Oxygen Carrier State of the Science Meeting

Participating Agencies:

Department of Defense (DoD),
Combat Casualty Care Research Program (CCCRP) and
Joint Program Committee 6 (JPC-6)

Biomedical Advanced Research and
Development Authority (BARDA)

National Heart, Lung, and Blood Institute (NHLBI)

US Food and Drug Administration (FDA)
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<th>Definition</th>
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<tbody>
<tr>
<td>AC</td>
<td>Assembly centers</td>
</tr>
<tr>
<td>ADME</td>
<td>Absorption, distribution, metabolism, and excretion</td>
</tr>
<tr>
<td>AE</td>
<td>Adverse event</td>
</tr>
<tr>
<td>AGE</td>
<td>Arterial gas embolism</td>
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<tr>
<td>AHSP</td>
<td>Alpha-hemoglobin-stabilizing protein</td>
</tr>
<tr>
<td>AIM</td>
<td>Apoptosis inhibitor of macrophages</td>
</tr>
<tr>
<td>ANH</td>
<td>Acute normovolemic hemodilution</td>
</tr>
<tr>
<td>Asp</td>
<td>Aspartate</td>
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<tr>
<td>ARDS</td>
<td>Acute respiratory distress syndrome</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>BNAO</td>
<td>Blood is not an option</td>
</tr>
<tr>
<td>CABG</td>
<td>Coronary artery bypass grafting</td>
</tr>
<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>CAO</td>
<td>Coronary artery occlusions</td>
</tr>
<tr>
<td>CAT</td>
<td>Catalase</td>
</tr>
<tr>
<td>CDH</td>
<td>Congenital diaphragmatic hernia</td>
</tr>
<tr>
<td>cDO₂</td>
<td>Critical oxygen delivery</td>
</tr>
<tr>
<td>CMRO₂</td>
<td>Cerebral metabolic rate of oxygen</td>
</tr>
<tr>
<td>CMV</td>
<td>Mechanical gas ventilation</td>
</tr>
<tr>
<td>CO</td>
<td>Carbon monoxide</td>
</tr>
<tr>
<td>COP</td>
<td>Colloid osmotic pressure</td>
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<tr>
<td>CPB</td>
<td>Cardiopulmonary bypass</td>
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<tr>
<td>Cys</td>
<td>Cysteine</td>
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<tr>
<td>DAMP</td>
<td>Damage-associated molecular pattern</td>
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<tr>
<td>DCLHb</td>
<td>Diaspirin cross-linked hemoglobin</td>
</tr>
<tr>
<td>DCS</td>
<td>Decompression sickness</td>
</tr>
<tr>
<td>DDFP</td>
<td>Dodecafluoropentane</td>
</tr>
<tr>
<td>DDFPe</td>
<td>Dodecafluoropentane emulsion</td>
</tr>
<tr>
<td>DO₂</td>
<td>Oxygen delivery</td>
</tr>
<tr>
<td>DOW</td>
<td>Died of wounds</td>
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<tr>
<td>ECLS</td>
<td>Extracorporeal life support</td>
</tr>
<tr>
<td>eIND</td>
<td>Emergency investigational new drug</td>
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<tr>
<td>GBM</td>
<td>Glioblastoma multiforme</td>
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<tr>
<td>GI</td>
<td>Gastrointestinal</td>
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<tr>
<td>GMP</td>
<td>Good manufacturing practices</td>
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<tr>
<td>GSH</td>
<td>Glutathione</td>
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<tr>
<td>GSSG</td>
<td>Oxidized glutathione</td>
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Hb  Hemoglobin  
HBOC  Hemoglobin-based oxygen carrier  
HEMS  Helicopter emergency medical services  
HES  Hydroxyethyl starch  
HIF  Hypoxia-inducible factor  
HIV  Human immunodeficiency virus  
HP  Heptaglobin  
HPX  Haemopexin  
HSA  Human serum albumin  
IAD  Intraoperative blood donation  
ICU  Intensive care unit  
IND  Investigational new drug  
IP  Intellectual property  
IV  Intravenous  
KIA  Killed in action  
LDH  Lactate dehydrogenase  
LVEF  Left ventricular ejection fraction  
NO  Nitrous oxide  
LPS  Lipopolysaccharide  
MAP  Mean arterial pressure  
MC  Medical centers  
metHb  Methemoglobin  
MRI  Magnetic resonance imaging  
MPO  Myeloperoxidase  
NIH  National Institutes of Health  
OALI  Oleic acid-induced lung injury  
OPB  Oxygen pre-breathing  
PAP  Pulmonary artery pressure  
PCI  Percutaneous coronary intervention  
PEG  Polyethylene glycol  
PEI  Polyethylenimine  
PFC  Perfluorocarbon  
PI  Principal investigator  
PLV  Partial liquid ventilation  
PO₂  Oxygen partial pressure  
PPHN  Persistent pulmonary hypertension  
pRBCs  Packed red blood cells  
R&D  Research and development  
RBCs  Red blood cells  
ROSC  Return of spontaneous circulation
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>RTR</td>
<td>Radiation, triage, treat, transport system</td>
</tr>
<tr>
<td>SAE</td>
<td>Serious adverse event</td>
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<tr>
<td>SAH</td>
<td>Subarachnoid hemorrhage</td>
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<tr>
<td>SCD</td>
<td>Sickle cell disease</td>
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<tr>
<td>SCI</td>
<td>Spinal cord injury</td>
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<tr>
<td>SOD</td>
<td>Superoxide</td>
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<tr>
<td>TBI</td>
<td>Traumatic brain injury</td>
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<tr>
<td>TCD</td>
<td>Transcranial Doppler</td>
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<tr>
<td>THb</td>
<td>Total hemoglobin</td>
</tr>
<tr>
<td>TLR-4</td>
<td>Toll-like receptor 4</td>
</tr>
<tr>
<td>TLI</td>
<td>Traumatic lung injury</td>
</tr>
<tr>
<td>TLV</td>
<td>Tidal liquid ventilation</td>
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<tr>
<td>TMZ</td>
<td>Tetrazolamide</td>
</tr>
<tr>
<td>tPA</td>
<td>Tissue plasminogen activator</td>
</tr>
<tr>
<td>TRISS</td>
<td>Trauma and injury severity score</td>
</tr>
<tr>
<td>TXA</td>
<td>Tranexamic acid</td>
</tr>
<tr>
<td>VO₂</td>
<td>Oxygen consumption</td>
</tr>
<tr>
<td>VV</td>
<td>Veno-venous</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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Oxygen Carrier SoS Meeting
Combat Casualty Care Research Program (CCCRP) | 6-8 February 2017 | Fort Detrick
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Oxygen Carrier SoS Meeting
Combat Casualty Care Research Program (CCCRP) | 6-8 February 2017 | Fort Detrick
Oxygen Carrier SoS Meeting
Combat Casualty Care Research Program (CCCRP) | 6-8 February 2017 | Fort Detrick

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HbO2 Therapeutics
Executive Summary

The JPC-6/CCCRP Oxygen Carrier State of the Science meeting took place 6-8 February 2017 at Fort Detrick, Maryland. Together with interagency partners from BARDA, NHLBI, and the FDA, these groups sought to bring experts in the field of hemorrhage and resuscitation in contact with trauma specialists, DoD scientific and programmatic experts, and regulatory officials to understand the current status of the science of oxygen carrier research and clinical trials and elicit the discussions necessary to move the field forward in the context of military medicine, specifically combat casualty care.

A representative from each agency within the partnership provided brief remarks detailing the purpose and interests of research supported by their institutes. Col Todd Rasmussen also provided an overview of the mission, vision, and investment strategy for the CCCRP.

A variety of sessions were focused on topics such as Unmet Medical Needs, Basic Chemistry and Physiology of Oxygen Carriers, and Clinical Needs. During each session, several presenters provided brief talks on different aspects of the session topic. Each session was followed by a question and answer session wherein meeting attendees and presenters discussed the historical and current issues and challenges and steps required to make advances associated with various facets of the oxygen carrier field in the context of hemoglobin (Hb)-based oxygen carriers (HBOCs) or perfluorocarbons (PFCs).

Highlights of the 3-day proceedings included a regulatory paths and issues panel focused on discussing the specific regulatory and clinical endpoint issues associated with conducting clinical trials of oxygen carrier products and presentations on historical and current clinical trials for oxygen carrier products.

Immediately following the scientific proceedings, a closed panel Government session took place. The purpose of this closed-panel session was to develop a strategy for the development of oxygen carriers in the mid- to long-term time range (5-10 years). Specific topics addressed included:

- Identification of the most promising opportunities, technologies, and recent advances related to oxygen carriers
- Identification of the most important barriers to oxygen carrier technologies
- Identification of key technologies and approaches recommended for research and development (R&D) investments for the DoD CCCRP in the next 5 years

Outcomes from the meeting and closed panel session are projected to be white papers and peer-reviewed publications providing scientific, regulatory, and/or clinical recommendations regarding the unmet needs of both the Services and civilian populations that could be addressed by the further development and use of oxygen carrier products.
Session I. Unmet Medical Needs

Unmet Military Medical Need: When RBCs Are Not an Option

LTC (P) Andre Cap, MD, PhD, US Army Institute of Surgical Research

Overview

LTC (P) Cap reviewed military needs for optimal trauma and hemorrhagic shock treatment to address bleeding as the number one cause of preventable death in combat. He noted the superiority of blood-based resuscitation but acknowledged that blood is not always available. He emphasized the need for whole blood substitutes that deliver oxygen without worsening coagulopathy.

Timing of Resuscitation Is Critical

During his review of combat death statistics from 2001 through 2011, LTC (P) Cap highlighted the contribution of hemorrhage to combat deaths. He reported that at least 10% of combat casualties are at a high risk of bleeding to death, noting that approximately 1,500 Service members had been estimated to bleed to death in Iraq and Afghanistan conflicts before reaching a hospital, indicating a failure of current pre-hospital therapy.

LTC (P) Cap discussed the duality of challenges in resuscitation, noting the oxygen debt must be paid but, at the same time, coagulopathy must be avoided. Ideally, these challenges are met by physical hemorrhage control, blood-based resuscitation, and rapid delivery to surgical care. He provided evidence from Joint Trauma System data that the definition of rapid surgical care is changing, as the “golden hour” following trauma is more properly viewed as the golden 30 minutes (Figure 1). He discussed recent research by Col Stacy Shackelford and colleagues demonstrating a six-fold increase in 24-hour survival and a three-fold increase in 30-day survival when transfusion was initiated during medical evacuations within 30-35 minutes of injury.

Changing Logistics of Casualty Care

LTC (P) Cap contrasted the extensive infrastructure available during recent conflicts in Afghanistan and Iraq with the current dispersed military presence worldwide. He cited dependence on helicopter evacuations to surgical care and a functional cold supply chain as approaches that are not possible for small units in remote areas. Instead, casualties will likely be transported by ground to a battalion aid station or transported by air in small fixed-wing craft with limited facilities. He reminded the audience of the vast distances in Africa (Figure 2) and the infrastructure challenges on that continent.
**Tactical Combat Care Risk Mitigation**

The best-case scenario for tactical combat care is provision of whole blood through means such as the 75th Ranger regiment’s Ranger O-Low Titer program, which banks type O blood with low titers of anti-A/B blood group antibodies. LTC (P) Cap noted that this program works best at the...
platoon level or above. It requires pre-deployment blood typing and testing for transfusion-transmitted diseases, with requisite equipment and trained personnel needs. In the absence of whole blood, dried plasma meets the needs of volume expansion and avoidance of coagulopathy but does not offer oxygen carrying capability. Crystal and colloid blood extenders have additional limitations beyond their inability to carry oxygen in that they cause hemodilution, coagulopathy, and depletion of Factor VIII. LTC (P) Cap concluded his presentation by emphasizing the military need for a relatively stable product to support resuscitation by providing oxygen without causing coagulopathy.

Unmet Civilian Medical Need: When RBCs Are Not an Option

Don Jenkins, MD, University of Texas Health Science Center, School of Medicine
Military Health Institute

Overview

Dr. Jenkins built upon LTC (P) Cap’s remarks on the military-relevant needs for blood extenders by indicating the importance of these products in the civilian realm as well. He highlighted the risk posed by hemorrhage in civilian trauma scenarios, noting that patients requiring 10 or more units of transfused blood are at a high mortality risk. Rural and remote areas of the US suffer from logistical challenges in access to blood transfusions, as demonstrated by the statistic that 40% of the US population lives in rural areas, but 60% of deaths occur in rural areas.

Dr. Jenkins used his experience as a trauma surgeon in the upper Midwest and southwest Texas to illustrate the challenges faced by rural providers and to suggest that development of programs for pre-hospital transfusion using HBOCs could save many lives.

Restricted Access to Transfusion Impacts Mortality in Rural US

Blood transfusion can be viewed as a predictor of injury mortality. Dr. Jenkins explained that there is 40% mortality in patients if a massive transfusion is required, thus bleeding is clearly a major problem. The physiology of bleeding to death in the civilian sector is not much different from that in the military; it takes just 22 minutes to bleed to death from a penetrating injury or approximately 28 minutes from a blunt injury, demonstrating that the time between injury and aid is just as critical in civilians as in Service members.

The area most at risk in the US is austere, rural America. While 40% of the US population lives in rural America, 60% of deaths occur there. As a result of limited resource availability and a lack of plasma availability, these patients experience a modified transfusion strategy far different from those with hospital transit times less than 30 minutes. As depicted in Figure 3, many hospitals in Minnesota, Wisconsin, and Iowa are “50 miles from nowhere,” with the majority of the state having to travel over 100 miles for accessibility to the health system.
Additionally, in many of these areas, emergency departments close at 10 p.m. If help is required after 10 p.m., a nurse at the hospital must call a doctor. Clearly, maintaining blood supply is important in this situation, but the blood must also be available during hospital transit. Mayo Helicopter Emergency Medical Services (HEMS) blood product use in the pre-hospital setting was examined from 1 January 2015 through 31 May 2016. There was a total of 2,968 transits during that time, but only 11% of the patients received blood products during transit. However, nearly all patients received transfusions once they arrived at the hospital.

Dr. Jenkins described the Southwest Texas Regional Advisory Council for Trauma that is tasked with maintaining the regional trauma system for 22 counties and a land mass of 26,000 square miles. The program includes five rotary wing HEMS organizations, and patients are taken to three different Level 1 trauma centers. He noted that Minnesota healthcare system is less well-equipped. In the last 18 months, the University Health Services trauma center has administered 64 massive transfusions. The typical patient was 40 years old and presented with blunt injury. The mortality rate was 76%. Almost all (95%) patients were transported by ground although their injuries were serious enough to warrant air transport. Experts suggested that patients were dying because no blood products were brought to the point of injury. The council was shocked by this unmet need, and a pre-hospital blood transfusion program has been implemented in South Texas.
Logistical Needs in the Rural/Remote US

In some cases, blood is not available at the point of injury. In these instances, Dr. Jenkins indicated the ideal oxygen carrying alternative would be stable at room temperature, non-immunogenic and free of transmissible diseases, have a long half-life and long shelf life, provide good oxygen delivery, and avoid immunosuppression. Blood scarcity is not unique to rural America. The product could benefit individuals in other locations, including austere or remote combat environments, space, Antarctica, US stockpiles, and areas with endemic transmissible diseases like Zika virus or human immunodeficiency virus (HIV). Newer developed oxygen carriers should avoid the prior side effects of hypertension, hemoglobinuria, stroke, MI, and jaundice.

Dr. Jenkins ended his presentation by stating that it is important to understand the unmet medical needs and logistics of resuscitation. If both air travel and a blood product containing the necessary elements for survival were accessible, it would be a monumental. He explained that in the US there are close to 180,000 deaths each year. The huge, unmet need of adequate blood resuscitation in preventable hemorrhage scenarios results in 25%-30% of those 180,000 deaths. He commented that creating a functional, economical, and safe alternative to red blood cells (RBCs) for transfusion would be transformative in pre-hospital, shipboard, critical access hospitals, and in times of crisis.

Potential Use of HBOCs in Mass Casualties

Chad Hrinda, MS, EMT, GC-WMD, US Department of Health and Human Services

Overview

Mr. Hrinda provided an overview of the logistical challenges posed by a mass casualty situation, using a nuclear detonation as an example. He explained that infrastructure damage would hinder the response time of first responders, who would be faced with a wide variety of injuries. Limited resources would be available to stabilize casualties at the point of injury, and the products used would need to have the ability to be rapidly administered, be easy to use, and have a high therapeutic index. Because of these stipulations, current iterations and procedures using HBOCs may be more appropriate for definitive rather than point-of-injury care in mass casualty situations.

Characteristics of Mass Casualty Situations

Nuclear detonations have the potential to cause immense traumatic injury in hundreds of thousands of casualties that could benefit from intervention with oxygen transporters. After a nuclear detonation, infrastructure damage would likely hinder medical responders, who would face a complex spectrum of injuries (radiation exposure, trauma, and thermal burns) with limited resources due to the necessity of a complex coordination of effort and sheer volume of injuries.

Mr. Hrinda explained that, due to the large number of victims, a crisis standard of care paradigm would be applicable. A nuclear detonation in a major city would result in about 260,000 patients who might benefit from transfusions, causing a supply issue. Additionally, although 13 minutes
is the critical window of response time, it would be nearly impossible to establish a response in that time.

The concept of operations response after a nuclear detonation is centered on the expected activities of the exposed population. A common triage approach is important to achieve fairness in resource allocation when resource adequacy will vary greatly across the response areas. It may require the standards of care to change from “conventional” to “crisis” or to treating those “most likely to survive” first approach. To provide the greatest effectiveness in response, MCMs will need to provide benefit without extensive medical interventions. Mr. Hrinda described how patients would be likely to coalesce into different radiation, triage, treat, transport system (RTR) categories around the blast site, with each RTR group exhibiting different injuries:

- **RTR 1** – Combined injuries of trauma, burn, and radiation
- **RTR 2** – Primarily radiation exposure
- **RTR 3** – Limited injuries

The response timeline begins with stabilization and resuscitation activities with an end goal of definitive care. Medical countermeasure considerations for stabilization and resuscitation are those that are immediate, easy to use, and have high therapeutic index. Relative to field countermeasures, those for definitive care would have a lower degree of immediacy and may incorporate a higher level of expertise and a lower therapeutic index. In the hypothetical blast scenario, field centers would be set up to take care of the injured in tiers further out from the blast site epicenter. As depicted in Figure 4, countermeasures would be developed for the health care professionals to provide care for patients that require field stabilization, screening, assessment, or definitive care. Patients would be treated and then moved to other levels of care.
Mr. Hrinda concluded his presentation by explaining that a nuclear denotation medical response would require immediate actions and field stabilization focused on splinting and hemostasis. To be most effective in these situations, oxygen transporters would need to be universal and easily administered. The benefits of oxygen transporters or substrates increase as they behave like whole blood better and provide hemodynamic stability. Increasing volume, transporting oxygen carbon dioxide or wastes, and hemostatic potential are all important characteristics to oxygen transporters. Utility for oxygen transporters may also exist in the emergency room and definitive care settings where blood demand would be high.
Session II. Basic Chemistry and Physiology of Oxygen Carriers

HBOC: Human Physiology and Adverse Effects

Aryeh Shander, MD, FCCM, FCCP, Englewood Hospital Medical Center

Overview

Hb levels are the current standard used to measure anemia, but this condition is more complex than just low Hb. Increased oxygen extraction and transport as well as functional capillary density are critical to oxygen delivery to the tissues. RBC transfusion is the gold standard of care to combat anemia; however, this is not the only solution to facilitate oxygen delivery to the tissue. HBOCs may have the potential to save the lives of those patients for whom RBC transfusion is not an option. Significant cooperation must be established among industry supporters, independent researchers, and regulatory agencies (FDA) to test and validate these products.

