Basic mechanisms of gas transport and past research using perfluorocarbons

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Key words

Perfluorocarbons, gas solubility, oxygen, nitrogen, carbon dioxide, nitric oxide, solubility, review article

Abstract

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Perfluorocarbon compounds have been utilized either in pure (neat) form or as emulsions suspended in aqueous fluids. These man-made chemicals possess a unique physical property allowing them to dissolve much more of the respired gases (oxygen, nitrogen and carbon dioxide) than any water-based system. Understanding the basic physical chemistry surrounding these emerging medical technologies will assure they are utilized to maximum benefit for mankind. It is clear they should not simply be viewed as 'blood substitutes' but rather as enhanced gas-transport pharmaceuticals.

Introduction

Carbon fluoride chemistry had its early beginnings in the 1930s–40s, during which time a unique feature of carbon halide bonds was discovered – a very high-energy ionic attachment. When a carbon chain or cyclic structure is completely substituted with halogens the ability of other carbon compounds to attack or change the parent structure is greatly limited.[1,2] They become, to a very great extent, inert and unable to be changed. The carbon-fluoride bond is unique in having the highest energy of any organic bond, 120 kcal·mole^{-1}. Solid perfluorocarbon (PFC) coatings are utilized to make non-stick pans. Liquid perfluorocarbon oils have other very useful properties.

During the Manhattan Project, it was discovered that such pure PFC oils were inert insulators. Uranium and plutonium could be stored safely in containers of PFC oils without fear of degradation and/or reaction. One would suppose unplanned reactions between uranium and plutonium could be a bad thing! A serendipitous observation occurred during such storage of radioactive material when it was noted that a tremendous amount of oxygen (O_2) dissolved in the oil. That observation went uninvestigated until the late 1960s and early 1970s, when a group of physiologists suggested that perhaps such oils could be used for medical purposes. LC Clarke, the famous physiologist and inventor of electrodes for pressure and biochemical measurements, along with Geyer and Galon, began experimenting with such PFC liquids.[1–4] They quickly found that tremendous amounts of dissolved O_2 and other respiratory gases could be harbored in equilibrium in such PFC oils. The now classic demonstration of rodents spontaneously breathing oxygenated PFC created outcries both of animal cruelty and fascination. Goldfish could swim in the water above the PFC and, as long as the PFC was in contact with 100% O_2, it seemed animals could live with liquid-PFC breathing for long periods, emerge and survive. This 'scientific trick' was picked up by Hollywood in the movie *The Abyss*. This was not just science fiction, as the producer had turned to advice from several excellent scientists such as Thomas Shaffer and Marla Wolfson, who were in liquid PFC-breathing research.[5–8] *The Abyss* did, however, spawn conjecture that perhaps such human liquid-PFC breathing could either be used as a way to escape from a disabled submarine or work as a new technology for deep sea exploration. Today, science fiction may be yet closer than we had previously believed.

Physiology of PFC usage

This article, however, is intended to discuss the physiology and physical chemistry whereby PFCs, either as pure oils or in intravenous emulsions, can enhance and change mammalian gas exchange.[9] The understanding of how these technologies work may well soon change medicine and mankind's future; but it is only through a careful understanding of their capabilities and limitations that science, not science fiction, will move them to technological reality.

Respiratory gases are transported both by active chemical binding and by passive solubility in fluids.[10] O_2 and CO_2 bind to haemoglobin as well as other metalloproteins throughout the body. The reactions are complex and widely reviewed elsewhere. What is important with regards to PFCs is the fact that, although erythrocytes carry vast amounts of O_2 within them, it is dissolved O_2 that ultimately fuels the energy production in target mitochondria. The red cells create a microenvironment of high-pressure O_2 immediately outside of their cellular membrane. Within Angstroms from their surface, the dissolved O_2 levels drop.[11] A popular myth exists that somehow cells pull O_2 from erythrocytes. It is rather that red cells, through biochemical changes in their cellular pH and 2,3 diphosphoglycerate (2,3-DPG), chloride ion, etc, release more O_2 thereby increasing dissolved O_2 in the local environment. The closer a red cell approaches the wall of a blood vessel the higher the local O_2 concentration gradient for cellular uptake. Total

Table 1
Oxygen content equations with and without perfluorocarbons present; note the dramatic increase in dissolved O_2 when PFC is present[9] (reproduced with permission with minor modification)

O_2 content equation	$Cao_2 = [1.36 \times Hb_{(conc)} \times Hb\ Sat] + [0.0031 \times PaO_2]$	Cao_2: total oxygen content; Hb_{conc}: haemoglobin concentration; Sat: saturation; Pao_2: arterial partial pressure of oxygen
O_2 content equation with PFC present	$Cao_2 = [1.36 \times Hb_{(conc)} \times Hb\ Sat] + (0.0031 \times PaO_2) + (0.1432 \times PaO_2 \times \beta)$	β: Fluorocrit (percentage of whole blood taken up by PFC particles – Oxycyte)
O_2 content equation done a different way with a second-generation PFC − Perflubron	$O_2\ blood = (Y \times O_{2max}) + (4.7 \times 10^{-3} \times V_{RBC} \times P) + (2.9 \times 10^{-3} \times V_{plasma} \times P)$	Y: relative saturation of Hb; O_{2max}: maximum O_2-carrying capacity of Hb (100% saturation; ml O_2.100 ml^{-1} blood) and equals $0.45 \times$ %haematocrit; V_{RBC}: fractional volume of the red blood cell; V_{plasma}: fractional volume of the plasma; P: total ambient pressure. A 1 g.PFC per kg BW dose added to the blood produces a 30% increase in total O_2 in the blood (all present and available for metabolism, since it is dependent only on Henry's law)
Gradient of O_2 from an erythrocyte (Hb) to the mitochondria	$VO_2 – DO_2 (PcO_2 – PmitO_2)$	VO_2: O_2 uptake; DO_2: O_2 diffusing capacity; PcO_2: average capillary partial pressure O_2; $PmitO_2$: average mitochondrial partial pressure O_2

