Veterinary Anaesthesia and Analgesia, 2006, **33**, 368–380 doi:10.1111/j.1467-2995.2005.00280.x

RESEARCH PAPER

Effects of isovolemic resuscitation with hemoglobin-based oxygen carrier Hemoglobin glutamer-200 (bovine) on systemic and mesenteric perfusion and oxygenation in a canine model of hemorrhagic shock: a comparison with 6% hetastarch solution and shed blood

B Driessen*† DVM, Dr Med Vet, Diplomate ACVA, ECVPT, **JS Jahr**†‡ MD, Diplomate ABA, **F Lurie**§ MD, PhD & **RA Gunther**¶** MA, PhD

*Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, PA, USA

†Department of Anesthesiology, David Geffen School of Medicine, University of California-Los Angeles, Los Angeles, CA, USA

‡Department of Anesthesiology, Charles R. Drew University of Medicine and Science, Los Angeles, CA, USA

§John A. Burns School of Medicine, University of Hawaii, Honolulu, HI, USA

¶School of Medicine, University of California, Sacramento, CA, USA

**Department of Surgery, University of California-Davis Medical Center, Sacramento, CA, USA

Correspondence: B. Driessen, Department of Clinical Studies, University of Pennsylvania, School of Veterinary Medicine, New Bolton Center, 382 W. Street Rd, Kennett Square, PA 19348, USA. E-mail: driessen@vet.upenn.edu

Abstract

Objective To study Hemoglobin glutamer-200 bovine (Hb-200), 6% hetastarch (HES) and shed whole blood (WB) resuscitation in canine hemorrhagic shock.

Study design Prospective laboratory investigation.

Animals Twelve adult dogs [29 ± 1 kg (mean ± SD)].

Methods Anesthetized dogs were instrumented for recording systemic and mesenteric hemodynamic parameters and withdrawal of arterial, mixed and mesenteric venous blood, in which hematological, oxygenation, blood gas and acid–bases variables were determined. Recordings were made before [baseline (BL)], after 1 hour of hypovolemia and immediately and 3 hours post-resuscitation with 30 mL kg^{-1} of either Hb-200, HES, or WB.

Results Blood withdrawal (average 34 ± 2 mL kg^{-1}) caused significant hemodynamic changes, metabolic acidosis and hyperlactatemia characteristic for hemorrhagic shock. Only WB transfusion restored all variables. Hemoglobin glutamer-200 bovine infusion returned most hemodynamic parameters including cardiac output and mesenteric arterial blood flow to BL but increased mean arterial pressure above BL ($p < 0.05$). However, Hb-200 failed to restore total Hb and arterial oxygen content (CaO_2), leaving systemic (DO_2I) and mesenteric O_2 delivery (DO_2Im) below BL ($p < 0.05$). Nevertheless, acid–base variables recovered completely after Hb-200 resuscitation, and met-hemoglobin (Met-Hb) levels increased ($p < 0.05$). Hetastarch resuscitation returned hemodynamic variables to or above BL but further decreased total Hb and CaO_2, preventing recovery of sDO_2I and mDO_2I ($p < 0.05$). Thus, systemic and mesenteric O_2 extraction stayed above BL ($p < 0.05$) while acid–base variables recovered to BL, although slower than in Hb-200 and WB groups ($p < 0.05$).

Conclusions and clinical relevance Resuscitation with Hb-200 seemed to resolve metabolic acidosis and lactatemia more rapidly than HES, but not WB;

yet it is not superior to HES in improving DO_2I and DO_2Im. The hyperoncotic property of solutions like Hb-200 that results in rapid volume expansion with more homogenous microvascular perfusion and the ability to facilitate diffusive O_2 transfer accelerating metabolic recovery may be the key mechanisms underlying their beneficial effects as resuscitants.

Keywords colloid oncotic pressure, dog, hemoglobin glutamer-200 bovine, hemoglobin-based oxygen carrier, hemorrhagic shock, perfusion and oxygenation.

Introduction

In dogs, as in other species, perfusion of splanchnic organs diminishes rapidly during hemorrhagic shock (Slater et al. 1975; Schoenberg et al. 1985; Schlichtig et al. 1991). Particularly, the intestine suffers severe hypoperfusion during progressive hemorrhage when blood is increasingly redistributed to more vital organs, such as heart and brain. As a result, oxygen (O_2) supply dependence begins earlier in the intestine than in the rest of the body especially exposing this organ to the risk of irreversible hypoxic damage (Nelson et al. 1987; Fink 1991). Sustained lag of O_2 delivery behind O_2 demand in intestinal tissues has been associated with serious sequelae, which include loss of intestinal mucosal barrier integrity, bacterial translocation and subsequent initiation of the septic inflammatory response syndrome (Fink 1991; Shoemaker et al. 1992; Fiddian-Green et al. 1993; Pastores et al. 1996). Thus, rapid restoration of splanchnic tissue oxygenation constitutes an important goal of hemorrhagic shock resuscitation. It is for this reason that Shoemaker et al. (1992) proposed the technique of 'supranormal' O_2 delivery resuscitation as they found this method of treatment to prevent or at least reduce the development of severe infectious complications, multiple system organ failure and possibly fatality.

In the early phase of hemorrhagic shock treatment, blood or red blood cell (RBC) concentrates are often not available for volume replacement, thereby delaying urgently needed improvement of O_2 delivery particularly to splanchnic organs. In this period, infusion of allogeneic and xenogeneic, stroma-free, solutions of hemoglobin-based oxygen carriers (HBOCs) may prove superior to conventional treatment with crystalloids or non-oxygen carrying colloids (e.g. dextran or starch solutions), as these products combine volume expanding and O_2 carrying capacities and are readily available without any need for time-consuming cross-matching (see for recent reviews: Creteur et al. 2000; Klein 2000; Standl 2001; Winslow 2000, 2003).

Hemoglobin glutamer-200 bovine (Hb-200, Oxyglobin; Biopure, Cambridge, MA, USA) is an ultrapure solution of highly polymerized bovine hemoglobin (13 g dL^{-1}) licensed for treatment of anemia in dogs (Rentko 2000). While Poli de Figueiredo et al. (2001) demonstrated that infusion of very small volumes of this HBOC (4 mL $kg^{-1} \leq 10\%$ of shed blood) restores only arterial pressures in dogs subject to hemorrhagic shock but does not improve systemic or mesenteric blood flow, a study by Driessen et al. (2001a) revealed that hypovolemic resuscitation with a higher volume of 10 mL kg^{-1} of Hb-200 (approximately equal to 30% of shed blood) was more efficacious by improving many hemodynamic parameters including mesenteric arterial blood flow. However, the higher Hb-200 volume still failed to restore both sDO_2I and DO_2I while normovolemic resuscitation with shed blood did (Driessen et al. 2001a). As numerous investigators have shown that treatment of hypovolemic shock based on O_2 transport rather than hemodynamic parameters is associated with improved outcome (Scaela et al. 1990; Shoemaker et al. 1991; Moore et al. 1992; Young et al. 1997), we hypothesized that a larger dose of Hb-200 would eventually increase O_2 delivery systemically but also to splanchnic organs and thus represent an alternative to whole blood (WB) or RBC resuscitation. In the present study, we therefore analyzed the effects of isovolemic resuscitation with Hb-200 on systemic and mesenteric perfusion and oxygenation in a canine model of controlled hemorrhagic shock and compared the results with treatment protocols using equal volumes of a non-oxygen carrying plasma expander (6% hetastarch; HES) and shed WB.

