Eur J Appl Physiol (2007) 101:571–576
DOI 10.1007/s00421-007-0528-2

ORIGINAL ARTICLE

Hyperbaric oxygen pretreatment reduces the incidence of decompression sickness in rats

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Accepted: 9 July 2007 / Published online: 3 August 2007
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Abstract We have previously hypothesised that the number of bubbles evolving during decompression from a dive, and therefore the incidence of decompression sickness (DCS), might be reduced by pretreatment with hyperbaric oxygen (HBO). The inert gas in the gas micronuclei would be replaced by oxygen, which would subsequently be consumed by the mitochondria. This has been demonstrated in the transparent prawn. To investigate whether our hypothesis holds for mammals, we pretreated rats with HBO at 304, 405, or 507 kPa for 20 min, after which they were exposed to air at 1,013 kPa for 33 min and decompressed at 202 kPa/min. Twenty control rats were exposed to air at 1,013 kPa for 32 min, without HBO pretreatment. On reaching the surface, the rat was immediately placed in a rotating cage for 30 min. The animal's behaviour enabled us to make an early diagnosis of DCS according to accepted symptoms. Rats were examined again after 2 and 24 h. After 2 h, 65% of the control rats had suffered DCS (45% were dead), whereas 35% had no DCS. HBO pretreatment at 304, 405 and 507 kPa significantly reduced the incidence of DCS at 2 h to 40, 40 and 35%, respectively. Compared with the 45% mortality rate in the control group after 24 h, in all of the pretreated groups this was 15%. HBO pretreatment is equally effective at 304, 405 or 507 kPa, bringing about a significant reduction in the incidence of DCS in rats decompressed from 1,013 kPa.

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Keywords Diving · Gas bubbles · Gas micronuclei

Introduction

A sudden or excessively rapid reduction in the ambient environmental pressure, such as on abortion of an underwater dive, during high altitude flight or the escape from a disabled submarine, can seriously increase the risk of decompression sickness (DCS) (Parker et al. 2000). It is widely accepted that DCS is caused by the formation of bubbles in supersaturated tissues (Bennett and Elliott 1993). In its less severe form, it may appear as joint pain, but the more severe type of DCS includes chokes (the pulmonary manifestation) and neurologic dysfunction, which may end in death if not treated. It is also accepted that bubbles grow from pre-existing gas micronuclei (Evans and Walder 1969; Vann et al. 1980; Tikuisis and Gerth 2003).

We have previously hypothesised that exposure to hyperbaric oxygen (HBO) before diving on air would result in oxygen replacing the resident inert gas in these micronuclei, to be subsequently consumed by the mitochondria. This would shrink and eliminate gas micronuclei having the potential to grow and form bubbles on sudden or too rapid decompression. This approach is totally different from other methods such as denitrogenation, in which the oxygen pre-breathe is performed before decompression to flush out the inert gas loaded in the tissues during the dive. The hypothesis was examined using the transparent prawn, in which HBO pretreatment before saturation with inert gas at high pressure reduced the number of bubbles following fast decompression from 203 kPa (Arieli et al. 2002). We also found that pretreatment at 405 kPa O_2 for 5 min was the optimal pressure-time combination for decompression from

203 kPa (Ertracht et al. 2005), and that this pretreatment is valid for decompression from 203, 304, and 405 kPa (Arieli et al. 2007b).

Past attempts to eliminate gas micronuclei used short compression to very high pressures that are not applicable to humans (Vann et al. 1980; Daniels et al. 1984; McDonough and Hemmingsen 1984). Denitrogenation required a very long exposure time, lasting several hours (Webb and Pilmanis 1999; Latson et al. 2000; Pilmanis et al. 2003). If a short pretreatment could be found which took only minutes rather than hours and was effective in the mammal, it might be possible to establish a protective protocol for human use.

In the present study, we evaluated the risk of DCS in rats decompressed from 1,013 kPa, and investigated the effectiveness of various HBO pretreatments in reducing this risk.

