401. BASIC SCIENCE AND CLINICAL PRACTICE IN BLOOD TRANSFUSION: NOVEL PRODUCTION AND USE OF BLOOD PRODUCTS | DECEMBER 2, 2016

Erythromer (EM), a Nanoscale Bio-Synthetic Artificial Red Cell: Proof of Concept and *In Vivo* Efficacy Results

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Abstract

BACKGROUND: There is need for an artificial oxygen (O_2) carrier for use when: stored blood is unavailable or undesirable. To date, efforts to develop hemoglobin (Hb) based oxygen carriers (HBOCs) have failed, because of design flaws which do not preserve physiologic interactions of Hb with: O_2 (they capture O_2 in lungs, but do not release O_2 effectively to tissue) and nitric oxide (NO) (they trap NO, causing vasoconstriction). EM design surmounts these weaknesses by: encapsulating Hb, controlling O_2capture/release with a novel 2,3-DPG shuttle and attenuating NO uptake through shell properties.

METHODS: The EM prototype and its lyophilized form were analyzed: (1) structurally (dynamic light scattering (DLS), transmission electron microscopy (TEM) and atomic force microscopy (AFM)), as well as for: (2) payload retention (Drabkin), (3) biocompatibility (ex vivo complement activation), (4) O_2 affinity (p50, Hill n, Adair), (5) rheology (cone and plate viscometer in rabbit plasma), (6) NO consumption (chemiluminescence), (7) pharmacokinetic (PK) profile (tracking ^{99m}Tc-labeled EM in rats), and (8) in vivo O_2delivery (two rodent models: hemorrhagic shock [rats, instrumented for tissue pO_2] and hemodilution [bioluminescent HIF-1α reporter mice]).

RESULTS: EM was structurally stable (size: 175±10 nm; polydispersity: 0.26±0.0 by DLS, confirmed by TEM and AFM; zeta potential: 12±2 mV). After 3 months storage, we observed nominal change (<10%) in size, zeta potential, or polydispersity. CH50 (complement activation) results were indistinguishable from negative controls and we observed no impact on plasma viscosity (1:10 and 1:5 dilution). p50 was calculated to be 21.46±2.75 Torr (control RBC p50: 23.63±1.84); EM Hill & Adair also similar to control RBC. Two compartment PK modeling in rats resulted in good fit, with distribution $t_{1/2}$=26.2±3.6 min and elimination $t_{1/2}$=300±12 min (R^2>0.96); which is likely to translate to a $t_{1/2}$ in humans of ~ 3h. EM NO sequestration varied as a function of shell crosslinking and was below the rate observed for RBCs. In our hemorrhagic shock model in fully instrumented SD Rats (400g), 40% blood volume was removed; animals were then resuscitated with an equal volume of EM (N=6) or normal saline (N=6). EM was suspended at 40 wt/vol%, [Hb]=4mM. EM infusion rapidly stabilized hemodynamics. During the 1st hour, we observed resolution of both lactic acidosis (3.2±1.5 v 8.2±2.1 mM) [for EM and NS, respectively, throughout] and elevated AV O_2difference (24±11 v 67±23%) as well as improved brain pO_2 (30.5±1.4 v 17.2±1.3 Torr); p<0.05, RMANOVA, for all. Hemodilution model:Un-instrumented, HIF-1α (ODD) luciferase mice underwent hemodilution (70% v/v) with pentastarch, fresh blood (autotransfusion controls), or EM [N=6, all groups];Hb target nadir was reached (5 mg/dL). To detect whole body luciferase expression, D-luciferin (50 mg/kg, IP) was injected, then serial images were obtained (IVIS, Living Image). HIF-luc radiance was significantly higher in the HES group than in autotransfusion and EM groups, which did not differ (p<0.01, RMANOVA).

CONCLUSIONS: The ErythroMer prototype has passed rigorous initial *ex vivo* and *in vivo* "proof of concept" testing and bench testing, which suggests this design surmounts prior challenges (by HBOCs) in emulating normal RBC physiologic interactions with O_2 and NO. In models of major bleeding/anemia, EM reconstitutes normal hemodynamics and O_2 delivery, observed at the system, tissue, and cellular level. EM potential for extended ambient dry storage has significant implications for portability and use. Next steps include formulation scaling, detailed study of pharmacokinetics, biodistribution and safety, as well as evaluation in large animal models of hemorrhagic shock.