Methods

Animals

Twelve healthy, adult, mongrel dogs [28.6 ± 0.7 kg (SD); four female, eight male] with no clinically detected heart disease were studied after approval by the University of California-Davis Campus Animal Care and Use Committee and in compliance with the Guide for the Care of Laboratory Animals

(National Institutes of Health publication 86–23, revised 1985), using a model that has been described previously by Driessen et al. (2001a,b). The animals were fasted over night but had free access to water up to 2 hours prior to induction of anesthesia.

Animal preparation and instrumentation

Dogs were premedicated intramuscularly (IM) with oxymorphone (0.04 mg kg^{-1} Numorphan; Endo Laboratories, Chadds Ford, PA, USA) and atropine (0.02 mg kg^{-1}; Phoenix Scientific Inc, St Joseph, MO, USA), followed by percutaneous catheterization of the cephalic vein for administration of drugs and continuous infusion of lactated Ringer's solution (LRS; Abbott Laboratories, Chicago, IL, USA). The LRS infusion rate was initially set at 10 mL kg^{-1} $hour^{-1}$ for the first hour after induction of anesthesia and then reduced to 5 mL kg^{-1} $hour^{-1}$ for the remainder of the experiment. Anesthesia was induced with intravenous (IV) administration of propofol 2–4 mg kg^{-1} (Propoflo; Abbott Laboratories) and diazepam 0.5 mg kg^{-1} (Diazepam; Abbott Laboratories), followed by orotracheal intubation and maintained using a balanced anesthesia protocol, including isoflurane (Isoflo; Abbott Laboratories) and fentanyl citrate (Fentanyl CII; Elkins-Sinn Inc., Cherry Hill, NJ, USA), to minimize potential confounding hemodynamic effects (Ilkiw 1999). During animal preparation and instrumentation isoflurane in O_2 was delivered at an end-tidal concentration of 0.7–1.1%, and fentanyl infused at a rate of 0.7 μg kg^{-1} $minute^{-1}$ that followed an initial IV bolus of fentanyl 10 μg kg^{-1} (Ilkiw 1999). The animals were mechanically ventilated to ensure an arterial partial pressure of carbon dioxide ($PaCO_2$) in the range of 35–45 mmHg (4.6–6.0 kPa). End-tidal partial pressure of CO_2 ($P_{ET}CO_2$), end-tidal concentration of isoflurane (ISO_{ET}), and inspired O_2 concentration (F_iO_2) were continuously monitored using a Datex 254 airway gas monitor (Datex, Helsinki, Finland).

Further instrumentation included placement of catheters into both femoral arteries for blood withdrawal and determinations of systemic arterial pressures. An 8-Fr balloon-tipped flow-directed thermodilution pulmonary arterial catheter (OptiQ, Abbott Laboratories) was also inserted via the jugular vein and floated into the pulmonary artery under direct monitoring of pressure traces for measurements of mean pulmonary arterial pressure (MPAP), central venous pressure (CVP), pulmonary artery occlusion pressure (PAOP), core body temperature and cardiac output (CO). The pulmonary arterial catheter was connected to a cardiac output computer (Critical Care Systems QVUE, Oximetrix 3, Abbott Laboratories) for continuous CO monitoring. Cardiac output was also assessed at Baseline (BL), post-hemorrhage (PH), resuscitation I (R I) and resuscitation II (R II) by thermodilution in triplicate using 10 mL of saline at room temperature. Body temperature was maintained between 38 and 39 °C by means of a heating pad and circulating warm air blanket (Bair Hugger Model 505; Augustine Medical Inc., Eden, MN, USA).

Dogs were splenectomized following a midline laparotomy to prevent release of sequestered RBCs during sympathetic stimulation and to reduce the interindividual variability in the dogs' responses to sudden blood loss (see also Driessen et al. 2001b). Subsequently, the cranial mesenteric artery (CMA) was identified and exposed just distal to its origin at the abdominal aorta by bluntly dissecting through surrounding mesenteric and perivascular tissue. Approximately 1 cm distal to its origin, the CMA was encircled by a 4-mm Doppler transit time flow probe (Transonics, Ithaca, NY, USA), which then was connected to a 2-channel ultrasonic blood flow meter (T201; Transonics). A tributary of the cranial mesenteric vein (CMV) was cannulated with a 20-gauge catheter, which was then advanced into the CMV 4–5 cm to allow for mesenteric venous blood sampling.

Measured variables

Measured variables included heart rate (HR), mean arterial blood pressure (MAP), CVP, PAOP, CO (as determined by thermodilution) and mesenteric arterial blood flow (MAF). Arterial (a), mixed-venous ($\bar{v}$) and mesenteric venous (mv) blood samples were collected intermittently from the femoral artery, pulmonary artery and CMV respectively. Immediately after collection, blood samples were sealed and stored on ice. Subsequently, arterial O_2 saturation (S_aO_2) and mixed venous total hemoglobin (Hb_{total}) and Met-Hb concentrations were measured using a co-oximeter (Model IL 482; Instrumentation Laboratories, Lexington, MA, USA) and arterial, mixed-venous and mesenteric venous O_2 contents (CaO_2; $C\bar{v}O_2$, $CmvO_2$) were directly measured in duplicate using an oxygen-specific electrode ($LEXO_2CON$-K; Hospex

Fiberoptics, Chestnut Hill, MA, USA) to avoid the necessity to calculate O_2 contents based on partial pressures of O_2 and SO_2 values. Subsequently, packed cell volume (PCV) was measured by means of centrifugation of capillary tubes. Plasma hemoglobin (Hb_{plasma}; after centrifugation) concentrations were measured using a HemoCue hemoglobin photometer (HemoCue AB, Ångelholm, Sweden). Mixed-venous and mesenteric venous lactate ($Lactate_{\bar{v}}$ and $Lactate_{mv}$) concentrations were determined in duplicate by means of a lactate analyzer (Model 1500; YSI Inc., Yellowsprings, OH, USA). Mixed-venous and mesenteric venous pH ($pH\bar{v}$; pHmv) and partial pressures of CO_2 ($P_{\bar{v}}CO_2$; $P_{mv}CO_2$) were analyzed with a blood gas analyzer (Model 170; Corning Medical, Medfield, MA, USA). Blood gas values were corrected for the body temperature of the animals at the time of sampling. Mixed-venous and mesenteric venous standard base excesses ($SBE\bar{v}$; SBEmv) were calculated by the blood gas analyzer. All laboratory analyzers used for determinations of oxygenation parameters were validated for use with HBOCs (Jahr et al. 2001, 2002, 2003, 2004).

Cardiac index (CI), systemic O_2 delivery index (DO_2I) and systemic and mesenteric O_2 extraction ratios (O_2Ex and O_2Exm respectively) were calculated using standard formulae, as previously reported (Driessen et al. 2001a,b). Mesenteric arterial blood flow index (MAFI) was calculated as MAFI/body surface area (BSA) and mesenteric O_2 delivery index (DO_2Im) as $CaO_2 \times MAFI$.