Defining Anemia

Dr. Shander began his presentation by highlighting the deficiencies of using Hb levels as a primary measure of anemia. The response to anemia is complex and individualized, he explained, and maintaining functional capillary densities is critical to oxygen delivery to the tissue. Restoring oxygen is the only treatment for anemia, but RBCs are not the only solution for oxygen delivery. Dr. Shander noted that HBOCs appear to have the potential to provide lifesaving treatment in situations where RBC transfusion is not an option. However, he noted functional capillary density cannot be addressed with products currently on the market; other agents are necessary.

The Hb concentration defined as anemic by the World Health Organization (WHO) is used more to guide intervention and therapy then to define it. Hb levels are used to estimate RBC volume. A study challenging this convention determined that cell mass does not correlate with Hb concentration (Jacob et al., Blood Transfus, 2012). Dr. Shander suggested using genetic markers to define anemia may improve diagnosis.

The Body’s Response to Anemia

An optimal level of Hb is necessary for organ function. Dr. Shander explained how anemia is associated with an increased risk of tissue/organ injury. As Hb concentration decreases, a hypoxic cell response begins which leads to anemic organ injury. Further decrease in Hb concentration to low Hb levels result in tissue hypoxia. The central mechanism underlying these phenomena is the impairment of oxygen delivery to the organs resulting from reduced Hb to oxygen affinity. Each organ has a varied amount of reserved oxygen and that reserve decreases as it is consumed. The heart and brain consumes has a larger basal oxygen extraction and
therefore has a smaller reserve of oxygen capacity. Different organs respond differently to decreases in red cell mass and oxygen content.

The compensatory response to anemia involves changes in microcirculatory, mitochondria ATP generation, and gene expression. Dr. Shander introduced hypoxia-inducible factor (HIF) and explained that its activation, along with Hb oxidation, are prompted by anemic conditions. HIF is the master regulator of hypoxic cellular adaptation; it shifts the process of necrosis to apoptosis. The compensatory tissue response and HIF stimulation are regulated by the oxygen extraction ratio. The oxygen extraction ratio is the ratio of oxygen consumption (VO₂) to global oxygen delivery (DO₂). At the point of critical DO₂ (cDO₂) the maximum oxygen extraction ratio is reached; beyond this point, any further increase in VO₂ causes tissue hypoxia and anaerobic metabolism. At this point, oxygen delivery is oxygen supply independent (DO₂n). As DO₂ increases, the whole body extraction ratio decreases. These points are illustrated below in Figures 5 and 6. There are various physiological parameters related to Hb used to measure hemodynamics in patients. Different values predict morbidity and mortality.

Figure 5. The Relationship Between Whole Body Oxygen Consumption (VO₂) and Whole Body Oxygen Delivery (DO₂)
Dr. Shander described the clinical consequences of anemia, including increased mortality, renal injury, cerebral dysfunction and injury, increased myocardial injury, and worse outcomes in spine and joint surgery. Treatment of these conditions with blood carries risks, which include infectious agents (bacterial or viral) and noninfectious agents (transfusion reactions, medical errors, iron overload, and transfusion-related injuries or immunosuppression). Additionally, RBC transfusion does not always improve tissue oxygenation. One study revealed that the change in oxygen extraction was not statistically significant following blood transfusion, putting to question the use of the oxygen extraction ratio as a transfusion trigger (Mung’ayi et al., *African Journal of Emergency Medicine*, 2014). Another study revealed that RBC transfusions do not associate with an improvement in tissue oxygenation in patients with sepsis and Hb levels less than 9 grams per deciliter (Mazza et al., *Clinics* (Sao Paulo) 2005).

**Plasma Alternatives**

Dr. Shander emphasized that scientists have been studying and attempting to emulate the oxygen-carrying capacity of blood for decades. Although its capacity as an oxygen carrier is understudied, plasma can act as a carrier when saturated with oxygen and can access areas of the tissue where blood delivery of oxygen is difficult such as microcapillary dense regions. Dr. Shander believed that the full potential of plasma as an oxygen carrier has not been explored and that plasma may be modified to deliver oxygen when RBCs are not a transfusion option.

Dr. Shander hypothesized that if plasma can be sustained and enriched with oxygen, it could present an excellent option for the delivery of oxygen to the tissues. He also mentioned that agents are available that have the potential to change the solubility of oxygen in plasma and help maintain physiologic oxygen pressure in the blood. Many discontinued HBOC products from different classes (cross-linked Hb, polymerized Hb, and conjugated Hb) were presented. One
discontinued in the United States but approved for use in South Africa, HBOC-201 (Hemopure) and one in Phase 1 trials in 2014, Sanguinate, were highlighted. HBOCs have many advantages over banked RBCs such as: a longer shelf life, ambient storage temperature, universal, and high oxygen extraction potential. HBOCs also effect tissue oxygen tension that result in a larger oxygen transport area, increased plasmatic flow of a Hb molecule, improved RBC or plasma flow (decreased viscosity), and increased microcirculatory perfusion. In addition, HBOCs can increase oxygen concentration without have the ability to increase oxygen concentration without reversing the hypoperfusion generated pre-transfusion.

Dr. Shander concluded his presentation by reiterating his key points: that anemia is not just low Hb, the response to anemia is complex and individualized, and, in terms of oxygen delivery, RBCs are not the only solution.

Oxidative Toxicity of Hb: Ways and Means for Control?

Abdu Alayash, PhD, DSc, Laboratory of Biochemistry and Vascular Biology, Office of Blood Research and Review, Center for Biologics Evaluation and Research, FDA

Overview

Recent animal studies have confirmed the involvement of oxidative reactions in triggering inflammatory responses. Oxidative pathways, subsequent radical migration, and loss of heme all play roles in the oxidative toxicity of Hb. To minimize the redox activities of HBOCs, intervention strategies including co-administration of reductants, antioxidants, or protein/heme scavengers are being explored. Another protective strategy against oxidative by-products that scientists are pursuing is the re-engineering of oxidative stability based on revolutionary, stable, mutant Hbs.

The Oxidative Toxicity of Hb

Dr. Alayash’s presentation focused on past experiences with Hb/HBOC oxidative regulatory pathways. Dr. Alayash explained that the oxidative toxicity of Hb and the consequences of its redox side reactions are difficult to study in living systems. However, he emphasized that recent animal studies have confirmed the involvement of oxidation reactions in the initiation of inflammatory responses, demonstrating that these pathways and their relevance to HBOCs must be understood to generate a viable HBOC product.

Dr. Alayash summarized the mechanisms of Hb oxidation and regulation (Figure 7). Briefly, the oxidation of Hb occurs naturally both inside and outside of the RBC due to its redox-active iron. Inside the RBC, a multitude of enzymatic and non-enzymatic mechanisms exist to slow Hb oxidation. In the early genesis of RBCs, a built-in mechanism, alpha-Hb-stabilizing protein (AHSP), specifically locks the more damaging alpha subunits in a non-reactive stable form. The enzymatic machinery is efficient at maintaining Hb in the ferrous functional forms; only 1%-3% of Hb is converted to non-functional ferric Hb.
For RBCs outside the circulation, Hb can undergo uncontrolled oxidation leading to the formation of its most highly oxidative forms of ferryl Hb and ferryl radicals. Several plasma-derived proteins (e.g., haptoglobin and haemopexin) have the capability to regulate Hb oxidative pathways. When the system is overwhelmed, the oxidative forms of Hb are reactive towards other biological molecules, and, eventually, the Hb molecule itself will self-destruct and lead to the loss of heme. Oxidative heme can contribute to altered cell metabolism, mitochondrial dysfunction, and inflammation, including activation of Toll-like receptor 4 (TLR-4). Now that it is well known that oxidative heme triggers inflammatory responses, it has accordingly been termed a DAMP (damage-associated molecular pattern) molecule. Dr. Alayash has explored the DAMP effect in the context of HBOCs and found that some HBOCs are more prone to oxidation than others.

**Interventions in the Oxidative Pathway**

Dr. Alayash presented study results from the examination of the effects of the oxidative pathway on Hb after exchange transfusion in guinea pigs, determining that almost 50% of Hb transitioned to an oxidized form, similar to results from experiments in a sheep model.
Dr. Alayash then went on to describe two possible strategies to counteract oxidative pathways for Hb and HBOCs: the use of antioxidants (e.g., protein and heme scavengers haptoglobin and haemopexin) and molecular engineering of oxidative stability.

Haptoglobin is designed to inactivate dimers of Hb to facilitate the clearance of heme by macrophages. Importantly, haptoglobin docks on the Hb alpha subunit, then folds over and binds to the hotspot on the beta subunit. Heme is released from Hb in an hours-long process. Dr. Alayash explored the use of haptoglobin as a heme scavenger in a sickle cell mouse capillary vaso-occlusion model. In this model, mice were infused with Hb, and the number of vessels occluded in the presence of haptoglobin and haemopexin were determined. Haptoglobin and haemopexin were both found to be effective in removing heme.

Dr. Alayash then provided an example of the treatment of a Jehovah’s Witness patient, who, for religious reasons, was unable to accept blood transfusion. Treatment with HBOC-201, a synthetic Hb-based oxygen carrier, and ascorbic acid, to reverse cardiac hypoxia, (Fitzgerald et al., Medical Journal of Australia, 2011, 194:471-473) induced oxidative stability. The patient was considered unlikely to survive without a transfusion. Using a slow infusion of HBOC-201 and ascorbic acid over the course of several days, the patient recovered and was discharged. No vasoactive side effects were associated with the treatment, although ascorbic acid toxicity in the absence of RBCs is a risk that could be associated with continued use of this treatment.

Dr. Alayash introduced Hb providence variants and explained that Hb was examined for mutations or variants. Three fractions of Hb were isolated from a patient’s blood. They varied at position 82 in the beta 3 chain, with the presence of either a lysine, asparagine or aspartate (Asp). The variants were investigated for stability and Asp82 was found to provide the most stability. The proximity of Asp82 to Cys93 may contribute to the extraordinary stability. This mutation was incorporated into Hb and was found to provide considerable stability as well as good oxygenation characteristics. The ultimate goal would be to create an oxidatively stable HBOC.

Dr. Alayash concluded his presentation by reiterating some of his key points:

- Oxidative pathways and heme loss play a central role on the oxidative toxicity of Hb
- Oxidation products of Hb induce mitochondrial dysfunction
- Re-engineering oxidatively stable Hb may provide a protective strategy
- Co-administration of reductants, antioxidants, or heme scavengers may minimize redox activities of HBOCs (e.g., haptoglobin)

HBOC: Chemistry and Potential Modifications to Address Effects

John Olson, PhD, Rice University

Overview

Dr. Olson provided an overview of critical structural and biochemical properties that need to be considered in the development of HBOCs. He outlined a strategy for engineering recombinant HBOCs using a combination of comparative and systems biology approaches toward optimizing critical properties of heme proteins that included enhancement of ligand binding parameters,
inhibition of nitrous oxide (NO) scavenging, minimization of auto-oxidation, inhibition of hemin loss, and enhancement of apoprotein stability.

**Acellular Hb Toxicity**

Dr. Olson briefly explained the molecular mechanisms of acellular Hb toxicity. When RBCs lyse, Hb diffuses up to and into the endothelium and results in NO dioxygenation, leading to vasoconstriction. Auto-oxidation and hydrogen peroxide production then occur, leading to oxidative stress and membrane damage. He noted that the most detrimental step is likely the release of heme, which leads to inflammation. Compensatory strategies to remove heme from the blood include scavenger pathways facilitated by haptoglobin, which bind and stabilize heme dimers until they can be consumed by macrophages.

**Targeting Hb Toxicity**

Dr. Olson used recombinant technology aimed at engineering Hb molecules capable of mitigating the side effects of cellular toxicity. To do this, amino acids would have to be changed to optimize key properties of Hb molecules (Figure 8). Five strategies that may be used to optimize these key Hb properties are:

1. Engineering of ligand binding parameters to optimize efficient oxygen transport or gas sensing
2. Inhibiting NO dioxygenation (scavenging) to prevent loss of NO as a signaling molecule and hypertension
3. Minimizing auto-oxidation, reducing reactions with hydrogen peroxide, and preventing oxidative stress
4. Inhibiting hemin loss, which leads to apoprotein unfolding and inflammation
5. Enhancing apoprotein stability to inhibit unfolding and denaturation and enhance heterologous holoprotein production in bacteria

Dr. Olson explained that the tools to accomplish strategies 1 and 2 delineated above already exist. Oxygen can be retained with additional hydrogen bonds, and in this way Hb can be altered to achieve the desired P50, the oxygen tension at which Hb is 50% saturated. In terms of NO scavenging, after Hb deoxygenates NO, NO is expelled quickly from the binding pocket. The binding pocket can be engineered in such a way that NO is captured, which would reduce the requirement for NO scavenging.

Critical obstacles were encountered in addressing strategies 3 and 4. For the third strategy, the mechanisms of oxidative reactions are known to try to prevent auto-oxidation; water gets into the binding pocket and is oxidized. An obvious approach would involve occluding the binding pocket via the substitution of a large amino acid residue to keep the water out of the pocket. A problem Dr. Olson encountered was that this approach did not work with Hb because release of heme from the binding pocket is very slow. Haptoglobin binds to the dimer and speeds up auto-oxidation, which in turn minimizes NO scavenging.
Dr. Olson then provided a simple solution for limiting hemin loss by stabilizing binding between Hb and haptoglobin to prevent the release of heme from Hb. Lastly, Dr. Olson detailed the use of recombinant Hb proteins in *Escherichia coli* to determine the folding patterns of the globin chains that would make the apoprotein molecule most stable and resistant to denaturation. Unfortunately, recombinant hemoglobin production costly, heterogenous, and contaminated with lipopolysaccharide antigens.

Dr. Olson concluded his presentation by stating that simple singular ideas are not the best approach for designing an HBOC. Because the biochemical and structural foundations behind the Hb mechanism of action are known, they should be exploited in concert to create an enhanced HBOC.

### Potential Novel Molecules

**Anirban Sen Gupta, PhD, Case Western Reserve University**

**Overview**

Dr. Sen Gupta provided an outline of key characteristics to consider when formulating a novel HBOC. Strategic engineering approaches included the use of recombinant, chemically modified,
cross-linked and polymerized, or encapsulated Hb (Figure 9). Important design considerations for the development of an effective HBOC were (1) the size of the molecule, which should be large to avoid extravasation and renal clearance, (2) qualities that may reduce NO-scavenging, and (3) the molecular stability and half-life of the molecule in circulation. Novel HBOC molecules should also incorporate RBC-mimetic redox and effector functionalities to allow physiologically relevant reversible oxygen loading and off-loading equilibrium kinetics.

Dr. Sen Gupta believed that investigators in the HBOC field needed to work together to ensure that the best HBOCs are developed. He emphasized that data sharing across basic science, preclinical, clinical, and commercial development stages is imperative to facilitate optimization of novel HBOCs. He also mentioned that design optimization could be application-specific rather than attempting to create a product generalizable to many different resuscitation scenarios (e.g., pre-hospital, in-hospital).

**Recombinant Hb**

Dr. Sen Gupta reviewed the challenges associated with generating recombinant mutant HBOC molecules, which included mimicking the functional properties of human and bovine Hb but minimizing immunogenicity, toxicity, and infectious risks. Dr. Sen Gupta discussed studies of three recombinant Hbs: Hb-Prisca, Hb-Minotaur, and Hb-Polytaur. Hb-Prisca was developed in *E. coli*, but yield issues prevented extensive study of this molecule. Hb-Minotaur, a recombinant integration of α-human and β-bovine chains, provided a much higher yield. Additionally, Hb-Minotaur could be polymerized via the cysteine residue at β9, resulting in the final iteration of
the product, Hb-Polytaur. This recombinant Hb displayed the desirable traits of reduced NO-scavenging, significant molecular stability, physiologically comparable oxygen-binding curves relative to human Hb, reduced auto-oxidation, and thermodynamics and kinetics similar to cross-linked Hb. These recombinant Hbs are still in in vitro testing. Dr. Sen Gupta noted a significant drawback to using recombinant Hbs is the production cost to facilitate in vivo applications.

**Chemically Modified Hb**

Dr. Sen Gupta provided examples of several chemically modified Hbs, including HemoTech, Core-Shell, and Hb-HSA. HemoTech is a bovine Hb with intramolecular ATP cross-linking and glutathione (GSH) conjugation targeted toward polymerizing Hb with pharmacologically active molecules to avoid the toxicity risks of common aldehyde cross-linkers. HemoTech is currently in a clinical proof-of-concept study in sickle cell anemia subjects, and results reported so far are highly promising.

Core-Shell is composed of covalent core–shell structured protein clusters of bovine Hb and human serum albumin (HSA) cross-linked via Hb surface lysines to HSA cysteine-34 using α-succinimidyl-ε-maleimide. This HBOC was designed to be large to avoid extravasation and charged to avoid glomerular clearance in addition to transporting oxygen. Core-Shell was reported to show higher oxygen affinity than native Hb, which could potentially lead to a thermodynamic imbalance in oxygen loading/off-loading. This molecule is currently being tested in vitro, but there are concerns that the chemicals used for cross-linking might cause toxicity issues in vitro.

Hb-HSA has a similar design to Core-Shell, but in addition to α-succinimidyl-ε-maleimide cross-linking, the molecule has Flavin or Platinum nanoparticles bound to the drug-binding pockets of HSA to increase the antioxidant capabilities of the molecule. This molecule is also currently being tested in vitro and has similar thermodynamic issues to Core-Shell.

Figure 10 summarizes the large number of cross-linked and polymerized Hb products produced. Many products advanced to clinical trials but were then suspended due to adverse effects. The newer designs are highlighted in green and are now in the clinical stages. Significant challenges remain for these products, which require rigorous risk versus benefit analysis, stringent tracking of adverse effects from preclinical through clinical testing, and open dialogue and data sharing among investigators on study outcomes.
Encapsulated Hbs

Dr. Sen Gupta explained that the three major types of Hb encapsulation are polymer membranes, liposomes, and polymersomes. Encapsulating Hb protects the molecule in circulation in an RBC-mimetic way and avoids extravasation and renal clearance. These are emulsion-induced membrane-bound particles or self-assembled vesicles formed from amphiphilic molecules that can encapsulate Hb potentially along with antioxidant enzymes and other effector molecules. It has been researched extensively in vitro and preclinical models have demonstrated the excellent promise of encapsulated Hb in transfusion applications. Drawbacks for this approach include the cost of scaling up, potential systemic safety concerns, and modes or timelines of clearance from the body.

Dr. Sen Gupta then provided examples of different encapsulation strategies and their drawbacks and benefits. Micelle-based encapsulated Hbs protect Hb in circulation in an RBC-mimetic way and avoid extravasation, renal clearance, etc. Another example of encapsulated Hb is co-precipitation and cross-linked complexation. Hb is co-precipitated with CaCO3 or MnCO3, stabilized by cross-linking, and further complexed with anionic proteins like HSA. Based on these reaction conditions, nano- or microparticles can be achieved. With both the micelle and co-precipitated packaging, only very preliminary in vitro characterization studies have been completed. The reports on these systems suggest cytocompatibility, opsonization, oxygen-binding, and hemocompatibility. Because only in vitro studies have been completed, it is too early to analyze efficacy of these molecules.

Another type of encapsulated Hb was manufactured to contain Hb-loaded microparticles that mimic the biconcave discoid shape, size, and flexibility of RBCs, as well as flexibility to maximize hemodynamic circulation and function. Results reported from in vitro and in vivo...
proof-of-concept studies provide information on particle manufacturing rather than oxygen-transport capabilities. However, the mechano-biological design aspects may provide additional refinement in the manufacturing of HBOCs.

Erythromer is another encapsulated Hb. This Hb contains hydrophobic tails conjugated to the well-known amphiphilic polymer polyethylenimine (PEI). Molecules self-assemble with rHb, diphosphoglycerate, and antioxidant agents in a reverse-micelle process to result in Erythromer. Initial \textit{in vitro}, \textit{ex vivo}, and \textit{in vivo} preclinical findings demonstrate mimicry of RBC oxygen transport but a rapid clearance of about 3 hours. This may present a problem for use in pre-hospital stabilization.

