Perfluorocarbon in Delayed Recompression with a Mixed Gender Swine Model of Decompression Sickness

William A. Cronin; Aaron A. Hall; Charles R. Auker; Richard T. Mahon

INTRODUCTION: Perfluorocarbons (PFC) are fluorinated hydrocarbons that dissolve gases to a much greater degree than plasma and hold promise in treating decompression sickness (DCS). The efficacy of PFC in a mixed gender model of DCS and safety in recompression therapy has not been previously explored.

METHODS: Swine (25 kg; $N = 104$; 51 male and 53 female) were randomized into normal saline solution (NSS) or PFC emulsion treatment groups and subjected to compression on air in a hyperbaric chamber at 200 fsw for 31 min. Then the animals were decompressed and observed for signs of DCS. Afterwards, they were treated with oxygen and either PFC (4 cc · kg^{-1}) or NSS (4 cc · kg^{-1}). Surviving animals were observed for 4 h, at which time they underwent recompression therapy using a standard Navy Treatment Table 6. After 24 h the animals were assessed and then euthanized.

RESULTS: Survival rates were not significantly different between NSS (74.04%) and PFC (66.67%) treatment groups. All swine that received recompression treatment survived to the end of the study and no seizures were observed in either PFC or NSS animals. Within the saline treated swine group there were no significant differences in DCS survival between male (75.00%, $N = 24$) and female (73.08%, $N = 26$) swine. Within the PFC treated swine, survival of females (51.85%, $N = 27$) was significantly lower than males (81.48%, $N = 27$).

DISCUSSION: In this large animal mixed gender efficacy study in DCS, PFC did not improve mortality or spinal cord injury, but appears safe during recompressive therapy. Gender differences in DCS treatment with PFC will need further study.

KEYWORDS: spinal cord injury, perfluorocarbon, decompression sickness, recompression.

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Significant reductions in ambient pressure, such as those experienced in diving, high altitude operations, and space exploration, present a risk of decompression sickness (DCS). Though the pathophysiology of DCS is not fully characterized, there is general (though not universal) consensus that its occurrence is mediated by the presence of gas bubbles resulting from a decrease in ambient pressure.[11] Such gas bubbles appear to be associated with mechanical obstruction, venous congestion, endothelial dysfunction, inflammation, and coagulation activation, all of which may lead to the syndromic condition of DCS.

One feared complication of DCS is neurological injury. Up to 40% of DCS cases that come to medical attention have neurological involvement, particularly involving the spinal cord.[3] In general, spinal cord injury from DCS (SCI-DCS) is initially treated with recompression therapy to potentially decrease the size of any existing bubbles as well as enhance tissue oxygenation.[26] Theoretically this would prevent further injury and potentially allow for preservation and rescue of ischemic tissue. Yet, in SCI-DCS cases in which objective motor impairment is noted at presentation, approximately 30% have residual severe disability after 1 mo.[10] Modalities to improve SCI-DCS outcomes are certainly desired.

One potential therapeutic agent to treat SCI-DCS is emulsified perfluorocarbons (PFC). Perfluorocarbons are a class of inert fluorinated hydrocarbons that dissolve gases to a much greater degree than plasma.[22] In the neat state fluorocarbons are both

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hydrophobic and lipophobic and must be emulsified to facilitate intravenous delivery. Oxycyte™ (Tenax Therapeutics, Inc., Morrisville, NC) is a second-generation PFC product composed of submicron particles of perfluoro(tert-butylcyclohexane) (FtBu) in a 60% w/v concentration with egg phospholipid emulsifier. FtBu is a saturated alicyclic perfluorocarbon with molecular formula $C_{10}F_{20}$ and molecular mass of 500.08. The oxygen-carrying capacity of FtBu (the PFC component of Oxycyte) is 43 mL O_2/100 mL blood at sea level.[21] The small-emulsified particles coupled with increased oxygen solubility serve to enhance plasma oxygen transport.[22] Emulsified PFC, when used with supplemental oxygen, increases blood oxygen content[20] and improves microvascular oxygen transport,[6] as well as increases tissue oxygen levels in traumatic SCI.[19]

