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Research report

Antiepileptic drugs prevent seizures in hyperbaric oxygen: A novel model of epileptiform activity

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ABSTRACT

Breathing oxygen at sufficiently elevated pressures can trigger epileptiform seizures. Therefore, we tested the hypothesis that pre-treatment with FDA-approved antiepileptic drugs could prevent seizure onset in hyperoxia at 5 atmospheres absolute. We selected drugs from two putative functional categories, Na^+-channel antagonists and GABA enhancers, each administered intraperitoneally at four doses in separate groups of C57BL/6 mice. The drugs varied in efficacy at the doses used. Of the five tested Na^+-channel antagonists, carbamazepine and lamotrigine more than tripled seizure latency compared to values seen in vehicle controls. Primidone, zonisamide and oxcarbazepine were less effective. Of the four GABA reuptake inhibitors, tiagabine and vigabatrin also increased seizure latency by more than three times control values; valproic acid was less effective, and the GABA synthesis promoter gabapentin was intermediate in effectiveness. We infer that Na^+-channel function and GABA neurotransmission may be critical targets in the pathophysiology of CNS O_2 toxicity. Because these essential components of neuronal excitation and inhibition are also implicated in the pathogenesis of other seizure disorders, including generalized epilepsy, we propose that, at some level, common pathways are involved in these pathologies, although the initiating insults differ. Furthermore, hyperoxic exposures are not known to cause the spontaneously-recurring seizures that characterize true clinical epilepsy. Nonetheless, experimental studies of hyperbaric oxygen toxicity could provide new insights into molecular mechanisms of seizure disorders of various etiologies. In addition, the neuropathology of hyperbaric oxygen is particularly relevant to the hypothesis held by some investigators that oxidative stress is an etiological factor in clinical epilepsies.

1. Introduction

Oxygen is a convulsant when breathed for a sufficient time at partial pressures of 2.5 atmospheres absolute (ATA) or above, and latency to onset of epileptiform patterns on the EEG and tonic-clonic neuromotor responses is inversely proportional to inspired Po_2. This has been well established in human and animal studies that have been conducted for many years, primarily to devise procedures for protecting personnel exposed to hyperbaric oxygen (HBO_2) in certain occupational and therapeutic environments (Balentine, 1982; Behnke et al., 1935; Clark and Thom, 2003; Donald, 1947a,b; Paton, 1967). Vulnerable groups include military and civilian divers, crew attempting escape from a disabled submarine and patients undergoing hyperbaric oxygen therapy.

Although many drugs have been developed to prevent seizures of various etiologies, particularly those of broad clinical relevance, none have been specifically formulated to protect against seizures in HBO_2, and it is unlikely that pharmaceutical companies would undertake the research needed to do so, since the human population at risk is small. Up to now, the only reliable methods

Abbreviations: HBO_2, hyperbaric oxygen; ATA, atmospheres absolute; AED, antiepileptic drug; CBZ, carbamazepine; GBP, gabapentin; LTG, lamotrigine; OXC, oxcarbazepine; PRM, primidone; TGB, tiagabine; VGB, vigabatrin; VPA, valproic acid; ZNS, zonisamide.

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for avoiding neurotoxicity in HBO_2 have been to limit the dose and duration of hyperbaric oxygenation.

Although the events or conditions that initiate seizures in hyperbaric oxygen and in other seizure disorders may differ, the final common pathways and ultimate molecular targets may be similar. Indeed, some investigators have proposed that oxidative stress plays a contributory role in epilepsy (Patel, 2004; Pearson et al., 2015; Zsurka and Kunz, 2015). We reasoned, therefore, that antiepileptic drugs (AEDs) could prevent or delay seizures of CNS O_2 toxicity and as a corollary, AEDs with known mechanisms-of-action might be used as investigative probes to further elucidate events that evoke hyperoxic seizures, although the true mechanisms of action of some of these drugs are not completely understood and may not fit neatly into their nominal functional classes. Furthermore, if a broad range of AEDs are found to prevent or delay seizures in HBO_2, and if there are parallels between the ictal events in HBO_2 and those seen in other seizure disorders, the hyperbaric model could elucidate mechanisms of various seizure-related disorders, including those due to the acquired and idiopathic epilepsies, traumatic brain injury and other causes. However, it must be understood that most animal models used in epilepsy research only simulate the seizures of epilepsy rather than epilepsy itself (Loscher, 2011), and this would apply to HBO_2 as well.

In this study we assessed the ability of nine FDA-approved AEDs, administered individually, to increase seizure latency in mice exposed to 100% oxygen at 5 ATA for 60 min. This level and duration of hyperoxia reliably elicits seizures in this species in a reasonable period of time (60 min or less) without producing a significant degree of direct cardiopulmonary injury (Demchenko et al., 2007).

Since abnormal propagation of excitatory neurotransmission and attenuation of inhibitory neurotransmission are established pathogenic factors in clinical epilepsies (Jefferys, 2010) and are also assumed to be factors in CNS O_2 toxicity (Colton and Colton, 1982; Dean et al., 2003; Demchenko and Piantadosi, 2006), we chose to evaluate the protective efficacy in HBO_2 of AEDs from two functional classes relevant to excitatory and inhibitory functions: sodium-channel antagonists and GABA transmission enhancers. We compared our findings with published values for the efficacy of the same AEDs in a well-established animal model of epileptic seizures, maximal electroshock (MES).

