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Carbamazepine, But Not Valproate, Displays Pharmacoresistance In Lamotrigine-Resistant Amygdala Kindled Rats

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Abstract

The voltage gated sodium channel (VGSC) blocker lamotrigine (LTG), when administered during kindling acquisition, leads to the development of resistance to LTG. The present study aimed to assess whether LTG-resistant amygdala-kindled rats display subsequent resistance to the VGSC blocker carbamazepine (CBZ) and the broad-spectrum antiepileptic drug (AED) sodium valproate (VPA). Two groups of male Sprague Dawley rats received either 0.5% methylcellulose (MC) or LTG (5mg/kg, i.p.) one hour before each amygdala kindling stimulation. Treatments were stopped once both the groups were fully kindled. Two days later, both groups were challenged with a higher dose of LTG (15 mg/kg, i.p.) to verify LTG-resistance in the experimental group (i.e., LTG-pretreated rats). The efficacy of CBZ and VPA was then evaluated in both groups. A higher dose of LTG blocked fully kindled seizures in the vehicle-treated rats but not seizures in the LTG-treated group. The mean seizure score, of the control group (1.2 ± 0.3) was significantly lower ($p < .05$) than that of the LTG-treated population (3.5 ± 0.7 ; $n=8$). A lower percent of the population in the control group was observed to display a generalized stage 4-5 seizure compared to the experimental group (i.e., those that received LTG during kindling acquisition) (28.5% vs 62% respectively). Interestingly, CBZ (10, 20, and 40 mg/kg) displayed a dose-dependent anticonvulsant effect in the vehicle-kindled group, but was less effective in LTG-treated animals. In contrast, VPA (300 mg/kg) effectively blocked the behavioral seizure and decreased the afterdischarge duration (ADD) in both vehicle and LTG groups. These findings suggest that the LTG-resistant, amygdala-kindled rat may represent a novel model of pharmacoresistant epilepsy.

Keywords

epileptogenesis; animal epilepsy model; antiepileptic drugs; therapy resistance; kindling acquisition

1. Introduction

More than 30% of patients with partial epilepsy are resistant to traditional antiseizure drugs (ASDs) (Kwan and Brodie, 2000). Thus, there is a definite need for pharmaco-therapies that

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will effectively manage seizures in that population of patients with pharmacoresistant epilepsy. Development of ASDs for the efficacious treatment of pharmacoresistant epilepsy patient has proven particularly difficult in part because of a lack of suitable preclinical animal models. An animal model that mimics human pharmacoresistant epilepsy may provide a useful tool to study the etiology of intractable seizures, as well as support the development of more effective therapeutic strategies.

Kindling is a well established experimental model of secondarily generalized partial seizures and can display characteristics of pharmacoresistant epilepsy (Loscher, 1997; White, 2003). The fully expressed kindled seizure represents a permanent alteration of CNS function and is associated with various morphological changes, including, but not limited to, neuronal cell loss and mossy fiber sprouting (Cavazos et al., 1994; Cavazos and Sutula, 1990). During kindling, pathologic plastic changes are observed to occur early in the epileptogenic phase of kindling acquisition. Some of these include altered gene-expression in individual neurons, loss of specific neuronal populations, and rearrangement of synaptic connections (Modi, 1999; Loscher and Brandt, 2010). Kindling is also unique in that most of the voltage-gated sodium channel blockers show different profiles of therapeutic effectiveness in blocking the different stages of seizure evolution (Post, 2004). For example, LTG does not block the development of kindling acquisition but it is highly effective against the fully expressed kindled seizure. Further, other sodium channel blockers such as PHT and CBZ also do not retard kindling acquisition but are highly effective against the fully kindled seizures (Post, 2004). On the other hand valproate is efficacious in preventing kindling development and blocking fully kindled seizures (Post, 2004).