In conjunction with supplemental oxygen, the perfluorocarbon emulsion Oxycyte has demonstrated survival benefit in juvenile male swine[14] at 5 cc · kg^{-1} and improved spinal cord pathology[12] at 3 cc · kg^{-1} when used to treat DCS. As an adjunct therapy, it is critical that PFC be compatible with standard recompression therapies such as U.S. Navy Treatment Table 6, which uses hyperbaric oxygen. However, the enhanced oxygen-carrying capacity of PFC raises the concern of seizures related to oxygen toxicity during the use of hyperbaric oxygen (HBO) encountered during standard recompression treatments. To further study PFC efficacy in DCS, as well as gain understanding of potential toxicity in recompression therapy, we tested the dual hypothesis that 4 cc · kg^{-1} PFC would improve mortality in a mixed gender swine model of DCS with no associated adverse events (such as seizure) while receiving delayed recompression therapy.

METHODS

Animals

All experiments were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 1996. 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 Federal regulations governing the protection of animals in research. The institution's animal care facility is fully AAALAC accredited and the veterinary staff is familiar with our 20–30 kg swine model.

A balanced group of juvenile unneutered male and female Yorkshire swine ($N = 104$) from a single vendor (Thomas Morris Inc., Reisterstown, MD) were housed in free running cages at our animal care facility, where they acclimated for 5 d prior to any procedures. They were fed standard pig chow twice daily (2–2.5% bodyweight; Quality Lab Prod, Elkridge, MA) with free access to water.

Procedure

The animals were trained to walk on a treadmill (T-2000, GE Healthcare, Milwaukee,WI) in three sessions starting at least 2 d before hyperbaric exposure. Each session was defined as complete when the animal walked comfortably for 5 min at 1 mph, but never exceeded 15 min.

On the day prior to hyperbaric exposure, the animals were placed in a Panepinto sling and sedated with diazepam (intramuscular, 0.25 mg · kg^{-1}; Abbott, North Chicago, IL). An ear vein was catheterized with an 18-gauge 2″ angiocatheter and secured with tape. Each animal was recovered in the sling until fully awake and able to ambulate and was then returned to its holding pen.

The animals were block randomized prior to hyperbaric exposure. Each block included four animals. The four animals included two from each gender with either Oxycyte (PFC) or normal saline solution (NSS).

We followed a DCS-inducing dive profile to 200 fsw for 31 min. This profile was developed in our lab and has a historical control mortality of approximately 45% with evidence of spinal cord pathology.[16] Briefly, the animals underwent a nonlinear compression profile to 200 fsw with a "bottom time" of 31 min. Bottom time was defined as the time from reaching 200 fsw until time leaving bottom pressure. After 31 min of bottom time, decompression was initiated at 30 fsw · min^{-1} until surface pressure was reached and the chamber door opened.

The animals were taken out of the chamber, removed from their kennels, and placed in a Panepinto sling. The ear vein catheter was used to administer 0.25 mg · kg^{-1} diazepam and the animals were observed for up to 60 min for signs of cutis marmorata ("skin bends") as previously described.[2] Skin bends have been shown to reliably precede the onset of severe DCS in 20-kg swine.[5]

At the onset of cutis marmorata, the animal was given 100% oxygen by snout cone (Smith Medical North America, Wausesha, WI) and randomized to receive either 4 cc · kg^{-1} intravenous PFC ($N = 54$) or an equivalent dose of normal saline solution (Baxter Healthcare Corporation, Deerfield, IL) ($N = 50$). The animals were continuously observed for signs of distress, including thrashing or vocalization, which were treated with additional diazepam (0.125 mg · kg^{-1} up to 2 mg · kg^{-1}). During the hour observation, oxygen saturation and pulse were measured by tail pulse oximetry (Oxisensor II, Nelcor Puritan Bennett, Pleasanton, CA). After 1 h, supplemental oxygen was discontinued and an animal assessment was performed. Based on the animal's condition, it was either returned to the holding pen or remained in the Panepinto sling. Any mortality was confirmed by the principle investigator.