Methods

Animals

Eighty male rats (Sprague-Dawley strain) weighing 261–306 g were used. The experimental procedure was approved by the Israel Ministry of Defence Animal Care Committee, and the rats were handled in accordance with the principles of laboratory animal care.

Probability of DCS

Animals were exposed to air at high pressure to produce about an 85% incidence of DCS, using the algorithm suggested by Lillo and Parker (2000). The probability of DCS was calculated using the equation (Lillo and Parker 2000):

$$\text{Probability(DCS)} = \text{dose}^n/(\text{dose}^n + P_{50}{}^n) \qquad (1)$$

where $P_{50} = 4.67$ (the dose at which there is a 50% probability of DCS), and $n = 6.48$. The dose was calculated by the equation (Lillo and Parker 2000):

$$\text{dose} = \text{Pti}_{N_2} - 1 \qquad (2)$$

The partial pressure of inert gas in the tissues (Pti_{N_2}) was calculated according to the equation (Lillo and Parker 2000):

$$\text{Pti}_{N_2} = (\text{Pamb}_{N_2} - \text{Pt0}_{N_2}) \times (1 - e^{-0.068t}) + \text{Pt0}_{N_2} \qquad (3)$$

where Pamb_{N_2} is the ambient partial pressure of the inert gas, Pt0_{N_2} is the partial pressure of the inert gas before the exposure, and t is the exposure time to the ambient pressure. From these equations, it was calculated that exposure to 80 msw (912 kPa) for 44 min would result in an 80% risk of DCS, and to 90 msw (1,013 kPa) for 32 min, an 85% risk. After preliminary trials we selected the latter option, because the former failed to achieve the anticipated results. Exposure duration was different for experimental and control rats, and we therefore designed the exposure so that the final nitrogen saturation would not deviate by more than 2% from 100% saturation.

From the same model (Lillo and Parker 2000), we found that prolonging the exposure of rats pretreated with oxygen by a further 1 min would result in equalization of the inert gas tension in their tissues with that of the control rats (based on the Pti_{N_2} calculations).

Experimental system and procedure

Exposure cage

Exposures were conducted in a double-walled metal cage (41 × 17 × 21 cm), as described previously (Arieli et al. 2005). A transparent wall on one side of the cage and the top cover enabled continuous observation of the animal. Thermoregulated water was pumped through the double wall to control the ambient temperature, which was kept in the range 25–28°C. The incoming gas flowed through a metal container attached to the cage wall for temperature equilibration before entering the cage. A thermistor (Telethermometer YSI 400A, Yellow Springs Inc., Yellow Springs, OH, USA) was inserted through the top of the cage for continuous monitoring of the temperature inside.

Experimental system

The exposure cage was placed in a 150-l hyperbaric chamber (Roberto Galeazzi, La Spezia, Italy), as described previously (Arieli et al. 2005).

A pneumatically operated cylindrical cage, which could be rotated at a speed of 3 m/min (Arieli et al. 2007a), was used to diagnose DCS by observing the animals' gait and behaviour following the exposure.

Experimental procedure

Two animals at a time were placed in the exposure cage, which was placed in the hyperbaric chamber. Pressure was increased linearly (at 101 kPa/min) to the desired pressure, with oxygen (experimental group) or air (control group). Experimental rats were pretreated with HBO at the desired pressure for 20 min for the assumed diminution of gas micronuclei. The oxygen was then switched to air for the

experimental rats, and pressure was increased linearly (at 101 kPa/min) to 1,013 kPa for both experimental and control animals. Rats remained at this pressure breathing air for 33 or 32 min (experimental or control group, respectively) to achieve an 85% risk of DCS (Lillo and Parker 2000). Immediately thereafter, the animals were subjected to rapid decompression at 202 kPa/min. A schematic diagram of this procedure is provided in Fig. 1. Following decompression, rats were immediately placed inside the cylindrical cage rotating at ~3 m/min for 30 min. This method of assessing DCS is based on past experience (Lillo et al. 1997; Kayar et al. 1998; Arieli et al. 2007a). The motion pattern of the rat in the cage enabled us to make an early diagnosis of DCS according to the following symptoms: walking difficulties, abnormal breathing patterns, forelimb and/or hind limb paralysis, rolling in the cage, convulsions, and death. Rats were checked again after 2 and 24 h (Lillo and Parker 2000). For the purpose of data analysis, the decompression results were scored as “No DCS”, “DCS”—when the abovementioned symptoms (excluding death) were observed, or “Death”—when DCS symptoms culminated in death.

