US Navy Experience With Research on, and Development of, Hemoglobin-Based Oxygen Carriers

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Following 9/11 and the advent of the wars in Iraq and Afghanistan, the Naval Medical Research Center (NMRC, Silver Spring, MD) embarked on an effort to develop an improved resuscitation fluid for use in the prehospital phase of care when blood is not available for transfusion. The long-range goal of this program was to develop a multifunctional blood substitute that had oxygen-carrying, hemostatic, and anti-inflammatory capabilities. A shorter-term goal was to deliver an oxygen-carrying resuscitation fluid to both military and civilian trauma medical communities via clinical trial(s) leading to approval from the US Food and Drug Administration (FDA).

Trauma is the leading cause of mortality among young adults and causes significant disability among survivors.[1–4] Exsanguinating hemorrhage causing hemorrhagic shock (HS) has been reported to account for 28% of combat deaths overall[5] and for the preponderance of civilian and potentially salvageable combat deaths (60% vs. 33% and 6% for tension pneumothorax and airway obstruction, respectively).[6] In Operation Iraqi Freedom (Iraq war) and Operation Enduring Freedom (Afghanistan war), 83% to 87% of "potentially survivable" fatalities were caused by hemorrhage occurring before hospital arrival in 55% of cases.[7] In the event of traumatic cardiopulmonary arrest, mortality remains unacceptably high (84–98%).[8] Traumatic anemia as a result of blood loss has been correlated directly with mortality.[9]

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After efforts to secure an airway, ventilate, and control compressible hemorrhage in casualties with HS, intravenous fluids are required to restore hemodynamics and tissue perfusion compatible with survival. However, standard intravenous fluids do not carry or transport oxygen; rather, they dilute blood oxygen content and thus often fail to treat HS adequately. For casualties with severe HS (e.g., Advanced Trauma Life Support Class III and IV), blood transfusions, which maintain or restore blood oxygen content, can be life-saving pending definitive surgical stabilization. However, deployment of blood transfusion capability is logistically costly and complex, requires significant additional training, and thus is rarely available in the civilian prehospital arena or forward military medical units.

Based on the recommendations of a panel of trauma experts, the NMRC down-selected hemoglobin-based oxygen carrier (HBOC-201; Hemopure, Biopure Corporation) for further development and use in a clinical trial. This selection was based on a number of factors, including universal compatibility, long-term stability without refrigeration, a readily available source and demonstrated manufacturing capability, and agreement to independent government sponsorship of a clinical trial.

Estimates of the efficacy of HBOC-201 for resuscitation from trauma were derived from preclinical studies in swine models of controlled and uncontrolled hemorrhage that indicated an overall 75% reduction in mortality from severe hemorrhage and HS. The magnitude of the survival benefit provided by HBOC-201 (vice asanguinous fluid resuscitation) is greatest in more severe hemorrhage/HS models and less in mild-to-moderate hemorrhage models. Estimates of risk were derived from a variety of previous nontrauma clinical trials, primarily a large trial of HBOC-201 for transfusion avoidance in orthopedic surgery. The NMRC believed that risk in a trauma population would likely be lower than in this study because subjects in this study were exposed to more HBOC-201 and for longer periods than the prehospital trauma resuscitation would entail and that the hospitalized orthopedic population was, on average, older and presumably had more comorbid disease than would be anticipated in a trauma population.

The NMRC initially proposed a clinical trial targeting a civilian trauma population with HS meeting the following criteria: systolic blood pressure (SBP) <90 mm Hg, a revised trauma score (RTS) >1 and ≤ 6.5, and an estimated mortality of 34%. Because the target population was anticipated to be unconscious, in need of immediate intervention, and possibly

without family members present, the trial was proposed under an Exception from Informed Consent (EIC, 21CFR50.24). The NMRC postulated that there was prospect of direct benefit to the subjects (conservatively estimating a 15% improvement in mortality vice the 75% improvement observed in swine models). The NMRC also postulated that risk was reasonable and likely lower than the 7.7% group difference in serious adverse events (SAEs; HBOC-201 versus standard of care) observed in the orthopedic trial because post hoc subgroup analysis of that trial indicated that group differences in AEs, cardiac SAEs, and peak SBP elevations were lower than the overall population values in stable trauma, hypotensive (SBP <90 mm Hg), or younger (<70 years) subgroups.[10]

The FDA placed the proposed trial on clinical hold (CH) primarily due to benefit:risk considerations in the setting of an EIC trial. Over multiple resubmissions to attempt to respond to the CH issues and the convening of an FDA Blood Products Advisory Committee meeting, the NMRC successively raised the estimated mortality of the target population (ultimately to 93%) by narrowing the RTS criteria and (at the Blood Products Advisory Committee's suggestion) altered the trial design from a large phase 3 trial (n = ~975) to a smaller phase 2 trial (n = ~300). However, the FDA continued the CH status. Following a suggestion made by the FDA, the NMRC crafted a separate contingency trial proposal to be considered for performance in theater, with the FDA's acknowledgment that data collection would necessarily be limited. However, this trial could be preconsented, obviating the need for EIC. The FDA also placed that contingency proposal on CH.

An FDA letter to the NMRC stated, "Most of the SAEs … are consistent with the hypothesis that they result from the vasoactive properties of HBOC-201." Based on that assessment, the NMRC embarked on a preclinical program to develop a "vasoactivity-attenuated" formulation of HBOC-201. The NMRC and a number of collaborators at institutions in the United States and Europe have investigated both intrinsic and extrinsic modifications to the HBOC-201 formulation for their ability to decrease the vasoactivity.[11,12] Intrinsic modifications include decreasing the amount of hemoglobin tetramer, increasing the molecular weight of HBOC-201, or altering the P_{50}. Based on the hypothesis that the vasoactivity of HBOCs is a result of their propensity to scavenge nitric oxide (NO), extrinsic modifications focused on the addition of potential NO donors. Models used included hemorrhaged or top-loaded rodent (mouse and rat), awake and instrumented top-loaded swine, intravital microscopy of rat muscle and mesentery, controlled hemorrhaged swine, and uncontrolled hemorrhaged swine.

Based on data collected in preliminary screening studies, the NMRC decided to concentrate on addition of the NO donor nitroglycerin (NTG). Feasibility studies in a swine uncontrolled hemorrhage model (from liver laceration) indicated that coinfusion of NTG with HBOC-201 successfully attenuated the elevation in systemic and pulmonary blood pressures during the first two of three 10-minute infusion periods; the attenuating effect was diminished during the third coinfusion. Additional and unanticipated benefits are that (1) survival, already improved by HBOC-201 alone (vice Hextend resuscitated controls), improves even further when NTG is coinfused with HBOC-201; and (2) tissue oxygenation (per transcutaneous oxygen monitoring) also improves with the coinfusion of NTG.

The concept of adding NTG, a vasodilator, to an HS resuscitation regimen is counterintuitive; but, results in our model are promising, both in terms of vasoactivity attenuation (the *raison d'être* for the study) and the improved tissue oxygenation and survival we observed. Current and future studies will investigate the use of HBOC-201 and NTG coinfusion in swine models of polytrauma (HS plus traumatic brain injury) and vascular injury (e.g., aortic or other arterial tear). Other studies will determine whether NTG can be mixed in the same container as HBOC-201 to obviate the clinically cumbersome approach of coinfusion in a prehospital setting.

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