Surviving animals were placed into a Plexiglas box (30″ x 42″ x 38″; manufactured in-house) positioned within our Multiple Large Animal Chamber 4 h after DCS onset (+ up to 15 min). Recompression therapy was then initiated with a standard U.S. Navy Treatment Table 6 (USN TT6). The USN TT6 provides hyperbaric oxygen therapy with a maximum pressure of 60 fsw for a total time under pressure of 288 min; 240 min on 100% oxygen interspersed with 48 min on air.[17] Routine animal assessments occurred before and after USN TT6 with a focus on ensuring animal comfort. No gait assessment was performed at these time points.

Spinal cord DCS is manifested as paresis/paralysis and sensory deficits.[1] A reliable indicator of hind limb function is the swine's ability to walk. We incorporated the Tarlov Scale, a recognized standard, developed specifically for spinal cord pathology in swine.[23] All surviving animals ($N = 73$) were assessed for their ability to stand 24 h postdive. If able to stand (Tarlov score 3), the animal was placed onto the treadmill and the speed gradually increased in 0.2-mph increments over approximately 30 s until the animal was able to walk at 1 mph for 5 min. This earned a Tarlov score of 5. If the animal was able to walk but unable to achieve 1 mph, the subject was scored a Tarlov 4 (weak walk). Other Tarlov scores were assigned as: complete paralysis of hind limbs, 0; minimal movement of hind limbs, 1; and able to stand with assistance only, 2. After this 24-h assessment animals underwent euthanasia.

Statistical Analysis

All data were compiled into Excel spreadsheets, then imported into Graphpad Prism 5 for statistical analysis. Mortality incidence was evaluated by a Fisher's exact test and a two-sample, one-sided test for equality of proportions. Tarlov score and predive weights were compared using two-way ANOVA with post hoc Bonferroni's test.

RESULTS

Swine ($N = 104$) were randomized into NSS ($N = 50$ total, 24 male, 26 female) or PFC ($N = 54$ total, 27 male, 27 female) treatment groups and subjected to the dive profile. Weights within the NSS group (25.65 $\pm$ 0.50 kg males; 25.25 $\pm$ 0.37 kg females) were not significantly different from the weights in the PFC group (24.87 $\pm$ 0.36 kg males; 25.96 $\pm$ 0.33 kg females) [$F(1,100) = 0.01$, $P = 0.94$]. Weights between genders were similarly not significantly different [$F(1,100) = 0.79$, $P = 0.37$, two-way ANOVA].

Survival rates were not significantly different between NSS (74.04%) and PFC (66.67%) treatment groups ($P = 0.52$, two-sided Fisher's exact test) (**Fig. 1**). All swine that received recompression treatment survived to the end of the study and no seizures were observed in either PFC or NSS animals (**Fig. 2**).

Gender effects on DCS survival within each treatment group were examined. Within the saline treated swine group there were no significant differences in DCS survival between male (75.00%, $N = 24$) and female (73.08%, $N = 26$) swine ($P = 1.00$, two-tailed Fisher's exact test) (**Fig. 3A**). Within the PFC treated swine, survival of females (51.85%, $N = 27$) was significantly lower than males (81.48%, $N = 27$) ($P = 0.04$, two-tailed Fisher's exact test) (**Fig. 3B**).

Surviving swine ($N = 72$) had neurological function testing, which was graded using the modified Tarlov scoring system (0–5), 24 h following recompression therapy. There were no significant differences between mean Tarlov scores in the NSS Male (4.176, $N = 18$), NSS Female (3.947, $N = 19$), PFC Male (3.500, $N = 22$), and PFC Female (3.429, $N = 14$) groups [$F(1,68) = 0.03$, $P = 0.86$; Treatment (NSS x PFC), $F(1,68) = 1.71$, $P = 0.20$; Gender $F(1,680) = 0.11$, $P = 0.74$, two-way ANOVA] (**Fig. 4**).