O_2-carrying capacity can be calculated from a standard equation (Table 1).[9] That equation takes into account, but downplays, dissolved O_2 in plasma. Indeed, in most medical teaching the content of dissolved O_2 is disregarded, yet it is dissolved O_2 that the mitochondria actually utilize. Therefore, erythrocytes function as a bank of stored O_2 that continuously overpressurizes the aqueous plasma fluid such that the net flow of O_2 is to the mitochondria of metabolizing cells. As blood courses through tissues, it exchanges O_2 between venous and arterial blood, as well as driving it into tissues. The levels of various gases within tissues is noted in Table 2.[12] The plasma not only is a conduit for gas movement but a resistor, as its capacity for gas solubility is quite limited (Figure 1).[13]

Understanding that the plasma gap functions as a resistor brings to light how PFCs may well be utilized for the future.[13] First, one has to understand the physics of gas solubility in PFCs. Henry's Law states that "*at a constant temperature, the amount of gas dissolved in a liquid is directly proportional to the partial pressure of that gas in equilibrium with that liquid.*"[9] Every fluid has an inherent solubility coefficient for every gas, dependent upon relative molecular polarity and molecular size. Water is a highly polar molecule, whereas lipids tend to be considerably less polar. Most fats, however, still have a large number of protons (hydrogen atoms) as side chains and, therefore, are relatively polar. Once a hydrocarbon molecule has all its available valences substituted with halogens (preferably fluoride), then the resultant carbon-based oil becomes highly non-polar. PFC gas solubility is noted in Table 3.[14]

Pure PFC can carry large amounts of O_2 dissolved at 101.3 kPa.[14] Even more soluble than O_2 is carbon dioxide (CO_2), and nitrogen (N_2) is somewhat less soluble in PFC than is O_2. However, N_2 is highly insoluble in water. Remember the O_2 solubility in plasma is 0.0031 ml.100ml^{-1}, whereas for a PFC emulsion (not pure PFC) the solubility of O_2 is 50-fold higher (Table 1). These facts can be utilized in making gas solubility and content equations (Table 1).

Figure 1
Representation of a tissue bore with a capillary running through it; the cylinders inside the capillary represent red blood cells separated by plasma gaps; various resistances to O_2 movement are indicated, with corresponding PO_2 within the plasma, vessel wall and tissue[13] (adapted with permission)
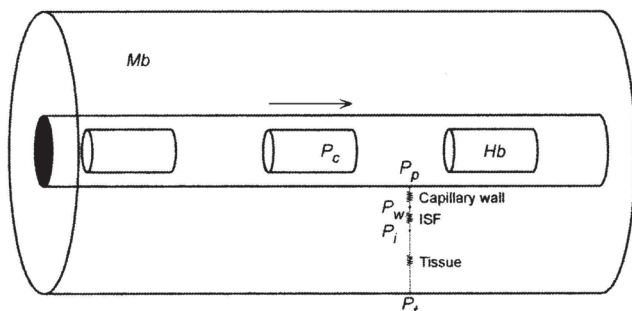
Table 2
Gas partial pressures in various parts of the human body; note the low tissue PO_2 and that all tissues are saturated with N_2 at sea level

Sample site	O_2	CO_2	N_2	H_2O	Total
Inspired air	158	0.3	596	5.7	760
Expired air	116	32	565	47	760
Alveolar air	100	40	573	47	760
Arterial air	100	40	573	47	760
Venous blood	40	46	573	47	706
Tissues	≤30	≥50	573	47	700

Table 3
O_2 solubility at 101.3 kPa in different pure perfluorocarbons; note that these PFC emulsions are between 20–60% PFC whereas modern emulsions are 40–60% PFC; one is limited in usage of the products to approximately 2–5% fluorocrit[9]

Compound	Solubility (mlO$_2$·100ml^{-1} of compound)
Perfluorodihexyl ether	55.42
Perfluorodibutyl sulfur tetrafluoride	48.02
Perfluorotriisobutylamine	44.37
Perfluoro-(*N*-ethylmorpholine)	50.10
Perfluoro-*N*,*N*-dipropylmethylamine	52.60
Perfluorotriethylamine	53.86
Perfluoro-*N*-methylpiperidine	41.33
Perfluoro-*N*-methylmorpholine	37.57
Perfluoro-*N*,*N*-dimethyl-*N*-hexylamine	51.51
Perfluoro-*N*-butylmorpholine	50.59
Perfluoro-4-(*N*,*N*-dimethyl-2-aminoethyl)-morpholine	45.07
F-Tertbutylperfluorocyclohexane	43.00

Pure PFC has been utilized to enhance O_2 delivery in the lungs.[5–8] Small amounts have been nebulized, causing a coating of PFC in the alveoli and terminal bronchi. These experiments seemed to use the PFC as a surfactant, although the enhanced O_2 solubility may have enhanced gas delivery. Also, fully filling the tracheo-bronchial tree with pure PFC and creating liquid-PFC breathing has been accomplished in both animal and human studies. This works to enhance gas transport, and data from respiratory distress syndromes suggest that it may have a clinical application there in the future. Some studies have suggested that by using liquid-PFC breathing, possibly in conjunction with intravenous PFC, decompression sickness could be averted.

