kg] administered over a 30-minute period; 6 dogs/group). Data were obtained before (30 minutes after splenectomy [baseline]) and after a 60-minute shock period and at 30, 60, 120, and 180 minutes after HBOC administration. *At a given time point, values are significantly ($P < 0.05$) different from all other time points but not different between treatment groups. †At a given time point, values for the 2 treatment groups are significantly ($P < 0.05$) different.

Results

Macrovascular variables—All data were normally distributed and are reported as mean ± SD. There were

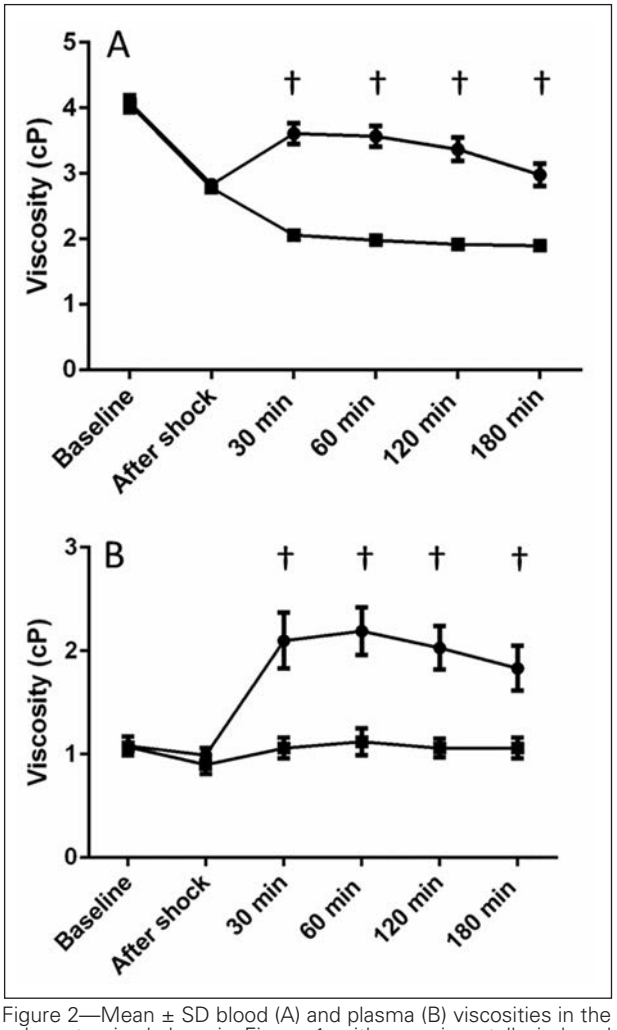

Figure 2—Mean ± SD blood (A) and plasma (B) viscosities in the splenectomized dogs in Figure 1 with experimentally induced hemorrhagic shock that received treatment with standard or hyperviscous HBOC. *See* Figure 1 for key.

no significant differences between groups at baseline (30 minutes after dogs underwent instrumentation and splenectomy) for any macrovascular variable. As expected, hemorrhagic shock resulted in tachycardia, metabolic acidosis, and decreased $Scvo_2$ (**Tables 1 and 2**) and increased O_2ER (**Figure 1**) in all dogs but with no significant differences between the groups. Following HBOC administration (at 30 and 60 minutes after resuscitative fluid administration), all blood gas variables and macrovascular variables except for central venous pressure were not significantly different, compared with baseline values. Blood and plasma viscosities were significantly higher in dogs treated with hyperviscous HBOC at all posttreatment time points (**Figure 2**). At 1 or more time points after HBOC administration, the group that received hyperviscous HBOC had significantly higher SVRI, MAP, and O_2ER as well as decreased CI, Po_2 and Pco_2 of central venous blood, and $Scvo_2$; there were no other significant differences in macrovascular variables between the 2 groups.