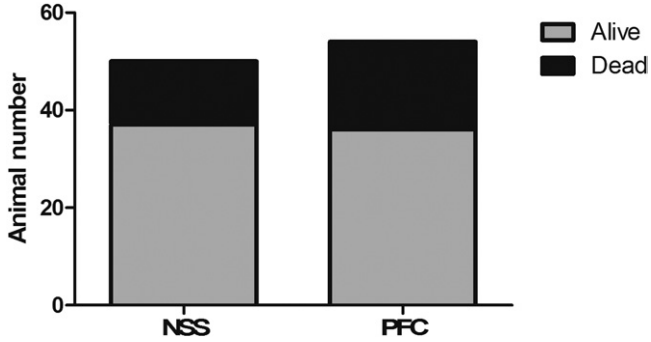

Fig. 1. Mortality. Bar graph depicting survival outcomes in the combined (male+female) PFC and NSS treatment groups; there was no significant difference in survival rates ($P = 0.5206$, two-sided Fisher's exact test).

DISCUSSION

Based on literature review this may be the first large animal mixed gender study evaluating the efficacy of PFC in the treatment of DCS. We found that PFC, at a dose of 4 cc $\cdot$ kg^{-1}, did not improve overall mortality. However, there appeared to be a difference in mortality based on gender. Additionally, we demonstrated that delayed recompression after receiving PFC was not associated with an increased incidence of seizures.

The precise use of PFC in prevention and treatment of DCS is not yet fully clarified. In rapid decompression from hyperbaric conditions, perfluorocarbons have been shown in animal models to increase nitrogen elimination, improve oxygen carrying capacity, and improve oxygen delivery.[20,22,27] Dromsky first demonstrated the role of PFC in mitigating DCS in a large animal model, when PFC was infused prior to the onset of

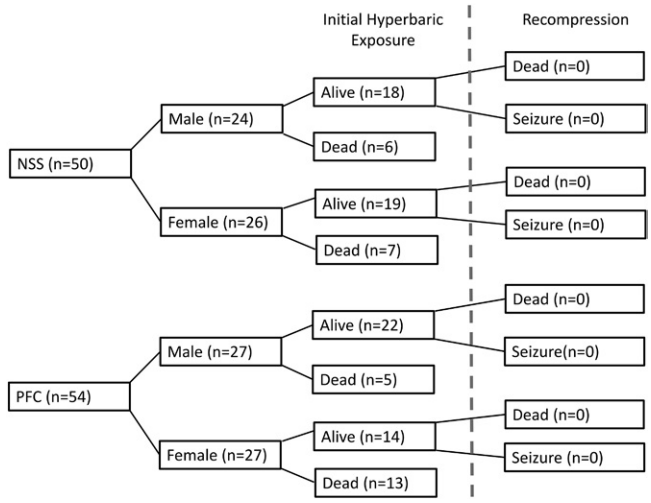

Fig. 2. Subgroup mortality and recompression. Branch diagram portraying the mortality for each subgroup after initial hyperbaric exposure to the left of the dotted line. The mortality and seizure incidence for the recompression treatment are demonstrated to the right of the dotted line.

