

Blood substitutes

Artificial oxygen carriers: perfluorocarbon emulsions

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Perfluorocarbon emulsions are being clinically evaluated as artificial oxygen carriers to reduce allogeneic blood transfusions or to improve tissue oxygenation. Perfluorocarbon emulsions are efficacious in animal experiments, and in humans they are well tolerated and at least as successful to reverse physiologic transfusion triggers than autologous blood. Perfluorocarbon emulsions may be used in the future in the concept of augmented acute normovolaemic haemodilution. In this concept relatively low preoperative haemoglobin levels are targeted during preoperative normovolaemic haemodilution and a perfluorocarbon emulsion is given to augment oxygen delivery during surgery when low endogenous haemoglobin levels are expected. The autologous blood is subsequently retransfused in the postoperative period when the patient's oxygenation is provided primarily by the endogenous haemoglobin. Additional uses of perfluorocarbon emulsions will include treatments of diseases with compromised tissue oxygenation such as cerebral or myocardial ischaemia, air embolism and emergency or trauma surgery as long as no allogeneic blood is available.

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Introduction

Artificial oxygen carriers aim at improving oxygen transport and oxygen unloading to the tissue. Artificial oxygen carriers may thus be used as an alternative to allogeneic blood transfusions or to improve tissue oxygenation and function of organs with marginal oxygen supply. The present article describes the currently evaluated perfluorocarbon emulsions, in order to summarize their efficacy, discuss potential side effects and illustrate potential future applications.

Background of perfluorocarbon emulsions

Perfluorochemicals are chemically inert synthetic molecules that consist primarily of carbon and fluorine atoms, and are clear, colourless liquids. They have the ability to physically dissolve significant quantities of many gases including oxygen and carbon dioxide. Perfluorochemicals are hydrophobic, and are therefore not miscible with water. Perfluorochemicals thus have to be emulsified for intravenous use. With sophisticated technology, it is possible to generate a stable perfluorocarbon emulsion with exceptionally small particles (median diameter $<0.2\mu\text{m}$) [1].

First-generation perfluorocarbon emulsions as well as Russian or Chinese products have been described in a previous review [1]. Western development of second-generation perfluorocarbon emulsions has focused on linear perfluorocarbon molecules such as perfluorooctyl bromide (C8F17Br, also referred to as perflubron), perfluorodecyl bromide (C10F21Br) and perfluorodichlorooctane (C8F16Cl2) [1]. Using solely 3.6% w/v egg yolk phospholipid, Alliance Pharmaceutical Corporation (San Diego, California, USA) developed a concentrated (60% w/v) and stable perfluorocarbon emulsion, Oxygent, which is composed of 58% w/v perfluorooctyl bromide and 2% w/v perfluorodecyl bromide, with an average particle size of 0.16–0.18 μm diameter. Hemagen/perfluorocarbon (St Louis, Missouri, USA) developed another concentrated (40% v/v) perfluorocarbon emulsion, Oxyfluor, using perfluorodichlorooctane, egg yolk phospholipid and triglycerid with an average particle size of 0.22–0.25 μm diameter.

After intravenous administration, the droplets of the perfluorocarbon emulsion are taken up by the reticuloendothelial system (RES). This uptake into the RES

determines the intravascular half life [1–3]. Intravascular half life is dose-dependent, and for Oxygent was found to be 9.4 ± 2.2 h for a dose of 1.8 g/kg [4]. After the initial uptake of the perfluorocarbon emulsion into the RES the droplets are slowly broken down, and the perfluorocarbon molecules are taken up in the blood again (bound to blood lipids) and transported to the lungs, where the unaltered perfluorocarbon molecules are finally excreted via exhalation. At present no metabolism of fluorocarbon molecules is known in humans [1–3].

Safety profile

Oxygent and Oxyfluor both underwent extensive safety testing. The results of most of these studies are not published in the scientific literature, but they form the basis for regulatory authorities to allow clinical testing. Common side effects such as delayed febrile reaction and flu-like symptoms seem to be related to the normal activity of phagocytic cells of the RES. The magnitude of these side effects depend on the particle size of the emulsion. Smaller particles (0.1–0.2 μm diameter) appear to be less detectable for the RES than larger particles (>0.2 μm diameter) and cause only mild temperature increases and mild flu-like symptoms in relatively few individuals [1,3,5].

