Pyridoxine Does Not Prevent Hyperbaric Oxygen-Induced Seizures in Rats

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Abstract—Normobaric supplemental oxygen can prolong seizures not caused by hyperbaric oxygen therapy. In addition, hyperbaric oxygen therapy can cause seizures. The mechanism of hyperbaric oxygen-induced seizures is unknown. We hypothesized that pretreatment with pyridoxine may delay the onset of hyperbaric oxygen-induced seizures, recognizing that pyridoxine is already an antidote for some epileptogenic poisons such as isoniazid and monomethylhydrazine. Therefore, rats were pretreated with intraperitoneal injections of pyridoxine at 48, 24, and 2 h before undergoing hyperbaric oxygen (HBO) treatment at 3 atmospheres absolute with 100% oxygen and were compared to a control group of HBO-treated rats for time to onset of seizures. There was no difference in onset of seizure time between the pyridoxine-treated group of rats and the control rats. Supplemental pyridoxine pretreatment did not alter the time to onset of seizures during HBO treatment in this study. © 2006 Elsevier Inc.

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INTRODUCTION

Normobaric supplemental oxygen can prolong seizures not caused by hyperbaric oxygen therapy (1). In addition, hyperbaric oxygen therapy can cause seizures (2).

Hyperbaric oxygen (HBO) therapy produces high plasma and tissue concentrations of dissolved oxygen for treating ill and injured patients. Some medical indications for HBO include carbon monoxide poisoning, decompression sickness, gas gangrene, problem wounds, and radiation tissue damage (3). Although HBO is relatively safe, seizures caused by central nervous system (CNS) toxicity are difficult to predict and remain an important complication of HBO (2). The mechanism by which hyperbaric oxygen causes seizures is still unknown, even though these seizures were first described by Paul Bert in 1878 (4). The recommendation for preventing HBO-induced seizures is to intermittently switch the patient from pure oxygen to air (5). The scientific mechanism for this recommendation is not elucidated (6).

One possible mechanism for HBO-induced seizures could be decreased gamma-aminobutyric acid (GABA) concentrations in the brain. GABA is the major inhibitory neurotransmitter in the brain and is formed from glutamate by the action of a rate-limiting step involving glutamate decarboxylase (GAD), a vitamin B6 (pyridoxine)-dependent enzyme. Wood and co-workers implicate the inhibition of GAD as a mechanism of HBO-induced seizures after finding decreased levels of GABA and inhibition of GAD activity in rat brains just before the
onset of HBO-induced convulsions (7). Wood et al. have shown that the greater and more rapid decrease in brain GABA concentrations during HBO, the more rapidly the seizures develop (8). Furthermore, Geddes and Wood found that the concurrent administration of pyridoxine with pro-convulsant drugs to mice significantly lengthens the time to onset of seizures induced by the convulsant drug alone at atmospheric conditions of oxygen (9). In normal rats, GAD is unsaturated with respect to the cofactor pyridoxal phosphate, and providing increased amounts of pyridoxine hydrochloride enhances brain GABA levels (10). Midazolam, which indirectly enhances GABA activity at GABA receptors and alters electroencephalogram (EEG) activity, has its activity on the EEG attenuated in a similar manner by both HBO and flumazenil (11).

Although the work of Wood and others noted in the previous paragraph generally supports the hypothesis that decreased GABA concentrations in the brain may predispose to hyperbaric oxygen-induced seizures, the work of Faiman and others does not support this hypothesis (12–14). Faiman et al. found that treatment with disulfiram, a central nervous system oxygen protectant, does not prevent the decrease in brain concentrations of GABA, and yet, disulfiram does protect against hyperbaric oxygen-induced convulsions (12,13). In addition, Faiman et al. find that pretreatment with gabaculine markedly increases brain GABA levels but this has no protective effect in preventing hyperbaric oxygen-induced seizures (13).

At this time, no single existing hypothesis explains the mechanism of HBO-induced seizures. Faiman et al. said, “Thus our findings cannot be regarded as conclusively eliminating the role of GABA in hyperbaric oxygen-induced convulsions” (12).

Pyridoxine is an essential cofactor in the production of many neurotransmitters, including GABA. It is an antidote (FDA category A) for certain poisonings involving hydrazine derivatives such as isonicotinic acid hydrazide (INH), an antituberculosis medication, and monomethylhydrazine, found in rocket fuel and the toxic mushroom Gyromitra esculenta. Pyridoxine is beneficial in treating theophylline-induced seizures in an animal model (15). Currently, the administration of pyridoxine is generally recommended not only for neonatal seizures, but also for any seizure with an onset before 2 years of age, because pyridoxine-dependent seizures are not widely recognized (16).

Because precise molecular mechanisms of HBO-induced seizures have not been elucidated, because GABA has not been conclusively eliminated as having some role in HBO-induced convulsions, and because pyridoxine is an inexpensive, readily available, relatively safe treatment for other epileptogenic poisons, we decided to conduct a pilot study to see if pretreatment with pyridoxine could prevent hyperbaric oxygen-induced seizures. This study was done as a pilot study to determine if a fixed dose of pyridoxine would be promising for dose ranging studies. This study is not a dose ranging study and is not a study that examines molecular mechanisms or hypotheses of HBO-induced seizures.