For single comparison variables, there were no significant differences between groups with regard to the mean total amount of blood removed to induce and maintain the shock state (52.7 ± 9.3 mL/kg for dogs that received hyperviscous HBOC vs 50.5 ± 5.4 mL/kg for dogs that received standard HBOC; $P = 0.63$) or mean total amount of crystalloid solution administered throughout the course of the experiment (33.3 ± 7.4 mL/kg for dogs that received hyperviscous HBOC vs 35.6 ± 8.1 mL/kg for dogs that received standard HBOC; $P = 0.62$). Over the course of the experiment, dogs that received standard HBOC produced significantly ($P = 0.03$) more urine (122 ± 32 mL) than did dogs that received hyperviscous HBOC (81 ± 21 mL). There was no significant difference in the volume of HBOC administered to reach an MAP of 65 mm Hg (145 ± 65.5 mL for dogs that received hyperviscous HBOC vs 118 ± 23 mL for dogs that received standard HBOC; $P = 0.39$) or the time until an MAP of 65 mm Hg was attained (6.62 ±

Table 3—Microvascular variables determined for the buccal mucosa (BM) and jejunal serosa (JS) in dogs 30 minutes following splenectomy (baseline), at 60 minutes after induction of hemorrhagic shock by means of controlled hemorrhage, and at 30, 60, 120, and 180 minutes after administration of a standard or hyperviscous HBOC (bolus [30 mL/kg] administered over a 30-minute period; 6 dogs/group).

Variable	HBOC treatment group	Baseline	After shock period	Time after HBOC administration (min)			
				30	60	120	180
TVD-BM (mm/m²)	Standard	13.62 ± 2.6	10.40 ± 4.4*	13.28 ± 2.6	12.93 ± 3.0	13.06 ± 2.4	12.18 ± 3.1
	Hyperviscous	14.53 ± 2.3	10.94 ± 3.4*	13.43 ± 2.4	13.03 ± 2.3	11.34 ± 2.5	13.48 ± 2.0
TVD-JS (mm/m²)	Standard	13.14 ± 1.8	9.67 ± 2.3*	12.69 ± 1.8	11.62 ± 2.1	11.65 ± 2.0	11.88 ± 2.2
	Hyperviscous	10.00 ± 2.7	9.28 ± 3.0*	10.60 ± 2.6	12.10 ± 2.1	12.17 ± 3.7	11.15 ± 2.4
PVD-BM (mm/m²)	Standard	14.43 ± 2.7	6.76 ± 3.7*	13.58 ± 2.8	12.84 ± 3.9	13.4 ± 2.9	12.75 ± 5.3
	Hyperviscous	14.80 ± 3.0	6.88 ± 5.8*	12.00 ± 5.5	10.74 ± 4.0	10.3 ± 3.2	13.04 ± 2.6
PVD-JS (mm/m²)	Standard	12.70 ± 2.8	5.21 ± 2.5*	10.47 ± 4.7	8.42 ± 3.0	8.78 ± 2.9	7.55 ± 3.5
	Hyperviscous	9.80 ± 2.5	4.80 ± 3.6*	9.32 ± 5.0	11.10 ± 2.6	11.12 ± 3.2	11.05 ± 2.9
PPV-BM (%)	Standard	81.88 ± 8	63.19 ± 26*	81.74 ± 12	78.32 ± 17	84.07 ± 9	80.83 ± 28
	Hyperviscous	83.64 ± 9	51.23 ± 33*	70.41 ± 34	68.77 ± 22	77.1 ± 20	87.45 ± 10
PPV-JS (%)	Standard	84.35 ± 8	39.24 ± 20*	77.38 ± 31	67.0 ± 16	70.50 ± 20	60.61 ± 28
	Hyperviscous	89.12 ± 7	42.74 ± 30*	79.50 ± 31	84.7 ± 11	74.12 ± 23	91.03 ± 7
MFI-BM	Standard	2.53 ± 0.43	1.71 ± 0.78*	2.60 ± 0.32	2.38 ± 0.73	2.50 ± 0.47	2.47 ± 0.98
	Hyperviscous	2.63 ± 0.47	1.43 ± 0.92*	2.31 ± 0.95	2.16 ± 0.82	2.39 ± 0.55	2.34 ± 0.51
MFI-JS	Standard	2.61 ± 0.32	1.05 ± 0.73*	2.29 ± 0.70	2.06 ± 0.73	2.20 ± 0.51	1.92 ± 0.58
	Hyperviscous	2.65 ± 0.36	1.11 ± 0.80*	2.38 ± 0.74	2.41 ± 0.37	2.42 ± 0.56	2.57 ± 0.56

Data are reported as mean ± SD.
*Value is significantly ($P < 0.05$) different from all other time periods, with no difference between treatment groups.
MFI = Microvascular flow index. PPV = Proportion of perfused vessels. PVD = Perfused vessel density. TVD = Total vessel density.

