Vigabatrin prevents seizure in swine subjected to hyperbaric hyperoxia

Aaron A. Hall, Colin Young, Michael Bodo, and Richard T. Mahon

Undersea Medicine Department, Naval Medical Research Center, Silver Spring, Maryland

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Vigabatrin prevents seizure in swine subjected to hyperbaric hyperoxia. J Appl Physiol 115: 861–867, 2013. First published May 30, 2013; doi:10.1152/japplphysiol.00221.2013.—Oxygen is the most widely used therapeutic strategy to prevent and treat decompression sickness (DCS). Oxygen prebreathe (OPB) eliminated DCS in 20-kg swine after rapid decompression from saturation at 60 feet of seawater (fsw). However, hyperbaric oxygen (HBO) has risks. As oxygen partial pressure increases, so do its toxic effects. Central nervous system (CNS) oxygen toxicity is the most severe side effect, manifesting as seizure. An adjunctive therapeutic is needed to extend OPB strategies to deeper depths and prevent/deal with seizures onset. The Food and Drug Administration-approved anti-epileptic vigabatrin has prevented HBO-induced seizures in rats up to 132 fsw. This study aimed to confirm the rat findings in a higher animal model and determine whether acute high-dose vigabatrin evokes retinotoxicity symptoms seen with chronic use clinically in humans. Vigabatrin dose escalation studies were conducted 20-kg swine exposed to HBO at 132 or 165 fsw. The saline group had seizure latencies of 7 and 11 min at 165 and 132 fsw, respectively. Vigabatrin at 180 mg/kg significantly increased seizure latency (13 and 27 min at 165 and 132 fsw, respectively); 250 mg/kg abolished seizure activity at all depths. Functional electroretinogram and histology of the retinas showed no signs of retinal toxicity in any of the vigabatrin-treated animals. In the 250 mg/kg group there was no evidence of CNS oxygen toxicity; however, pulmonary oxygen toxicity limited HBO exposure. Together, the findings from this study show that vigabatrin therapy is efficacious at preventing CNS oxygen toxicity in swine, and a single dose is not acutely associated with retinotoxicity.

Hypothesis: Vigabatrin therapy is efficacious at preventing CNS oxygen toxicity in swine, and a single dose is not acutely associated with retinotoxicity.

Divers breathing compressed air absorb metabolically inert, nonconsumable gas (predominantly nitrogen) in proportion to their ambient pressure, incurring what is known as a decompression obligation. This state requires a slow ascent to surface pressure [1 atmosphere absolute (ATA)], allowing sufficient time to exhale the excess gas. In situations where safe decompression ascent profiles are not followed, the additional gas enters a supersaturated state that can result in bubbles forming in the vasculature and tissue upon rapid ascent. These bubbles cause a host of pathological endpoints, including inflammatory cascades, occlusive/ischemic phenomenon, and mass/compressive effects that are collectively known as decompression sickness (DCS) (1, 16).

Breathing 100% oxygen at depth is one method to rapidly reduce the decompression obligation and DCS risk via the concept known as isobaric denitrogenation (1). However, hyperbaric oxygen (HBO) breathing is limited by the risk for oxygen toxicity that can manifest in both the central nervous system (CNS) and pulmonary system. For example, a period as brief as 10 min of HBO prior to rapid decompression from saturation (a state where the body can absorb no additional gas at a given pressure) at 5 ATA decreased DCS mortality by 54% in 20-kg swine (14). However, seizure activity, presumably related to CNS oxygen toxicity, was noted in 44% of the animals. This unacceptably high seizure risk illustrates the limitations of this otherwise beneficial decompression strategy in emergency situations.

To extend the benefits of HBO to deeper operational depths, a successful seizure mitigation strategy must be implemented. In Sprague-Dawley rats exposed to HBO at 5 ATA Tzuk-Shina et al. (17) demonstrated that vigabatrin significantly increased seizure latency at all doses studied (50, 180, and 250 mg/kg). In fact, 180 mg/kg vigabatrin prevented seizures completely at 5 ATA during a 50-min experimental period.

Vigabatrin is a selective irreversible antagonist of the GABA transaminase (GABA-T) enzyme. It is indicated as mono-therapy for infantile spasm and adjunct therapy for highly refractory complex partial seizures (18). Vigabatrin use is limited clinically by the occurrence of progressive peripheral retinopathy associated with chronic use longer than 3 mo (4, 10). Vigabatrin use in conjunction with HBO (which is associated with acute retinal impairment) has not been assessed, to our knowledge, with regards to retinal health from a structural or functional standpoint (2).

