Decompression sickness in the rat following a dive on trimix: recompression therapy with oxygen vs. heliox and oxygen

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Arieli R, Svidovsky P, Abramovich A. Decompression sickness in the rat following a dive on trimix: recompression therapy with oxygen vs. heliox and oxygen. J Appl Physiol 102: 1324–1328, 2007. First published December 28, 2006; doi:10.1152/japplphysiol.01195.2006.—Trimix (a mixture of helium, nitrogen, and oxygen) has been used in deep diving to reduce the risk of high-pressure nervous syndrome during compression and the time required for decompression at the end of the dive. There is no specific recompression treatment for decompression sickness (DCS) resulting from trimix diving. Our purpose was to validate a rat model of DCS on decompression from a trimix dive and to compare recompression treatment with oxygen and heliox (helium-oxygen). Rats were exposed to trimix in a hyperbaric chamber and tested for DCS while walking in a rotating wheel. We first established the experimental model, and then studied the effect of hyperbaric treatment on DCS: either hyperbaric oxygen (HBO) (1 h, 280 kPa oxygen) or heliox-HBO (0.5 h, 405 kPa heliox 50%-50% followed by 0.5 h, 280 kPa oxygen). Exposure to trimix was conducted at 1,110 kPa for 30 min, with a decompression rate of 100 kPa/min. Death and most DCS symptoms occurred during the 30-min period of walking. In contrast to humans, no permanent disability was found in the rats. Rats with a body mass of 100–150 g suffered no DCS. The risk of DCS in rats weighing 200–350 g increased linearly with body mass. Twenty-four hours after decompression, death rate was 25% and 20% with HBO and heliox, respectively.

METHODS

Animals

White male Sprague-Dawley rats were used. The experimental procedure was approved by the Israel Ministry of Defense Animal Care Committee, and the rats were handled in accordance with internationally accepted humane standards.

Exposure Cage and Experimental System and Procedure

Exposure cage. The exposure cage was a metal, double-walled cage (25 × 11 × 12 cm). One wall for observation of the animal and the top cover, which could be opened, were made of Plexiglas. Thermoregulated water was pumped through the double wall to control the ambient temperature. 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 Instrument, Yellow Springs, OH) was inserted through the top of the cage.

Experimental system. The exposure cage was placed in a 150-liter hyperbaric chamber (Roberto Galeazzi, La Spezia, Italy). The flow of trimix, oxygen, or heliox through the cage was controlled by a needle valve and by observation of a flowmeter situated inside the hyperbaric chamber. The outgoing gas exiting via a bypass tube into the atmosphere of the hyperbaric chamber. A small portion of the outgoing gas was directed out of the hyperbaric chamber (this was controlled by another needle valve), passed through a flowmeter, and sampled for oxygen concentrations by an oxygen analyzer (Servomex, Sussex, UK). Water hoses were connected to ports in the hyperbaric chamber and to the ports in the exposure cage for recirculation of the thermoregulated water (C/H Temperature Controller Bath and Circulator 2067, Forma Scientific, Marietta, OH). The temperature inside the exposure cage was kept at 27°C.

A pneumatic rotating cylindrical cage was constructed from two commercial running wheels (diameter 21 cm, width 21 cm). A door was cut in each running wheel to enable the rat to be placed inside. The engine of a pneumatic drill was adjusted with transmission wheels and a rubber band to run the cylindrical cage, while the rotation rate was controlled using a pressure gauge. The cage was designed to operate both in normobaric and hyperbaric conditions.

DCS is not common in trimix diving (5, 21). There are no general validated decompression procedures (7) and no specific recompression treatment for DCS resulting from trimix diving. There has been no investigation of hyperbaric recompression in rats with DCS after trimix diving. Because the load of two inert gases is involved, the efficacy of recompression treatment could vary depending on whether oxygen or heliox is used. The purpose of the present study was to validate a rat model for DCS following decompression from a trimix dive and to compare recompression treatment using oxygen or heliox.

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However, because of a limited supply of trimix, it was not used inside the hyperbaric chamber.

