OXYGEN THERAPEUTICS: CURRENT ISSUES AND NEW CHALLENGES

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The blood transfusion needs are still a topical question, especially in emerging threat of blood hypovolemic or extra-circulating situations, and a diminish of blood availability had led to develop 'blood substitute or OXYGEN THERAPEUTICS' in the North America, in Japan, in China and now in Europe. Although the development of these blood substitutes has been an active field of interest over two or three decenies[1], these compounds are not yet suited to replace blood because of their limited retention time in the circulation, the HBOC unwanted vasoconstrictive effects, and uncertainty about their oxygen transport effectiveness[2]. However, without a need for cross-matching, less strict storage conditions, and an increased shelf life, haemoglobin solutions and PFC emulsions represent promising resuscitation fluids, especially in emergency situations outside the hospitalinwhich resuscitation of trauma victims is required. Because of an impending short fall of allogeneic blood products within the next decades and ongoing problems (transfusion reactions, immunomodulating side effects, bacterial risk, viral and prion transmission)[3] associated with relevant testing and storage costs, the development of alternatives has been intensified during the last 15 years. Although the transmission's risk of pathogen agents in transfused blood is minimal in Europe, United States and other developed countries, the incidence is still high in other areas of the world, such as sub-Saharan Africa and South East Asia. Besides military need and infection agents that drive the commercialization of oxygen therapeutics, other factors are potential new viruses and other emerging infectious agents, and unexpected civilian blood shortages. This alternative oxygen therapeutics can potentially replace blood transfusion in clinical instances in which banked blood is unavailable or unsafe. Most of the

candidate solutions have been based on hemoglobin, derived from animals, outdated banked human blood, or recombinant systems. Other solutions are based on perfluorocarbons.

Hemoglobin based Oxygen carriers: The HBOC solutions have been produced from outdated human or bovine blood and so carry a potential contamination with viruses or prions. Free human Hb presents limits when injected, because of the short retention time in vessels (Hb dissociation into monomers), low molecular mass, high oncotic pressure, increase of vascular pressure, low oxygen release to tissues (P50)[4]... leading to undesirable effects including vasoconstriction, kidney and liver dysfunctions and gastrointestinal distress.This is why we need to modify the heme conformation. Modern chemically modified purified Hb (human or bovine) is a multi-step process to create conjugated, cross-linked, polymerized or encapsulated form of Hb, allowed HBOCs stabilization.[4] This chemically way to modify haemoglobin chains solved the initial problems of nephrotoxicity of H BOC resulting from the presence of free haemoglobin chains in the blood, and extended retention time in the vasculature.[5] However, until now, successful application of hemoglobin solutions has been limited, mainly by their vasoconstrictive properties. Several mechanisms may explain the vasoconstrictive effect of HBOCs, especially by scavenging of the vasodilator NO and by extravasation of hemoglobin[6–7] increased endothelin synthesis,[8] sensitization of adrenergic receptors,[4,8] and precapillary oxygen autoregulation have been suggested.[2] The effects of these chemically modified haemoglobins on arterial pressure indicated that scavenging of NO by haemoglobin is the primary cause of vasoconstriction. Itis unclear whether vasoconstriction by haemoglobin solutions is beneficial or deleterious to microvascular oxygenation.[9] Although a rise in blood pressure could increase microvascular blood flow, whereas arteriolar vasoconstriction can decrease it, one of the main blood regulation parameters is depending of the HBOC solution viscosity, especially throw the shear stress applied to the vesselwalls. That's why a high intrinsic viscosity of the solution is required to maintain a peripherical vasodilatation increasing the recruitment of functional blood vessel density.[10] Additionally, vasoconstriction could alter the distribution of blood flow between and within organs, inducing ischemic tissue area.[11] Besides clinical studies have shown that peri-operative use of different HBOCs (Hemopure, PolyHeme, Hemolink and HemAssist)[12-16] can reduce the num ber of homologous RBC units and increase the avoidance rate of hom ologous transfusion in emergency bleeding, vascular, cardiac and non-cardiac surgery. However, HBOC-201 (Hemopure) is the only substance licensed for the treatment of patients w ith acute peri-operative anemia in South Africa.[14,17]

