REVIEW ON ARTIFICIAL BLOOD

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Abstract-The idea of using a blood substitute instead of human blood for transfusing patients is not new. In the 17th century, Sir Christopher Wren suggested that ale, wine, and even opium could be used as substitutes for human blood. It was not until 1901 that the modern age of blood transfusion began with the discovery of human blood group antigens by Karl Landsteiner. He categorized human blood into A, B, and C (later renamed O) groups, and a year later the AB group was added to the list. These studies were critical to understanding why blood transfusions had failed thus far. Ottenberg in 1913 was the first to apply blood group serology to transfusion practice. Even with Ottenberg's description of blood compatibility testing, blood transfusions were severely limited because of a lack of suitable anticoagulants and storage methods. The First and Second World Wars precipitated progress on these two fronts, at last allowing blood transfusion to become a standard part of medical treatment. there are several reasons for developing blood substitutes. Human red blood cells have exacting storage requirements designed to prolong clinical effectiveness and to reduce the risk of bacterial contamination. This substantially limits the availability of blood at disaster sites or on the battlefield.

Keywords-**human blood,transfusion,anticoagulants,contamination**

I.INTRODUCTION

The idea of using a blood substitute instead of human blood for transfusing patients is not new. In the 17th century, Sir Christopher Wren suggested that ale, wine, and even opium could be used as substitutes for human blood. It was not until 1901 that the modern age of blood transfusion began with the discovery of human blood group antigens by Karl Landsteiner. He categorized human blood into A, B, and C (later renamed O) groups, and a year later the AB group was added to the list. These studies were critical to understanding why blood transfusions had failed thus far[1]. The First and Second World Wars precipitated progress on these two fronts, at last allowing blood transfusion to become a standard part of medical treatment. there are several reasons for developing blood substitutes. Human red blood cells have exacting storage requirements designed to prolong clinical effectiveness and to reduce the risk of bacterial contamination. This substantially limits the availability of blood at disaster sites or on the battlefield. Blood substitutes with less stringent storage requirements would be valuable in these situations. In addition, blood substitutes are more amenable to sterilization to remove infectious pathogens, and they do not require cross-matching because they do not harbor blood group antigens[2]. Donor blood shortages make blood substitutes attractive for the short-term replacement of erythrocytes during surgery. In the 1980s, the realization that the human immunodeficiency virus (HIV) could be transmitted through blood transfusion provided renewed impetus for the development of "disease-free" blood substitutes. Before the development of a specific test for HIV, the risk of transfusion-associated acquired immunodeficiency syndrome was about 38 per 100,000 transfused patients[3].

Researchers interested in developing blood substitutes (dubbed artificial blood) have predominantly concentrated on mimicking the oxygen-carrying capacity of hemoglobin. However, in addition to being able to transport oxygen, an ideal blood substitute would also (i) require no cross-matching or compatibility testing, (ii) be suitable for long-term storage (preferably at room temperature), (iii) be able to survive in the circulation for several weeks (the intravascular "dwell" time) before being cleared by the kidney, (iv) be free of side effects, (v) be free of pathogens, and (vi) not only transport but also effectively deliver oxygen to tissues.

II.ARTIFICIAL BLOOD SUBSTITUTES

For many reasons, hemoglobin, modified to prolong its circulation time, seems to be the optimal choice for a cell-free O2 carrier (blood substitute) because of its capacity to reversibly bind O2 in the lung and release it in tissue. After refining methods to prepare highly purified hemoglobin solutions and to chemically or genetically modify hemoglobin to overcome renal toxicity and to prolong retention time, several unwanted effects were observed in human clinical trials[4]. These included
symptoms referable to the GI tract, elevated pancreatic enzymes and hypertension, presumed to be the result of vasoconstriction. Studies on the mechanism of vasoconstriction induced by hemoglobin, using new techniques to investigate the microcirculation have led to a surprising new paradigm for the design of safe and effective solutions. These include increased O2 affinity (low P50) and increased viscosity and oncotic pressure. These second-generation solutions hold greater promise for clinical development.

The study reported here was designed solely to determine the safety and tolerance in normal men of these SFH solutions. The solutions were weIl tolerated in 6 of the 8 subjects[5]. In 2 subjects abdominal pain occurred; one episode was mild and transient and one moderately severe. The efficacy, however, of hemoglobin as an oxygen-transporting plasma expander in humans can be determined only in patients whose vascular volumes are depleted and hematocrits are low (hemorrhagic shock). Such an efficacy of these SFH solutions has been demonstrated in our laboratory in dogs who have been acutely bled and hemodiluted down to a hematocrit of 5% or less. Infusion of albumin or dextran does not maintain life at these low hematocrits, whereas the dogs do survive when infused with these hemoglobin solutions even at hematocrits as low as 1%.5lt is such intriguing observations which have maintained the interest of investigators over the years in hemoglobin solution as a potential plasma expander, despite its known limitations[6]. One of these limitations is the short half-life (tVz) of the hemoglobin molecule in the plasma outside the erythrocyte.

