Original articles

A rat model of chronic moderate alcohol consumption and risk of decompression sickness

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Abstract

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Introduction: This study aimed to establish if chronic, moderate, pre-dive alcohol consumption had any affect upon susceptibility to decompression sickness (DCS) in rats.

Methods: A treatment group of 15 rats were given water containing 12 mL·L^{-1} of ethanol for four weeks. Controls (n = 15) were given water. Both groups were compressed with air to 1,000 kPa, followed by staged decompression. An additional 30 control rats from a similar previous experiment were added, raising the control-treatment ratio to 3:1.

Results: Rats in the treatment group consumed the equivalent of an 80 kg man drinking 2 L of 5% alcohol by volume beer per day, which is three times the recommended daily limit for men. Overall, comparing the treatment group with the combined control groups neither weight (P = 0.23) nor alcohol consumption (P = 0.69) were associated with DCS.

Discussion: We observed that chronic, moderate alcohol consumption prior to compression was neither prophylactic nor deleterious for DCS in young, male rats.

Key words

Animal model; decompression illness; risk factors; hyperbaric research; regression analysis

Introduction

Decompression sickness (DCS), occurs after ascending from depth, when previously dissolved gas is liberated in the form of bubbles rather than harmlessly diffusing out of a diver's tissues and blood.[1] Prior alcohol consumption is often cited as a risk factor for DCS, though evidence for this is lacking. The history of this caution dates back to 1874 when Dr Alphonse Jaminet, physician for the construction of the Eads Bridge over the Mississippi River at St Louis, rejected 25 of 133 (19%) job applicants for intemperance.[2] More recently in an editorial in this journal, it was stated that "*almost certainly excessive alcohol intake contributed to one case of neurological decompression sickness at a SPUMS Annual Scientific Meeting some years ago*",[3] though it remains equally possible that the case could have been worse were it not for the previous night's consumption.

In the UK, the recommended daily benchmark for alcohol consumption is 30–40 mL of pure alcohol per man.[4] Surveys of British recreational divers have consistently found that around 25% exceed the recommended weekly threshold and around 16% report regular binge drinking, commonly defined as twice the Government's recommended daily benchmark.[4–6] Both chronic and acute alcohol consumption may, therefore, be as common among divers as in non-divers. Whether acute or chronic, how habitual alcohol consumption affects risk of DCS remains unclear. Case-series reviews of decompression illness have not shown higher consumption among injured divers compared with either uninjured divers or non-divers.[6–10] Acute administration of alcohol following decompression has been shown to be beneficial in preventing or treating DCS in rabbits and humans. The aim of this study was to establish if chronic, moderate, pre-dive alcohol consumption has any affect in rats upon their susceptibility to DCS.

Methods

Male Sprague-Dawley rats (n = 30) were obtained from Janvier SAS (Le Genest St Isle, France) at age 10 weeks. Before commencing the experiment, the rats were housed for one week in the Faculty of Sciences and Techniques Vivarium in standard conditions (mean temperature 21.2°C ± 0.2 SD, relative humidity 27% ± 16% SD, 12-hour light:dark cycle, 0700–1900 h), during which they had access to rat chow ad libitum. Rats in the treatment group (n = 15) were then given 50 mL of water per day containing 12 mL·L^{-1} of ethanol (12 mL of alcohol was mixed with 988 mL of tap water to form 1 L) for 28 or 29 days, depending on the day of compression. Fifty mL just exceeds the typical water consumption measured during previous experiments. Control rats (n = 15) were given water only ad libitum. The rats were weighed each week and on the day of diving before being compressed in a 170-litre hyperbaric chamber (Comex, Marseille, France). All dives commenced in the morning after 0800 h. Hydration was withdrawn from both groups 30 mins before compression.

The 15 alcohol-treated rats in this study are a convenience sample of vehicle-control animals from separate studies yet to be reported. The duration of alcohol treatment was

Table 1
Mean weight (SD) at compression and number (*n*) of rats with decompression sickness (DCS) by group; * $P < 0.05$; † Not significant compared to the combined control group

	Control groups		Alcohol group
	Previous	Current	
	(*n* = 30)	(*n* = 15)	(*n* = 15)
Mean weight (g)	455 (27)*	414 (14)*	434 (15)†
No DCS (*n*)	6	6	5†
Survived DCS (*n*)	4	2	2†
Dead DCS (*n*)	20	7	8†

based on the requirements of these other studies. Fifteen water-only control rats were contemporaneous with these alcohol-treated rats. An additional 30 control rats from a previous experiment were added to the 15 control rats in this study, thus reducing the number of animals used while still raising the control to treatment ratio to 3:1. These additional control rats were the same age, sex and strain, housed under the same housing/cage/light/food/water/humidity/temperature conditions and underwent the same compression/decompression protocol and observation period, at the same time of day. This study and the previous experiment were approved by the French Ministry of Agriculture and the Universite de Bretagne Occidentale Animal Research Ethics Committee (R-2011-FG-01).

