Effects of intravenous perfluorocarbon and oxygen breathing on acute decompression sickness in the hamster

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Lynch PR, Krasner LJ, Vinciquerra T, Shaffer TH. Effects of intravenous perfluorocarbon and oxygen breathing on acute decompression sickness in the hamster. Undersea Biomed Res 1989; 16(4):275–281—Anesthetized female hamsters (Mesocricetus auratus) were divided into 3 experimental groups with 16 animals in each group. After control arterial blood pressure and ECG recordings, the animals were placed in a hyperbaric chamber for 30 min at 7 ATA and then decompressed directly to the surface at a rate of 60 fsw/min. After their removal from the chamber, animals were either not treated (group 1); given i.v. saline while breathing 100% oxygen (group 2), or given i.v. oxypherolperfluorochemical (Fluosol-43) perfusion emulsion while breathing 100% oxygen (group 3). Thirty minutes after decompression, all but one of group 1 had died (a 6% survival rate). Group 2 had a 62% survival rate and group 3 had a 94% survival rate. Perfluorochemicals were observed to reduce the number of bubbles formed, enhance bubble disappearance, and reduce dysrhythmias.

Decompression sickness (DCS) can be devastating, resulting in permanent disability or death. While immediate recompression to the depth of relief remains the primary treatment, other types of therapy have been proposed for those who fail to respond and when there is no compression chamber available (1). The purpose of recompression treatment is to decrease bubble size, increase the resolution of gas bubbles, and increase the oxygenation of tissues. Once the diagnosis of DCS is made, immediate emergency treatment is to administer 100% oxygen and crystalloids i.v. until the patient reaches a recompression chamber (2).

For treating severe acute decompression sickness generated in the hamster (3), we have hypothesized that the perfluorochemical, Fluosol-43, having a high solubility for nitrogen, would absorb this gas in situ and decrease bubble formation (4). (Perfluorotributylamine manufactured as Fluosol-43 by Green Cross Corporation, Osaka, Japan. Distributed as Oxypherol-E.T. by Alpha Therapeutic Corporation, Los Angeles.)
les, CA.) Our hypothesis rests on the knowledge that perfluorocarbon solutions such as Fluosol-43 are capable of delivering more oxygen to the tissues than most other volume expanders, decreasing blood viscosity, and possibly reducing intravascular coagulation.

MATERIALS AND METHODS

Animal preparation

Forty-eight female golden hamsters (Mesocricetus auratus) weighing between 80 and 140 g were anesthetized with an i.p. injection of 50 mg/kg of sodium pentobarbital. They were then placed in the supine position on a lucite animal board. A tracheostomy was immediately performed on each animal using PE-200 polyethylene tubing. A polyethylene PE-10 catheter was placed in the femoral vein, attached to a two-way stopcock and used for the injection of additional anesthesia or plasma expanders. The contralateral femoral region was surgically exposed to visually observe blood movement and bubbles in the femoral vein and the femoral artery. A common carotid artery was catheterized with PE-50 polyethylene tubing and connected to a Statham db-23 pressure transducer for recording arterial blood pressure. Subcutaneous needle electrodes were placed into the limbs for recording the ECG. A control recording of the arterial blood pressure and ECG (lead II) were made on an Electronic for Medicine VR-6 polygraph. All surgically exposed sites were kept moist with mammalian Ringer's solution.

Experimental protocol

Once the surgery was completed and a baseline graphic tracing was obtained, the animals were given a small dose of additional anesthesia (0.2 ml s.c.). The hamsters were then placed in a CGS Scientific Corporation hyperbaric chamber. The pressure was raised in the chamber to 7 ATA, equivalent to approximately 60 m or 200 ft of sea water, for 30 min. This saturates the hamster tissues with nitrogen (5). The pressure was then released in the chamber over 3 min until reaching 1 ATA (sea level). The chamber door was opened and the animal was removed to the binocular microscope stage for direct observation of the femoral vascular region. The animal's ECG leads and arterial catheters were attached to the amplifiers so that blood pressures and ECGs could be recorded. Recordings of blood pressure and lead II of the ECGs were made every 5 min over the next 35 min. Then all surviving animals were killed for a detailed postmortem examination.

