Neurotoxicity Research (2019) 36:788–795
https://doi.org/10.1007/s12640-019-00063-5

ORIGINAL ARTICLE

Increased Antiseizure Effectiveness with Tiagabine Combined with Sodium Channel Antagonists in Mice Exposed to Hyperbaric Oxygen

Ivan T. Demchenko[1,2] · Sergei Yu Zhilyaev[2] · Olga S. Alekseeva[2] · Alexander I. Krivchenko[2] · Claude A. Piantadosi[1,3,4] · Heath G. Gasier[5]

Received: 6 February 2019 / Revised: 24 April 2019 / Accepted: 9 May 2019 / Published online: 30 May 2019

Abstract

Hyperbaric oxygen (HBO_2) is acutely toxic to the central nervous system, culminating in EEG spikes and tonic-clonic convulsions. GABA enhancers and sodium channel antagonists improve seizure latencies in HBO_2 when administered individually, while combining antiepileptic drugs from different functional classes can provide greater seizure latency. We examined the combined effectiveness of GABA enhancers (tiagabine and gabapentin) with sodium channel antagonists (carbamazepine and lamotrigine) in delaying HBO_2-induced seizures. A series of experiments in C57BL/6 mice exposed to 100% oxygen at 5 atmospheres absolute (ATA) were performed. We predicted equally effective doses from individual drug-dose response curves, and the combinations of tiagabine + carbamazepine or lamotrigine were tested to determine the maximally effective combined doses to be used in subsequent experiments designed to identify the type of pharmacodynamic interaction for three fixed-ratio combinations (1:3, 1:1, and 3:1) using isobolographic analysis. For both combinations, the maximally effective combined doses increased seizure latency over controls > 5-fold and were determined to interact synergistically for fixed ratios 1:1 and 3:1, additive for 1:3. These results led us to explore whether the benefits of these drug combinations could be extended to the lungs, since a centrally mediated mechanism is believed to mediate hyperoxic-induced cardiogenic lung injury. Indeed, both combinations attenuated bronchoalveolar lavage protein content by ~ 50%. Combining tiagabine with carbamazepine or lamotrigine not only affords greater antiseizure protection in HBO_2 but also allows for lower doses to be used, minimizing side effects, and attenuating acute lung injury.

Keywords Antiepileptic drugs · CNS oxygen toxicity · Drug synergy · Isobolographic analysis · Neuroprotection

Abbreviations

HBO_2	Hyperbaric oxygen
ATA	Atmospheres absolute
AEDs	Antiepileptic drugs
CBZ	Carbamazepine
LTG	Lamotrigine
TGB	Tiagabine
GBP	Gabapentin

✉ Heath G. Gasier
heath.gasier@usuhs.edu

[1] Center for Hyperbaric Medicine and Environmental Physiology, Duke University Medical Center, Durham, NC, USA

[2] Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg, Russia

[3] Department of Anesthesiology, Duke University Medical Center, Durham, NC, USA

[4] Department of Medicine, Duke University Medical Center, Durham, NC, USA

[5] Department of Military and Emergency Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD, USA

Introduction

Breathing oxygen at partial pressures ≥ 2.0 atmospheres absolute (ATA) is common in hyperbaric oxygen (HBO_2) therapy and possible during diving, but can become acutely toxic to the central nervous system (CNS oxygen toxicity) culminating in tonic-clonic seizures (Bert 1943; Demchenko et al. 2007). The appearance of hypersynchronous high-amplitude (slow waves) discharges from electroencephalogram (EEG) recordings in HBO_2 suggests there are similarities between seizures

induced by HBO_2 and epilepsy (Cohn and Gersh 1945; Harel et al. 1969; Rucci et al. 1967; Stein and Sonnenschein 1950; Voronov 1964). Since partial seizures can spread and produce generalized tonic-clonic seizures (Goldenberg 2010), and because subcortical and/or cortical EEG profiles have been inconsistent within and between studies (Harel et al. 1969; Rucci et al. 1967; Sonnenschein and Stein 1953), the origin of seizures caused by HBO_2 remains unknown. Nonetheless, antiepileptic drugs (AEDs) used in the management of clinical epilepsy should afford similar antiseizure protection in HBO_2 allowing for the oxygen dose to be safely increased in patients being treated with HBO_2, in divers, and in the event that a submarine becomes disabled and crewmembers are exposed to increased atmospheric, and oxygen, pressure.

In an earlier study, our team explored the pretreatment of mice with AEDs to extend seizure latencies in extreme HBO_2 (5 ATA) (Demchenko et al. 2017). Studied were γ-aminobutyric acid (GABA) enhancers (tiagabine, gabapentin, vigabatrin, and valproic acid) and sodium channel blockers (carbamazepine, lamotrigine, primidone, zonisamide, and oxcarbazepine). GABA enhancers were tested because HBO_2 leads to a reduction in brain GABA and glutamic acid decarboxylase (GAD) activity (Gasier et al. 2017; Wood and Watson 1963; Wood et al. 1967), and decreased GABA levels are positively associated with seizure latency (Wood et al. 1969). Moreover, vigabatrin protects against oxygen-induced seizures in rats (Tzuk-Shina et al. 1991) and swine (Hall et al. 2013). High-frequency and repetitive firing of action potentials at seizure onset in HBO_2 suggests that sodium channel blockade may also protect against oxygen-induced seizures. Support for this stems from reported seizure protection in rats pretreated with carbamazepine and exposed to HBO_2 at 5 ATA (Reshef et al. 1991), and from two case reports describing efficacy of phenytoin treatment of focal status epilepticus induced by HBO_2 therapy in a patient (Seckin et al. 2011), and for prevention of generalized seizures in a 27-year-old male being treated with HBO_2 for cerebral gas embolism (Weaver 1983). Because multiple AED therapy is used for optimal seizure control in epileptic patients (Czuczwar and Borowicz 2002), combining AEDs may also enhance seizure protection in HBO_2.

