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HYPERBARIC OXYGEN-INDUCED SEIZURES CAUSE A TRANSIENT DECREMENT IN COGNITIVE FUNCTION

L. DOMACHEVSKY,[a]* L. RACHMANY,[b] Y. BARAK,[b] V. RUBOVITCH,[b] A. ABRAMOVICH[a] AND C. G. PICK[b,c]

[a] *Israel Naval Medical Institute, IDF Medical Corps, Box 89, Rambam Health Care Campus, P.O. Box 9602, 3109601 Haifa, Israel*

[b] *Department of Anatomy and Anthropology, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel*

[c] *Sagol School of Neuroscience, Tel Aviv University, Tel Aviv, Israel*

Abstract—Hyperbaric oxygen-induced seizures are classified as brief, generalized tonic–clonic seizures. They are believed to cause no residual cognitive damage, although this has not been investigated in depth. In the present study, we examined whether hyperbaric oxygen-induced seizures cause impairment of behavioral and cognitive abilities. Cognitive status was assessed using four behavioral tests: Y-maze, novel object recognition, the elevated plus maze, and a passive avoidance task. Three time intervals were examined: 24 h, and 7 and 30 days after the seizures. We found transient impairment of performance in the compressed group on three tests (the novel object recognition paradigm, the Y-maze paradigm, and the passive avoidance task). On the elevated plus maze test, the impairment persisted. The time interval to the appearance of deficits and to eventual recovery was not the same for the different tests. We conclude that hyperbaric oxygen-induced seizures result in transient impairment of performance on behavioral tests in a mouse model. Further investigation is required to establish the mechanism and location of injury, and to determine whether the performance decrement on the elevated plus maze test represents permanent damage or transient damage with slow resolution. These new findings should be taken into account when planning hyperbaric oxygen treatments, to ensure that the chosen protocol is therapeutic yet minimizes the risk of CNS oxygen toxicity.

Key words: behavioral tests, convulsions, neurologic damage, oxygen toxicity.

INTRODUCTION

The central nervous system (CNS) is a major site of oxygen toxicity when breathing oxygen at high pressure. Prolonged exposure to 100% oxygen at a pressure of more than 3 atmospheres absolute (ATA) will result in CNS oxygen toxicity (Lambertsen, 1965), whose neurologic manifestations include a variety of symptoms and signs (Butler and Thalmann, 1986; Donald, 1992). Non-specific symptoms include nausea, dizziness, abnormal sensations such as tingling around the lips, headache, disorientation, lightheadedness, and a feeling of apprehension. Specific symptoms include blurred vision, tunnel vision, and tinnitus. Signs of CNS oxygen toxicity include respiratory disturbances, eye twitching, twitching of the lips, mouth and forehead, and seizures. There is no consistency in the order of appearance of symptoms and signs prior to the development of seizures. Also referred to as hyperbaric oxygen (HBO)-induced seizures, these are the most disturbing signs of CNS oxygen toxicity, although they are reversible, disappearing on reduction of the partial pressure of the inspired oxygen. They are believed to cause no residual cognitive damage (Lambertsen, 1965), a belief which has led most researchers to focus on the mechanisms of CNS oxygen toxicity and to abandon the question of its long-term effect on the brain.

Behavioral tests are well established as a sensitive method for assessing brain damage, often providing the only evidence of brain injury in cases where other clinical examinations are normal. In a mouse model of minimal traumatic brain injury without motor or sensory damage, Zohar et al. (2011) used these tests to demonstrate significant and irreversible long-term deficits in behavioral and cognitive abilities. The time interval to the appearance of these deficits varied among the different tests.

Over the years, there have been a number of investigations of the possible association between seizures and behavioral and cognitive impairment. Dodrill (2002) explored the neuropsychological effect of seizures on mental abilities by conducting a selective review of the world literature. Overall, he found a certain connection between seizures and adverse cognitive change. In particular, he noted a statistically significant relationship between the number of seizures and lower scores on tests of cognitive ability, changes in intelligence corresponding to changes in seizure frequency, better performance over time in normal subjects than in those with epilepsy, and loss of mental ability in patients with uncontrolled seizures. There is a consensus that the most common change observed is loss of memory (Dodrill, 2004).

The understanding that brief seizures might cause brain damage has evolved only in the past few decades. The damage in such cases is usually more subtle, and

*Corresponding author. Tel: +972-4-8693040; fax: +972-4-8693240.
E-mail address: liranura@gmail.com (L. Domachevsky).
Abbreviations: ATA, atmospheres absolute; CNS, central nervous system; HBO, hyperbaric oxygen.