Haptoglobin is a plasma glycoprotein that scavenges Hb, reduces dissociated Hb toxicity, facilitates macrophagic endocytosis of dissociated Hb, and has other protective functions. Haptoglobin-Hb complexes offer native protection that could be utilized in reducing the damage and risks with HBOC. There are currently ongoing \textit{in vitro} and \textit{in vivo} (preclinical) studies.

In conclusion, Dr. Sen Gupta explained that it is well known that tetrameric Hb needs to be stabilized. Recombinant, cross-linked, polymerized, encapsulated, and a combination of methods can all potentially increase Hb stability, reduce rapid clearance, reduce NO scavenging, and can incorporate necessary enzymes and effector molecules. Dr. Sen Gupta emphasized the need for establishing standardized pre-clinical and clinical evaluation criteria that encompasses comparative studies, \textit{in vitro} / \textit{ex vivo} metrics, animal models, risk vs. benefit analysis. Along with detailed data sharing across basic science, preclinical, clinical, and commercial development stages to facilitate design optimization of novel HBOC technologies.

Discussion

Presenter and meeting attendee comments and questions addressed the following topics related to Sessions I and II:

- **Hb/Haptoglobin interactions:** A question on the stringency of the Hb-haptoglobin interaction and ways to alter the binding dynamic was posed. It was explained that haptoglobin binds Hb irreversibly to Hb dimers and tetramers, regardless of whether the molecule is cross-linked. Ways to alter the dynamic were not discussed, except in the capacity that an equation would have to be devised to examine this issue.

- **NO scavenging:** The origin of the NO deoxygenation effect was discussed. Some experts suggest it is derived from skeletal muscle whereas others believe it comes from the heart. It was noted that adding hemoglobin after NO release from the binding pocket cannot prevent NO loss.

- **National stockpiles:** The meeting participants discussed the existence of strategic reserves for mass casualty reserves, the locations of which are classified. It was noted that during recent larger casualty events, hospitals ran out of platelets, so it would be a critical advantage to be able to add an oxygen carrier product to these stockpiles.

- **Ideal HBOC:** It was questioned whether the ideal HBOC will mimic RBCs or provide a short-term therapy for a bridge to standard medical care. The overall consensus was the goal is an HBOC that can provide a bridge.
• **Optimal P50:** The presenters and meeting participants discussed the design of HBOCs and whether optimizing an HBOC’s P50 is a pressing concern. One presenter felt this issue was a secondary concern relative to toxicity.

• **Heme oxidation and loss:** The possibility of co-inducing a catalase to reduce autoxidation of heme was raised, but one presenter pointed out that this would not alleviate heme loss, because only hydrogen peroxide would be eliminated. This rationale was based on the high stability of the ferrous iron bond, whereas ferric Hb is easily hydrolyzed. It was mentioned that keeping Hb in its reduced form is the ideal goal.

### Session III. HBOC Clinical Trials – Lessons Learned

Overview of Baxter’s Hb Therapeutics (Trauma) Program

**Ken Burhop, PhD, Corporate Vice President/Chief Scientific Officer, Integra Lifesciences**

**Overview**

Before covering historical background regarding clinical trials testing diaspirin cross-linked Hb (DCLHb, also known as HemAssist), Dr. Burhop recapped the primary benefit of HBOCs, which is their ability to reduce the need for a blood transfusion, and issues associated with previous clinical trials. He encouraged the meeting participants to remember that new approaches, including recombinant technologies, can improve known first-generation HBOC issues.

Dr. Burhop indicated that DCLHb was tested in both preclinical and clinical trials. Preclinical trials yielded promising results, but one of the clinical trials was terminated prematurely based on ethical concerns. Additional difficulties encountered in the clinical trials, including variations in the health status of patients randomized into trial arms and the efficacy and safety of comparing an unapproved product with HBOCs, may have also contributed to their lack of success.

**DCLHb Preclinical**

Dr. Burhop began his presentation by explaining that he has contributed extensive DCLHb preclinical research in over 15 species. He catalogued the issues experienced during clinical trials of DCLHb that led to their failure although DCLHb was an excellent oxygen transporter, non-immunogenic, and perfused tissues efficiently. The overall conclusions from these trials were that DCLHb produced hemodynamic effects such as increased blood pressure, induced moderate gastrointestinal symptoms, and caused enzyme elevations and myocardial lesions in some species.

Myocardial lesions produced by the DCLHb solution were detected in monkeys and pigs but not dogs or rats. This effect was not observed in cardiac surgery patients, but extensive testing for the presence of lesions in humans was prevented by ethical concerns. Based on this possible
outcome, great care had to be taken in determining dosage in humans calculated from animal dose response curves. Vasoactivity was also a concern. The study results revealed greater NO-scavenging and higher blood pressure in animals receiving DCLHb.

Heart lesions and enzyme increases were key issues from pre-clinical studies. Although these adverse symptoms were present, regulators approved progression into clinical trials as long as the dose was kept as low as possible. The administration volume had to be low so not to be considered as a blood substitute and the official name of the program was changed from “Blood Substitutes” to “Hemoglobin Therapeutics.”

**DCLHb Clinical Trials**

Dr. Burhop performed a study of DCLHb in US perioperative surgery trials, which demonstrated positive results (25% avoidance and 20% reduction in packed RBCs [pRBCs]). In a cardiac surgery trial, DCLHb was successfully used as a bridge to RBC transfusion.

Testing of DCLHb in two trauma trials, one in the US and the second in Europe, was scheduled. The US trial was terminated early at the behest of the Data Monitoring Committee because there was a relative increase in mortality among patients receiving DCLHb compared with controls. The Baxter Phase III trial in the US investigating the efficacy of DCLHb in traumatic hemorrhagic shock had a predicted trauma and injury severity score (TRISS) mortality rate of 35.5% in the controls, but mortality was much lower (17.4%). Mortality for subjects receiving DCLHb was estimated at 43% but was found to be 46%.

Many issues were encountered with the first-generation HBOC:

- Vasoactivity (increase in blood pressure)
- Heart lesions
- Extravasation of Hb
- Relatively short half-life
- Serum enzyme increases
- Increase in pancreatic enzymes
- Gastrointestinal (GI) dysmotility
- Hb/endotoxin interaction
- Oxygenation of tissues

The use of a recombinant molecule has the potential to eliminate some of these issues. However, in European trials where treatment was administered en route to the hospital, there was no difference between treatment and controls groups in mortality. This highlighted two important considerations for designing an HBOC trauma trial: (1) proper randomization of patients into treatment and control arms and (2) the need to identify differences between treating patients on-scene versus in a hospital.
**DCLHb Clinical Confounding Factors**

Dr. Burhop explained that there were several confounding factors in the US trial. (1) The baseline mortality was different between the trial arms, and there was a higher than expected number of poor responders in the treatment group. (2) Improper administration of DCLHb, which has twice the blood volume expansion effects of albumin can lead to adverse effects. Patients in the US study received a larger volume of fluid, which may have led to adverse effects. (3) Dosing issues: Patients administered DCLHb frequently appeared to improve clinically after DCLHb infusion, but then would crash hours later. Among the patients who died within 24 hours of infusion, DCLHb-treated patients received less blood and fluids. Dr. Burhop hypothesized that this puzzling effect may have been due to patients appearing to be more improved than they actually were, or the positive effects of DCLHb being short-lived. There may have also been a delayed adverse effect.

Dr. Burhop summarized the possible reasons the patients responded poorly to DCLHb resuscitation:

- The patients in the treatment group may have been sicker than controls
- There may have been an enrollment bias, effect, or issue
- Possible adverse interactions with concomitant therapy
- Suboptimal DCLHb dosing
- Lack of appreciation for duration of patient response
- Possible adverse side effect(s) of DCLHb

Dr. Burhop closed his presentation by reiterating that HBOCs may be able to reduce the need for blood transfusion and that using modern tools and design strategies could yield a next-generation HBOC that overcomes the shortfalls of previous iterations.

**Northfield - Trauma**

**Steven Gould, MD, The Gould Consulting Group, LLC**

**Overview**

Dr. Gould highlighted the main feasibility challenge associated with planning trauma trials, the difficulty of enrolling an adequate number of subjects with appropriate characteristics to reach statistical power to support a generalizable conclusion. He provided the Northfield trial, a US multicenter trauma trial that compared PolyHeme to crystalloid treatment and RBCs, as an example of this feasibility challenge that many HBOC trials face. The results of the Northfield trial failed to demonstrate non-inferiority of PolyHeme relative to controls. Dr. Gould suggested that unconventional approaches to clinical trial design may be the best path forward for HBOC use. Using subjects who refuse transfusion, volunteer for elective surgery, or are in situations where RBCs are not available may all be options for HBOC clinical trials.
The Northfield Trial

The goal of the Northfield trial was to develop a product with life-sustaining capability that could be used to treat bleeding and shock in patients for a prolonged timespan onsite and in transit until patients could transition to definitive care. However, the trial encountered feasibility issues centered on logistics, resources, and obtaining patient consent and had to be reconfigured as a US urban multicenter trauma trial in the pre-hospital setting that did not ideally match the conditions of prolonged transport or lack of RBC availability.

Dr. Gould indicated that the biggest lesson learned from this trial was that investigators need to ask appropriate questions when designing a clinical trial:

- What is the *right* question?
- What is the *appropriate* endpoint?
- What is the *appropriate* analysis?
- What is the *relevant* answer?

Dr. Gould discussed these important study considerations and issues in the context of the Northfield clinical trial. He noted that the trial was not set up for success. The intended treatment population would have long transport times and limited access to RBCs. Conversely, the clinical trial populations had short transit times and early access to RBCs. The trial also analyzed the benefits of PolyHeme administration relative to treatment with a combination of Crystalloid and RBCs, which may not have been an appropriate comparison. The treatment of bleeding and shock was to start in the field and then continue in the hospital with a primary efficacy endpoint at 30-day mortality and a dual superiority and non-inferiority margin of 7%. Given the short transit times and early access to RBCs for the patients, it may have been beneficial to consider whether they were relevant to the study. The non-inferiority outcomes are displayed in Figure 11. The 30-day trial revealed a delta of just over 7% with a 95% confidence interval in the intent-to-treat group. Dr. Gould questioned the appropriateness of the chosen non-inferiority margin, noting that if they had chosen 8%, PolyHeme would have demonstrated a benefit. He was uncertain on the best procedure to be used to identify the correct inferiority margin for use in a given clinical trial.
Dr. Gould ended his presentation by arguing that a well-designed and adequately powered trauma trial is not possible. He noted that a simulated setting may be necessary to answer the questions most pertinent to HBOC use in a trauma setting, but ethical considerations, relevant endpoints, analysis population, and correct extrapolation of benefits would all present issues in this context as well.

**Clinical Trials of Hemopure (HBOC-201) for Surgery: Lessons Learned**

**Colin Mackenzie, MD, Emeritus Professor, University of Maryland School of Medicine**

**Overview**

Dr. MacKenzie showcased HBOC-201 (also known as Hemopure®) as an attractive alternative to RBCs, because it is cell free and requires no refrigeration or cell typing. However, he cautioned that clinicians require training in the unique characteristics and proper use of HBOCs to maximize their benefits. HBOC-201 is advantageous in that it is better able than colloid treatment to increase Hb blood concentration. The use of HBOC-201 in the clinic had the ability to reduce the amount of blood needed for transfusion, and up to 2 units of HBOC-201 could be used without risk of a serious adverse event (SAE). Although there are some potential adverse effects that could be associated with HBOC use, the risk of death can be reduced if HBOCs are used in scenarios where blood transfusion is not an option. Consequently, Dr. MacKenzie argued that appropriate controls for comparison against HBOC treatment would be those who do not receive RBCs.
**HBOC-201 Advantages and Use**

Dr. MacKenzie described his experience with HBOCs as a Principal Investigator (PI) at the Shock Trauma Center in Baltimore, MD, as well as in expanded access tele-Consultant support for novice users of Hemopure®.

Dr. MacKenzie stressed the importance of educating clinicians on the key differences between HBOC-201 and RBCs, which include an increased P₅₀ (40 mm Hg versus 27 mm Hg for RBCs), a half-life of 19-20 hours that necessitates HBOC-1 be replenished more frequently than blood, and peak erythropoietin, iron, and reticulocyte counts that occur 3 days post-infusion. Dr. MacKenzie then enumerated several advantages of HBOC-201, including the lack of a need for refrigeration, reconstitution, defrosting, special IV lines, or filters. HBOC-201 also has a shelf-life of 3 years at room temperature, will not communicate any known transmissible diseases, and does not present a hemodilution risk for patients. HBOC-201 was determined to be better than colloid or crystalloid, but not RBCs, at increasing total Hb concentrations in the blood of patients.

**HBOC-201 Clinical Trials**

Dr. MacKenzie stated that 22 HBOC-201 Phase I to Phase III clinical trials have been conducted to date. He presented results from two of those trials, HEM-0114 and HEM-115. HEM-0114 was a Phase II prospective randomized clinical trial wherein the transfusion-sparing ability for 28 days of up to 7 bags of HBOC-201 relative to 2 units of pRBCs was assessed. A significant blood-use reduction was found in the 83 patients randomized to HBOC-201 compared to the 77 receiving RBCs for non-cardiac surgery. There was an increase in non-serious adverse events (AEs) in the HBOC-201 patients, but no difference in SAEs was detected.

The HEM-115 Phase III prospective, randomized clinical trial consisted of 688 patients, 350 of whom were randomized to HBOC-201. HBOC-201-treated patients showed an identical incidence of SAEs relative to controls. Dr. MacKenzie emphasized that hemodilution and crystalloid fluid mismanagement contributed to the incidence of SAEs in the HBOC-201 treatment arm and stressed the importance of avoiding excess crystalloid volume overload and undertreatment with HBOC-201.

Despite 70% of the patients having cardiovascular disease, myocardial infarction and mortality were similar between the two groups, indicating that HBOC-201 treatment alone is a poor predictor of cardiac ischemic events. In a comparison of HBOC-201 treatment alone versus pRBCs or pRBCs with HBOC-201 treatment, clinicians were able to avoid transfusing blood in 96% of patients randomized to HBOC-201 for 24 hours and 70% of patients for 1 week.

Dr. MacKenzie highlighted the opportunity for the use of HBOC-201 in shock trauma scenarios. Seventy-two percent of patients that need transfusions require 2 units of pRBCs, and 8.3% will receive administration of un-crossmatched pRBC units.

Dr. MacKenzie concluded that it was important to evaluate the risk versus benefit in untreated acute anemia separately from untreatable acute anemia. HBOC-201 could be used as a RBC transfusion avoidance solution and in compassionate use cases. In the latter context, in one trial, 41.8% of admitted patients with a mean Hb concentration of 4g/dl were discharged without
transfusion after treatment with HBOC-201. In both pre-hospital and prolonged field care settings, HBOC-201 could be life-saving.

Sangart’s MP4OX Development in Trauma: Lessons Learned

Peter Keipert, PhD, KEIPERT Corp Consulting

Overview

MP4OX was designed to minimize vasoactivity and enhance perfusion of ischemic tissues. The preclinical trauma models of shock resuscitation demonstrate that MP4OX effectively reverses lactic acidosis and reduces mortality when administered as an adjunct to the standard of care. SANGART’s Phase II trauma protocol included lactic acidosis to identify patients who were more likely to benefit from MP4OX treatment. MP4OX Phase II trauma studies showed positive efficacy trends for improved clinical outcomes but did not achieve statistical significance.

MP4OX Properties

Dr. Keipert introduced the HBOC, MP4OX, developed by SANGART. MP4OX (MalPEG-Hb) consists of a human Hb conjugated to polyethylene glycol (PEG) polymers at β93-cysteine residues and thiolated surface lysine residues. The molecule has a large molecular volume with a PEG hydration shell surrounding it. MP4OX exhibits enhanced regeneration of NO through its nitrate reductase activity. The physical properties of MP4OX were designed to minimize vasoactivity and enhance perfusion of ischemic tissues, as depicted in Figure 12. Key properties include low P50 activity and high colloid osmotic pressure (COP).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb concentration (g/dL)</td>
<td>4.3</td>
</tr>
<tr>
<td>Vehicle</td>
<td>Ringer’s lactate</td>
</tr>
<tr>
<td>P50 (mm Hg)</td>
<td>~ 5</td>
</tr>
<tr>
<td>n (Hill coefficient)</td>
<td>1.2</td>
</tr>
<tr>
<td>COP (mm Hg)</td>
<td>~ 50</td>
</tr>
<tr>
<td>Viscosity (cP)</td>
<td>2.5</td>
</tr>
<tr>
<td>Plasma T½ (hours)</td>
<td>~ 20 - 36 (dose; blood loss)</td>
</tr>
</tbody>
</table>

Figure 12. Physical Properties of MP4OX
**MP4OX Preclinical Trial**

Dr. Keipert presented data from MP4OX administration in a preclinical swine trauma model of high mortality, traumatic hemorrhagic shock resuscitation. In all treatment groups, which included Ringer’s acetate, MP4OX, Stroma-free Hb, and Pentastarch, there was an initial decrease in lactate, but only MP4OX effectively reversed lactate levels, indicating support of aerobic metabolism. MP4OX significantly reduced mortality compared to the other treatment arms.

**MP4OX Clinical Trial**

Dr. Keipert described results from two clinical studies. The first was a lactic acidosis trauma trial by Dr. Karim Brohi wherein the inclusion criteria for the trial included a physiological biomarker of increased lactate rather than using a blood pressure of less than 90. The study revealed that the duration of elevated lactate due to occult hypoperfusion is correlated with increased mortality after trauma, and if elevation of lactate is reversed within 12 hours, mortality is similar to patients who do not have elevated lactate levels.

Dr. Keipert then introduced the SANGART MP4OX trauma clinical trial. Prior HBOC trauma studies used hypotension as a primary indicator of shock to randomize patients. SANGART’s key inclusion criterion was a prospective lactate level of at least 5 millimolar, and only patients with a documented oxygen debt were randomized. Data from the SANGART Phase IIA pilot study revealed no significant differences between the MP4OX group and the control group, although the MP4OX group exhibited an increased likelihood of being discharged from the hospital relative to the control group. In a SANGART Phase IIB trial featuring patients with severe hemorrhage and lactic acidosis located at multiple international centers, a total of 313 patients were dosed, but no statistical differences in mortality between MP4OX-treated and control patients were found nor was there a difference in patients discharged alive at 28 days. Overall, the efficacy endpoints were lower in the MP4OX-treated group, but not significantly. The conclusion of the study was that MP4OX was well tolerated in high-risk trauma patients.

A critical outcome from the SANGART MP4OX trial was information gleaned on lactate clearance as a biomarker. The study exhibited two types of responder populations: slow responders and rapid responders. The slow responders had a lactate level that was still above 5 millimolar at 60 minutes post-MP4OX treatment, which correlated with a 25% increase in risk of death. Utilization elements (e.g. ICU stay, time to discharge) will paradoxically increase as more of these patients survive. The rapid responders still exhibited abnormal lactate levels but were less than 5 millimolar (but still greater than 2.2 millimolar) at 60 minutes post-treatment. In this group, more rapid restoration of optimal tissue perfusion is expected to shorten intensive care unit (ICU) stays and reduce the overall in-hospital time for patients. Death was rarely documented in this group, so it is not expected in future trials that any demonstrable improvement in survival would occur in rapid responders treated with MP4OX.

Dr. Keipert concluded by emphasizing the lessons learned from the MP4OX studies. The most important lessons were to seek and heed regulatory and scientific advice and maintain standard of care practices for resuscitation in the study design. It is also imperative to perform proper dose-ranging for efficacy and safety and use an appropriate physiologic biomarker to identify
and monitor the patient population of interest. He recommended using doses that mimic nonclinical animal models to reach similar plasma levels.

Hemosol – Cardiac Surgery: Lessons Learned

Gerson Greenburg, MD, PhD, Professor of Surgery, Emeritus, Brown University

Overview

Dr. Greenburg introduced Hemosol, an HBOC product investigated for its potential as a blood-saving approach. He also enumerated critical factors that should be considered when designing and conducting a clinical trial, including the need to address issues brought forth by regulatory agencies and the consideration that preclinical results are unlikely to predict the potential AEs and SAEs in clinical trials. He also noted that 1:1 randomization is necessary and that it is important to avoid the use of “matched references” or “historical controls.” In clinical trials, it is imperative that differences in clinical standards or practices are explored, that a control solution appropriate to the objective is selected, and that populations with high risks for AE and SAEs are avoided. Root cause analysis can be utilized to understand AE and SAE causation.