**Experimental procedure.** The rat was placed in the exposure cage inside the hyperbaric chamber. The animal was unrestrained, and it could move about freely inside the cage. The pressure in the chamber was raised at a rate of 180 kPa/min. During this procedure, until the desired pressure was reached, the gas mixture flowing through the cage was trimix. For each bottom pressure, the trimix yielded a nitrogen pressure of 405 kPa and an oxygen pressure of 142 kPa, with the balance helium. The rat was observed through a window in the hyperbaric chamber for any signs of distress. After decompression to atmospheric pressure, the experimental rats were given hyperbaric treatment, whereas control rats were left untreated. The rat was freed from the exposure cage and placed inside the cylindrical cage rotating at a perimeter speed of \( \sim 3 \text{ m/min} \) for 0.5 h. This was established to be a sufficient length of time for almost all cases of DCS to become evident (12). The rat was observed for signs of decompression sickness while walking for 0.5 h, as well as at 2 and 24 h after decompression (using the rotating cylindrical cage for a short period). When obvious difficulties were observed while walking in the rotating wheel, the rat was removed from the wheel for further observation. Signs of DCS according to Lillo and Parker (13) consisted of walking difficulties, abnormal breathing patterns, forelimb and/or hindlimb paralysis, rolling in the rotating wheel, convulsions, and death. Outcome was divided into three categories: “No DCS”; “DCS,” DCS excluding death; and “Death,” when signs of DCS culminated in death.

**Experimental Protocol**

**Series A.** In series A, we established the experimental model. We determined the exposure protocol, and we tried to find a combination of exposure time, depth, and decompression rate that would yield the desired percentage of DCS. The initial exposures were calculated using the algorithm suggested by Lillo and Parker (13) to result in 85% DCS. Later on, we changed the parameters of depth, exposure time, and decompression rate to achieve an \( \sim 50\% \) risk of DCS. We also tested the effects of body mass and repeated exposure on the risk of DCS. There were four phases in this series. 1) We established the experimental model. In the initial exposures (\( n = 17 \)), the body mass range was 150–400 g, bottom pressure 910–1,320 kPa, and decompression rate 100–1,000 kPa/min. 2) We conducted 78 exposures of 18 rats weighing 250–350 g to pressures from 1,110 to 1,320 kPa, with a decompression rate of 25–100 kPa/min. Rats were used once a week, provided they had no signs of DCS before the next exposure. From the results, we selected a pressure of 1,110 kPa, 30-min bottom time, and a decompression rate of 100 kPa/min as our model for the investigation to follow. 3) The effect of body mass on the risk of DCS was established in 72 rats by a 30-min exposure to 1,110 kPa and decompression at a rate of 100 kPa/min. 4) The effect of repeated weekly exposures was further studied on 44 rats weighing 250–350 g exposed to 1,110 kPa for 30 min and decompressed at a rate of 100 kPa/min. A rat that suffered DCS was not used for any further exposures.

**Series B.** In series B, we studied the effect of hyperbaric hyperoxic treatment on DCS. Each protocol in series B was a consequence of the results obtained in previous protocols. In this series, we used rats in the weight range 250–350 g. The hyperbaric exposure was at 1,110 kPa (100 m of seawater) for 30 min, and decompression was carried out at a rate of 100 kPa/min. Hyperbaric treatment after decompression was either hyperbaric oxygen (HBO) at 280 kPa for 1 h (similar to US Navy Table 6, Ref. 16) or heliox-HBO, 0.5 h at 405 kPa on heliox (He-O\(_2\) mixture, 50%–50%) followed by 0.5 h at 280 kPa on oxygen (similar to Table Cx 30, Ref. 10). There were five protocols. 1) Twenty rats were used for a control exposure, in which there was no treatment after decompression. *Protocols 2 and 3* tested the effect of HBO treatment immediately after a trimix dive or on the following trimix exposure, respectively. 2) Twenty rats were treated by HBO immediately after decompression from the hyperbaric exposure. Rats that survived without any residual signs of DCS were exposed to trimix again after being given a week to recover. 3) The rats that survived the control exposure (*protocol 1*) without signs of DCS were left for a week before a further trimix exposure followed by immediate HBO treatment. Because immediate HBO treatment had a 100% success rate, in *protocols 4 and 5* the treatment was given after a 5-min delay for the purpose of comparing HBO and heliox. 4) Twenty rats were treated by HBO 5 min after decompression to the surface from the trimix exposure. 5) Twenty rats were treated by heliox-HBO 5 min after decompression to the surface from the trimix exposure.