This study compared the time to onset of seizures in a group of rats pretreated with pyridoxine versus a group of rats not pretreated with pyridoxine. All rats in each group received HBO until they seized. Our hypothesis was that rats pretreated with pyridoxine before HBO would have a longer time period until their onset of seizures vs. the controls.

**METHODS**

All experiments were performed in accordance with the National Institutes of Health animal care guidelines and were approved by the Institutional Animal Care and Use Committee and the Research Committee. Young male Sprague-Dawley rats (156–270 g) were purchased weekly from Charles River (Wilmington, MA) through Harlan Vendors (Indianapolis, Indiana) by University Animal Care. Rats were housed in a climate-controlled room (22–24°C), were kept on a 12-h light/dark cycle, and were fed Teklad 4% Mouse/Rat Diet (Harland Teklad, Madison, WI) that routinely contains pyridoxine, 9.4 mg/kg, and had access to water ad libitum. Rats were randomly assigned by a computer-generated random numbers program (Biometry Unit, Cancer Center) to receive either pyridoxine (American Pharmaceutical Partners, Schaumburg, IL) 71 mg/kg by intraperitoneal injection or diluent normal saline injection. Each rat received either a total of three 71 mg/kg injections of pyridoxine or three equivolumetric injections of normal saline, at 48 h, 24 h, and 2 h before HBO in a Sechrist monoplace hyperbaric chamber (Sechrist Industries, Anaheim, CA). Individual rats were placed in a transparent Plexiglas container and received HBO at 3 atmospheres absolute until the animal seized, ending the experiment; the time to seizure was noted. Every rat in either group received HBO at 3 atmospheres absolute until it seized. The investigator who observed the rats during HBO and during the rats’ seizures was blinded as to what type of injections the rats had received.

The total number of animals required was statistically estimated to be 9 rats per group to allow detection of a 1 h difference in mean time until onset of seizure with > 80% power. Therefore, 9 rats were randomized to the pyridoxine pretreatment group and 9 rats were randomized to the saline (control) pretreatment group. Data analysis was performed using a two-tailed non-paired T
test to determine if the latency time to seize between the two groups was different. An alpha level of significance was set at $p \leq 0.05$.

### RESULTS

The results are shown in Table 1. As can be seen, there is little difference in the mean time to onset of seizure, with more variability in the control rats. The mean, standard deviation, standard error, and median time to onset of seizure for each pretreatment group are shown in Table 1. The 95% confidence interval for the difference in the means between the treated and control rats was $(-30.4$ to $34.4)$ minutes. Thus, there is no statistically significant effect of pyridoxine pretreatment on the time to onset of seizure ($p = 0.90$). Based on the observed standard deviation of the mean in the control animals after 9 rats were treated, this study had $> 90\%$ power to detect a 1 h difference in mean time until onset of seizure between the pyridoxine pretreatment and control groups (assuming a one-sided significance level of 0.05).

### DISCUSSION

The present study indicates that supplemental pyridoxine, in the manner described above, does not prolong the seizure-free period during HBO. Although a single, smaller intramuscular dose of pyridoxine had been shown to be ineffective using a similar experimental model, we had hypothesized that multiple, larger supplemental doses of pyridoxine would be more effective in delaying hyperbaric oxygen-induced seizures (17). One case report suggests that pyridoxine, functioning independently as a cofactor of enzymes, may act as a neuromodulator to reduce the epileptogenic excitation of neurons (18).

There is little guidance as to the appropriate dose of pyridoxine to be used. The recommended initial dose of pyridoxine to be given to a human with seizures caused by ingesting an unknown amount of INH is 5 grams for a 70-kg person (71 mg/kg) (19). Children with pyridoxine-dependent seizures receive an oral maintenance dose of pyridoxine ranging from 2 mg to 300 mg per day, with most patients requiring 20 mg to 100 mg per day to remain seizure-free (20). In light of the above facts, it seemed promising to test the hypothesis that 2 days of pyridoxine supplementation in rats (71 mg/kg each day) with an additional 71 mg/kg pyridoxine dose just before experimentation, would prolong their seizure-free period during HBO.

There are a number of limitations to this study. It is possible that different strains of rats might be more susceptible to changing levels of pyridoxine. We chose to study this research question in Sprague-Dawley rats. It is also possible that suboptimal doses of pyridoxine were used. A diet containing pyridoxine (9.4 mg of pyridoxine per kilogram of feed) for both treatment and control groups could have been a confounding variable, but we chose this study methodology because we did not want to study rats with pyridoxine-deficient diets. Finally, EEG was not used to verify onset of seizure time in the rats. However, the rats’ convulsions were easily observed.

In conclusion, providing supplemental pyridoxine to rats in this experimental model does not alter their susceptibility to oxygen toxicity during HBO therapy.

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### REFERENCES