Crafty air carrier

By Michael J. Haas, Senior Writer

A team led by Boston-area researchers has shown that infusion of oxygen-loaded microparticles could keep the blood of asphyxiated rabbits oxygenated, preventing organ damage and death.¹ Although the method could help treat hypoxemia resulting from acute airway obstruction or damage, future studies will have to show that the microparticles are safe and have the biochemical properties of endogenous hemoglobin.

Current strategies for treating hypoxemia include insertion of a breathing tube through the throat and mechanical ventilation. However, those procedures can take several minutes to implement, and even a few minutes of hypoxemia can cause lactic acid buildup (lactic acidosis), which triggers tachycardia, seizures, coma, brain and other organ damage, cardiac arrest or death.

Therapeutics to treat hypoxemia include oxygen-absorbing perfluorocarbons such as perflubron² as well as human hemoglobin-based carriers such as Sangart Inc.'s MP4OX. MP4OX is an oxygenated, pegylated, hemoglobin-based colloid that is in Phase IIb testing to treat lactic acidosis caused by hemorrhagic shock in severely injured trauma patients.

However, perfluorocarbon- and hemoglobin-based carriers must be reoxygenated by circulation through the lungs and thus are ineffective when normal respiration has been compromised by tracheal damage or airway blockage.

The Boston team was inspired to develop a new, rapid method to treat hypoxemia by the experience of team leader John Kheir when he treated a patient with pneumonia.

When the patient experienced an abrupt onset of hypoxemia as a result of pneumonia complications, Kheir inserted a breathing tube and placed her on a heart-lung bypass machine. But during the 15–20 minute period required for these procedures, her hypoxemia caused “profound brain injury, and she subsequently died,” Kheir told *SciBX*.

Frustrated by the lack of technologies for rapid treatment of hypoxemia, “I formed a team with the capabilities of creating a solution to this problem by manufacturing oxygen gas-filled microparticles,” he said.

Kheir is a staff physician in the Cardiac Intensive Care Unit at **Boston Children's Hospital** and an instructor of pediatrics at **Harvard Medical School**.

Kheir's team began by developing microparticles composed of a phospholipid-copolymer shell loaded with free oxygen and formulated in Plasma-Lyte—an i.v. fluid similar to plasma. The suspensions contained

up to 90% oxygen by volume, remained stable at 4 °C for up to 100 days and released their cargo when exposed to a low-oxygen environment such as that found in venous blood.

Although 90% oxygen-by-volume suspensions would carry the maximum possible payload, the team found any suspension containing more than 70% oxygen by volume did not adequately mix with whole human blood, thereby slowing the subsequent transfer of oxygen cargo to hemoglobin.

In *ex vivo* deoxygenated human red blood cells, 70% by-volume suspensions delivered their oxygen cargo and reoxygenated hemoglobin within 4 seconds compared with 71 seconds for 90% by-volume suspensions.

Thus, the team chose suspensions containing 70% oxygen by volume for the rest of the experiments, including studies of the microparticles in normal rabbits that had moderate hypoxemia induced by ventilation on low-oxygen air.

The group infused the hypoxemic rabbits with microparticles for two minutes, during which time the fraction of arterial hemoglobin that was oxygenated rose from 65%–70% to near-normal levels of more than 90%. The microparticles also reversed the hypoxemia-induced increase in blood pressure in the rabbits.

Finally, the team tested the microparticles in a rabbit model of airway obstruction-induced asphyxia, in which a surgically implanted tracheal cuff was clamped shut to cut off the supply of inhaled oxygen. In this model, continuous infusion of microparticles during 15 minutes of asphyxiation normalized blood levels of oxyhemoglobin and decreased acidosis, signs of liver injury and the incidence of cardiac arrest and increased survival compared with free vehicle infusion.

The treatment caused no observable complications or side effects, such as pulmonary embolisms, tachycardia or hypotension.