Preclinical Trial Considerations

Dr. Greenburg opened his presentation by emphasizing the potential blood-saving approach of using Hemosol in cardiac surgery as a hemodiluent to preserve and extend blood bank supplies. He then went on to describe factors that need to be considered in designing preclinical experiments for product testing, including the investigation of possible side effects and toxicity, use of the correct preclinical model for the type of product and investigative approach (e.g., using a small animal model of cardiac surgery may be difficult and inappropriate). Dr. Greenburg emphasized that, regardless of the preclinical model chosen, it would be unlikely to predict AEs and SAEs. He noted that regulatory agencies may be the only organizations with enough comparative data to ensure that the research model is appropriate and determine whether there are any indications of side effects.

Hemosol Clinical Trials

Dr. Greenburg then moved on to discuss important considerations in the design of Phase I safety and efficacy clinical trials. He stated that, historically, regulators were concerned about the occurrence of myocardial ischemia and infarction, elevations in blood pressure, and any effects on renal and pulmonary function in patients. He also believed that ADME (absorption, distribution, metabolism, and excretion) in humans also needs to be explored in Phase I clinical trials. Dr. Greenburg noted that appropriate patient populations must be selected and should include normal human volunteers, compromised patients, and individuals of both sexes. He also emphasized that the study staff and trial location should have established experience in conducting clinical trials. Another component of clinical trials highlighted was using a balanced model of 1:1 patient enrollment and using a dose escalation model.

Dr. Greenburg then introduced his multicenter, randomized, double-blinded trial for efficacy and safety of Hemosol. An equal volume of 10% pentastarch was used as a control. The study involved an interoperative autologous blood donation (IAD) immediately before
cardiopulmonary bypass. Two hundred ninety-nine patients were matched to 150 historical controls. The primary endpoint, RBC transfusion avoidance, was attained. In the patients administered Hemosol, 56% of the patients received RBCs, as compared to those who received pentastarch (76%) and reference controls (95%). The decrease in the use of RBC products was a positive benefit for the blood bank.

Dr. Greenburg then discussed another study, the COSTART 5th edition Hemosol study to determine the occurrence of SAEs. Every patient in the study exhibited at least one AE. SAE occurrence was 4.3% in Hemosol-treated individuals versus 3.3% in pentastarch-treated individuals. Hypertension was found in 65% of the patients administered Hemosol and 35.8% of the patients administered pentastarch, but Dr. Greenburg emphasized that this was possibly a side effect of transfusion in general. Myocardial infarctions were significantly higher in the patients treated with Hemosol.

Dr. Greenburg recapped the lessons learned from the COSTART study, namely, that transfusion avoidance is a valid endpoint for efficacy studies, but blood alternative clinical trials using cardiac surgery patients may not be viable based on their underlying increased risk of mortality and the risk of other surgical complications.

Dr. Greenburg concluded by commenting that, in his opinion, a randomized controlled trial is insufficient to understand the basis for the observed AEs and SAEs and that it is important to avoid the use of patient populations at high risk for an AE or SAE. He also thought it is important to use root cause analysis to understand the cause of AEs or SAEs.

Discussion

Presenter and meeting attendee comments and questions addressed the following topics related to Session III:

- **Blinding and adaptive design trials:** A question about successful blinding during clinical trials was posed. The group discussed the inherent bias of investigators to under-report in the control group and over-report in the treatment group. Double blinding is difficult but could eliminate this problem. However, a recurrent issue with trials in hypertonic saline trials, which were blinded, was that patients were given the product, improved, and then later crashed. It was suggested that adaptive design trials might prove useful in HBOC studies. When a positive effect is seen, the protocol needs to change for how care is administered.

- **Surgical vs. trauma trials:** Trauma trials are difficult because they are skewed and there is no good way to identify patients that will die, and blinding these trials is equally problematic. Only 5% of trauma patients will ever get blood, so a way to select patients for trauma trial would be essential. One would assume better controls in a surgical setting, but a conundrum is that the military wants to use it. A 20-year-old on the battlefield is not the same as a 40- to 50-year-old civilian in a car accident. It was pointed out that products are approved for general medical use, not just military use. Patients of any age and any form of morbidity should be able to use the product. Ethical considerations with conducting either surgical or trauma trials exist. Patients can’t be denied blood administration.
• **Historical control use:** An FDA representative noted that earlier proposals to the FDA involved the use of historical controls. There was uncertainty over whether there was enough recent data to allow for this idea.

• **Delivering oxygen to the microcirculation:** An audience member pointed out that it is critical to oxygenate tissues after traumatic hemorrhagic events, but that hemoglobin delivery may not be able to access and reoxygenate the microcirculation. Starch was mentioned as a therapeutic means to help this process, but better alternatives are necessary.

### Session IV. Additional Clinical Directions

**Organ Preservation**

**Robert J. Porte, MD, PhD, Professor of Surgery, University Medical Center Groningen**  
**The Netherlands**

**Overview**

Dr. Porte presented rationale for using machine perfusion of donor organs to address unmet needs for organ transplants. He detailed the ability of machine perfusion to recondition organs using a combination of hypothermic conditions, controlled rewarming, and normothermic conditions. Dr. Porte explained how the ability of HBOCs to support oxygenation under all temperature conditions makes them superior to RBCs for machine perfusion-based reconditioning. He introduced a Phase I clinical trial of the HBOC Hemopure in machine perfusion to recondition suboptimal donor livers *ex vivo*.

**Addressing the Shortage of Organs for Transplant**

Dr. Porte began his presentation by reminding the meeting attendees that the limiting factor in organ transplantation is the shortage of donor organs relative to the number of waiting recipients (Figure 13). The transplant field has moved to include older donors and those who had chronic disease or succumbed to cardiac arrest, in contrast to the classical case of a brain-dead donor. However, many donated organs are discarded due to concerns about their suitability for successful transplantation.
Dr. Porte then discussed the drawbacks and benefits of various modes of organ preservation. The current model for organ preservation is static cold storage. Dr. Porte discussed the many advantages of preserving organs using *ex vivo* perfusion to supply oxygen and nutrients. The ability to reduce ischemia and reperfusion injury and prolong preservation times could result in an increase in the number and quality of donor organs. He noted potential gains in transplantable organs via the ability to perform pharmacological preconditioning and restoration of organs before transplant, for example, by removing excess fat. In addition, machine preservation allows better ex situ assessment of organ viability before transplant, he said. Dr. Porte noted that use of machine that include a pump, gas exchanger, and tubing to replicate the heart, lungs, and blood vessels is more complex and costly than static cold storage. He added that if organs are to be perfused at (sub)normothermic (body) temperature, an oxygen carrier will be needed in the perfusion solution.

**Organ Temperature Control Issues in Resuscitation**

Dr. Porte reviewed the considerations for resuscitation temperatures in organ transplant protocols. When organs are perfused at hypothermic temperatures of 10°C to 12°C, the metabolic rate and oxygen demand are low, and ATP can be restored if oxygen is available. At low temperatures, a crystalloid solution alone can carry sufficient oxygen to meet organ needs. If perfusion is performed at normothermic temperatures (35°C to 37°C), the organs can maintain full metabolic function, which would allow the clinician to assess organ function. However, normothermic perfusion is more expensive, potentially riskier, and requires an oxygen carrier. With the use of machine perfusion, surgical teams could weigh the costs, risks, and benefits to choose a combination of hypothermic and normothermic perfusion during the transplant timeline that best meet the needs of a particular transplant scenario (Figure 14).
Experience with Machine Perfusion of Livers for Transplant

Dr. Porte cited the first trial of hypothermic machine perfusion of livers for transplant in humans performed at Columbia University, which established the efficacy of this technique. This trial did not employ oxygenation, however. Oxygenation of perfusion solutions at low temperatures cannot be achieved with RBCs because cell membranes are non-fluid at low temperatures and will clog capillaries. Thus, HBOCS are an excellent solution for oxygenation at low temperatures or for situations where both hypothermic and normothermic solutions will be used in the perfusion protocol.

Dr. Porte and colleagues performed a proof-of-principle experiment in which discarded donor livers, which experienced at least 6 hours of ischemia, were subjected to ex vivo normothermic oxygenated perfusion using either RBCs or Hemopure (HBOC-201). Hemopure performed well in supporting glucose metabolism and outperformed RBCS in supporting blood flow (Figure 15).
The success of this experiment paved the way for a Phase I clinical trial currently open in The Netherlands that uses Hemopure in machine perfusion to recondition suboptimal donor livers \textit{ex vivo}. The protocol calls for hypothermic oxygenated perfusion to resuscitate mitochondria and restore cellular ATP, followed by controlled oxygenated rewarming from 10°C to 37°C and an additional 2 to 6 hours of normothermic perfusion. Livers that meet viability criteria after this treatment will be used in human transplants. Dr. Porte noted that because Hemopure is used only \textit{ex vivo} and flushed from the donor liver before transplant, no FDA approval is required for its use. He concluded by pointing out that the use of machine perfusion to recondition organs will create a need for perfusion solutions that will exceed the supply of donor blood, a need that HBOCs can well supply.

Wound Healing

\textbf{Franck Zal, PhD, MBA, Chief Executive Officer, Hemarina}

\textit{Overview}

An aging population with concurrent chronic diseases has increased the need for advanced wound management. Approximately 60 million people worldwide are affected by chronic wounds every year. Dr. Zal summarized current approaches combining antimicrobial and chemical matrices to provide a warm moist environment for wound healing. He focused on the need to supply oxygen to wounds at appropriate partial pressures and introduced the Hb, M-101, derived from the marine lugworm \textit{Arenicola marina}, as a viable solution to those needs. This large extracellular Hb molecule has a very high affinity for oxygen and is capable of delivering oxygen to wounds while remaining embedded in the wound dressing. A proof-of-principle experiment in \textit{db/db} diabetic mice subjected to punch wounds demonstrated the efficacy of a collagen dressing carrying M-101 in hastening wound healing.

![Figure 15. Comparison of RBC and Hemopure for Normothermic Liver Machine Perfusion](image)
Wound Management Overview

Dr. Zal opened his presentation with an overview of approaches to wound management. He noted that the traditional approach to wound management focused on keeping wounds dry to support drainage and reduce infection risk, but the current trend is the use of moist dressings incorporating antibiotics and biomaterials. He explained that the general aging of the population and prevalence of chronic diseases such as diabetes create a growing need for management of bedsores, ulcers, and other chronic wounds. Dr. Zal stated that chronic wounds annually affect about 350,000 people per year in France, 4 million people in the US, and over 60 million people worldwide. Based on the US market cost of $2.8 billion, the worldwide market for wound treatment is likely tens of billions of dollars. 1 amputation is carried out every 30 seconds on a diabetic patient while 20% of diabetics will present with a foot ulcer during their lifetime.

Wound dressings can combine chemicals such as antimicrobials, alginates, or hydrocolloids; biologicals such as growth factors or engineered skin; and devices such as gauze or hydrogel sheets, according to Dr. Zal. These components are at different levels of maturity, as shown in Figure 16. The dressings should support an oxygen supply to the wound without resulting in excessive air exposure, which would result in drying and inhibition of healing.

Figure 16. Components of Wound Management Products According to Class and Maturity
Oxygen Supply in Wound Healing

There is evidence that partial pressures of oxygen are greatly reduced in wounds and that oxygen availability may be rate-limiting in wound healing. Dr. Zal noted that this need must be balanced against potential negative effects of excess oxygen. Use of a specific oxygen carrier can provide the appropriate oxygen balance. The M-101 Hb from the marine lugworm Arenicola marina is the oxygen carrier used in Hemarina’s wound dressing. This high molecular weight extracellular Hb has a $P_{50}$ of 6.5 millimeters of mercury. This high affinity for oxygen enables M-101 to create an oxygen gradient that releases oxygen into the wound. Oxygen supplied to the wound by M-101 enables the destruction of bacteria resistant to local antibiotic therapies. Oxygen is also vital for many cellular functions including cell repair and regeneration. M-101 can be incorporated in wound dressings to supply oxygen in a protected, moist environment.

Development and Testing of Oxygen-Carrying Wound Dressing

Dr. Zal explained that M-101 was found to be compatible with different matrices. It could be embedded in an alginate matrix to maintain moisture or in hydrocolloid or hydrogel matrices to support the debridement phase of wound healing. A collagen matrix was selected for a proof-of-principle trial. The db/db diabetic mouse exhibits impaired wound healing. Groups of db/db mice were subjected to a punch wound and then treated with two applications of a saline compress, collagen alone, or collagen embedded with M-101. The inclusion of M-101 resulted in accelerated wound healing. Animals that received Hemarina (0.5 g/L, 5g/L, or 50 g/L) were all completely healed at day 20 but not the compress saline treated group. The largest implication of accelerated wound healing by Hemarina is that infection problems often occur in the first days of treatment. This conclusion was supported by histological examination that revealed an increased depth of granulated tissue and immunostaining to show increased levels of the angiogenesis marker CD31 in the M-101-treated mice (Figure 17).
Collectively, these results suggest that oxygen dressings such as those that incorporate M-101 are a promising technology and have the potential to speed the wound healing process by delivering the correct amount of oxygen to cells and protect against bacterial infection.

Sickle Disease

Abe Abuchowski, PhD, Chief Executive Officer, Prolong Pharmaceuticals, LLC

Overview

The abnormal Hb produced in sickle cell disease is subject to deoxygenation and polymerization, resulting in the characteristic sickled shape of affected RBCs. The resulting reduction in oxygen transport, microvascular blockage, and accelerated turnover of RBCs contribute to comorbidities of sickle cell patients. The bovine Hb-based SANGUINATE™ is designed to “unsickle” the deoxygenated RBCs, thus prolonging their life, and deliver oxygen downstream of blocked blood vessels. Phase II trials of SANGUINATE™ for vaso-occlusive crisis and sickle cell-related leg ulcers are in progress.

Sickle Cell Disease

Dr. Abuchowski introduced the physicochemical characteristics of the altered Hb produced in sickle cell disease. A mutation in the beta globin gene causes the Hb to polymerize under low oxygen conditions. The polymerized Hb can no longer carry oxygen and distorts RBCs into the characteristic sickle shape resulting in constant anemia. In addition to the loss of oxygen-carrying capabilities, the sickled RBCs block the microvasculature, causing pain and organ
damage as oxygen delivery is interrupted. In addition, sickled blood cells are turned over more rapidly, leading to anemia. Treatment for sickle cell disease must address the sickling of RBCs as well as restoring oxygen supply to tissues and organs.

**SANGUINATE™ Properties and Clinical Testing**

Prolong Therapeutics received orphan drug designation for the use of SANGUINATE™ in treating comorbidities of sickle cell disease. Dr. Abuchowski explained that SANGUINATE™ is composed of Hb from a genetically defined strain of cows, PEGylated for stability, and incorporates carbon monoxide to act as an anti-inflammatory agent and inhibit vasoconstriction. SANGUINATE™ does not bind NO or form metHb and is stable for up to 2 years at storage temperatures between 2°C and 25°C.

*In vitro* studies demonstrated that SANGUINATE™ decreased the expression of inflammatory cytokines and cell surface proteins in response to lipopolysaccharide stimulation of RBCs from normal controls and sickle cell patients (Figure 18). When RBCs from sickle cell patients were deoxygenated, treated with SANGUINATE™ *in vitro*, and subjected to imaging flow cytometry, restoration of RBC circularity (aka “unsickling”) was demonstrated. In addition, when sickle cell patients in vaso-occlusive crisis were treated with SANGUINATE™ and their blood was analyzed by flow cytometry, there was evidence for restoration of RBC circularity (Figure 19).

![Figure 18. Anti-Inflammatory Effects of SANGUINATE™ In Vitro](image)
Dr. Abuchowski reported that there are three current clinical studies of SANGUINATE™. A Phase II study in the hospital setting is recruiting patients in vaso-occlusive crisis. These may be sickle cell patients or patients for whom blood is not an option. A similar Phase II study for vaso-occlusive crisis in an ambulatory setting is a randomized, placebo-controlled test of a single SANGUINATE™ dose that aims to enroll 24 adult patients. Preliminary observations were that the four patients randomized to SANGUINATE™ reported a reduction in their pain score index. An escalating repeated dose open label Phase II study of SANGUINATE™ for leg ulcers in sickle cell patients completed enrollment and results are being analyzed. SANGUINATE™ was well-tolerated. There was a slight improvement in vascular status, but no evidence for clinically meaningful changes in patient status. Dr. Abuchowski stated that SANGUINATE™ will be tested in other sickle cell comorbidities.
Traumatic Brain Injury

Paula Moon-Massat, DVM, Naval Medical Research Center

Overview

Dr. Moon-Massat reviewed the rationale for the benefit of brain tissue oxygenation following traumatic brain injury (TBI). Improved neurological outcomes are seen if the duration of hypoxemia can be reduced and the balance between increased cerebral perfusion pressure and decreased cerebral edema is maintained. She noted that oxygen delivery and fluid balance needs can be met by HBOCs, making them a good choice for TBI treatment.

HBOC Studies in TBI

Citing the absence of clinical data on the use of HBOCs for TBI, Dr. Moon-Massat stated that a clinical trial of DCLHb was performed about 20 years ago, and the results appeared beneficial. Although one might expect that trials of HBOCs for trauma such as those discussed at this meeting could provide information about TBI applications, she noted that all those trials excluded subjects with TBI. Dr. Moon-Massat stated that there have been two cases of compassionate use of HBOCs in humans with TBI. She pointed out that a recently completed Phase II trial of SANGUINATE™ for patients at risk of delayed cerebral ischemia after acute aneurysmal subarachnoid hemorrhage might provide useful information, as one outcome measure is brain tissue oxygen tension.

Dr. Moon-Massat reported that a review of the literature from 2002 through 2016 identified 11 preclinical studies of HBOCs that were focused on TBI, and an additional 11 trials on trauma or surgery that had collected data on brain function. Unfortunately, only two trials focused on TBI in the absence of polytrauma. Data from all the trials were combined and analyzed for trends. The HBOCs studied in these trials were HbO2 Therapeutics’ HBOC-201 and HBOC-301, Sangart’s MP4CO-NP, Hemarina’s M-101, OXYVITA’s Oxyvita and Oxyvita C, Synzymes’s PNPH, and Prolong Pharmaceuticals’ SANGUINATE™.

HBOC Benefits in TBI

A comparison of survival across multiple trauma only and trauma plus TBI trials suggested that brain injury did not increase mortality risk. White et al. demonstrated directly that there was no additional risk associated with TBI in a study of HBOC-mediated fluid resuscitation of uncontrolled hemorrhage in swine (Shock, 2013, 39(2):210-219). In both rat and swine, supporting increased brain tissue oxygen tension and increased cerebral perfusion pressure offered a survival benefit. Dr. Moon-Massat reported that HBOCs were beneficial under conditions of hypercapnia and when fewer fluids were provided. She noted that in three trauma studies, injured animals were resuscitated sufficiently to be removed from ventilators, an indication of survival benefit.

As noted by other presenters, HBOC-stimulated vasoconstriction is a concern. Dr. Moon-Massat noted that while many studies demonstrated HBOC-induced vasoconstriction in skeletal muscles, lung, and heart, the brain appeared unaffected. She cited her own research on HBOC-201 in swine (Mongan et al. 2009, Trauma 67(1):51-60), which demonstrated adequate cerebral blow...
flow even in the presence of peripheral vasoconstriction (Figure 20). Dr. Moon-Massat concluded by stating that the limited clinical and preclinical data available suggested a value for HBOCs in TBI.

