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LABORATORY INVESTIGATIONS

Effects of haemoglobin-based oxygen carrier Hemoglobin glutamer-200 (bovine) on intestinal perfusion and oxygenation in a canine hypovolaemia model

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The objective of this investigation was to study the effects of the first marketed haemoglobin-based oxygen carrier, Hemoglobin glutamer-200 (bovine) (Hb-200) (Oxyglobin®) on splanchnic perfusion and oxygenation in a canine model of acute hypovolaemia. Twelve anaesthetized dogs [mean weight 30.8 (S.D. 1.4) kg] were instrumented for recordings of heart rate (HR), mean arterial pressure (MAP), central venous pressure (CVP), cardiac output and cranial mesenteric arterial (CMA) and venous blood flows (CMV). Total and plasma haemoglobin (Hb), oxygen content and saturation, lactate concentration, pH and blood gases were analysed in arterial, mixed venous and mesenteric venous blood samples. Measurements were made before (baseline) and after 1 h of haemorrhage, after which animals were resuscitated with either shed blood (controls) or Hb-200 until HR, MAP and CVP returned to prehaemorrhage levels. Recordings were repeated immediately and 3 h after termination of fluid resuscitation, after which organ specimens were obtained for microscopic examination. Haemorrhage (average 32 ml kg^{-1}) reduced MAP to 50 mm Hg, increased HR and systemic vascular resistance (SVR), and was accompanied in both the systemic and the splanchnic circulation by significant decreases in blood flow, Hb content and oxygen delivery (DO_2), and lactic acidosis. In controls, all variables recovered to baseline after isovolaemic resuscitation with shed blood. In dogs resuscitated with a small volume of Hb-200 (10 ml kg^{-1}), HR, MAP, CVP and CMA and CMV blood flows returned to baseline. However, cardiac output, total Hb, oxygen content and systemic and mesenteric DO_2 remained depressed while SVR increased further. Mesenteric and systemic acid–base status recovered in both groups, and there was no difference in microscopic tissue damage between groups. Thus, Hb-200 reconstituted splanchnic perfusion and oxidative metabolism in spite of pronounced systemic vasoconstriction and insufficient restoration of CO and DO_2; it may improve diffusive oxygen transport in the microvasculature by virtue of haemodilution and its high efficiency in the uptake and release of oxygen.

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Severe haemorrhage is associated with redistribution of cardiac output, increasing oxygen delivery to some vital organs (brain, heart) but reduced delivery to others, such as the gut.[1 2] Mesenteric ischaemia is a major factor involved in impaired function of the intestinal mucosal barrier[3] and may contribute to the initiation of the septic inflammatory

response syndrome.[4,5] Therefore, restoration of splanchnic perfusion in addition to the normalization of global perfusion is an important goal of resuscitation in hypovolaemia.[4]

Allogeneic and xenogeneic, stroma-free, ultrapurified haemoglobin solutions combine volume-expanding and oxygen-carrying capacities, making them potentially superior candidates for volume resuscitation in hypovolaemic patients.[6–9] However, most haemoglobin-based oxygen carriers (HBOCs) exert profound vasoconstrictive actions.[6–8] While a vasopressor effect may be advantageous for the body as a whole during hypovolaemic shock,[7] it might prolong the impairment of regional blood flow and oxygen delivery.[10–12] Animal studies are inconclusive, some providing evidence for improved gut perfusion[11,13–15] while others do not.[10–12]

Hemoglobin glutamer-200 (bovine) (Hb-200) (Oxyglobin®; Biopure, Cambridge, MA, USA) is an ultrapure solution of highly polymerized bovine haemoglobin.[6] It has been approved by the US Federal Drug Administration for the treatment of anaemia in dogs.[16] Hb-200 shares most of the properties of HBOC-201 (Hemopure®; Biopure),[6] the proposed human oxygen carrier currently under Phase III clinical investigation. In humans, both diaspirin-crosslinked human haemoglobin and polymerized bovine haemoglobin solutions have been associated with mild to moderate increases in plasma transaminases and pancreatic enzymes, and gastrointestinal symptoms.[7] Likewise, dogs may develop abdominal discomfort, peritoneal effusion and pancreatitis after infusion of Hb-200.[16] These signs may reflect impaired gut perfusion, considering the significant vasoconstrictive action of bovine haemoglobin solutions in both dogs and man.[17–19] We studied the effects of Hb-200 on mesenteric perfusion and oxygenation in a canine model of acute haemorrhage and compared the results with effects on the systemic circulation.

Methods

Twelve healthy, adult, mongrel dogs [mean weight 30.8 (SD 1.4) kg; seven female, five male) were studied after approval by the 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).

Animal preparation and instrumentation

Dogs were premedicated with oxymorphone 0.02 mg kg^{-1} i.m. and atropine 0.02 mg kg^{-1} i.m., and the cephalic vein was then catheterized percutaneously for continuous infusion of lactated Ringer's solution at the rate of 10 ml kg^{-1} h^{-1} throughout the preparation and instrumentation period and for administration of drugs. Anaesthesia was induced with propofol 2–4 mg kg^{-1} i.v. and diazepam 0.5 mg kg^{-1} i.v., and the animals were then intubated orotracheally and maintained with a balanced anaesthesia protocol, including isoflurane and fentanyl, to minimize potential confounding haemodynamic effects.[20] During animal preparation and instrumentation, isoflurane in oxygen was delivered at an end-tidal concentration of 0.8–1.2%, and fentanyl was infused at the rate of 0.7 μg kg^{-1} min^{-1} after an initial i.v. bolus of 10 μg kg^{-1}.[20] The lungs were ventilated mechanically with an anaesthesia ventilator (Model 2000; Hallowell EMC, Pietsfield, MA, USA), using tidal volumes (V_T) of 12–15 ml kg^{-1} and a respiratory rate of 9–11 breaths per minute to ensure an arterial partial pressure of carbon dioxide (Pa_{CO_2}) in the range of 35–45 torr (4.6–6.0 kPa). End-tidal partial pressure of carbon dioxide PE'_{CO_2}, end-tidal concentration of isoflurane (ISO_{ET}) and inspired oxygen concentration (FI_{O_2}) were monitored continuously using a Datex 254 airway gas monitor (Datex, Helsinki, Finland).