The purpose of this research was to examine the antiseizure effectiveness of combining GABA enhancers with sodium channel antagonists in mice exposed to HBO_2 at 5 ATA. By employing effect-based and dose-effect-based analytical approaches, we determined whether the combination of AEDs from different functional classes affords greater antiseizure protection than individual AEDs, and if the pharmacodynamic interactions between two AEDs were greater (synergy), equal (additivity), or less (antagonism) effective than the effects of each alone (Berenbaum 1977; Foucquier and Guedj 2015). A secondary objective was to determine whether the most effective AED combinations can also attenuate acute lung injury, since CNS oxygen toxicity and acute cardiopulmonary damage are linked (Demchenko et al. 2011).

Materials and Methods

Animals

Experiments were performed on conscious C57BL6 mice (19–25 g) at the Center for Hyperbaric Medicine and Environmental Physiology, Duke University Medical Center (Durham, NC, USA), and at the Institute of Evolutionary Physiology and Biochemistry, Russian Academy Sciences (St. Petersburg, Russia), ~ half at each facility. All animal procedures were approved independently by the Institutional Animal Care and Use Committee of Duke University and the Ethical Review Board of the Institute of Evolutionary Physiology and Biochemistry. AEDs, pretreatment time, hyperbaric exposures, experimental design, assessments of animal responses, and statistical methods were similar at both institutions.

Experimental Design

In order to establish dose-response curves, an initial set of experiments were conducted in mice (373 mice; $n = 10–12$/group ×34) pretreated with either carbamazepine, lamotrigine, tiagabine, or gabapentin 60 min prior to HBO_2 exposures. For each drug, 8–9 doses were considered for testing based upon the drugs pharmacokinetics, our previous observations (Demchenko et al. 2017), and whether drug doses produced adverse effects prior to HBO_2. In a second set of experiments and in order to determine the combined effectiveness of AEDs, 66 mice ($n = 8$/group ×8) were pretreated with different dosing strategies of either tiagabine + carbamazepine or tiagabine + lamotrigine and exposed to HBO_2 for 90 min. These AED combinations were selected based on the results obtained from experiment 1, and because AEDs with different mechanisms of action are believed to have the greatest potential for interacting synergistically (Deckers et al. 2000). In a third set of experiments, 33 mice ($n = 5–6$/group ×6) were used to determine the type of interaction in HBO_2 using isobolographic analyses (see below). In a final set of experiments, 4 groups of mice ($n = 8$/group) were used to assess whether pretreatment with the most effective combined doses of tiagabine + carbamazepine or tiagabine + lamotrigine attenuated acute lung injury in mice exposed to HBO_2 for 90 min.

In the dose-response experiments, carbamazepine and lamotrigine were dissolved in DMSO and tiagabine and gabapentin were dissolved in 0.9% NaCl. In experiments two-four, tiagabine + carbamazepine or tiagabine + lamotrigine were dissolved in 0.9% NaCl + 10% DMSO. Control mice received the combined vehicle. All

Table 1 Effects of AEDs in various doses on seizure latency compared with vehicle in HBO_2

AED trade name (vehicle)	AED dose (mg kg^{-1})	Mice (*n*)	Mean seizure latency (min ± SEM)	Motor impairment (%)
0.9% NaCl +10% DMSO		12	13.5 ± 1.6	0
Carbamazepine (CBZ)	1	10	15.3 ± 2.3	0
Tegetrol®	5	12	22.4 ± 2.7*	0
	7	10	33.6 ± 3.4*	0
FDA approved in 1968	10	12	34.8 ± 3.2*	0
	12	10	40.1 ± 4.1*	0
Sodium channel blocker	15	12	43.8 ± 4.4*	0
	20	10	44.9 ± 4.3*	10
	25	12	45.5 ± 4.6*	20
	50	10	Not tested	80
Lamotrigine (LTG)	1	10	16.1 ± 2.8	0
Lamictal®	2	12	28.2 ± 3.3*	0
	5	12	35.7 ± 4.2*	0
FDA approved in 1994	10	12	41.0 ± 3.4*	0
	15	10	42.8 ± 4.1*	0
Sodium channel blocker	20	12	44.0 ± 4.1*	0
	25	10	44.8 ± 4.9*	10
	40	10	Not tested	70
Tiagabine (TGB)	0.5	10	15.6 ± 2.2	0
Gabatril®	1	12	22.1 ± 3.9*	0
	2	12	31.8 ± 4.6*	0
FDA approved in 1997	4	10	37.7 ± 3.9*	0
	6	12	43.6 ± 3.8*	0
GABAergic	9	12	46.9 ± 3.3*	0
	12	10	49.1 ± 4.7*	20
	20	10	Not tested	60
Gabapentin (GBP)	10	12	16.8 ± 2.5	0
Neurotonin®	20	12	20.3 ± 2.7*	0
	30	12	25.3 ± 4.7*	0
FDA approved in 1993	50	12	28.3 ± 3.7*	0
	100	10	32.0 ± 3.2*	0
GABAergic	300	10	37.3 ± 4.2*	0
	500	10	39.7 ± 4.9*	20
	1000	10	Not tested	70

Vehicle or AEDs were administered (IP) 60 min before exposing conscious mice to HBO_2 at 5 ATA for 60 min. Adverse side effects to AEDs were determined at 1 ATA (air breathing) using the rotarod test. If > 20% of mice exhibited altered motor coordination or balance, the dose was not tested in HBO_2. *$p < 0.05$ vs. vehicle

drugs and were administered intraperitoneally in a volume of 0.005 mL · g body $weight^{-1}$.

