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Review Article

RECENT INNOVATIONS IN DELIVERY OF ARTIFICIAL BLOOD SUBSTITUTE: A REVIEW

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ABSTRACT

Focus of present review is to explore various approaches involved in delivery of artificial blood substitutes for last three decades. Delivery of artificial blood substitutes eg. Hemoglobin Based Oxygen Carriers (HBOCs), non hemoglobin based Perfluorocarbon (PFCs) and diffusion rheological compound such as Drag Reducing Polymer (DRG) by vesicular approaches (liposomes, pegylated liposomes, nanocarriers etc.,) and non vesicular approaches (solution, emulsions, microemulsions) are discussed with their merits and demerits. Delivery of artificial oxygen carriers by HBOCs and PFCEs in the form vesicular and non vesicular forms will gain its importance in field of pharmaceutical technology and clinical medicines in the coming years.

Keywords: HBOCs, liposomes, Microemulsions, Nanocarriers, PFCEs, Polymerosomes

INTRODUCTION

Role of delivery system in development of blood substitute

Since 1980s as danger of HIV transmission by blood transfusion has come to light, an increased interest is being given to blood substitutes. Blood substitutes will help to overcome issues of finding the right blood type, transfusing pathogens, hepatitis and the most dreaded HIV AIDS and will always be available unlike the shortage associated with natural blood. Unlike natural blood the only function provided by synthetic blood substitutes will be gas transportation, that is providing oxygen to cells and carry our exchange of carbon dioxide. This becomes the one critical thing to be dealt with when large amount of blood is lost due to massive trauma. Modified hemoglobin from human, animals, or recombinant origin and fluorocarbon emulsions have been the two main approaches used to exploit artificial blood substitutes. Hemoglobin approach is most appealing because it most directly mimics the mechanism of oxygen in natural blood. Genetic engineering, chemical cross linking, attaching synthetic protective coating is used in artificial blood substitutes to mimic the function of red blood cells and reduce the potential toxicity of hemoglobin as contained in cells. Some unwanted issues like, vasoconstriction caused by hemoglobin based blood substitutes due to scavenging of nitric oxide, hydrophobicity of fluorocarbons leading to short residence time in organs cause hindrance too.

Perflurocarbons show their potential to work by getting back circulated from the RES system, with the help lipoproteins and chylomicrons, to the lungs and thereby are excreted from there itself. Limitations associated with toxicity induced by the perflurocarbons are the driving force for finding better blood substitutes that overcome these problems.

Encapsulation of hemoglobin was the first technique used for artificial blood delivery. Instability, environmental degradation, toxicity of phospholipids and rapid degradation during storage are certain obstacles which prevent use of perflurocarbons. It is therefore necessary to use biodegradable membranes which are easily excreted.

Liposome-encapsulated hemoglobin (LEH) is a novel artificial oxygen carrier that has been developed for clinical applications to enable long-term storage and subsequent transfusion. The liposomal surface is modified by polyethylene glycol to improve biocompatibility and to avoid aggregation of particles. The LEH particles (mean diameter: 230 nm) are extremely small compared to erythrocytes, approximately 1/30 the size [5–7]. LEH is expected to carry oxygen into vital organs via collateral routes during ischemia because its size may allow O_2 delivery beyond the plasma obstruction to areas where erythrocytes seldom reach.

Several studies have demonstrated that LEH has beneficial effects on the sequelae of brain ischemia and hemorrhage. LEH reduced the size of cerebral infarction in rats subjected to photochemically induced thrombosis (PIT) of the middle cerebral artery (MCA) [8, 9] and also in primates treated with MCA occlusion (MCAO) [10]. Transfusion of LEH rescued lethal progressive hemodilution and improved hemodilution-induced metabolic acidosis in rats [11]. Acellular hemoglobin has been reported to act as nitric oxide (NO) scavengers and to have constrictive effects on peripheral vessels [13, 14]. The LEH used in the present study has been reported to have no NO scavenging action with lethal progressive hemodilution in model rats [11] and is not believed to have pressor effects. However, the effects of LEH on hemodynamics remain controversial.

Dendrimers are definite globular sized nano structures which are chemically stable, flexible, having low cytotoxicity and a hydrophilic exterior to mimic blood plasma. These varied characteristics make them a good choice in the field of blood substitutes eg. Poly amidoamino dendrimers (PAMAM)[15].