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necessitates sensitive tests for its detection. It is interesting to note that in two studies where patients had no seizures over a 5-year period, test performance either remained the same or improved (Dodrill and Wilensky, 1992; Äikiä et al., 1999).

HBO-induced seizures are categorized as brief, generalized tonic–clonic seizures. It would therefore appear reasonable to employ sensitive tests for the detection of brain damage in HBO-induced seizures. In the present study, we examined whether HBO-induced seizures cause impairment of behavioral and cognitive abilities. We used the novel object recognition test, the Y-maze paradigm, the elevated plus maze, and a passive avoidance task in an animal model. Three time intervals were examined: 24 h, and 7 and 30 days after seizures.

EXPERIMENTAL PROCEDURES

Animals

The experimental protocol was approved by the Ethics Committee of the Sackler School of Medicine, in compliance with the guidelines for animal experimentation of the National Research Council (Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011). We used the smallest possible number of animals necessary to achieve statistical significance, and every effort was made to minimize their suffering.

Male ICR mice weighing 30–40 g were kept five per cage in a constant 12-h light/dark cycle at room temperature (22 ± 2 °C). Food (Purina rodent chow) and water were available *ad libitum*.

Study groups

Ninety-two mice were used in the study. Three animals died during the experiments. Forty-five experimental mice were exposed to a pressure of 6 ATA breathing oxygen to induce seizures. These animals were randomly assigned to three groups which performed behavioral tests 24 h after the appearance of HBO-induced seizures (compressed: immediate), 7 days post-seizures (compressed: 1 week), or 30 days post-seizures (compressed: 30 days). The remaining 47 mice, which were not exposed to HBO, served as control groups (control: immediate, control: 1 week, and control: 30 days).

Hyperbaric exposure

Mice were placed in a double-walled, metal exposure cage measuring 13 × 25 × 25 cm. One side of the cage is made of Perspex, enabling continuous observation of the animal. The mouse was able to move about freely inside the cage. The exposure cage was closed and placed inside a 150-liter hyperbaric chamber (Roberto Galeazzi, La Spezia, Italy), which was then sealed. At this stage, the pressure was raised to 6 ATA at a rate of 1 ATA per minute with oxygen flowing through the exposure cage. The mouse has no problem with pressure equalization of the middle ear, and any changes in its behavior can be seen through the Perspex wall.

The mouse was observed during this exposure to high-pressure oxygen until the appearance of the first clinical seizures, at which point the pressure was reduced and the mouse released. HBO-induced seizures are tonic–clonic in nature. The tonic phase begins as forelimb flexion, progressing to a generalized tonus or a generalized clonic convulsion in which both forelimbs and hindlimbs are involved. A postictal period is seen once seizures subside.

Neurological assessment

The neurological status of each of the experimental mice was assessed using an extensive battery of tests, 1 and 24 h following the seizure. The tests included hind-leg flexion reflex (when raised by the tail), righting reflex (falling on all four legs after a short drop from a legs-up position), corneal reflex (blinking response), secretory signs (around the mouth and eye), strength, a beam balance task, a beam-walking coordination task, and exploration and locomotor activity tests, as described previously (Zohar et al., 2003).

Behavioral tests

Behavioral tests were conducted 24 h, and 7 and 30 days after compressing the animals. Cognitive status was assessed using four behavioral tests: Y-maze, novel object recognition, the elevated plus maze, and a passive avoidance task. The passive avoidance task was the final test in the series due to its aversive nature.

Novel object recognition paradigm. An object recognition task was used to evaluate recognition memory, as previously described by Messier (1997). This task is based on the tendency of rodents to discriminate between a familiar and a novel object. Twenty-four hours before the test, mice were individually habituated to an open field box (59 × 59 × 20 cm) for 5 min. During the acquisition phase, two identical objects (A and B) were positioned symmetrically within the arena for 5 min. These objects were sufficiently heavy and high to ensure that the mice could neither move them nor climb over them. At 24 h after acquisition phase training, one of the objects (A or B randomly) was replaced by a novel one (C), and exploratory behavior was again analyzed for 5 min. After each session, the objects were thoroughly cleaned with 70% ethanol to prevent odor recognition. Exploration of an object was defined as rearing on the object or sniffing it at a distance of less than 2 cm, and/or touching it with the nose. Successful recognition was reflected by preferential exploration of the novel object. Discrimination of visual novelty was assessed by a preference index (Dix and Aggleton, 1999): (time near the ‘‘new’’ object − time near the ‘‘old’’ object)/(time near the ‘‘new’’ object + time near the ‘‘old’’ object).

Y-maze paradigm. The Y-maze test was used to assess spatial memory. The maze is composed of three