Experimental protocol

Following completion of the surgical procedure, the inspired O_2 concentration was reduced to that of ambient air (F_iO_2: 0.21–0.22) and anesthesia maintained with a decreased end-tidal concentration of 0.7–0.8% isoflurane and a reduced infusion rate of fentanyl (0.4 μg kg^{-1} $minute^{-1}$) to compensate for potential changes in fentanyl and isoflurane pharmacokinetics during hypovolemia (Egan et al. 1999; Driessen et al. 2001b). After these adjustments the dogs were allowed to stabilize for 45 minutes (Equilibration period), after which all measurements (BL) were taken. Subsequently, approximately 40% of the dogs' blood volume (total blood volume: 85 mL kg^{-1}), was withdrawn from the femoral artery over 30 minutes until an MAP of about 50 mmHg was reached. Shed blood was collected in citrate containing bags and weighed. Additional small amounts of blood were drawn to maintain the arterial blood pressure at 50 mmHg for 60 minutes. At the end of the hypovolemic period, all measurements were repeated (PH) and the dogs were randomly allocated to receive 30 mL kg^{-1} of either 6% hetastarch in 0.9% sodium chloride (HES Hespan; Abbott Laboratories), hemoglobin glutamer-200 (Hb-200 Oxyglobin, Biopure; composition described in Table 1 of Driessen et al., 2001a), or shed WB. Solutions were infused at a rate of 30 mL kg^{-1} $hour^{-1}$. All measurements were repeated immediately (R I) and 3 hours (R II) after termination of volume resuscitation. Animals were euthanized following the last measurements with an overdose of potassium chloride without regaining consciousness.

Statistical analysis

Results are given as arithmetic mean ± SD. All data recorded in this study were continuous measures with normal distributions. Statistical evaluation of data included a two-way repeated measures analysis of variance (ANOVA), with treatment group and time as the two factors, followed by use of a *post hoc* test that depended on multiple comparisons *versus* BL (Dunnett's method). We used ANOVA for repeated measures, followed by Tukey's Studentized range test, to assess differences between the three study groups at each time point. The level of statistical significance was set at $p < 0.05$.

Results

No statistically significant differences in systemic and mesenteric hemodynamic, oxygenation and acid–base parameters (except plasma lactates) measured prior to hemorrhage (BL) were observed between groups (see Tables 1–3 and Figs 1–3). The depth of anesthesia (as judged by jaw tone, eye reflexes and pinch withdrawal reflexes), ventilation and arterial O_2 saturation of hemoglobin remained unchanged throughout the experiment.

During the hemorrhage period 33.2 ± 1.9, 33.4 ± 1.7 and 36.1 ± 4.9 mL kg^{-1} of blood were withdrawn in the HES, Hb-200 and WB groups, respectively, to reduce MAP to approximately 50 mmHg. The average blood volume withdrawn (34.2 ± 1.7 mL kg^{-1}) corresponded to 40% of the estimated circulating blood volume (i.e. 85 mL kg^{-1}; Dellenback et al. 1969). Hemorrhage caused an average decrease in Hb_{total} and CaO_2 by

Table 1 Effects of hemorrhage and subsequent fluid resuscitation on mixed-venous blood parameters

Variable	Study group	BL	PH	R I	R II
PCV (%)	HES	44 ± 2	38 ± 3*	23 ± 4*	24 ± 3*‡
	Hb-200	40 ± 3	33 ± 2*	19 ± 2*§	18 ± 1*§‡
	WB	44 ± 1	35 ± 1*	41 ± 1†	43 ± 1§†
Hb_{total} (g dL^{-1})	HES	15.1 ± 1.0	12.5 ± 0.6*	7.6 ± 0.8*‡	8.0 ± 1.0*‡
	Hb-200	14.9 ± 1.0	11.4 ± 0.7*	11.5 ± 0.7*‡	10.4 ± 0.9*‡
	WB	15.7 ± 0.4	12.0 ± 0.4*	13.6 ± 0.5*	14.8 ± 0.3§†
Hb_{plasma} (g dL^{-1})	HES	0 ± 0	0 ± 0	0 ± 0	0 ± 0‡
	Hb-200	0 ± 0	0 ± 0	5.4 ± 0.2*§‡	4.5 ± 0.1*§‡
	WB	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Met-Hb (%)	HES	0.8 ± 0.1	1.0 ± 0.5	0.7 ± 0.2†	0.6 ± 0.2†
	Hb-200	1.0 ± 0.3	0.8 ± 0.2	1.7 ± 0.3*§‡	2.7 ± 0.2*§‡
	WB	1.1 ± 0.3	0.8 ± 0.2	1.0 ± 0.2†	1.0 ± 0.1†

Packed cell volume (PCV), total hemoglobin (Hb_{total}) and free plasma hemoglobin (Hb_{plasma}) and met-hemoglobin (Met-Hb), in dogs at baseline (BL; end of equilibration), 60 minutes post-hemorrhage (PH), and immediately [resuscitation I (RI)] and 3 hours [resuscitation II (R II)] following resuscitation with 30 mL kg^{-1} of either 6% hetastarch solution (HES), hemoglobin glutamer-200 (Hb-200), or whole blood (WB). Values are mean ± SD of four dogs in each group. Significant differences from baseline values: *$p < 0.05$; from Hb-200 group: †$p < 0.05$; and from WB group: ‡$p < 0.05$; from HES group: §$p < 0.05$.

Table 2 Effects of hemorrhage and subsequent fluid resuscitation on cardiovascular parameters

Variable (mmHg)	Study group	BL	PH	R I	R II
MPAP	HES	14 ± 4	10 ± 2	19 ± 3†	20 ± 2*‡
	Hb-200	17 ± 1	11 ± 3	30 ± 4 *§‡	21 ± 3‡
	WB	14 ± 1	11 ± 1	19 ± 4†	13 ± 2§†
PAOP	HES	6 ± 1	3 ± 1*	9 ± 1*‡	6 ± 1
	Hb-200	8 ± 1	5 ± 1*	13 ± 1‡	10 ± 2‡
	WB	5 ± 1	4 ± 1	5 ± 2	4 ± 1†
CVP	HES	3 ± 2	1 ± 0	5 ± 2	4 ± 1†
	Hb-200	8 ± 2	2 ± 2*	11 ± 1§‡	7 ± 0§‡
	WB	6 ± 1	2 ± 1*	7 ± 1†	4 ± 0†

Mean pulmonary arterial pressure (MPAP), pulmonary occlusion pressure (PAOP), and central venous pressure (CVP), in dogs at baseline (BL; end of equilibration), 60 minutes post-hemorrhage (PH), and immediately [resuscitation I (RI)] and 3 hours [resuscitation II (R II)] following resuscitation with 30 mL kg^{-1} of either 6% hetastarch solution (HES), hemoglobin glutamer-200 (Hb-200), or whole blood (WB). Values are mean ± SD of four dogs in each group. Significant differences from baseline values: *$p < 0.05$; from Hb-200 group: †$p < 0.05$; and from WB group: ‡$p < 0.05$; from HES group: §$p < 0.05$.

approximately 22% and 26%, respectively (Table 1 and Fig. 2) and changes in systemic as well as mesenteric hemodynamic and oxygenation parameters and lactic acidosis, characteristic of hypovolemic shock (Tables 2 & 3; Figs 1–3) and similar to those previously reported (Driessen et al. 2001a, 2003). Systemic and DO_2Im indexes were diminished by an average of 70% and 64%, respectively, and $C\bar{v}O_2$ and CO_2 were reduced by an average of 72% and 59%, respectively, following hemorrhage (Fig. 2; Table 3). Oxygen extraction in the systemic and mesenteric vascular bed increased to average 2.9 and 3.0 times the BL value (Table 3), however, for unclear reasons, there were significant group differences, with animals of the HES group revealing greater $C\bar{v}O_2$ and $CmvO_2$ values and, hence, lower O_2Ex and O_2EXm values than the Hb-200 and WB groups (Table 3).

