Chapter 8
NIH/FDA/DOD Interagency Working Group on Oxygen Therapeutics

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8.1 Introduction

Research and development in the field of oxygen therapeutics has made substantial progress in the past two decades. However, significant challenges have been encountered, as highlighted at an NIH/FDA/HHS workshop in 2008 (Silverman and Weiskopf 2009a, 2009b). The combination of challenges, negative perceptions, and current difficulties in obtaining adequate funding for continued research and product evaluation led the NIH/FDA/DOD to convene a working group of experts to examine the future of oxygen therapeutics in Boston in July 2011. The group examined the medical needs for oxygen therapeutics and outlined the basic and applied research needed to continue drug development for these clinical applications. This document summarizes the opinions of the working group.

A full list of the Working Group members are provided at the end of the article.

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8.2 Need

The Working Group's consensus is that there is a substantial unmet medical need for oxygen therapeutics and that lives could be saved if safe products were available. Oxygen carriers would be particularly valuable for patients in one or more of the categories discussed below: (a) "bridge to transfusion"; (b) alternative to red blood cell transfusion; (c) novel opportunities where red blood cell transfusion is not an established therapy.

8.2.1 "Bridge to Red Blood Cell Transfusion"

This is an area where red blood cell transfusion is indicated and will remain necessary, but compatible red blood cells are not available in a timely manner. Examples are: (a) pre-hospital use in remote areas; (b) military in-theater use with long evacuation times, or complete unavailability of red cells; (c) patients who are difficult to cross-match and require red cells before a satisfactory cross match can be established and compatible units identified; (d) patients with rare blood types awaiting arrival of a compatible unit available from a distant location; (e) mass casualties.

8.2.2 Alternative to Red Blood Cell Transfusion

This category is identified for use similar to red cell use, but when red cells cannot be used or when an oxygen therapeutic could be more satisfactory: (a) patient refusal to accept red cell transfusion (e.g. religious objection); (b) massive transfusion, to conserve the stored red cell resource, transfusing red cells only after hemostasis has been established; (c) treating anemic patients, who are transfused repetitively over a substantial period of time, in order to diminish immunological sensitization (e.g. sickle cell disease, thalassemia); (d) developing countries where red cell availability for transfusion is exceedingly limited or not available. In these regions no blood banking system may exist or the blood bank may have poor quality control (e.g. high viral infection rate).

8.2.3 Novel Opportunities Where Red Cell Transfusion is not an Established Therapy

1. Treatment of acute ischemia when red cell transfusion would not be used ordinarily, including (a) myocardial ischemia due to arterial insufficiency; (b) cerebral ischemia due to major cerebral artery insufficiency; (c) acute limb

ischemia due to arterial insufficiency with reduced blood flow; (d) traumatic brain injury (e) sickle cell crisis, to prevent or treat areas of limited blood flow; (f) spinal cord ischemia; (g) reconstructive surgery- free or attached flaps.
2. Other novel opportunities include, for example: (a) carbon monoxide poisoning; (b) organ preservation (transplantation, cardiac surgery, etc.); (c) a component of a multi-functional resuscitation fluid.

8.3 Animal Models of Toxicity

Although current generation HBOCs were tested in a large number of well-defined animal studies, including models of hemorrhagic shock and anemia in normal animals, clinical trials with these products have been noted to have side effects that were not predicted by pre-clinical tests (Silverman and Weiskopf 2009, 2010). Examples of these serious adverse effects included excess morbidity, cardiac mortality, and pancreatitis, which were often associated with pre-existing conditions (Silverman and Weiskopf 2009, 2010). Consequently, the interagency working group strongly recommends that better animal models of various human disease states are needed to evaluate potential toxicity of oxygen therapeutics either as individual molecules or as a class of compounds (it should not be assumed that a particular side effect is associated with the entire class of compounds). The group believes that in the future, both as individual drugs, and classes of drugs, oxygen therapeutics should be evaluated in the presence of common clinical conditions. The working group believes two important and common disease pathways, endothelial dysfunction and oxidative stress, may have contributed to serious adverse events in prior clinical HBOC trials.

8.3.1 Models of Endothelial Dysfunction

It has been known for decades that free hemoglobin avidly scavenges nitric oxide (NO). In the late 1990s, the HBOC field recognized that dioxygenation of NO by oxyhemoglobin was the major underlying cause of the rapid increase in blood pressure in animals and patients after administration of acellular hemoglobin. Major efforts were made to reduce the rate of this reaction by site-directed mutagenesis (Doherty et al. 1998) or by reducing in vivo extravasation by increasing HBOC molecular weight (Lieberthal et al. 1999; Knudson et al. 2003; Gould and Moss 1996; Nelson et al. 1992). It became clear that the vasoconstrictor and hypertensive effects of acellular hemoglobins and HBOCs, (Silverman and Weiskopf 2009, 2010) and gastro-intestinal symptoms in conscious humans (Viele et al. 1997) are due to scavenging NO, and that these side effects were potentiated