![Figure 20. Blood Flow Change from Baseline in Swine Transfused with HBOC-201 (HBOC) or Human Serum Albumin (HSA) Compared to Untransfused Controls](image)

Discussion

Presenter and meeting attendee comments and questions addressed the following Session IV-related topics:

- **Use of HBOCs in organ transplantation:** The ability to use a single HBOC-containing solution during both hypothermic and normothermic organ perfusion offers many advantages and was shown in practice to allow storage of a liver for more than 18 hours before transplant. Perfusion of livers with RBCs up to 6 hours without hemolysis was possible.

- **M-101 properties:** The high affinity of M-101 for oxygen allows this marine Hb to concentrate oxygen from the air at normal atmospheric pressures and deliver it to wounds. Hyperbaric oxygen would not be required in combination with M-101-containing wound dressings.

- **SANGUINATE™ properties:** The bovine Hb in SANGUINATE™ is more stable and has a higher affinity for oxygen than human Hb, which allows SANGUINATE™ to
Oxygen Carrier State of the Science Meeting
Combat Casualty Care Research Program (CCCRP) | 6-8 February 2017 | Fort Detrick, MD

extract oxygen from RBCs and deliver it preferentially to hypoxic tissue. Use of this product requires a hemoglobin level below 5.

Session V. Addressing Clinical Issues and Use

HBOCs – Cardiovascular
Borja Ibanez, MD, PhD, FESC, Centro Nacional de Investigaciones (CNIC)

Overview

HBOC-201 has mild effects on mean systemic arterial pressure and vascular resistance but no effect on coronary physiology. The use of HBOC-201 as a full blood substitute meets the heart’s metabolic demands. In a myocardial infarction setting, modified HBOC-201 outperforms blood during early phases of reperfusion. HBOC-201 might be used as a delivery platform to avoid the neutralizing actions of blood.

Dr. Ibanez explained that his interests lie in myocardial diseases: myocardial ischemia, myocardial reperfusion, heart failure, myocardial diffuse fibrosis, and pulmonary hypertension. His laboratory is a translational research group with areas of research in systems biology, large animal study, clinical trials, and basic research.

Studies Involving HBOC and the Myocardium

Dr. Ibanez went on to discuss the effects of HBOC on the myocardium. He began with a study involving an IV infusion of HBOC-201 in coronary artery disease (CAD) patients. The study involved 45 patients undergoing percutaneous coronary intervention (PCI) who were infused with solutions of colloidal, HBOC-201, or colloidal and HBOC-201. The effects of HBOC infusion included moderately increased systemic mean blood pressure, moderately decreased cardiac output, an increase in pulmonary vascular resistance, and a moderate increase in systemic vascular resistance and in blood pressure. HBOC-201 significantly increased MAP (mean arterial pressure) while exhibiting no effect on coronary flow and on the diameter of coronary arteries. Other AEs were identified in the HBOC-201 patients, but most were non-serious.

Dr. Ibanez then described another study where HBOC-201 was infused distal to a coronary occlusion in pigs. A fixed volume of HBOC-201 (12 grams per deciliter) was used, and different rates and temperatures were tested in the animals. Coronary artery occlusions (CAOs) were induced by inflating an intracoronary balloon catheter; preoxygenated HBOC-201 (12 g/dL) was infused distally through the central lumen of the balloon catheter. Animals underwent consecutive 3-minute CAOs interspersed by 30 minutes of reperfusion, accompanied by different HBOC-201 infusion rates (0, 15, 23, 30, 40, and 50 ml/minute) and two infusion temperatures (18°C or 37°C) in random order. Myocardial systolic shortening was reduced with no treatment. Systolic shortening in the occluded area increased significantly with increasing infusion rates and plateaued at 50 ml/minute.

Dr. Ibanez presented another study by the same group of researchers on stable CAD patients undergoing PCI with a stent in the proximal left anterior descending artery. HBOC-201 (13 grams per deciliter) was infused distal to the stent at a rate of 48 ml/minute or 17 gr Hb. There
was no difference in the measured parameters between baseline and HBOC-201 treatment. The administration of HBOC protected left ventricle function preservation as well as left ventricle end-diastolic pressure and cardiac output and prevented arrhythmia/ischemia. The overall conclusion of the study was that the myocardium was preserved.

Dr. Ibanez then moved on to his study testing bloodless reperfusion with HBOC-201 in acute myocardial infarction in pigs. He used a fixed volume of 600 mL HBOC-201 (72g Hb), and then varied the infusion rates and duration. In the first group, assessment was done at 12 minutes; subsequent time points were 17, 30, and 60 minutes. In most cases a moderate decrease in hemodynamics was observed as the duration increased. The effect in arrhythmia induction increased with duration of infusion. The long-term effect on myocardial performance was stunning: at 15 minutes post-infusion, a significant decrease in the left ventricular ejection fraction (LVEF) in all time groups was observed, but most dramatically in the 60-minute group. Seven days post infusion the LVEF level remained significantly lower in the 60-minute group.

Dr. Ibanez explained that the flow of HBOC-201 can be extended for 12 or 17 minutes without any adverse effects, so he utilized this time range to test HBOC reperfusion. Ischemia was induced for 45 minutes followed by HBOC-201 reperfusion for 12 or 17 minutes, then subsequent blood flow restoration. The 12- and 17-minute reperfusion groups displayed identical LVEF and infarct size compared to control groups. Dr. Ibanez then tried to improve HBOC-201 by reducing pH and adding glucose and insulin. This also had no significant effect on systemic hemodynamic parameters.

Dr. Ibanez expressed interest in using a vehicle for improved gene delivery enabling an increase in gene transduction. Blood contains neutralizing antibodies, so trials of cardiac gene therapy thus far have failed. One option would be flushing the heart with HBOC to make gene therapy possible, and his results presented today show that heart function will not be impaired under such conditions.

Dr. Ibanez concluded by emphasizing important points of his presentation. He emphasized that HBOC-201 meets the heart’s metabolic demand and results in no detrimental effect on myocardial performance. In a myocardial infarction setting, modified HBOC-201 outperforms blood during early phases of reperfusion. Lastly, HBOC-201 might be useful as a delivery platform permitting gene therapy treatments.

HBOC Use in South Africa, Including Guidelines

Lewis Levien, MB, Bch, PhD, Sunninghill Hospital

Overview

HBOCs can be used to delay or avoid RBC transfusion in acute anemia. This is especially important in areas where blood availability is a major problem. Physician training and understanding in the use of HBOCs are critical for its success.

HBOC Trial in South Africa

Dr. Levien explained how South Africa was an ideal location for HBOC use based on a confluence of financial restrictions and health factors, including HIV and blood
Dr. Levien used results from Phase III RBC infusion clinical trials as a guide for designing Hemopure trials in South Africa. His observations of AEs and SAEs in clinical centers revealed that the rate of fluid infusion was an important factor. Centers with low AEs and SAEs used slow delivery methods, ideally between 2-10 milliliters/minute.

Dr. Levien described the initial guidelines for his study examining the use HBOC instead of RBCs in acute surgical anemia. A slow rate of infusion (start at 2 to 10 milliliters/minute) was utilized and a second unit was to follow on completion of the first if native Hb was expected to fall below 8 grams/deciliter.

The Hemopure dosing guidelines were to treat symptomatic anemic, non-hypovolemic patients if their plasma Hb concentration was greater than 0.3 grams per deciliter. It was unusually effective if RBC Hb levels were greater than 7 grams per deciliter. One bag (30 grams/250 milliliters) was to be administered over 3 hours, and additional doses were given to treat signs and symptoms, with a maximum dose of 7 bags.

An initial 80 patients went through all the rigor of the clinical trial; subsequently there were 256 patients that followed based on the findings of the first 80. Once full commercial usage was implemented, 266 additional patients were added. In total, information on 602 patients was obtained with a range of 1-19 units.

Lessons Learned

Dr. Levien discussed the important lessons learned from the study. One lesson was that physicians needed to be reminded of the normal physiology of Hb and HBOCs. One important fundamental of training was to remind the physicians that these were not RBCs. HBOC increases the efficiency of on-loading and off-loading of oxygen in RBCs. Understanding this distinction from RBCs was essential to correct administration of the product. Cardiac SAEs often occurred when the physician was unfamiliar with and reluctant to use HBOCs. In patients presenting with severe anemia, the physician would often only use HBOCs as a last resort after large volumes of crystalloid or colloid had already been administered, which resulted in fluid overload in an already anemic patient and an increased risk of SAEs and AEs. Conventional measures could be followed to reduce fluid overload and facilitate patient recovery.

Additional knowledge gained from the trial was that the rate of fluid administration was critical to success. Most AEs correlated with a rapid rate of infusion. Some patients experienced HBOC-related hypertension that could be managed by reduced rates of infusion or controlled with the administration of a calcium channel blocker or IV nitrate.

Dr. Levien concluded by emphasizing that HBOC-201 can be used to delay or avoid RBC transfusion in acute anemia. It is highly suitable as an alternative to RBCs when blood is not an option or readily available and as an oxygen carrier in cases of acute blood loss during patient transport. Lastly, he noted that guidelines for clinical use of HBOC-201 were recently published (Transfusion; 2016).
Addressing Unmet Needs with HBOCs When RBCs Are Not an Option, What Data Do We Have?

Richard Weiskopf, MD, Professor Emeritus, University of California San Francisco

Overview

No clinical trials are available that have directly tested HBOCs for when RBC transfusion is not an option. Data from expanded access databases has possibilities and deficits. A single patient use would provide limited information, but a large series of expanded access may provide more information. For some circumstances, expanded access information may be the only source of data available for the outcome of interest. Existing data strongly indicate that use of an HBOC decreases mortality due to acute anemia.

Challenges Facing HBOC Clinical Trials

Dr. Weiskopf began his presentation by providing an overview of the difficulties of testing HBOCs in real-life scenarios where they could have the most therapeutic impact. These difficulties included (1) insurmountable feasibility and logistical challenges in designing clinical trials testing HBOCs in prolonged field care scenarios; (2) ethical and logistical considerations that prevent pre-hospital trials with prolonged transit times in the US; and (3) low numbers of in-hospital patient populations eligible for HBOC trials (e.g., patient refusal of blood transfusion, inability of timely blood cross-matching). Logistics, statistical power, and ethical issues would also be major impediments to an in-hospital trial.

Dr. Weiskopf emphasized that the HBOC community must band together to maximize the information gleaned from currently available clinical trials and research data. He posed the idea of using an expanded access database, which is a type of database used when investigating an unapproved drug, biological, or device for patients with serious or life-threatening diseases where no comparable or satisfactory alternative therapy is available.

Expanded Access Database

The potential benefits for the use of such a database must outweigh the risk, and treatment cannot interfere with a clinical investigation that could support approval of a therapeutic. Dr. Weiskopf mentioned most companies might not want to participate in such a program because of its high-risk, high-reward nature.

He went on to propose the question of whether anything can be learned from an expanded access program. Little can be learned from individual patients, but a group of patients gathered together in a database could offer the potential for greater insights, although limitations would still exist. These limitations include the absence of a contemporaneous randomly allocated control group, homogeneous population, treatment protocol with specific initiation, dose and timing, mandated uniform efficacy and safety data collection, and verification of collected data.

Dr. Weiskopf explained that information from an expanded access database should be utilized similarly to a retrospective database analysis where randomized control trials are not possible, an inadequate number of available patients exists relative to the required study size, there is a wide
geographic distribution of relatively few patients, or there are unrealistic required resources or ethical issues.

He introduced an example of a prospective data analysis study by Dr. Keyvan Karkouti examining whether the incidence of stroke is related to severity of anemia before bypass surgery. This database analysis included perioperative data from 10,949 patients who underwent cardiopulmonary bypass surgery from 1999 to 2004 at a hospital center. A direct association between the degree of hemodilution during surgery and risk of stroke was determined. In this case, a randomized trial was not possible and the expanded access database represented the best information available.

Dr. Weiskopf also used a data repository to perform a correlative analysis to confirm the results of a clinical trial performed in healthy young adults illustrating that low Hb impairs cognition. He suggested an expanded access program as a tool to be used when a randomized control trial is not possible due to an inadequate number of available patients to significantly power a study or the ideal patient population has a large degree of geographic dispersion.

Dr. Weiskopf presented additional examples of studies using an expanded access program. He began by introducing Dr. Jeffrey Carson’s retrospective cohort study examining the relationship between Hb levels and survival without transfusion in surgical patients. The data spanned a time range from 1981 to 1994 and were from patients who refused transfusion (about 90%) and patients who were unable to receive RBCs for other logistical reasons. Dr. Aryeh Shander then repeated the retrospective analysis with surgery patients between 2003 and 2012. Dr. Shander’s data are superimposed on Dr. Carson’s data and show human physiology has not changed in 30 years (Figure 21).

![Figure 21. Mortality of Acute Severe Untransfused Anemia Surgical Patients](image)
Dr. Weiskopf then introduced the use of a database in Dr. Andrei Beliaev’s study in New Zealand. This was a multicenter observational study using 103 untransfused Jehovah’s Witness patients. Dr. Weiskopf then compared this to Dr. Nicole Guinn’s study in the US at Duke University Medical Center and found that these data were highly correlative (Figure 22).

![Figure 22. Mortality of Acute Severe Untransfused Anemia in Hospitalized Patients](image)

The results are consistent; the lower the nadir Hb, the lower the survival. Dr. Weiskopf also examined the mortality of acute severe untransfused anemia patients from all hospitalized patients relative to surgical patients. A similar trend was established, with more rapid decline in survival in total hospitalized patients (Figure 23).

![Figure 23. Mortality of Acute Severe Untransfused Anemia in Hospitalized Patients](image)
Dr. Weiskopf concluded by emphasizing the importance of patient selection. A drug should not be given to a patient when death is inevitable, it would not be an accurate study. He also emphasized some of his presentations key points. No clinical trials have tested directly HBOCs for when RBC transfusion is not an option. While data from an expanded access databases have limitations, for some circumstances, they may be the only information available for the outcome of interest.

Discussion
Presenter and meeting attendee comments and questions addressed the following Session V-related topics:

- **HBOC toxicity:** The question of whether anyone believes HBOCs as a class are cardiotoxic was raised. The consensus was the term “class” was not a good way to ask the question. Cardiac lesions have been detected in monkeys in pigs, suggesting HBOCs may be cardiotoxic in these models although no functional deficits were identified. No massive infarctions occurred but microscopic lesions did occur. It was unclear whether these results could be extrapolated to humans. There are not yet enough data to determine whether HBOCs are cardiotoxic as a class.

- **Expanded access database:** Clarity on the expanded access program idea was requested along with whether propensity matching would be a possibility with such a database. In a certain population, such a database might be good enough to show that the drug used had increased survival, but the database used should have similar inclusion/exclusion criteria for the study enrollment population. If the database cannot be used for the certain population, then it will be difficult, if impossible, to analyze the data. Propensity matching can be done with the New Zealand and Duke University data.

- **HBOC-related myocardial infarctions:** The mechanism for HBOC causing myocardium infarctions was questioned. It was clarified that no functional deficit was determined due to the infarctions. The mechanism was most likely due to the extra oxygen consumption. A meta-analysis revealed a 1.5% increase in mortality in all HBOC studies when compared to RBC, and an order of magnitude greater when compared to care without RBCs.

- **Current HBOC use in South Africa and US:** The current use in South Africa is similar to that in the US. It is available for compassionate use cases, but it is not marketed. 1435 units have been used. HBOCs are used in rural areas where transport is an issue.

- **HemAssist meta-analysis:** An audience member asked whether data for HemAssist use was excluded in Dr. Weiskopf’s re-analysis of meta-analysis data. He responded that he could not recall whether it was included but that controls had a mortality of 6% and HBOC mortality was 7.5%.
SANGUINATE™: When Blood Is Not an Option
Abe Abuchowski, PhD, Prolong Pharmaceuticals, LLC

Overview

SANGUINATE™ is an intravenous (IV) therapeutic agent designed to treat hypoxia resulting from an ischemic or anemic event. The polyethylene glycosylated bovine carboxy-Hb has a non-human Hb oxygen core, an oxygen transfer agent, and a carbon monoxide-releasing molecule (Figure 24). The three components provide targeted oxygen delivery while inhibiting vasoconstriction and inflammation.

![Figure 24. SANGUINATE™ Composition and Mechanisms of Action](image)

Dr. Abuchowski highlighted the following SANGUINATE™ therapeutic properties:

- Reverses the effects of vascular ischemia by reducing inflammation and vasoconstriction
- Improves perfusion and oxygenation of hypoxic tissue
- Does not bind nitrogen dioxide
- Does not form metHb (metHb) in vivo
- Acts as a potent colloid
- It is stable for 2 years at 2°C-25°C

Dr. Abuchowski presented the anti-inflammatory effects of SANGUINATE™ on lipopolysaccharide (LPS)-challenged sickle cell disease (SCD) whole blood, reporting decreased
levels of RNA for various inflammatory cytokines and protein levels of inflammatory cell surface markers (Figure 25). He noted that SANGUINATE™ uses an active oxygen transfer mechanism, which is unique compared to any previously developed molecule, for enhanced oxygen delivery from RBCs to hypoxic sites. Furthermore, a comparison between SANGUINATE™ and Hextend® in the resuscitation of hemorrhagic rats demonstrated the superiority of SANGUINATE™ in enhanced blood flow, pressure, and oxygen delivery to tissues immediately and after 8 hours. In a more lethal model with post-resuscitation survival, SANGUINATE™ demonstrated enhanced tissue oxygenation and mean arterial pressure and a dramatic reduction in lactate, as compared with Hextend®.

Figure 25. Anti-Inflammatory Effects of SANGUINATE™ in Pretreated Normal and SCD RBCs

Several ongoing clinical trials for the safety and efficacy of SANGUINATE™ were outlined by Dr. Abuchowski, including the effect of a single dose in patients at risk of delayed cerebral ischemia following an acute aneurysmal subarachnoid hemorrhage. The results of this study demonstrated an unexpected decrease in the cerebral metabolic rate of oxygen (CMRO₂), despite the rise in oxygen delivery. Dr. Abuchowski stated that these results suggested prior irreversible cerebral injury and noted that since the CMRO₂ declined further at 24 hours, it is unlikely to be an effect of the infusion.

Dr. Abuchowski briefly summarized results from clinical trials with “blood is not an option (BNAO) patients,” who represent a heterogeneous population treated with SANGUINATE™. He presented data from single and repeat-dose SANGUINATE™ exposure in 94 BNAO patients, highlighting that 75% of patients received 3 or fewer units, whereas 25% received 4 or more units, and he stated that no SAEs were reported as result of SANGUINATE™ infusion (Figure 26). Dr. Abuchowski reported positive results for several outcome measures in those treated with SANGUINATE™, including improved cognition, renal function, and cerebral blood flow. He highlighted that SANGUINATE™ treatment reduced or eliminated the need for administration of pressors to elevate arterial blood pressure. Collectively, the findings from SANGUINATE™ studies support its mechanism of action for improved oxygenation and reduced inflammation.
Dr. Abuchowski outlined many in-progress or planned SANGUINATE™ clinical trials that could expand the potential of the product beyond use for tissue oxygenation, including SCD, delayed graft function, and delayed cerebral ischemia. He also revealed plans for a Phase III, multicenter trial that will compare SANGUINATE™ to the standard of care for BNAO patients as well as a non-inferiority trial comparing SANGUINATE™ to RBCs. A fully good manufacturing practices (GMP)-compliant, state-of-the-art facility will be utilized in manufacturing SANGUINATE™.

Dr. Abuchowski concluded his presentation by stating that a fully GMP-compliant facility capable of producing 1,000,000 units per year will be used to manufacture the product and that multiple locations in the US and abroad are secured to assure a continued supply and isolation of infrastructure.
Current Projects and Future Directions

Gregory P. Dubé, PhD, Vice President, Research & Development, Hemoglobin Oxygen Therapeutics, LLC

Overview

Dr. Dubé provided a brief history of HBOC-201, highlighting its enormous potential in therapy. He noted that HBOC-201 is the most studied HBOC in the field and the only one compared to RBCs in randomized clinical trials. HBOC-201 has been approved in South Africa for the treatment of acutely anemic surgery patients, with more than 2,000 patients treated to date.