However, other actual developments of HBOCs come from microorganisms, with modified bacteria, fungiand even some plants, avoid cultural/ethical objections, are virus and other blood components free, and have potentially large-scale production but high costs. In Europe, we are not able to produce any sort of modified hemoglobin. That's why, through

the 6th PCRD program, European teams have the opportunity to develop together a modified hemoglobin solution, call **"Europe-blood substitute"**.[18] The project will span 3 years of intensive research into the development of a technological baseline for producing blood substitute components (novel haem proteins) using micro-organisms, such as bacteria and fungi. The Euro Blood Substitutes Consortium involved in 12 academ ic and industrial partners, including University of Nottingham – UK, University of Essex – UK, Technical University of Denmark, University of Nancy – France, Semmelweis University – Hungary, University of Rome – Italy, University of Parma – Italy, University of Milano – Italy, University of Lund – Sweden, LCC Engineering & Trading GmbH –Switzerland, Alligator Bioscience, AB – Sweden, Scottish National Blood Transfusion Service–UK and Sanquin Bloodbank – Netherlands.

The aim of this new solution conception is to control the amount of vasoconstriction through recombinant manipulation.[19-20] It allows the use of a compound with vasoconstrictive properties that match theconditions in which it will be used.

The *EuroBlood-Substitutes* Project will provide a technological baseline for use of micro-organisms as cell-factories for the production of a much needed, effective blood substitute.[18] Such new blood substitutes will, in principle, benefit all European citizens and the European biotechnology industry. The project has received major funding since the potential for production of blood substitutecomponents from micro-organisms is huge. Such technology has the capacity for the production of 'tailor-made' blood substitutes with novel properties. Research by the consortium is to focus on the use of two micro-organisms, both ofwhich are already widely used as 'cell factories' for synthesizing commercially-important pharmaceuticals.

Other way to produce hemoglobin solution is from transgenic animals,[2,18] where human Hb genes are introduced into developing animal eggs; animal then is able to produce Hb when matures, allowing potential supply of large volumes of Hb. Some recent studies suggest the possibility of using worm Hb to develop a blood substitute after successfully extracting Hb from two common worms. The worm Hb has performed well in pre-clinical testing, maintaining normal oxygen-carrying capacity and causing no allergic reactions in mice[21] .

Perfluorocarbons: (PFCs) are formally derived from their hydrocarbon analogues by replacing all hydrogen atoms by fluorine atoms. PFCs were first synthesized in the 1920s, but were popularized by a spectacular experiment reported by Clark and Gollan,[22] in which mice survived even completely immersed inPFC liquids saturated with oxygen at atmospheric pressure. PFCs are not miscible in water,and must therefore be prepared as emulsions using a variety of surfactants. The new generation of PFC emulsion used eggs lecithin as emulsions.[23] Unlike Hb, oxygen dissolution in PFCs is made according to a linearly partial pressure of oxygen. This require from patients receiving PFCs to breathe 100% oxygen. PFCs can be

manufactured in large quantities, are not subject to oxidation, do not participate in free radicalreactions, and cannot scavenge nitric oxide or bind carbon m onoxide.[24-26] Moreover PFCs are not metabolized, excreted from the body via the lungs in exhalation, and therefore present no metabolite-related toxicities.[27,28] However, complement activation and reduced platelet function are major clinical problems in patients receiving PFC-based products.[28] *Oxygent*® is an exciting prospect in the field of blood substitute technology,[29] producing a PFC-based product (perfluoroctyl bromide (C8F17Br)). Its clinical European phase III trials are currently under reconstruction to focus on a use of the product as an alternative to donor blood in patients undergoing surgery after setbacks in recent clinical trials.[30,31] Recently, Alliance and Co announced collaboration agreements between Europe and South Korea.[29]

From these researches, new generations of Oxygen therapeutics are emerging, designed specifically to target oxygen to the peripherical tissues.[9,32] It is possible that one or more of the earlier products may be approved by regulatory agencies soon.

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