Further instrumentation included placement of catheters into the dogs' lateral saphenous vein and both femoral arteries for drug and fluid administration, blood withdrawal, and determination of systemic arterial pressures. An 8-Fr balloon-tipped, flow-directed thermodilution pulmonary arterial catheter (OptiQ; Abbott Laboratories, Chicago, IL, USA) was inserted via the jugular vein and floated into the pulmonary artery under direct monitoring of the pressure traces for measurements of central venous pressure (CVP), pulmonary artery occlusion pressure (POP), core body temperature and cardiac output. The pulmonary arterial catheter was connected to a cardiac output computer (Critical Care Systems QVUE, Oximetrix 3; Abbott Laboratories, Chicago, IL, USA) for continuous monitoring of cardiac output. Cardiac output was also assessed 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, Eden, MN, USA).

Dogs were splenectomized after a midline laparotomy to prevent release of sequestered red blood cells during sympathetic stimulation. 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. The CMA was encircled by a 4 mm Doppler transit-time flow probe (Transonics, Ithaca, NY, USA) approximately 1 cm distal to its origin. Using a similar technique, the triangle between portal and cranial mesenteric vein (CMV) was identified, and the CMV was encircled with a 6 mm Doppler transit-time flow probe (Transonics) 1–2 cm upstream to the CMV–portal junction. Flow probes were connected to a two-channel ultrasonic blood flow meter (T201; Transonics). Finally, a tributary of the CMV was cannulated with a 20 gauge catheter, which was then advanced into the CMV 4–5 cm upstream of the flow probe to allow mesenteric venous blood sampling.

Measured variables

Measured variables included heart rate (HR), mean arterial blood pressure (MAP), CVP, POP, cardiac output (as determined by thermodilution), and CMA and CMV blood flow. Arterial, mixed venous and mesenteric venous blood samples were collected intermittently from the femoral artery, right atrium and CMV respectively. Immediately after collection, blood samples were sealed and stored on ice. Subsequently, mixed venous (vHb_{total}) and mesenteric venous total haemoglobin ($mvHb_{total}$), and mixed venous plasma met-haemoglobin (Met-Hb) concentrations were measured in these samples using a Nova co-oximeter (Nova Biomedical, Waltham, MA, USA), and arterial, mixed venous and mesenteric venous oxygen contents (Ca_{O_2}, Cv_{O_2} and Cmv_{O_2} respectively) were measured directly in duplicate using an oxygen-specific electrode ($LEXO_2CON$-K; Hospex Fiberoptics, Chestnut Hill, MA, USA). Mixed venous and mesenteric venous lactate concentrations were determined in duplicate by means of a lactate analyser (Model 1500; YSI, Yellowsprings, OH, USA). Mixed venous and mesenteric venous pH (pH_v, pH_{mv}) and partial pressures of carbon dioxide (Pv_{CO_2} and Pmv_{CO_2} respectively) were analysed with a blood gas analyser (Model 170, Corning Medical, Medfield, MA). Blood gas values were corrected for the body temperature of the animals at the time of sampling. Mixed venous and mesenteric venous oxygen saturation (Sv_{O_2}, Smv_{O_2}) and standard base excesses (SBE_v, SBE_{mv}) were calculated by the blood gas analyser. All laboratory analysers used for determinations of oxygenation variables were validated for use with HBOCs.[21]

Body surface area (BSA, in square metres) was determined as $(10.1 \times [\text{body weight in grams}]^{2/3})/10\ 000$. Systemic vascular resistance (SVR) was calculated as $([MAP - CVP] \times 79.9/(\text{cardiac output/BSA})$; systemic oxygen delivery (sD_{O_2}) as $Ca_{O_2} \times$ cardiac output; mesenteric oxygen delivery (mD_{O_2}) as $Ca_{O_2} \times$ CMA blood flow; systemic oxygen consumption (sV_{O_2}) as $(Ca_{O_2} - Cv_{O_2}) \times$ cardiac output; mesenteric oxygen consumption (mV_{O_2}) as $(Ca_{O_2} - Cmv_{O_2}) \times$ CMA blood flow; systemic oxygen extraction ratio ($sExv_{O_2}$) as sV_{O_2}/sDv_{O_2}; and mesenteric oxygen extraction ratio ($mExv_{O_2}$) as mV_{O_2}/mD_{O_2}.

Experimental protocol

After completion of the surgical procedure, the infusion of lactated Ringer's solution was discontinued and the inspired gas switched from a source of 100% oxygen to medical air (FI_{O_2} 21–22%). For the remainder of the experiment, anaesthesia was maintained with end-tidal concentrations of isoflurane 0.7–0.8% [corresponding to 0.5–0.6 times the minimum alveolar concentration of isoflurane in dogs (~1.32 vol%)][22] and an infusion of fentanyl at a reduced rate of 0.4 μg kg^{-1} min^{-1} to take into account the lack of further surgical stimulation and to compensate for potential changes in fentanyl pharmacokinetics during hypovolaemia.[23] After these adjustments the dogs were allowed to stabilize for 45 min (equilibration period), and then all measurements (baseline) were taken. Subsequently, approximately 40% of the dogs' blood volume, which was estimated as 85 ml kg^{-1} body weight,[24] was withdrawn simultaneously from the lateral saphenous vein and femoral artery until an MAP of about 50 mm Hg was reached; shed blood was collected in citrate-containing bags and weighed. Additional small amounts of blood were removed to maintain the blood pressure at 50 mm Hg for 60 min. Cardiac output decreased by more than 50% from baseline during haemorrhage. At the end of the hypovolaemic period, all measurements were repeated (post-haemorrhage) and then dogs were allocated randomly to receive either shed blood at the rate of 30 ml kg^{-1} h^{-1} (control group) or Hb-200 (for details see Table 1) at the manufacturer's recommended infusion rate of 10 ml kg^{-1} h^{-1} (corresponding to 1.3 g kg^{-1} h^{-1} of bovine haemoglobin). Transfusion of shed blood or Hb-200 was discontinued once HR, MAP and CVP had returned to baseline and stabilized. All measurements were repeated immediately and 3 h after fluid resuscitation had been terminated. Animals were euthanized without regaining consciousness after the last measurements by means of an overdose of potassium chloride, and underwent post-mortem necropsy.

