Hemoglobin-vesicles as artificial oxygen carriers: interactions with ligand molecules in the production process and in blood circulation

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Hb-vesicles (HbV, 250 nm) are artificial O2 carriers, and the safety and efficacy as a transfusion alternative have been clarified in detail. Hb binds not only O2 but also CO and NO very strongly. In some conditions HbCO dissociates easily. The interactions of HbV with these gaseous ligands are important both in the production process and in blood circulation.

HbCO is resistant to heating and it enables pasteurization at 60°C for 10 hours to guarantee the utmost safety from infection. The purified HbCO is concentrated to ca. 40mg/ml and encapsulated with a lipid bilayer membrane without protein denaturation. The vesicular surface is decorated with PEG. HbCO can be easily converted to HbO2 by photoradiation in an aerobic condition. Finally O2 is completely removed and the resulting deoxy-state HbV can be stored at room temperature for over 2 years.

HbV does not induce vascular constriction in contrast to mouse Hbs. This difference presumably relates to the reduced uptake of endogenous NO and CO owing to the large dimension and the cellular structure of HbV. Recently, we tested intravenous injection of exogenous CO-bound HbV into rats, and found out that CO was released quite promptly in 3 hours with a cytoprotective effect at reperfusion. This indicates a possibility of a new clinical application of HbV in addition to its use as a transfusion alternative.

Conclusion: Safety of HbV in long term survival was proven in shock-resuscitation model.

Liposome-Encapsulated Hemoglobin: Prospects for Clinical Application

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Liposome-encapsulated hemoglobin (TRM-645) is developed as a RBC substitute for transfusion (Tx) as well as oxygen therapeutics. Its liposome capsule makes typing and screening (T/S) unnecessary and stable for months. Viral filtration/inactivation makes it safe for blood-borne infection. These characteristics make TRM-645 ideal for ubiquitous storage for an emergency use in and out of hospital.

Conclusion: TRM-645 has been proved protective in ischemic stroke (2 ml/kg), in myocardial ischemia, to accelerate surgical wound healing (0.4 ml/kg) as O2 therapeutics.

O2 Therapeutics.

TRM-645 has been proved protective in ischemic stroke (2 ml/kg), in myocardial ischemia, and to accelerate surgical wound healing (0.4 ml/kg) as O2 therapeutics.

OR.

Among 2079 surgical cases who ordered T/S in 2003, only 28% actually received Tx, rendering 72% of T/S unnecessary. Furthermore, only 154 (7.4%) required 9 units or more, suggesting that more than 92% of T/S might have been unnecessary if TRM-645 is used for the initial 8 units (1040 mL) of Tx.

Conclusion.

TRM-645 may be useful in a wide range of ischemic disease as an O2 therapeutics as well as a blood substitute especially in areas with a high prevalence of blood-borne infection and in underpowered or underdeveloped Tx service.
There is an evident need for blood substitutes; however, currently tested Hb-based oxygen carriers have toxicity and efficacy problems. These products were developed before the recognition of Hb's intrinsic toxicity. To diminish intrinsic toxic effects of Hb, Texas Tech University scientists have developed and patented a novel concept of "pharmacologic cross-linking" and formulated an effective free Hb-based blood substitute product. This novel blood substitute, HemoTech, that was licensed to HemoBioTech, Inc. for commercial development, is composed of purified bovine Hb, cross-linked intramolecularly with o-ATP and intermolecularly with o-adenosine, and combined with reduced glutathione (GSH). The idea behind the use of o-adenosine was to counteract the vasocostrictive and pro-inflammatory properties of Hb with the activation of adenosine receptors, which would produce vasodilatation and reduce inflammatory reactions. The concept of conjugation of Hb with GSH was to introduce more electronegative charges onto the surface of Hb, which would block Hb's transglomular and transendothelial passage, and would make it less visible to phagocytes. In addition, GSH shields Hb from reactive oxygen species and glutaricate. The reaction with o-ATP stabilizes the Hb tetramer, but the reaction with o-adenosine allows the formation of Hb low molecular weight polymers with uniform electronegative charge. HemoTech was subjected to preclinical testing and proof of medical concept. The results of these studies are favorable, indicating that HemoTech has vasodilatory activity and can reduce vasocostriction that follows hemorrhage, has erythropoietic activity and produces no adverse nephrotoxic, neurotoxic, oxidative, inflammatory or apoptotic reactions. These findings indicate that one may design a non-toxic and efficacious free Hb-based blood substitute product, pharmacologic cross-linking of the Hb molecule is necessary. Now, HemoTech has entered the regulatory process for commercial development in the U.S.