This study investigated the efficacy of vigabatrin to prevent HBO-induced seizures in a 20-kg swine model. Further investigations were made to determine the potential secondary mechanisms of action underlying vigabatrin’s efficacy and to determine the effects of acute vigabatrin therapy coupled with hyperbaric oxygen on retinal health.

Materials and Methods

The methods reported here were conducted according to the principles set forth in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 2011). Before commencing, our Institutional Animal Care and Use Committee reviewed and approved all aspects of this protocol. The institutional animal care facility is fully AAALAC accredited, and the veterinary staff is familiar with our swine model for DCS.

Animals. Thirty-three male Yorkshire swine (25.34 ± 2.1 kg) were housed in free-running cages at the animal care facility on site for 5 days before any procedures. They were fed 2–2.5% of body weight twice daily (Lab Diet Mini-Pig Grower, Quality Lab Products, Elkridge, MA) and water was provided ad libitum.

Surgical preparation. Ear vein catheter placement was conducted 1 day prior to hyperbaric exposure to allow for full recovery from anesthesia (intramuscular ketamine/xylazine, 20 mg/kg Ketaject and 2 mg/kg Xyla-Ject, respectively; Phoenix Pharmaceutical, St. Joseph, MO). Full ambulation after recovery was verified before return to the holding pen where the animals remained overnight.

Infusion preparation. Vigabatrin was custom synthesized (Cayman Chemical, Ann Arbor, MI), dissolved in normal saline solution (0.9%...
NaCl, Hospira, Lake Forest IL), and filter sterilized using a 0.22-µm filter prior to infusion.  

**Predive preparation.** On the day of hyperbaric exposure the animals were placed in a Panepinto sling, fitted with electroencephalogram (EEG) electrodes, and randomized to receive either vigabatrin (50, 180, or 250 mg/kg in 50 ml/kg saline) or normal saline (50 ml/kg) 4 h prior to HBO administration. The 4-h time interval between dosing and hyperbaric exposure was based on the experimental setup described by Tzuk-Shina et al. (17) and reflects the intraperitoneal administration route used. Although the intravenous route used in this study leads to a more rapid distribution of vigabatrin, its efficacy is in part due to the accumulation of GABA. Therefore, to remove the potential confound of less time for GABA to accumulate, the 4-h time interval was maintained.  

**EEG electrode placement.** By using a hand clipper and electric shaver, an area was prepared over the frontal and parietal bones, and the exposed skin was rubbed with isopropyl alcohol. Disposable prewired (Grass Technologies/Astro-Med, Warwick, MA) or SF405 (Kendall/Medi-Trace, Tyco Healthcare, Mansfield, MA) self-adhesive Ag/AgCl electrodes were placed over the parietal bones bilaterally (bipolar derivation), close to the lateral edges, between the ears and eyes. A ground electrode was positioned on the frontal bone, centered between the eyes.

**ECG electrode placement.** Preparation and electrodes were identical to that described for EEG. Placement was bilaterally, behind the shoulder, lateral to the vertebrae ~4–7 cm above the ribs 5–7 (bipolar derivation) and the ground electrode ~2 cm lateral to the vertebrae on right side, between ribs 12 and 13.

**Diving chamber.** The diving chamber is a 1,260-liter hatched cylinder (Bethlehem Steel, Bethlehem, PA) with hull penetrations for electrical wires and gas inflow/outflow; it is equipped with O₂ delivery capability and temperature and humidity sensors, as well as a platform for insertion of a cart/sling for holding the swine. Pressure/depth was monitored by a CPG 2500 Digital Pressure Gauge (Mensor, San Marcos, TX) and recorded together with EEG and ECG. Animals were fitted with a snout cone mask (Smith Medical North America, San Marcos, TX) and recorded together with EEG and ECG. After the animal was positioned inside, the chamber was sealed and pressurized using air at the rate of 30 feet/minute. If animals expired due to pulmonary oxygen toxicity, the chamber was decompressed at 30 feet/minute. Upon reaching surface the hatch was opened and the animal was allowed to recover for 30 min in the chamber with 100% O₂ and maintained until behavioral signs of a seizure were observed (tonic-clonic muscle contraction) and epileptiform EEG activity was evident. At seizure onset the inspired gas was switched back to air for 3 min, then the chamber was decompressed at 30 feet/minute. If animals expired due to pulmonary oxygen toxicity, the chamber was decompressed at 30 feet/minute and latency was recorded to the final respiration. Upon reaching surface the hatch was opened and the animal was allowed to recover for 30 min in the chamber with the door opened. Electroretinogram (ERG) recordings were then performed in situ within the chamber.