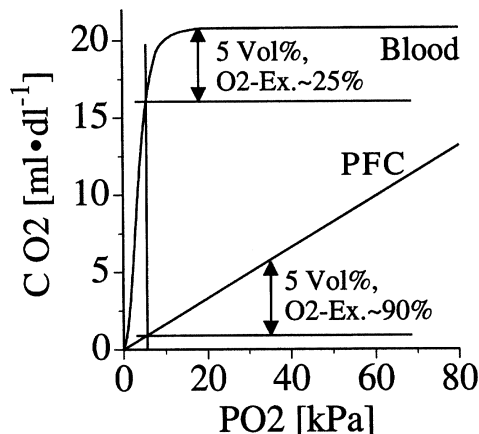
Once in the RES, excretion depends on vapor pressure and lipid solubility of fluorocarbons. Elimination half-time is 3–4 days for perfluorooctyl bromide and 8 days for perfluorodichlorooctane. Because fluorocarbon molecules are inert to biochemical degradation, they diffuse back into the blood where they dissolve in plasma lipids. These lipids transport the perfluorocarbon molecules to the lungs where they are excreted by exhalation.

At higher doses (1.7 g/kg Oxyfluor, 1.8 g/kg Oxygent) a transient decrease in platelet count was observed 2–3 days after dosing, with recovery by 7–14 days [1,6]. With Oxygent, however, the decrease in platelet count was mild (10–20%) and no drug-related effects on platelet function were observed. Furthermore, plasmatic coagulation was not compromised and template bleeding time was not prolonged by 1.8 g/kg Oxygent [1,6]. In addition, there was no complement activation [7]; no suppression of humoral or cell-mediated immune function; no haemodynamic effects or vasoconstriction; no changes in liver, lung or renal function; and no clinically relevant effects on blood chemistry [1].

Oxygen transport

Oxygen transport characteristics of perfluorocarbon emulsions are fundamentally different from those of blood (Fig. 1). Blood exhibits a sigmoidal oxygen dissociation curve. In contrast, perfluorocarbon emulsions are characterized by a linear relationship between oxygen partial pressure and oxygen content. Elevated arterial oxygen partial pressures are thus beneficial to maximize the oxygen transport capacity of perfluorocarbon emulsions.

Figure 1



Perfluorocarbon emulsion and blood-based oxygen transport: oxygen dissociation curve of native human blood (Blood) and a perfluorocarbon (PFC) emulsion. Note that 5 volume % (5 Vol%) of oxygen can be offloaded by blood as well as by a PFC emulsion. With a PFC emulsion, higher arterial partial oxygen tension values are required. Note also that PFC emulsion-transported oxygen is more completely offloaded than blood-transported oxygen, resulting in an approximate oxygen extraction (O₂-Ex.) ratio of 90% for the PFC emulsion, as compared with approximately 25% for blood, assuming a normal mixed venous partial oxygen tension of approximately 40 mmHg. C O₂, oxygen content; PO₂, partial oxygen tension. Modified according to Looker *et al* [24] and Keipert *et al* [11].

Ventilation with 100% oxygen may raise concerns regarding oxygen toxicity. During relatively short exposure times (<8 h), however, no evidence of oxygen toxicity was detected [8] and the earliest signs of oxygen toxicity were observed only after 18 h [9].

Red blood cells are flexible, disk-shaped cells approximately 7–8 μm in diameter and are packed with highly concentrated hemoglobin. In arterioles red cells fill most of the vessel diameter with a relatively small plasma phase near the vessel wall where smaller cells such as platelets (1 μm in diameter) and small particles concentrate (near-wall particle excess phenomenon). In the capillaries the distance between red blood cells increases, producing significant intercellular plasma gaps, and capillaries are found that are perfused by plasma only.

Due to the small size (<0.2 μm in diameter) perfluorocarbon emulsion particles mainly flow in the peripheral plasma layer in larger vessels [1,10]. In the microcirculation, perfluorocarbon emulsion particles perfuse even the tiniest capillaries (4–5 μm in diameter), where no red blood cells may flow under certain conditions. It is precisely this area in which perfluorocarbon emulsions exert their greatest effects, because they augment local oxygen delivery much more than would be expected from the increase in oxygen content in the arterial blood (large

vessel with red blood cells) [1]. Another important aspect that determines the efficacy of perfluorocarbon emulsions is the fact that all oxygen carried by the perfluorocarbon is in the dissolved state, resulting in a higher oxygen partial pressures in the microcirculation and thereby augmenting the driving pressure for the diffusion of dissolved oxygen into the tissue.

