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The effect HBOC-201 and sodium nitrite resuscitation after uncontrolled haemorrhagic shock in swine☆

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ABSTRACT

Background: Development of Haemoglobin-based oxygen carriers (HBOCs) as blood substitutes has reached an impasse due to clinically adverse outcomes attributed to vasoconstriction secondary to nitric oxide (NO) scavenging. Studies suggest haemoglobin exhibits nitrite reductase activity that generates NO and N_2O_3; harnessing this property may offset NO scavenging. Therefore, the effects of concomitantly infusing sodium nitrite ($NaNO_2$) with HBOC-201 were investigated.

Methods: Swine underwent uncontrolled liver haemorrhage before receiving up to three 10 min 10 ml/kg infusions of HBOC-201 (HBOC) with or without concurrent $NaNO_2$ (5.4 μmol/kg [LD $NaNO_2$] or 10.8 μmol/kg [HD $NaNO_2$]) or 6% Hetastarch (HEX) with or without HD $NaNO_2$ during "prehospital" resuscitation (15, 30 and 45 min after injury). Definitive surgical care occurred at 75 min; anaesthetic recovery at 120 min. Animals were euthanised at 72 h.

Results: $NaNO_2$ temporarily reduced systemic and pulmonary blood pressure increases from HBOC in a dose-dependent fashion. There was no significant effect between groups in indices of tissue oxygenation or survival. Adverse clinical signs requiring humane euthanasia occurred with highest frequency after HBOC + HD $NaNO_2$ (3 of 4 pigs) and HBOC + LD $NaNO_2$ (2 of 4 pigs). Gross evidence of pulmonary congestion was observed in 5 of 8 swine receiving a HBOC and $NaNO_2$ combination compared to 1 of 16 swine receiving HBOC alone, HEX alone, or HEX + $NaNO_2$. Gross lesions correlated with histological evidence of pulmonary oedema and congestion, and in 2 of 4 HBOC + HD $NaNO_2$ pigs, pulmonary fibrin thrombi also were found. No other pig had similar evidence of thrombi. Asymmetric pre-resuscitation cardiac index was a potential confounder.

Conclusions: A significant interaction between $NaNO_2$ and HBOC-201 ameliorated HBOC-201 vasoconstrictive effects, consistent with HBOC possessing a nitrite reductase activity that generates vasodilator NO equivalents. Results were relatively equivalent in survival and markers of tissue oxygenation. The highest dose of $NaNO_2$ was the most effective in reducing HBOC-associated pulmonary and systemic vasoactivity but also with the highest incidence of adverse events. In this model, the transient nature of $NaNO_2$ in offsetting HBOC-201 vasoconstriction makes it less clinically promising than anticipated and the combination of $NaNO_2$ and HBOC appear to increase the risk of pulmonary complications in a dose-dependent fashion independently of haemodilutional effects on haemostatic components.

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Introduction

Haemoglobin-based oxygen carriers (HBOCs) have been under development as oxygen-carrying resuscitative fluids for several decades.[25] HBOC-201 (haemoglobin glutamer-250 [bovine]; Hemopure®, Biopure [now OPK Biotech], Cambridge, MA) has theoretical potential to benefit trauma casualties because animal models show that it delivers oxygen to tissues, expands intravascular volume, stabilises haemodynamics with low-volume resuscitation, and increases tissue oxygenation in haemorrhagic shock.[14,19,21–24] In addition, it is particularly suited for pre-hospital resuscitation because it is universally compatible, does not require refrigeration, and is as easy to administer as standard fluids[20]

However, HBOC-201 and other HBOCs have been associated with adverse side effects. Vasoconstriction has been suggested as the underlying cause of secondary adverse events (i.e., myocardial infarct, stroke) in human trials and concern over this vasoactivity has hampered the development of HBOC-201 for trauma use.[18] The vasoactivity is thought to be primarily generated by the tetrameric form of HBOC and is due to the nitric oxide (NO) scavenging effects of the haemoglobin,[2,12,15] as determined by the highly conserved dioxygenation reaction of oxyhaemoglobin with NO, forming methaemoglobin (metHb) and nitrate (Eq. (1)).

$$HbO_2 + NO \rightarrow MetHb + NO_3^- \quad (1)$$

Normally, haemoglobin is compartmentalised within the RBC. This compartmentalisation generates major diffusional barriers for NO: an unstirred layer around the RBC, an intrinsic membrane barrier to NO, and a cell-free zone along laminar flowing blood. These three diffusional barriers reduce the reaction rate of NO with intracellular haemoglobin by 1000-fold. During infusion of cell-free HBOCs, these barriers can be disrupted and even small amounts of plasma haemoglobin can impair NO-dependent vasodilation and cause vasoconstriction. This effect is only partly moderated by increasing the molecular size of the HBOC.[21]