**Statistical Analysis**

A logistic model was used to compare the three treatments and the control group in series B proved to be unsolvable. The Fisher exact test was therefore used to compare pairs of groups for the frequency of “no DCS” and “DSC+Death.” Differences between the groups were examined for statistical significance at 2 and 24 h postdecompression.

**RESULTS**

**Time Course of DCS in Rats**

The course of the development of DCS is summarized in Table 1. The data are from 40 rats that had at least 1 symptom of DCS. Death occurred during the final stage of decompression in six rats. Peak incidence of death was during the 30-min period of walking (12 animals), mostly in the early stages. Four animals died in the interval between the end of the initial 30-min observation period and the examination conducted at 2 h. Only three animals were found dead 24 h after decompression. All of the remaining symptoms were more frequent during the initial 30-min observation period. The prevalence of all symptoms decreased markedly 2 h after decompression, and none was seen at 24 h. In no case was the first appearance of a symptom later than the 30-min period of walking.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Immediately After Decompression</th>
<th>During 30 min Walking</th>
<th>2 h After Decompression</th>
<th>24 h After Decompression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td>15</td>
<td>45</td>
<td>55</td>
<td>63</td>
</tr>
<tr>
<td>Hindlimb paralysis</td>
<td>10</td>
<td>45</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Walking difficulties</td>
<td></td>
<td>23</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Abnormal breathing patterns</td>
<td>3</td>
<td>25</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Rolling in the rotating wheel</td>
<td>5</td>
<td>18</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Convulsions</td>
<td></td>
<td>20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are prevalence (in %) given for 4 stages of observation. Each number expresses the percentage of the whole group of 40 rats; only death is a cumulative percentage. DCS, decompression sickness.
The risk of DCS in rats having a body mass of 200–350 g increased linearly with body mass. The outcome of treatment given after a 5-min delay (HBO, protocol 4; heliox, protocol 5) is shown in Fig. 5 for three points in time at which we observed the animals: 5 min after decompression, 95 min after decompression at the end of calculation of risk for this exposure. The trend noted for a reduced risk of DCS with an increasing number of exposures was not significant (Fisher exact test). The probability of DCS+Death was 53% in the first exposure, declining to 38% in the third exposure and 25% in the fourth (only 4 rats). Each case of DCS+Death relates to a different rat.

Series B

In the control group (protocol 1), by the end of the 30-min period of walking, eight rats had no DCS, four had signs of DCS, and eight had died. At 24 h, all four animals with signs of DCS had recovered. No DCS was recorded in 20 rats given HBO treatment immediately after decompression (protocol 2). When these rats were used a week later for a further trimix exposure, nine were dead at 2 h postdecompression. Of the 20 rats exposed to trimix as a control group (protocol 1), by 2 h, 8 had died and 3 had DCS. A week later, none of the 12 surviving animals had any discernible signs of DCS, and after exposure to trimix followed by immediate HBO treatment, they continued to exhibit no signs of DCS (protocol 3). When the 20 rats treated by HBO after the first exposure were compared with the 20 control rats, a beneficial effect of immediate HBO treatment was found at 2 h (P < 0.0001) and at 24 h (P < 0.003).

When 5 min separated decompression to the surface and HBO treatment (protocol 4), four rats died and six developed DCS within those 5 min. After 1 h, one of the six rats with DCS had died, two remained with DCS, and three had recovered. At 24 h, the last two animals with DCS had also recovered. Twenty-four hours after decompression, 40% of animals from the control trimix exposure had died (protocol 1, Fig. 4), compared with only 25% when HBO treatment was given after a 5-min delay (protocol 4, Fig. 4). The reduced number of deaths when treatment was given after a 5-min delay was not significantly different from the control group at 2 h or at 24 h. The results for the 30-min postdecompression period following protocol 1 (the control exposure) are also shown in Fig. 4. It can be seen that by 24 h, the four rats with DCS had recovered.