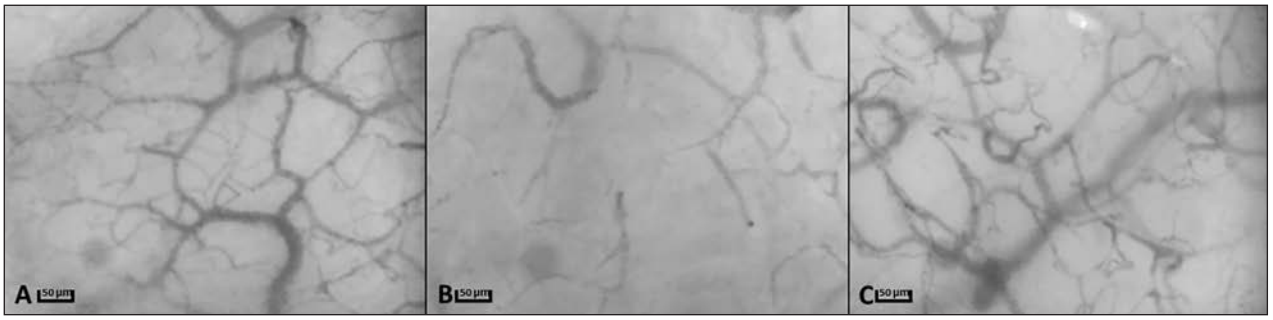

Figure 3—Photomicrographs of the microcirculation of the buccal mucosa in 1 of the 6 splenectomized dogs in Figure 1 with experimentally induced hemorrhagic shock that received treatment with hyperviscous HBOC. Images were obtained by means of sidestream dark-field microscopy before (30 minutes after splenectomy [baseline; A]) and after a 60-minute shock period (B), and at 30 minutes after HBOC administration (C). Bar = 50 μm.

Figure 4—Photomicrographs of the microcirculation of the jejunal serosa in the same dog in Figure 3 with experimentally induced hemorrhagic shock that received treatment with hyperviscous HBOC. Images were obtained by means of sidestream dark-field microscopy before (30 minutes after splenectomy [baseline; A]) and after a 60-minute shock period (B), and at 30 minutes after HBOC administration (C). Bar = 50 μm.

2.1 minutes for dogs that received hyperviscous HBOC vs 4.62 ± 0.8 minutes for dogs that received standard HBOC; $P = 0.06$).

Microvascular variables—All videos were assessed for quality and clarity of the recording. Some video recordings had to be removed from the analysis because of suboptimal quality, which was not apparent at the time of acquisition. The number of recordings that had to be removed at each time point included 3 at baseline, 3 after the 60-minute shock period, 1 at the 30-minute time point, 4 at the 60-minute time point, 2 at the 120-minute time point, and 5 at the 180-minute time point (18/216 [8%] video recordings). All dogs had at least 2 videos of excellent quality for each time point.

With regard to microvascular variables, no significant differences between the hyperviscous HBOC and standard HBOC treatment groups were noted at baseline or after the 60-minute hemorrhagic shock period (**Table 3**). Perfused vessel density was significantly ($P < 0.05$) lower in both treatment groups after the shock period, compared with the respective baseline value. This was evident on gross inspection of the videomicroscope images (**Figures 3 and 4**) as well from determination of perfused vessel density with vascular analysis software. In both treatment groups, microvascular flow index, total vessel density, and proportion of perfused vessels were also significantly ($P < 0.05$) decreased from baseline for both the buccal mucosa and jejunal serosa after the shock period. Following HBOC administration, there were no significant differences in microvascular variables between dogs that received standard HBOC or hyperviscous HBOC; similarly, there were no significant differences in microvascular variables between baseline and the 30-, 60-, 120-, or 180-minute posttreatment time points for either group. The coefficient of variation for intraobserver variability for all microvascular variables was within the accepted limits of < 10%.[22]