in animals with endothelial dysfunction. Possible dysfunction models include mice fed a high fat diet for 6 weeks, (Yu et al. 2010) diabetic mice (db/db), (Yu et al. 2010) or lambs with partial inhibition of NO synthesis by chemical blockade (Baron et al. 2012). In addition to regulating vascular tone, excess plasma hemoglobin may produce inflammation and platelet activation (Villagra et al. 2007; Boretti et al. 2009). Thus, the working group strongly advocates that HBOCs be studied in animal models of endothelial dysfunction to search for pulmonary and systemic vasoconstriction, and alterations of coagulation (including platelet activation), and inflammation. Coronary occlusion and pro-coagulant models, some of which have been studied for the effects of NO supplementation, could also be used to test the safety (also an area of potential therapeutic efficacy) of these molecules in diseased states (Schmidt et al. 2001). Effects of oxygen therapeutics on other pathways influencing endothelial-blood interactions, vascular reactivity and inflammation/platelet activation should be evaluated.

8.3.2 Models of Oxidative Stress

Auto-oxidation of HBOCs causes the release of superoxide, which in turn rapidly dismutates to hydrogen peroxide and reacts with the newly formed methemoglobin to produce destructive protein-based radicals. All of these reactive oxygen species (ROS) are capable of producing tissue injury through lipid oxidation and protein degradation. This oxidative stress may be compounded by vasoconstriction and stimulate inflammatory responses, including the release of additional endogenous oxidant molecules. Methemoglobin itself is relatively unstable and rapidly loses heme. Free heme in turn can generate more ROS and, if present in high amounts, leads to iron overload pathology. Therefore physiological models examining oxidant stress, especially in animals without sufficient capacity for the production of anti-oxidants should be examined. There are a number of such animal models, including those that lack the ability to produce reducing molecules, such as ascorbic acid (e.g., guinea pigs, unlike most rodents, lack the ability to synthesize ascorbate) (Buehler et al. 2007; Butt et al. 2011). Other models for study include knockout mice without ascorbate synthesis (Koike et al. 2010).

Perfluorocarbon-based oxygen carriers are molecularly distinct from HBOCs, and have different pharmacodynamic, pharmacokinetic, and biologic activities. Thus, they may need to be studied in different animal models. Perfluorocarbons have high solubility for many gases, including oxygen. Further work on the basic mechanisms of oxygen delivery and alterations of NO signaling with perfluorocarbons would be beneficial. Animal models should include those mimicking common human diseases that might be expected in patients receiving these novel molecules. Studies of the effects of perfluorocarbons on microvascular and endothelial cell biology should be included in their development plans. Potential therapeutic areas for PFCs that might not pertain to HBOCs owing to the physical

properties of PFCs include treatment of gas embolism and decompression sickness. Research should include efforts to understand the mechanism by which PFCs induce transient thrombocytopenia.

8.4 Developing New Hemoglobin-based Oxygen Transporting Materials

Safety concerns have been identified in preclinical and clinical testing of HBOCs (Silverman and Weiskopf 2009, 2010) suggesting that further development of HBOCs and/or improved formulations are needed. We anticipate that studies in the additional animal models described above will help to identify further improvements in HBOC engineering and/or improved formulations and additives.

8.4.1 Further Engineering of HBOCs

The study group recommends further research to develop entities designed to:

1. Lower rates of nitric oxide scavenging via inhibition of the dioxygenation reaction.
2. Inhibit extravasation into blood vessel walls.
3. Suppress damaging oxidative reactions.
4. Reduce heme loss and unfolding of HBOC protein.
5. Eliminate complement activation in normal animals and animal models of common human diseases.
6. Enhance pharmacokinetic properties, including extending the intra-vascular half-life.
7. Improve shelf life at room temperature.
8. Reduce oncotic pressure at high hemoglobin concentrations.
9. Enhance the O_2-carrying capacity of individual Hb molecules.

8.4.2 Improved Formulations and Additives

The committee recommends further research to define any potential benefit for HBOC prototypes that may include entities that

1. Enhance production of NO from exogenous or added nitrite by augmenting the anaerobic nitrite reductase activity of deoxyhemoglobin.
2. Restore endothelial NO with bioactive molecules that release free NO or generate bioactive NO metabolites.

3. Are loaded with CO to reduce oxidative cellular metabolism and induce vasodilation.
4. Utilize co-administration of anti-oxidants or haptoglobin (Boretti et al. 2009) to reduce or eliminate oxidative pathway activation and heme release.

8.5 Improved Understanding of the Effect of the Oxygen Therapeutics on Tissue PO_2

Optimal development and evaluation of novel oxygen therapeutics requires improved methods to measure in vivo the PO_2 in vital tissues and organs in both animal models and humans. Such advances would provide the parameter of most importance to the therapeutic goal of administering oxygen therapeutics.

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