The hemoglobin is rapidly removed from the plasma by two mechanisms: (I) complex formation with plasma haptoglobin and rapid absorption of this complex by the reticuloendothelial system; dissociation of some of the excess uncomplexed plasma hemoglobin (free Hb) into its subunit dimers and monomers and filtration of these subunits through the glomerulus into the urine. Measurements of the in vivo tlJzs of infused SFH preparations have ranged from 2 hr in the monkey! to 4 to 5 hr in the dog. 10 Although the plasma t1Iz was not measured in the present study, the subjects' gross hemoglobinuria had completely disappeared by 6 to 10hr after infusion, which suggests a plasma tVz of only a few hours[7].

One of the most important functions of blood is to solubilize and distribute oxygen within the body. As such, it is vital that this property is replicated (safely) by any artificial blood product. In this paper, we describe the facile synthesis of a series of simple diblock polymers capable of self-assembling into micellar structures at concentrations around 3×10-3 mg/mL. Using a dissolved oxygen meter, we were able to demonstrate that aqueous solutions of these aggregated structures could retain oxygen and release it (into the aqueous bulk phase). The increased oxygen retention was quantified by measuring the rate of oxygen release and its half-life. These experiments indicated that oxygen retention/binding was dependent on the fluorine concentration. 19F NMR experiments on a micellar solution saturated with oxygen showed small upfield shifts in the fluorine peaks, which provided qualitative evidence that indicated oxygen binding occurred within the fluorine region of the polymer aggregates. Using a modified enzyme/glucose oxidation assay, we were able to establish that the aqueous oxygen concentrations were 33% higher in a solution of polymer. Our prototype design was relatively simple and would include a solubilizing PEG block as a macroinitiator for an ATRP synthesis involving a monomer rich in fluorine. The synthesis is shown in Scheme 1 and involves the initial functionalization of Me PEG-2000 1 with α-Bromoisobutyryl bromide(BIBB) 2 to generate the macroinitiator 3. This was then reacted with various amounts of 2,2,2- Trifluoro ethyl methacrylate 4 in the presence of CuCl and the ligand N, N,N',N'',N''-Pentamethyl diethylenetriamine (PMDETA), to generate a series of fluorinated polymers (PEG-poly 2,2,2-trifluoroethyl methacrylate/PEG-PTFEMA) 5 with different degrees of polymerization (DP). GPC using RI detection generated chromatograms that showed a degree of bimodal character and higher than expected polydispersity's. However, this is typical of diblock polymers that possess polymer blocks with very different refractive indices[8]. this is much lower than the refractive index of poly(ethyleneglycol), which is 1.47. This leads to a significant underestimation of the fluorinated polymers signal intensity and an exaggeration of the apparent contamination by the PEG macro-initiator.

Polyhaemoglobins (PolyHb) and perfluorochemicals are in advanced phase III clinical trials and conjugated hemoglobin's in phase II clinical trial. New recombinant human hemoglobin with no vasoactive is being developed. A soluble macromolecule of PolyHb–catalase–superoxide dismutase is being studied as an oxygen carrier with antioxidant properties. New artificial red blood cells that are more like RBC are being developed. One is based on hemoglobin lipid vesicles. A more recent one is based on nano-dimension artificial red blood cells containing hemoglobin and RBC enzymes with membrane formed from composite copolymer of polyethylene glycol–polylactic acid. Their circulation time is double that of PolyHb.
As early as 1957, this author prepared encapsulated hemoglobin to form artificial red blood cells . In 1964, the same author showed the possibility of cross-linking hemoglobin to form PolyHb . In 1968, another group showed that intramolecularly cross-linked tetrameric hemoglobin no longer has the renal toxicity of free hemoglobin. Thus, the basic ideas of modified hemoglobin, although very crude, were all there in the 1960s. Unfortunately, except for some military interest, there was little academic, industrial or public interest in these ideas until the crisis of HIV in donor blood. Unfortunately, a product cannot be ready for clinical use without years of research and development followed by time consuming clinical trials. Thus, despite more than 10 years of intense efforts there is still no blood substitute ready for routine use in the Western world. Even when the first-generation oxygen carriers become available for routine use, they are only good for some clinical conditions. Much more still needs to be done to develop new generations of blood substitutes that can be used in other conditions. It is true that effective screening tests for HIV and hepatitis C have markedly decreased the risk of infection from donor blood to negligible numbers. However, can we be sure that there will not be another unknown agent in the future? If so, it will take time to develop suitable screening tests and even more time to develop the new generations of blood substitutes needed. Furthermore, with an increasing ageing population there is an increasing demand on the short supply of donor blood. In addition, increasingly more sophisticate and extensive surgical procedures and more frequent international conflicts also place an unrealistic demand on the short supply of donor blood. The first generation oxygen carriers have already demonstrated the feasibility of red blood cell substitutes as follows. These oxygen carriers can be ultrafiltered and pasteurized to remove microorganisms including HIV and other viruses. Because these oxygen carriers do not have blood group antigens, cross-matching and typing are not required before use. This saves time and facilities and allows on-the-spot transfusion especially in emergency situation. Furthermore, these blood substitutes can be stored for more than 1 year even at room temperature for one type[9]. These first generation oxygen carriers already have a number of important applications. However, there is an urgent need to develop the new generations of blood substitutes to broaden the potential areas of applications. However, even if we start to do this seriously and immediately, it will still take much time for these to be ready.