Most PFC utilizations in commercial development have focused upon intravenous emulsion technology.[9] PFC, an oil, is immiscible with plasma, therefore, micro-particles of fat emulsions (micelles) have been created.[15–17] At least one technology utilizes perfluorododecapentane, which has a boiling point close to body temperature.[18] This is injected in liquid form and flashes instantly into micro-gas particles, leading to enhanced gas transport within the particles. However, the rest of this article will focus upon the biophysics and chemistry of the emulsions in preparation today.[19–21] Modern-day emulsions are made from egg yolk phospholipids, quite similar to propofol and intralipid. The particles closely range around 0.2 microns in diameter, in comparison to an erythrocyte which is 5–8 microns. The micelles are considerably denser than other formed elements of the blood, and they will separate out in low-flow or standing fluids. However, with normal blood flow the PFC micelles are dispersed between red cells and also pushed to the walls of the blood vessels. The plasma gap essentially is replaced with micelles forming a gas-conduit bridge from

Figure 2
Human sickle red cells in an artificial capillary; A: normal plasma, B: PFC has been added; note the granular appearance of the PFC micelles. The dramatically increased solubility of O_2 in these micro particles overcomes the resistance of the plasma gap when PFC is added to whole blood[9] (reproduced with permission)
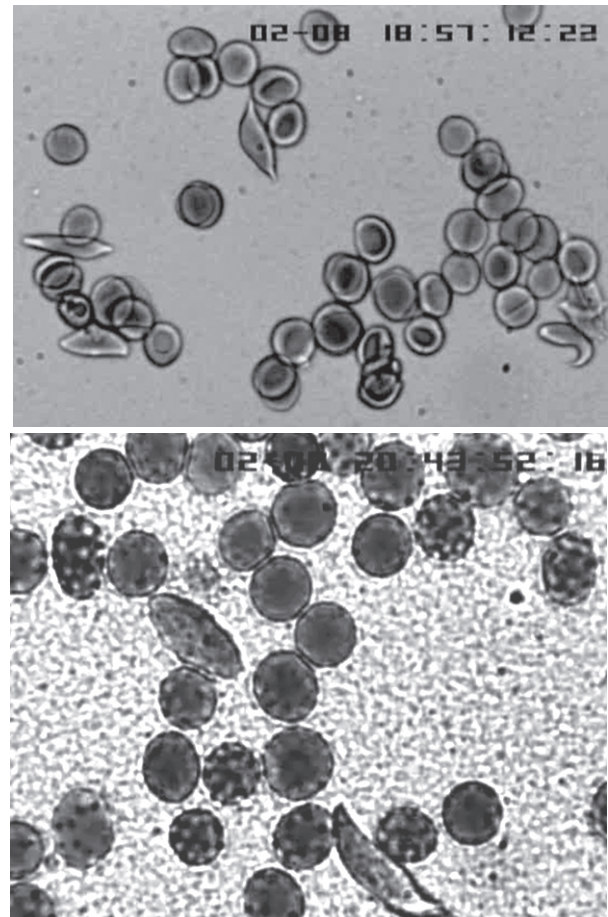
red cells to endothelium and vice versa (Figures 1 and 2). If one considers that the solubility of respiratory gases is up to 50 times higher than in plasma, then this bridge of microparticles allows for rapid gas transfer. Diffusion speed appears to be enhanced by PFC micelle presence, but in reality it is simply a function of enhanced solubility of gas within each micelle and the close proximity of each micelle that makes gas movement appear to speed up.[9]

Because gas molecules in PFC micelles are not chemically bound but are held through enhanced solubility, every molecule of O_2 is available for metabolic utilization. In whole blood, haemoglobin has a complex interaction between each added O_2 molecule in the four haem moieties, as well as being modulated by pH, chloride ion, and 2,3–DPG. Therefore, under normal physiologic conditions, the maximum 21 volumes per cent of O_2-carrying capacity can only release from haemoglobin approximately 5–6 volumes per cent of O_2 (tissue demand). PFC micelles are

rapidly in equilibrium with any microenvironment they inhabit. It is gas solubility according to Henry's Law and the relative gas partial pressures in those tissue/blood or lung microenvironments that determine the content of gases dissolved within them at any one time. PFC can be effective as a third compartment of gas-carrying capacity within the blood stream. The amount of added potential gas-carrying capacity can be calculated (Table 1).

However, gas delivery from the red cells should be thought of as the most important physiologic contribution of PFC. Using normal and low haematocrit blood, adding PFC emulsions increases the mass transfer coefficient by 14% or more. Convective gas movement (forward propulsion of blood) and diffusive gas movement are considerably different.[13] With normal whole blood, convective movement must be present, otherwise tissues become hypoxic very quickly and withdraw all available O_2 from the microcirculation. In several recent studies, it has been shown that, with PFC present in the microcirculation at low or no-flow convective states, tissue O_2 delivery remains present. This must be due to the massively enhanced diffusion effects.