Experimental protocol

A different oxygen pressure was selected for pretreatment in each of three experimental protocols. In *Series A*, 20 rats were pretreated with HBO at 304 kPa for 20 min. In *Series B*, 20 rats were pretreated with HBO at 405 kPa for 20 min. In *Series C*, 20 rats were pretreated with HBO at 507 kPa for 20 min. After HBO pretreatment, each rat was compressed to 1,013 kPa for 33 min and then decompressed. In the *Control* protocol, 20 rats were compressed to 1,013 kPa for 32 min without HBO pretreatment, and then decompressed. Each rat was assigned to one exposure only. Before any exposure, the animal was placed in the rotating cage to ensure a normal motion pattern.

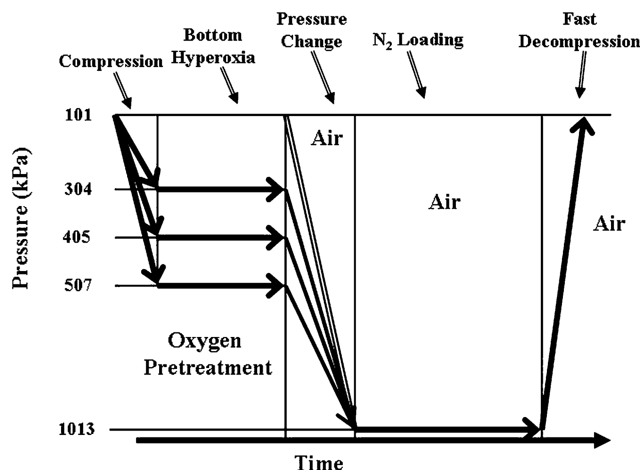

Fig. 1 General scheme of the exposure. *Black arrows* represent experimental exposures, and the *double-lined arrow* represents control

Data analysis and statistics

Similarity of outcome in the different groups was examined using the Fisher exact test and the χ^2 test.

Results

No symptoms of oxygen toxicity were observed in any of the animals following HBO pretreatment. Figure 2 shows the results of exposure to 1,013 kPa, in the control group and after each of the three HBO pretreatment protocols, with regard to the three outcome categories: no symptoms of DCS; symptoms of DCS without death within the first 30 min and up to the 2-h observation point; symptoms of DCS culminating in death within the first 30 min and up to the 2-h observation point.

Although the model predicted an 85% incidence of DCS (including death), in the control group these two categories comprised 65%. All cases of DCS occurred within 30 min of decompression, except for one which occurred in the 2-h observation period. When DCS symptoms appeared, the clinical state usually deteriorated and most of the affected rats died before the end of the 30-min observation period. When obvious signs of DCS appeared, the rat was removed from the rotating cage. Figure 2 shows that up to 2 h post

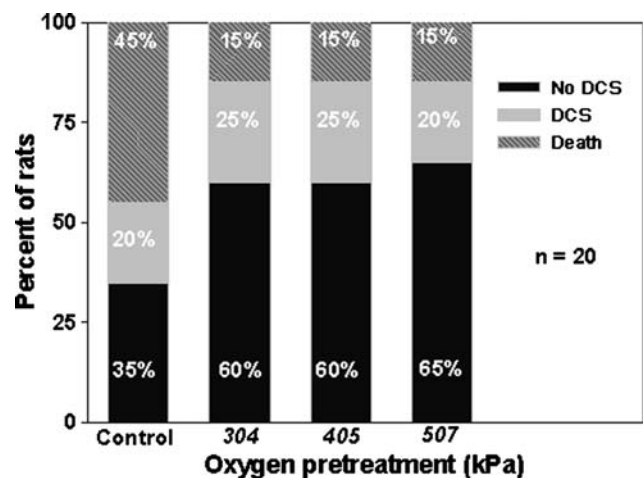

Fig. 2 Incidence of decompression sickness in control and experimental (HBO-pretreated) rats during the 30-min walking period and up to 2 h after decompression. *No DCS* denotes rats with no sign of DCS; *DCS* denotes DCS symptoms (excluding death); *Death* denotes DCS symptoms culminating in death

