Short communication

Pre-hydration strongly reduces decompression sickness occurrence after a simulated dive in the rat

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Key words
Hydration; Animal model; Rat; Diving deaths

Abstract
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Introduction: Hydration status is considered a parameter likely to influence the risk of decompression sickness (DCS), but scientific evidence is scarce and conflicting. This experiment aimed to analyse the influence of pre-hydration on DCS occurrence in a rat model.
Methods: Intra-peritoneal injections of saline solution were administered to rats (NaCl 0.9% 0 ml (Control), 0.1 ml (Group 1), or 1 ml·100g^{-1} body mass (Group 2) at each of 24 h, 12 h, and 30 min prior to simulated air dives (45 min at 1,010 kPa; compression and decompression rates 101 kPa·min^{-1}; stops 5 min at 202 kPa, 5 min at 160 kPa, 10 min at 130 kPa). Evaluation of DCS occurrence and severity was made after decompression.
Results: Pre-dive hydration reduced severe DCS from 47% (Control) to 29% (Group 1) and 0% (Group 2), and increased the proportion of animals without any signs of DCS from 40 (Control) to 57% (Group 1) and 93% (Group 2); Chi^2 $P = 0.041$.
Conclusions: This experiment demonstrated that pre-hydration can drastically reduce the DCS occurrence in an animal model. In the context of scuba diving, this result highlights the importance of elucidating the mechanisms linking hydration status and DCS risk.

Introduction

During scuba diving, the tissues of divers are progressively loaded with inert gases and during the fall in ambient pressure when ascending and reaching the surface, tissue gas supersaturation may lead to bubble formation. Tissue and circulating bubbles are considered to be the primary trigger of decompression sickness (DCS). Circulating bubbles are nevertheless a poor predictor of DCS[1] and the mechanisms of DCS are far from being fully understood. During the last decade, preconditioning strategies (exercise, sauna, preoxygenation, vibration, chocolate or hydration) have been investigated as potential means of reducing the risk of DCS in divers.[2,3]

The question of the link between the hydration status and the risk of DCS was raised 70 years ago in the context of altitude DCS[4], but the literature on this issue is still scarce and conflicting. Dehydration has been proposed as a risk factor in few DCS case reports.[5,6] However, in others, it has been proposed as a possible cause of bubble reduction after diving, both in the case of pre-dive exercise[7] and in experimental dehydration in rats.[8] On this point, animal studies find either no effect of dehydration on DCS in a murine model,[8,9,10] or an increased DCS occurrence in dehydrated rabbits and swine.[11,12] Interestingly, in a human study using infrared-ray dry sauna a reduction of circulating bubbles was associated with a moderate dehydration after a simulated dive.[13] There are, as far as we know, only three papers concerning pre-hydration. Two were performed on swine. One study failed to reduce neurological DCS after crystalloid infusion.[14] The other study was designed to evaluate the effect of methylprenidsolone on DCS, not to assess the effect of prehydration.[15] In that study an intravenous infusion of saline appeared to strongly reduce the occurrence of DCS and death, but only after comparison with a historical control group without a saline infusion. In humans, a study established that pre-hydration could reduce circulating bubbles after diving.[16]

In this context, there is a clear need to assess the influence of the hydration level on DCS occurrence after a dive. Consequently, in order to investigate the effect of pre-hydration on DCS occurrence intra-peritoneal (IP) injections of saline solution were adminstered to ratsprior to simulated air dives and we evaluated the occurrence and severity of DCS.

Table 1
Characteristics of the studied 15 male Sprague-Dawley rats. IP = intra-peritoneal

Parameter	Control	Group 1 IP injection 0.1 mL.100g^{-1}	Group 2 IP injection 1 mL.100g^{-1}	*P*-value
n	15	14	14	–
Age (weeks)	12	12	12	–
Weight (g) mean (SD)	410 (48)	442 (51)	423 (55)	> 0.05

Methods

ANIMALS

Animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and with the approval of the Université de Bretagne Occidentale Ethics Committee for Animal Experimentation (approval no. 01462.02). This study complies with recognized ethical standards and national/international laws.