Since acute post-decompression alcohol administration has been shown to increase survival from DCS in rabbits, we selected a compression/decompression profile that resulted in > 50% mortality among control rats, expecting this proportion to fall significantly in the treatment group.[11] Compression with air to 1,000 kPa occurred at the rate of 100 kPa·min^{-1}. Maximum pressure was maintained for 45 minutes followed by decompression at 100 kPa·min^{-1} to 200 kPa. Decompression was thereafter staged with 5 min at 200 kPa, five min at 160 kPa and 10 min at 130 kPa.

This protocol has been shown to produce DCS signs in a predictable proportion of male Sprague-Dawley rats aged 10 to 11 weeks.[12,13] Following decompression, the rats were immediately removed from the chamber and observed for signs of DCS for one hour. The classification used was 0 – No observable DCS (*n*DCS); 1 – respiratory distress or paralysis (*s*DCS); 2 – death within one hour (*d*DCS). Two observers agreed the diagnosis in each case. Time of death was recorded as occurring at 0 minutes if observed when the chamber was opened or at time since surfacing in all other cases. The observation period ended at 60 minutes and mortality or survival noted.

ANALYSIS

Data were analysed using SAS ver 9.3 (SAS, Cary, North Carolina). Distribution of DCS between the two water-only groups was assessed for significant difference using cumulative logistic regression. The modelled probability (p) of a DCS outcome of state $\leq j$ (*n*DCS or *s*DCS), is shown in Equation 1:

$$Ln\left(\frac{p_j}{1-p_j}\right) = \underline{\alpha} + \beta_1 Group \quad (1)$$

where $\underline{\alpha} = [\alpha_1, \alpha_2]$ intercepts, α_1 for the logit of the probability of *n*DCS and α_2 for the logit for either *n*DCS or *s*DCS. The probability of *d*DCS is 1 – (probability of *n*DCS + probability of *s*DCS); *Group* was either of the two water-only groups. Differences in weight between groups were tested for significance by the Kruskal-Wallis test of Wilcoxon (rank sum) scores. Significance of alcohol consumption and weight upon DCS were assessed using a second cumulative logistic regression model (Equation 2) with p, j and $\underline{\alpha}$ as described above. Weight on the day of compression was included since weight is known to have a significant effect upon likelihood of DCS:[13]

$$Ln\left(\frac{p_j}{1-p_j}\right) = \underline{\alpha} + \beta_1 Alcohol + \beta_2 Weight \quad (2)$$

Alcohol was the control (0) or treatment (1) group and *Weight* was in grams. Significance in either regression model, determined by Maximum Likelihood Wald chi-square test for coefficient difference to a value of zero, was accepted at $P < 0.05$.

Table 2
Odds ratios for the two regression models combined; CI – confidence interval

Parameter	Point estimate	95% Wald CI	*P*-value
New water-only group vs. old (*n* = 45)	0.43	0.12, 1.40	0.15
Weight (per g; *n* = 45)	1.01	0.99, 1.03	0.23
Alcohol (*n* = 60; 0 vs. 1)	1.26	0.40, 3.94	0.69

Results

Twelve of the 15 rats consumed a mean 1.3 mL·kg^{-1} (1.0 g·kg^{-1}) of alcohol per rat per day (consumption was not measured for three of the rats), the mean weights (SD) and DCS outcomes are shown for each group in Table 1.

Distribution of DCS across *n*DCS, *s*DCS or *d*DCS did not significantly differ between control groups (Equation 1, χ^2 test $P = 0.15$). Weight at compression was significantly different between control groups ($P < 0.0001$), but was not significantly different between the combined control groups and the alcohol group ($P = 0.50$). Overall, comparing the treatment group with the combined control groups (Equation 2) neither weight (χ^2 $P = 0.23$) nor alcohol consumption (χ^2 $P = 0.69$) were associated with DCS. Odds ratios with confidence intervals are shown in Table 2.

