Dodecafluoropentane (DDFPe) and Decompression Sickness-Related Mortality in Rats

Ryan L. Sheppard; David P. Regis; Richard T. Mahon

BACKGROUND: Perfluorocarbon (PFC) formulations can be a useful adjunct treatment for decompression sickness (DCS) when staged decompression procedures cannot be followed due to time constraints or lack of equipment. The benefit of PFC treatment is believed to result from its ability to transport more dissolved gas than can be transported by blood alone. Dodecylfluoropentane (DDFPe) is a unique nanodroplet compound that expands into a gaseous state when exposed to physiological temperatures, resulting in a higher dissolved gas-carrying capacity than standard PFC formulations.

METHODS: We investigated the efficacy of DDFPe in reducing morbidity and mortality in a rat model of severe DCS. Male Sprague-Dawley rats (250-280 g) were compressed to 210 fsw for 60 min before rapid decompression. Animals were immediately injected with 2% DDFPe (0.07 ml · kg^{-1}, 0.5 ml · kg^{-1}, 1.0 ml · kg^{-1}) or saline, and were transferred to a 100% O_2 environment for 30 min.

RESULTS: Of the animals in the saline group, 47% (18/38) did not survive the decompression event, while ~98% (46/47) of the animals in the DDFPe group did not survive. Of the animals that died during the observation period, the saline group survived on average 89% longer than DDFPe treated animals. Seizures occurred in 42% of the DDFPe group vs. 16% in the saline group. Histological analysis revealed the presence of large, multifocal gas emboli in the liver and heart of DDFPe treated animals.

CONCLUSIONS: We conclude that DDFPe is not an effective nonrecompressive treatment for DCS in rodents.

KEYWORDS: DCS, diving, rodent, perfluorocarbon.

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Though the mechanisms of decompression sickness (DCS) are not fully known, one of the necessary conditions is a change in the ambient pressure sufficient to reach a state of tissue supersaturation with inert gas (most commonly nitrogen). Once tissue supersaturation is reached, the formation of intravascular and tissue-based bubbles is possible. It is believed that DCS is mediated by the presence of these gas bubbles, resulting in endothelial dysfunction, inflammation, and hemostatic changes.[12] The standard treatment for DCS is aimed at decreasing existing bubbles, eliminating inert gas, and improving oxygen delivery to tissue beds. In general, this is accomplished by recompression in a hyperbaric chamber followed by a slow, controlled decompression in conjunction with hyperbaric oxygen. Unfortunately, such hyperbaric chambers have limited portability and require qualified and experienced personnel to operate safely. Consequently, depending on the geographic location and availability of resources, scenarios such as a disabled submarine rescue may not allow for staged decompression procedures on such a scale. In these cases nonrecompressive (i.e., those not requiring the use of a hyperbaric chamber) treatment strategies are needed. Intravenous perfluorocarbons (PFC) treatment is one such nonrecompressive strategy.

PFC formulations are fluorinated hydrocarbons that can dissolve and transport greatly increased levels of oxygen and other respiratory gases over plasma alone.[16,19] PFC emulsions have been shown to increase inert gas elimination and oxygen delivery in tissues[9,18,27] and to decrease morbidity

From the Naval Medical Research Center, Silver Spring, MD.

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Address correspondence to: Diana Temple, M.P.H., Undersea Medicine Department, Naval Medical Research Center, 503 Robert Grant Ave., Silver Spring, MD 20910; diana.temple@med.navy.mil.

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and mortality in animal models after a decompressive event.[2,4,13] Dodecafluoropentane (DDFPe) is a unique PFC emulsion that consists of nanodroplets which expand into a gaseous state when reaching physiological temperatures, but do not appear to form actual microbubbles in vivo at room temperature.[7] In vitro, DDFPe's oxygen carrying capacity more than doubles when temperatures are increased from 21°C to 37°C, and DDFPe has been shown to carry significantly more oxygen than other PFC formulations.[7] Additionally, this expansion into a gaseous state at physiological temperatures confers a small intravenous dosing schedule when compared to other emulsified PFCs.

