ORIGINAL ARTICLES

Effects of recombinant-hemoglobin solutions rHb2.0 and rHb1.1 on blood pressure, intestinal blood flow, and gut oxygenation in a rat model of hemorrhagic shock

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The vasoconstriction induced by hemoglobin-based oxygen carriers (HBOCs), mainly a result of nitric oxide (NO) scavenging, until now has limited the application of HBOCs as resuscitation fluids. In this study, we tested the hypothesis that the new modified recombinant-hemoglobin solution rHb2.0, with a 20 to 30 times lesser NO-scavenging rate, would minimize vasoconstriction without adverse effects on microvascular oxygenation. Responses were compared with those to rHb1.1, a recombinant-hemoglobin solution with a wild-type NO-scavenging rate, as well as an oncotically matched albumin solution. In a fixed-pressure (40 mm Hg) rat model of hemorrhagic shock and resuscitation, rHb2.0 and albumin both restored mean arterial pressure (MAP) to baseline values, whereas rHb1.1 increased MAP to 27% above the baseline value. Mesenteric vascular resistance after resuscitation with rHb2.0 was 57% less than that with rHb1.1. rHb2.0 was found to have 55% greater intestinal oxygen delivery (Do_{2int}) and resulted in a 27% lower oxygen-extraction rate than did rHb1.1 after resuscitation. Intestinal microvascular Po_2, determined on the basis of oxygen-dependent quenching of palladium-porphyrin phosphorescence, revealed no difference between rHb2.0 and rHb1.1. The findings of this study confirm that the well-known pressure effect of HBOCs is caused by their effect on the NO-scavenging rate; recombinant modification of this rate did not increase MAP during resuscitation compared with baseline values. Although systemic vasoconstriction was absent, intestinal vasoconstriction almost negligible, and Do_{2int} greater after resuscitation with rHb2.0, the effect of rHb2.0 on pH, base-excess and microvascular Po_2 levels after resuscitation were comparable to those achieved with the use of the albumin solution. (J Lab Clin Med 2005;145:21–32)

Abbreviations: C_aO_2 = arterial oxygen content; $C_{mv}O_2$ = mesenteric venous oxygen content; COP = colloid osmotic pressure; DCLHb = diaspirin cross-linked hemoglobin; Do_{2int} = intestinal oxygen delivery; HBOC = hemoglobin-based oxygen carrier; HSA = human serum albumin; MAP = mean arterial pressure; MVR = mesenteric vascular resistance; NO = nitric oxide; P_{50} = oxygen half-saturation pressure; Po_2 = partial oxygen pressure; Q_{sma} = superior mesenteric-artery blood flow; rHb1.1 = recombinant hemoglobin 1.1; rHb2.0 = recombinant hemoglobin 2.0; Vo_2int = intestinal oxygen consumption

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Dr Raat is supported by grant LSBR006 from the Landsteiner Stichting voor Bloedtransfusie Research.

Submitted for publication December 29, 2003; revision submitted April 28, 2004; accepted for publication May 10, 2004.

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0022-2143/$ – see front matter

doi:10.1016/j.lab.2004.05.017

Although the development of HBOCs has been an active field of interest over the years,[1] these compounds are not yet suited to replace blood because of their limited retention time in the circulation, their unwanted vasoconstrictive effects, and uncertainty about their oxygen transport effectiveness. However, without a need for cross-matching, less strict storage conditions, and an increased shelf life, hemoglobin solutions represent promising resuscitation fluids, especially in emergency situations outside the hospital in which resuscitation of trauma victims is required.