Discussion

Rats in our treatment group consumed a mean of 1.3 mL kg^{-1} (1.0 g·kg^{-1}) of alcohol per day in a 1.2% alcohol tap-

water solution. In terms of raw alcohol consumption, this is the equivalent of an 80 kg man drinking 2 L of Fosters beer per day, which is three times the recommended daily limit for men,[4] although it must be noted that the metabolic rate of ethanol likely differs between the two species. Rats are naturally nocturnal and consumption mostly took place at night. The lights came on at 0700 h and the rats would usually settle down to sleep. Compression commenced later in the morning after alcohol and/or water were withdrawn at least 30 mins before. Therefore, it is unlikely that any rats were under the influence of alcohol at the time of compression, though this was not measured. This mimics the human population of interest, namely divers who drink chronically and then sleep before diving.

This is a relatively moderate dose of alcohol that has been shown to induce various behavioural and physiological responses in adult rats such as anxiolytic effects,[14] and raised acylated and total ghrelin levels in peripheral blood.[15] Far higher doses (5–10 $g \cdot kg^{-1} \cdot day^{-1}$) are commonly used experimentally; however, our rats were conveniently selected for this analysis from two separate, unrelated experiments, wherein they were the vehicle-control groups (unpublished data).[17] Higher doses or longer chronic ethanol consumption may well alter the risk of DCS but that remains to be investigated.

Investigating the effect of acute alcohol treatment, 32 New Zealand rabbits were compressed to 608 kPa for 30 minutes and then returned to one atmosphere.[11] Sixteen rabbits were given alcohol-saline injections (25% ethanol by volume, 3 $mL \cdot kg^{-1}$) immediately following decompression and 16 were injected with saline only.[11] All the rabbits given alcohol survived decompression whereas half the control rabbits died within 10–35 minutes.

In a pilot study, four marine fishery divers and two salvage divers who presented with acute DCS after rapid decompression were given 50–75 mL dry alcohol in a glucose drink soon after symptoms began. Sixty minutes later, four of the divers had fully recovered and all had fewer detectable circulating VGE.[16] The authors suggested "*it may be that inexpensive wine, which contains alcohol, could be an effective substitute for compression if administered shortly after the onset of acute DCS*".[16]

Four potential mechanisms have been proposed whereby ethanol may protect against DCS:[11]

- by enhancing the solubility of nitrogen in blood (the solubility of nitrogen is ten-fold greater in ethanol than in either blood or water);
- lowering the surface tension of bubbles by a factor of three and thereby acting as a de-frothing agent;
- reducing the adherence, aggregation and coagulation of platelets;
- increasing vasodilation and, thus, accelerating gas washout.

All four of these mechanisms require a blood alcohol concentration and, given the low dosage and delay between removing access to fluids and compression, it is unlikely the rats in this study had a blood alcohol level sufficiently high during decompression for the above mechanisms to play a role. This may approximate a similar circumstance to divers who drink regularly every night, sleep and then arrive at the dive site with a low or zero blood alcohol concentration. However, extrapolation from rats to the human condition can only be tentative.

Furthermore, if the addition of ethanol to the circulation does enhance nitrogen solubility then, in the case of chronic alcohol consumption, (as in this study), the degree of solubility enhancement would be symmetrical, whereas in the rabbit studies, with post-decompression ethanol solution injection, the addition of ethanol to the blood after decompression would create asymmetrical gas uptake and washout.

A limitation of our study is that we used a compression/decompression profile known to elicit a predictable proportion of three DCS outcomes[13] and to target slower tissues in the rat.[17] Therefore, a profile that generates DCS by targeting faster compartments, for example, by rapid ascent or ascent to altitude, may produce different results. Another potential limitation of this study is that the treatment and control groups arrived at the vivarium at age 10 weeks and, therefore, chronically consumed alcohol throughout sexual maturation. Since both alcohol and hormones are known to have cardiovascular effects we caution that a similar study using older animals, or females, may yield different results. The effects of chronic, higher doses of and/or longer exposure to ethanol remain to be investigated.

In conclusion, though acute alcohol consumption may prevent HPNS in rats,[18] ameliorate symptoms of experimental DCS in rabbits[11] and/or may prove an effective treatment in humans,[16] we report that chronic, moderate alcohol consumption prior to compression was neither prophylactic nor deleterious for DCS in young adult male rats.

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