DDFPe enhances nitrogen elimination in oxygen breathing anesthetized swine[9] and reduces mortality in a severe anemia model by increasing oxygen tension.[10] Though not previously evaluated for efficacy in treatment of DCS, DDFPe may facilitate greater oxygen delivery to, and nitrogen removal from, tissue beds that are obstructed by bubble formation or other vessel occlusion, preventing red blood cell migration. The goal of this study was to assess the viability of DDFPe as an adjunct treatment for DCS in rodents following rapid decompression. We surmised that DDFPe would reduce the incidence and severity of DCS in a rat model following rapid decompression.

METHODS

Animals

The study protocol was reviewed and approved by the Walter Reed Army Institute of Research/Naval Medical Research Center Institutional Animal Care and Use Committee in compliance with all applicable Federal regulations governing the protection of animals in research. All animals were maintained under the surveillance of veterinary staff and this facility is AAALAC accredited. Male Sprague-Dawley rats ($N = 47$ DDFPe and $N = 38$ saline) weighing 250-280 g were pair-housed at the animal care facility and maintained on a 12-h light/dark cycle with free access to standard rodent chow and water.

Procedures

After 7 d acclimation, animals were fitted with tail-vein catheters immediately prior to beginning the experiment. Animals were inserted into individual lanes of a five-lane custom built steel mechanical running wheel and the entire device was positioned inside a small animal hyperbaric chamber. As previously described,[15] the wheel was fitted to an exterior motor and kept at a constant 3.6 $m \cdot min^{-1}$ pace to ensure similar activity level and respiratory rate between animals. The hyperbaric chamber was pressurized at a rate of 60 $fsw \cdot min^{-1}$ to a depth of 210 fsw. The animals were maintained at 210 fsw, 21% O_2, and 28°C for 1 h (long enough to reach tissue saturation of inert gas). After 1 h the chamber was rapidly decompressed and the animals were immediately removed and injected via tail vein catheter with either chilled saline or a chilled 2% DDFPe formulation (NuvoxPharma, Tucson, AZ) at one of three doses: 0.07 $ml \cdot kg^{-1}$, 0.5 $ml \cdot kg^{-1}$, or 1.0 $ml \cdot kg^{-1}$. All injections were normalized to 1 ml total volume.

Immediately following injection each animal was transferred to a Plexiglas holding box ventilated with 100% oxygen and closely observed for up to 30 min. The 30-min observation period was based on extensive experience with this dive protocol demonstrating that animals surviving the first 30 min after surfacing typically have a full recovery within the next 24 h. Signs and symptoms indicative of DCS induced by this dive protocol include the following: rapid and/or heavy respiration, slumped posture, ataxia, fasciculation of the digits, recumbent position, paralysis, and seizure. Necropsies were performed immediately after confirmation of death, while survivors of the 30-min observation period were euthanized via decapitation prior to necropsy. Tissue samples included heart, liver, lungs, kidney, stomach, intestines, adipose, and skeletal muscle. Harvested tissues were removed and fixed in 10% neutral buffered formalin before being trimmed, sectioned at 5-6 mm thickness (minimum of five sections per tissue, per animal), and stained with hemotoxylin and eosin. Emboli area was calculated via NIH ImageJ software (National Institutes of Health, Bethesda, MD) with vasculature and sectioning artifacts excluded from the analysis. Sections were analyzed by a board-certified veterinary pathologist using an Olympus B-50 light microscope.

Statistical Analysis

A 1-way ANOVA and Student's *t*-test using PRISM (GraphPad Software, Inc., La Jolla, CA) were used for comparison of means between saline and DDFPe groups. All values are expressed as means ± SE, except for bodyweights, which are expressed as means ± SD. Values of $P < 0.05$ were considered statistically significant.

RESULTS

The average weight for animals in the DDFPe and saline groups was 267.2 ± 8.78 g and 266.4 ± 8.73 g, respectively. Extensive experience with this dive protocol in our laboratory indicates that we could anticipate ~50% mortality in rats, with mortality being defined as not surviving the 30-min observation period. Mortality in the saline group was approximately 47% while mortality in the DDFPe group was significantly higher at approximately 98% [$F(4,127) = 19.42$, $p < 0.001$] (**Fig. 1A**). Mortality by dose was as follows: 0.07 $ml \cdot kg^{-1}$ = 100% ($N = 28$), 0.5 $ml \cdot kg^{-1}$ = 92% ($N = 13$), 1.0 $ml \cdot kg^{-1}$ = 100% ($N = 6$) (**Fig. 1B**). Of the animals that did not survive the observation period, death in the DDFPe group occurred significantly faster than in the saline group [218 ± 17 s vs. 362 ± 43 s, respectively; $t(62) = 3.86$, $p < 0.001$] (**Fig. 2A**). Tonic-clonic seizures were observed in 42% of the animals in the DDFPe group, but in only 16% of the saline group [$t(83) = 2.748$, $p = 0.007$] (**Fig. 2B**). None of the animals from either group manifesting this symptom of neurological DCS survived the observation period. To rule out DDFPe toxicity alone as the cause of death, 10 animals were administered 1.0 $ml \cdot kg^{-1}$ of 2% DDFPe as described