Histopathology

Immediately after death, specimens from the lung, kidney, liver, pancreas, mesenteric lymph nodes, duodenum, jejunum, ileum, colon and caecum were taken for microscopic examination, and transferred to 10% neutral buffered formalin solution. Tissues were then processed routinely to paraffin blocks and 5 μm sections were stained with haematoxylin and eosin. Interpretation was performed by a pathologist (SMG), who was blinded to the study groups.

Table 1 Specifications of Hemoglobin glutamer-200 (bovine) (Hb-200). All data except colloid oncotic pressure and P_{50} provided by manufacturer. [a]Measured with Colloid Oncometer 4420 (Wescor, Logan, UT, USA). P_{50} is the oxygen tension at which the polymerized bovine haemoglobin molecule is half saturated with oxygen.

Polymerized bovine haemoglobin	13 g dl^{-1}
NaCl	113 mmol $litre^{-1}$
KCl[1]	4 mmol $litre^{-1}$
$CaCl\text{-}2H_2O$	1.4 mmol $litre^{-1}$
NaOH	10 mmol $litre^{-1}$
Na-lactate	27 mmol $litre^{-1}$
N-acetyl-L-cysteine	200 mg dl^{-1}
Osmolality	300 mosm kg^{-1}
pH	7.8
Colloid oncotic pressure[a]	42 torr (5.6 kPa)
P_{50}[39]	34 torr (4.5 kPa)
Unpolymerized haemoglobin	< 5%
Molecular weight of ~50% of haemoglobin polymers	65–130 kDa
Molecular weight of ≤10% of haemoglobin polymers	>500 kDa
Free glutaraldehyde	<3.5 μg ml^{-1}
Endotoxin	<0.05 EU ml^{-1}

Table 2 Effects of haemorrhage and subsequent resuscitation with autologous blood (control) or Hemoglobin glutamer-200 (bovine) (Hb-200) on systemic haemodynamic variables. Variables determined were heart rate (HR), cardiac output (CO), mean arterial pressure (MAP), central venous pressure (CVP), pulmonary arterial occlusion pressure (POP) and systemic vascular resistance (SVR). Values are mean (SEM) for six dogs in each group. Resuscitation I=immediately after transfusion of autologous blood; Resuscitation II=3 h after transfusion of autologous blood. Significant differences from baseline values: *$P<0.05$; **$P<0.01$. Significant differences between control and Hb-200 group: $^{\dagger}P<0.05$; $^{\dagger\dagger}P<0.01$

Variable	Resuscitation group	Baseline	After haemorrhage	Resuscitation	
				I	II
HR (min^{-1})	Control	113 (6)	211 (8)**	122 (14)	96 (5)
	Hb-200	104 (7)	195 (9)**	127 (13)	139 (28)†
CO (litre min^{-1})	Control	3.4 (0.3)	1.5 (0.3)**	3.8 (0.3)	3.8 (0.2)
	Hb-200	3.0 (0.2)	1.3 (0.2)**	2.3 (0.2)*††	1.8 (0.2)**††
MAP (mm Hg)	Control	95 (4)	54 (1)**	83 (8)	99 (4)
	Hb-200	87 (6)	48 (3)**	92 (3)††	80 (13)
CVP (mm Hg)	Control	4 (1)	3 (2)	6 (2)	6 (1)
	Hb-200	3 (1)	0 (1)	4 (2)	3 (1
POP (mm Hg)	Control	5 (1)	4 (1)	6 (1)	7 (1)
	Hb-200	3 (1)	2 (1)	3 (1)	3 (2)
SVR (dynes s^{-1} cm^{-5})	Control	2303 (362)	3239 (631)**	1611 (128)*	1998 (177)
	Hb-200	2307 (144)	3100 (281)**	3169 (207)*††	3549 (286)*††

Statistical analysis

Results are given as arithmetic mean (SEM). Statistical evaluation of data within each group (i.e. testing for differences between time points) included analysis of variance (ANOVA) for repeated measures. When ANOVA indicated significant differences, statistical testing was followed by comparisons between baseline and time points using a Student's *t*-test for independent samples and *post hoc* Bonferroni correction. Statistical evaluation of data between both groups was performed using an ANOVA for repeated measures followed by the *t*-test. $P<0.05$ was taken to be statistically significant.

Results

Haemodynamic, oxygenation and acid–base variables

No statistically significant differences in systemic and mesenteric haemodynamic, oxygenation and acid–base variables measured before haemorrhage (baseline) were observed between groups (Tables 2–4; Figs 1–3). The depth of anaesthesia (as judged by jaw tone, palpebral reflex and lack of motor response to intermittent toe pinch stimulation), ventilation and arterial oxygen saturation remained unchanged throughout the experiment.

Approximately 40% of the estimated blood volume[24] was withdrawn in the control and Hb-200 groups [31.6 (4.1) and 31.5 (4.1) ml kg^{-1} respectively] to reduce MAP to the set point of approximately 50 mm Hg. Haemorrhage resulted in a decline in vHb_{total} and Ca_{O_2} of approximately 20 and 18% respectively (Table 3). Dogs in both groups responded to the acute blood loss as expected and to the same extent (Tables 2–4; Figs 1–3). Cardiac output (Table 2), CMA blood flow, CMV blood flow (Fig. 1), systemic oxygen delivery (sD_{O_2}) and mesenteric oxygen delivery (mD_{O_2}) (Fig. 2) decreased on average by 56, 50, 43, 65 and 58% respectively. HR and SVR increased by an average of 87 and 38% respectively (Table 2). Central venous pressure and POP decreased immediately after haemorrhage, but at the end of the hypovolaemic period, when post-haemorrhage values were recorded, this effect was not statistically significant (Table 2). sDv_{O_2} and mDv_{O_2} were equally diminished after haemorrhage (Fig. 2), and mixed venous and mesenteric blood oxygenation variables were signifi-