The outcome of treatment given after a 5-min delay (HBO, protocol 4; heliox, protocol 5) is shown in Fig. 5 for three points in time at which we observed the animals: 5 min after decompression, 95 min after decompression at the end of

Series A

1) In our initial efforts to find a trimix exposure that would yield 85% decompression sickness, we used Lillo and Parker’s algorithm (13). However, we were unable to produce DCS using the exposure time calculated for 910 and 1,110 kPa. From these initial trials, it became clear that rats weighing <200 g are highly resistant to DCS, whereas heavy rats, ~400 g in weight, are very sensitive. We also noted that a slow decompression rate protected rats from DCS, and that almost no DCS occurred after decompression from 910 kPa.

2) We therefore exposed rats having a body mass in the range 250–350 g to pressures >1,100 kPa, followed by decompression at >25 kPa/min. The results are shown in Fig. 1. All three DCS categories are represented in the pressure and time ranges tested. Exposure for 30 min to 1,110 kPa with a decompression rate of 100 kPa/min gave ~50% DCS+Death, and it was selected for further assessment. 3) The percentage of DCS+Death in 72 rats with varying body mass is shown in Fig. 2. Rats with a body mass of 100–150 g had no DCS at all. The risk of DCS in rats having a body mass of 200–350 g increased linearly with body mass (P < 0.0001). In the DCS-sensitive body mass range, the risk increased by 8% for each 100-g increase in body mass. The effect of repeated exposures is shown in Fig. 3. Some of the rats were given HBO treatment after the first exposure, and are therefore excluded from the

Fig. 1. Results from different trimix (a mixture of helium, nitrogen, and oxygen) exposures (bottom time, pressure, and decompression rate). Symbols are clustered around their pressure-time point to be discernible. DCS stands for any symptom of decompression sickness, excluding death.

Fig. 2. Percentage of DCS+Death as a function of body mass. Exposure condition is shown, and the number of rats in each body mass group is given above the symbol.

Fig. 3. Percentage of DCS+Death as a function of exposure number. Exposure condition and weight range are shown. The number of rats in each exposure is given above the symbol, and the mean weight in italics below the symbol.
轮的两处理方法的结局相似（虽然没有显著的不同），并且它可能带来一个轻微的，不显著的优势于控制状态。接受过高氧治疗的动物的DCS发生率更高（11% 与 9%），这可能也与适应有关。立即高氧处理作为第二暴露，DCS的患病率在经历高氧治疗后有较大的优势（在控制状态下，DCS的患病率是0.003）。在高氧或氦氧处理，DCS在给予5分钟后发生。在每种处理组的每个类别中，DCS的患病率可见于适当的条形图段。

DISCUSSION

在我们的实验模型中，我们强调了体重对敏感性的重要性于DCS。在大鼠中，体重敏感性是低的，150 g体重的范围是200-350 g（一个范围是150 g）。图2的斜率大于我们数据的斜率。我们的发现也同意Lillo et al. (12)的报道，他们指出所有的DCS病例发生在第一30分钟。目前对DCS过程的描述可能用于进一步的研究。与人类不同，没有永久的残疾被发现于大鼠，而且无疑在他们适应DCS的反应有不同。

在我们对实验模型的讨论中，我们强调了适应的重要性。Smith et al. (15)研究给小鼠高氧暴露到689 kPa空气30分钟，并且指出了当死亡发生时，通常在30分钟内。这与我们的数据一致。我们的发现也同意Lillo et al. (12)的报道，他们指出所有的DCS病例发生在第一30分钟。目前对DCS过程的描述可能用于进一步的研究。与人类不同，没有永久的残疾被发现于大鼠，而且无疑在他们适应DCS的反应有不同。

体重相关的敏感性可能由增加的脂肪组织引起，这使得存储更多的惰性气体，而且由于在特定的代谢率和组织灌注，镇流到更轻的气体气流，于重大的影响。

在许多先前的研究中，氧气治疗被用作高氧后时间。当其他研究中，DCS的患病率在经历高氧治疗后的动物数是稍微增加（11对9），这可能也与适应有关。在每个类别中，DCS的患病率可见于适当的条形图段。

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DISCLOSURES

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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