Apart from sodium channel blockers, other mechanistically distinct class of AEDs have also been shown to exert differential effects on kindling acquisition and secondarily generalized seizures. For example, the NMDA receptor antagonist CGX-1007 blocked the expression of fully kindled seizures but did not modify the acquisition of kindling (Barton and White 2004). In contrast, the NMDA receptor antagonist MK-801 has been shown to modify kindling acquisition, but to be ineffective against the fully kindled seizure (McNamara et al., 1988). Further, the AMPA receptor antagonist GYKI 52466 and the mixed AMPA receptor and GluR5 kainate receptor antagonist LY 293558 do not prevent kindling acquisition. (Rogawski et al., 2001). In addition, the M current activator EZG has also been shown to possess potent antiseizure activity against both focal and generalized seizures in the amygdala kindled rat (Tober *et al.*, 1996) and to retard kindling development in the rapid hippocampal kindled rat (Mazarati et al., 2008) Postma et. al (2000) described a phenomenon in an animal model of kindling where treatment with a low dose of LTG during amygdala kindling acquisition leads to the subsequent resistance to an even higher dose of LTG in the fully kindled rat (Postma et al., 2000). They referred to this as contingent tolerance and suggested that early exposure to an inadequate dose of LTG during the critical period of kindling acquisition might lead to pharmacoresistance. However they did not utilize their model to evaluate the effectiveness of other AEDs. We subsequently validated this finding in the pentylenetetrazole (PTZ) kindled rat; thus confirming Postma's original observation in amygdala kindled rats. Furthermore, we extended Postma et al, original finding in the LTG-resistant PTZ-kindled rats to include CBZ and phenytoin (PHT) (Srivastava et al., 2003). These findings are consistent with the criteria for a model of pharmacoresistant epilepsy as defined by NINDS model workshop (Stables et al., 2003) i.e., demonstrated resistance to two or more AEDs.

CBZ is one of the most commonly used AEDs in the treatment of human temporal lobe epilepsy (TLE) and is thought to exert its anticonvulsant activity through a use-dependent block of voltage-gated sodium channels (Ragsdale and Avoli 1998). *In vivo* animal data also suggest a strong inhibitory effect of CBZ on spontaneous motor seizures in rats in both the

chemical-induced (kainate and pilocarpine) model of TLE and the electrically-induced status epilepticus spontaneous recurrent seizure model (Grabenstatter et al., 2007; Leite and Cavalheiro, 1995, Nissinen and Pitkanen, 2007). Electroencephalographic (EEG), behavioral, and pathological features of spontaneous seizures in these animal models resemble those of complex partial seizures which are often pharmacoresistant. In an animal model of kindling, CBZ blocks the expression of fully kindled seizures but is ineffective in blocking the development of kindling (Post, 2004). However, despite sharing a common mechanism of action with PHT, no cross resistance was observed between PHT and CBZ in an animal model of drug-resistant kindling; i.e., the PHT-resistant rat. In this model, the afterdischarge seizure threshold was significantly increased by CBZ in both PHT-responders, and PHT-nonresponders (Loscher 1991).

CBZ was found to be ineffective at therapeutic concentrations in *in vitro* studies using brain slices from kainate-treated rats (Smith et al., 2007). In this study CBZ (100 μ m) did not abolish spontaneous burst (SB) activity recorded in the medial entorhinal cortex of slices from kainic acid treated rats whereas it dose-dependently reduced the SB activity in slices from control rats (Smith et al., 2007). In another study, use-dependent block of sodium channels by CBZ was found to be decreased in brain slices obtained from pilocarpine-treated spontaneously epileptic rat (Remy et al., 2003b). Similarly, *in vitro* studies on tissues obtained from human TLE patients have also observed loss of use-dependent block of sodium channels by CBZ (Remy et al., 2003a). Therefore, despite efficacy in models of spontaneous seizures, kindling, and in-vitro control tissues, CBZ was ineffective in LTG-resistant PTZ kindled rats and in an in-vitro model of pharmacoresistant seizures (Smith et al., 2007; Remy et al., 2003b; Jandova et al., 2006). These findings emphasize the need to further evaluate the role of CBZ in other models characteristic of pharmacoresistant epilepsy. The present study was designed to assess the effect of CBZ and VPA in a new model of pharmacoresistance; e.g., the LTG-resistant- amygdala kindled rat.

2. Methods

2.1. Animals

Adult male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 225-250g were group housed in Plexiglas cages in a temperature- and humidity- controlled facility. Lighting was maintained on a constant 12:12 light: dark cycle. Rats were acclimatized to this facility for at least one week before implantation of a bipolar electrode into the basolateral amygdala. Rats were allowed access to standard laboratory chow and tap water *ad libitum*. Animal care and use was in accordance with the guidelines set by National Institute of Health and the University of Utah Institutional Animal Care and Use Committee in an AAALAC approved facility.