decompression, 65% of the control rats suffered DCS, with a mortality rate of 45%. Thirty-five percent of the control rats had no DCS. After HBO pretreatment at 304, 405 and 507 kPa, the percentage of affected animals was reduced to 40, 40, and 35%, respectively.

There was no significant difference between the three HBO-pretreated groups, therefore they were combined into one and compared with the control group. HBO pretreatment significantly reduced the incidence of DCS and death due to DCS ($P < 0.025$, χ^2, a 2 × 3 table). Most of the rats with DCS symptoms which survived the 30-min observation period recovered completely after 2 h and resumed normal walking, as previously described (Arieli et al. 2007a). The distribution of the affected rats summarised at 24 h is shown in Fig. 3. Twenty-four hours post decompression, all rats with symptoms of DCS had recovered, and at this point there were now only rats without DCS or rats which had died. Mortality from DCS in the HBO-pretreated rats (15%) was significantly lower than the 45% mortality rate in the control group ($P < 0.01$, χ^2, a 2 × 2 table).

Discussion

The present study shows that HBO pretreatment prior to inert gas saturation provides a considerable degree of protection against DCS. There was no difference in the effect of the three HBO pretreatment pressures (304, 405, and 507 kPa) on the incidence of DCS in rats. However, in comparison with the control group, in all three pretreatment groups there was a considerable increase in the percentage of rats without any DCS symptoms, and a considerable reduction in the incidence of death resulting from DCS. This reinforces our hypothesis that HBO pretreatment before inert gas loading reduces the risk of DCS, most probably by eliminating gas micronuclei. Replacement of the inert gas in the micronuclei by oxygen probably reaches a peak at 304 kPa, and thus increasing the pretreatment pressure beyond this level will not alter the results or lead to a further reduction in the risk of DCS.

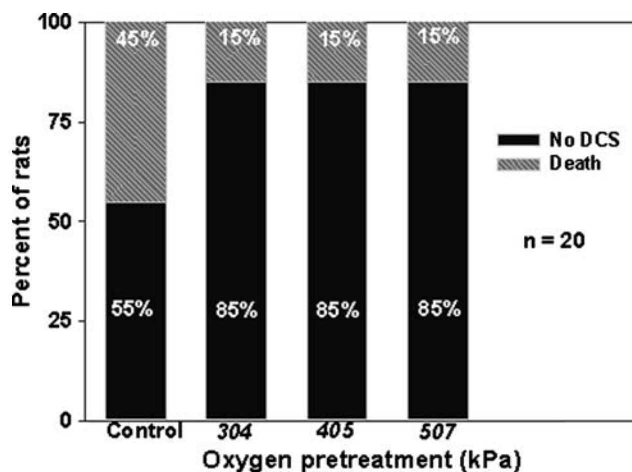

Fig. 3 Incidence of decompression sickness in control and experimental (HBO-pretreated) rats summarised at 24 h after decompression. Other symbols as in Fig. 2

Our HBO pretreatment has the potential to provide divers with a safety margin when faced with the risk of having to make a fast ascent to the surface, or when there is a chance they may not be able to keep to the dive plan. It also has immense potential as a preparatory measure before escape from a disabled submarine or high altitude flight. In previous studies on large mammals and man, an extended period of hyperoxia (about 10 h) was required to reduce the risk of decompression sickness (Webb and Pilmanis 1999; Latson et al. 2000). This may be related to the different treatment methods we employed. The duration of the hyperoxic exposure was shorter in the present study (20 min), and we now continue to investigate how far it can be shortened and still remain effective. We are also studying whether oxygen pretreatment pressures lower than 304 kPa might be effective in reducing the incidence of DCS in rats. Altogether, the present study has shown that a short pretreatment with HBO (20 min) seems to provide effective protection against DCS, with a very low risk of DCS or central nervous system (CNS) oxygen toxicity. This would appear to hold promise of a much shorter and more effective oxygen exposure than previously envisaged.