Present review describes vesicular and non vesicular approaches for effective delivery of blood substitutes.

Vesicular approaches

Liposomes and pegylated liposomes

The cellular structures of liposomes has been exploited as a carrier for drugs, genes, enhancing blood retention, targeting a site because of encapsulation by membranes which prevent the material against degradation and enhance bio-distribution. Drugs in liposome forms such as AmBisome® and Doxil®, are in clinical trials [18]. Hemoglobin encapsulated in sterile liposomes made from chloroform, HSPC, cholesterol, negatively charged DMPG and alpha- tochopherol formulated by Farmetal *et al,* showed oxygen carrying capacity of 20%, a half life of 15 -20 hrs as measured in mice. These liposome have a phospholipid bilayer, with cholesterol molecules added for increased rigidity and mechanical stability which then encloses a stroma free haemoglobin solution and 2,3 DPG or inositol hexaphosphate as a gelatinous fluid.[20]

Use of cell-free Hb solutions is associated with many deterious side effects and to overcome these, liposome-encapsulated hemoglobin (LEHb)-based artificial blood substitutes are being used since it has no blood group antigens and can therefore be stored for a longer time. [21-22] Plain liposomes undergo deformation and destruction when exposed to fliud stress so Chung et al. [23], in a LEHb study, demonstrated that 10–20% of the encapsulated Hb in LEHb dispersions is released when disspersion is subjected to a well-defined flow field.

Use of UV irradiation and redox inhibitors cause polymerization of unsaturated phospholipids incorporated in the vesicle membrane and provides mechanical strength to the vesicle. [24-25]

Instead of introducing covalent linkages between the hydrophobic tails, stabilization of the liposomes can also be carried out by coating the liposomes with help of polymers. [26-30]

Akama *et. al* increased the mechanical strength of the membrane of Hb encapsulated vesicles, by introducing actin matrix in the inner aqueous core of the vesicles and these are used as a potential artificial blood substitute. [31] The sub micron size of the liposomes makes it by pass the RES system and inhibits recognition of the opsonins. [32] Because of the smaller size as compared to RBC s also enables them to pass through blockages, clots in cases of stroke and heart attack.[33]

Efficacy and quality of liposomes can be further increased by modifying them with polyethylene glycol (PEG), which makes the liposomes even more stable, increase the half lilfe, induce water soluble property, lower immunogenicity and antigenicity along with site specific targeting. [34-35]

HbV, a cellular oxygen carrier has high concentration of hemoglobin encapsulated in phospholipid bilayers membrane with PEG and is advantageous as the oxygen affinity can be easily manipulated by changing the concentration of allosteric effector such as pryidoxal 5'-phosphate.[36] PEG coating and tailored size of 250nm enhances time of blood circulation as compared to other vesicles (t1/2 for cell-free Hb and PEGylated Hb in rats of 1.5 and 10.9 hr, respectively[37], Encapsulation of Hb helps to suppress renal excretion but eventual capturing by phagocytes in the mononuclear phagocyte system (MPS) does take place in case of these HbV [38]. These unique features of increased circulation time, as reported in rats, mouse and hemorrhagic rat model, of the vesicles make them comparable to RBCS. [39-44]

Instead of conjugation of multiple copies of PEG-5k which imparts an enhanced molecular volume to the vesicle, PEG chains gives a low density atom in PEG shell relative to protein core and the chain increases the hydrodynamic drag on the molecule and slows the sedimentation rate. The same influence is seen with PEG-Hb when used as a blood substitute.[45]

Dendrimers

Dendritic polymer having a well defined nanoscopic size of 1 nm, compromises of fluorocarbon and hydrophilic moieties. Compatibility with plasma is due to discrete well-defined globular shapes, flexibility, chemical stability low cytotoxicity; and hydrophilicity of exterior makes it a major step forward in the field of blood substitutes eg Poly amidoamino dendrimers (PAMAM).[46]

Polymersomes

Vindico NanoBioTechnology Inc. (Vindico) is developing a hemoglobin-based cellular oxygen therapeutic (blood substitute), *Nano-Heme*, based on its proprietary nanoparticle-based delivery platform known as polymersome. Polymersomes are synthetic polymer vesicles that are formed in nanometric dimensions which can efficiently encapsulate oxygen-carrying proteins such as hemoglobin (Hb). The lead Nano Heme formulation comprises of a diblock copolymer comprising hydrophilic polyethylene oxide (PEO) and hydrophobic polycaprolactone (PCL). It demonstrates all the characteristics of ideal oxygen therapeutic, such as tunable oxygen binding capacity, uniform and small size, viscosity and oncotic pressure characteristics similar to human blood as well a s ease of mass production and storage. Encapsulation of Hb inside polymersome core protects surrounding tissues and blood components from direct contact with Hb and it also allows for the use of less expensive animal Hb[47]