Discussion

In the present study, as expected, hemorrhagic shock resulted in profound hemodynamic derangements in the splenectomized dogs. After the shock period, dogs received either a hyperviscous or standard HBOC as a resuscitative measure. Although administration of hyperviscous HBOC resulted in increased plasma and blood viscosities, compared with the effects of treatment with standard HBOC, there were no clinically relevant differences between the treatment groups. However, HBOC administration in either group resulted in recovery of most macrovascular and all microvascular variables such that they were not significantly different from baseline values.

At 30 minutes after HBOC administration, plasma viscosity in the dogs that received hyperviscous HBOC was 2.19 cP, similar to the ideal target viscosity determined in another study of resuscitative hyperviscous fluid therapy in hamsters with experimentally induced hemorrhagic shock.[6] Although a plasma viscosity of 2.2 cP was shown to be beneficial in that study, it is possible that the degree of viscosity enhancement needed to alter microvascular perfusion is different in dogs, thereby resulting in no significant differences in microvascular variables between the treatment groups in the present study.

Although viscosity enhancement of the HBOC did not appear to offer any advantage with regard to improving microcirculatory perfusion in the present

study, it is interesting to note that most microcirculatory variables were restored to baseline values (obtained 30 minutes after dogs underwent instrumentation and splenectomy) in both treatment groups. In the study dogs, there was no significant reduction in microvascular flow and no development of systemic hypertension, compared with findings at baseline, which is contrary to data in multiple published reports of HBOC use.[1–3,23,24] These differences may be a consequence of the severity of shock induced by the experimental design of the present study because loss of vasomotor tone is a component of severely decompensated shock.[25] The use of inhalation anesthesia could also contribute to lack of vasoconstriction because sevoflurane directly relaxes vascular smooth muscle.[26] This effect could also explain the lack of significant change in SVRI following hemorrhage in the dogs of the present study. However, sidestream dark-field microscopy revealed marked decreases in all microvascular variables after the shock period, despite the unchanged SVRI. Given that SVRI is a calculated variable, it may not be reflective of the microcirculation, especially if shunting or regional decreases in microvascular perfusion have occurred.

Interestingly, the dogs that received the hyperviscous HBOC in the present study had increased SVRI at the 120- and 180-minute posttreatment time points, compared with findings for the dogs that received the standard HBOC, although those values were not significantly different from the baseline values for hyperviscous HBOC-treated dogs. Given that resuscitation with hyperviscous fluids is purported to promote vasodilation and improve flow through the microcirculation, there should have been a reduction in SVRI rather than an increase. At each of those 2 time points, the dogs that received hyperviscous HBOC had significantly decreased CI, compared with the CI for the dogs that received the standard HBOC; this was presumably attributable to increased resistance to flow secondary to the increased blood viscosity.[27,28] The SVRI cannot be directly measured, but rather it was calculated by use of an equation as follows: ([MAP – central venous pressure] × 80)/CI. Many variables influence SVRI; however, it is directly proportional to the radius of the blood vessels and indirectly proportional to blood viscosity. In the present study, any vasodilation provided by the administration of the hyperviscous HBOC may have been offset by the viscosity-associated increase in resistance and decease in CI, with the net effect being an increase in calculated SVRI. This idea is substantiated by the fact that there was no evidence of significant reduction in microvascular variables in the hyperviscous HBOC treatment group, compared with baseline values or values for the standard HBOC treatment group, which would be expected if vasoconstriction was the underlying cause for increased SVRI.

