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Experimental paper

Sodium nitroprusside ameliorates systemic but not pulmonary HBOC-201-induced vasoconstriction: An exploratory study in a swine controlled haemorrhage model☆

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ABSTRACT

Background: Vasoconstriction is a side effect that may prevent the use of haemoglobin based oxygen carrier (HBOC) as blood substitute. Therefore, we tested the hypothesis that the NO donor, sodium nitroprusside (SNP), would mitigate systemic and pulmonary hypertension associated with HBOC-201 in a simple controlled haemorrhage swine model. *Methods*: After 55% estimated blood volume withdrawal through a venous catheter, invasively anesthetized and instrumented animals were resuscitated with three 10 ml/kg infusions of either HBOC-201 or Hextend (HEX) with or without 0.8 μg/kg/min SNP (infused concomitantly via different lines). Haemodynamics, direct and indirect measures of tissue oxygenation, and coagulation were measured for 2 h. *Results*: Haemorrhage caused a state of shock manifested by hypotension and base deficit. HBOC-201 resuscitation resulted in higher systemic ($p < 0.0001$) and pulmonary ($p < 0.002$) blood pressure than with HEX. Elevation of systemic ($p < 0.0001$) but not pulmonary ($p > 0.05$) arterial pressure was attenuated by co-infusion of SNP, without significant group differences in haemodynamics, tissue oxygenation, platelet function, coagulation, methaemoglobin, or survival ($p > 0.05$). *Conclusion*: In swine with haemorrhagic shock, co-administration of the NO donor, SNP, effectively and safely reduces HBOC-201-related systemic but not pulmonary vasoactivity. Interestingly, co-administration of the vasodilator SNP with HEX had no deleterious effects in comparison with HEX alone.

1. Introduction

Haemorrhagic shock (HS) compromises vital organ perfusion, impairing tissue oxygenation despite standard pre-hospital resuscitation fluids. In experimental models of severe HS, resuscitation with some haemoglobin-based oxygen carriers (HBOC) decreases lactic acidosis and improves tissue oxygenation, haemodynamics, and survival.[1–6] This is of particular interest when blood is unavailable.[2] However, adverse events, including myocardial infarctions, related to systemic and pulmonary vasoconstriction were reported in HBOC clinical trials.[7,8] Indeed, increased afterload from systemic vasoconstriction, and ventilation-perfusion mismatch causing blood deoxygenation and decreased venous return and cardiac output from pulmonary vasoconstriction could theoretically contribute to cardiac ischemia. HBOC vasoactivity mechanisms have been put forth, including vasoconstriction due to hyperoxia (high P50), endothelin release, adrenergic receptor activation, and arachidonic acid interaction, but nitric oxide (NO) scavenging by extracellular HBOC is considered the primary mechanism.[9–13]

NO is synthesized from L-arginine by endothelial-derived nitric oxide synthase (eNOS). Freely diffusing through cell layers, NO acts as a biological vasoregulator, regulating smooth muscle dilatation via the guanyl cyclase/cGMP pathway. NO has a short half-life and concentration is regulated by iNOS.[14] HBOC infusion (presumably) reduces blood NO levels, manifesting clinically as systemic and pulmonary hypertension. To offset NO scavenging and its consequences, we and others have investigated various approaches, including reduction of HBOC-201 tetrameric/dimeric haemoglobin, increasing mean molecular weight, co-infusion of drag reducing polymers, and pharmacologic repletion of NO with L-arginine, sodium nitroprusside (SNP), sodium nitrite ($NaNO_2$), nitroglycerine

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Table 1
Dose regimen for the experimental treatment groups.

Time window for fluid infusion	SNP dose regimen μg/kg/min			Total dose
	T15-T30	T30-T45	T45-T60	μg/kg
	0.8	0.8	0.8	24

n	Treatment group	Abbreviated
4	HBOC-201 only	HB
8	HBOC + SNP 0.8 μg/kg/min	HB-SNP
4	Hextend only	HEX
4	Hextend + SNP 0.8 μg/kg/min	HEX-SNP

(NTG), and inhaled NO (INO).[15–21] HBOC-201 modifications and L-arginine co-infusion were ineffective and high-dose $NaNO_2$ caused unexpected adverse events.[15–19] NTG is efficacious in HS in swine and INO is efficacious in topload studies in mice and awake sheep.[9,22] SNP, used clinically to treat hypertensive crisis, has potent arterial vasodilatory properties.[23] In vascular strip preparations, SNP potently reverses HBOC-201 vasoactivity.[20]

In contrast with in-hospital setting where titratable dosing is possible, our broad concept is to investigate the efficacy/safety of fixed dosing of NO donors (e.g., SNP) in the pre-hospital setting where titration would rarely be possible. This initial study tested a fixed SNP dose in a 55% estimated blood loss (EBV) haemorrhage model before full evaluation in more complex uncontrolled haemorrhage models. This model has been used in our laboratory to screen resuscitation fluids and reliably induced pathophysiologic manifestations of HS in swine that were improved after HBOC-201 resuscitation.[6,17,24] The aim of this study was to assess SNP's effectiveness for neutralizing HBOC-201-induced systemic and pulmonary vasoconstriction in a simple HS swine model.