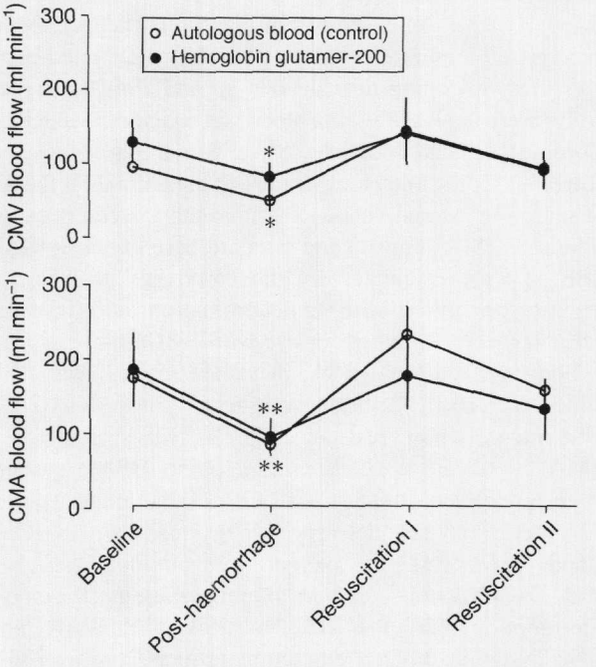

Fig 1 Cranial mesenteric arterial (CMA) and venous (CMV) blood flow in anaesthetized dogs at baseline (end of equilibration), 60 min after haemorrhage (end of hypovolaemia) and immediately (Resuscitation I) and 3 h (Resuscitation II) after transfusion of autologous blood (control) and Hemoglobin glutamer-200 (bovine). Values are mean (SEM) for six dogs in each group. Significant differences from baseline values: *$P<0.05$; **$P<0.01$.

Table 3 Effects of haemorrhage and subsequent resuscitation with autologous blood (control) or Hemoglobin glutamer-200 (bovine) (Hb-200) on systemic and mesenteric oxygenation variables and total haemoglobin. Variables measured were arterial blood oxygen content (Ca_{O_2}), arterial oxygen saturation (Sa_{O_2}), mixed venous blood total haemoglobin content (vHb_{total}) and oxygen content (Cv_{O_2}), oxygen saturation (Sv_{O_2}), mesenteric venous blood total haemoglobin content ($mvHb_{total}$) and oxygen content (Cmv_{O_2}), and oxygen saturation (Sv_{O_2}, Smv_{O_2}). Systemic and mesenteric oxygen consumption (sV_{O_2}, mV_{O_2}) were calculated. Values are mean (SEM) for six dogs in each group. Resuscitation I=immediately after transfusion of autologous blood; Resuscitation II=3 h after transfusion of autologous blood. Significant differences from baseline values: $^{*}P<0.05$; $^{**}P<0.01$. Significant differences between control and Hb-200 group: $^{\dagger}P<0.05$; $^{\dagger\dagger}P<0.01$. Significant differences between mixed venous and mesenteric venous blood: $^{\ddagger}P< 0.05$; $^{\ddagger\ddagger}P< 0.01$.

Variable	Resuscitation group	Baseline	After haemorrhage	Resuscitation	
				I	II
Ca_{O_2} (ml dl^{-1})	Control	19.2 (0.6)	16.5 (1.2)*	18.7 (0.8)	19.0 (0.6)
	Hb-200	20.3 (0.5)	16.1 (0.8)**	16.8 (0.6)*†	16.9 (0.8)*†
Sa_{O_2} (%)	Control	97.9 (0.6)	96.5 (1.1)	97.7 (0.5)	97.5 (0.6)
	Hb-200	95.9 (0.5)	95.2 (0.5)	96.5 (0.5)	96.7 (0.5)
vHb_{total} (g dl^{-1})	Control	14.5 (1.8)	11.8 (1.9)**	13.8 (1.5)	14.5 (1.6)
	Hb-200	15.2 (1.3)	11.8 (1.3)**	11.5 (0.9)**†	12.3 (2.0)*†
Cv_{O_2}(ml dl^{-1})	Control	16.0 (0.7)	7.4 (2.1)**‡	15.2 (0.9)	16.4 (0.9)
	Hb-200	15.6 (0.7)	6.6 (1.0)**‡	9.4 (0.6)**†‡	9.6 (1.4)**††
Sv_{O_2} (%)	Control	81.7 (0.6)	37.9 (2.4)**‡‡	82.2 (1.2)	78.6 (1.5)
	Hb-200	74.1 (3.1)	38.8 (5.1)**‡‡	59.5 (1.6)*†‡‡	46.6 (7.8)*†‡
sV_{O_2} (ml min^{-1})	Control	108 (44)	134 (29)	131 (21)	96 (24)
	Hb-200	137 (12)	122 (16)	168 (16)	135 (28)
$mvHb_{total}$ (g dl^{-1})	Control	14.7 (1.6)	11.8 (1.3)**	13.6 (1.6)*	14.7 (1.8)
	Hb-200	15.4 (1.8)	12.0 (1.3)**	11.8 (0.6)**†	12.1 (1.6)**†
Cmv_{O_2} (ml dl^{-1})	Control	16.4 (0.7)	9.3 (1.6)**	15.8 (1.0)	16.4 (0.9)
	Hb-200	15.6 (0.7)	8.6 (0.5)**	10.9 (0.6)**††	10.8 (1.4)**††
Smv_{O_2} (%)	Control	87.3 (2.9)	55.8 (9.7)**	85.9 (1.2)	80.3 (3.6)
	Hb-200	79.4 (4.2)	49.4 (3.1)**	70.0 (2.8)*†	60.0 (10.4)*†
mV_{O_2} (ml min^{-1})	Control	5.0 (1.2)	5.6 (1.1)	6.4 (1.5)	3.8 (0.7)
	Hb-200	5.9 (1.2)	7.5 (2.2)	12.0 (3.6)	7.4 (2.2)

cantly reduced (Table 3). The mixed venous oxygen content (Cv_{O_2}) and saturation (Sv_{O_2}) decreased rapidly by an average of 56 and 51% respectively, and in parallel but to a lesser degree, mesenteric venous oxygen content (Cmv_{O_2}) and saturation (Smv_{O_2}) declined by 50 and 37% respectively (Table 3). Systemic (sV_{O_2}) and mesenteric (mV_{O_2}) oxygen consumption did not change in either group during hypovolaemia; however, oxygen extraction in the systemic and mesenteric vascular bed increased to 2.9 and 2.6 times the baseline value respectively (Table 3; Fig. 3). Oxygen extraction was significantly greater in the systemic than mesenteric circulation (Fig. 3). There was no difference between groups in this increase in oxygen extraction ratios, and there was also no difference between groups in the degree of ischaemia-induced lactate acidosis that developed during the post-haemorrhage phase (Table 4).

