Hemoglobin-based Oxygen Carriers for Reversing Hypotension and Shock

"NO" Way, "NO" How?

EFFORTS to develop a blood substitute, especially for therapy of hemorrhagic shock, have been ongoing. Most clinical trials have focused on hemoglobin-based oxygen carriers (HBOCs) rather than perfluorochemical derivatives. Although HBOCs are purified, synthesized, or modified hemoglobins from human, bovine, or recombinant sources, they most often originate from human senescent blood.[1,2] Traditional thinking supports the idea that HBOCs may facilitate volume expansion and stabilize shock patients until erythrocytes become available. Unfortunately, it is not that simple.

Hemoglobin is an oxygen-carrying transport molecule physiologically contained in erythrocytes and evolutionarily designed as such. However, outside erythrocytes, the molecules function quite differently, with an altered affinity for oxygen in a different milieu of the circulation.[3,4] Unmodified hemoglobin solutions have different oxygen affinities when outside red cells; the chains have much higher oxygen affinity than do native or cross-linked hemoglobins.[3,4] As a result, purified hemoglobins have been modified by polymerization, cross-linking, or pegylation to improve oxygen delivery, increase their half-life, and alter their safety profile. The oncotic pressure exerted by these solutions varies widely. Each HBOC has been uniquely modified to improve its oxygen-carrying and -delivery capabilities and contains different amounts of hemoglobin as dosed. However, despite these physiochemical modifications, most HBOCs significantly scavenge nitric oxide (NO). This process is thought to account for many of the adverse effects and outcomes associated with these agents.[1] HBOCs were never intended to have this property. Their use for resuscitation or intraoperative treatment was based on their volume replacement and oxygen-carrying capacity, which is the basis of the current study in ANESTHESIOLOGY.[5]

In the endothelium, throughout the vascular arterial bed, NO is important in maintaining vascular tone. Under physiologic conditions and within the erythrocyte membrane, hemoglobin is mostly insulated from the vascular endothelium. However, once released, free hemoglobin and HBOCs are potent NO scavengers. Because of this mechanism, most HBOCs have been shown to cause systemic and pulmonary artery vasoconstriction, though an oxygenated polyethylene glycol–modified hemoglobin molecule (MP4OX [formerly known as Hemospan®]; Sangart, Inc., San Diego, CA) is thought to have enhanced NO synthesis (NOS) as a result of nitrite reductase properties because of β-chain cysteine pegylation in the 93 position.[1,6–8]

Thus, one of the major drawbacks in studying HBOCs for the treatment of hemorrhagic shock is that they avidly bind NO, causing pulmonary and systemic vasoconstriction.[8–10] The ferric group of iron in hemoglobin is the primary mechanism by which NO is scavenged. This is also why, in part, inhaled NO as used in clinical concentrations has pulmonary artery vasodilating specificity with minimal effects on the systemic vasculature: scavenging occurs *via* erythrocyte hemoglobin.[11] HBOCs increase pulmonary and systemic blood pressures, that may, in turn, impair autoregulation of vascular flow in organ systems, as demonstrated in animal models. This process may account for adverse events seen clinically with HBOCs.[2] Furthermore, endothelial dysfunction may enhance vasoconstriction, as in the case of patients with atherosclerotic vascular disease.[12]

In ANESTHESIOLOGY this month, Olofsson *et al.*[5] report a clinical study of MP4OX as a low-volume, oxygen-therapeutic agent that is intended to deliver oxygen to ischemic tissue.[5] The authors used this oncotically active agent to prevent hypotensive episodes and also to determine its safety in a surgical setting. Olofsson *et al.*[5] observed 367 patients from 18 sites in six countries during elective primary hip arthroplasty with spinal anesthesia. Patients were randomized to receive MP4OX or hydroxyethyl starch (HES130/0.4) at induction of spinal anesthesia. A second dose was made available if a predefined drop in systolic blood pressure occurred.

The proportion of patients with one or more hypotensive episode(s), the primary endpoint of the study, was significantly lower in the MP4OX group (66.1%) *versus* HES 130/0.4 controls (90.2%). However, when compared with controls, more MP4OX-treated patients experienced adverse events (72.7 *vs.* 61.4%), including nausea (20.8 *vs.* 12.0%) and hypertension (4.4 *vs.* 0.5%). None of the hypertensive adverse events observed occurred during the operative and 6-h postoperative recovery period.