Polymeric shell

Encapsulation of hemoglobin in polymeric shells, which are suspension of fine particles of artificial blood substitutes, avoids drawbacks such as nephrotoxicity, activation of complement C3a associated with liposomes as carriers for delivery of artificial blood substitutes. In addition to its ability to carry poorly soluble agents, hemoglobin encapsulated in polymeric shells has high binding capacity for oxygen. Key difference between protein microspheres and polymeric shells is that in former case they don't have proteins shells rather proteins are dispersed throughout volume of microspheres. Polymeric shells are completely degraded by proteolytic enzymes as the polymer present is protein, which result in minimal side effects as compared to current formulation. Coating of polymeric shells with PEG result in increase circulation time maintain high levels of artificial blood substitutes [48]. Number of biocompaptible materials such as natural and synthetic proteins, ploypeptide or oligopeptide etc. having sufficient sulfhydryl or disulfide group for crosslinking are used successfully formulating polymeric shells. Recently new proteins such as albumin and hemoglobin are exploited for formation of polymeric shells. By encapsulating hemoglobin with in core of fluorocarbons it possible to deliver large amount of oxygenfor short periods as in case tissue ischemia and tumor therapy.

Block copolymer approach

Circulation time of polymerized hemoglobin can be increased by encapsulating them with in micellar system forming amphiphilic block copolymer having size range between 30-100 nm in diameter. The hydrophobic core of the polymer micelle entrap hydrophobic hemoglobin protein, while water soluble corona primarily composed of poly (ethylene glycol)) provides a steric barrier to protein absorption and avoids clearance by the reticuloendothelial system (RES) [49] fig.3 Block copolymer entrapping hemoglobin]

Non vesicular approaches

Solution

Since hemoglobin solutions have capability to transport oxygen and because of their oncotic activity they are therefore tested to be used as artificial blood substitutes and plasma expander respectively. Reversible oxygenation property of these solutions makes them choice for rapid initial treatment of hypovolemia and tissue hypoxia [50]. Tetramers free hemoglobin solution having advantage of having half lives nearly 12-24 hrs and avoiding vasoconstriction, renal toxicity, hemoglobinuria or the other problems associated with the intravenous administration of synthetic and semi synthetic oxygen carriers and blood substitutes, can be used in patients suffering from hypovolmic shock during surgery and trauma[51]. Stroma free hemoglobin solution has been found to overcome the problems of toxicity, shelf life and stability by many folds [52]. Aqueous solutions have additional benefits such as presence of polysaccharide oncotic agent which makes them ideal to be used as synthetic plasma expanders.

Emulsions

Novel emulsions having novel surfactants such as Alkyl phosphorylcholine or alkylglycerophosphoryl choline are exploited as oxygen transport agents and artificial cell or red blood substitutes. Fluorocarbon emulsions is only class has been investigated as artificial blood substitutes over the years [53]. Fluorocarbon liquid is dispersed as physiologically acceptable emulsion as they cause vascular obstruction and death when injected intravenously [54, 55, 56]. For last one decade there is continuous research around different part of world toward developing new emulsifying agents that provide emulsion having great stability and broader utility in many industries including medical and non-medical fields.

Perfluorocarbon emulsions (PFC'S)

Literature review suggest that number of scientist have reported that fluorocarbon compound emulsion may possibly be used as artificial blood substitute for mammals and as perfusion fluid for preservation of internal organ to be transplanted, particularly as substitute infusion fluid capable of transporting oxygen [57]. Size of particle plays important role in toxicity and efficacy of fluorocarbon emulsions, as the particle size is increased beyond.03µm toxicity increases and retention time of particle in blood stream decreases [58, 59]. Apart from particle size, PFC'S having 9 to11 carbon atoms should

be used as a material for artificial blood substitutes in order to be completely eliminated from the from body after parental administration[60]. Excepetionally higher molecular weight PFC for eg. Perflurotributylamine, can be used in combination with commercial plasma expander such as dextran or hydroxyl ethyl starch, or modified gelatin solution to deliver oxygen and act as artificial blood substitutes. Other PFC such as perflurodecalin emulsion cannot be used in combination with plasma expander because of formation of precipitates and increases chance of instability [61]. Oxyctye™ is perflurocabon emulsion under clinical trials [62].