**EEG and ECG recordings.** Wideband AC amplifiers (Grass, Quincy, MA) were used for EEG and ECG recordings; a 0.5- to 35-Hz and 60-cycle notch filter setting was used. The EEG signal was collected by a Dell PC (Round Rock, TX) and an analog-digital converter card (PCI-6052E, National Instruments, Austin, TX) with 16-bit resolution and 200-Hz sampling rate. DataLyser software was used to record the data.

**ERG recordings.** A full-field ERG was constructed in accordance with approved international standards (3) consisting of the following components: 1) Grass PS33 Plus Photic Stimulator with single stimuli-
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Fig. 1. Vigabatrin dose dependently increases seizure latency in swine at 6 ATA hyperbaric oxygen (HBO). Bars represent mean ± SE latency to seizure in swine (n = 3 per group) treated with saline or vigabatrin at doses of 50 mg/kg, 180 mg/kg, and 250 mg/kg, 4 h prior to 6 ATA HBO exposure. The 250 mg/kg bar represents mean ± SE latency to mortality; these animals did not exhibit signs of seizure during the entire hyperbaric exposure. Seizure was assessed by EEG with visual confirmation. *Significance (P < 0.05) from saline-treated animals; #significance (P < 0.05) from 180 mg/kg treated animals as determined by one-way ANOVA with post hoc Bonferroni’s test. x-Axis represents dose of vigabatrin in mg/kg, y-axis represents seizure latency in minutes.

R-wave). The time interval between peaks was referred to as the NN interval. The result is given as a frequency distribution histogram, peak-to-peak graph as well as numbers for the following values: 1) number of peaks found, 2) mean, 3) SD, 4) median, 5) minimum, and 6) maximum. To characterize HRV, we used SD values of each group. To achieve this, we made a reference analysis, using an ECG simulator (214A patient simulator, Dynatech Nevada, Carson City, NV) with fixed frequency of 80 beats/min, 0.5 mV. The length of measured periods were 1) 114.995 s; peak-to-peak distance 0.750 ± 0.0004 s; 153 peaks; FFT 1.329 Hz = 79.74 beats/min; and 2) 326.52 s; peak-to-peak distance 0.750 ± 0.0003; 434 peaks; FFT 1.332 Hz = 79.92 beats/min.

Statistical analysis. All data were compiled into Excel spreadsheets then imported into Graphpad Prism 5 for statistical analysis. Seizure latency, HRV, and ERG results were compared using one-way ANOVA with post hoc Bonferroni’s test. Two-way ANOVA with post hoc Bonferroni’s test was used to compare seizure latency between animals subjected to 5 vs. 6 ATA. A power analysis for sample size determination for the efficacy studies was conducted based on seizure latency in saline-treated historical controls exposed to 6 ATA HBO.

RESULTS

Vigabatrin dose dependently increases seizure latency in swine exposed to 6 ATA HBO. Vigabatrin was previously shown to increase seizure latency in rats exposed to 5 ATA HBO. To compare the efficacy of vigabatrin in a large animal model of HBO exposure swine (n = 3 per group) were treated at doses of 50, 180, and 250 mg/kg and compared with saline controls. Swine were pressurized to 6 ATA breathing air then switched to >90% oxygen. Seizure was assessed by EEG with visual confirmation. Vigabatrin pretreatment significantly increased seizure latency in the 180 mg/kg (13.30 ± 1.3 min, P < 0.05) and 250 mg/kg (39.00 ± 3.1 min, P < 0.05) groups, but not the 50 mg/kg group (8.61 ± 1.2 min, P > 0.05) compared with saline controls (8.09 ± 0.4 min; Fig. 1). The latency time reported in the 250 mg/kg group was latency to mortality; no seizures were observed with this group. Postmortem analysis of these animals determined that death was due to

Fig. 2. Vigabatrin increases seizure latency depending on depth of HBO. Seizure latency is known to vary based on HBO depth. To determine whether vigabatrin efficacy on seizure latency was similarly depth dependent, swine were randomized to receive saline infusion (vehicle control) (n = 5), 50 mg/kg vigabatrin (n = 3), or 180 mg/kg vigabatrin (n = 3). A significant difference was observed (P = 0.0042) between the seizure latencies in swine pressurized to 5 ATA compared with swine pressurized to 6 ATA at different doses of vigabatrin as determined by 2-way ANOVA. x-Axis represents dose of vigabatrin in mg/kg, y-axis represents seizure latency in minutes.

