Physiological Properties of Blood Substitutes

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Blood substitutes (modified hemoglobin solutions, perfluorocarbon emulsions) serve as artificial oxygen carriers and are alternatives to blood transfusions. Hemoglobin solutions mimic the sigmoidal oxygen dissociation curve of natural blood. Perfluorocarbon emulsions exhibit a linear relation between PO2 and dissolved oxygen. The most advanced substances may enter medicine in few years.

Natural blood is a near-perfect solution in the body of the person in whom it originated. If transfused to other individuals, allogeneic blood is limited in its efficacy to improve O2 consumption and outcome in severely ill patients (3) and is associated with side effects (8). Such side effects include transmission of infectious diseases, immunosuppression resulting in a higher incidence of postoperative infections, transfusion-related acute lung injury, and significant costs (8). In addition, intermittent blood shortages limit the availability of allogeneic blood (8). As a result, there is a variety of reasons to search for alternatives.

Artificial O2 carriers are alternatives to allogeneic blood transfusions and aim at enhancing O2 delivery to improve tissue oxygenation and function of organs with marginal O2 supply. The purpose of the present article is to describe the currently evaluated artificial O2 carriers, to summarize their efficiency, and to discuss side effects.

Artificial O2 carriers can be grouped into modified hemoglobin (Hb) solutions and perfluorocarbon (PFC) emulsions. The native human Hb molecule needs to be modified to decrease O2 affinity and to prevent rapid dissociation of the native α2-β2 tetramer into α-β dimers. This has been reviewed in detail previously (9, 14). Intravascular half-life is 10–14 h for most Hb solutions as well as for the perfluorobron emulsion, the only PFC emulsion in clinical testing (10, 14). One Hb solution even has a half-life of 24–48 h (14).

The O2 transport characteristics of modified Hb solutions and PFC emulsions are fundamentally different (Fig. 1). The Hb solutions exhibit a sigmoidal O2 dissociation curve similar to blood that may be right shifted, allowing a more complete O2 offloading in the organism (Fig. 2). In contrast, the PFC emulsions are characterized by a linear relationship between PO2 and O2 content. Hb solutions thus provide O2 transport and unloading capacity similar to blood. This means that at a relatively low arterial PO2, substantial amounts of O2 are already being transported. In contrast, relatively high arterial PO2 is necessary to maximize the O2 transport capacity of PFC emulsions.

Hemoglobin solutions

Physiological efficacy of hemoglobin solutions. Efficacy of Hb solutions to transport and unload O2 has been shown in a variety of shock models and at extremem hemodilution (ref-erenced in Ref. 9). Sheep tolerated extreme hemodilution to a hematocrit of 2.4 ± 0.5% only when a polymerized bovine Hb solution was used as replacement fluid but not when colloids devoid of O2 carrying capacity were used. All animals surviving acute hemodilution also survived the following 25 days without evidence of renal or hepatic dysfunction (12).

In a rat model of hemorrhage and surgical trauma, Xu et al. (15) furthermore demonstrated that treatment with α-α-diaspirin cross-linked Hb improved wound healing, enhanced hepatic cell proliferation, and, most importantly, decreased splanch- nical bacterial translocation compared with transfusion of fresh autologous blood. Hb solutions have also been used in resuscitation from hemorrhagic shock (1). In awake sheep bled to a base deficit of −5 to −10 meq/l, infusion of α-α-diaspirin cross-linked Hb restored base deficit at a similar rate to the infusion of autologous blood. In summary, there is no doubt that modified Hb solutions improve tissue oxygenation and function in a variety of pathophysiological conditions.

Side effects of hemoglobin solutions. Since the breakdown of the native α2-β2 Hb tetramer into α-β dimers is largely prevented by genetic modification or chemical modification, nephrotoxicity is no longer a side effect of these solutions (11).

Vasoconstriction results in an increase in systemic and pulmonar y artery pressures. This has been observed with all modified Hb solutions evaluated so far. The mechanisms involved include nitric oxide (NO) scavenging, endothelin release, and a sensitization of peripheral α-adrenergic receptors (9, 14). NO produced by the endothelial cells reacts with the Fe2+ in the guanylyl cyclase located in the smooth muscle cells of the vessel wall to modulate the vascular tone toward vasodilation. It has been speculated that, in particular, unpolymerized Hb molecules may penetrate into the interstitial space of the subendothelial layers of vessel walls. Extravascular Hb at this location would be perfectly positioned to scavenge NO and thus to shift vasomotor tone toward vasoconstriction. Although there are no studies directly proving the presence of exogenous Hb molecules within the interstitial spaces of blood vessels, there are studies documenting extravasation of Hb molecules (9, 14).