Preclinical efficacy of second-generation perfluorocarbon emulsions

A perflubron emulsion (Oxygent) was assessed in a variety of haemodilution studies. Keipert *et al* [11] applied perflubron emulsion in dogs after acute normovolemic haemodilution (ANH) at a haematocrit of 10%. With the application of Oxygent, cardiac output tended to increase and a massive rise in mixed venous oxygen partial pressure and mixed venous saturation was observed. The percentage of metabolized oxygen that originated from endogenous haemoglobin decreased with the application of Oxygent, indicating that the oxygen transported by Oxygent is preferentially metabolized, due to its excellent oxygen unloading characteristics [11].

An increase in mixed venous oxygen partial pressure indicates improvement in global oxygenation status [12], rather than shunting of oxygen from arterioles directly into venules (i.e. oxygen bypassing the microcirculation). This view is substantiated by a study that demonstrated an increase of oxygen consumption in a maximally working, in-situ gastrocnemius muscle preparation in anesthetized and haemodiluted dogs after Oxygent administration [12]. It is also substantiated by the fact that increases in mixed venous oxygen partial pressure are generally associated with an Oxygent-dependent improvement in tissue oxygenation, as demonstrated in the heart, brain, muscle, gut and liver [13,14]. Furthermore, Holman *et al* [15] tested Oxygent in severely haemodiluted dogs undergoing cardiopulmonary bypass. Dogs treated with increasing doses of Oxygent survived cardiopulmonary bypass progressively better than did control animals.

Oxygent may also be beneficial as an adjunct to resuscitation. In a porcine model of near fatal haemorrhage, Oxygent treatment in addition to standard resuscitation decreased mortality from 43 to 13% [16]. Infusion of oxygenated Oxygent into the aortic arch also improved outcome in another resuscitation model [17].

Mixed venous oxygen partial pressure was higher in Oxygent-treated animals after ANH to a haemoglobin of 7 g/dl than in control animals [14,18], and measures of left ventricular systolic and diastolic contractile function were found to be improved after Oxygent administration at a haemoglobin level of 3 g/dl [19]. This might be explained by an augmented oxygen delivery through very narrow capillaries where Oxygent particles may penetrate better

than the relatively large red blood cells, and thereby improve local tissue oxygenation more than red blood cells [1]. These studies thus indicate that Oxygent indeed transports and unloads oxygen into the areas where it is needed most.

Clinical efficacy of second-generation perfluorocarbon emulsions

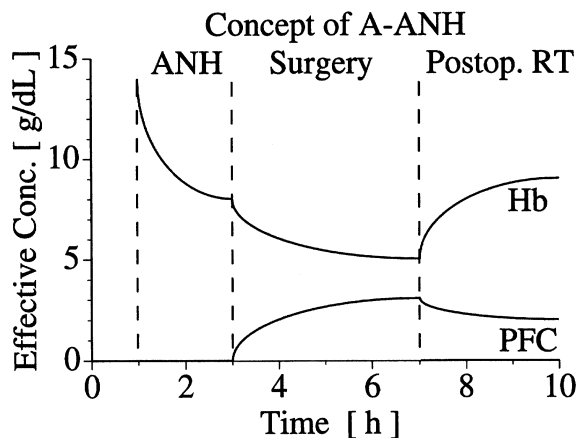
Oxygent has also been used in humans [6,20,21,22]. ANH to a haemoglobin concentration of approximately 9 g/dl was performed [20]. During surgery Oxygent (0.9 g/kg) was administered when a blood transfusion was deemed necessary by the anaesthesiologist, which occurred at a haemoglobin concentration of approximately 8 g/dl. Mixed venous oxygen tension and mixed venous oxygen saturation both increased significantly after Oxygent administration, and cardiac output was stable. Although only relatively little oxygen was transported by perflubron emulsion (approximately 1%), 5% of the metabolized oxygen was transported by Oxygent, again indicating that Oxygent-transported oxygen is preferentially metabolized [11,20].