O_2 diffusion is important for normal metabolic function. The first generation PFC that garnered approval for treatment of myocardial ischaemia during balloon angioplasty was not easy to use commercially and was withdrawn from the market. Today, a third-generation compound, (Oxycyte™, Oxygen Biotherapeutics Inc, USA) is being tested for a wide range of tissue ischaemia indications, including traumatic brain and spinal cord injury, organ preservation, carbon monoxide poisoning and cardiopulmonary resuscitation.

N_2 is highly insoluble in whole blood and tissue. Rapid changes in ambient pressure can cause supersaturation leading to formation of a gas phase in blood and tissues. PFC has been shown to increase xenon (another highly insoluble gas) movement out of striated muscle by well over 100%. In multiple experiments using PFC infusions, air embolism effects have been reduced.[17,20,22–30] These studies show dramatic reductions in organ effects. The speed of bubble resolution has been shown to be increased by PFC presence, but the entire story is not simply the increased solubility of N_2 in PFC.[30] Bubbles sticking and interacting with endothelial cells are decreased, perhaps by the surfactant effects of the PFC as well as its emulsifiers. PFC also has under-investigated, independent anti-inflammatory effects and may preserve endothelial cell function. It is entirely possible that PFC may change the stress induced upon endothelial cells when a bubble is present in the microcirculation and that the glycocalyx itself is better preserved.[31] Whilst PFCs speed up the dissolving of N_2, perhaps the more important effect is on O_2 delivery to tissues that would otherwise become hypoxic from the blood vessel blockade due to bubble formation.

CO_2 is far more soluble in PFC than in either whole blood or plasma, and, when PFC is present, cerebral blood flow increases, perhaps 10% or more. Whether this is due to fluxes in CO_2 or whether other mechanisms are at play is not established.[9] Either way, in models of neurologic ischaemia, traumatic brain and spinal cord injury and stroke, PFC appears to have a unique neurological protective effect.

Another important gas in the microcirculation is nitric oxide (NO). NO exerts so many different cellular function-controlling events that it is hard to generalize everything it does. NO is probably very highly soluble in PFC micelles. Only a small number of studies to date have looked at NO effects in the presence of PFC. It appears that PFC at first works as a NO sink, but once it is equilibrated, it then may act as a NO donor.[32] To date, no one has tried attaching NO donors within the micelle itself or pre-equilibrating the emulsions with NO before infusion. Just as PFC micelles enhance movement of O_2 from erythrocytes, so might such micelles enhance the movement of NO from endothelial cells to erythrocytes. Normally haemoglobin is a NO binder and, being encased in the red cells streaming through the centre of capillaries, it is held away from endothelial cells. If PFC enhances NO movement from endothelial cells to haem proteins, one might expect to see hypertension, but this is not described as a side effect of PFC infusions.

Conclusion

In the future, it is likely that an intravenous PFC will become approved as a treatment for tissue ischaemia. Today, a phase IIb double-blind, placebo-controlled, large (128 patients), dose-escalation trial of PFC for the treatment of civilian closed-head injury is underway. Animal studies in blast-induced traumatic brain injury are showing efficacy, and that programme is expanding quickly due to its military importance. The use of PFC infusions for sickle cell crisis and carbon monoxide poisoning is being researched as are other indications. In a second article, the use of PFCs for decompression illness will be reviewed. Suffice it to say that success in such a treatment is dependent upon understanding of the physico-chemical means by which PFC emulsions can carry both O_2 and N_2. The future investigation of enhanced delivery/removal of respiratory gases by PFC will almost certainly encompass a more basic understanding of the physiology of CO_2 and NO fluxes when PFC is present. To look way into the future, the use of liquid-inhaled PFC may yet find a medical usage. It does offer the possibility of being used as a method to create or enhance suspended animation as well as for individual organ preservation.[33] Work and discussions are on-going with space agencies to understand how this might be possible.[34] PFC as a tool for medical application, temporarily changing the way that respiratory gases are transferred within the body, is very exciting.

Disclosure

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The potential role of perfluorocarbon emulsions in decompression illness

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Key words

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Abstract

(Spiess BD. The potential role of perfluorocarbon emulsions in decompression illness. *Diving and Hyperbaric Medicine*. 2010;40:28-33.)
Decompression illness (DCI) is an occasional occurrence in sport, professional, and military diving as well as a potential catastrophe in high-altitude flight, space exploration, mining, and caisson bridge construction. DCI theoretically could be a success-limiting problem in escape from a disabled submarine. Perfluorocarbon emulsions (PFCs) have previously been investigated as 'blood substitutes' with one approved by the United States Food and Drug Administration for the treatment of myocardial ischaemia. PFCs possess enhanced (as compared to plasma) respiratory gas solubility characteristics, including oxygen, nitrogen and carbon dioxide. This review examines approximately 30 years of research regarding the utilization of PFCs in gas embolism as well as experimental DCI. To date, no humans have been treated with PFCs for DCI.