Clinical experience with hemoglobin solutions. Allogeneic blood transfusion may be reduced with the use of α-β-diaspirin
 discontinued due to side effects and even increased, was reduced by the use of an Hb solution (2). Despite emergency surgery the number of allergenic blood transfused compared with none in the control group. Also, in the α-packed red blood cells. At hospital discharge, 19% of patients 100% of patients randomized to the control group required blood transfusions until the first postoperative day. In contrast, in the linked Hb group, 59% of patients had no need of allogeneic blood. The excellent O₂ unloading characteristics of this PFC emulsion (referenced in Ref. 7).

Perflubron emulsion was also assessed in severely hemodiluted dogs undergoing cardiopulmonary bypass. Without the use of catecholamines, dogs treated with increasing doses of perflubron emulsion survived cardiopulmonary bypass progressively better than control animals. Also, brain tissue oxygenation may be improved by a combined treatment with perflubron infusion and 100% O₂ ventilation, notably more than with the 100% O₂ ventilation alone (referenced in Ref. 7).

Perflubron emulsion may also be beneficial as an adjunct to resuscitation. In a porcine model of near-fatal hemorrhage, perflubron emulsion treatment in addition to standard resuscitation decreased mortality from 43 to 13%. Although this difference did not reach statistical significance because of a low number of observations (n = 15 total), it was felt by the authors (see Ref. 7) that the additional O₂ provided by perflubron emulsion was beneficial. Also, in a dog model of ventricular fibrillation, the additional direct infusion of oxygenated perflubron emulsion into the aortic arch improved the chances of spontaneous return.

PFC emulsions

PFCs are carbon-fluoride compounds characterized by a high gas-dissolving capacity (O₂, CO₂, and other gases), low viscosity, and chemical and biological inertness (9). PFCs are virtually not miscible with water. The first-generation PFCs, such as Fluosol (Green Cross), used a poloxamer-type Pluronic F-68 as an emulsifier, which, however, has the potential to cause anaphylaxis. The second-generation PFCs use egg yolk phospholipids as emulsifier, which are well tolerated except in patients with an egg allergy (4, 9). The remainder of this report is restricted to the only PFC emulsion in advanced clinical testing, the perflubron emulsion (14).

Manufacturing an emulsion with very specific characteristics is a great technological challenge, because only droplets of a very specific size (~0.16 µm diameter) are well tolerated. The spectrum of side effects also critically depends on the size distribution of the droplets; the narrower the distribution around the target size, the lesser the side effects. With the development of a stable 60% (58% perfluorooctyl bromide and 2% perfluorodecyl bromide) emulsion, there is now a relatively highly concentrated emulsion that is clinically well tolerated (4).

Physiological efficacy of perflubron emulsions. After intravenous application, the droplets of the emulsion are taken up by the reticuloendothelial system (RES). This uptake into the RES determines the intravascular half-life (4, 9), which is ~10 hours after a 1.8 g/kg perflubron emulsion dose (referenced in Ref. 10). After the initial uptake of the PFC emulsion into the RES, the droplets are slowly broken down, and the PFC molecules are taken up in the blood again (bound to blood lipids) and transported to the lungs, where the unaltered PFC molecules are finally excreted via exhalation. At the present time, no metabolism of PFC molecules is known in humans (4, 9).

Perflubron emulsion was assessed in a variety of hemodilution studies. During hemodilution, the expected increase in cardiac output was observed. With the application of the perflubron emulsion, cardiac output tended to increase further and a massive rise in mixed venous Po₂ and mixed venous saturation was observed. The percentage of metabolized O₂ originating from endogenous Hb decreased with the application of perflubron emulsion, indicating that the O₂ transported by perflubron emulsion is preferentially metabolized, most likely because of the excellent O₂ unloading characteristics of this PFC emulsion (referenced in Ref. 7).

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of circulation, and when this occurred, it was achieved earlier than with standard resuscitation (referenced in Ref. 7).

In addition, mixed-venous \( P_O2 \) was higher in perflubron emulsion-treated animals after hemodilution to a Hb of 7 g/dl than in control animals, and measures of left ventricular contractility were also found to be improved after perflubron emulsion administration at a Hb level of 3 g/dl (referred to in Ref. 7). This might be explained by an augmented \( O_2 \) delivery through very narrow capillaries where perflubron emulsion particles (<0.2 \( \mu \)m in diameter) may penetrate more easily than the relatively large red blood cell (7–8 \( \mu \)m in diameter) and thereby increase local tissue oxygenation, including the myocardium (4).