Our definition of survival included those animals that were breathing on their own after 30 min decompression, based on previous experimental evidence that hamsters breathing on their own 0.5 h after decompression had a strong probability of surviving (3). Animals were randomly selected and placed into 1 of 3 experimental groups after they were removed from the hyperbaric chamber. Group 1: Sixteen animals received no therapeutic support (i.e., they were not placed on a respirator or given any fluids after being removed from the chamber). Group 2: Sixteen animals were given 1.5 ml of physiologic saline (0.9%) i.v. and placed on a respirator that delivered 100% oxygen. Group 3: Sixteen animals were attached to the small animal respirator which
delivered 100% oxygen and were given 1.5 ml of the perfluorocarbon (PFC) Fluosol-43 i.v.

Data reduction and analysis

The Fisher Exact Test was used to determine survivability. Two-way analysis of variance (ANOVA) with repeat measures was used to determine the significance of progressive cardiovascular responses. Statistical significance was defined as $P < 0.01$.

RESULTS

The results of the experimental program clearly support our hypothesis that Fluosol-43 will reduce the mortality caused by the severe form of acute DCS seen in this hamster model (4). Using the Fisher Exact Test we found that the group 1 animals were different from group 2, with a $P < 0.01$; group 1 animals were different from group 3 with a $P < 0.01$, and finally group 2 animals were different from group 3 with a $P < 0.01$ (Fig. 1). Thus the differences between groups are all statistically significant.

Severe DCS in this hamster model is associated with major changes in cardiovascular dynamics as evidenced by heart rate, blood pressure, and electrocardiographic changes. Heart rate decreased by at least 50 beats/min following decompression in all experimental groups (Fig. 2 left). Immediately after decompression mean heart rate decreased significantly ($P < 0.01$) from the control period. Group 1 heart rates continued to decline throughout the next 16 min.

Blood pressure decreased in all of the animals after decompression. In group 1 animals this decline, like that of the heart rate, was rapid and few animals were alive 15 min after decompression. Animals in groups 2 and 3 maintained lower blood pressures than they had during the control period ($P < 0.01$) (Fig. 2 right).

Group 1 animals had an immediate increase in T wave amplitude of the ECG, followed by a brief tachycardia, multifocal ectopic beats that eventually lead to a 2:1 or 3:1 heart block. An S-T segment deviation was seen in 2 animals that survived, 1 for 20 min and the other for 30 min. Group 2 animals had multifocal ectopic beats,

Fig. 1. Survival data from groups 1, 2, and 3.
increased T wave amplitude, widening of the QRS complex, and second degree heart block. The animals that died in group 2 had a profound bradycardia. Group 3 animals had distinctly fewer ectopic beats and the majority (11 hamsters) had nothing but a sinus rhythm following decompression. One animal in group 3 had a 2:1 heart block.

Group 1, which received no therapeutic treatment after hyperbaric exposure, had a massive number of bubbles in the femoral veins and in a few minutes in the femoral arteries (4). The animals’ respiration became labored, they began to gasp, and in a few minutes stopped breathing. Only 1 animal in this group was still breathing 35 min after decompression. All group 2 animals, which received saline and 100% oxygen following decompression, had bubbles in their femoral veins. All but 1 animal eventually had bubbles in the femoral arteries. Surviving hamsters (n = 10) of group 2 had no femoral arterial bubbles when observed 35 min after decompression. Group 3 animals all had bubbles initially in the femoral vein, but femoral arterial bubbles were never seen in 6 of the 16 PFC experiments. In those animals (n = 10) in which bubbles were observed in the femoral artery following decompression, the bubbles all disappeared within 30 min with only 1 exception. One animal in this series died in the first 9 min.

In postmortem findings, group 1 animals all had massive bubbles in their gastrointestinal blood vessels, liver vessels, coronary arteries, right atrium, and right ventricle; 8 animals also had a few bubbles in the left ventricle. The lungs appeared congested or hemorrhagic in 6 hamsters. The 1 surviving hamster in group 1 had no bubbles in the coronary arteries or left ventricle 35 min after decompression. Group 2 hamsters that died before 35 min all had observable gas bubbles in the gastrointestinal vessels, livers, coronary vessels, and right and left ventricles. Those animals in group 2 listed as survivors had no bubbles in the coronary vessels or left ventricles when the postmortems were performed 35 min after decompression. Group 3 hamsters, with 1 exception (the animal that died), had a few isolated bubbles in the gastrointestinal vessels, livers, and right ventricles, but no bubbles were observed in either the coronary vessels or left ventricles. The 1 animal that died in group 3, 9 min after decompression did have massive bubbles in the femoral vein and femoral artery.
DISCUSSION