Forty-three male Sprague-Dawley rats, 12 weeks old on the day of the experiment, were obtained from Janvier SAS (Le Genest St Isle, France). Animals were housed individually in a cage in an environmentally controlled room (temperature 21°C (SD 1), 12–12 h light-dark cycle) and were fed daily with 20–25 g of standard rat chow and water *ad libitum*. The animals were randomly assigned into three groups of 14 to 15 animals: air diving with no hydration (the animals were exposed to the simulated dive without treatment, Control); air diving with low hydration treatment (intra-peritoneal injection of NaCl 0.9% 0.1ml·100g^{-1} body mass at each of 24 h, 12 h, and 30 min before the simulated dive, Group 1); and air diving with high hydration treatment (intraperitoneal injection of NaCl 0.9% 1ml·100g^{-1} body mass at each of 24 h, 12 h, and 30 min before the simulation, Group 2). Number, age, sex and weight of the rats in the three groups are given in Table 1. There was no significant difference in the weight of rats between groups.

DIVING PROTOCOL

The dive protocol applied in the present study is routinely used in the lab and is known to induce DCS in 63% (SD 4) of cases[17] (in rats of identical strain, age, sex and weight). Each rat was positioned in a 130 L steel hyperbaric chamber, always at the same hour to avoid interference by biological rhythms. Air was used as the breathing mixture. The animals were compressed at a rate of 100 kPa·min^{-1} to 1,000 kPa absolute pressure (90 metres' seawater [msw] equivalent) and remained at that pressure for 45 min (Figure 1). Decompression then followed at a rate of 101 kPa·min^{-1} with three decompression stops: 5 min at 202 kPa (10 msw), 5 min at 160 kPa (6 msw) and 10 min at 130 kPa (3 msw). Total hyperbaric exposure duration was 83 min.

DCS ASSESSMENT

Following hyperbaric exposure and decompression, the rats were observed for two hours for the appearance of four standard DCS symptoms: respiratory distress; walking difficulty; paralysis and/or convulsions. Animals were scored as having DCS only when one or more of these four symptoms appeared. The trinary classification of no DCS, mild DCS (one or more of the four symptoms but without death) and death was applied.

STATISTICS

The weight of rats was presented as mean (SD). The difference in the weight of rats between groups was evaluated with one way-ANOVA (Statistica 13.3) and considered significant when $P < 0.05$. The difference between the ratios of DCS morbidity among groups was analysed with a Chi-Square test (Statistica 13.3) and considered significant when $P < 0.05$.

Results

After the dive without pre-hydration, the percentage of severe and mild DCS occurrence were 47% and 13% respectively. In the same condition 40 % of the animal experienced no DCS after decompression. Hydration before the dive significantly reduced the proportion of severe DCS (to 29% and 0% in the groups receiving injections of 0.1 ml·100g^{-1} and 1 ml·100g^{-1} of saline respectively) and significantly increased the proportion of animals without any signs of DCS (to 57% and 93% in the same groups respectively; Chi^2 $P = 0.041$) (Figure 2).

Discussion

The goal of this study was to analyse the link between pre-hydration and DCS occurrence in a murine model. The results clearly show that in our conditions, intraperitoneal injection of saline solution of either 0.1 or 1 ml·100g^{-1} body mass 24 h, 12 h and 30 minutes before a simulated dive significantly reduced DCS occurrence from 60% to 7%. This