The results demonstrate that the technology could provide rapid, short-term oxygenation to patients with acute airway obstruction or lung failure, or during lengthy intubation procedures, to prevent organ damage and cardiac arrest, the team wrote in its report in *Science Translational Medicine*.¹

The group included researchers from **Columbia University**, **Harvard University**, the **Medical University of South Carolina**, the **University of Colorado at Boulder** and formulation CRO **Particle Sciences Inc.**

Airing differences

For the technology to clear clinical and regulatory hurdles, future studies should show that the microparticles can deliver oxygen and maintain blood pressure in the way endogenous hemoglobin does, said David Platt, chairman, director, CEO and CFO of **Boston Therapeutics Inc.**

Indeed, the blood-substitute space is littered with failed hemoglobin-based products such as PolyHeme from now-defunct Northfield Laboratories Inc., HemAssist from **Baxter International Inc.** and Optro rHb1.1 from Somatogen Inc., now part of Baxter.

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—John Kheir,
Boston Children's Hospital

“To date, the FDA has not approved any blood substitutes or artificial oxygen carriers because they do not have all of the critical attributes of hemoglobin” contained in red blood cells, Platt said. Among those attributes are the ability to deliver about 250 mL/min of oxygen—the normal amount carried by arterial blood in a healthy person—and the ability to scavenge the right amount of nitric oxide (NO) required to maintain proper blood pressure, he said.

“Kheir’s team will need to show their microparticles meet these two criteria or they don’t have any chance of clinical success,” Platt said.

Boston Therapeutics’ Ipoxyn, a stabilized glycoprotein containing oxygen-rechargeable iron, is in preclinical testing to treat hypoxia.

Kheir said the microparticles can deliver the amount of oxygen that a human requires. He noted that in the rabbit models of asphyxia, the microparticles delivered 4 mL/min of oxygen per kg of body weight—the equivalent of 200–250 mL/min in an average adult human.

Furthermore, “a major advantage of the microparticles is that they do not interact with the nitric oxide system” and thus do not affect blood pressure by scavenging too much or too little NO, he said.

Both Platt and Jennifer Johnson, cofounder and COO of **NuvOx Pharma LLC**, said the size of the microparticles presented a potential safety problem.

Johnson noted that the Kheir team’s microparticles averaged about 4 µm in diameter—about half the size of red blood cells—but a small fraction of the particles were 10 µm or larger. Particles of that size “could block capillaries and cause stroke, pulmonary embolism or heart attack,” she said. “It is not likely the FDA would approve particles with such a wide distribution in size” because of those safety issues.

Evan Unger, cofounder, president and CEO of NuvOx, suggested testing the safety of the microparticles in dogs, which “tend to be much more sensitive than other species to the size distribution of intravenous microparticle formulations.”

NuvOx’s lead compound, NVX-108, a submicroemulsion of the perfluorocarbon dodecafluoropentane (DDFP), is in preclinical testing to treat fetal hypoxia, nitrogen narcosis, stroke-related hypoxic brain damage and other indications involving hypoxia.

Platt also noted that platelets and artificial oxygen carriers such as perfluorocarbons can form aggregates that cause blood clots or deposit in the tissues with long-term toxicity issues. He wanted to know where in the body the microparticles go and how long they take to clear.

“Until the longevity of the breakdown products of the microparticles are known, it is difficult to suggest a use for them, other than a very short-lived, short-term intervention,” he said.

Johnson agreed. Assuming the team could reduce and control the microparticles’ size, “they would seem best suited for short-term oxygenation when the patient is unable to breathe.”

Added Unger, “They seem least suited for treatment longer than about 15 minutes in a nonbreathing patient, because this would require multiple doses and would not prevent the accumulation of carbon dioxide or help remove it.”

Kheir said his team is running safety studies to determine the *in vivo* clearance of the spent or degraded microparticles. The team also is testing the oxygen-loaded microparticles in animal models of cardiac arrest, he said.

He added that the technology could be used to deliver other gases to the bloodstream for therapeutic or diagnostic purposes but declined to describe specific applications.

The findings reported in the paper are patented. The team plans to develop the microparticles through Phase I and then seek a partner or licensing deal.

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