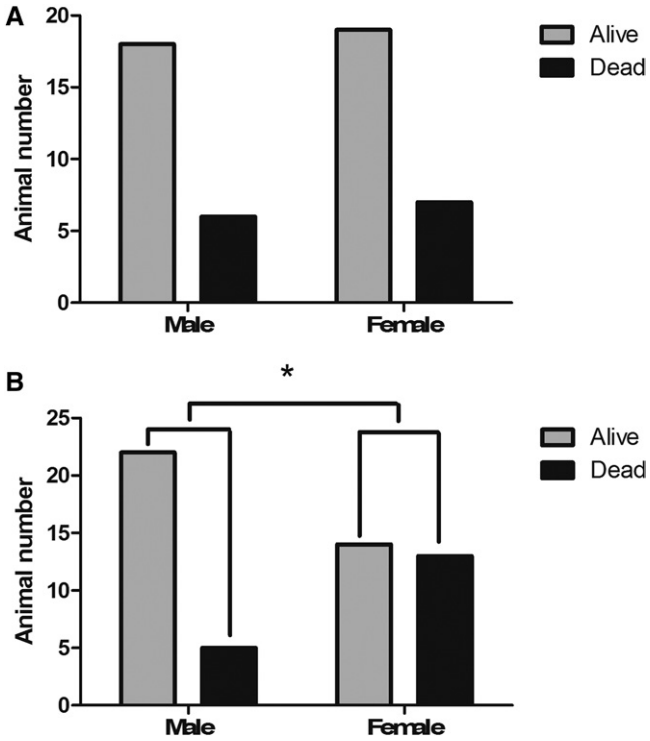

Fig. 3. Gender effects on mortality. A) Bar graph demonstrating DCS survival outcome between male and female swine within the NSS treatment group; there were no significant differences observed ($P = 1.0000$, two-tailed Fisher's exact test). B) Bar graph demonstrating DCS survival outcome between male and female swine in the PFC treatment group. Asterisk depicts significant difference between male and female swine groups ($P = 0.0418$, two-tailed Fisher's exact test).

DCS.[9] Some criticism of that work for using historical controls was later addressed in a randomized control trial evaluating oxygen prebreathe and the use of PFC where PFC administered prior to DCS onset dramatically reduced mortality from 96% in controls to 29% in PFC treated animals.[7] This body of work established a significant beneficial role when PFC was used as a preventive measure. PFC used after DCS onset appears to also provide a benefit, albeit less robust. Oxycyte specifically has been shown to decrease mortality (5 cc · kg^{-1}) and improve

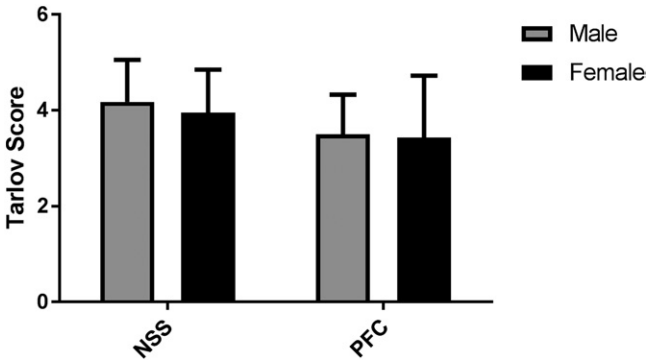

Fig. 4. Neurological deficit. Bar graph depicting mean (± 95% confidence interval) Tarlov scores for the male and female subgroups of the NSS and PFC treatment groups; there was no significant difference in Tarlov scores observed ($P = 0.1950$, two-way ANOVA with post hoc Bonferroni's test).

spinal cord injury (3 cc · kg^{-1}) when used therapeutically after DCS onset.[15,16] To our knowledge PFC combined with recompression therapy has not previously been studied. We used a 4-h delay to study the treatment of DCS with PFC, which could potentially be used at the site of occurrence followed by a transport to what is considered standard of care treatment with recompression therapy.

We chose the 4 cc · kg^{-1} dose to further expand on our previous work involving doses of 3 cc · kg^{-1} and 5 cc · kg^{-1}. The lack of mortality benefit in this balanced mixed gender study is not wholly surprising, as previous studies have suggested a dose-response relationship in the treatment of DCS that is not fully characterized. What we found surprising was an apparent gender difference in survival. Reasons for this can only be speculative at this point, but it is plausible that gender differences in disease state and/or gender differences in drug effects are contributing factors.