HBO_2 Exposures

Four to six freely-moving mice per group were placed in separate cages in a hyperbaric chamber and compressed to 5 ATA O_2 at 0.7 ATA min^{-1}. The HBO_2 exposures lasted up to 60 min for the animals treated with AEDs separately and up to 90 min for those treated with AED combinations. Chamber temperature and relative humidity were maintained at 23 ± 0.5 °C and 60 ± 2%. All mice were continuously monitored for signs of CNS HBO_2 toxicity, and in some instances, video recordings were made for later review. HBO_2 exposures were concluded when mice exhibited tonic-clonic seizures.

Adverse Side Effects to AED Doses

The rotarod test was used to determine whether individual and combined AEDs altered motor coordination and balance in mice breathing room air at 1 ATA (Dunham and Miya 1957). Sixty minutes following administration of AEDs, mice

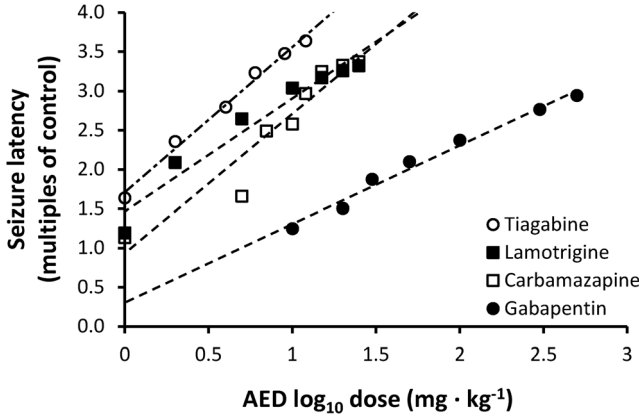

Fig. 1 Dose-dependent effects of the four AEDs tested (**a**), and regression lines for the calculation of equally effective doses (**b**) on seizure latencies in mice exposed to HBO_2 at 5 ATA for 60 min

were placed on a rod (3-cm diameter) that rotated at a constant speed of 6 rpm. The AED dose was considered to cause adverse side effects if the mice were unable to remain on the rod for at least 2 min (Borowicz and Czuczwar 2005). If more than 20% of mice failed the rotarod test, that specific dose was not tested in HBO_2.

Acute Lung Injury

Immediately following HBO_2 exposures and euthanasia, bronchoalveolar lavage fluid was collected to determine total protein content, an index of acute lung injury, as previously described (Demchenko et al. 2007). In brief, a tracheostomy was performed and 1 mL of phosphate-buffered saline was slowly injected into both lungs, slowly removed, repeated, and then frozen at − 80 °C until analysis of protein levels using the bicinchoninic acid assay.

Analysis of Combined AEDs

Experimentally derived dose-response data are traditionally used to determine the ED_{50}, in this instance, the dose that protects 50% of the animals from developing oxygen-induced seizures. Because this approach relies on an all-or-nothing response, we previously determined a more uniform standard for comparing AEDs in HBO_2 could be obtained by predicting equally effective doses (EQD_x) from dose-response curves using linear regression analysis (Demchenko et al. 2017). The EQD_x reflects the dose that would increase seizure latency by a fixed multiple of the mean seizure latency of controls. In the present study, AED doses that increased seizure latencies over vehicle controls by multiples of 2 ($EQD_{2\times}$), 2.5 ($EQD_{2.5\times}$), 3 ($EQD_{3\times}$), and 3.5 ($EQD_{3.5\times}$) were calculated using log probit analysis (Litchfield Jr. and Wilcoxon 1949). The 95% confidence limits were transformed into the standard error of the mean (SEM) (Luszczki et al. 2003; Porreca et al. 1990). With these EQD_x, we first used an effect-based strategy to compare combined $EQD_{2\times,2.5\times,3\times,\text{ and }3.5\times}$ for tiagabine + carbamazepine and tiagabine + lamotrigine to each of its individual component $EQD_{2\times,2.5\times,3\times,\text{ and }3.5\times}$ (Foucquier and Guedj 2015). The highest single agent allowed for comparisons to be made between the effect of combined AEDs (E_{AB}; A = tiagabine and B = carbamazepine or lamotrigine) and the individual AED that demonstrated the highest effect, max (E_A, E_B) (Lehar et al. 2007). Next, we determined the type of interaction between tiagabine + carbamazepine and tiagabine + lamotrigine using isobolographic analyses with modification. Based on the results obtained from effect-based testing, $EQD_{3\times}$ was used to calculate the additive doses of combined AEDs that should theoretically increase seizure latency over controls by a factor of 3 ($EQD_{3\times\text{-add}}$) for three fixed-dose ratio combinations (1:3,