Introduction

Decompression illness (DCI) is an incompletely defined clinicopathological diagnosis in humans with a wide spectrum of presenting signs and symptoms.[1,2] The disease is caused by gas bubble formation/movement in tissues and within the vascular tree or by gas forced into the circulation from pulmonary barotrauma. These gas bubbles cause either primary direct tissue destruction or secondary events from decreased blood flow (oxygen delivery), endothelial cell dysfunction, inflammation, coagulopathy/thrombosis and many other effects. The readership is familiar with many of the manifestations and difficulties with the diagnosis of DCI and is referred elsewhere for review.[1–3]

Mankind lives and works most often in a narrow range of ambient gas pressures. The gas column above us functions as a fluid, exerting continuous equal pressure to all parts of the body. Gases are soluble in tissues and blood, based upon Henry's law. At 101.3 kPa (1 bar) the human body is saturated, with all respiratory gases in equilibrium with the partial pressures of each gas. Seventy per cent of the body is made up of water, therefore the relative solubility coefficients for respiratory gases in water versus fat (oils) determine the total amount of gas dissolved in aqueous media or tissues at any one time.[4,5] It is through a sudden decrease in ambient pressure that tissues and blood potentially become supersaturated with gases. Supersaturation leads to bubble formation. The respiratory gases leave their dissolved state when some, as yet undefined, parameter allows for a small nidus of micro-bubble formation to occur.[6] It has been suspected that micro-particles allow for the original formation of micro-bubbles.[6] Once formed the micro-bubbles grow, potentially rapidly, as local, supersaturated gases move from tissue and blood into the gaseous phase of the micro-bubble.

Growth of a bubble is dependent upon gas composition, internal/external pressures and the surface tension of the bubble itself.[6] Bubble dynamics is an entire study unto itself, but, suffice to say, particularly within the blood stream, bubbles are rapidly coated with proteins that themselves then have complex interactions with tissues, cells and the micro-environment.[6–7] DCI is often thought to be a disease of diving, with inadequate times for gas equilibration between various faster and slower equilibrating tissue categories. However, any rapid reduction in ambient pressure may cause DCI and as man ventures into ever more unique

environments (deep ocean, high altitude and space) the potential for DCI will be ever more challenging.

To date, the standard of care has been to treat suspected DCI with high inspired oxygen (O_2) and, as rapidly as possible, with recompression therapy.[2,8] Recompression therapy requires specialized, expensive, bulky, not easily transported equipment, as well as trained personnel. Transport from remote accident/dive sites to an available treatment chamber may require many hours, involve difficult logistics and therein further delay treatment, as well as altitude exposure during air transport. A robust, easily administered, effective, non-toxic therapy that could begin as soon as DCI was suspected would, therefore, represent a dramatic advance in treatment. The remainder of this article will explore work performed to offer a potential alternative/supplement to existing recompression therapy.

Prior PFC studies

Perfluorocarbons are organic-based oils that have complete substitution of the carbon hydrogen bonds with fluoride.[9,10] Respiratory gases are non-polar molecules whereas water is a highly polar molecule. The gas solubility coefficient for gases is dependent upon relative polarity between the gas and the solution as well as molecular size. Organic fluids, for example fats such as membranes, have a higher solubility coefficient for respiratory gases than water. Plasma (60% of blood) is water-based, with some proteins and fat micelles. Blood supplies O_2 to cells through a complex interaction of erythrocytes (acting as an O_2 bank) and dissolved O_2 in plasma. Nitrogen (N_2) and other insoluble gases have a limited capability of being carried in blood and plasma. PFCs, because of their change in molecular polarity, change gas solubility dramatically. PFCs have a 50- to 60-fold enhanced O_2 solubility as compared to aqueous media. Their ability to dissolve N_2 may be even greater. The use of PFC emulsions (stable intravenous solutions) offers an attractive possibility for the treatment/prevention of DCI. The preceeding article in this issue describes how PFCs act as gas transporters.[11]

Early work with PFC emulsions utilized a relatively weak (less concentrated) set of emulsions. FC-43 and Fluosol-DA 20% were 10% v-v of PFC as compared to solutes and emulsifying agents. Fluosol-DA 20%, a Japanese-manufactured emulsion, was the first 'blood substitute' FDA-approved PFC for human usage.[12] FC-43 had a longer half-life and was utilized for animal studies only. These were investigated in the 1970s and 80s as potential 'artificial blood' compounds. A series of cases of use in humans of Fluosol-DA 20% for patients refusing blood transfusion was heralded and its usage for coronary ischemia made it commercially available.[12] The emulsion itself was cumbersome (supplied frozen and not completely emulsified) and for that reason it was withdrawn from the market in 1994.

In DCI, the earliest work was in rodent models.[13–15] Work in hamsters and rats showed that the use of these agents could potentially prolong life after usually lethal experimental dives. Neither of the groups that did the early work in these rodent models followed up with larger animal models or proposed going to human trials. This initial success did spur other work, but the focus was more on arterial gas embolism (AGE) in heart surgery than on the treatment of DCI in dive accidents.

Treatment of venous (femoral vein) gas embolism (VGE) in rabbits was investigated as a model of surgical gas embolism prevention, and provided early experimental evidence that PFCs could perhaps absorb air.[16] In both continuous (0.25ml·kg^{-1}·min^{-1}) and bolus air embolism models, the instillation of FC-43 dramatically prolonged life in 100% O_2-breathing animals. Both venous and arterial partial pressures of O_2 were better in the PFC groups, whilst central venous pressure was lower in the PFC, O_2-breathing group, suggesting partial resolution of bubble size. In the PFC group breathing room air, the average time to death was the same as in those who did not receive PFC. Of interest, that particular PFC emulsion was stabilized by a unique emulsifier. The use of emulsifier alone did not enhance prolongation of survival.[16] Other studies using larger animal AGE and VGE models showed promise in terms of prevention of organ damage (stroke and myocardial infarction as well as death).[17–20]

An awake-rat, DCI survival study with compression to 690 kPa (6.8 bar) for a short bottom time also demonstrated that PFC infusion immediately after surfacing, but prior to the full development of DCI, dramatically decreased lethality by 24 hours (control 11/12 versus PFC 4/12).[21] The numerical reduction in lethality is not the entire story. Those that died of DCI in the untreated group did so very quickly whereas even those that did die of DCI after PFC treatment and 100% oxygen breathing did so much later in their time course. These first rodent experiments were focused on the efficacy of PFCs for a much more prevalent problem, AGE and VGE in surgery, particularly cardiac surgery, rather than on DCI.

