Chemistry & Biology
Innovation

Blood Relatives: Artificial Oxygen Carriers between Promise and Concern

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DOI 10.1016/j.chembiol.2007.10.003

Massive bleeding during childbirth kills more than 100,000 women worldwide each year. Blood loss kills a vast number of accident victims before they can reach a trauma center. Loss of blood is also blamed for 50% of deaths on the battlefield. Prompt transfusion of blood could prevent most of these deaths. Unfortunately, since the life-giving fluid has to be kept refrigerated and needs to be typed and cross-matched before use, it is rarely available in ambulances or in combat situations. And with a growing population accompanied by a decline in blood collection, even the best-equipped hospitals are not immune to shortages. Any catastrophic event such as an earthquake or a terrorist attack could rapidly exhaust available supplies.

Blood shortages in developing countries are particularly acute, according to the World Health Organization. These nations account for more than 80% of the world's population, yet collect less than 35% of the global blood supply. Safety standards there are often lower as well. The United States and other developed countries have come a long way since the 1980s, when thousands of hemophiliacs died from receiving HIV-tainted blood products. But more than 70 developing countries still don't test all their donated blood for HIV, hepatitis, or syphilis. "Blood is in short supply, and the blood that is available is often unsafe," says Harvey Klein, who heads the department of transfusion medicine at the National Institutes of Health (NIH). "A substitute that doesn't need typing and doesn't transmit disease would be invaluable."

These considerations give new urgency to the search for a blood substitute—or more correctly, an artificial oxygen carrier. Scientists have known since the 19th century that a protein in red blood cells (RBCs), hemoglobin, is essential for oxygen transport. Early attempts in the 1930s to transfuse a solution of purified cell-free hemoglobin largely failed. It was only in the 1950s that scientists discovered the protein's molecular structure: a tetramer consisting of four polypeptide chains, each equipped with a heme unit that can bind to an oxygen molecule. With about 250 million of these oxygen-hungry molecules packed into each of the 25 trillion RBCs in a human body, hemoglobin is the key to an amazingly effective system that delivers the right amount of oxygen to tissues at all levels of activity.

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Outside the RBC, however, the hemoglobin molecule is less well behaved: it enters blood vessel walls, causing over-oxygenation and reduction of the nitric oxide level that maintains blood vessel walls in a relaxed state. The result is vasoconstriction—a narrowing of arteries and capillaries that causes blood pressure to shoot up. Free hemoglobin also generates reactive oxygen species such as hydrogen peroxide and superoxide ions that damage cells and tissues. Furthermore, hemoglobin molecules outside RBCs rapidly break down into dimers highly toxic to the kidney, as was observed in the first human safety trial of free hemoglobin in 1978.

To make a more stable compound, a common strategy is to crosslink reactive amino groups that lie on the hemoglobin molecule's surface. Crosslinks *within* the molecule can prevent it from breaking down into toxic dimers, but not from getting into blood vessel walls. Products based on this approach, such as Baxter's HemAssist, have fared poorly in human trials and have largely been abandoned. Crosslinks *across* molecules, on the other hand, result in large polyhemoglobin aggregates that don't infiltrate vascular walls as easily. Examples of these so-called second-generation oxygen carriers are Northfield Laboratories' PolyHeme and Hemosol's Hemolink, both based on human hemoglobin, and Biopure's Hemopure, based on bovine hemoglobin. All three products have shown mixed results in human trials; while they reduce the need for blood transfusion, they seem to increase the risk of cardiovascular and other problems. "The good news is that they deliver oxygen effectively," says Klein. "But they show disturbing evidence of toxicity."

In addition to posing safety concerns, the products are challenging to manufacture because they require extraction, purification, and modification of hemoglobin. Further, the raw material in most cases is either cow's blood, which might raise disease concerns, or expired human blood, which is dwindling in supply.

Fluorocarbon emulsions are a potential alternative to hemoglobin. Developed initially during the Manhattan Project as an inert buffer material for handling corrosive uranium isotopes, fluorocarbons turned out to be good solvents for oxygen. In a dramatic demonstration, scientists showed in 1966 that mice immersed in an oxygen-saturated fluorocarbon liquid could survive for up to ten minutes. As oxygen carriers, fluorocarbons offer several advantages: they are nontoxic, use easily available raw materials, and are simple to manufacture. "We just put a handful

of chemicals together and make it into an emulsion,'' says Thomas Drees, president and CEO of Sanguine, a fluorocarbon manufacturer. ''The process is not capital or labor intensive.''

Although less toxic than hemoglobin, fluorocarbons are not problem free. They can provoke the complement system, affect platelet function, and potentially cause strokes. And unlike hemoglobin-based products, which can deliver oxygen very effectively under physiologic conditions, fluorocarbon-based carriers require the patient to breathe oxygen-enriched air. ''The bulk of evidence suggests that a hemoglobin-based carrier is more likely to come to market,'' says Klein.