Perfluorocarbons are shown to be promising clinical blood substitutes. Infusion of Fluosol into rabbits produced hypoxemia, neutropenia, thrombocytopenia and pulmonary leukostasis. The adverse reactions to Fluosol are probably mediated by C activation and steroid premedication may prevent them in susceptible patients. Fluosol-DA and Pluronic F-68 was added to minimally heparinized normal human plasma. Incubation was done for an hour at 37° C. Fluosol was removed by centrifugation. For complete activation, zymosan was incubated in parallel with plasma and removed by centrifugation. Fluosol was incubated with plasma that had been decomplemented by heat or EDTA or with plasma. Fluosol incubated plasma was added to suspension of granulocytes

provoked aggregation. The aggregating activity failed to develop when Fluosol was incubated with plasma that had been decomplemented by heat or EDTA or with plasma. The component of Fluosol-DA is responsible for C activation.

The exposure of polymorphonuclear cells to clinically relevant concentrations of Fluosol-DA inhibits PMN migration, due to inhibition of adhesion. This inhibitory effect is due to the detergent, Pluronic F-68. Artificial blood substitutes containing Pluronic f-68 may compromise the ability of PMN to prevent or control microbial infections. Granulocytes are prepared. The PMN is suspended in the basal eagle's medium with Earle's salts. The preparation is stored and complete Fluosol-DA was prepared. The PMN were incubated with either FDA, stem emulsion without PF, or components of FDA. Incubation was performed at 37° C in a 5% co2 incubator for an hour. FDA did not increase cell loss or LDH release during incubation period. Fluosol did not affect the trypan blue dye exclusion. FDA did not decrease the amount of degranulation in response to phagocytosis of OZ. FDA did not inhibit the bactericidal capability of PMN. FDA severely inhibited both the random migration and the chemotaxis of PMN to both synthetic peptides and activated serum[10]. Fluosol-DA was responsible for neutrophil migration inhibition.

Perfluorocarbons are biologic inert synthetic materials, which are highly hydrophobic and tend to self-aggregation. These PFCs can act as 19_F magnetic resonance imaging agents and oxygen carriers. The construction of the fluorinated carriers will improve the stability of the carriers and provide them with additional functions. PFCs are more hydrophobic and stable because of their tendency to segregate from the surrounding environment. The paper shows the fluorination of the carriers, such as the liposomes and micelles, was able to improve their stability and serum resistant capability both in vitro and in vivo. It also shows that fluorination of the liposome vastly prolonged the circulation lifetime[11]. To enhance the circulation time of the liposome, a PEGylated liposome was constructed. The construction of the fluorinate carriers will be a promising strategy to overcome the limitations of systemic delivery for conventional nano-carriers.

III.CONCLUSION

New testing and screening procedures have rendered the donor blood supply increasingly safe. For example, the risk of transfusion associated HIV infection is now estimated to be as low as 1 per 835,000 transfused patients. Similarly, the risk of transfusion-associated infection with hepatitis C virus (HCV) is between 1 per 300,000 and 1 per 600,000, compared with an incidence of 1 per 103,000 in the early 1990s before a test for HCV became available. As the safety of the donor blood supply continues to improve, there must be careful consideration of the advantages of blood substitutes over donated blood, particularly given that new blood substitutes potentially carry unknown risks. Yet a shortage of donor blood for transfusion still advocates for the development of readily available blood substitutes. However, there is still a long way to go before artificial blood can replace real blood in routine transfusions. Most important, the intravascular dwell times of blood substitutes need to be increased, the cost of these products needs to be competitive, and difficulties with obtaining and processing sufficient amounts of these compounds must be overcome.

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