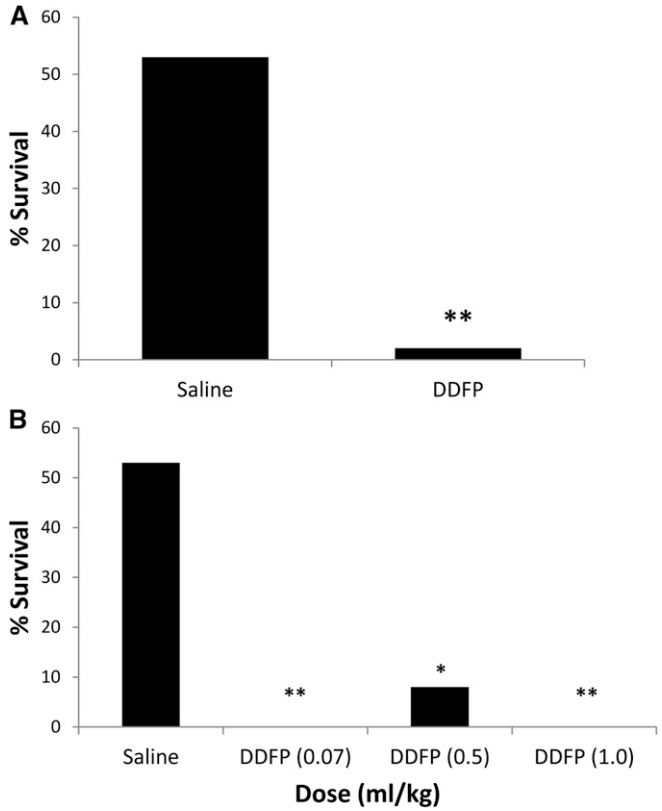

Fig. 1. Effects of DDFPe on mortality in a rat model of DCS A) overall and B) by dose of DDFPe. Animals were treated with saline or a 2% DDFPe solution with doses ranging between 0.07-1.0 ml • kg^{-1} immediately upon surfacing. Asterisks indicate significant difference from saline controls; *$P < 0.05$, **$P < 0.001$.