Whereas the infusion of 10 ml kg^{-1} Hb-200 was as effective as a three times larger volume of autologous blood (average 32 ml kg^{-1}) in returning HR, MAP and CVP to prehaemorrhage levels (Table 1), this treatment, in contrast to shed blood transfusion, failed to restore other systemic haemodynamic, blood and oxygen variables (Figs 1–3; Tables 2 and 3). In the Hb-200 group, cardiac output, vHb_{total}, Ca_{O_2}, Cv_{O_2}, Sv_{O_2} and sD_{O_2} all remained significantly below and SVR and systemic oxygen extraction remained significantly above baseline values throughout the entire post-resuscitation period (Figs 1–3; Tables 2 and 3). Systemic oxygen consumption (sV_{O_2}) was not significantly affected, though a trend for an increase was recognized in

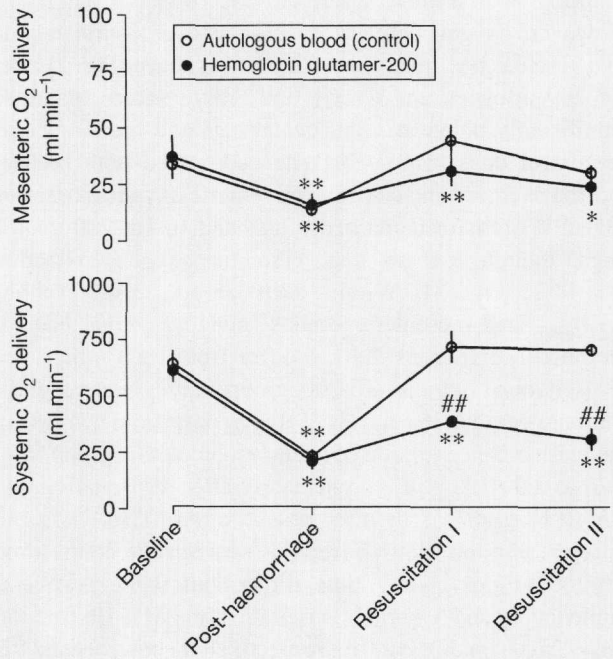

Fig 2 Systemic and mesenteric oxygen delivery in anaesthetized dogs at baseline (end of equilibration), 60 min after haemorrhage (end of hypovolaemia), and immediately (Resuscitation I) and 3 h (Resuscitation II) after transfusion of autologous blood (control) or Hemoglobin glutamer-200 (bovine). Values are mean (SEM) for six dogs in each group. Significant differences from baseline values: $^{*}P<0.05$; $^{**}P<0.01$. Significant differences between control and Hb-200 groups: $^{\#\#}P<0.01$.

Table 4 Effects of haemorrhage and subsequent resuscitation with autologous blood (control) or Hemoglobin glutamer-200 bovine (Hb-200) on blood acid–base variables. Variables determined were mixed venous pH (pH_v), partial pressure of carbon dioxide (Pv_{CO_2}), standard base excess (SBE_v) and lactate ($Lactate_v$), and mesenteric venous pH (pH_{mv}), partial pressure of carbon dioxide (Pmv_{CO_2}), standard base excess (SBE_{mv}) and lactate concentration ($Lactate_{mv}$). Partial pressures of carbon dioxide are also given in SI units (kPa, square brackets). Values are mean (SEM) for six dogs in each group. Resuscitation I=immediately after transfusion of autologous blood; Resuscitation II=3 h after transfusion of autologous blood. Significant differences from baseline values: *P<0.05; **P<0.01

Variable	Resuscitation group	Baseline	After haemorrhage	Resuscitation	
				I	II
pH_v	Control	7.35 (0.01)	7.16 (0.02)**	7.24 (0.02)**	7.36 (0.03)
	Hb-200	7.36 (0.01)	7.22 (0.02)**	7.28 (0.01)**	7.29 (0.06)
Pv_{CO_2}(torr)	Control	43.0 (1.7)	61.1 (2.2)**	50.7 (2.1)**	41.7 (2.3)
(kPa)		5.7 (0.2)	8.1 (0.3)	6.8 (0.3)	5.6 (3.1)
	Hb-200	40.4 (1.2)	56.4 (2.4)**	48.3 (1.2)**	47.2 (4.2
		5.4 (0.2)	7.5 (0.3)	6.4 (0.2)	6.3 (0.6)
SBE_v (mmol litre^{-1})	Control	–1.7 (0.4)	–8.3 (0.9)**	–6.6 (1.4)*	–1.2 (1.5)
	Hb-200	–2.2 (0.6)	–6.0 (0.6)**	–4.0 (0.5)*	–4.2 (2.6)
$Lactate_v$ (mmol litre^{-1})	Control	1.41 (0.17)	3.61 (0.38)**	2.19 (0.10)**	1.53 (0.24)
	Hb-200	1.18 (0.23)	2.87 (0.50)**	2.09 (0.18)**	2.57 (1.45)
pH_{mv}	Control	7.37 (0.02)	7.19 (0.02)**	7.26 (0.02)**	7.37 (0.03)
	Hb-200	7.35 (0.01)	7.22 (0.01)**	7.29 (0.01)**	7.30 (0.07)
Pmv_{CO_2}(torr)	Control	41.8 (1.8)	55.4 (3.8)**	47.0 (1.3)**	41.5 (1.5)
(kPa)		5.6 (0.2)	7.4 (0.5)	6.3 (0.2)	5.5 (0.2)
	Hb-200	41.0 (1.9)	55.0 (1.4)**	48.4 (1.2)**	46.4 (4.7
		5.5 (0.3)	7.3 (0.2)	6.5 (0.2)	6.2 (0.6)
SBE_{mv} (mmol litre^{-1})	Control	–0.9 (0.4)	–7.9 (0.7)**	–6.2 (1.0)*	–1.0 (1.3)
	Hb-200	–2.3 (1.0)	–5.9 (0.7)*	–3.9 (0.4)*	–4.3 (2.9)
$Lactate_{mv}$ (mmol litre^{-1})	Control	1.29 (0.24)	3.34 (0.47)**	2.01 (0.12)*	1.62 (0.27)*
	Hb-200	1.34 (0.27)	2.77 (0.56)**	2.14 (0.08)*	3.33 (1.42)**