Though gender differences in DCS have been hypothesized, no conclusive evidence has been demonstrated. In the only prospective human DCS study (altitude DCS), Webb et al. demonstrated that it was body mass index and maximal oxygen consumption (but not gender) that correlated with actual DCS risk.[25] Interestingly, they showed that women on hormonal contraceptives were at higher risk for DCS in the last 2 wk of their menstrual cycle than those not on such therapy. More germane to the current study, in a large animal model of DCS treated with PFC, we recently showed that DCS onset is quicker, cardiovascular changes are more severe, and that Oxycyte increased pulmonary artery pressure more in juvenile female swine compared to juvenile male swine.[13]

Despite the question regarding PFC efficacy raised by this current work, we have contributed to the understanding of PFC safety in recompression therapy. The increased gas carrying properties of PFC raise concerns of HBO related toxicity, particularly central nervous system toxicity as manifested by seizure. In a rodent study of PFC, risk of seizure appeared dose-dependent with a nonsignificant decrease in hyperbaric oxygen seizure latency at a 3 cc · kg^{-1} dose and a significant decrease in latency at 6 cc · kg^{-1}.[8] Yet, in a swine model, PFC at a 5 cc · kg^{-1} dose did not decrease seizure latency with 5 ATA oxygen.[15] Neither of these studies involved decompression or DCS.

With respect to seizures from hyperbaric oxygen in humans, Plafki reported 4 seizures in over 11,000 HBO treatments at up to 250 kPa (15 msw).[18] The Navy formerly used an oxygen tolerance test at 280 kPa (2.8 ATA), where oxygen toxicity (presumably not all convulsive) was noted in 1.9% of tested subjects.[4] There is much less data on central nervous system toxicity during recompressive therapy for DCS. Weaver reported no cases of central nervous system toxicity using a USTT6 during 118 therapies in a monoplace chamber, while 4 cases in 200 treatments were recorded in a survey of multiplace chambers.[24] In our study no animal (control or PFC) that survived to delayed recompression therapy with a USTT6 manifested seizures. The number of animals receiving PFC and recompression in our study does not compare to 11,000 patients reported by Plafki or the several hundred subjects noted by

Butler,[4] but does lend some reassurance regarding the safety of recompression therapy after PFC has been administered.

As in any work, limits exist in the design and results. Though the possible increased mortality in female swine with DCS treated with PFC is concerning, the study was not powered adequately to specifically address that question. We believe that this question is most easily addressed by a single gender (female) swine study with a dive profile and dose of PFC known to have mortality benefit.[16] Additional limits include the lack of pharmacokinetics that might have demonstrated a correlation with gender. Furthermore, we did not perform pathological analysis of the spinal cords, which may have yielded further insight into the effect of PFC. Lastly, our historical mortality rate in this model was not realized, further diminishing the power of these results.

In this first large animal mixed gender efficacy study of DCS therapy, PFC appears safe during recompressive therapy in animals surviving DCS. Although this work adds to the overall body of literature supporting PFC as a nonrecompressive option to prevent and treat DCS, nuances such as gender differences remain and will need further study prior to human use.