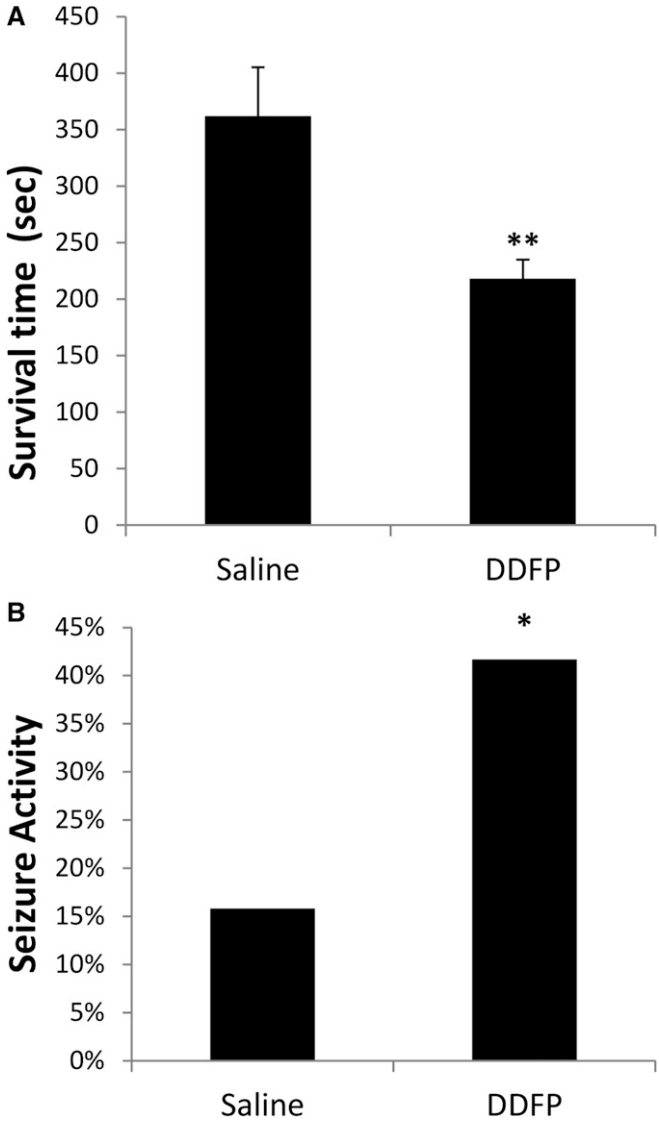

Fig. 2. A) Time of death (post-surfacing) for animals that did not survive the 30-min observation period. B) Incidence of seizures in saline and DDFPe treated animals. Seizures were visually confirmed by experienced veterinary technicians. *$p < 0.05$; **$p < 0.001$.

above, but the hyperbaric chamber was not pressurized. All animals survived and showed no observable signs of distress or discomfort (data not shown).

Necropsies were performed by a board certified veterinary pathologist. No gross abnormalities were apparent in the lungs, kidneys, stomach, intestines, adipose, or skeletal muscles of any of the animals; however, atypical blanching and bubble formation was noted in the liver and heart of multiple DDFPe treated animals. Upon histologic examination significant lesions were noted in the liver, consisting of multifocal to coalescing foci of hepatic sinusoidal dilations with notable compression of adjacent hepatocytes (**Fig. 3**). Sinusoidal dilations were noted across the lobes and affected 5–10% of total liver parenchyma. In contrast, no significant hepatic sinusoidal dilations were noted in the saline treated animals (Fig. 3). Sections from all five hepatic lobes were H&E stained and total dilation area (excluding vasculature and sectioning artifacts) was quantified. Dilation area in the DDFPe sections was found to be nearly twofold greater than in the saline sections [$t(11) = 2.648$, $p = 0.023$] (**Fig. 4**). Similar sinusoidal dilations were observed in the cardiac muscle (**Fig. 5**); however, the effect was not as pronounced as in the liver sections and the presence of significant artifacts and sectioning damage in the cardiac tissue prevented an accurate quantification of emboli area as was done for the liver sections.

DISCUSSION

To our knowledge, this is the first study to directly examine the efficacy of DDFPe in preventing DCS-related mortality in rats. Though we hypothesized that prophylactic administration of DDFPe would be protective against DCS following rapid decompression, DDFPe was associated with a marked increase in mortality from DCS in all doses studied. Additionally, DDFPe treatment was also associated with a significant increase in clinically apparent seizures compared to saline only, and animals that seized subsequently expired rapidly (< 30-60 s).

Though DDFPe had a significantly negative impact on survival after rapid decompression, administration of a 1.0 ml • kg^{-1} dose resulted in no apparent negative effect on animals that were not exposed to decompression stress. This is not