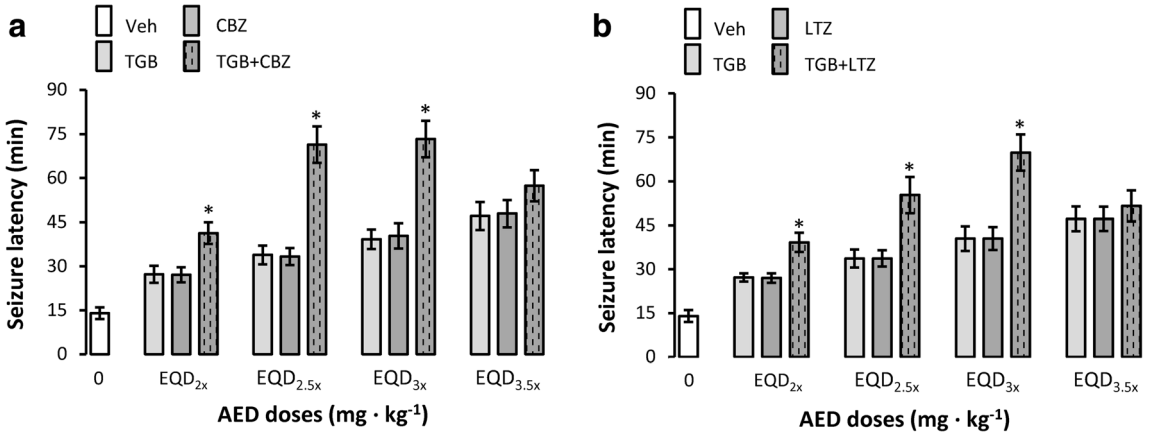

Fig. 2 Effects of combined AEDs on seizure latency in mice exposed to HBO_2 at 5 ATA for 90 min. Equally effective doses of tiagabine (TGB) + carbamazepine (CBZ) (**a**) and tiagabine + lamotrigine (LTG) (**b**) were calculated from regression analysis and administered prior to HBO_2 exposures. The doses, EQD_x (mg kg^{-1}), tested for TGB + CBZ included $EQD_{2\times}$ (1.3 + 4.6), $EQD_{2.5\times}$ (2.6 + 8.6), $EQD_{3\times}$ (4.8 + 16.4), and $EQD_{3.5\times}$ (9.4 + 30.2), and for TGB + LTG included $EQD_{2\times}$ (1.3 + 3.1), $EQD_{2.5\times}$ (2.6 + 6.4), $EQD_{3\times}$ (4.8 + 13.2), and $EQD_{3.5\times}$ (9.4 + 27.6).*Combined AED effects > highest single agent, $p < 0.05$

Table 2 Theoretical fixed-dose ratios (F) tested in HBO_2 to determine the type of interaction

AEDs and F	$EQD_{3\times\text{-add}}$	+ 1.5 ×	− 1.5 ×	− 2 ×	− 2.5 ×
TGB + CBZ					
3:1	3.6 + 4.1	5.4 + 6.15	2.4 + 2.7	1.8 + 2.05	1.44 + 1.64
1:1	2.4 + 8.2	3.6 + 12.3	1.6 + 5.6	1.2 + 4.1	0.96 + 3.28
1:3	1.2 + 12.3	1.8 + 18.4	0.8 + 12.3	0.6 + 6.2	–
TGB + LTG					
3:1	3.6 + 3.3	5.4 + 4.9	2.4 + 2.2	1.8 + 1.65	1.44 + 1.32
1:1	2.4 + 6.6	3.6 + 9.9	1.6 + 4.5	1.2 + 3.3	0.96 + 2.64
1:3	1.2 + 9.9	1.8 + 14.8	0.8 + 12.3	0.6 + 6.2	–

Values are drug doses (mg kg^{-1}) TGB + CBZ or TGB + LTG in. *TGB*, tiagabine; *CBZ*, carbamazepine; *LTG*, lamotrigine; *$EQD_{3\times\text{-add}}$*, the theoretical additive doses of TGB + CBZ or TGB + LTG that increases seizure latency over vehicle control by a factor of 3. $EQD_{3\times\text{-add}}$ for each fixed-dose ratio was increased by 1.5 × and decreased by 1.5, 2, and 2.5 ×. For fixed-dose ratio 1:3, − 2.5 × was not tested. Pretreated mice were exposed to HBO_2 at 5 ATA for 90 min, and seizure latencies were recorded. Dose-response curves were then generated to determine $EQD_{3\times\text{-mix}}$, the experimentally determined doses of TGB + CBZ or TGB + LTG that increase seizure latency over vehicle control by a factor of 3

1:1, and 3:1) (Borowicz et al. 2002; Porreca et al. 1990). For each fixed-dose ratio, the combined drug doses corresponding to $AED_{3\times}$ were increased by 1.5 × and decreased by 1.5, 2, and 2.5 × in order to maintain the desired mass ratio (1:3, 1:1, and 3:1), and subsequently tested in HBO_2. Dose-response curves were generated from these data, and the experimental or actual $EQD_{3\times\text{-mix}}$ values were calculated with their SEMs. If $EQD_{3\times\text{-mix}}$ is less than, equal to, or greater than $EQD_{3\times\text{-add}}$, the combined AED interaction is considered synergistic, additive, or antagonistic, respectively (Matsumura and Nakaki 2014).

Statistical Analysis

A two-way ANOVA was used to compare mean seizure latencies between each AED-dose combination with vehicle controls. For effect-based strategy analysis, a *t* test was used to compare the combined EQD_x with its most effective single component (highest single agent) (Foucquier and Guedj 2015). For isobolographic analysis, a *t* test was used to compare experimentally derived $EQD_{3\times\text{-mix}}$ values with their respective theoretical additive $EQD_{3\times\text{-add}}$ values (Tallarida 2001; Tallarida 2012). A one-way ANOVA was used to compare whether combined AEDs using $EQD_{3\times}$ attenuated acute lung injury. Values of $p < 0.05$ were considered statistically significant.