Following volume resuscitation with either 30 mL kg^{-1} of HES, Hb-200 (13 g dL^{-1} Hb) or

© 2006 Association of Veterinary Anaesthetists,

Table 3 Effects of hemorrhage and subsequent fluid resuscitation on oxygenation parameters

Variable	Study group	BL	PH	R I	R II
$C\bar{v}O_2$ (mL dL^{-1})	HES	16.7 ± 1.4	7.3 ± 0.8*†‡	7.8 ± 1.0*‡	7.8 ± 0.7*‡
	Hb-200	14.4 ± 1.8	3.6 ± 0.8*§	9.8 ± 0.7*	8.8 ± 0.6*‡
	WB	16.0 ± 0.7	4.0 ± 0.3*§	13.0 ± 0.5*§	14.0 ± 0.7§†
$CmvO_2$ (mL dL^{-1})	HES	16.7 ± 1.0	9.7 ± 0.7*†‡	10.0 ± 1.7*‡	8.5 ± 0.7*‡
	Hb-200	16.0 ± 0.9	5.1 ± 0.3*§	9.7 ± 0.6*‡	9.9 ± 0.8*‡
	WB	17.2 ± 0.8	5.7 ± 0.9*§	14.8 ± 0.8§	16.1 ± 0.5§†
O_2Ex	HES	0.20 ± 0.04	0.56 ± 0.02*†‡	0.31 ± 0.11	0.37 ± 0.03*
	Hb-200	0.28 ± 0.07	0.75 ± 0.03*§	0.27 ± 0.03	0.29 ± 0.01§
	WB	0.23 ± 0.07	0.75 ± 0.02*§	0.29 ± 0.03	0.26 ± 0.04§
O_2Exm	HES	0.20 ± 0.03	0.41 ± 0.03*†‡	0.32 ± 0.11	0.31 ± 0.03*†‡
	Hb-200	0.18 ± 0.05	0.64 ± 0.01*§	0.27 ± 0.03	0.19 ± 0.02§
	WB	0.18 ± 0.03	0.62 ± 0.06*§	0.17 ± 0.05	0.16 ± 0.03§

Mixed-venous and mesenteric-venous oxygen content ($C\bar{v}O_2$ and $CmvO_2$ respectively), and systemic and mesenteric (m) oxygen extraction ratio (O_2Ex) in dogs at baseline (BL; end of equilibration), 60 minutes post-hemorrhage (PH), and immediately [resuscitation I (RI)] and 3 hours [resuscitation II (R II)] following resuscitation with 30 mL kg^{-1} of either 6% hetastarch solution (HES), hemoglobin glutamer-200 (Hb-200), or whole blood (WB). Values are mean ± SD of four dogs in each group. Significant differences from baseline values: *$p < 0.05$; from Hb-200 group: †$p < 0.05$; and from WB group: ‡$p < 0.05$; from HES group: §$p < 0.05$.

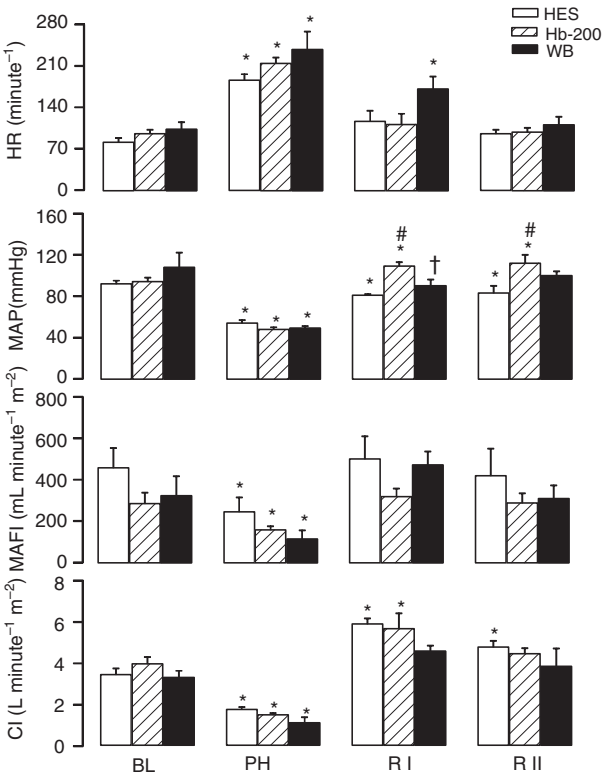

Figure 1 Heart rate (HR), mean arterial pressure (MAP), mesenteric arterial blood flow index (MAFI) and cardiac index (CI) in dogs at baseline (BL; end of equilibration), 60 minutes post-hemorrhage (PH) and immediately [resuscitation I (RI)] and 3 hours [resuscitation II (R II)] following resuscitation with 30 mL kg^{-1} of either 6% hetastarch solution (HES), hemoglobin glutamer-200 (Hb-200), or whole blood (WB). Values are mean ± SD of four dogs in each group. Significant differences from BL group: *$p < 0.05$ and from HES group: #$p < 0.05$.

WB (12.8 ± 0.8 g dL^{-1}), significant differences in hemodynamic, hematological, oxygenation and acid–base parameters were apparent between groups (Tables 1–3; Figs 1–3). Only shed blood transfusion restored all hemodynamic, hematological, oxygenation and acid–base variables back

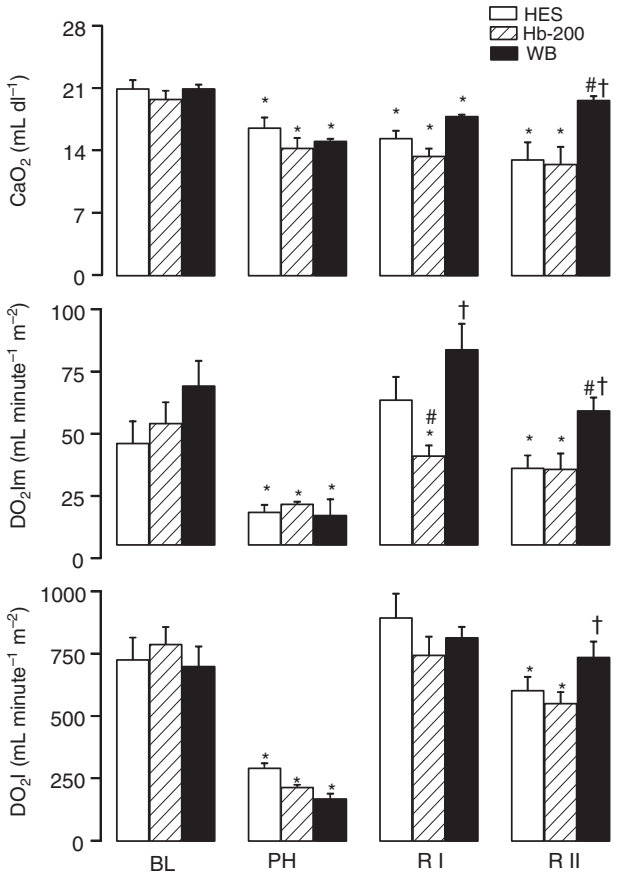

Figure 2 Systemic and mesenteric (m) oxygen delivery index (DO_2I) and arterial oxygen content (CaO_2) in dogs at baseline (BL; end of equilibration), 60 minutes post-hemorrhage (PH) and immediately [resuscitation I (RI)] and 3 hours [resuscitation II (R II)] following resuscitation with 30 mL kg^{-1} of either 6% hetastarch solution (HES), hemoglobin glutamer-200 (Hb-200), or whole blood (WB). Values are mean ± SD of four dogs in each group. Significant differences from BL group: $^{*}p < 0.05$, from HES group: $^{\#}p < 0.05$; and from Hb-200 group: $^{\dagger}p < 0.05$.