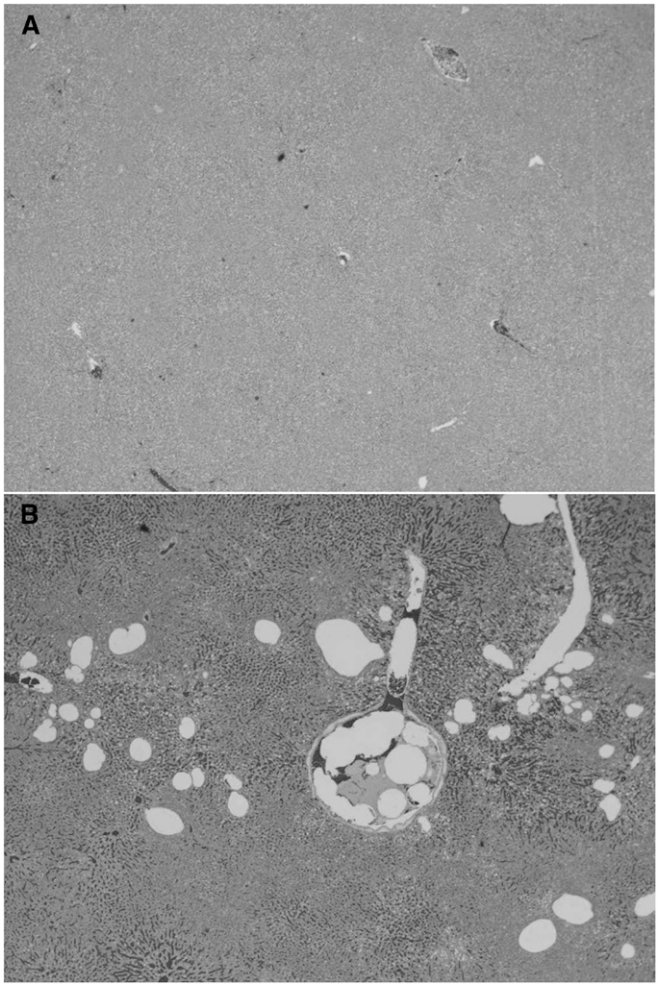

Fig. 3. HE stained liver sections (20×) from A) saline and B) 1.0 ml • kg^{-1} DDFPe treated animals.

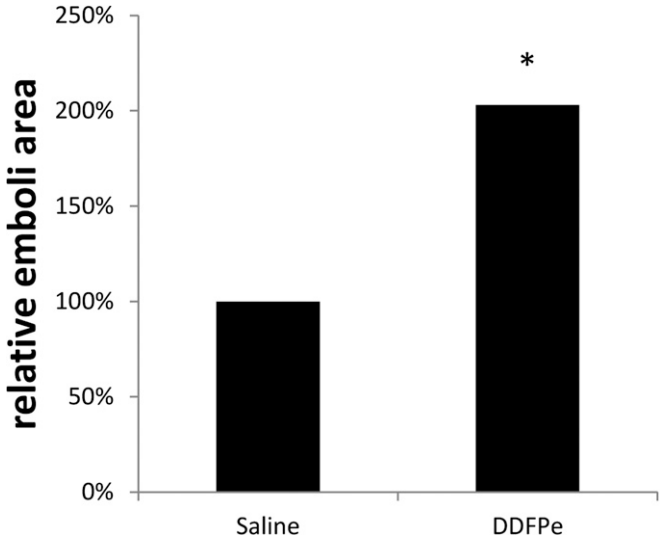

Fig. 4. Total area of sinusoidal dilations in the liver of DDFPe treated animals relative to saline treated controls. H&E sections were scanned and stained vs. unstained areas quantified via ImageJ software. Vessel, tear, and sectioning artifact areas were manually identified and excluded from quantification. *$p = 0.023$.

surprising as DDFPe has an established safety record as an ultrasound contrast agent in humans,[5,17] and other conditions where the therapeutic value of DDFPe is being leveraged are unlikely to experience such extreme atmospheric pressures (and subsequent depressurization) as was used in this study. Additionally, the oxygen carrying capacity of DDFPe has shown promise in animal models of anemia,[10] carbon monoxide poisoning,[21] radiation-resistant tumor sensitization,[8] and stroke[1,26] with no evidence of untoward effects. Our results also contradict those obtained from studies of other PFC formulations in swine and rodent models of DCS that demonstrated improvements in mortality and morbidity.[11,14,20]

While we did not conduct an extensive pathological analysis into cause of death for each animal, symptoms of cardiopulmonary and neurological DCS were consistently present, and histological analysis demonstrated markedly increased levels of tissue gas emboli in DDFPe treated animals. This suggests that the animals died with an increased total body bubble load. It is likely DDFPe served as micronuclei for bubble formation, leading to an increase in bubble load. Relying on only changes in ambient pressure, bubble formation in saturated liquid requires a change in pressure on the order of 100-1000 atm.[6] The presence of small (< 10 micron) gaseous particles has been demonstrated to serve as a nidus for overt bubble formation and experimental strategies aimed at decreasing micronuclei (such as breathing oxygen or using a brief compression prior to a dive) have decreased bubble formation in humans and DCS in animals.[22]

The 250-nm size of the DDFPe along with the expansion into a gaseous pocket at physiological temperatures alone would seem to qualify this compound as a micronuclei. Further growth of micronuclei would then be anticipated in the supersaturated state of decompression, where ambient gas partial pressure is exceeded by tissue gas partial pressure. This situation makes it feasible for the gas pockets of DDFPe to grow in vivo during decompression and also explains why no growth of these pockets is observed without decompression.