Both classes of product have shown mixed results in clinical trials. HemAssist showed promise in early studies, but in Phase III trials it seemed to increase mortality rates. Baxter suspended a Phase III trial of the product in 1998 because of adverse outcomes. Hemolink fared similarly: although one Phase III trial demonstrated its effectiveness in reducing blood transfusion needs, another Phase IIb trial of the product was suspended in 2003 because of increased incidence of heart problems. A Phase III trial completed in Europe in 2002 showed that Oxygent, a fluorocarbon-based carrier made by Alliance, could effectively reduce blood transfusion needs during surgery. However, another Phase III trial of this product in the U.S. ended early in 2001 because of an apparent increase in the risk of stroke. In a Phase III trial of Hemopure completed in 2002, nearly 60% of surgery patients getting the product did not need any blood transfusions at all. However, safety concerns caused FDA to reject Biopure's 2006 proposal for a Phase III trial of the product for hemorrhagic shock. (At present Hemopure is approved in South Africa; in the U.S, a low-grade version is available for veterinary use.) And in a recently completed Phase III trial by Northfield Laboratories, trauma victims infused with PolyHeme, which performed well in earlier, smaller human studies, fared worse than controls who got saline followed by blood. Such setbacks have landed many of the manufacturers in serious financial difficulty.

Many of these failures can be attributed to poor study design, says Jonathan Jahr, an anesthesiologist at the University of California, Los Angeles, who has led five clinical trials of oxygen carriers. Jahr points out that no pathway currently exists for independent investigators to obtain and study these products, and he urges the Food and Drug Administration (FDA) and the NIH to rectify this situation. ''Otherwise, companies rush into clinical trials without a product that was validated in the first place,'' he says.

Some companies express frustration with regulatory policies. ''Safety standards kept creeping up as you went towards a trial,'' says David Bell, vice president of drug development at Hemosol, referring to the suspended 2003 Hemolink clinical trial. ''You started to move away from the true benefit of the product and started focusing on the risks.'' For instance, regulators may require a product designed to resuscitate trauma victims in the field, where blood is typically unavailable, to be at least as safe as blood, since it could potentially be used ''off-label'' in a different setting. Such requirements are a challenge for the industry, agrees Jahr. ''A head-to-head comparison with blood is the worst-case scenario for an oxygen carrier,'' he says. Regulatory officials, however, argue that their task is complicated by the scientific complexity of oxygen carriers. Inherent product toxicities and lack of adequately predictive animal models make it ''difficult to judge the relative benefits and risks of proposed trials,'' says Jay Epstein, director of the Office of Blood Research and Review at the FDA.

While existing oxygen carriers await regulatory approval, some companies are making ''third-generation'' products that modify hemoglobin in new ways. Examples are Hemosol's HRC 101, which attaches a starch polymer to the hemoglobin molecule; Sangart's Hemospan, which affixes strands of polyethylene glycol to the molecule; and Oxyvita's Oxyvita, a polymerized hemoglobin made without crosslinks. While HRC 101 and Oxyvita are in the preclinical stage, Hemospan is in Phase III trials in Europe for use during surgery. According to the manufacturers, preliminary results are promising: the new products are less toxic and don't constrict blood vessels as much. ''We've tuned the oxygen release parameters so that the capillaries are open and flowing and oxygen is delivered to tissue,'' says Robert Winslow, Sangart's president and CEO.

What will be the commercial potential for an oxygen carrier, once it is licensed? That depends on the application, says Eugene Trogan, biotechnology analyst for the investment banking firm Morgan Joseph. For situations where blood is unavailable, such as field resuscitation, Trogan estimates the U.S. market to be about $150 million per year. But if the product replaces blood in the 1.8 million transfusions that are given each year, the market could exceed a billion dollars, he says. A longer shelf life and the lack of a typing/cross-matching requirement would, in theory, make such a product attractive. To access this larger market, however, the product would need to be at least as safe as blood. It would also need to last for a reasonably long time in the human circulation; current products, unfortunately, work only for a few days, whereas a RBC circulates for nearly four months. ''This field has a tremendous potential in the marketplace,'' says Trogan. ''The problem is that it has been mired with clinical difficulties.''

Leading oxygen carrier manufacturers have spent $150–$500 million in research and development, Trogan estimates. Given this outlay, the products may cost $500–$1000 per unit, 2–4 times more than blood. The price tag may deter their use where they are most needed: in the developing world, where blood is often either unavailable or unsafe. ''If you can't afford to test for HIV, you probably can't afford to buy a blood substitute,'' says Klein.

Despite past setbacks, manufacturers of oxygen therapeutics remain, well, sanguine. They are confident that their products will soon surmount the scientific and regulatory hurdles and succeed commercially. ''It's a very exciting time,'' says Winslow. ''I think we are getting close to the finish line here.''

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