Microemulsions

Small size of Microemulsions (0.1µm) makes them to pass through the small capillaries and therefore can exist as a more stable single phase preparations they do not cause crenation or hemolysis of RBCs [63].Microemulsions, are formed spontaneously by adding suitable surfactants (or a surfactant + a cosurfactant) in appropriate proportions to a non miscible mixture of water and oil , contrarily to classical emulsions. Surfactants having peculiar property of forming micellars aggregates in water and oils (hydrocarbons or fluorocarbons) results in stable microemulsions. Synthesis of a novel microemulsion system requires mixed fluorinated and hydrogenated oil C_8F_{17} CH_2CH CH C_4H_9 along with biocompatible hydrogenated surfactant, Montanox 80 which increased solubility of oxygen more than Fluosol-DA which has been withdrawn from market two decade ago as an oxygen transporter in biomedical applications. Light scattering studies showed that the system was composed of small sized aggregates which should in principle be compatible with blood. After intraperitoneal and intravenous injection in rats, or mice toxicity of the microemulsions was found to be negligible or well ttolerated. Therefore PFC'S shows great promise artificial blood technology. [64]

Miscellaneous Approaches

Recombinant Technology for delivery of blood substitutes

Recombinant human gelatin like protein having isoelectric point near to 8 is an ideal method method to control the clearance of artificial blood substitutes. Slow gelling due to hydroxypropline content results in of the composition which allows the use of high molecular weight protein which helps to maintain suitable colloidal osmotic pressure. These can also prevent risk of anaphylactic shock which exists with most of commercially available formulations. [65]

Cross-linking technique for delivery of blood substitutes

An ideal blood substitute should have acceptable erythrocyte sedimentation rate (ESR) and excretion rate (EXC) values eg. Dextran -Hemoglobin (Hb-Dx) conjugates having a molecular weight between 50kd and 500kd. Other linkers can be used such as acyl phosphate ester benzene pentacarbooxylate and PEG Various modified Crosslinked hemoglobin can therefore be classified in to three main categories which includes intermolecularly cross bridged Hb tetramer or inter and intermolecular cross bridged Hb polymer and Hb surface decorated with inert polymer such as PEG. All three classes are classified as modified hemoglobin which function primarily by preventing glomerular filtration of of acellular Hb and hence eliminate nephrotoxicity problem which is mainly associated with unmodified hemoglobin. [66].

Diaspirin cross-linked hemoglobin (DBBF-Hb) represents a useful model to explore possible correlations between structural-functional alterations and toxicity of hemoglobin-based blood substitutes, as it has been extensively evaluated in vitro and in animal models, [67]

Expected Outcomes

The ability of hemoglobin solution to be oncotically active and transport oxygen suggests that they would be desirable for a resuscitation fluid where rapid initial treatment of hypovolemia and tissue hypoxia is required. But the drawback is in order to function as an adequate resuscitation fluid; hemoglobin solutions must be capable of maintaining tissue oxygenation for prolonged period of time. Also the blood fraction plasma (BSP), which is a physiologically balanced colloidal solution, and fulfills many of the requirements of a blood volume expander, cannot be safely used for this purpose. The high incidence and the risk of transmitting homologous serum hepatitis associated with plasma are so great, that its use is no longer warranted. [68]

Problems associated with hemoglobin based blood substitutes such as Cell free tetrameric Hb, stroma free hemoglobin (Hb) solutions or perflurocarbons (PFCs) are nephrotoxicity and antigenicity. One solution to the problems associated with hemoglobin based blood substitutes regardless of the source is the encapsulation of hemoglobin within a liposome, PEGylated transfersomes, dendrimers or polymerosomes. These have the potential to reduce the toxicity of hemoglobin whether in the tetrameric, cross-linked, polymerized, or recombinant forms as vesicular these systems have been proven to reduce the toxicity of a number of bioactive agents.