Results

Individual AED Dose-Responses to HBO_2

All control mice pretreated with saline/DMSO exhibited neuromotor responses in HBO_2 that progressed from intensive grooming and twitching (stage I) to rhythmic paroxysms in the face and body (stage II), and then to tonic-clonic convulsions (stage III). The mean time of HBO_2-induced generalized convulsions was 13.5 ± 1.8 min. Pretreatment with the four tested AEDs 60 min prior to HBO_2 significantly delayed the onset of generalized seizures when the following doses were used: carbamazepine (5–25 mg kg^{-1}), lamotrigine (2–25 mg kg^{-1}), tiagabine (1–12 mg kg^{-1}), and gabapentin (20–500 mg kg^{-1}) (Table 1). Dose limits for each AED were documented during rotarod testing; thus, the following AED doses were not examined in HBO_2: carbamazepine

Table 3 Interactive antiseizure effectiveness of combined AEDs in mice exposed to HBO_2

AEDs	F	$EQD_{3\times\text{-add}}$ (mg kg^{-1})	$EQD_{3\times\text{-mix}}$ (mg kg^{-1})	Interaction
TGB + CBZ	3:1	7.7 ± 0.09	5.3 ± 0.05*	Synergy
	1:1	10.6 ± 1.10	6.9 ± 0.08*	Synergy
	1:3	13.5 ± 1.70	9.7 ± 0.90	Additivity
TGB + LTG	3:1	6.9 ± 0.08	4.8 ± 0.06*	Synergy
	1:1	9.1 ± 1.10	6.1 ± 0.08*	Synergy
	1:3	11.1 ± 1.20	12.1 ± 1.30	Additivity

Values are means $EQD_{3\times\text{-add}}$ and $EQD_{3\times\text{-mix}}$ ± SEM. *TGB*, tiagabine; *CBZ*, carbamazepine; *LTG*, lamotrigine; *F*, fixed-dose ratio. *$p < 0.05$

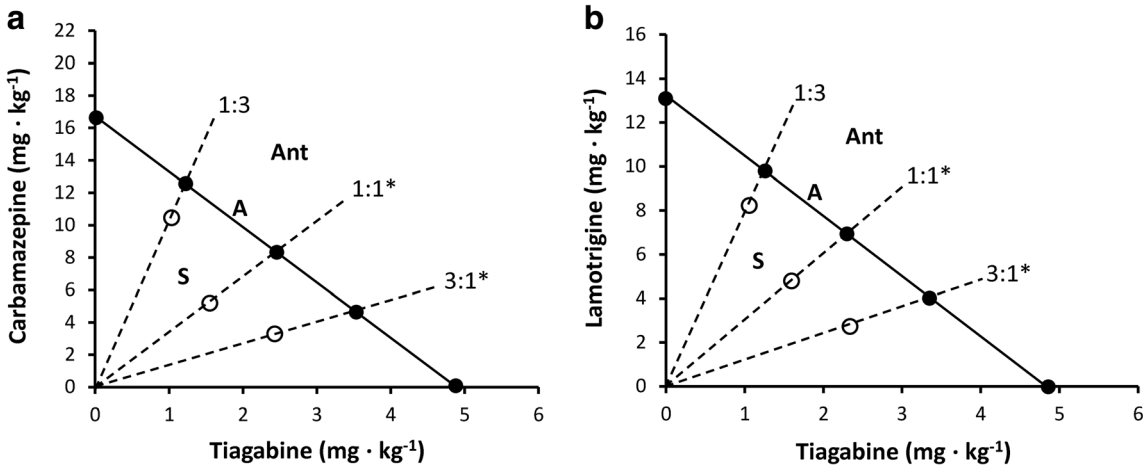

Fig. 3 Isobolograms illustrate the interactions between carbamazepine and tiagabine (**a**) and lamotrigine and tiagabine (**b**) for three fixed-dose ratios—1:3, 1:1, and 3:1 (dotted lines). The additive doses of drug combinations ($EQD_{3\times\text{-add}}$) that should theoretically increase seizure latency over controls by 3-fold (*filled circles*) were compared with the experimentally obtained ($EQD_{3\times\text{-mix}}$) values determined from the dose-response curves of the drug combinations administered in the same fixed-dose ratios for 4–5 dose combinations (Table 2) (*open circles*). Plotted data points represent mean $EQD_{3\times\text{-add}}$ and $EQD_{3\times\text{-mix}}$. Antagonist (Ant), $EQD_{3\times\text{-mix}} > EQD_{3\times\text{-add}}$; additive (A) for fixed dose ratio 1:3, $EQD_{3\times\text{-mix}} = EQD_{3\times\text{-add}}$; *synergy (S) for fixed dose ratios 1:1 and 3:1, $EQD_{3\times\text{-mix}} < EQD_{3\times\text{-add}}$

(50 mg kg^{-1}), lamotrigine (40 mg kg^{-1}), tiagabine (20 mg kg^{-1}), and gabapentin (1000 mg kg^{-1}).

Dose-response curves for tested AEDs were transformed to linear regression lines ($R^2 \geq 0.95$ for all AEDs) in order to predict EQD_x (Fig. 1). Since the $EQD_{3.5\times}$ for gabapentin exceeded a dose that could be tested in HBO_2 due to side effects, only tiagabine was considered for combination testing with the sodium channel antagonists at $EQD_{2\times}$–$EQD_{3.5\times}$.