The dogs that received hyperviscous HBOC in the present study had decreased $Scvo_2$ and Po_2 of central venous blood and increased O_2ER at several time points, compared with findings for the dogs that received standard HBOC. These findings are similar to those of our previous study,[5] in which resuscitative fluid therapy with hyperviscous LRS in dogs with hemorrhagic shock was evaluated. Increased oxygen extraction ratio may reflect poor resuscitation of the hyperviscous HBOC-treated group because $Scvo_2$, Po_2 of central venous blood, and O_2ER are indirect indicators of tissue perfusion and oxygen consumption. Alternatively, if the hyperviscous HBOC-treated group had less microvascular shunting and better perfusion, those changes may represent repayment of the oxygen debt that was accumulated during the shock period. Results of a recent study[29] in humans with sepsis indicated that the death rate among patients with high $Scvo_2$ was higher than that among patients in which $Scvo_2$ was considered normal, presumably because of microvascular shunting or mitochondrial dysfunction in the patients with high $Scvo_2$.

There were several limitations to the present study. The dogs were administered inhalation anesthesia and because of the effects of sevoflurane on vasomotor tone, the use of this drug may have resulted in falsely increasing the baseline values of the microvascular variables as well as blunting the microvascular response to shock and resuscitation. The present study also involved a fixed-pressure, controlled hemorrhage procedure, which may limit the clinical application of these results. This might be especially true in patients in hemorrhagic shock that may have ongoing bleeding or comorbidities such as trauma. However, performing laparotomy and splenectomy in the study dogs introduced some degree of tissue injury and inflammation—processes that can have a major impact on physiologic response to hemorrhagic shock—and thereby more closely approximated the effects of trauma.[30,31]

Because of the impact of intestinal motility, the duration of the microcirculatory video recordings obtained in the present study was only 5 seconds, as opposed to the recommended 20 seconds' duration. The resulting shorter video recordings may have led to overestimation of microvascular variables if flow was intermittent during a 20-second period rather than the 5-second period captured for analysis. In addition, all video recordings appeared to be of adequate technical quality at time of acquisition, but during analysis, several videos were deemed unacceptable because of excessive motion or pressure artifacts. Ultimately, 18 video recordings were removed from the analysis, which resulted in some dogs only having 2 video recordings available for analysis at certain time points.

Last, the sample size was small, with only 6 dogs in each treatment group. Although power calculations performed prior to beginning the study indicated that 6 dogs/group would be adequate to find a 10% difference (with a value of $P < 0.05$) between the groups, it is possible that use of a larger number of dogs may have resulted in detection of significant differences between the groups.

On the basis of the results of the present study, it appears that resuscitative fluid therapy with a hyperviscous HBOC does not offer advantages over resuscitative fluid therapy with the standard solution in splenectomized dogs with hemorrhagic shock. After both treatments, most macro- and microvascular variables returned to preshock baseline values. As such, there was no evidence of HBOC-induced vasoconstriction after

administration of either the hyperviscous or standard solution as revealed by direct imaging of microcirculatory vessels. Compared with dogs that received the standard HBOC, dogs that received the hyperviscous HBOC had significantly lower Scv_{O_2} at several posttreatment time points and significantly higher O_2ER at the 180-minute posttreatment time point, which could indicate less shunting and improved homogeneity of flow; however, this was not reflected in the microvascular variables. Although further study may be warranted, the findings of the present study suggest that administration of viscosity-enhanced HBOC does not appear to result in improved resuscitation or microvascular perfusion, compared with the effects of a standard HBOC solution, in splenectomized dogs with experimentally induced hemorrhagic shock.

a. Microscan, MicroVision Medical, Amsterdam, The Netherlands.
b. Oxyglobin, Biopure, Cambridge, Mass.
c. Datex, Instrumentarium Corp, Helsinki, Finland.
d. Passport 2, Datascope Corp, Fairfield, NJ.
e. LiDCO, LiDCO Ltd, London, England.
f. ABL 725, Radiometer America, Westlake, Ohio.
g. 4420, Wescor, Logan, Utah.
h. DV-II+ viscometer, Brookfield Engineering Laboratories, Middleboro, Mass.
i. ADA Vascular Analysis, MicroVision Medical, Amsterdam, The Netherlands.
j. SigmaStat, version 3.5, Systat, San Jose, Calif.

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