the Hb-200 group immediately after resuscitation. Unlike in the systemic circulation, blood flow in the CMA and CMV returned to baseline after Hb-200 infusion (Fig. 1). However, oxygen delivery did not recover completely in this vascular bed (maximum 82% of baseline) (Fig. 2) after Hb-200 infusion, and Cmv_{O_2} and Smv_{O_2} values remained significantly below baseline recordings and measurements in control dogs (Table 3). When compared with prehaemorrhage values and controls, mesenteric oxygen extraction (35–37%) remained markedly elevated in Hb-200-treated dogs, though less so than systemic oxygen extraction (43–44%; Fig. 3). Within each group, mixed venous (vHb_{total}) and mesenteric venous ($mvHb_{total}$) total haemoglobin concentrations did not differ from each other after resuscitation, but the Hb-200 group values were significantly lower than in controls (Table 3) despite an increase in the plasma concentration of haemoglobin in this group from zero to 2.9 (0.1) g dl^{-1} immediately after resuscitation and 2.5 (0.1) g dl^{-1} 3 h after resuscitation. There was no difference in the Met-Hb fraction between the control and Hb-200 groups at any time throughout the experiment; immediately and 3 h after resuscitation, the Met-Hb fraction was 0.2 (0.0) and 0.3 (0.0)% respectively in controls and 0.3 (0.1) and 0.4 (0.1)% in Hb-200-treated dogs.

After resuscitation, the control and Hb-200-treated animals did not differ in their mesenteric or systemic acid–base status (Table 4). At the end of the observation period, all acid–base variables listed in Table 4 had returned to prehaemorrhage levels, except the lactate concentration in mesenteric venous blood of both groups.

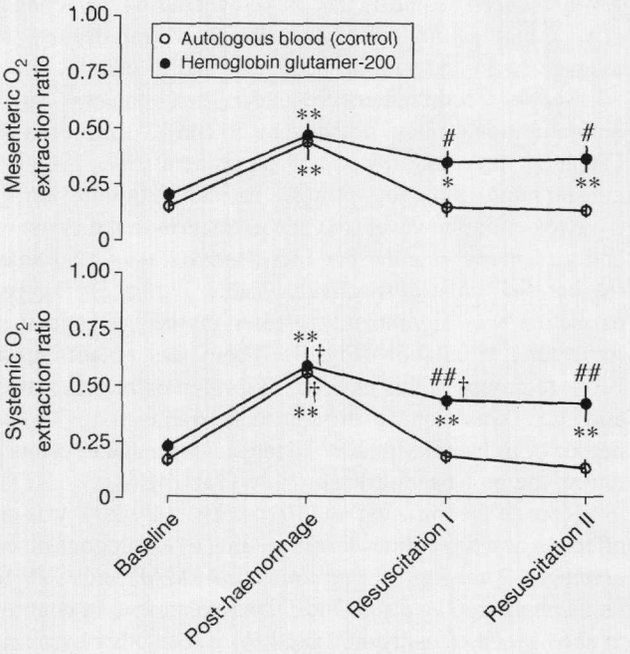

Fig 3 Systemic and mesenteric oxygen extraction ratio in anaesthetized dogs at baseline (end of equilibration), 60 min after haemorrhage (end of hypovolaemia), and immediately (Resuscitation I) and 3 h (Resuscitation II) after transfusion of autologous blood (open circles, control) or Hemoglobin glutamer-200 (bovine) (closed circles). Values are mean (SEM) for six dogs in each group. Significant differences from baseline values: *P<0.05; **P<0.01. Significant differences between control and Hb-200 groups: $^{\#}P$<0.05; P<0.01. Significant differences between systemic and mesenteric oxygen extraction: $^{\dagger}P$<0.05.

Table 5 Histomorphological changes after haemorrhage and subsequent resuscitation with autologous blood (control) or Hemoglobin glutamer-200 bovine (Hb-200). Immediately after the experiment, necropsy specimens were taken for microscopic examination from the intestine, including duodenum, jejunum, ileum, colon and caecum, mesenteric lymph nodes, pancreas, liver, kidneys and lungs. A blinded examiner then evaluated the tissues. Values given are the number of dogs in the control group (resuscitation with autologous blood) and Hb-200 group (resuscitation with Hemoglobin glutamer-200 bovine]) in which the microscopic changes recorded were found

Tissue	Morphological changes	No. of dogs per group	
		Control	Hb-200
Intestines	Acute epithelial necrosis/sloughing	6	5
	Congestion of lamina propria	0	1
	Submucosal haemorrhage and/or congestion	1	1
Mesenteric lymph node	Scattered medullary erythrophagocytosis	2	5
Pancreas	Focal areas of acute necrosis	0	1
Liver	Mild periportal neutrophilic infiltration	0	2
	Sinusoidal congestion with neutrophilic infiltrates	1	1
	Portal bile duct stasis	0	1
	Multifocal subcapsular necrosis with haemorrhage and neutrophilic infiltrates	1	0
Kidney	Mild chronic interstitial nephritis	0	1
	Congested glomeruli	0	1
Lung	Interstitial congestion	2	2

Histopathological findings

On necropsy, no macroscopically abnormal findings were recorded in any of the visceral organs examined except hyperaemia of intestinal organs and congestion of mesenteric blood vessels in both groups. Histological changes were observed in necropsy specimens from all dogs and are listed in Table 5. However, the only tissues that consistently showed changes were from the gastrointestinal tract. In both groups, the gastrointestinal tract had acute mucosal damage in all or parts of the duodenum, jejunum, ileum, colon and caecum, consisting of epithelial cell necrosis and sloughing. The occurrence of red blood cells in the cytoplasm of phagocytes residing in the mesenteric lymph nodes was the only morphological lesion with a markedly higher incidence in Hb-200-treated dogs.