Figure 1
Dive protocol for *in vivo* diving simulations of rats

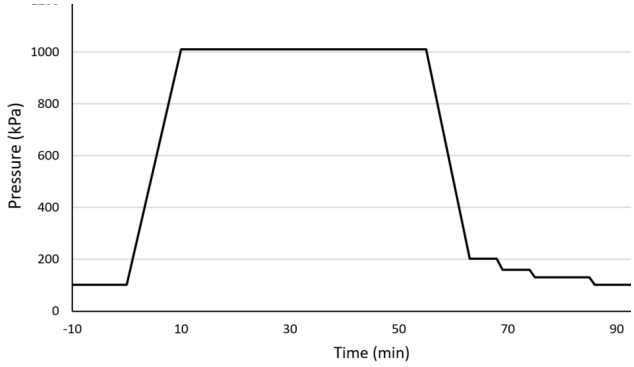
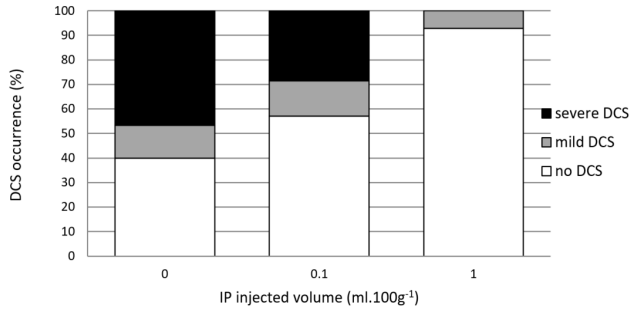


Figure 2
Morbidity of DCS in rats after *in vivo* simulated dives



result is consistent with the study in swine that suffered from the absence of a proper control group[15] and with the study in humans that did not assess DCS but used circulating bubbles as a proxy of decompression stress.[16] In that study, where decompression bubbles were measured after a 30-min dive at 30 msw, oral prehydration (with 1,300 ml of isosmotic saline-glucose beverage) significantly reduced venous bubbles when compared to a control group.[16]

Three main mechanisms are proposed in the literature to explain the effect of the hydration status on the risk of DCS.

1. *Surface tension (ST):* low ST is known to facilitate the formation of bubbles and dehydration may decrease ST. But in the case of pre-dive hydration, no change in plasmatic ST was observed by Gempp et al.[16] while circulating bubbles were reduced.

2. *Vasoconstriction:* large fluid intake can lead to gastric distention, peripheral sympathetic mediated vasoconstriction and consequently reduce the inert gas intake during the dive.[2] However, in the present experiment pre-hydration was performed via intraperitoneal injection; therefore this hypothesis is unlikely.

3. *Prevention of hypovolaemia:* scuba diving is known to induce hypovolaemia, and potentially, reduction of tissue microperfusion, inert gas removal and an increased risk of DCS.[11] The hypovolaemia is a consequence of immersion and not of hyperbaric exposure. Since the animals were exposed to pressure in a hyperbaric chamber, this hypothesis is an inadequate explanation of results in the present experiment.

Unfortunately, this experiment was designed only to evaluate DCS occurrence and mortality after the dive. The high percentage of dead animals in the control group prevented any biological analyses from being performed and did not allow exploration of the mechanism of a reduction of DCS after pre-hydration. Nevertheless, in the human experiment,[16] pre-dive hydration caused reduction in circulating bubble formation, and it is plausible that a reduction in bubble formation could explain the protective effect of pre-hydration on rats. It must be acknowledged that notwithstanding these results, there has been no demonstration of an effect of pre-hydration on DCS risk *per se* in human subjects. Furthermore, the question of the scalability of the fluid loading in humans is relevant since excessive fluid loading in divers must be avoided as it could lead to an increased risk of immersion pulmonary oedema. However, from an experimental point of view, the modulation of DCS occurrence via the manipulation of the hydration status can be a very interesting tool to develop our understanding of this multifactorial disease.

Regarding the assessment of DCS, animals were observed by two operators with significant experience, and only unambiguous symptoms (i.e., respiratory distress, walking difficulty, paralysis, convulsions or death) were considered for determination of the presence of DCS. More ambiguous signs such as, for example, paresthesia, prostration or agitation were not used because of the risk of subjectivity. This method was used in our previous studies.[18,19] Although it may probably underestimate the incidence of DCS in our model, we think that it helps prevent biases resulting from subjectivity and, therefore, allows comparison between groups.

Conclusions

Pre-hydration significantly reduced DCS occurrence in a rat model. The result can only be extrapolated to human diving with caution due to uncertainty over scalability and since hyper-hydration in the latter setting could lead to an increased risk of immersion pulmonary oedema. This finding nevertheless identifies the potential importance of hydration status in DCS risk and highlights the need for further experiments to explore the underlying mechanisms of this effect.

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Conflicts of interest and funding

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