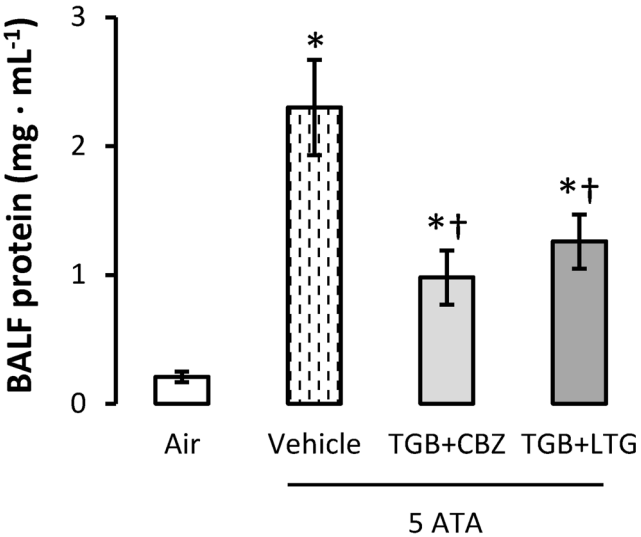

Fig. 4 Total bronchoalveolar lavage fluid (BALF) protein (an index of lung injury) from mice pretreated with vehicle, tiagabine + carbamazepine ($EQD_{3\times}$ = 4.8 + 16.4 mg kg^{-1}) or tiagabine + lamotrigine ($EQD_{3\times}$ = 4.8 + 13.2 mg kg^{-1}) and exposed to HBO_2 at 5 ATA for 90 min. Significantly different from mice breathing room air* or vehicle controls†, $p < 0.05$

Antiseizure Effectiveness of Combined AEDs in HBO_2

The combined effects of tiagabine + carbamazepine or lamotrigine at $EQD_{2\times}$, $EQD_{2.5\times}$, and $EQD_{3\times}$, but not $EQD_{3.5\times}$, were significantly greater than the effects documented with the highest single agent (Fig. 2a, b). AED combinations at $EQD_{2.5\times}$ and $EQD_{3\times}$ delayed seizure latency over controls by ~5-fold. Motor side effects occurred in a significant proportion (40–70%) of the mice at $EQD_{3.5\times}$.

The $EQD_{3\times\text{-add}}$ for tiagabine + carbamazepine and tiagabine + lamotrigine was calculated for fixed ratios 1:3, 1:1, and 3:1 (Table 2), and compared with the $EQD_{3\times\text{-mix}}$ values for the same fixed-dose ratios (Table 3). For both AED combinations, $EQD_{3\times\text{-mix}}$ was significantly < $EQD_{3\times\text{-add}}$ for fixed-dose ratios 1:1 and 3:1 (all dose combinations), and similar for 1:3 (all dose combinations), indicative of synergy in the former and additivity in the latter, depicted in an isobologram (Fig. 3).

Combined AEDs Attenuate Acute Lung Injury in HBO_2

In mice pretreated with vehicle and exposed to HBO_2 for 90 min, bronchoalveolar lavage fluid protein was 11-fold greater than control mice breathing room air at 1 ATA (Fig. 4). Pretreatment with tiagabine + carbamazepine or tiagabine + lamotrigine attenuated this increase by 57 and 45%, respectively.

Discussion

To our knowledge, this is the first investigation to systematically explore the potential use of AED combinations from different functional classes to protect against HBO_2-induced seizures. We

report three novel findings. First, the combination of the GABA enhancer tiagabine with either of the sodium channel antagonists tested, carbamazepine or lamotrigine, led to substantial increases in seizure latencies compared with individual agents, and since lower single-doses were used, motor function was not impaired. Second, the pharmacodynamic interaction of tiagabine + carbamazepine or lamotrigine for fixed ratios 1:1 and 3:1 was synergistic, and additive for 1:3. Third, the maximally effective doses of tiagabine + carbamazepine or lamotrigine reduced the magnitude of acute lung injury by ~ one-half.

Combination drug therapies offer the potential of improved effectiveness with fewer side effects. The most commonly used methods for establishing whether combined drug effects are superior to the single components are effect-based and dose-effect-based approaches, the latter allowing for the interaction to be determined when drugs have nonlinear dose-response curves and intercepts that deviate from zero (Foucquier and Guedj 2015). The reasoning for *the highest single agent* approach was to first establish combined drug doses that were efficacious since we did not use the traditional ED_{50} of each drug. The doses that increased seizure latency over controls 3-fold ($EQD_{3\times}$) were determined to be the most effective when combined, and used to determine the type of interaction. Interestingly, we previously determined there to be good qualitative agreement between $EQD_{3\times}$ and ED_{50} (Demchenko et al. 2017).

The types of pharmacodynamic interactions are influenced by each drug mechanism(s) of action. Tiagabine is a potent and selective inhibitor of GABA transporter 1 (GAT1), thus the reuptake of GABA and a promoter of GABAergic neurotransmission and prolongation of inhibitory post-synaptic potentials (Suzdak and Jansen 1995). Carbamazepine inhibits voltage-gated sodium channels, reducing the inward sodium current and membrane potential and subsequent glutamate release (Jo and Bean 2014). Lamotrigine binds to inactivated sodium channels, limiting repetitive and sustained neuronal firing without affecting normal synaptic conduction, and also decreases voltage-gated calcium currents; both effects are thought to contribute to decreased presynaptic neuronal glutamate release (Cheung et al. 1992; Coulter 1997; Stefani et al. 1997). When mice were pretreated with tiagabine + carbamazepine or lamotrigine with fixed-ratio combinations of 1:1 or 3:1, but not 1:3, and exposed to HBO_2, the AEDs acted synergistically, indicating that provision of an equal or greater amount of a GABA enhancer with a sodium channel antagonist, at least at the ratios tested, enhances antiseizure activity more than the individual AEDs. These data highlight the potential of maintaining GABA levels while simultaneously inhibiting glutamatergic neurotransmission to protect against oxygen-induced seizures.