Discussion

In a canine model of acute haemorrhage, we studied the restoration of systemic and gut perfusion and oxygenation, as well as morphological changes in the visceral organs after resuscitation with either autologous blood or an HBOC. Our data indicate that hypovolaemic resuscitation with Hb-200 reconstitutes mesenteric perfusion and oxidative metabolism in the early post-resuscitation period (Fig. 1; Tables 3 and 4) despite pronounced vasoconstriction in the systemic vasculature accompanied by low cardiac output and persistence of lower than normal systemic and mesenteric oxygen delivery (Table 2; Fig. 2).

The canine model of acute hypovolaemia used in this study is well characterized.[25–26] We decided to use a haemorrhage that decreased mean arterial pressure to 50 mm Hg, which was achieved with moderate blood loss (about 40% of circulating blood volume) in the control and study groups.[24] This method ensures a severe but not necessarily lethal insult that was similar to many clinical situations of acute blood loss. Under most clinical circumstances, fluid resuscitation is guided by measurements of HR, MAP and CVP.[27] We therefore used the return of these clinically most commonly measured variables to baseline as the endpoint for volume resuscitation, followed by a 3 h period for continued monitoring of the dogs.

In both groups of animals, all systemic haemodynamic, oxygenation and acid–base variables measured before haemorrhage were well within the normal range reported for dogs (Tables 2–4).[2 25 26] Likewise, mesenteric blood flow and oxygenation data at baseline were in accordance with those reported in dogs not undergoing laparotomy (Fig. 1; Table 3),[28 29] indicating an intact splanchnic circulation before haemorrhage. In the dog, progressive haemorrhage induces strong activation of the sympathetic nervous system and the subsequent redistribution of blood flow causes hypoperfusion in many organs, including the pancreas.[2 3 30] Also, gastrointestinal perfusion diminishes with haemorrhage, initially in proportion to the reduction in cardiac output,[2 30] but later out of proportion to the decrease in cardiac output as blood loss becomes more severe.[3] However, this decrease is primarily mediated by the activation of local renin–angiotensin mechanisms.[3] We observed similar haemodynamic responses to haemorrhage in the present experiment. MAP and cardiac output decreased substantially, and HR and SVR increased significantly (Table 2). Mesenteric arterial blood flow and oxygen delivery decreased in both groups to about the same extent as cardiac output (Figs 1 and 2; Tables 2 and 3), which is consistent with findings in pigs with similar haemorrhages.[11 15] Gut oxygen consumption was maintained because of a marked increase in oxygen extraction (Table 3; Fig. 3). The corresponding decrease in mesenteric venous oxygen saturation (Smv_{O_2}) was similar to that measured non-invasively in dogs after haemorrhage,[29] thus excluding a major effect of the preceding surgical procedure on gut blood flow and oxygenation. In both the systemic and splanchnic circulation, increased oxygen extraction failed to

compensate entirely for insufficient oxygen delivery, and anaerobic metabolism therefore occurred. However, the resulting metabolic acidosis (Table 4) was mild enough that a possible further impairment of mesenteric blood flow due to any vasoconstrictive action of Hb-200 in the splanchnic system was likely to be recognized as a further deterioration in the acid–base variables.

Stroma-free haemoglobin solutions appear to be superior resuscitation fluids because they carry, deliver and release oxygen in a cooperative manner, and are highly oxygen-saturated at ambient oxygen pressures.[7 9] This applies particularly to bovine haemoglobin solutions (Hb-200, HBOC-201), which have a relatively high haemoglobin content (13 g dl^{-1}), low oxygen affinity and hence high oxygen off-loading capacity [P_{50} 34 torr (4.5 kPa)], and a relatively long plasma half-life (>20 h).[6 7 18] Furthermore, exercise physiology studies have suggested that these solutions have an efficacy ratio three times greater than erythrocyte-borne haemoglobin, on the basis of increments and decrements in oxygen diffusion capacity.[6] However, our data challenge this view. In contrast to isovolaemic resuscitation with autologous blood (controls), we did not find restoration of global oxygen delivery to prehaemorrhage levels by Hb-200, but instead significant systemic vasoconstriction and a lower cardiac output after administration of a volume one-third of that of blood loss (Table 2). Furthermore, total haemoglobin and arterial oxygen contents remained at the low post-haemorrhage level (Table 3). Thus, animals in the study group were systemically under-resuscitated despite HR, MAP and CVP returning to baseline. Cardiac output probably remained lower because of the increase in total peripheral vascular resistance caused by Hb-200 rather than because of volume underloading, because indicators of right (CVP) and left (POP) ventricular preload returned to baseline in this group also (Table 2). Significant decreases in cardiac output resulting from pronounced vasoconstriction have also been reported in dogs[19] and humans[18 31] undergoing isovolaemic haemodilution with polymerized bovine haemoglobin solutions. It is thought that scavenging of the endothelium-derived relaxing factor, nitric oxide, is the chief mechanism by which stroma-free haemoglobin elicits vasoconstriction, but the release of endothelin-1 and interaction with adrenoceptors and inositol triphosphate pathways may also be involved in this effect.[8] Unexpectedly, global tissue hypoxia resolved after low-volume resuscitation with Hb-200, as indicated by evaluation of mixed venous acid–base status (pH, SBE, lactate concentration).

Our data provide no evidence that Hb-200 compromised gastrointestinal perfusion and oxygenation significantly. CMA and CMV perfusion, unlike cardiac output, returned to the prehaemorrhage level after low-volume resuscitation with Hb-200 (Fig. 1). Blood flow in the CMA and CMV increased immediately after resuscitation by an average of 90 and 78% respectively from post-haemorrhage levels. Cardiac output increased to nearly the same extent (average 88%) (Table 2), suggesting that now the stroke volume was distributed in favour of the splanchnic system, which was probably the result of a lack of vasoconstrictive action by Hb-200 in this vascular bed. Total haemoglobin concentrations were similar in mesenteric and mixed venous blood (Table 3), which argues against a greater haemodilutional effect in the mesenteric vasculature and against increased flow through larger numbers of capillaries carrying just plasma. Hemoglobin glutamer-200 seems to behave similarly to diaspirin-crosslinked haemoglobin (DCLHb), pyridoxalated haemoglobin polyoxyethylene conjugate and stroma-free human haemoglobin solution, all of which improve intestinal blood flow after acute haemorrhage.[11 13–15] The systemic and regional haemodynamic effects of Hb-200 resemble particularly those of DCLHb, which increases vascular resistance in skeletal muscle but not in the gastrointestinal system, liver, kidney brain, heart or skin.[32] In contrast, recombinant human haemoglobin (rHb1.1), bovine fumaryl-crosslinked haemoglobin (ββHb) and αα-crosslinked haemoglobin (ααHb) reduce intestinal perfusion as much as perfusion of other organs.[10–12] Therefore, the effects of HBOCs on the splanchnic circulation may depend on the chemical modification technique used for tetramerization.