In severe anemia, oxygen consumption is limited by its delivery to the tissues. Dr. Dubé explained that in order to increase oxygen consumption, the systemic concentration must be increased to allow for a higher extraction ratio, and ultimately a higher Hb concentration. He stated that the primary purpose of HBOCs is to increase the total hemoglobin (THb) concentration. Dr. Dubé presented the effects of resuscitation fluids on the change in THb concentration in anemic patients. He highlighted the change in THb after administration of HBOCs, noting that THb concentration is dependent on HBOC treatment.

Dr. Dubé next presented results from a PolyHeme clinical trauma trial that revealed decreases in THb concentrations for those treated with PolyHeme several hours post-treatment (Figure 27), suggesting that additional resuscitation for these individuals was required. Results from the PolyHeme trauma trial suggested the following:

- HBOCs are not equivalent to pRBCs
- THb concentration is an important determinant of mortality in hemorrhagic trauma
- HBOC should be started immediately if warranted by blood loss
- A crossover to RBCs should be made as soon as they are available

![Figure 27. THb Concentration Trends Downward After Resuscitation with PolyHeme](image)

Dr. Dubé stated that clinical development plans include a hemorrhagic trauma trial sponsored by The Alfred Hospital, with collaboration with Royal Melbourne Hospital and Ambulance Victoria in Australia. He noted that the study will be initiated upon the termination of an ongoing tranexamic acid (TXA) trial. The key inclusion criteria will include a shock index of greater than 1.0 and pre-hospital transport times greater than 20 minutes. The exclusion criterion is the
presence of a penetrating TBI. The clinical plans also include a postpartum hemorrhage observational study in South Africa as well as a pre-hospital, randomized civilian trauma trial. Dr. Dubé reviewed clinical development plans for an in-hospital PolyHeme trial when blood is not an option in the US, which includes the conversion of 5-10 expanded access protocols into a single multisite investigational new drug (IND), a patient population with a religion-based objection to blood products, and Hb of less than 7 grams per deciliter as a study condition. He also noted pursuing non-anemia indications in \textit{ex vivo} normothermic oxygenated machine perfusion and transplantation of human extended criteria donor livers. Dr. Dubé noted multiple donor organ research collaborations for liver, kidney, and heart transplantation, as well as potential applications in other areas such as myocardial infarction and cardiac pulseless electrical activity resuscitation.

Oxyglobin\textsuperscript{®}, or HBOC-301, was introduced by Dr. Dubé as a product similar to HBOC-201. HBOC-301 is the first oxygen therapeutic product to be FDA- and the European Medicines Agency-approved for veterinarian use in treatment of canine anemia of any cause. The product has been used to treat more than 150,000 animals with an excellent safety record. In summary, Dr. Dubé reiterated that, when treating severe anemia, it is important to consider both HBOC and THb concentrations. He emphasized the pursuit of a variety of indications for HBOCs in unmet clinical needs.

HEMARINA\textsuperscript{®}

Franck Zal, PhD, HERMARINA\textsuperscript{®}

Overview

Dr. Zal introduced HEMARINA\textsuperscript{®}, the biotechnology company founded in 2007 with a focus on the development of universal marine oxygen carriers extracted from the marine lugworm, \textit{Arenicola marina}, for therapeutic indications. HEMARINA\textsuperscript{®} has centers in Morlaix and Paris, France and in Boston, Massachusetts. Dr. Zal indicated that extracellular marine Hb has a high molecular weight and the potential to carry roughly 40 times more oxygen relative to human Hb, is compatible with all blood types, and has activity independent of any cofactor.

Dr. Zal indicated that this company focuses on organ preservation and wound dressing. He introduced HEM\textsubscript{2}life\textsuperscript{®} as an additive to organ preservation solutions, HEMHealing\textsuperscript{®}, an oxygenating dressing for hypoxic and chronic wounds, and HEMOXYCarrier\textsuperscript{®}, a therapeutic oxygen carrier for ischemic pathologies.

HEMOXYCarrier\textsuperscript{®} is a 3,600 KiloDalton extracellular Hb molecule 250 times smaller than an RBC capable of binding up to 156 oxygen molecules. Dr. Zal reviewed the molecule’s structure and functional properties, noting the presence of 156 globin and 42 structural chains, and highlighted its enhanced oxygen affinity. In addition to its intrinsic superoxide dismutase-like activity and anti-oxidative properties, Dr. Zal highlighted the following features of HEMOXYCarrier\textsuperscript{®}:

- The lack of immunogenic or allergenic effects
- The absence of vasoconstriction or hypertensive influence
- Oxygen binding and release is dependent on the partial pressure of the oxygen gradient
A brief overview of the production process for *Arenicola marina* and industrial production of *Arenicola marina*-derived products was provided by Dr. Zal. He noted that the products can be easily reconstituted with deionized water.

Dr. Zal went on to delineate the different clinical applications of HEMO2life®. The first application of HEMO2life® (Hemarina-M101, or M101) in organ and tissue preservation was in a large white pig kidney autotransplantation model. Dr. Zal presented cell protection data, demonstrating lactate dehydrogenase (LDH) release and metabolic activity for 24-hour cold-stored LLC-PK1 cells. He reported decreased LDH and increased metabolic activity in cells treated with higher concentrations of M101, indicative of product-enhanced cell preservation (Figure 28). In a kidney transplantation model, the HEMO2life® treatment group displayed decreased creatinemia (Figure 29) and tissue fibrosis.

![Figure 28. HEMO2life® Enhances Cell Preservation](image-url)
Dr. Zal indicated that HEMO2life® is under evaluation as an additive to organ preservation solutions for kidney transplantation in the OXYOP clinical trial in six clinical centers in France. He presented OXYOP data for auto-transplantation of pig kidneys and human kidney transplantation, highlighting the significant decreases in creatinemia in both scenarios with HEMO2life® supplementation (Figure 30). Dr. Zal noted that HEMO2life® is a class III medical device and is hopeful it will be on the market by the end of 2017. He highlighted that the product is implemented in lung, heart, Langerhans islets, and liver preservation.

The HEMOXYSraxier® mechanism of action was reviewed by Dr. Zal, highlighting its implementation in ischemia and reperfusion. He stated that the product improves the microcirculation oxygenation and ischemic lesions and emphasized that this effect is not
diminished by vasoconstriction or microcirculation impairment, as seen with other HBOC products. Dr. Zal explained that HEMOXYCarrier® possesses antioxidative properties, which limit reactive oxygen species formation once reperfusion occurs. He identified the following as priorities for HEMOXYCarrier® use:

- SCD
- Stroke
- TBI
- Elective and emergency surgery
- Adjuvant in cancer therapy

Dr. Zal indicated that several strong proof-of-concept outcomes were obtained during preclinical trials for HEMOXYCarrier® in vivo, highlighting the improved oxygenation of hypoxic tissue following IV administration of the product, including brain tissue oxygenation in a rat TBI model. He noted that a similar effect is expected in tissues submitted to ischemia during vaso-occlusive crises in SCD, which could prevent further acute events and irreversible neuronal damage. Preclinical trial data also indicated that HEMOXYCarrier® had nonsignificant vasoactivity at both the macro- and microcirculatory levels and promising results from biodistribution analyses.

Dr. Zal reviewed the upstream process results for HEMOXCell® targeted toward improving oxygenation of cells used in HBOC production. Dr. Zal reviewed several solutions to date, including the utilization of new cell lines with reduced sensitivity to oxygenation. In conclusion, Dr. Zal presented the effects of HEMOXCell® on Chinese hamster ovary cells, demonstrating increased cell density, cell viability, and protein production.

Discussion

Presenter and meeting attendee comments and questions addressed the following Session VI-related topics:

- **Hb reactivity and CO binding to SANGUINATE™**: It was noted that SANGUINATE™ is vasoactive, and that it maintains vasculature to prevent vasoconstriction. It was explained that CO is immediately taken up by RBCs upon its release from the molecule. Once SANGUINATE™ hits the circulation, this process occurs in the blood stream within a matter of minutes.

- **Clinical development plans**: During discussions regarding the clinical development plans for the in-hospital trial when blood is not an option, it was indicated that the focus is anticipated to be on mortality in the trauma trials, specifically 30-day and 70-day mortality. A follow-up question queried the length of transit time for the Australian trial, prompting the response that the Australian trial is expecting transit times more than 30 minutes, or around 50 minutes. It was noted that stratification by transit time may be strategic, given the importance of transit time to outcomes.

- **THb versus mortality in PolyHeme trauma trials**: Concerns were raised with respect to the THb versus mortality in the PolyHeme trauma trial data, which demonstrate increased Hb concentrations in the control population. It was clarified that the data are an
average and that some populations most likely had much lower Hb in the control population in comparison with the PolyHeme population.

- **Study design for OXYOP clinical trials:** Queries with respect to the study design for the kidney transplantation in the OXYOP clinical trial were noted. It was clarified that 60 kidneys in kidney failure were grafted for *ex vivo* studies, followed by the addition of roughly 30 mL of product. It was noted that some of the kidneys were washed and some were not, which depended upon the availability of the surgeon’s time during the procedure. During discussions regarding the degree of rigorous investigation of the protein in use, it was clarified that the primary protein sequence has been examined and published, and no immunogenicity effects have been detected in animals.

- **Lugworm Hb use in tumor environment:** A question on the kinetics and efficacy of IV administration of lugworm Hb for oxygenation of tumors was raised. It was clarified that tumor cells were marked with a glut1 marker and then injected *in vivo*. Tumor oxygenation was demonstrated, and oxygen transfer to the tissue was confirmed.

- **HEMOXYCarrier® biodistribution and clearance from body:** It was noted that no toxic interactions were detected, and that these data, as well as the crystalloid structure of the molecule, have been published. Briefly, the molecule is 156 monomers connected by disulfide bridges.

### Session VII. Regulatory Paths and Issues

**Panel Discussion**

**Panelists:**

RADM Carmen Maher, FDA; Dr. Victor Baum, FDA; Dr. Steven Gould, The Gould Consulting Group; Dr. Bruce Spiess, University of Florida, College of Medicine; Dr. Aryeh Shander, Englewood Hospital and Medical Center; and Dr. Toby Silverman, Parexel International

**Overview**

During this discussion, it was emphasized that the core focus of the meeting is the reappraisal of oxygen carriers as adjuncts for resuscitation for prolonged field care. The main interest remains in the mitigation of the underlying toxicity mechanisms. Historically, the FDA’s perspective was that a study of trauma in hemorrhagic shock was necessary. However, the ability to obtain informed consent was an issue, which led to the necessity for hospital studies. The following fundamental questions were raised:

- Is there a scientific consensus that trauma studies with delays in blood transfusions are infeasible?

- Are there credible bases to extrapolate from studies in other settings outside of the hospital, where oxygen carriers can be compared to other resuscitation methods?
• With respect to the patients who will not accept blood products, should there be a one-arm prospective study with a historical control?

Discussion

Presenter and meeting attendee comments and questions addressed the following Session VII-related topics:

• **Feasibility, logistics, and ethics of conducting oxygen carrier trauma trials:** With consideration of the current environment and history of short transit times in urban settings, concerns regarding ethics, feasibility, and logistics of conducting a trauma trial were expressed. A few of the panelists believe that, given the evidence suggesting a trend to better survival with early intervention, a trauma trial may be feasible with the appropriate endpoints. It was noted that a primary outcome of 30-day mortality decreases a study’s chance of producing positive outcomes. From a DoD perspective, it was noted that feasibility of conducting a clinical trial featuring long transport times that meets ethical guidelines would be logistically impossible. It would be difficult to obtain approvals to mount a study where most patients with long transport times are spread out over vast distances across the country.

• **Delivery of oxygen carrier products to rural environments:** Concerns were expressed regarding the difficulty of delivering the products to rural sites where injury has occurred, as well as the issue of properly training the personnel caring for patients to administer the product. After further discussion, several of the panelists expressed strong skepticism regarding the feasibility of a trauma trial for the utilization of oxygen carrier products for pre-hospital use that can be a potential study with a reasonable population.

• **The importance of non-randomized data in making informative, clinical decisions:** Such data include experience on available situations, which are carefully recorded, tracked, and analyzed, and become evidence. However, it was noted that the challenge of variation in data aggregation becomes problematic. Additionally, the uncertainty of the benefits and risks associated with the use of a product in another setting, although the use of the product is legal, was acknowledged. From the perspective of the Office of Counterterrorism and Emerging Threats, it was noted that within the context of public health, electronic health records and advances in technology that can be coupled to generate more data and are being investigated. It was noted that these techniques can be of great value for this discussion.

• **Use of maternal hemorrhage populations in oxygen carrier clinical trials:** It was questioned whether data provided from maternal hemorrhage could influence or be used in oxygen carrier clinical trials. It was noted that although this is a potential surrogate population, the required level of trial and data management may not be available in remote locations, which would be problematic.

• **Use of non-US clinical trial data for FDA approval:** It was noted that nothing prevents the FDA from utilizing non-US data in approval processes, except for two constraints: the evidence must be ethically and scientifically analyzable. The extent of FDA concern was questioned regarding a study that may be presented in a foreign country, assuming the study is successful, performed at a reputable institution, and is comparable to the US, yet its population does not reflect that of the US with respect to the
various minorities being different. The response indicated that a judgement call would be engaged, noting that it comes down to the sub-setting and how comparable it is to the US. The importance of assessing such factors was highlighted, and it was noted that efforts would be made in distinguishing the relevance. An additional approach is to grant the approval, followed by conducting a well-structured Phase IV trial, depending on the questions to be addressed, in populations where variation is expected.

- **Expanded access program use:** A comment was provided regarding the use of electronic health records, noting that real-world evidence should be considered when identifying the data points of interest for extraction from the records. It was noted that data points are to be extracted from a Phase IV trial, followed by an assessment of whether, from system to system, the data can be extracted, highlighting that not all electronic health record systems are well-integrated.

- **Use and design of early efficacy HBOC clinical trials:** The efficacy of early drug intervention in some of the HBOC clinical trials was mentioned, and it was noted that this model is available in emergency room settings where data collection can be properly accomplished. It was questioned whether field-based trials would be required for FDA product approval if a similar model can be repeated to show clinical benefit in a hospital setting. An FDA representative stated that non-inferiority or superiority would need to be presented, noting that it comes down to the question of therapeutic potential. It was indicated that the FDA would most likely not reject a trial with this design. However, ethical concerns regarding conduction of a trial in the hospital, as well as one in the field without the availability of RBCs, were evident.

- **Use of historical controls in oxygen carrier clinical trial design:** Given the natural history of severe anemia and refusal of transfusions, as well as when transfusions are available, it was questioned whether a trauma trial is adequate in terms of comparing the use of these products for when blood is not an option with historical controls. It was noted that historical controls were previously addressed and required a large treatment effect. Additionally, it was mentioned that the clinical data are quite poor. Limitations regarding the absence of a treatment protocol and lack of information for optimal patient treatment in hospitals for expanded access were noted. Inclusion of an emergency IND (eIND) into the industry sponsor expanded access program was recommended for better control in patient treatment and follow-up. Inquiries regarding the use of natural history data, as well as the need for a prospective one-arm study to understand the care applied and if it matches the controls were brought forward. It was noted that the general principle would be to have a well-designed prospective, single-arm study to provide oxygen carriers to population matched with the historical cohort in cases where blood is not available. However, approval for hospital use and extrapolation to the field were questioned.

- **Inclusion of mortality endpoints in clinical trials:** The feasibility of conducting an adequately sized clinical trial including mortality endpoints was discussed. It was noted that based on the power analysis, a powered study for mortality would not be feasible.

- **Inclusion of a rescue arm in clinical trials:** It was mentioned that if substantial knowledge exists regarding the life-saving potential of HBOC for patients refusing blood products, then a prospective, randomized trial should be conducted, at least as a rescue
arm. However, statistical difficulties with the inclusion of a rescue arm were noted, and it was determined that this option is not feasible.

- **Non-inferiority to blood as validation for the therapeutic use of oxygen carriers:** The panelists and meeting attendees discussed whether validation of oxygen carrier products as non-inferior to blood was sound rationale for use of these products when blood is not an option. The importance of balancing risks with benefits in medical centers with the intent of use was stated. It was also noted that if this line of thought was to be pursued, data from past trials could be reanalyzed to determine non-inferiority if detailed records were available. In response, it was explained that expanded access data records may be grossly inadequate. Those treating patients and their sponsors were encouraged to obtain adequate data and send the data to the FDA to allow for review in a meaningful fashion.

- **Military versus on-label use of products:** In discussions regarding consideration for oxygen carriers to be brought to Warfighters with the intent to gather information from this population, it was noted that the policy is to use on-label products and that military-only use is not favored.

- **Animal HBOC data:** It was questioned whether the animal population would be an appropriate means to collect additional data, since there is an approved HBOC for animal use. It was noted that the animal studies did not predict the toxicity issues seen in humans. However, depending on how closely the animal model mimics the real situation, additional animal data would be helpful in addressing risk mitigation. Additionally, it was noted that the animal efficacy rule is implemented only when human efficacy studies are not feasible or ethical.

### Session VIII. Perfluorocarbons

**Perfluorocarbon Enhanced Gas Transport: Overview**

*Bruce D. Spiess, MD, FAHA, Department of Anesthesiology, University of Florida*

**Overview**

At the start of the session, Dr. Spiess introduced PFC oxygen carriers. He noted that much can be learned from PFCs and the changes they illicit in mammalian physiology. Although he felt that PFC emulsions could be a definitive therapy for tissue ischemia, he also mentioned their therapeutic potential in trauma or resuscitation. Early development of PFCs was pursued by Green Cross in Japan, and the only artificial oxygen carrier approved by the FDA is the PFC Fluosol-DA-20%. Dr. Spiess emphasized that PFCs should not be viewed as a blood substitute, but rather as an enhanced oxygen carrier between RBCs and target tissues, that can complement HBOCs. He noted that oxygen transport via PFCs is fundamentally different than Hb-based oxygen transport and that PFCs can deliver oxygen with little or no flow of blood in microcirculation. Additionally, PFCs can transport other gases, such as nitrogen and NO. Dr. Spiess noted many treatment indications for PFCs, including air embolism (e.g., from a blast...
injury), decompression sickness (DCS), cerebral arterial gas embolism, carbon monoxide poisoning, and acute respiratory distress syndrome (ARDS).

**Critical Oxygen Delivery: The Sweet Spot**

**Kevin R. Ward, MD, Center for Integrative Research in Critical Care, University of Michigan**

**Overview**

During his presentation, Dr. Ward provided an overview of critical aspects of oxygen delivery and noted that these considerations are applicable to both PFCs and HBOCs. He encouraged the audience to think of blood as an organ, particularly as it is related to combat casualty care. Blood is made up of several components and can fail like other organs do. Oxygen carriers should be evaluated on their ability to prevent and reduce the effects of hemorrhage and oxygen debt, such as endotheliopathy and coagulopathy (Figure 31).

![Figure 31. The Effects of Oxygen Debt on Cellular Processes](image)

**Repaying the Oxygen Debt**

Dr. Ward reviewed the biphasic relationship between oxygen delivery and consumption and noted that many things can occur while VO2 is maintained. However, at a certain point, oxygen delivery no longer meets the demands of the tissue, and the VO2 becomes delivery independent. Oxygen uptake varies among individuals and represents a moving target. Dr. Ward described indirect calorimetry methods to measure oxygen debt and noted that it can take 60 minutes to return to normal VO2 and “repay” the oxygen debt after 4 minutes of exercise. The extent of organ compromise, failure, or death after an injury depends on the time it takes to repay the oxygen debt. Dr. Ward cautioned that the choice of marker to monitor oxygen debt and resuscitation is important, noting that lactate is commonly used but may show normalized levels...
before the oxygen debt is fully repaid. He also noted that it is important to understand what part of oxygen debt is related to coagulopathy. A better understanding of the role of oxygen carriers could lead to a reduced need for blood components to address oxygen debt and the related manifestations (Figure 32).