**Side effects of PFC emulsions.** Volunteers experienced mildest flu-like symptoms with myalgia and light fever (4), but with a recent modification of the perflubron emulsion, this no longer represents a clinically relevant problem. In addition, a 15–20% decrease in platelet count 2–4 days after dosing that returns to normal by day 7 is regularly observed (10). Traditional coagulation tests, including bleeding time, however, were unaffected by perflubron emulsion (4).

**Clinical experience with PFC emulsions.** Perflubron emulsion has also been used in humans (13). Acute normovolemic hemodilution to a Hb concentration of ~9 g/dl was performed preoperatively. During surgery, perflubron emulsion (0.9 g/kg) was administered when a blood transfusion appeared necessary. Mixed venous \( O_2 \) tension and mixed venous \( O_2 \) saturation both increased significantly after perflubron emulsion administration, and cardiac output was stable. Although only relatively little \( O_2 \) was transported by perflubron emulsion (~1%), 5% of the metabolized \( O_2 \) originated from perflubron emulsion-transported \( O_2 \), again indicating that perflubron emulsion-transported \( O_2 \) is preferentially metabolized (13).

Recently, the results of a large prospective randomized multicenter study on the use of perflubron emulsion in orthopedic surgery were presented (10). In this study, the patients were hemodiluted preoperatively to a Hb level of 9 g/dl. After the patients had reached a predefined transfusion trigger, they were randomized into four groups: A, standard of care (retransfusion of 450 ml of autologous blood at an unchanged FiO2 of 0.4); B and C, perflubron emulsion (0.9 or 1.8 g/kg, respectively) with colloid to a total 450 ml with ventilation with an FiO2 of 1.0; and D, infusion of 450 ml of colloid with ventilation with an FiO2 of 1.0. Perflubron emulsion at 1.8 g/kg was most successful in reversing transfusion triggers in 97% of patients, compared with 60% in the control group. Transfusion trigger reversal lasted significantly longer (80 min) in the perflubron emulsion 1.8 g/kg group than in the control and colloid groups (55 and 30 min). Thus physiological transfusion triggers may be treated at least as successfully with perflubron emulsion as with autologous blood colloids. This illustrates the remarkable potency of perflubron emulsion to deliver readily available \( O_2 \) to those areas of the body where the extra \( O_2 \) is most needed.

### Future uses of Hb solutions and PFC emulsions

Besides the use of PFC emulsions to reduce allogeneic blood transfusions in surgery, there are numerous other potential future indications based on their potential to augment tissue oxygenation. Such future indications will likely include treatment and prevention of cerebral ischemia, stroke, cardiopulmonary bypass-related cerebral adverse events, spinal cord ischemia, myocardial ischemia due to acute infarction, percutaneous coronary angioplasty, acute limb ischemia, emergency surgery and trauma when no allogeneic blood is available (10), and decompression sickness. Other applications include the use of PFC emulsions to augment tumor oxygenation to render tumors more sensitive to radiation and chemotherapy, prevention or treatment of sequelae of arterial embolism, and finally improved organ preservation for subsequent organ transplantation (referenced in Ref. 4).

Optimal use of Hb solutions and PFC emulsions to reduce allogeneic blood transfusion requirements may consist of a combination of acute normovolemic hemodilution (ANH) with the application of an artificial \( O_2 \) carrier during the operation, a procedure termed Augmented ANH (Fig. 3) (7). Augmented ANH is a concept in which patients will undergo ANH to relatively low hematocrit levels preoperatively. During the operation, when the hematocrit decreases further because of surgical blood loss and concomitant colloid or crystalloid replacement, artificial \( O_2 \) carriers will be administered to maintain tissue oxygenation. As a consequence, lower hematocrit levels can be safely tolerated. Toward the end of the operation, the autologous blood harvested during ANH will be retransfused. This will result in relatively high hematocrit levels in the postoperative period, and \( O_2 \) delivery will again be provided by endogenous red blood cells. Therefore, greatly elevated arterial \( P_O2 \) values are not necessary in the postoperative period, and the relatively short half-life of all artificial \( O_2 \) carriers (<24 h) will not compromise their success in reducing perioperative allogeneic blood transfusion requirements (Fig. 2).
We apologize to all those that made great contributions to the development of artificial oxygen carriers whose work was not directly referenced in this text. Because of space constraints, Refs. 3, 13, and 14 are cited as review articles containing the majority of papers discussed but not directly referenced in this manuscript.

References