All previous studies investigating the protective role of oxygen in DCS undertook to reverse the process of inert gas loading by denitrogenation, a procedure designed to wash out the inert gas from the tissues just before the start of decompression (Webb and Pilmanis 1999; Latson et al. 2000; Pilmanis et al. 2003; Mahon et al. 2006). This is in contrast to the method presented here, in which oxygen is used to flush the tissues even before loading of the inert gas. Our HBO pretreatment was designed for nuclei shrinkage as distinct from denitrogenation. It may well be that the procedure of denucleation can be combined with denitrogenation, and thus achieve even greater protection. This is currently under investigation in our laboratory. Oxygen denucleation may also explain why 10 h of decompression on oxygen from saturation at 1.5 ATA resulted in a 13% incidence of DCS in humans, whereas after 4 h of oxygen breathing at bottom pressure and 6 h decompression on oxygen, subjects ended up without any DCS (Latson et al. 2000). Another explanation for the same phenomenon may be that rapid decompression on oxygen produces large numbers of bubbles, compared with

decompression after denitrogenation by an oxygen prebreathe (Flook 2004).

We used HBO pretreatment to reduce the number of bubbles that grow during decompression, and thus reduce the risk of DCS. It has been shown that oxygen has an additive effect on the risk of DCS, albeit much lower than that of nitrogen (<40%) (Weathersby et al. 1987; Parker et al. 1998; Lillo and Parker 2000). It is unlikely that residual oxygen will be left to serve as extra inert gas in the tissues after a 33-min exposure to air at 1,013 kPa, and it should not therefore act as an additional inert gas in the growth of bubbles during decompression. Furthermore, oxygen plays a protective role against lipid peroxidation in the rat brain and prevents the adherence of polymorphonuclear leukocytes to the brain endothelium (Thom 1993), a phenomenon which follows bubble production and aggravates DCS.

The present investigation supports the hypothesis that bubbles originate from preexisting micronuclei, as has been shown in our previous studies and those of others, and that HBO pretreatment may reduce the effective micronuclei (Evans and Walder 1969; Vann et al. 1980; Daniels et al. 1984; Latson et al. 2000; Arieli et al. 2002; Tikuisis and Gerth 2003; Arieli et al. 2007b). A recent investigation (Landolfi et al. 2006), similar to our previously published study on the prawn (Arieli et al. 2002) but conducted for the first time on humans, examined HBO pretreatment prior to nitrogen loading and decompression. It was claimed that this pretreatment with oxygen at 160 kPa reduced the number of bubbles after decompression from 4 ATA. Although the statistical analysis was criticized, the idea presented is similar to our own and warrants further assessment. Our short HBO pretreatment at higher pressures than this in the rat did not result in any signs of CNS oxygen toxicity, which is in agreement with the finding in goats that short exposure to very high oxygen pressure did not cause CNS oxygen toxicity (Blogg et al. 2002). The mechanism of DCS is similar in small and large animals (Kindwall 1962; Lin 1981). The HBO pretreatment procedure for denucleation may therefore be a major step towards reducing the risk of DCS in humans, although the HBO exposure period may well be longer in humans. We are currently in the process of planning further human and animal experimentation.

Acknowledgments The authors thank Richard Lincoln for skillful editing of the manuscript, Dorit Shteinberg for the statistical analysis, and Menashe Brodezki for technical support. This study was supported in part by a grant from the Public Committee for the Allocation of Estate Funds, Ministry of Justice, Israel. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or as reflecting the views of the Israel Naval Medical Institute.

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