Recently, the results of two large prospective randomized multicenter studies on the use of Oxygent in orthopaedic and genitourinary surgery were presented [21,22]. In the orthopaedic study [21], 147 patients undergoing hip replacement and spine surgery were haemodiluted preoperatively to a hemoglobin level of 9 g/dl. After the patients had reached a predefined transfusion trigger, they were randomized into four groups: standard of care (retransfusion of 450 ml autologous blood at an unchanged fractional inspired oxygen of 0.4); Oxygent (0.9 or 1.8 g/kg) with colloid to a total 450 ml with ventilation with an fractional inspired oxygen of 1.0; and infusion of 450 ml colloid with ventilation with an fractional inspired oxygen of 1.0. Oxygent (1.8 g/kg) was most successful in reversing transfusion triggers in 97% of patients, as compared with 60% in the control group. The duration of transfusion trigger reversal in the Oxygent 1.8 g/kg group was significantly longer (80 min) than in the control and colloid groups (55 and 30 min, respectively). In the study that including 109 patients undergoing genitourinary surgery [22], similar results were achieved. Thus, physiologic transfusion triggers may be treated at least as successfully with Oxygent as with autologous blood of colloids. This illustrates the remarkable potency of Oxygent to deliver readily available oxygen to those areas in the body in which the extra oxygen is needed most.

Future uses of perfluorocarbon emulsions

Optimal use of perfluorocarbon emulsions in the future may consist of a combination of ANH preoperatively with application of an artificial oxygen carrier such as a perfluorocarbon emulsion during the operation, a procedure termed 'augmented ANHSM' [Roth DJ, Keipert PE, Faithfull NS, Zuck TF, Riess JG: Facilitated oxygen

Figure 2



The concept of augmented acute normovolemic haemodilution (A-ANH) is divided into three periods (a-c). (a) Preoperative ANH with conventional volume replacement without the use of an artificial oxygen carrier such as a perfluorocarbon emulsion. Preoperative ANH targets relatively low hemoglobin levels, close to the individual transfusion trigger. (b) During surgery when the haemoglobin concentration is expected to fall further due to surgical blood loss, a perfluorocarbon emulsion will be used to augment oxygen-offloading capacity to the body. Note that total oxygen offloading capacity from combined haemoglobin-based and perfluorocarbon emulsion-based oxygen transport is maintained during surgery at the level reached after preoperative ANH (ie above the individual transfusion trigger, despite low haemoglobin concentrations towards the end of surgery). (c) Postoperative retransfusion (Postop. RT) of ANH blood increases the haemoglobin concentration above the individual transfusion trigger. Therefore, the decreasing contribution of PFC emulsion-based oxygen transport will not adversely affect overall oxygenation of the organism.

delivery in conjunction with hemodilution. US Patent #5,451,205 (issued September 19, 1995) and European Patent #EP 0627 913 B1 (issued April 4, 1998) (Fig. 2). Augmented ANH is a concept in which patients undergo ANH to relatively low haemoglobin levels preoperatively. During the operation, when the haemoglobin concentration decreases further due to surgical blood loss and concomitant colloid or crystalloid replacement, perfluorocarbon emulsions in conjunction with 100% oxygen ventilation is administered to enhance oxygen delivery and improve tissue oxygenation. As a consequence, lower levels of haemoglobin concentration can be safely tolerated. Towards the end of the operation, the autologous blood harvested during ANH is retransfused. This will result in a relatively high haemoglobin concentration in the postoperative period and oxygen delivery will again be provided by endogenous haemoglobin. Therefore, greatly elevated arterial partial oxygen tension values are not necessary in the postoperative period and the relatively short half-life of perfluorocarbon emulsions (<24h) will not compromise their success in reducing allogeneic blood transfusion requirement (Fig. 2).

For the concept of augmented ANH, it is important not to compromise blood coagulation during the surgical procedure [1,6], otherwise blood loss might be enhanced and thus allogeneic blood savings limited. Therefore, a careful selection of colloids is necessary to avoid blood coagulation becoming compromised [23].

Apart from the use of perfluorocarbon 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 probably include treatment and prevention of cerebral ischaemia, stroke, cardiopulmonary bypass-related cerebral adverse events, spinal cord ischaemia, myocardial ischaemia due to acute infarction, percutaneous coronary angioplasty, acute limb ischaemia, emergency surgery and trauma as long as no allogeneic blood is available [21], and decompression sickness. Other applications include the use of perfluorocarbon emulsions to augment tumour oxygenation to render them more sensitive to radiation and chemotherapy, to prevent or treat sequelae of air embolism, and finally to improve organ preservation for subsequent organ transplantation (referenced in [1]).

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