2. Materials and methods

The experiments were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals" Institute of Laboratory Animals Resources, National Research Council, National Academy Press, 1996. The study was approved by the WRAIR/NMRC Institutional Animal Care and Use Committee and all procedures were performed in an animal facility approved by the Association for Assessment and Accreditation for Laboratory Animal Care International.

2.1. Animal model

The 55% EBV controlled haemorrhage model and animal monitoring have been described by our group and is summarized in Supplementary material.[6,24] Animals were randomly allocated to three treatment groups as described in Table 1.

2.2. Treatment groups and fluid infusion

HBOC-201 (HB) or Hextend (HEX) (see Supplementary material for composition)[24] was infused through the femoral vein at 10 ml/kg while SNP or saline was administered concomitantly but separately via right jugular vein. SNP-treated animals received three fixed dose infusions of 0.8 μg/kg/min. This initial dose regimen was selected based on standard clinical dosing for treatment of malignant hypertension (0.3–2 μg/kg/min);[23] a limited pilot dose-escalation study supported the dose selection. At T60, all swine entered the "hospital phase" and were eligible to receive autologous blood transfusions for Hb < 7 g/dL or saline for hypotension (MAP < 60 mmHg) at T60, T75, T90 and T105. At 2 h, the animals were euthanized.

2.3. In vitro measurements

All functional laboratory assays were performed at T0, 15, 60 and 120 min including Complete Blood Count (CBC) (Pentra 60C+; Horiba ABX Diagnostics, Irvine, CA), coagulation parameters including prothrombin time (PT) and partial thromboplastin (PTT) (Stat-Compact, Diagnostica Stago, Parsippany, NJ), thromboelastography (gamma, Rotem Inc., Durham, NC) and ADP-induced aggregation (Aggregometer, Chronolog, PA). Non-invasive Trans Cutaneous Oxygenation (TCOM) was continuously measured with a TCM4 Tina monitor (Radiometer, Copenhagen, Denmark).[4] Blood gas parameters, methaemoglobin and indirect oxygenation parameters were measured by blood gas analysis (ABL-750, Radiometer, Copenhagen, Denmark) at T0, T15, T30, T45, T60, T90, T105 and T120. Blood gas values were used to calculate derived parameters such as oxygen consumption (VO_2), delivery (DO_2) and extraction (see Supplementary material).

2.4. Statistics

Results are presented as mean ± standard deviation (SD). Survival rates and time-to-event data were analyzed with Fisher exact and Kaplan Meier tests. For data collected over time, results were analyzed using repeated measure analysis of variance (ANOVA) to examine group differences of continuous variables. The Kenward–Roger method was used to estimate Degree-of-Freedom of the model; covariance structure was assumed to be compound symmetry. Tukey's method was used for adjusting *p*-value for multiple comparisons. Normal probability plot of the residuals was used to test model assumptions. Data were assumed to be normally distributed. Possible transformation was used to stabilize variance. Baseline values were excluded from group/time analyses. $p \leq 0.05$ was considered significant.

3. Results

3.1. Baseline

There were no significant group differences in baseline pig weights (30.9 ± 2.2 kg) or any measured parameters (MAP, MPAP, HR, CO, lactate, glucose, BE, CBC, and thromboelastography; $p > 0.05$, ANOVA). Haemorrhage caused hypotension and negative base excess (BE) ($p < 0.001$) in all groups prior to fluid resuscitation (T15) ($p > 0.05$, ANOVA). Of the 20 animals, one survivor in the HB-SNP group was excluded because of technical problems with monitoring/recording of vital parameters.

3.2. Survival

Survival was similar for all groups: 4/4 with HB, 7/7 with HB-SNP (8/8 including the excluded animal), 3/4 with HEX and 4/4 with HEX-SNP ($p = 0.266$; Fisher's exact). With HEX, the animal death occurred at 51 min post-haemorrhage.