Fig. 3. Vigabatrin administration induces a dose-dependent increase in heart rate variability (HRV) in swine prior to dive. A: mean standard deviation of the NN interval following administration of saline (0), 50, 180, or 250 mg/kg vigabatrin 4 h prior to diving. *Significance (P < 0.05) from saline (0) control as determined by one-way ANOVA with post hoc Bonferroni’s test. B: mean NN interval following administration of saline (0), 50, 180, or 250 mg/kg vigabatrin (n = 8, 5, 3, and 4, respectively) 4 h prior to diving. There was no significant difference in the mean NN interval observed between groups (P = 0.431) as determined by one-way ANOVA with post hoc Bonferroni’s test. x-Axis represents dose of vigabatrin in mg/kg, y-axis represents the standard deviation of the NN interval in seconds (A) or the NN interval in seconds (B).
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Fig. 4. Vigabatrin treatment showed no histological evidence of retinal toxicity. Histological examination (structural examination) assessed retina integrity in swine subjected to HBO and vigabatrin at various doses. Representative photomicrographs of the retinas from swine exposed to saline (A) or vigabatrin at a 50 mg/kg (B), 180 mg/kg (C), or 250 mg/kg (D) concentration in conjunction with HBO. *, outer nuclear layer; †, photoreceptor layer; #, ganglion cell layer.
ECG waveforms demonstrated a dose-dependent increase in parasympathetic tone that partially mediated the efficacy observed. Histological and functional analysis of the retina showed no significant changes associated with a single high dose of vigabatrin in conjunction with hyperbaric oxygen.

There is a paucity of effective clinical treatments for seizures evoked by HBO exposure. Although several therapies increase seizure latency in rodents, there are no reports in the literature where these findings have been replicated in a clinically relevant large animal model or human. Vigabatrin, an irreversible GABA-T inhibitor was shown to significantly increase seizure latency in rats exposed to 5 ATA hyperbaric oxygen (17). The primary endpoint of the current study was to validate these findings in the 20-kg swine. Our finding that vigabatrin significantly increased latency is, to our knowledge, the first report of its efficacy in a large animal model of HBO-induced seizure.

Interestingly, although vigabatrin did increase seizure latency in the swine, higher doses were required (180 and 250 mg/kg) than those used in the rat study (50 mg/kg) or in clinical use (~45 mg/kg). GABA-T enzyme expression is not restricted to the CNS, demonstrating particularly high expression in the visceral organs such as the liver (12). This peripheral GABA-T may act as a larger sink because the swine has a larger liver-to-brain ratio than the rat, requiring more vigabatrin to increase latency. Clinically, lower doses of vigabatrin are used to control seizure in patients with infantile spasm or complex partial seizure. However, human GABA-T is more sensitive to vigabatrin than GABA-T from other species, a finding that likely explains the dosing discrepancy seen in this study (12).

The latency to seizure after HBO exposure is highly depth dependent. In this study seizure latency in the vigabatrin-treated animals at the 180 mg/kg dose was significantly reduced when the exposure depth to HBO was increased from 5 to 6 ATA, although brain GABA-T and GABA levels should have been equivalent because of identical pre-dive treatment. GABA pools have been shown to be depleted in the brains of rats exposed to HBO (9). Furthermore, this depletion has been described to occur in a gradual manner during the HBO exposure and is dependent on nitric oxide (NO) signaling (7). Therefore, if NO-dependent GABA depletion occurred at an increased rate in the animals exposed to 6 ATA O2 compared with 5 ATA O2 this would likely explain the difference in vigabatrin efficacy seen between these two groups.