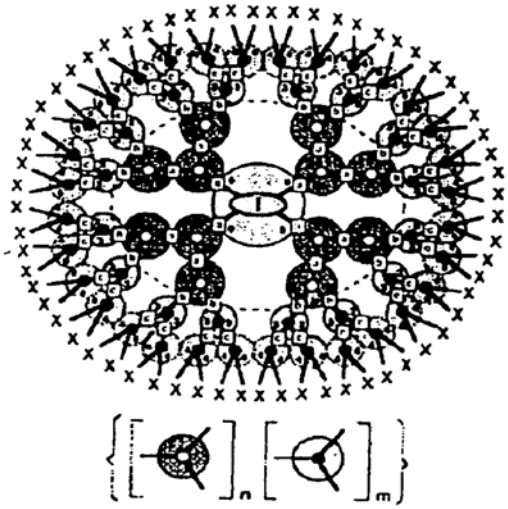

Fig. 1: Dendrimers molecule (Radially layered branch cell organization)

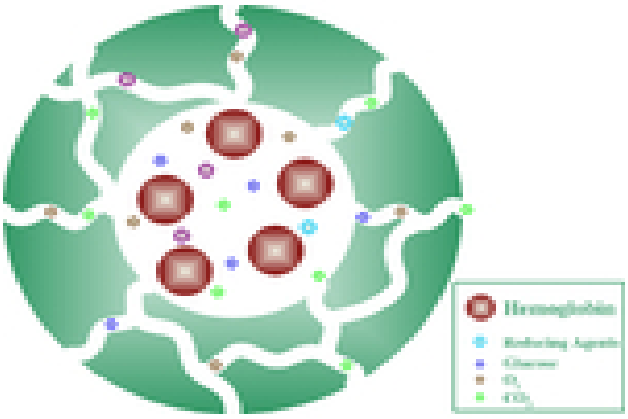

Fig. 2: polymerosomes

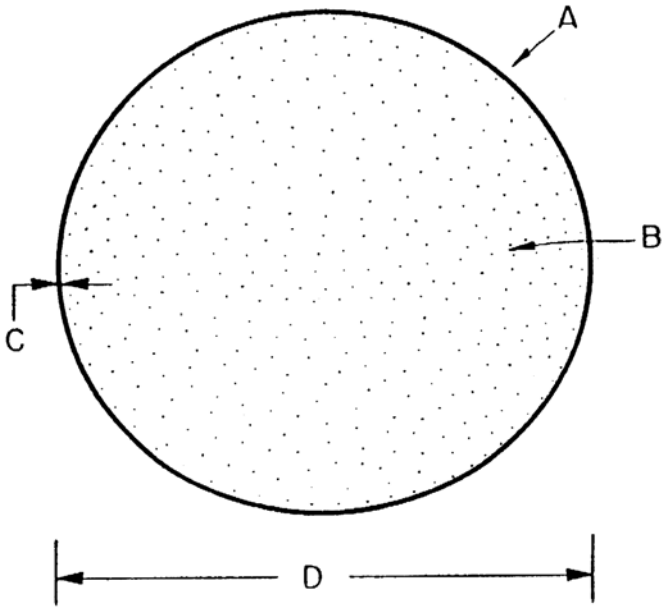

Fig. 3: A:-Insoluble disulfide cross linked polymeric shell (B) Interior of polymeric shell containing fluorocarbon containing dissolved oxygen. C:- Thickness of polymeric shell(5-50nm) D:- Diameter of polymeric shell(0.1-20µm)

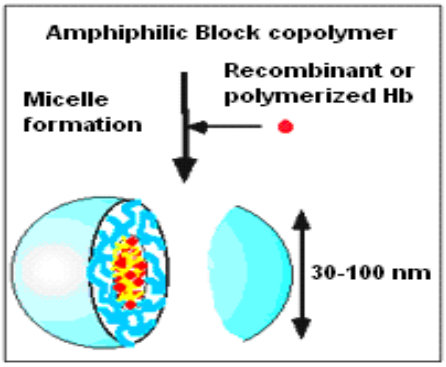

Fig. 3: Biodegradable micelles

CONCLUSION

Although, potentially life saving the use of blood products can be associated with short and long term complications. In addition to the traditional product, synthetic and hormonal treatments are being developed that may be applicable in the emergency department. Available products and the appropriate indication for these products will be reviewed. Vesicular approaches therefore seems to be a promising approaches for the design of a universal oxygen carrier, since it has no blood group antigens on its surface, non-immunogenic, increased circulation time and could be stored for long periods of time.

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