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REFERENCES

1. Blick G, Durh MD. Notes on divers paralysis. BMJ. 1909; 2(2556):1796–1798.
2. Broome JR, Dick EJ Jr. Neurological decompression illness in swine. Aviat Space Environ Med. 1996; 67(3):207–213.
3. Brubakk AO, Neuman TS, editors. Bennet and Elliott's phyisology and medicine of diving, 5th ed. London (UK): Saunders Elsevier Science; 2003:580.
4. Butler FKJ, Knafelc ME. Screening for oxygen intolerance in U.S. Navy divers. Undersea Biomed Res. 1986; 13(1):91–98.
5. Buttolph TB, Dick EJ Jr, Toner CB, Broome JR, Williams R, et al. Cutaneous lesions in swine after decompression: histopathology and ultrastructure. Undersea Hyperb Med. 1998; 25(2):115–121.
6. Cabrales P, Tsai AG, Frangos JA, Briceno JC, Intaglietta M. Oxygen delivery and consumption in the microcirculation after extreme hemodilution with perfluorocarbons. Am J Physiol Heart Circ Physiol. 2004; 287(1):H320–H330.
7. Dainer H, Nelson J, Brass K, Montcalm-Smith E, Mahon R. Short oxygen prebreathing and intravenous perfluorocarbon emulsion reduces morbidity and mortality in a swine saturation model of decompression sickness. J Appl Physiol. 2007; 102(3):1099–1104.
8. Demchenko IT, Ahiyaev SY, Moskvin AN, Piantadosi CA, Allen BW. Autonomic activation links CNS oxygen toxicity to acute cardiogenic pulmonary injury. Am J Physiol Lung Cell Mol Physiol. 2011; 300(1):L102–L111.
9. Dromsky DM, Spiess BD, Fahlman A. Treatment of decompression sickness in swine with intravenous perfluorocarbon emulsion. Aviat Space Environ Med. 2004; 75(4):301–305.
10. Gempp E, Blatteau JE. Risk factors and treatment outcome in scuba divers with spinal cord decompression sickness. J Crit Care. 2010; 25(2):236–242.
11. Madden LA, Laden G. Gas bubbles may not be the underlying cause of decompression illness – the at-depth endothelial dysfunction hypothesis. Med Hypotheses. 2009; 72(4):389–392.
12. Mahon RT, Auker CR, Bradley SG, Mendelson A, Hall AA. The emulsified perfluorocarbon Oxycyte improves spinal cord injury in a swine model of decompression sickness. Spinal Cord. 2013; 51(3):188–192.
13. Mahon RT, Cronin WA, Bodo M, Tirumala S, Regis DP, Auker CR. Cardiovascular parameters in a mixed-sex swine study of severe decompression sickness treatment with the emulsified perfluorocarbon Oxycyte. J Appl Physiol. 2015; 118(1):71–79.
14. Mahon RT, Dainer HM, Nelson JW. Decompression sickness in a swine model: isobaric denitrogenation and perfluorocarbon at depth. Aviat Space Environ Med. 2006; 77(1):8–12.
15. Mahon RT, Hall A, Bodo M, Auker C. The intravenous perfluorocarbon emulsion Oxycyte does not increase hyperbaric oxygen-related seizures in a non-sedated swine model. Eur J Appl Physiol. 2013; 113(11):2795–2802.
16. Mahon RT, Watanabe TT, Wilson MC, Auker CR. Intravenous perfluorocarbon after onset of decompression sickness decreases mortality in 20-kg swine. Aviat Space Environ Med. 2010; 81(6):555–559.
17. Naval Sea Systems Command. U.S. Navy Diving Manual, Revision 6, Change A. Washington (DC): U.S. Government Printing Office; 2008.
18. Plafki C, Peters P, Almeling M, Welslau W, Busch R. Complications and side effects of hyperbaric oxygen therapy. Aviat Space Environ Med. 2000; 71(2):119–124.
19. Schroeder JL, Highsmith JM, Young HF, Mathern BE. Reduction of hypoxia by perfluorocarbon emulsion in a traumatic spinal cord injury model. J Neurosurg Spine. 2008; 9(2):213–220.
20. Smith CR, Parsons JT, Anu J, Spiess BD. The effect of intravenous perfluorocarbon emulsions on whole-body oxygenation after severe decompression sickness. Diving Hyperb Med. 2012; 42(1):10–17.
21. Spiess BD. The potential role of perfluorocarbon emulsions in decompression illness. Diving Hyperb Med. 2010; 40(1):28–33.
22. Spiess BD. Perfluorocarbon emulsions as a promising technology: a review of tissue and vascular gas dynamics. J Appl Physiol. 2009; 106(4):1444–1452.
23. Tarlov IM. Spinal cord compression: mechanism of paralysis and treatment. Springfield (IL): Thomas Publishing; 1957:147.
24. Weaver LK. Monoplace hyperbaric chamber use of U.S. Navy Table 6: a 20-year experience. Undersea Hyperb Med. 2006; 33(2):85–88.
25. Webb JT, Kannan N, Pilmanis AA. Gender not a factor for altitude decompression sickness risk. Aviat Space Environ Med. 2003; 74(1):2–10.
26. Yarborough OD, Behnke AR. The treatment of compressed air illness using oxygen. J Ind Hyg Toxicol. 1939; 21:213–218.
27. Zhu J, Huyllet JB, Somera L, Barbee RW, Ward KR, et al. Intravenous perfluorocarbon emulsion increases nitrogen washout after venous gas emboli in rabbits. Undersea Hyperb Med. 2007; 34(1):7–20.