It has recently been appreciated that haemoglobin possesses a nitrite reductase activity (Eq. (2)) and a nitrite anhydrase activity (Eq. (3)) that can convert nitrite to vasodilatory NO and N_2O_3, respectively.

$$NO_2^- + HbFe^{2+} + H^+ \rightarrow NO + HbFe^{3+} + OH^- \quad (2)$$

$$2NO_2^- + HbFe^{II} + H^+ \rightarrow HbFe^{II} + N_2O_3 + OH^- \quad (3)$$

Consistent with such an activity, multiple research groups have found that deoxygenated haemoglobin and deoxygenated RBCs incubated with nitrite can generate NO gas, increase aortic ring cGMP, inhibit cytochrome *C* oxidase of the mitochondrial electron transport chain, and vasodilate the circulation.[3,4,6–8,10,11] Further supporting this mechanism, Yu and colleagues infused low concentrations of nitrite with high concentrations of murine haemoglobin, and reported that nitrite interacted with the haemoglobin to limit vasoconstriction.[27] Similar nitrite reductase activity of nitrite with HBOC-201 has been reported in a mouse model of haemorrhage.[24] The reaction of nitrite with deoxyHb appears to generate vasodilatory NO and N_2O_3 and thus has potential to replete NO scavenged by HBOC-201.

We therefore hypothesised that similar findings might occur in a large animal (swine) model where multiple infusions of HBOC-201 would be required for prehospital resuscitation of uncontrolled bleeding. Separate but concurrent administration of $NaNO_2$ might reduce HBOC-201-associated vasoactivity by providing a source of NO that is mainly released during hypoxia without having adverse effects on tissue oxygenation and survival.

Table 1
Treatment groups.

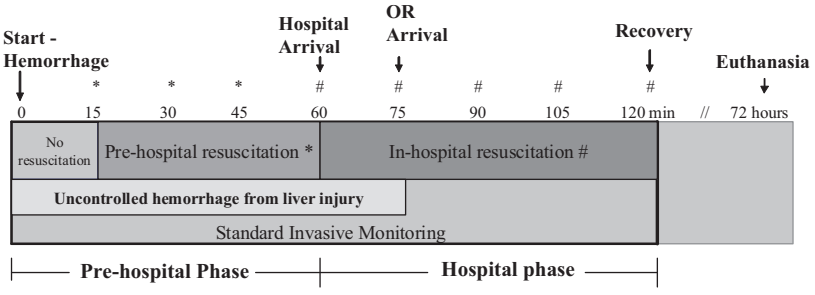
Group	Resuscitation fluid	*N*
HBOC	10 ml/kg HBOC-201[a]	6
HBOC + LD $NaNO_2$	10 ml/kg HBOC-201[a] + 5.4 μmol/kg $NaNO_2$[b]	4
HBOC + HD $NaNO_2$	10 ml/kg HBOC-201[a] + 10.8 μmol/kg $NaNO_2$[b]	4
HEX	10 ml/kg HEX[c]	6
HEX + HD $NaNO_2$	10 ml/kg HEX[c] + 10.8 μmol/kg $NaNO_2$[b]	4

[a] HBOC-201: Haemoglobin glutamer-250 (bovine); Hemopure® solution; 13 g/dL Hb (Biopure Corporation, Cambridge, MA).
[b] $NaNO_2$: sodium nitrite (Fisher Scientific, Pittsburg, PA) reconstituted with 5% mannitol by the pharmacy at NIH prior to injection.
[c] HEX Injection, USP: HEX® Solution; 6% hetastarch in lactated electrolyte (Hospira, Inc., Lake Forest, IL).

Materials and methods

The experiments reported herein were conducted in compliance with the Animal Welfare Act and in accordance with 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 Institutional Animal Care and Use Committee (IACUC) and all procedures were performed in an animal facility approved by the Association for Assessment and Accreditation for Laboratory Animal Care International (AAALAC).

With the exception of a different protocol timeline (Fig. 1) and treatments for resuscitation (Table 1), the experimental design was very similar to that previously described.[9] Yorkshire swine ($n = 24$; 27 ± 0.6 kg, mean ± sem; Animal BioTech Industries, Danboro, PA) were assigned randomly to resuscitation groups prior to each experiment (Table 1). Blinding to treatment occurred up until infusion of test product (i.e., after liver injury had been completed),



* Pre-hospital resuscitation: HBOC-201, HEX, HBOC-201 + $NaNO_2$ or HEX + $NaNO_2$

\# In-hospital resuscitation: Blood or 0.9% saline

Fig. 1. Experimental design.