Vigabatrin administration inhibits GABA-T systemically, leading to a global increase in GABA. Direct injection of

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**Fig. 5.** Vigabatrin treatment caused no functional evidence of retinal toxicity. Electroretinograms (ERGs) clinically monitor retinal health in patients who use vigabatrin for seizure management. ERG changes precede vision field defects in vigabatrin patients and were used to assess swine treated with saline (n = 6) or vigabatrin at 50 mg/kg (n = 4) and 250 mg/kg (n = 4) doses within 1 h of surfacing. A: ERG waveform conducted in the swine annotated to demonstrate typical a-wave and b-wave amplitude and latency measurements. B: mean (±SE) a-wave amplitude for swine treated with saline (0), 50, or 250 mg/kg vigabatrin. C: mean (±SE) a-wave latency for swine treated with saline (0), 50, or 250 mg/kg vigabatrin. D: mean (±SE) b-wave amplitude for swine treated with saline (0), 50, or 250 mg/kg vigabatrin. E: mean (±SE) b-wave latency for swine treated with saline (0), 50, or 250 mg/kg vigabatrin. One-way ANOVA with post hoc Bonferroni’s test compared a-wave and b-wave amplitude and latency. No significant differences were observed. For A, x-axis represents time in ms, y-axis represents amplitude in μV. For B–E, x-axis represents dose of vigabatrin in mg/kg, and y-axis represents peak amplitude in μV (B, D) or time to peak in ms (C, E).
GABA intraperitoneally is associated with increased seizure latency in mice exposed to HBO (9). In these same experiments it was demonstrated that the blood-brain barrier does not open during HBO-induced seizure, suggesting that peripheral GABA may play an important role in HBO-induced seizure. Peripheral GABAergic signaling has also been associated with parasympathetic (PNS) tone in a variety of mammalian species. This is supported by HRV analysis (an index of autonomic tone) of predive ECG recordings that demonstrated a dose-dependent increase in HRV indicative of increased PNS tone. Determining the relative contributions of peripheral vs. central GABA signaling on HBO-induced seizures is outside of the scope of the current study; however, from a practical standpoint the HRV finding provides a potentially noninvasive method to gauge vigabatrin dosing efficacy prior to HBO exposure. HRV as a surrogate marker of vigabatrin efficacy would be extremely useful because of the species differences in efficacy observed in this study.

The greatest contraindication to clinical vigabatrin use is the development of a progressive retinotoxicity that begins at the periphery and expands inward causing tunnel vision. Multiple electroretinographic findings have been reported in a variety of species (humans, rabbits, and rats) given chronic doses of vigabatrin. The most common finding is reduced photopic (light adapted) b-wave amplitude (13). Others less frequent, include oscillatory potential decrease, reduction in response to 30-Hz flicker, and reduction in scotopic (dark adapted) b-wave amplitude. Light microscopic changes in the retina include disruption of the outer nuclear layer with displacement of these nuclei into the photoreceptor layer, loss of ganglion cells in the ganglion cell layer initially at the periphery (5, 8, 11, 15), and atrophy of the retina in general. However, these functional and histological changes have been associated only with chronic use (>3 mo) of vigabatrin and have never been assessed when vigabatrin therapy is used in conjunction with HBO. To our knowledge this is the first report describing the effects of a single dose of vigabatrin on swine retinal structure and function. On the basis of the ERG and histology findings in this study, primary retinopathy does not occur as a consequence of a single large dose of vigabatrin in conjunction with HBO. This conclusion is largely in agreement with clinical findings describing retinopathy as a side effect associated with vigabatrin and have never been assessed when vigabatrin is used in conjunction with HBO. This conclusion is largely in agreement with clinical findings describing retinopathy as a side effect associated with vigabatrin and have never been assessed when vigabatrin is used in conjunction with HBO. To our knowledge this is the first report describing the effects of a single dose of vigabatrin on swine retinal structure and function. On the basis of the ERG and histology findings in this study, primary retinopathy does not occur as a consequence of a single large dose of vigabatrin in conjunction with HBO.

In summary, a single dose of vigabatrin delivered 4 h prior to HBO exposure provided protection from CNS oxygen toxicity in swine, albeit at higher concentrations than previously described in rats. In addition, no animals exposed to vigabatrin demonstrated acute functional or histological evidence of retinal toxicity. Vigabatrin treatment was also associated with an increase in parasympathetic tone that served as a useful marker of vigabatrin efficacy. Taken together, these findings suggest that vigabatrin is a promising candidate for the treatment of CNS oxygen toxicity.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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