A model of gas saturation in muscle tissue examined radioactive xenon washout. Xenon is an inert and relatively insoluble gas.[22] Dogs were allowed to breathe radioactive xenon until such time as they had saturated all their striated muscle tissues. They were then given either a saline control volume expander or an equal volume of PFC emulsion and the speeds of off-gassing of the xenon and muscle washout were examined. It was calculated that, given the dose of the PFC utilized, its relative amount in a stable emulsion, volume of distribution, etc, that the speed of muscle removal of xenon would be increased by 77%. In actuality the speed of xenon washout exceeded that and was increased 109%. It should be noted that this did not come anywhere near a 30- to 50-fold increase in solubility that could be calculated for xenon in pure PFC alone. However, because the amounts of PFC in stable emulsions are limited and the amount of

emulsion added to the circulating volume is again limited, the washout speeds achieved are more modest.

The findings of PFC-enhanced survival after experimental air-dive DCI went under-appreciated for about a decade. In the late 1990s, the PFC literature was reviewed and a large animal (swine) saturation dive model with >85% lethality perfected. These investigators had used this dive profile as a standardized tool with which to investigate a number of interventions. The United States Navy, in developing this large swine model, has utilized it as a more reliable reflection of human physiology than rodent models. In a now landmark study, 20–25 kg swine were compressed to 485 kPa (4.8 bar) for 22 hours (thought to be a N_2 saturation dive).[23] From that depth, they were rapidly decompressed (202.6 kPa·min^{-1}). The day prior to diving, the swine had an indwelling catheter placed for intravenous or PFC infusion after surfacing. Upon surfacing, the animals were immediately removed from the chamber and placed in plastic cages breathing either room air or $\geq 95\%$ O_2. Three groups were studied: a control group which received volume expansion but breathed room air; another group with volume expansion and enhanced O_2 and the experimental group which received 6 ml·kg^{-1} of PFC (Oxygent™, Alliance Pharmaceuticals Inc, San Diego, California) within 10 minutes of emergence. The results were dramatic, with a reduction in lethality from 85% to 15%. Of interest, this reduction of lethality was similar to that seen in the rodent experiments. Of great interest was the observation that any animals that did succumb in the PFC-treated group died a sudden death. Such animals were up and around their enclosures walking, eating and drinking normally then suddenly collapsed and died as though an air embolism had obstructed a coronary artery. Those who breathed room air or O_2 succumbed to neurological (ataxia and paralysis) and cardiopulmonary DCS (pulmonary oedema and tachypnoea). Thus, not only was there a quantitative difference in lethality, but also a clear difference in the physiological path leading to death.

Other work in AGE had shown that PFCs infused to a cardiopulmonary bypass (CPB) machine prior to either routine CPB or a massive cerebral AGE could dramatically reduce the effects of embolism. AGE is a near-universal event in CPB for heart surgery. Massive AGE, however, is a rare and devastating event, whereas micro air emboli happen in every case. Retinal angiography has shown that temporary occlusion of arterioles occurs in most CPB cases and that by 45 minutes after surgery these occlusions have passed.[24] With a first-generation PFC infusion in dog models of CPB, up to 95% of the microvascular obstructions from AGE could be prevented.[25] In the massive air embolism model, PFC infusion prior to AGE prevented cerebral strokes, attenuated electroencephalographic insult and actually increased brain blood flow. Furthermore, in some unique studies of retinal endothelial permeability after AGE, it was shown that, by using PFC prior to an AGE, endothelial integrity could be maintained.[26,27] Clearly PFC

pre-treatment protected the vasculature from the effects of transiting air. Later work examining present-day PFCs and isolated, cultured endothelial cells has supported this earlier CPB work.[28] Other research has shown that PFC given prior to AGE decreases a bubble's dwell time in the pre-capillary arterioles. Furthermore, they have shown that air bubbles, when touched to the cell membrane of cultured endothelial cells, will cause a programmed cell death for those cells. In the presence of PFC, this effect of killing endothelial cells is largely prevented for reasons that are still not clear. Eckmann has also shown that bubble dissolution time is sped up when PFC is present in the microcirculation. This work was conducted using a Russian PFC formulation very similar to the weak, first-generation FC-43 and Fluosol-DA 20%.[29]

Essentially the work by Dromsky et al could be viewed as either pre-treatment or very early treatment/prevention of DCI. The swine, when surfaced immediately, received intravenous PFC. The AGE and VGE experiments were carried out with the same idea of pre-treatment. In a series of studies trying to understand the physiology of PFC, VGE and DCI models in rabbits and then in swine were utilized. In these experiments, animals were highly instrumented and mechanically ventilated. The respiratory exhaled gases were continuously monitored with a mass spectrometer recording breath by breath end-tidal N_2 concentrations. In the rabbit study with PFC pre-treatment and VGE carried out in exactly the same manner as the work from the early 1980s, those animals that had PFC introduced into their circulation had a higher peak exhaled N_2 and a quicker return to baseline (no detectable N_2, Figure 1).[30] It is interesting to note that, with PFC present, prior to femoral vein air embolism, there exists a small but noticeable amount of N_2 coming out of the animal. This may be due to enhanced N_2 tissue washout because the PFC is stored in its vials with argon gas, not N_2. The conclusion was that PFC partially increased the speed of N_2 elimination through the lungs.