to BL 3 hours following resuscitation. Infusion of Hb-200 returned most hemodynamic parameters including mesenteric arterial blood flow (MAFI) to BL but increased MAP above BL (Table 2; Fig. 1). All three MPAP, PAOP and CVP were significantly greater with Hb-200 when compared with the WB group (Table 2). In contrast to WB transfusion, Hb-200 infusion failed to restore Hb_{total} and CaO_2 and, as a result, DO_2I and DO_2Im as well as $C\bar{v}O_2$ and $CmvO_2$ remained significantly below BL (Table 1; Fig. 2). Nevertheless, in both the systemic and mesenteric system all measured acid–base variables recovered to BL values following resuscitation with Hb-200 as they did following WB infusion. In the HES group, HR and MAFI returned to BL, yet MAP remained significantly below and CI significantly above BL following treatment. As in the Hb-200 group, MPAP was higher than in the WB group 3 hours following resuscitation. Being a non-Hb carrying solution, HES further decreased Hb_{total} and CaO_2 PH, thereby preventing complete recovery of DO_2I and DO_2Im (Table 1; Fig. 2). As a result, $C\bar{v}O_2$ and $CmvO_2$ remained significantly below and O_2Ex and O_2Exm significantly above BL (Table 3). Nonetheless, in the HES group systemic and mesenteric acid–base variables recovered to BL, yet slower than in the other two groups (Fig. 3).

At either R I or R II post-resuscitation, both the HES- and Hb-200-treated animals did not differ significantly with respect to Hb content (although Hb_{total} did not further decrease from PH values in the Hb-200 group while it did in the HES group and Hb_{total} was about 34% and 23% higher in the Hb-200 when compared with HES group at R I and R II respectively), CaO_2, or DO_2I and DO_2Im (Table 1; Fig. 2). However, systemic and particularly mesenteric O_2 extraction as well as acid–base parameters returned significantly faster to normal values following Hb-200 when compared with HES infusion (Table 3; Fig. 3).

Administration of Hb-200 produced Met-Hb, reaching approximately 3% of total Hb at 3 hours post-resuscitation, while neither HES nor WB infusion were associated with increases in Met-Hb levels (Table 1).

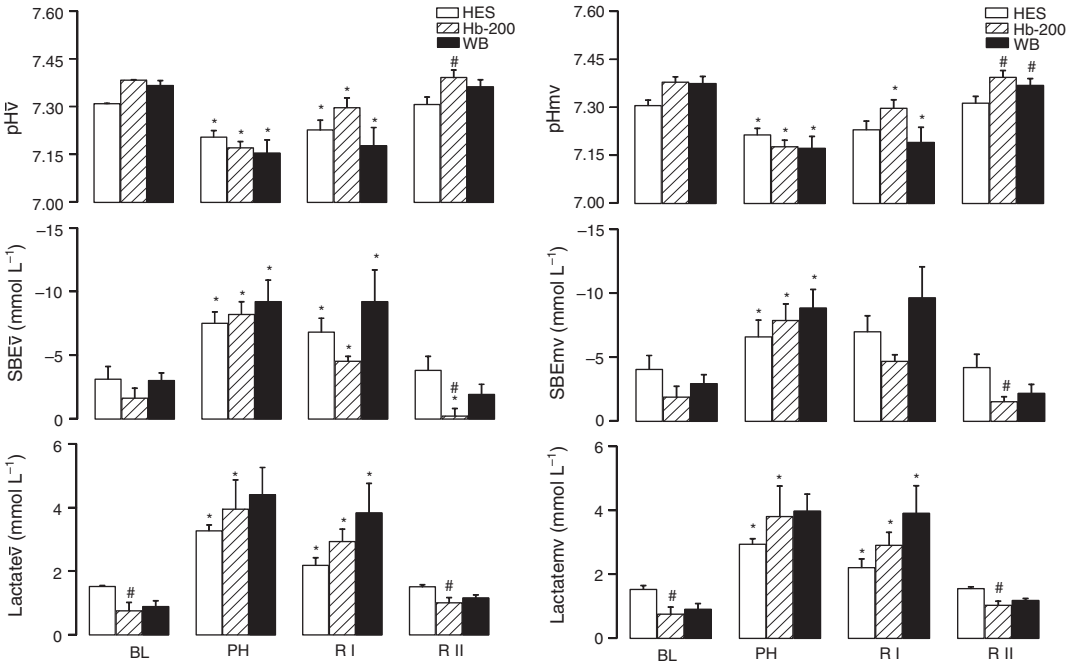

Figure 3 Mixed-venous ($\bar{v}$) and mesenteric venous (mv) acid–base variables in dogs at baseline (BL; end of equilibration), 60 minutes post-hemorrhage (PH) and immediately [resuscitation I (RI)] and 3 hours [resuscitation II (R II)] following resuscitation with 30 mL kg^{-1} of either 6% hetastarch solution (HES), hemoglobin glutamer-200 (Hb-200), or whole blood (WB). Values are mean ± SD of four dogs in each group. Significant differences from BL group: $^{*}p < 0.05$, from HES group: $^{\#}p < 0.05$; and from Hb-200 group: $^{\dagger}p < 0.05$.

Discussion

Isovolemic resuscitation with Hb-200 failed to restore O_2 delivery in the splanchnic as well as systemic circulation PH, despite providing an Hb load similar to WB transfusion (13.0 *versus* 12.8 g dL^{-1}). While returning mesenteric arterial blood flow and cardiac output back to BL (Fig. 1), Hb-200 did not increase Hb_{total} and hence CaO_2 3 hours PH compared with WB. As a result, DO_2I and DO_2Im was not significantly different from resuscitation with non-oxygen carrying colloid HES (Table 1, Fig. 2). This observation coincides with findings in a previous study (Driessen et al. 2003) and reflects the strong oncotic properties of this HBOC [colloid oncotic pressure (COP) 43 mmHg (5.7 kPa); Driessen et al. 2003], causing marked hemodilution by fluid shifting from the interstitial into the intravascular space. Indicators of cardiac preload (CVP, PAOP) immediately increased post-resuscitation along with fluid mobilization (Table 2). This was more prominent with the HBOC than HES solution, mirroring differences in oncotic forces between Hb-200 and HES [43 *versus* 36 mmHg (5.7 kPa *versus* 4.8 kPa); Driessen et al. 2003]. Despite failing to restore DO_2I and DO_2Im HBOC infusion reestablished acid–base homeostasis, which is considered the best predictor for survival in hemorrhagic shock (Hannon et al. 1990). Splanchnic as much as systemic metabolic acidosis and lactatemia resolved completely following Hb-200 administration, similar to the effects of WB transfusion, which returned Hb_{total} to BL (Table 1, Fig. 3). While infusion of HES as non-oxygen carrying colloid eventually resolved shock-induced metabolic acidosis, Hb-200 seemed to reverse tissue hypoxia more readily. In agreement with this assumption, O_2 extraction in both systemic and mesenteric circulation remained significantly elevated 3 hours post-HES resuscitation, indicating that at least in some tissues O_2 debt was not yet completely paid back (Table 3).