The dramatic increase in DCS mortality demonstrated in this study indicates that a cautious approach is warranted when leveraging the oxygen carrying capacity of DDFPe where a significant change in ambient pressure is anticipated. In developed countries, patient transport to a tertiary treatment center after initial resuscitation is common.[25] In altitude-related DCS, intravascular bubble formation is quite rare in humans below 15,000 ft elevation, but appears to occur in ~50% of those ascending to 21,200 ft.[24] Further, the presumed generation of micronuclei during exercise (while breathing air) immediately prior to ascent significantly increases the amount of intravascular bubbles detected.[3] Thus it is possible that DDFPe could lower the threshold altitude for bubble formation in the unpressurized, air-transported patient, which may be of particular concern for military casualties, who are often evacuated in

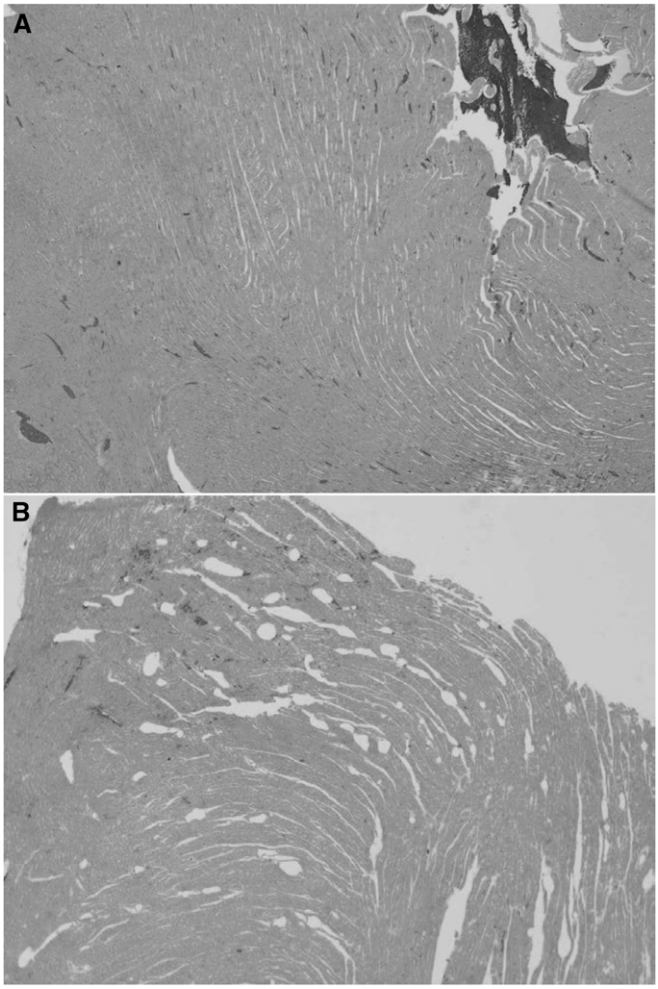

Fig. 5. HE stained heart sections (20×) from A) saline and B) 1.0 ml · kg^{-1} DDFPe treated animals.

unpressurized aircraft. Further studies are necessary to investigate the clinical implications of such a scenario.

Similarly, the high incidence of seizures is not fully explained in this work. Suspicion for large bubble formation in the central nervous system (CNS), leading to seizure activity, is possible.[23] Alternatively, the increased oxygen delivery capacity of DDFPe could have made the animals prone to CNS oxygen toxicity. However, neither of these hypotheses is addressed in this study as we did not conduct a thorough histological examination of any CNS tissue.

In conclusion, DDFPe administered as a bolus injection immediately following rapid decompression from 210 fsw increases mortality and seizure incidence in a rat model of DCS. Further studies into the effects of DDFPe during changes in ambient pressure are warranted.

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Authors and affiliation: Ryan L. Sheppard, Ph.D., David P. Regis, M.D., and Richard T. Mahon, M.D., Naval Medical Research Center, Silver Spring, MD.

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