In a swine DCI project, highly instrumented anesthetized, spontaneously breathing animals were dry dived to 608 kPa (6 bar) for 30 minutes bottom time.[31] On surfacing, severe cardiopulmonary DCI developed. Animals were treated with PFC immediately upon removal from the dive chamber and then underwent controlled ventilation with 100% O_2. Although outwardly this study had some similarities to the earlier US Navy work (swine and treatment at surface), it had major differences (spontaneous versus controlled ventilation, anesthetized versus awake, and fully instrumented) and was pursued in an attempt to further understand the physiology of PFC in DCI. Those animals which received PFC had a slightly faster N_2 washout. At necropsy, most of the animals had large bubble loads still present in their pulmonary arteries and right heart, suggesting that even though the mass spectrometer had detected washout of N_2 DCI was very much present.[30] The swine study showed that the creation of bubbles in the venous circulation was greatly reduced when PFC was present

Figure 1
End-tidal N$_2$ (mmHg) over time for each breath with and without infused PFC after experimental venous air embolism[30] (with permission)
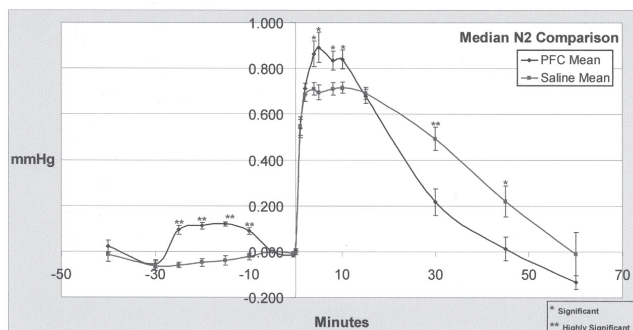
Figure 2
The automated embolic counts per minute in the internal jugular vein of swine[31] (with permission)
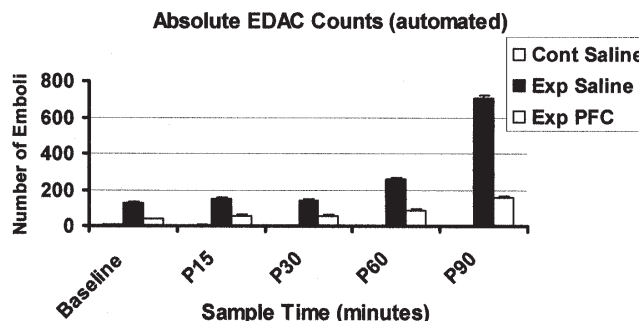
(Figure 2). Control saline is simply a measure of background noise, in that these animals did not undergo DCI. A new bubble-counting technology (EDAC, Luna Technologies, Hampton Roads, VA, USA) had been applied to the internal jugular vein. Using active sonar, this technology counts and can potentially characterize bubble size as emboli move past the sensor. This provides more quantitative information than standard ultrasound detectors. So, although both mass spectroscopy and the bubble-counting device showed promise in terms of the physiologic effects of PFC to blunt the response, the cardiac output and pulmonary artery pressures rose in the animals that received PFC. Swine have a species-specific pulmonary hypertensive response to the micro-particles of the PFC emulsion. This had been well described elsewhere but considerably confused the data. Even with that effect, there was a positive survival effect of PFC as has been the case in all the other, both large and small, animal studies.
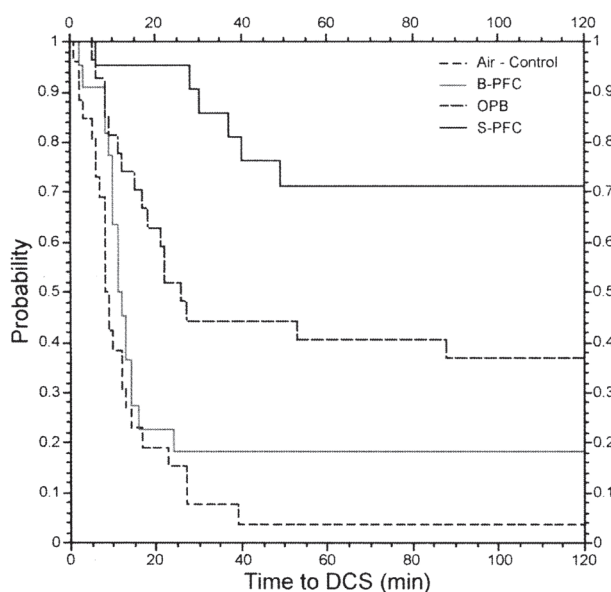
Because of the pulmonary vascular effect in swine, the next series of experiments switched to sheep, as they had little or certainly much less of a pulmonary vascular hypertensive response. Again, changes in exhaled N$_2$ were seen, but more important seemed to be the effects of whole-body O$_2$ delivery and utilization. Although N$_2$ washout curves were similar, the plateau that the animals reached showed more total N$_2$ removed in the PFC group. In animals that received the intravenous emulsion and breathed a helium-O$_2$ mixture, there was a slightly increased removal of N$_2$. The most important finding of this work was not the amount or speed of N$_2$ washout, but that animals with PFC had an increased O$_2$ delivery to tissues and an increased O$_2$ utilization.[32] The conclusion was that there is a combined effect of PFC in DCI, both decreasing bubble effects and, perhaps more importantly, increasing O$_2$ delivery to tissues at risk for ischemia.[32]