In extreme HBO_2, acute lung injury is mediated by excitation of the CNS and excessive sympathetic outflow that contributes to capillary damage in the lung (Demchenko et al. 2011). We propose that AEDs can prevent neuroexcitation and subsequent sympathoexcitation that results in right and left ventricular dysfunction, pulmonary hypertension, and alveolar-capillary membrane damage. In the final set of experiments designed to assess pulmonary damage, 7 of the 8 mice seized and had very high levels of bronchoalveolar lavage protein. In contrast, the incidence of tonic-clonic seizures was reduced to 25 and 38% when mice were pretreated with tiagabine + carbamazepine or lamotrigine, respectively, and protein levels were substantially reduced. Even so, some of the animals in the treatment groups still had elevated bronchoalveolar protein levels despite not seizing. This profile is consistent with previous reports by others (Wood et al. 1965) and our team (Demchenko et al. 2007). Two different mechanisms may be responsible for this phenomenon: *one*, neuronal nitric oxide production may continue to increase and this overrides AEDs inhibitory effects on sympathetic outflow (Demchenko et al. 2012), and/or *two*, some degree of baroreflex impairment is occurring secondary to increased systemic vascular resistance and mean arterial blood pressure (Demchenko et al. 2014). In the latter scenario, combining an adrenoceptor antagonist, e.g., propranolol, with the paired AEDs used herein should offer further protection against acute lung injury by preservation of cardiovascular function in extreme HBO_2 (Demchenko et al. 2011; Gasier et al. 2018).

Combining the FDA-approved AEDs tiagabine with carbamazepine or lamotrigine affords greater seizure protection in mice exposed to HBO_2 at 5 ATA than individual AEDs by acting additively or synergistically. An important and additional benefit is a reduction in acute lung injury. Combining AEDs allowed for single-doses to be tested at lower doses, and although we did not assess cognition, memory, or learning, motor function was not impaired. Combined AEDs from different functional classes offers promise for increasing the tolerance to higher HBO_2 doses.

Funding Information This research was supported by the Office of Naval Research Grant N00014-15-1-2072 (to C. A. Piantadosi) and the Russian Academy of Sciences, Program 18, Project 0132-2018-0011 (to I. T. Demchenko).

Compliance with Ethical Standards

All animal procedures were approved independently by the Institutional Animal Care and Use Committee of Duke University and the Ethical Review Board of the Institute of Evolutionary Physiology and Biochemistry

Conflict of Interest The authors declare that they have no conflict of interest.

Disclaimer The views expressed are those of H.G. Gasier and do not reflect the official position of the Uniformed Services University of the Health Sciences, United States Navy, or Department of Defense.

References

Berenbaum MC (1977) Synergy, additivism and antagonism in immunosuppression. A critical review. Clin Exp Immunol 28:1–18

Bert P (1943) Barometric pressure: researches in experimental physiology. Translated from the French by Hitchcock MA, Hitchcock FA. College Book Company, Columbus

Borowicz KK, Czuczwar SJ (2005) Influence of aminoglutethimide and spironolactone on the efficacy of carbamazepine and diphenylhydantoin against amygdala-kindled seizures in rats. Eur J Pharmacol 516:212–218

Borowicz KK, Swiader M, Luszczki J, Czuczwar SJ (2002) Effect of gabapentin on the anticonvulsant activity of antiepileptic drugs against electroconvulsions in mice: an isobolographic analysis. Epilepsia 43:956–963

Cheung H, Kamp D, Harris E (1992) An in vitro investigation of the action of lamotrigine on neuronal voltage-activated sodium channels. Epilepsy Res 13:107–112

Cohn R, Gersh I (1945) Changes in brain potentials during convulsions induced by oxygen under pressure. J Neurophysiol 8(3):155–160

Coulter DA (1997) Antiepileptic drug cellular mechanisms of action: where does lamotrigine fit in. J Child Neurol 12:S2–S9

Czuczwar SJ, Borowicz KK (2002) Polytherapy in epilepsy: the experimental evidence. Epilepsy Res 52:15–23

Deckers CL, Czuczwar SJ, Hekster YA, Keyser A, Kubova H, Meinardi H, Patsalos PN, Renier WO, Van Rijn CM (2000) Selection of antiepileptic drug polytherapy based on mechanisms of action: the evidence reviewed. Epilepsia 41:1364–1374

Demchenko IT, Welty-Wolf KE, Allen BW, Piantadosi CA (2007) Similar but not the same: normobaric and hyperbaric pulmonary oxygen toxicity, the role of nitric oxide. Am J Phys Lung Cell Mol Physiol 293:L229–L238

Demchenko IT, Zhilyaev SY, Moskvin AN, Piantadosi CA, Allen BW (2011) Autonomic activation links CNS oxygen toxicity to acute cardiogenic pulmonary injury. Am J Physiol Lung Cell Mol Physiol 300:L102–L111

Demchenko IT, Moskvin AN, Krivchenko AI, Piantadosi CA, Allen BW (2012) Nitric oxide-mediated central sympathetic excitation promotes CNS and pulmonary O_2 toxicity. J Appl Physiol 112:1814–1823

Demchenko IT, Gasier HG, Zhilyaev SY, Moskvin AN, Krivchenko AI, Piantadosi CA, Allen BW (2014) Baroreceptor afferents modulate brain excitation and influence susceptibility to toxic effects of hyperbaric oxygen. J Appl Physiol 117:525–534