While splanchnic blood flow returned to the prehaemorrhage level in Hb-200-treated animals, oxygen delivery to the gut did not, because of a lack of increase in total blood haemoglobin and, hence, arterial oxygen content (Fig. 2; Table 3). As a result, mesenteric oxygen extraction remained significantly elevated and mesenteric venous oxygen saturation decreased significantly after resuscitation (Fig. 3; Table 3). This effect was significantly less pronounced in the intestinal than in the systemic circulation, reflecting the better correction of mesenteric arterial than systemic blood flow and oxygenation. This finding coincides with the improvement by DCLHb of gut microcirculation and mucosal oxygen tension after haemorrhagic shock.[13 15] Increased oxygen extraction in both small bowel and peripheral tissues sufficed to resolve the anaerobic metabolism that had occurred during hypovolaemia and to return the acid–base status to baseline (Table 4). Only lactate values remained slightly elevated, but they were similar to those of the control group (Table 4).

Although blood flow in the intestinal mucosa was not measured directly, microscopic examination of intestinal tissues did not reveal evidence of greater ischaemic damage in Hb-200-treated dogs than in animals transfused with whole blood. The lesions observed were similar in frequency and severity in the two groups and compatible with hypoxic injury caused by haemorrhagic shock (Table 5).[33] Hence, the clinically observed signs of abdominal discomfort, elevated plasma transaminases and pancreatitis after administration of HBOCs[7 16] may not be associated with any further ischaemic/hypoxic insult caused by these solutions. However, because animals did not recover from anaesthesia, survived only 3 h after resuscitation and were

incompletely volume-resuscitated in the Hb-200 group, it remains speculative as to whether the microscopic changes seen in the gut and other visceral organs might have progressed with time, considering the long plasma half-life of bovine HBOCs (e.g. the $t_{1/2}$ of Hb-200 is 18–43 h).[6 16] Nonetheless, our results agree with previous studies in dogs, in which there were either no or only minor morphological changes in liver, kidney and lung biopsy specimens obtained 7 days[34] or 2 h[35] after HBOC infusion. The variable erythrophagocytosis within the mesenteric lymph nodes of both groups was probably related to manipulation during the experiments and, in the case of Hb-200, to uptake of the HBOC by the reticuloendothelial system.[6]

A surprising yet important finding of the present study was that systemic and regional tissue oxidative metabolism seemed to be reinstituted after low-volume resuscitation with Hb-200 despite sustained depression of cardiac output and low arterial oxygen delivery in both the systemic and the mesenteric circulation. Factors that may have contributed to this positive effect include better blood flow characteristics as a result of decreased blood viscosity, an increase in plasma oxygen transport capacity and improved oxygen diffusion from the blood into the tissues. Hemoglobin glutamer-200 has a viscosity three times lower that of than whole blood.[18] In addition, it has high colloid oncotic pressure (42 torr), which promotes haemodilution by intravascular fluid shifts. This was evident from a lack of increase in total haemoglobin after resuscitation despite an average increase of 2.9 g dl^{-1} in plasma haemoglobin concentration. Lower blood viscosity, and hence more homogeneous distribution of capillary flow, might have preferentially affected the perfusion of tissues in which vasoconstriction prevailed. In the intestinal mucosa, nutrient arterioles branch from their parent vessel at a right-angle. This microvascular pattern facilitates 'plasma skimming', a phenomenon characterized by a decrease in the effective haematocrit of blood as it passes through villous capillaries relative to the haematocrit measured in the systemic circulation.[3] As a result, the oxygen-carrying capacity of the blood may decrease selectively in the splanchnic microcirculation. The presence of an HBOC-like Hb-200 in blood plasma, however, will help minimize the impact of this effect on tissue oxygenation by virtue of its oxygen-carrying capacity. Theoretical analysis of oxygen release in the microcirculation,[36] as well as *in vitro*[37] and *in vivo* data,[17 18] suggest that the presence of free haemoglobin in plasma markedly increases the diffusion of oxygen from the blood into tissues. This appears to be even more important at a low haematocrit, when the spatial distance between erythrocytes increases and extracellular haemoglobin functions as a bridge for oxygen transfer from red blood cells to the endothelial membrane. The increased oxygen diffusion capacity of blood containing acellular bovine haemoglobin may be due to the low oxygen-binding affinity of bovine HBOCs (P_{50} 34 torr versus 28 for canine haemoglobin),[21] favouring oxygen release but not markedly affecting oxygen uptake[38], and their pronounced Haldane (carbon dioxide) and Bohr (pH) effects,[18] which augment oxygen off-loading, particularly in hypoxic tissues. Thus, it is likely that in Hb-200-treated animals the presence of cell-free plasma haemoglobin, (approximately 25% of total haemoglobin) strengthened the diffusive component of oxygen transport, thereby preserving tissue viability.

In summary, in dogs subjected to acute haemorrhage, low-volume resuscitation with Hemoglobin glutamer-200 (bovine) 10 ml kg^{-1} was inadequate to restore cardiac output and oxygen delivery but was as effective as isovolaemic whole-blood resuscitation (32 ml kg^{-1}) in returning splanchnic blood flow and acid–base status to the pre-haemorrhage levels. These results indicate that bovine haemoglobin-based oxygen carriers (i) may not impair splanchnic perfusion in spite of their systemically vasoconstrictive action and their ability to compromise cardiac output, and (ii) may improve capillary oxygen transport efficiency and hence oxygen uptake into tissues sufficiently to allow aerobic cell metabolism to be maintained even in situations of moderately reduced arterial oxygen content.

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