Dr. Ward concluded by saying that oxygen carriers should be evaluated on their ability to repay oxygen debt and reduce tissue hypoxia, reduce coagulopathy, and reduce the need for traditional blood components. He also noted that oxygen carriers present opportunities as carriers of other therapeutic gases or agents to modulate oxygen uptake.

Perfluorocarbons and Dissolved Oxygen-Enhanced Diffusion

Bruce D. Spiess, MD, FAHA, Department of Anesthesiology, University of Florida

Overview

Dr. Spiess described the potential for the enhanced diffusion of oxygen using oxygen carriers such as PFCs. Oxygen transport is not just a matter of how much oxygen is carried by Hb, but also includes the release of oxygen from Hb and delivery to the target tissues. Total oxygen content can be measured, but it is difficult to measure how easily the oxygen is released in the final delivery phase. PFCs have enhanced oxygen diffusivity and can overcome resistance to oxygen movement.

Hb as an Oxygen Buffer

Dr. Spiess discussed the hypothesis that Hb evolved as an oxygen regulator to detoxify oxygen rather than as an oxygen carrier and noted that Hb binding to oxygen is 100,000 times tighter than the release of oxygen. This allows Hb to act as a buffer and ensure that the tissue is not flooded with oxygen, which is toxic in high doses (Figure 33).
PFCs Enhance Solubility and Transport of Gases

Unlike HBOCs, PFCs are not protein-based and are centered on the carbon-fluorine bond, which is the highest energy carbon bond available. Dr. Spiess described the timeline of PFC development and mentioned that fluorinated oils were used in the Manhattan Project to buffer uranium-238 when physicists noticed large amounts of dissolved oxygen in the oils. This launched work into the use of oxygenated PFC liquids for breathing. Currently, PFCs for IV use are formulated in micelles surrounded by a phospholipid surfactant. Dr. Spiess emphasized that the total oxygen binding is less important than the enhanced diffusion of oxygen through plasma. PFCs can enhance oxygen diffusion through plasma up to 50-fold. In addition to enhancing oxygen solubility, PFCs also enhance the solubility of nitrogen and other non-polar gases, especially at cooler temperatures. PFCs are a fraction of the size of RBCs and can fill in between them, thereby increasing the gas transport surface area. Dr. Spiess briefly described how PFCs can attenuate reperfusion injury by reducing white cell rolling and adhesion to endothelial cells after ischemia and reperfusion. Finally, Dr. Spiess presented the advantages and disadvantages of PFC emulsions. The advantages of PFCs include easy transport of oxygen to the body, increased solubility of oxygen in plasma, and buffers factors like pH and temperature minimizing their effects in blood circulation. Some disadvantages of PFCs include allergic reaction to 1st generation emulsions, decreased platelet counts, high pulmonary pressure and flu-like symptoms.

Perfluorocarbons, Oxygen Transport and Microcirculation in Low Flow States

Ivo Torres, MD, PhD, US Army Institute of Surgical Research

Overview

Dr. Torres provided an overview of his work with PFCs as a transport mechanism for oxygen delivery to the microcirculation in both in vivo and in vitro systems. He detailed the risks and benefits of using PFCs as an oxygen carrier and presented promising results suggesting that
therapeutic PFC administration could benefit SCD patients and those with arterial gas embolism (AGE) or ischemia.

**In Vitro and In Vivo Models of PFC Oxygen Transport**

Dr. Torres began by describing an *in vitro* system to determine how much oxygen was offloaded with and without the use of PFCs (Figure 34). He found that normal human blood and blood from SCD patients showed increased oxygen extraction when mixed with PFCs.

Next, he presented studies looking at the effects of PFC on oxygen delivery in ischemia *in vivo* where perfusion and oxygenation were measured using phosphorescence quenching before and after injection of PFC. In addition, laser-based measurements captured the oxygen partial pressure (PO₂) values of the tissue and inside the vasculature. The data presented indicate that oxygenation can be restored with the use of PFCs after occlusion.

Dr. Torres also described work investigating the effects of arterial gas embolisms. Air bubbles were introduced into the femoral artery and tracked in the tissue. The appearance and disappearance of bubbles was measured over time, and the oxygenation of tissue locations was tracked as a result of bubble location. For studies of massive embolisms, the blood flow velocity and oxygenation were measured before and after PFC injection. Bubbles were reabsorbed as a function of PFC treatment, and blood flow was restored as were tissue and arteriolar PO₂ values (Figure 35).
Figure 35. Recovery of Tissue (top) and Arteriolar (bottom) PO\textsubscript{2} with PFC Treatment Following (Arterial Gas Embolism) AGE

Overall, Dr. Torres presented data suggesting that PFC emulsions can enhance oxygen transport in blood from healthy individuals and from patients with sickle cell disease. In addition, use of PFC emulsion showed improved oxygenation following localized tissue ischemia and appeared to increase air bubble absorption in models of arterial gas embolism leading to enhanced blood flow and oxygen delivery.

Effect of Intravenous Infusion with Perfluorocarbon Emulsions on Platelet Number and Function

Bruce D. Spiess, MD, FAHA, Department of Anesthesiology, University of Florida

Overview

To end Session VIII, Dr. Spiess discussed the regulatory hurdles to PFC use and concerns over potential effects of PFC emulsions on platelet count and function. Challenges and unknowns the PFC field still faces include the question of whether PFC use is associated with higher risk of stroke occurrence and platelet dysfunction or death.
Regulatory Hurdles in PFC Use

Dr. Spiess provided a summary of issues facing the use of PFC in human clinical trials. He noted that there are reports of decreased platelet counts in the days following infusion with PFC as well as reports of platelet function being compromised after administration of PFCs. One study in activated porcine platelets treated with the PFC perflubron reported that platelet aggregation stimulated by arachidonic acid was inhibited. However, these results were dose-dependent, and, upon stimulation with other agents that stimulate platelet aggregation (e.g., Fluosol and Intralipid), inhibition of platelet aggregation was found to be inconsistent in the presence of PFCs.

There were also concerns about higher levels of stroke in the PFC-treated group of a hemodilution trial in humans, although it was noted that the groups were treated unequally and it is unclear whether the risk of stroke is directly related to PFC use. FDA officials were concerned that PFC use had increased the risk of micro-thrombus emboli. Dr. Spiess believed that a confounding factor in this study may have been the use of Hetastarch as an euvoletic infusion in combination with PFC. It is unknown whether Hetastarch interacts with PFCs or whether these potential interactions could have deleterious health effects. Additionally, transcranial Doppler (TCD) signals, which are used to monitor the occurrence of cerebral arterial emboli, were observed with PFC infusion. However, Dr. Spiess noted that those do not necessarily correlate with PFC-platelet emboli.

Dr. Spiess described additional studies that have been performed in sheep, which is one of the most sensitive species to platelet perturbation. These studies showed a transient drop in platelets in the first 24 hours following PFC infusion (Figure 36), and platelet aggregation seemed unaffected. Dr. Spiess mentioned that despite meetings with the FDA, human trials with PFCs remain halted.

![Figure 36. The Change in Platelet Numbers in Sheep Following PFC Infusion](image-url)
PFC Panel Discussion

Presenter and meeting attendee comments and questions addressed the following Session VIII-related topics:

- **Comparison of HBOCs and PFCs:** Meeting attendees discussed the lack of direct comparisons between HBOCs and PFCs. It was noted that HBOCs and PFCs should be evaluated under the same conditions to determine how they compare in terms of oxygen delivery. It was also noted that these products will not affect diffusion of oxygen only enhance the transport and delivery of it. In addition, the combined use of HBOCs with PFCs could mitigate toxicity and enhance efficacy.

- **Potential effects of PFCs on physiology and the immune system:** Meeting attendees wondered about the effects of PFCs on the immune system. There has been some circumstantial evidence that shows a reduction in overall white cell activation after PFC administration without an increase in antibody formation. Despite being taken up by macrophages and monocytes, PFCs do not appear to activate these cells nor do they seem to increase antibody formation. In terms of affecting physiology, PFCs dissolve many gases including NO and there is evidence that PFCs interact with endothelial cells by rolling and interacting with the glycocalyx. However, it is unknown whether this affects the biology of microvessels.

- **Potential for PFC-mediated oxygen delivery in low/no flow conditions:** Meeting attendees were excited about the potential to rescue tissue oxygenation with PFC administration in patients with low or no blood flow.

- **Effects of PFCs on platelets:** It was noted that the baboon studies indicating possible effects on platelets were conducted in animals that had been used in other studies. No drop in platelet count was observed when the experiments were repeated with naive baboons.

### Session IX. Human Trials

**Perfluorocarbons: Early Work**

**Steven Hill, MD, University of Texas Southwestern Medical Center**

*Overview*

Dr. Hill introduced the early clinical work with PFCs, noting that PFC compounds have been known since the 1960s to be effective oxygen carriers and increase the solubility of oxygen in aqueous solutions. The only FDA-approved PFC oxygen carrier, Fluosol-DA, was developed by Green Cross Corporation of Japan, but the data describing the pharmacokinetics in humans are largely unavailable outside of company records.
Fluosol-DA Characteristics

The molecule is composed of a 7:3 ratio of perfluorodecalin to perfluorotipropylamine. The use of perfluorodecalin shortens the oxygen retention time, whereas perfluorotipropylamine increases the miscibility of the molecule. A solution carrying 20% Fluosol-DA is able to carry a 7.2% volume of oxygen at 37°C in contrast to whole blood with an average Hb concentration, which carries a 21% volume of oxygen at 37°C. The circulating half-life of this product is 24 hours, but the elimination half-life of perfluorodecalin is 7.2 days and that of perfluorotipropylamine is significantly longer. Fluosol-DA as an emulsion is unstable at room temperature and needs to be stored in a frozen state. For use it requires thawing and sonication to emulsify the solution however, the toxicity of the emulsifying agents limits the amount of infusion. Studies have shown the latter compound to be retained by the reticuloendothelial system for greater than 4 months.

In addition to uptake by the reticuloendothelial system, Fluosol-DA use is associated with other AEs. Patients infused with Fluosol-DA exhibit suppression of phagocytic mechanisms for more than 5 days after infusion. Fluosol-DA also activates complement in up to a third of patients due to its pluronic F-68 component.

Fluosol-DA Clinical Use

A summary of case reports on Fluosol-DA available in the literature indicates increased blood oxygen levels in patients administered Fluosol-DA; however, no outcome data are available. Fluosol-DA was approved for high-risk angioplasty; however, the formulation was difficult to use due to instability at room temperature and the toxicity of the emulsifying agents. The use of autoperfusing angioplasty catheters has rendered Fluosol-DA in angioplasty obsolete. Companies have since developed improved PFC formulations that can carry a higher percentage of oxygen, but FDA approval has not been granted.

Perfluorocarbons: Emulsions in Orthopedic Surgery

**Donat Spahn, MD, Institute of Anesthesiology, University Hospital Zürich, Switzerland**

Overview

Dr. Spahn summarized the knowledge gleaned from the use of perflubron emulsions in orthopedic surgical trials. He emphasized that there is an urgent need for PFC products in a variety of trauma-related scenarios (e.g., prolonged patient transfers or emergency room use). He also thought that PFCs can provide a bridge to or delay the need for whole blood transfusions in a variety of trauma or surgical scenarios (e.g. in the treatment of Jehovah’s Witness patients). Potential applications for PFC emulsions included cardiac, gut, and cerebral ischemia, sickle cell crisis, organ preservation, and oncological tumor oxygenation.
**Perflubron Emulsions in Orthopedic Surgery Clinical Trials**

Dr. Spahn provided an overview of studies investigating the use of the PFC perflubron emulsion Oxygent™ in orthopedic surgery. Once patients enrolled in the study reached transfusion trigger criteria, they were randomized into one of four treatment groups (Figure 37). Dr. Spahn noted that the use of Oxygent™ delayed blood transfusions in the orthopedic surgery patients. Although lower platelet counts were observed in the 1-5 days following administration, there was no trend toward more infections in patients administered with the perflubron emulsion. Dr. Spahn mentioned that an increase in fibrinogen 2-5 days post-surgery could contribute to the drop in platelet count.

A subsequent Phase III study was conducted with perflubron in patients undergoing major non-cardiac surgery with expected blood loss of 20-70 ml/kg. Patients were split into two treatment groups once transfusion trigger criteria were reached: one group received the standard of care, and the other received acute normovolemic hemodilution (ANH) augmented with perflubron. The oxygen carrier was given to offset the drop in Hb that accompanies ANH. Compared to the control group, the augmented ANH with perflubron resulted in a reduction of allogeneic blood transfusions in non-cardiac surgeries with a blood loss of 10 ml/kg or more. Dr. Spahn noted minimal AEs related to perflubron use, indicating that the only one of significance was a reduction in the number of platelets from postoperative day 1 to postoperative day 7 without coagulopathy. He believes that the use of PFCs during surgical interventions remains a promising strategy and has the potential for use in many different surgical and trauma scenarios.
Perfluorocarbon as a Therapy for Spinal Cord Injury and Traumatic Brain Injury
Bruce D. Spiess, MD, FAHA, Department of Anesthesiology, University of Florida, on behalf of Bruce Mathern, MD

Overview

Dr. Spiess discussed the use of PFCs in treating spinal cord injury (SCI) and TBI. He noted that PFCs have a unique potential to address issues in neurological tissues. For example, air bubbles are a common problem in cardiopulmonary bypass, and many patients exhibit neurological deficits after surgery. TBI is a large problem in civilian and DoD medicine, and the complexity of the injury poses challenges for treatment, from oxygen supply issues in the microvasculature to shearing of axons and edema (Figure 38).

Use of PFCs to Treat TBI and SCI

Dr. Spiess presented work using a blunt TBI model where treatment with PFCs rescued mitochondrial function by 40-60% and spared brain tissue by 40%-70%. Increased brain oxygenation was observed with PFCs in cases of subdural hemorrhage. Dr. Spiess described a severe traumatic brain injury trial, VCURES of 9 patients with a closed-head injury (non-penetrating trauma). Data from one patient in the study showed brain oxygen levels increasing for several days following PFC administration; the patient was alert and was discharged on day 16 after injury. Out of the nine patients receiving PFC seven survived (one patient withdrew from the study), demonstrating a decrease in mortality compared with a historical cohort with 40-60% mortality. All seven patients were neurologically intact at time of discharge and only 1
had mild impairment at 6 months following injury. Historically, there is usually a 40-60% neurologic deficit following this type of TBI and 10-15% of patients require a long care facility.

Studies of a rat blast TBI model indicated that animals receiving PFCs had better magnetic resonance imaging (MRI) data - reduced cytotoxic edema in the whole brain or hippocampus, and better balance and beam walking abilities than the control group. In SCI rodent studies, treatment with PFCs improved bladder function in rats with SCI and increased oxygen delivery to the spinal cord. PFC treatment also improved gross hindlimb function measured using the Basso, Beattie, and Bresnahan scale and angle of incline motor functions in a dose-dependent manner. Animals treated with PFC did show decreased lesion size and white matter sparing, qualitatively. Dr. Spiess noted that treatment with PFC reduced caspase-3 and TNF-α activity and ERK 1/2 expression, indicating a reduction in apoptosis. Overall, results suggest that PFCs could improve neurologic oxygenation.

Perfluorocarbons: Cardiac Surgery

Steven Hill, MD, University of Texas Southwestern Medical Center

Overview

Dr. Hill related outcomes from clinical trials run concurrently in the US and Europe to investigate the use perflubron in cardiac surgery. He noted that the studies did not achieve significance in their primary endpoints, but the results suggested that perflubron use may have prevented or delayed the need for blood transfusion. He concurred with earlier presenters that training in the appropriate administration of oxygen carriers was imperative to avoid AEs.

Use of Perflubron in Non-Cardiac and Cardiac Surgery

Dr. Hill provided an overview of simultaneous trials run in Europe and the US investigating PFC use to augment ANH in high-blood loss general surgery (Europe) and coronary artery bypass grafting (CABG) with cardiopulmonary bypass (CPB) (US). The European non-cardiac surgery study described by Dr. Spahn in his presentation was a part of the trials. In this single-blind randomized clinical trial, a perflubron emulsion was evaluated for its ability to supplement preoperative ANH for preventing or reducing the need for blood transfusion in patients undergoing general surgery. Findings revealed similar Hb concentrations in both the perflubron-treated and control groups, but there were more SAEs in the PFC group. Further, the use of perflubron helped avoid or reduce the amount of transfused blood required for these surgical patients.

In the Phase II cardiac surgery study, a perflubron emulsion was used to augment the harvesting of autologous blood. Dr. Hill mentioned that the primary endpoint in this study was the percentage of patients that reached a transfusion trigger during CPB, and no significant difference was observed between those receiving perflubron and the control group. However, there was a significant difference between the group receiving the higher dose of perflubron and controls by the end of surgery, and this was maintained 5 days post-surgery. There was a trend suggesting that use of perflubron was effective in avoiding triggers for transfusion, and Dr. Hill commented that the trial would have been positive with a different endpoint. In another trial
with Perfluorocarbon Emulsion, cerebral blood flow and cerebral emboli were measured for all
patients in a cardiac trial. Contrary to expectations, cerebral blood flow was increased in patients
treated with perflubron despite the increased oxygen-carrying capacity that accompanied the use
of PFCs. In addition, the number of cerebral emboli was also increased in the PFC group. Dr.
Hill noted that although follow-up studies were recommended, none have been performed to
date.

Russian Use of PFC – In Clinical Use Now

Maria Irwin, MD, PhD, University of Florida College of Medicine

Overview

Dr. Irwin provided historical background on the use of Perftoran as an anti-hypoxic and anti-
ischemic plasma extender in Russia. She emphasized that the data generated were not from
clinical trials, but were observations collected over the course of 30 years of application of this
emulsion. She cited the stability, safety profile, and lack of alternative therapeutics in the
absence of blood as sound justification for the administration of Perftoran in patients.

Perftoran Use in Russia

Dr. Irwin presented an overview of observational data from Russia where the PFC Perftoran has
been used and studied. Perftoran is a PFC that contains two oxygen-carrying moieties
formulated as an emulsion. She noted that there are no randomized clinical trials, and the reports
are only published in Russian; however, Perftoran was administered to over 900 patients during
clinical trials from the 1980s to 1990s. After it was approved by the Russian Pharm Committee
3,528 patients have been administered between 1997-2004.

Dr. Irwin reviewed a list of conditions appropriate for the use of Perftoran, emphasizing that this
treatment is not an artificial blood substitute but an anti-hypoxic and anti-ischemic plasma
expander. It improves oxygen delivery as well as microcirculation and blood rheology.
Perftoran can be administered intravenously as well as locally, for example, to irrigate wounds.

The major indication for Perftoran is microcirculatory ischemia, and it has also been used in
peripheral vascular disease and organ preservation during apnea testing. The overall rate of side
effects is reported between 1.8% and 8%, and Dr. Irwin noted that the complication rate is
related to improper storage and defrosting techniques. Trials evaluating Perftoran in trauma
reported faster normalization of tissue perfusion, resolution of metabolic acidosis, and improved
organ function in the PFC group. In cardiac surgery trials, Perftoran decreased arrhythmias,
improved contractility post-bypass, and increased pulse pressure. In cases of peripheral vascular
disease, Perftoran treatment improved tissue oxygenation (Figure 39), lowered lactate levels,
reduced blood viscosity and RBC trauma, and increased blood flow and skin temperature. Pain
relief and functional improvement were reported up to a few months after treatment. Dr. Irwin
noted that other indications include brain and spinal cord trauma, acute and chronic infections,
microvascular surgery, and sickle cell anemia. Overall, Perftoran has been in clinical use in
Russia for roughly 30 years with a good safety profile. Dr. Irwin concluded by saying that
further investigation into Perftoran is warranted given the history of use.
NuvOx Pharma Saves Lives with Nanotechnology Oxygenation

Evan Unger, MD, FACR, President and CEO NuvOx Pharma

Overview

NuvOx Pharma is a clinical stage pharmaceutical company that supports clinical Stage I and Stage II research and develops IV oxygen carrier therapeutics based on dodecafluoropentane (DDFP, C5F12) emulsion (DDFPe) technology. Potential indications of DDFPe include severe hemorrhage, radiation-resistant cancer, and TBI.