The i.v. injection of a commercially available PFC, Fluosol-43, clearly modifies the course of acute DCS in the hamster model. Figure 1 shows that perfluorochemically treated animals had a clear advantage in survival; 94% of the group 1 animals died, whereas 94% of the animals given Fluosol-43 i.v. plus 100% oxygen survived, with higher, more sustained blood pressures and distinct reduction or absence of arterial gas emboli. The PFC-treated hamsters had a remarkable absence of ectopic rhythms on the ECGs recorded. Although bubbles did occur in the femoral veins and occasionally in the femoral arteries, they disappeared rapidly.

Since perfluorochemicals were introduced by Clark and Gollan in 1966 (6) for use in successful liquid-breathing experiments in mice, these chemicals have been used for a variety of investigations into gas transport, particularly oxygen. Gollan and Clark (7) perfused rat hearts with oxygenated perfluorochemicals and kept them alive in vitro. Sloviter and Kamimoto (8), using emulsified FX-80 PFC with albumin, maintained rat brain function for several hours. Geyer et al. (9), using a solution containing emulsified fluorotributylamine, replaced almost all the blood of rats and they survived.

Perfluorochemicals are derived from hydrocarbons and other organic compounds in which fluorine atoms replace hydrogen atoms. Oxygen is highly soluble in most liquid PFC compounds (10). As much as 65% oxygen by volume can be dissolved in undiluted perfluorochemical media (9). Furthermore, in certain perfluorochemical compounds, carbon dioxide is at least twice and sometimes 3 times as soluble as oxygen (11). Most important to our interest is that nitrogen readily diffuses into perfluorochemicals. Nitrogen has a 24 and 30 times higher solubility in perfluorodecalin and perfluoropropylamine at 37°C than in plasma (11).

In DCS the ability of the blood to rapidly transport nitrogen from the tissues to the lungs without forming gas emboli may be the key to survival. Solubility of nitrogen in fluorocarbons is high (12–15). Cassuto et al. (13) used fluorocarbon infusions to remove nitrogen from subcutaneous air pockets in rats. Nitrogen has a solubility of 40–50% in pure perfluorocarbon emulsions (PFEs) (14). The speed of gas diffusion into the perfluorochemicals is not significantly different from normal gas diffusion into plasma, but the storage capacity is greater (15). Recently, Spiess et al. (16) demonstrated that volume expansion with PFC FC-43 provides protection from coronary air emboli in a spontaneously perfused dog model ventilated with 100% oxygen.

In our experiment, the PFE clearly reduced the incidence of dysrhythmias, which may have been related to tissue hypoxia caused by gas emboli. In another study it was shown that giving a dog PFE before a venous air embolism also offered protection. However, the PFE and 100% oxygen therapy were given before the embolization, and the protective effects were theorized to be related to the denitrogenation by FC-43 plus the increased oxygen-carrying capacity (14).

More recently, Spiess et al. (15) demonstrated that the survival of Sprague-Dawley rats was longer if the animals were treated with perfluorochemical (FC-43) after DCS (16). The PFE (FC-43) in sufficient quantities decreases blood viscosity. Solutions of PFE contain particles that are between 70 and 80 times smaller than erythrocytes, increasing the surface area for gas absorption. More gas can be absorbed into a volume of PFC than an equal volume of plasma. All of these factors may contribute
to the effectiveness of perfluorocarboxylate in the emergency treatment of severe DCS as well as air embolism (14–16).

The objectives of oxygen treatment for DCS are to reduce ischemia and assist in the removal of inert gases. The objectives of fluid therapy in DCS are to replace depleted volume, to restore hematocrit, to prevent blood sludging, and to improve tissue perfusion (2, 3). The reduced plasma volume in severe DCS may be related to the destruction of platelets and the release of thromboxane, which increases blood vessel permeability (17).

In conclusion, we have demonstrated that PFC FC-43 plus 100% oxygen therapy improves hemodynamic data and reduces the dysrhythmias seen in acute DCS. This FC-43 PFC is a commercial material that has been used in humans for fluid volume expansion when oxygen delivery was also a consideration (18).

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