The US Navy laboratories have continued working with their awake-swine, saturation-dive model of DCI. In an effort to 'prevent' DCI, PFC was given at depth ten minutes prior to surfacing.[33] Results for PFC given at depth were compared to those with PFC given on surfacing, as well as for a 10-minute period of 100% O$_2$ pre-breathing prior to rapid surfacing (Figure 3) . Both O$_2$ pre-breathing and PFC infusion at depth were better than no treatment, but the best was treatment at the surface with PFC and 100% O$_2$. What was missing from this study was a combination of O$_2$ pre-breathing and PFC at depth as well as O$_2$ breathing during the surfacing time period.

Breathing a high O$_2$ partial pressure combined with an enhanced O$_2$-carrying capacity and tissue delivery of O$_2$ carries the possibility of seizures at depth. It appears that concern is warranted. When the same group investigated grand mal seizure activity at 507 kPa (5 bar) with control versus saline infusion versus PFC infusion, they noted 0/26 seizures in the controls, 1/16 in the saline-infusion group and 7/16 in the PFC-infusion group.[34] In this study, all animals

Figure 3
The Kaplan-Meier curves for development of decompression sickness[33] (with permission)

breathed an enhanced O_2 mixture (46% N_2, 54% O_2). Even with these problems, there was no increase in death rate due to PFC. Indeed, PFC animals had the best survival statistics. Does this mean that DCI cannot be prevented with PFC at depth? To date, there has been no trial using other gas mixtures at depth with controlled levels of O_2, and perhaps helium as an inert gas for pre-breathing prior to assent along with PFC infusion. Also, it cannot be assumed that DCI victims who have previously received PFC infusions could not be recompressed.

Future research directions

Some key pieces of information remain to be investigated before PFC infusion, in conjunction with high FiO_2 breathing, can be recommended as an adjunctive or non-recompression treatment for DCI. If PFC were to be utilized, it is most likely that it would be utilized first in a group of victims who have a delayed or prolonged travel time to definitive recompressive therapy. That would make sense, in that the PFC treatment is portable and easy to administer, as is O_2 therapy. However, a major question remains in terms of how long after symptoms develop does PFC therapy retain efficacy? That question is now being investigated by the US Navy and our laboratory. If, for example, spinal cord AGE was encountered and symptoms had been present for 12–24 hours, would PFC infusion still be able to provide any central neurologic salvage? If a victim receives PFC and then either does not have complete resolution of their symptoms or develops symptoms later, could they safely be recompressed using a standard 284 kPa compression dive table? This question, and any increased risk in seizure activity, is presently being investigated.

A major challenge to regulatory approval of PFC therapy for DCI is the fact that it is essentially unethical to create experimental DCI in humans for the purpose of a randomized treatment trial. The groups working in this area have held discussions with the US FDA to invoke a 'two-animal rule' for approval of a new drug indication. This rule allows for proving efficacy with more than one species using the prescribed treatment, i.e., PFC intravenous infusion at the surface with enhanced O_2 breathing. Still, safety needs to be proven. If a DCI indication is allowed by regulatory agencies, the safety of the drug infusion will have to be proven both in human volunteers (divers at surface without DCI) and other human trials (traumatic brain injury trials, for example, wherein PFC treatment is underway).

Conclusions

There is considerable literature and scientific support for the use of a PFC intravenous infusion in conjunction with 100% FiO_2 breathing to treat/prevent DCI. Although the work has spanned nearly 30 years of animal experimentation, no human has yet to be treated for DCI with PFC. To do so safely today remains as yet unethical, since we do not know the consequences of such treatment in terms of

limiting standard treatment options. However, groups are working today on answering the remaining questions and the data from such studies look promising in terms of being able to recompress victims without undue risk of seizures. Perhaps in the not too distant future, PFC infusions could be utilized at the site of first contact/rescue when an intravenous line is placed to begin treatment prior to transport and recompression therapy.

It may well be possible and ethically appropriate to design a human trial of such therapy in a sub-group of patients for whom lengthy transport or delays to treatment are expected. Only once more is known about recompression with PFC circulating will it be possible to push the possibilities of human trials and/or change recompression tables depending upon N_2 off-gassing and alternative gas-mixture breathing. One motivation for developing PFC treatment of DCI is disabled submarine rescue, wherein a potential mass casualty event with DCI is at least a theoretical fear. PFC appears promising as a therapeutic option in such a disaster scenario, wherein it would be logistically difficult to have enough chambers and trained personnel on site at a DISSUB rescue mission. This is most likely to happen in remote parts of the world with politically difficult or hostile environments meaning that quick movement of medical teams and rescue vehicles into position may be difficult or impossible. Therefore PFC as a pre-treatment or early intervention at 101.3 kPa appears, at least scientifically, at this point to hold great promise.

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