Characteristics of DDFPe

Dr. Unger provided an analysis of characteristics of DDFPe molecules developed by NuvOx Pharma. DDFP is mixed with an emulsifier and additives to generate DDFPe. DDFPe carries over 100 times more oxygen per unit weight than other higher molecular weight fluorocarbon oxygen carriers, resulting in a lower dose needed for treatment. The emulsion can be injected
intravenously, has a terminal half-life of 90 minutes, and the DDFP and emulsifier components are cleared via exhalation and the hepatobiliary system, respectively.

![Figure 41. Phase Ib/II Glioblastoma Clinical Trial Using NVX-108](image)

**Clinical Use of DDFPe**

NuvOx Pharma has several DDFPe-based oxygen therapeutics in preclinical or clinical studies as seen in Figure 40. In addition, DDFP can be used as an ultrasound contrast solution. NuvOx uses DDFPe-based oxygen therapeutics to treat a variety of conditions, including radiation-resistant cancer, stroke, TBI, and hemorrhagic shock.

The DDFPe, NVX-108, is in a Phase Ib/II clinical trial to increase tumor oxygen levels in the hypoxic brain tumor, glioblastoma multiforme (GBM), thereby increasing tumor sensitivity to radiation therapy. Tumor oxygenation increased significantly (p=0.015) after injection of NVX-108 in patients with GBM, without significantly changing the oxygenation of normally oxygenating brain tissue. Eleven GBM patients were genetically tested to determine whether they would be responsive to tetrazolamide (TMZ) prior to treatment with NVX-108. NVX-108 was administered intravenously 30 times over a 6-week period as patients also received the standard of care chemo-irradiation treatment. Two patients with a genetic profile suggesting they would not respond to TMZ survived to 19 and 21 months. Another patient with a genetic profile indicating response to TMZ is still being monitored after 21.5 months survival (Figure 41).

An additional potential application for DDFPe formulations is cardiac stroke. Animal studies using another DDFPe, NVX-208, can extend the time for which tissue plasminogen activator (tPA) is an effective treatment for stroke from 3 hours to 9 hours. Repeat administration of NVX-208 in rabbits yields an 85% reduction in percent infarct brain volume. A Phase Ib trial has just started looking at repeat dosing of NVX-208 in humans for acute ischemic stroke.
Preclinical studies of another DDFPe in a hemorrhagic shock model, NVX-408, suggest this emulsion could be an effective alternative to the volume expander, Hextend®, when caring for wounded Soldiers in the field. Studies using pigs show 100% survival after 50% blood loss when given NVX-408 compared to 80% when given volume expander or 30% in the control animals. NuvOx Pharma has signed a licensing agreement with a Chinese company, Jiangsu Nhwa Pharmaceutical Co. Ltd, to develop NVX-408 as a drug for perioperative blood loss and hemorrhagic shock in China.

Perftoran/Vidaphor: Introduction into Western Medicine

**Gary Latson, MD, Texas A&M University Health Science Center, College of Medicine; Consultant, FluorO2 Therapeutics**

**Overview**

Perftoran, now known as Vidaphor, is similar in terms of biological activity to other PFCs, but has a much smaller particle size (Figure 42). It is one-third the size of Oxycyte or Oxygent. The smaller size is attributed to fewer side effects and less adverse effects on the immune system. Although the delivery capacity may be less than Oxygent the total surface area of the particles is greater which may result in greater facilitation of oxygen transport. Vidaphor has a favorable risk to benefit ratio and has been given to over 30,000 people, making it the most widely used PFC in humans (Figure 43). This product meets critical needs in many countries that do not have the resources or infrastructure to maintain a large blood supply.

The frequency of side effects is between 1.8 and 6% and include manageable allergic reactions (urticaria, pruritis), increase in heart rate, reduction of arterial pressure, headaches, and difficulty breathing. Its clinical application show effectiveness in anti-shock and anti-ischemic medicine and additional applications in acute or chronic anemia, cranial trauma, cardiac surgery and limb ischemia.
Manufacturing History of Perfloran/Vidaphor

Perfloran was developed by the Russian Academy of Sciences between 1979 and 1995. Clinical application of Perfloran was approved by the Ministry of Health of the Russian Federation in 1996 and industrial manufacturing began in 1997. In 2005, Perfloran was registered in Mexico as Perftec, a blood substitute. Shortly thereafter, manufacturing was moved out of Russia and to a GMP-compliant facility in the US. Once a small amount of investment capital is obtained, manufacturing of Perfloran, which was rebranded as Vidaphor, at FluorO2 Therapeutics can resume. Ultimately, US FDA approval is desired, however, extensive research still needs to be done and may include replicating key studies done in other countries. The most common indication for Perfloran/Vidaphor is blood loss and multiple organ dysfunction (Figure 44). When it is administered to patients with occlusion vessel disease, Perfloran has been shown to
increase circulation within 2 hours.

<table>
<thead>
<tr>
<th></th>
<th>VIDAPHOR</th>
<th>OXYCYTE</th>
<th>OXYGENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFC w/v</td>
<td>20%</td>
<td>60%</td>
<td>60%</td>
</tr>
<tr>
<td>Primary PFC</td>
<td>perfluorodecalin PMCHP</td>
<td>perfluoro tert-butylcyclohexane</td>
<td>perfluoroctyl bromide perfluorodecyl bromide</td>
</tr>
<tr>
<td>Emulsifier (%)</td>
<td>4% Kolliphor</td>
<td>4% egg yolk phospholipid</td>
<td>3.6% egg yolk lecithin</td>
</tr>
<tr>
<td>Mean Particle Size</td>
<td>0.07 microns</td>
<td>0.2 microns</td>
<td>0.17 microns</td>
</tr>
<tr>
<td>Dose</td>
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<td>3 ml/kg</td>
<td>3.5 mL/kg; 200-300 mL</td>
</tr>
<tr>
<td>Average</td>
<td>400 mL</td>
<td>~200 mL</td>
<td>1.8 – 2.7 g/kg</td>
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<tr>
<td>Half-life</td>
<td>14 hours</td>
<td>12 hours</td>
<td>9 hours</td>
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<tr>
<td>Stability</td>
<td>3 years frozen; 2 weeks at 2-8 °C</td>
<td>2 years at 2-8 °C</td>
<td>2 years at 2-8 °C</td>
</tr>
<tr>
<td>Exposures</td>
<td>30,000+</td>
<td>~41</td>
<td>~2000</td>
</tr>
<tr>
<td>Clinical Studies</td>
<td>1, 2, 3, post-market*</td>
<td>1, 2a, 2b</td>
<td>1, 2, 3 (1 of 2 completed)</td>
</tr>
<tr>
<td>Safety Profile</td>
<td>Flu-like symptoms; allergic reactions (incidence of 4-6%)</td>
<td>Flu-like symptoms; significant thrombocytopenia; significant elevation of LFTs; increased clotting times; marked decrease in ability to fend off Listeria</td>
<td>Flu-like symptoms; concern for stroke and neurological complications vs Placebo; bleeding complications; back pain; 20%-30% decrease in platelet activity</td>
</tr>
</tbody>
</table>

Figure 43. Comparison of Vidaphor with Oxyce and Oxygent

The Mexican government has indicated interest in a large initial purchase of Vidaphor, which could then be rapidly available to other Latin American countries due to reciprocal agreements. This would benefit countries that do not have an adequate blood supply or blood donors, lack the infrastructure to handle medical traumas, and/or have limited availability of vascular rehabilitation.
Perfluorocarbons in Trauma

Richard Mahon, MD, Naval Medical Research Center, Henry M. Jackson Foundation

Overview

Dr. Mahon began his presentation by noting the immense potential of PFCs for the treatment of many indications including hemorrhage, ARDS, pseudo-allergic complement activation, and ischemic reperfusion. He reported positive outcomes for the use of PFCs in a few of these disease contexts using model systems. He cautioned that animal model studies need to be carefully designed and extreme care must be taken in determining whether a given animal system is appropriate for testing the condition of interest.

PFC Use in Animal Models

Animal studies show that PFCs increase survival in hemorrhage, ARDS, and ischemia-reperfusion. Rats treated with hydroxyethyl starch (HES) and the PFC perflubron in a hemorrhage model show an increase in local cerebral glucose utilization, cerebral blood flow, and cerebral oxygen delivery compared to HES treatment alone. In a swine controlled hemorrhage model, at 120 minutes survival there was a 43% survival in the PFC treatment group.
and 12% survival in the control group. Oxygen delivery and pulmonary arterial pressure was also significantly increased in the PFC group. In a swine model of ARDS, the PFC Oxycyte was administered before or after oleic acid-induced lung injury (OALI). Increased blood oxygen content and improved lung histology was seen when Oxycyte was administered after OALI. On the other hand, pretreatment with Oxycyte resulted in decreased cardiac output, increased pulmonary hypertension, and ultimately increased mortality. Similarly, IV PFC administration improved functional capillary density and decreased leukocyte activation in a hamster window chamber model of ischemia reperfusion if administered after, but not before, ischemia. Another study using a swine model system to test cardiopulmonary hypersensitivity to PFC treatment showed that increased pulmonary hypertension is a liposomal dose-dependent effect involving complement activation.

Taken together, these studies indicate that using PFCs to treat hemorrhage, ARDS, and ischemia in humans is promising. Caution needs to be taken regarding model systems, with attention to increased pulmonary hypertension in swine models and particle size of the PFC. Some studies also indicate a need for attention to timing regarding delivery of PFCs and assessing experimental endpoints.

Perfluorocarbons in Decompression Sickness Therapy

Richard Mahon, MD, Naval Medical Research Center, Henry M. Jackson Foundation

Overview

It is necessary to have a realistic solution for responding to crisis situations involving disabled submarines. All submariners would be at risk of developing DCS, which is characterized by release of nitrogen from the tissues, bubble formation in the blood, pain in the joints, neurological deficits, and cardiovascular collapse. PFCs seem like a sensible solution to combat DCS.

PFC Applications for DCS

Studies have looked at using PFCs both for the prevention of and therapy for DCS. As a preventative measure for DCS, oxygen pre-breathing (OPB) in combination with IV administration of the PFC Oxygent, at the surface rather than at depth, results in increased survival and decreased incidence of severe DCS in a swine model system. In a therapeutic application, increased DCS survival was observed in rats treated with FC-43 and in swine given a 5 cc/kg dose of Oxycyte at the onset of DCS. Oxycyte given at 3 cc/kg did not show a decrease in mortality, but improved spinal cord pathology was seen at this dose (Figure 45).
A mixed gender animal study revealed a possible relationship between response to PFC therapy for DCS and gender. DCS treatment with Oxycyte followed by delayed recompression in a mixed gender swine study shows decreased survival of PFC-treated females compared to males when subgrouped by gender (Figure 46). No seizures were noted using the US Navy TT6 hyperbaric oxygen protocol in this study. Further investigation into survival differences in gender subgroups reveals that Oxycyte increased pulmonary artery pressure (PAP) in both males and females, but female swine had a more rapid onset of cutis marmorata and earlier onset to maximal PAP. Due to the increased PAP observed in swine and the need to further explore the gender response to PFCs, a male sheep model has been developed and shows a possible survival benefit when given 5 cc/kg of Oxycyte to treat DCS.
PFCs show a survival benefit in treatment of DCS and multiple animal models show a survival benefit when PFCs are used as a preventative measure for DCS. Using Oxybyte to treat DCS has shown no increase in seizures, acceptable platelet levels, and can be used prior to recompression. PFCs would be useful in crisis situations involving disabled submarines. The focus needs to be on obtaining a viable drug product to seek FDA approval and conducting human safety studies.

Inhaled Perfluorochemicals: Intrapulmonary Delivery for Lung Protection and Nasopharyngeal Aerosolized Delivery for Preferential Brain Cooling for Neuroprotection

Marla R. Wolfson, MS, PhD, Lewis Katz School of Medicine, Temple University

Overview

Dr. Wolfson summarized the applications for PFCs in cooling various organ systems in patients to reduce sequelae and complications associated with disease and injury scenarios. She provided evidence that intrapulmonary delivery of PFCs affords mechanoprotective and cytoprotective mechanisms to temporize the lung against acute lung injury, supports gas exchange, and provides an adjunctive delivery method for biologics. Using an intranasal aerosolized PFC, she also demonstrated that PFC treatment can support rapid and preferential brain cooling for neuroprotection without instrumentation encumbrances and systemic complications of whole body cooling.

Inhaled PFCs for Treatment of ARDS and Lung Injury

There are two mechanisms for the delivery of inhaled PFCs: intrapulmonary and nasopharyngeal. Intrapulmonary delivery of PFCs to treat respiratory distress in infants and adults, as well as traumatic lung injury (TLI), improves pulmonary compliance and provides adjunctive delivery of biologics compared to mechanical ventilation alone. Intrapulmonary delivery of PFCs includes tidal liquid ventilation (TLV) and partial liquid ventilation (PLV). Using TLV, all gas-liquid surface tension is removed in the lung and the lung is protected from inflation pressures. PLV is a form of liquid breathing where respiratory gases are exchanged using mechanical gas ventilation (CMV) while a PFC containing liquid is infused into the lungs.

PLV treatment shows improved lung function compared to CMV, but not as robust as TLV. A pre-term lamb model approximately equal to the human gestational age of 20 weeks shows PLV treatment improves arterial oxygen tension, respiratory compliance, and peak inspiratory pressure compared to CMV alone. The improvement was not as dramatic as with TLV treatment. Histological analysis reveals improved lung histology with PLV compared to CMV, but the alveoli were not as expanded and perfused as seen with TLV treatment.

A more user-friendly TLV system is needed to clinically treat infant and adult patients, and work is being done to improve TLV systems. A multicenter study published in 2008 shows promise for using TLV to treat acute lung injury in adult sheep. This oleic acid injury model demonstrated increased arterial oxygen tension, oxygen delivery, venous oxygenation, and decreased physiologic shunt in adult sheep. Improved respiratory compliance and ventilatory pressures were also observed.

PLV treatment shows decreased mortality and improved lung function in both infants and adults. Alliance Pharma became interested in a fluid respiratory support agent, LiquiVent.
A multicenter study looking at the effectiveness of LiquiVent to treat severe respiratory distress syndrome in infants was conducted as part of a Phase I-II study as a corporate-sponsored IND application. Premature infants less than 5 days old considered to be in extremis who failed other therapies were eligible for the study. PLV with perflubron (LiquiVent) was administered, and gas exchange and lung improvement was seen within 24 hours. Another study examined the use of PFCs to recruit available lung parenchyma in full-term infants with congenital diaphragmatic hernia (CDH) or ARDS who failed conventional treatment and were on extracorporeal life support (ECLS). Perflubron PLV (LiquiVent) was administered to the infants and was well tolerated. Lung recruitment and improved lung function were seen in infants treated with perflubron PLV. Some infants with ARDS recovered and became long-term survivors. In addition, an infant with meconium aspiration syndrome and refractory persistent pulmonary hypertension (PPHN) was treated with veno-venous ECLS (VV ECLS) for 23 days with no sign of improvement prior to perflubron PLV administration. Five days later, perflubron PLV was stopped and the infant was eventually extubated and fully recovered. In a Phase II adult ARDS trial, perflubron PLV resulted in decreased mortality and increased ventilator-free days in patients less than 55 years of age. In another large, multicenter adult trial of PLV treatment of adult ARDS no improvement in outcome was observed in patients treated with PLV compared to CMV. This study also brings attention to protocol concerns when conducting these studies. Careful consideration in study design and execution must be considered. It is critical to monitor multiple arms of a study when being conducted at different facilities. Errors in reading the meniscus for dosing were a concern. Furthermore, the ventilators needed to be disconnected for dosing in the PLV treatment groups but not in the CMV treatment group.

In addition to treating respiratory distress syndrome, intrapulmonary aerosolized PFC treatment shows promise for treating TLI. Intrapulmonary aerosolized PFC administration after TLI in a sheep model increased the partial pressure of oxygen in arterial blood and lung compliance, while decreasing shunt and pulmonary artery pressure. The inflammatory cytokines IL-6 and IL-8 were also decreased after intrapulmonary aerosolized PFC treatment (Figure 47). PFCs are also useful to treat conditions where tissue cooling is beneficial to survival, such as TBI and cardiac arrest.
Cooling Applications for PFCs in TBI

Nasopharyngeal delivery of PFCs has been used as an application to treat TBI. Nasopharyngeal delivery of PFCs involves spraying a volatile liquid into the nasal cavity and cooling occurs as the liquid evaporates. Animal studies of TBI and data collected from both animal studies and human trials of pre- and post-return of spontaneous circulation (ROSC) reveal that the earlier whole body cooling can be initiated, the better the outcome. TBI causes a metabolic surge and local heat production. Cooling is useful in treating TBI due to attenuation of inflammatory responses. Additionally, reduction in metabolic demand confers protection against ischemia and hypoxia. Current cooling methods include whole body surface cooling and invasive intravascular cooling. Both are slow, cumbersome systems compared to nasopharyngeal PFC cooling. Studies show that nasopharyngeal PFC cooling has a higher cooling rate than the cooling blanket and is therefore able to reach the necessary therapeutic hypothermic zone faster (Figure 48).
Increased microglial and astrocyte activation, increased ICP, increased coagulation time caused by TBI in sheep was reversed with the nasopharyngeal PFC cooling technique, RhinoChill. The earlier the cooling initiated post-TBI, the greater the attenuation.

When RhinoChill was used to treat pre- and post-ROSC in cardiac arrest patients, an increase in patient survival was seen. Furthermore, the desired therapeutic temperature of 34°C was reached 2 to 3 hours faster than other whole body cooling methods (Figure 49).
Moreover, the pre-ROSC IntraNasal Cooling Effectiveness (PRINCE) study shows that earlier, pre-ROSC administration of RhinoChill leads to increased overall and neurologically intact survival in patients (Figure 50). The Pre-hospital Resuscitation IntraNasal Cooling Effectiveness Survival Study (PRINCESS) is currently ongoing to further assess the benefits of early cooling in cardiac arrest.
Taken together, these data highlight the effectiveness of intrapulmonary administration or nasopharyngeal aerosolized delivery of PFCs. Inhaled PFCs may offer protection against acute lung injury and brain injury. It is important to realize that not all PFCs are the same. The PFC to use as a therapeutic should be carefully selected. Bromide containing PFCs for lung studies are useful to easily view lungs and determine whether treatment is beneficial.

Panel Discussion

Presenter and meeting attendee comments and questions addressed the following Session IX-related topics:

- **Use of oxygen carriers in ANH protocols:** The meeting attendees commented that both HBOCs and PFCs could be used for augmented hemodilution. However, it was also noted that proper training in the technique is very important to avoid complications and confounders in clinical trials data where oxygen carriers are being evaluated.

- **Challenges to performing clinical trials:** It was noted that complications in previous clinical trials evaluating PFCs have made it difficult to pursue follow-up studies with improved PFC formulations. Not only is it difficult to find investors, but careful site selection and coordination among sites are necessary to ensure procedures are being
performed consistently across study sites. The meeting attendees also emphasized the need to demonstrate proper training for a new treatment or device.

- **Availability of clinical data related to PFCs:** The meeting attendees agreed that data related to clinical use of PFCs (such as the Russian Perftoran data) need to be available and accessible to help bring PFCs into development.

- **Lack of approved PFC for DoD use:** It was noted that PFCs are in use in other countries, including Perftoran in Russia and Perfluorodecalin in China; however, the DoD is lacking a similar capability. Currently, it is not common for sailors to die from DCS but if that changes it could affect the use of PFC for civilian administration. This issue was addressed by noting that recently FDA has given approval for just military use with restrictions for use by non-military. The FDA

- **Immunostimulatory effects of PFCs:** Meeting attendees discussed whether the particle or micelle size of PFCs could have immunostimulatory effects. It was noted that particle size is important and effects can vary depending upon the size of the molecule. In a follow-up discussion, one attendee observed that particle sizes of less than .2 microns can avoid activating thromboxane secretion and complement activation. Using small particles of this size could help avoid the development of anaphylactic symptoms.