In the last decade, several HBOC compounds from different sources and with different properties have been developed.[1,2] Most of these compounds have been produced from outdated human or bovine blood and so carry the potential hazard of contamination with viruses or prions. Cross-linking of the individual hemoglobin chains solved the initial problems of nephrotoxicity of HBOC resulting from the presence of free hemoglobin chains in the blood, and extended retention time in the vasculature.[1] However, until now, successful application of hemoglobin solutions has been limited mainly by their vasoconstrictive properties.[3]

Several mechanisms may explain the vasoconstrictive effect of HBOCs. In addition to scavenging of the vasodilator NO and extravasation of hemoglobin,[3–5] increased endothelin synthesis,[6,7] sensitization of adrenergic receptors,[3,8] and precapillary autoregulation[1] have been suggested. Recently Doherty et al[9] tested in vivo a set of recombinant hemoglobins that vary in P_{50} and rates of reaction with NO but have similar rates of autoxidation. The effects of these recombinant hemoglobins on MAP indicated that scavenging of NO by hemoglobin is the primary cause of vasoconstriction. We have previously performed experiments in endothelial nitric oxide synthase (eNOS)-knockout mice showing that diaspirin cross-linked hemoglobin (DCLHb), a hemoglobin compound with properties similar to those of rHb1.1, is indeed causing vasoconstriction through inhibition of NO.[10]

It is unclear whether vasoconstriction by hemoglobin solutions is beneficial or deleterious to microvascular oxygenation.[11] On one hand, a rise in blood pressure could increase microvascular blood flow, but on the other hand, arteriolar vasoconstriction can decrease it. Additionally, vasoconstriction could alter the distribution of blood flow between and within organs.[12] That is why the ability to control the amount of vasoconstriction through recombinant manipulation may provide an important benefit. It allows the use of a compound with vasoconstrictive properties that match the conditions in which it will be used.

Hemoglobin solutions produced through the use of recombinant-DNA technology have at least 2 main advantages over traditional products isolated from outdated human or bovine blood. First, recombinant-hemoglobin solutions carry a reduced risk of viral or prion contamination because they are derived from a nonhuman source. Second, site-directed mutagenesis allows further modification of the human hemoglobin molecule to provide it with additional properties, making it optimally suited for use as an HBOC.

The HBOC rHb2.0, the first example of such an approach, has a 20 to 30 times lesser NO-scavenging rate than the previously developed recombinant-hemoglobin rHb1.1[13] with wild-type hemoglobin properties or other HBOCs that are developed from outdated human blood, such as DCLHb.[14,15] The modification of the rHb2.0 molecule also reduced the cooperativity of oxygen binding, but it is difficult to predict the consequences of this reduction on oxygen transfer to tissues. However, although the O_2-binding kinetics of rHb2.0 and the O_2 association/dissociation rates are different, its total oxygen-binding capacity has remained unchanged.

In this study, we tested the hypothesis that resuscitation with rHb2.0 would result in less vasoconstriction than would resuscitation with rHb1.1 in a model of fixed-pressure hemorrhagic shock in the rat. Furthermore, we hypothesized that the oxygen delivery and available tissue oxygen after administration of rHb2.0 are comparable to those of rHb1.1 and tested this hypothesis by measuring the gut microvascular Po_2 as determined with the use of the palladium-porphyrin phosphorescence technique.[16,17] Microvascular gut Po_2 is an important local parameter of the balance between oxygen delivery and utilization[18] that is not necessarily reflected by systemic parameters.[19,20] Redistribution of blood flow or shunting may deprive certain tissues of oxygen in favor of other tissues and therefore hide hypoxic pockets in tissue beds.[20] We chose the gut as the measuring site for its high sensitivity to decreases in oxygen delivery and its important role in maintaining a barrier function.[21]

METHODS

Animals. This study was approved by the animal-research committee of the Academical Medical Center at the University of Amsterdam. We included 30 experiments with male Wistar rats (Charles River, Maastricht, The Netherlands) with a mean ± SD body weight of 350 ± 26 g in this study.

Resuscitation fluids. Resuscitation fluids were provided by Baxter Hemoglobin Therapeutics (Boulder, Colo). rHb1.1, a recombinant human hemoglobin, as described by Looker et al,[13] was modified from native human hemoglobin in 2 ways to overcome the problem of a low P_{50} resulting from lack of 2,3-diphosphoglycerate (2,3-DPG) and to increase stability between the different globin chains as a means of reducing nephrotoxicity and increasing half-life in the circulation. In brief, rHb1.1 was produced by means of the expres-