Conventional wisdom is that to be effective, an HBOC must be able to increase the Hb concentration and thus O_2 transport capacity of blood (Rentko 2000; Kramer 2003). However, the present data in concert with results from similar investigations in the dog (Driessen et al. 2001a, 2003), hamster (Wettstein et al. 2003), pig (Noone et al. 1998; Van

Iterson et al. 1998; McNeil et al. 2001; Drobin et al. 2004) and sheep (Fischer et al. 1999) challenge this view. Assuming a more rapid reversal of anaerobic metabolism after Hb-200 than HES infusion despite a lack of increase in systemic arterial O_2 delivery, Driessen et al. (2003) suggested that the primary mode of action of HBOCs must be facilitation of O_2 transfer at the level of the microcirculation itself rather than enhancing arterial O_2 content and bulk O_2 transport to the microcirculation. This was not a new idea but was built on the early observation by Scholander (1960) that simple diffusion of O_2 is multiplied by the presence of Hb in solution, which was later confirmed in *in vitro* experiments with artificial capillaries (Page et al. 1998). Furthermore, a more homogeneous distribution of HBOC (when compared with RBCs) during the early phase of resuscitation when vasoconstriction still prevails is in support of this idea. Also the lower O_2 affinity of Hb-200 [P_{50} 34 mmHg (4.5 kPa)] *versus* native canine Hb [P_{50} 28 mmHg (3.7 kPa)] allowing for more rapid O_2 off-loading from the Hb molecule seems to favor the assumption of an augmented O_2 diffusion from the vascular to the tissue site when HBOCs such as Hb-200 are present (Driessen et al. 2003). However, the concept that low O_2 affinity (high P_{50}) of modified Hb promotes diffusive O_2 transport to tissues has been challenged. Recently, Winslow (2003) formulated a new paradigm for the development of second generation HBOCs according to which the ideal agent should exhibit a high O_2 affinity [low P_{50}: <10 mmHg (<1.3 kPa)], contain Hb at the lowest possible concentration, exert increased COP, have a long plasma half-life and act as O_2 transport agent rather than as an RBC substitute. This theorem was largely based on three findings. Firstly, McCarthy et al. (2001) using artificial capillaries demonstrated that in hypoxic/anoxic environments Hb solutions show the same pattern of O_2 off-loading independent of their O_2 affinity (P_{50}). Secondly, Intaglietta et al. (1996), conducting microscopic measurements of PO_2 in very small areas of the microcirculation in hamster skin, showed that increased diffusive O_2 transfer at the level of small arterioles, i.e. upstream to the microvascular bed and therefore called 'premature unloading of O_2', paradoxically decreases O_2 uptake by tissue because of vasoconstriction in precapillary arterioles (known as 'autoregulation theory'; Intaglietta 1999). Thirdly, Rohlfs et al. (1998) demonstrated that there is no correlation between nitric oxide (NO) binding characteristics of different HBOCs and their vasoactive properties.

Considering the vasoconstrictive action of HBOCs as the major obstacle to their introduction into clinical practice in human medicine, Winslow (2003) suggested that the low O_2 affinity of first generation HBOCs, which support 'premature unloading of O_2' in the microcirculation, is the primary cause of HBOC-induced vasoconstriction rather than NO scavenging by cell-free Hb as previously thought (see Creteur et al. 2000; Klein 2000; Standl 2000). Consistent with this idea are findings with a second generation HBOC that has recently been developed (Winslow et al. 1998; Conover et al. 1999; Wettstein et al. 2003; Drobin et al. 2004). In a swine hemorrhagic shock model resuscitation with several formulations of maleimide-polyethylene glycol-modified hemoglobin [MalPEG-Hb; modified Hb_{human} 4.2 g dL^{-1}, COP 49 mmHg (6.5 kPa), P_{50} 5.9 mmHg (0.8 kPa)] was not associated with significant increases in systemic vascular resistance but with full recovery of mean arterial pressure, cardiac output and almost complete return of acid–base variables (pH, base excess and lactate) to BL (Drobin et al. 2004). The authors interpreted the results as indicating that hemodynamic and metabolic resuscitation can be effectively accomplished with MalPEG-Hb, although they acknowledged that metabolic recovery was similar with 10% pentastarch as control solution.

Interestingly, the outcome in the present study using a first generation HBOC was nearly the same despite Hb-200's well-known vasoconstrictive property (Muir et al. 2000; Driessen et al. 2001a,b, 2003) and low O_2 affinity. Also resuscitation with recombinant hemoglobin solution [rHb1.1; modified rHb 5.0 g dL^{-1}, COP 42 mmHg (5.6 kPa), P_{50} 32 mmHg (4.3 kPa)], another first generation HBOC with potent vasoconstrictive activity (Resta et al. 2002), allowed a more rapid reversal of metabolic acidosis and lactic acidemia than treatment with a colloid/blood mixture in a canine hemorrhagic shock model (Siegel et al. 1997). It should be fair to assume that recovery of all measured acid base parameters in both the systemic and splanchnic circulation (Fig. 3) and return of CI and MAFI to BL values after Hb-200 infusion (Fig. 1) indicate resolution of global as well as regional hypoperfusion and reversal of anaerobic metabolism. Thus, one might wonder whether, as previously postulated by Intaglietta et al. (1996) and Winslow (2003), the combination of vasocon-

striction and 'premature O_2 unloading' from Hb really impairs downstream O_2 delivery to capillaries and hence tissue O_2 uptake. This question becomes even more pertinent as both MalPEG-Hb (Wettstein et al. 2003) and Hb-200 (Cheung et al. 2004) have been shown to restore microcirculatory functions measured by intravital microscopy. In addition, in the hamster experiments of Wettstein et al. (2003) only shed blood resuscitation returned PO_2 in skin tissue to near normal [19 ± 6 mmHg (2.5 ± 0.8 kPa)], yet without improving microcirculatory functions and acid base excess as much as MalPEG-Hb did; at the same time post-resuscitation tissue O_2 tension remained significantly lower than normal with 8 ± 3 and 5 ± 2 mmHg (1.1 ± 0.1 and 0.7 ± 0.3 kPa) in the MalPEG-Hb and 5% HES groups respectively. For these reasons, it seems rather unlikely that Hb concentration, O_2 affinity (P_{50}) and vasoconstrictive properties of currently tested HBOCs are key factors determining their efficacy as resuscitants in hemorrhagic shock. But how then can we explain that resuscitation with either one of the mentioned HBOCs (MalPEG-Hb, Hb-200, rHb1.1) leads to similar outcome? A feature that all three HBOCs share is a similarly high COP (49, 43 and 42 mmHg respectively), while viscosity properties are more variable [MalPEG-Hb: 2.2 cP; Hb-200: 1.8 cP; rHb1.1: 0.8 cP *versus* transfused native canine blood of 3.7 ± 0.3 cP ($n = 8$; personal communication of Dr A.T.W. Cheung)]. Therefore it may well be that the hyperoncotic effect of these solutions, favoring rapid volume expansion and more homogenous microvascular perfusion (Driessen et al. 2003; Wettstein et al. 2003), is a key mechanism behind their beneficial effects as resuscitants in hemorrhagic shock. This would also explain why starch solutions with slightly lower or higher COP (5 or 6% HES, 10% pentastarch) had overall similar effects on metabolic resuscitation in this and previous studies (Driessen et al. 2003; Wettstein et al. 2003; Cheung et al. 2004; Drobin et al. 2004), while in animals treated with stroma-free hemoglobin (SFH; COP 15 mmHg) acid–base parameters failed to return to BL (Drobin et al. 2004). It remains to be shown whether the advantage of HBOCs over conventional colloids, namely carrying O_2 and facilitating capillary O_2 transfer to peripheral tissues so that cells can recover from anaerobia more rapidly (Driessen et al. 2003), only comes to effect once hypoperfusion of the microcirculation has been overcome as a result of COP-driven fluid mobilization. Rapid metabolic resuscitation with HBOCs low in Hb content (e.g. MalPEG-Hb, rHb1.1) emphasizes how little of the free Hb component in plasma has to be present to augment diffusive O_2 transport at the microcirculatory level.