Demchenko IT, Zhilyaev SY, Moskvin AN, Krivchenko AI, Piantadosi CA, Allen BW (2017) Antiepileptic drugs prevent seizures in hyperbaric oxygen: a novel model of epileptiform activity. Brain Res 1657:347–354

Dunham NW, Miya TS (1957) A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharm Assoc Am Pharm Assoc 46:208–209

Foucquier J, Guedj M (2015) Analysis of drug combinations: current methodological landscape. Pharmacol Res Perspect 3:e00149

Gasier HG, Demchenko IT, Tatro LG, Piantadosi CA (2017) S-nitrosylation of GAD65 is implicated in decreased GAD activity and oxygen-induced seizures. Neurosci Lett 653:283–287

Gasier HG, Demchenko IT, Zhilyaev SY, Moskvin AN, Krivchenko AI, Piantadosi CA (2018) Adrenoceptor blockade modifies regional cerebral blood flow responses to hyperbaric hyperoxia: protection against CNS oxygen toxicity. J Appl Physiol 125:1296–1304

Goldenberg MM (2010) Overview of drugs used for epilepsy and seizures: etiology, diagnosis, and treatment. P T 35:392–415

Hall AA, Young C, Bodo M, Mahon RT (2013) Vigabatrin prevents seizure in swine subjected to hyperbaric hyperoxia. J Appl Physiol (1985) 115:861–867

Harel D, Kerem J, Lavy S (1969) The influence of high oxygen pressure on the electrical activity of the brain. Electroencephalogr Clin Neurophysiol 27:219

Jo S, Bean BP (2014) Sidedness of carbamazepine accessibility to voltage-gated sodium channels. Mol Pharmacol 85:381–387

Lehar J, Zimmermann GR, Krueger AS, Molnar RA, Ledell JT, Heilbut AM, Short GF 3rd, Giusti LC, Nolan GP, Magid OA, Lee MS, Borisy AA, Stockwell BR, Keith CT (2007) Chemical combination effects predict connectivity in biological systems. Mol Syst Biol 3:80

Litchfield JT Jr, Wilcoxon F (1949) A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther 96:99–113

Luszczki J, Swiader M, Czuczwar M, Kis J, Czuczwar SJ (2003) Interactions of tiagabine with some antiepileptics in the maximal electroshock in mice. Pharmacol Biochem Behav 75:319–327

Matsumura N, Nakaki T (2014) Isobolographic analysis of the mechanisms of action of anticonvulsants from a combination effect. Eur J Pharmacol 741:237–246

Porreca F, Jiang Q, Tallarida RJ (1990) Modulation of morphine antinociception by peripheral [Leu5]enkephalin: a synergistic interaction. Eur J Pharmacol 179:463–468

Reshef A, Bitterman N, Kerem D (1991) The effect of carbamazepine and ethosuximide on hyperoxic seizures. Epilepsy Res 8:117–121

Rucci FS, Giretti ML, La Rocca M (1967) Changes in electrical activity of the cerebral cortex and of some subcortical centers in hyperbaric oxygen. Electroencephalogr Clin Neurophysiol 22:231–238

Seckin M, Gurgor N, Beckmann YY, Ulukok MD, Suzen A, Basoglu M (2011) Focal status epilepticus induced by hyperbaric oxygen therapy. Neurologist 17:31–33

Sonnenschein RR, Stein SN (1953) Electrical activity of the brain in acute oxygen poisoning. Electroencephalogr Clin Neurophysiol 5:521–524

Stefani A, Spadoni F, Bernardi G (1997) Votage-activated calcium channels: targets of antiepileptic drug therapy. Epilepsia 38:959–965

Stein SN, Sonnenschein RR (1950) Electrical activity and oxygen tension of brain during hyperoxie convulsions. J Aviat Med 21:401–405

Suzdak PD, Jansen JA (1995) A review of the preclinical pharmacology of tiagabine: a potent and selective anticonvulsant GABA uptake inhibitor. Epilepsia 36:612–626

Tallarida RJ (2001) Drug synergism: its detection and applications. J Pharmacol Exp Ther 298:865–872

Tallarida RJ (2012) Revisiting the isobole and related quantitative methods for assessing drug synergism. J Pharmacol Exp Ther 342:2–8

Tzuk-Shina T, Bitterman N, Harel D (1991) The effect of vigabatrin on central nervous system oxygen toxicity in rats. Eur J Pharmacol 202: 171–175

Voronov IB (1964) Brain structures and origin of convulsions caused by high oxygen pressure (Hop). Int J Neuropharmacol 3:279–282

Weaver LK (1983) Phenytoin sodium in oxygen-toxicity-induced seizures. Ann Emerg Med 12:38–41

Wood JD, Watson WJ (1963) Gamma-aminobutyric acid levels in the brain of rats exposed to oxygen at high pressures. Can J Biochem Physiol 41:1907–1913

Wood JD, Stacey NE, Watson WJ (1965) Pulmonary and central nervous system damage in rats exposed to hyperbaric oxygen and protection therefrom by gamma-aminobutyric acid. Can J Physiol Pharmacol 43:405–410

Wood JD, Watson WJ, Ducker AJ (1967) Oxygen poisoning in various mammalian species and the possible role of gamma-aminobutyric acid metabolism. J Neurochem 14:1067–1074

Wood JD, Watson WJ, Murray GW (1969) Correlation between decreases in brain gamma-aminobutyric acid levels and susceptibility to convulsions induced by hyperbaric oxygen. J Neurochem 16: 281–287