

Perfluorocarbons and haemoglobin solutions : will they ever reach clinical practice ?

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Transfusion of packed red blood cells is limited by several factors. It appears that to maintain a haemoglobin (Hb) level in the range of 7-9 g/dL in critically ill patients is associated with a lower mortality than the use of liberal transfusion to increase the Hb levels (1). To face this problem, blood substitutes have been developed from the 1980's as resuscitation fluids, based on modified free Hb or perfluorocarbon (PFC) emulsions (2-4). As their primary function is oxygen delivery, the blood substitutes cannot replace the numerous functions of blood, and are better referred to as O₂ carriers.

The PFC are synthetic linear or cyclic perfluorinated hydrocarbons, with chemical inertness and lack of metabolism *in vivo*. They are dense transparent liquids with a low surface tension, non miscible in water: for intravascular use, they are administered as emulsions. They dissolve gases without covalent binding, and unload them easily. The quantity of dissolved O₂ is linearly related to the partial pressure, and the use of PFC emulsion thus needs high O₂ tensions (generally 100% O₂). Oxygent (Alliance Pharmaceutical-Baxter) is a second generation emulsion with 60% perflubron (weight/volume), ideal in terms of O₂ carriage, prepared with egg yolk phospholipids as surfactant and by emulsification techniques producing particles with diameter of $\pm 0.2 \mu\text{m}$, able to maintain perfusion of the capillaries during states of local vasoconstriction and ischemia (5-7). At an FiO₂ of ± 1.0 , Oxygent releases $\pm 10 \text{ ml O}_2/100 \text{ mL emulsion}$, around twice that of blood. Oxygent is stable for 1 to 2 years refrigerated, has a viscosity $\pm 30\%$ higher than blood but decreasing with dilution in the blood flow, and a short circulating lifetime of ± 6 hours. Oxygent was administered with the purpose of transfusion avoidance focusing on acute normovolemic hemodilution, in 5 phase II clinical trials (surgical patients) without serious adverse events and efficacy in the reversal of transfusion trigger (8). A phase III study in non cardiac surgery patients was completed in Europe. A phase III study in cardiopulmonary bypass in the USA was

halted for stroke imbalance. A new phase III study in general surgery is announced. A still unresolved question with the clinical use of PFC emulsions remains the long term effects due to the particle phagocytosis by macrophages.

The haemoglobin based oxygen carriers (HBOCs) are prepared from free Hb obtained from human or bovine erythrocytes, or from genetic engineering (recombinant Hb). They are devoid of antigenic properties and high purification eliminates acute toxicity. Free Hb retains O₂ carrying properties, is fully saturated by room air breathing, but has a greater affinity for O₂ than intracellular Hb (due to the loss of 2,3-diphosphoglycerate), delivering less O₂ to the tissues. For prolonging the intravascular half-life, and maintaining a normal O₂ affinity, Hb is modified by internal stabilisation of the tetrameric molecule, polymerisation, cross linking of dimers, conjugation with larger molecules, pyridoxylation, or encapsulation within synthetic lipid membranes (3, 4). The HBOCs have a reduced O₂ affinity compared to human Hb, promoting O₂ unloading into tissues. The limitations of HBOCs include a short plasma half-life of ± 16 hours, a vasopressor effect attributed to nitric oxide ($\bullet\text{NO}$) binding and a diffusion into endothelial cells and extravascular tissues followed by a catabolism into bilirubin leading to «jaundice-like» syndrome. HBOCs metabolic pathways are poorly characterised, and their susceptibility to oxidation producing metHb, further forming reactive O₂ species, is still insufficiently in-depth studied (9, 10).

The diaspirin cross-linked human Hb (DCLHb; HemAssist from Baxter) reached the phases II and III clinical trials (11, 12) but a phase III trial in severe traumatic haemorrhagic shock was halted at interim analysis for increased mortality in the treated patients, and the preparation of

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DCLHb was stopped (13, 14). Baxter is now focusing on second generation O₂ carriers based on recombinant Hb engineered to modify the Hb-NO interactions. Three HBOCs are still in clinical trials : ongoing phase II and III trials in trauma and aortic surgery with a human glutaraldehyde polymerised Hb (Polyheme ; Northfield Laboratories), completed phase II and III trials in surgical patients with a bovine glutaraldehyde polymerised Hb (Hemopure ; Biopure Corporation), completed phase II and III trials in cardiac and orthopedic surgery and chronic renal failure, and an ongoing trial in cardiac surgery with a human o-raffinose polymerised Hb (Hemolink : Hemosol Inc.). No severe adverse events were reported ; mild elevation of blood pressure and “jaundice-like” syndrome were observed in 20 to 50 % of the patients for Hemopure and Hemolink, but seemed lower for Polyheme. The haemodynamic effect of HBOCs, related to NO scavenging, is presented as a potential pharmaceutical tool in severe septic shock (12). Four oxygen carriers (a PFC emulsion and 3 HBOCs) are thus promising oxygen carriers for human use, with minor adverse effects and good oxygen transport and delivery capacities. The small size of their particles allows a closer contact with the microvessel walls, the bypass of constricted or partially obstructed capillaries, and the perfusion of ischemic tissues. But many questions remain unsolved on their *in vivo* catabolism, especially the reaction of modified Hb with O₂ and •NO. These questions need precise answers before future HBOCs (new recombinant Hb or encapsulated Hb) and PFC emulsions (encapsulation in biodegradable capsules) can become reliable alternatives to blood transfusion, especially in traumatic haemorrhagic shock patients, correcting the deficits of O₂ transport and restoring the volume (15).

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