Lack of O_2 supply to peripheral tissues during hemorrhagic shock leads to alterations in metabolic reactions producing hypoxanthine and activation of the enzyme xanthine oxidase. The degree of hypoxanthine production increases with the duration and severity of ischemia. During tissue reperfusion with O_2 carrying solutions (blood, RBC concentrates, or HBOCs) xanthine oxidase converts O_2 and hypoxanthine into superoxide (Chang 2003). As a result of superoxide production O_2 radicals are formed that can cause severe tissue injury. Under physiological conditions superoxide dismutase and catalase, both present in RBCs, will convert superoxide rapidly into hydrogen peroxide that is then converted into H_2O and O_2. However, first generation HBOCs such as Hb-200 do not contain these enzymes and thus are associated with an increased risk for ischemia-reperfusion injury, particularly in the intestine (Chang 2003). In addition, chemical modification of the Hb molecule in HBOCs not only affects O_2 and NO affinity, but also redox properties. Lacking any protective membrane or envelope, stroma-free deoxyHb is highly susceptible to oxidation leading to both the formation of Met-Hb but also highly reactive (cytotoxic) superoxide anions (D'Agnillo & Alayash 2000). This risk applies particularly to Hb-200 and hemoglobin glutamer-250 (HBOC 201, Hemopure, Biopure, Cambridge, MA, USA), as polymerization/cross-linking of the bovine Hb molecule with glutaraldehyde creates a greater accessibility of the heme pocket resulting in a high redox potential (Alayash et al. 2001). The recent study by Alayash et al. (2001) suggests that enhanced autooxidation and oxidative kinetics may not only affect the stability and functional half-life of these bovine HBOCs, but also trigger harmful oxidative side reactions. For this reason, we measured Met-Hb levels as an indicator of oxidative reactions following HBOC infusion. Three hours post-resuscitation the Hb-200-treated group showed significant increases in Met-Hb formation, approaching 3% of Hb_{total} (Table 1). Elevated Met-Hb levels have previously been observed. In a sheep blood exchange model with hemoglobin glutamer-250 (HBOC 201) Met-Hb formation amounted to 33 ± 7% of total circulating plasma Hb at 24 hours (Lee et al. 1995). Sprung et al. (2002) noted, in a

clinical study, a steady increase in Met-Hb from 1.3 ± 0.5% of total Hb immediately after administration of hemoglobin glutamer-250 to 3.7 ± 3.2% on the third post-operative day; plasma Hb content in these patients after infusion was lower than in the dogs of the present study and averaged 2.9 ± 1.1 g dL^{-1}. Considering the reported marked increase in Met-Hb levels over time, oxidative processes may indeed become a serious problem upon repeated administration of HBOCs such as Hb-200. Not only are the O_2 radicals formed in these reactions cytotoxic, but also the ability of an HBOC to oxygenate tissue becomes significantly impaired when Met-Hb accumulates to levels in excess of 10% of plasma Hb (Linberg et al. 1998).

In conclusion, in canine hemorrhagic shock isovolemic resuscitation with HBOCs such as Hb-200 does not improve arterial O_2 content or DO_2I and DO_2Im more so than HES, but may resolve systemic and mesenteric metabolic acidosis more rapidly than HES because of facilitated O_2 diffusion to tissues. The question as to the mechanism by which HBOCs are improving O_2 supply to the microcirculation and hence allowing for more rapid reversal of anaerobic cell metabolism is far from being resolved and warrants more detailed experiments in the future, also because animals were splenectomized, making this model somewhat less physiological, and thus extrapolation of the findings to the clinical situation slightly more difficult. Likewise, the long-term effects of oxidative reactions associated with HBOC infusion, particularly in case of repeated administrations, are poorly understood. While potentially superior to conventional colloids (HES), Hb-200 does not appear to be as effective as WB resuscitation.

Acknowledgements

The authors like to thank Jessica Davis and Linda Talken for their technical assistance during the study. We are also grateful to Dr Peter Moate, Section of Biostatistics, University of Pennsylvania, School of Veterinary Medicine, Department of Clinical Studies – New Bolton Center for providing advice in the statistical analysis of the data. This study was supported by intramural research funds of the Section of Anesthesia, Department of Clinical Studies-NBC, University of Pennsylvania, School of Veterinary Medicine and by NIH-BRG grant 1 R01 HL67432. Parts of this study were presented at the Association of Veterinary Anaesthetists' Autumn Meeting, Vienna, Austria, September 13–16, 2004.

References

Alayash AI, Summers AG, Wood F et al. (2001) Effects of glutaraldehyde polymerization on oxygen transport and redox properties of bovine hemoglobin. Arch Biochem Biophys 391, 225–234.

Chang TMS (2003) Future generations of red blood cell substitutes. J Int Med 253, 527–534.

Cheung ATW, Driessen B, Jahr JS et al. (2004) Blood substitute resuscitation as a treatment modality for moderate hypovolemia. Artif Cells Blood Substit Immobil Biotechnol 32, 189–207.

Conover CD, Linberg R, Lejeune L et al. (1999) PEG-hemoglobin as a resuscitation solution in the treatment of hypovolemic shock in the anesthetized rat. Artif Organs 23, 1088–1098.

Creteur J, Sibbald W, Vincent JL (2000) Hemoglobin solutions – not just red blood cell substitutes. Crit Care Med 28, 3025–3034.

D'Agnillo F, Alayash AJ (2000) Site-specific modifications and toxicity of blood substitutes. The case of diaspirin cross-linked hemoglobin. Adv Drug Deliv Rev 40, 199–212.

Dellenback RJ, Usami S, Chien S et al. (1969) Effect of splenectomy on blood picture, blood volume, and plasma proteins in beagles. Am J Physiol 21, 891–897.

Driessen B, Jahr JS, Lurie F et al. (2001a) Effects of haemoglobin-based oxygen carrier Hemoglobin glutamer-200 (bovine) on intestinal perfusion and oxygenation in a canine hypovolaemia model. Br J Anaesth 86, 683–692.

Driessen B, Jahr JS, Lurie F et al. (2001b) Inadequacy of low-volume resuscitation with hemoglobin-based oxygen carrier Hemoglobin glutamer-200 (bovine) in canine hypovolemia. J Vet Pharmacol Therap 24, 61–71.

Driessen B, Jahr JS, Lurie F et al. (2003) Arterial oxygenation and oxygen delivery after hemoglobin-based oxygen carrier infusion in canine hypovolemic shock: a dose-response study. Crit Care Med 31, 1771–1779.

Drobin D, Kjellstrom BT, Malm E et al. (2004) Hemodynamic response and oxygen transport in pigs resuscitated with maleimide-polyethylene glycol-modified hemoglobin (MP4). J Appl Physiol 96, 1843–1853.

Egan TD, Kuramkote Gong SG et al. (1999) Fentanyl pharmacokinetics in hemorrhagic shock. Anesthesiology 91, 156–166.

Fiddian-Green RG, Haglund U, Gutierrez G et al. (1993) Goals for resuscitation of shock. Crit Care Med 21(Suppl. 2), S25–S31.

Fink MP (1991) Gastrointestinal mucosal injury in experimental models of shock, trauma, and sepsis. Crit Care Med 19, 627–641.

Fischer SR, Burnet M, Traber DL et al. (1999) Plasma volume expansion with solutions of hemoglobin, albumin, and ringer lactate in sheep. Am J Physiol Heart Circ Physiol 276, H2194–H2203.

Hannon JP, Wade CE, Bossone CA et al. (1990) Blood gas and acid–base status of conscious pigs subjected to fixed-volume hemorrhage and resuscitated with hypertonic saline dextran. Circ Swhock 32, 19–29.