Another consistent finding in HBOC studies has been transient elevations in certain laboratory assays, including

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liver function tests and troponin.[13] Such was the case also in this study by Olofsson *et al.*[5] Despite this finding, however, there were no differences between study groups with regard to morbidity or serious adverse events. The authors[5] conclude that the adverse event profile does not support the use of MP4OX in routine, low-risk surgical patients.

Reports from an earlier phase 2 clinical trial of MP4OX enrolled patients undergoing orthopedic surgical procedures.[14] In that investigation,[14] patients were randomized to receive MP4OX or Ringer's acetate (30 patients per group). However, the earlier investigation by Olofsson *et al.*[14] was a safety study. Olofsson *et al.*[5] report that MP4OX mildly elevates hepatic enzymes and lipase and was associated with less hypotension and more bradycardia events.

There are ongoing controversies regarding the safety of HBOCs in clinical trials. One analysis in particular was criticized for including different therapeutic areas and HBOCs in the analysis given the inherent variability of the patients studied and differences between HBOCs.[9] Previous clinical trials by Baxter International, Inc. (Deerfield, IL) and Northfield Laboratories (Evanston, IL) have tested the use of HBOCs in trauma.[2,14] However, because hemorrhage and shock are the leading cause of civilian and battlefield mortality, there remain, in my view, potential applications of HBOCs in treating such injuries, as noted by the S. Armed Forces Blood Program.[15] In 2009, the results of a National Institutes of Health and U.S. Food and Drug Administration workshop were published regarding HBOC safety, addressing current status and future direction of these agents.[2,14]

Multiple HBOCs are available and have been evaluated in numerous clinical studies, as discussed. My experience with HBOCs was primarily with Hemopure® (Biopure Corporation, Cambridge, MA), a cross-linked hemoglobin polymer from bovine blood.[16,17] I also wrote an editorial for ANESTHESIOLOGY about diaspirin cross-linked hemoglobin, DCLHb (HemAssist; Baxter Healthcare Corporation, Deerfield, IL), as studied in clinical trials for coronary artery surgery.[7,18] However, adverse effects detected in other studies ended clinical investigations for this agent.[7] NO scavenging has consistently been held responsible for many of the adverse effects and events observed with HBOCs, including myocardial injury and infarction, gastrointestinal symptoms (*e.g.*, nausea), and ischemic injury in shock.[2,14] Heme itself may be toxic. In addition, MP4OX used in low volumes and having a reduced hemoglobin concentration may account for the low rate of reported adverse effects. It is noteworthy that the current Olofsson *et al.*[5] study reported adverse effects similar to those detailed in the workshop report by the National Institutes of Health and U.S. Food and Drug Administration.[2]

Clinical studies have also investigated agents that inhibit NOS, specifically the enzyme involved in NO production. These investigations report conflicting results, documenting beneficial and detrimental effects.[19] Because the precursor for NO is arginine, most NOS inhibitors are derivatives of arginine. A study in humans evaluated *L*-N-monomethyl-arginine, an NOS inhibitor, in 79 patients after acute myocardial infarction and cardiogenic shock, despite an open infarct-related artery.[20] Using incremental dosing, mean change in mean arterial pressure ranged from 4.8 to 11.6 mmHg at 15 min, but not at 2 h. However, cardiogenic shock is probably not a great model when evaluating NO inhibition. Using an *in vitro* human vasodilation model, we reported the effects of the NOS inhibitors methylene blue and N(G)-monomethyl-L-arginine, as well as prostaglandin inhibitors (indometacin).[21] We noted that inhibition of either NOS or prostaglandin pathways did not completely reverse vasodilation; both effects were required for mediator-induced vasodilation.[21] Thus, in severe vasodilatory shock, including anaphylactic shock, therapeutic approaches must be multimodal and target more than NO.

In summary, it is unlikely that we will have a NO-scavenging agent to treat hypotension or shock until the molecule is sufficiently safe for any benefits provided. However, Olofsson *et al.*[5] (and Sangart, Inc.) are to be congratulated on this new data—and for safety findings and information that may help future researchers who wish to investigate novel oxygen-therapeutic molecules for other, more appropriate clinical indications. The paradigm of using a molecule that scavenges NO as an adverse effect for a primary therapeutic endpoint is important. It may provide a useful path for future studies in this therapeutic area.

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