2. Results

2.1. Seizure activity in vehicle-treated mice

The 24 mice treated with vehicle (0.9% NaCl or DMSO) and exposed to HBO_2 at 5 ATA exhibited neuromotor responses that progressed in 2 or 3 stages, comparable to those of the Racine Scale (Racine, 1972). In stage I, restlessness, intensive grooming, slight tremors, twitching vibrissae and transient muscular spasms were observed. In stage II, persistent, rhythmic spasms appeared in the face and body along with bilateral forelimb clonus and escape behaviors. Stage III, if it occurred, was characterized by generalized tonic-clonic convulsions. Stages I and II were seen in 23 (>95%) of the vehicle-treated mice; one mouse was unresponsive. All three stages occurred in 17 (>70%) of these animals. In the remaining 7 (29%) vehicle-treated mice, stage III was not seen; instead, the signs of stage II suddenly disappeared and behavioral arrest was observed for the remainder of the 60-min exposure. For the purpose of evaluating the efficacy of AEDs, we defined the termination of seizure latency to coincide with either the first signs of stage II or with behavioral arrest, either of which would incapacitate human subjects and therefore mark the limit of safe exposure to HBO_2.

2.2. Seizure activity in AED-treated mice

Each of the tested AEDs delayed the onset of CNS O_2 toxicity in a dose-dependent fashion (Table 1, Figs. 1 and 2). However, almost all of these animals exhibited either signs of stage II or behavioral arrest at some point during a 60-min exposure. Stage III was observed in 47% of these animals, mostly those treated with lower doses. Of the Na-channel antagonists, carbamazepine (CBZ) and lamotrigine (LTG) were the most effective, increasing seizure latency by more than three times that observed in animals treated with vehicle; whereas primidone (PRM), zonisamide (ZNS) and oxcarbazepine (OXC) were least effective, and at the lowest doses used they failed to increase seizure latency significantly ($P \leqslant 0.05$), compared to vehicle controls.

Of the GABA transmission enhancers, the reuptake inhibitors tiagabine (TGB) and vigabatrin (VGB) were the most effective, increasing seizure latency by more than three times compared to vehicle controls, when administered at the highest doses. Valproic acid (VPA) was the least effective. The GABA-synthesis promoter gabapentin (GBP) was intermediate in effectiveness. Only VGB provided significant increases in seizure latency at the lowest dose.

2.3. Equally-effective doses

To provide a uniform standard for evaluating efficacy, we used linear regression analysis ($R^2 > 0.95$ in all cases) to predict an equally-effective dose (EQD) for each AED that would increase seizure latency by a fixed multiple of the mean latency observed in the corresponding vehicle controls (Fig. 3A and C). Because the Na^+-channel antagonists LTG and CBZ and the GABA enhancers VGB and TGB each increased latency by more than a factor of 3, we selected EQD_{3x} as a convenient standard for comparison. For the other 5 drugs, this parameter was estimated by extrapolation along the calculated lines of best fit. This procedure was validated by comparison with ED_{50}'s obtained using log Probit Analysis (Litchfield and Wilcoxon, 1949). Since the latter method assumes all-or-none responses, latency data from the first 40 min of each hyperbaric exposure were used, an interval in which some animals exhibited seizure activity and some did not. For each drug, we found good qualitative agreement between the EQD_{3x} and the ED_{50}.

3. Discussion

We have demonstrated that a range of FDA-approved AEDs, of two functional classes, can significantly delay seizure onset in extreme hyperoxia. Although other investigators have tested the protective efficacy of two such drugs in HBO_2, CBZ and VGB (Bitterman and Halpern, 1995; Hall et al., 2013; Harel et al., 1978; Reshef et al., 1991; Tzuk-Shina et al., 1991), we know of no study in which the anticonvulsant efficacies of multiple AEDs were compared in hyperbaric hyperoxia.

Among the Na^+-channel antagonists we tested, CBZ and LTG were the most effective in increasing seizure latency and exhibited the highest potencies, as shown in Fig. 3A. Clinically, CBZ is considered "first line anticonvulsant therapy for generalized tonic-clonic and partial seizures" (Reinikainen et al., 1987), whereas LTG is used as initial therapy for focal epilepsy, idiopathic generalized epilepsy and absence seizures in children (Biton et al., 2005; Coppola et al., 2004). Both of these drugs impose use-dependent blocks on neuronal sodium channels during rapid, repetitive, sustained firing (Clemens et al., 2007; Goldenberg, 2010; Vreugdenhil and Wadman, 1999). LTG may also inhibit neuronal synthesis of glutamate and aspartate, diminishing the release of these excitatory neurotransmitters (Lee et al., 2008).

The remaining Na^+-channel antagonists, OXC, ZNS, and PRM, were less effective in HBO_2 but still doubled seizure latency when