Ilkiw JE (1999) Balanced anesthetic techniques in dogs and cats. Clin Tech Small Anim Pract 14, 27–33.

Intaglietta M (1999) Microcirculatory basis for the design of artificial blood. Microcirculation 6, 247–258.

Intaglietta M, Johnson PC, Winslow RM (1996) Microvascular and tissue oxygen distribution. Cardiovasc Res 32, 632–643.

Jahr JS, Driessen B, Lurie F et al. (2001) Oxygen saturation measurements in canine blood containing hemoglobin glutamer-200 (bovine): in-vitro validation of the NOVA CO-Oximeter. Vet Clin Path 30, 39–45.

Jahr JS, Lurie F, Driessen B et al. (2002) The HemoCue®, a point of care B-hemoglobin photometer, measures hemoglobin concentrations accurately when mixed in vitro with canine plasma and three hemoglobin-based oxygen carriers (HBOC). Can J Anaesth 49, 243–248.

Jahr JS, Lurie F, Driessen B et al. (2003) Validation of the oxygen saturation measurements in a canine model of hemoglobin-based oxygen carrier (HBOC) infusion. Am J Ther 10, 21–28.

Jahr JS, Osgood SL, Rothenberg SJ et al. (2005) Lactate measurement interference by hemoglobin-based oxygen carriers (Oxyglobin®, Hemopure®, and Hemolink^{TM}). Anesth Analg 100, 431–436.

Klein GH (2000) The prospects of red-cell substitutes. N Engl J Med 342, 1638–1643.

Kramer GC (2003) Counterintuitive red blood cell substitute – polyethylene glycol-modified human hemoglobin. Crit Care Med 31, 1882–1884.

Lee R, Neya K, Svizzero TA et al. (1995) Limitations of the efficacy of hemoglobin-based oxygen-carrying solutions. J Appl Physiol 179, 236–242.

Linberg R, Conover CD, Shum KL et al. (1998) Hemoglobin based oxygen carriers: how much methemoglobin is too much? Artif Cells Blood Substit Immobil Biotechnol, 26, 133–148.

McCarthy MR, Vandegriff KD, Winslow RM (2001) The role of facilitated diffusion in oxygen transport by cell-free hemoglobins: implications for the design of hemoglobin-based oxygen carriers. Biophys Chem 92, 103–117.

McNeil JD, Smith DL, Jenkins DH et al. (2001) Hypotensive resuscitation using a polymerized bovine hemoglobin-based oxygen carrying solution (HBOC-201) leads to reversal of anaerobic metabolism. J Trauma 50, 1063–1075.

Moore FA, Haenel JB, Moore EE et al. (1992) Incommensurate oxygen consumption in response to maximal oxygen availability predicts postinjury multiple organ failure. J Trauma 33, 58–62.

Muir WW, Autran de Morais HS, Constable PD (2000) The effects of a hemoglobin-based oxygen carrier (HBOC-301) on left ventricular systolic function in anesthetized dogs. Vet Surg 29, 449–455.

Nelson DP, King CE, Dodd SL et al. (1987) Systemic and intestinal O_2 extraction in the dog. J Appl Physiol 63, 387–394.

Noone RB, Mythen MG, Vaslef SN (1998) Effect of αα-cross-linked hemoglobin and pyridoxalated hemoglobin polyoxyethylene conjugate solutions on gastrointestinal regional perfusion in hemorrhagic shock. J Trauma 45, 457–469.

Page TC, Light WR, McKay CB et al. (1998) Oxygen transport by erythrocyte/hemoglobin solution mixtures in an *in vitro* capillary as a model of hemoglobin-based oxygen carrier performance. Microvasc Res 55, 54–64.

Pastores SM, Katz DP, Kvetan V (1996) Splanchnic ischemia and gut mucosal injury in sepsis and the multiple organ dysfunction syndrome. Am Gastro 91, 1697–1710.

Poli de Figueiredo LF, Cruz RJ, Net AC et al. (2001) Initial management of severe hemorrhage with an oxygen-carrying hypertonic saline solution. Artif Organs 25, 922–927.

Rentko V (2000) Practical use of a blood substitute. In: Kirk's Current Veterinary Therapy XIII – Small Animal Practice (13th edn). Bonagura JD (ed.). Saunders, Philadelphia, USA, pp. 424–427.

Resta TC, Walker BR, Eichinger MR et al. (2002) Rate of NO scavenging alters effects of recombinant hemoglobin solutions on pulmonary vasoreactivity. J Appl Physiol 93, 1327–1336.

Rohlfs RJ, Bruner E, Chiu A et al. (1998) Arterial blood pressure responses to cell-free hemoglobin solutions and the reaction with nitric oxide. J Biol Chem 273, 12128–12134.

Scaela TM, Simon HM, Duncan AO et al. (1990) Geriatric blunt multiple trauma: improved survival with early invasive monitoring. J Trauma 30, 129–134.

Schlichtig R, Kramer DJ, Pinsky MR (1991) Flow redistribution during progressive hemorrhage is a determinant of critical O_2 delivery. J Appl Physiol 70, 169–178.

Schoenberg MH, Lunberg C, Gerdin B, (1985) Hemorrhagic shock in the dog. II studies on central hemodynamics and regional blood flow. Res Exp Med 185, 469–482.

Scholander P (1960) Oxygen transport through hemoglobin solutions. Science 131, 585–590.

Shoemaker WC, Appel PL, Kram HB (1991) Oxygen transport measurements to evaluate tissue perfusion and titrate therapy: dobutamine and dopamine effects. Crit Care Med 19, 672.

Shoemaker WC, Appel PL, Kram HB (1992) Role of oxygen debt in the development of organ failure, sepsis, and

death in high-risk surgical patients. Chest 102, 208–215.

Siegel JH, Fabian M, Smith JA et al. (1997) Use of recombinant hemoglobin solution in reversing lethal hemorrhagic hypovolemic oxygen debt shock. J Trauma 42, 199–212.

Slater GI, Vladeck BC, Bassin R et al. (1975) Sequential changes in distribution of cardiac output in hemorrhagic shock. Surgery 5, 714–722.

Sprung J, Kindscher JD, Wahr JA et al. (2002) The use of bovine hemoglobin glutamer-250 (Hemopure®) in surgical patients: results of a multicenter, randomized, single-blinded trial. Anesth Analg 94, 799–808.

Standl T (2001) Haemoglobin-based erythrocyte transfusion substitutes. Expert Opin Biol Ther 1, 831–843.

Van Iterson M, Sinaasappel M, Burhop K et al. (1998) Low-volume resuscitation with a hemoglobin-based oxygen carrier after hemorrhage improves gut microvascular oxygenation in swine. J Lab Clin Med 132, 421–431.

Wettstein R, Tsai AG, Erni D et al. (2003) Resuscitation with polyethylene glycol-modified human hemoglobin improves microcirculatory blood flow and tissue oxygenation after hemorrhagic shock in awake hamsters. Crit Care Med 31, 1824–1830.

Winslow RM (2000) Blood substitutes. Adv Drug Deliv Rev 40, 131–142.

Winslow RM (2003) Current status of blood substitute research: towards a new paradigm. J Int Med 253, 508–517.

Winslow RM, Gonzales A, Gonzales M et al. (1998) Vascular resistance and the efficacy of red cell substitutes in a rat hemorrhage model. J Appl Physiol 85, 993–1003.

Young JS, Fernandez M, Meredith JW (1997) The effect of oxygen delivery-directed resuscitation on splanchnic and hepatic oxygen transport after hemorrhagic shock. J Surg Res 71, 87–92.

Received 14 July 2004; accepted 6 November 2004