2.2. Stereotaxic electrode implantation Surgery

Under aseptic conditions, rats were anaesthetized with intraperitoneal injection of a cocktail of ketamine (120mg/kg) and xylazine (12mg/kg). A bipolar electrode (Plastic One, Roanoke, VA, USA) was implanted stereotactically in right basolateral amygdala using the coordinates AP -2.2, ML -4.7 DV -8.7 (Paxinos, 1998). Anterior-posterior and lateral measurements were from bregma whereas the ventral measurement was from the surface of the skull. Three to four skull screws were implanted to provide strength to the implants. Electrodes were fixed by dental acrylic and the wound was sutured with suture clips. Each animal received a single dose of a subcutaneous injection of 60,000 units of Bicillin. Neomycin ointment was then applied topically to the wound and rats were returned to their home cage to recover for a minimum of 7 days. The general health and body weights of each

animal were monitored daily and any animal showing signs of infection, lack of weight gain, or poor grooming habits was sacrificed.

2.3. Amygdala Kindling procedure

Two groups of male Sprague Dawley (n =8-10) rats were kindled according to the method described by Postma et al., (2000). Briefly, on the day of the experiment, rats were allowed to acclimatize in the recording chamber for 5 min before a baseline EEG (Biopack MP 100) was obtained. After obtaining a 10 sec base-line EEG, rats were stimulated once daily by a stimulator using a supra threshold stimulus (200 μ Amp for 50 Hz, 2 sec duration). They were observed for 180 sec after the stimulation and their seizure severity graded according to the Racine scale: 0 = no response; stage 1, grooming and hyperactivity; stage 2, head nodding and tremor; stage 3, unilateral fore-limb clonus; stage 4, clonus with rearing; stage 5, generalized clonic seizures with loss of righting reflex (Racine, 1972). The electrographic afterdischarge duration (ADD) was recorded for a period of 180 seconds. Seizure stage and ADD were used as indices of kindling development. Rats were considered to be fully kindled when they were observed to have 4-5 consecutive stage 4 or 5 seizures. Only those fully kindled rats were advanced for subsequent pharmacological treatment. Non-kindled animals were excluded from further evaluation.

2.4. Drug Treatment protocol

Prior to the first kindling stimulation, rats were randomized into two groups i.e., control and experimental. Rats in the vehicle-treated control group received 0.5% (W/V) methylcellulose (MC) intraperitoneally (i.p.) daily one hour before the amygdala stimulation until they were fully kindled (typically two weeks). Rats in the experimental group received LTG (5 mg/kg, i.p.) one hour before each amygdala stimulation. Amygdala stimulation and drug treatment was discontinued once rats in both groups were fully kindled. Two days following the last kindling session both groups were challenged with a higher dose of LTG (15 mg/kg, i.p.). The seizure score and ADD were recorded for each rat. Beginning two days after the last treatment (MC or LTG), rats from both groups were treated with CBZ (10, 20 or 40 mg/kg) or VPA (300 mg/kg). A minimum of a two day washout period was permitted between each drug treatments. The time to peak effect for LTG, CBZ and VPA, was 60 min, 30 min and 15 min, respectively. All drugs were suspended in 0.5 % MC.

2.5. Statistical analysis

Results are expressed as the mean seizure score $\pm$ standard error. The Fisher exact test was used to calculate the significance for the percent incidence of generalized behavioral seizures. The ADD was expressed as the mean $\pm$ the standard deviation. Statistical difference in the ADD was calculated by the Students t-test. The estimated median effective dose (ED50) and 95% confidence intervals for CBZ was calculated by Probit Analysis (Finney, 1971). A $p < 0.05$ was considered significant.

3. Results

3.1. Effect of LTG on kindling acquisition

As shown in fig 1, the presence of a low dose of LTG (e.g., 5 mg/kg) during kindling acquisition did not block kindling acquisition. In fact, the acquisition curve was shifted slightly to the left of that observed in control rats. These results are consistent with those described by Postma et al., 2000. At the conclusion of the acquisition period, the mean behavioral seizure score was 4.0 ± 1.5 and 4.1 ± 1.1 for the LTG-treated and vehicle control amygdala kindled rats respectively (Fig 1). In addition the mean time required to reach the fully kindled state for vehicle and LTG was not different between groups; i.e., 13.6 ± 0.3 and 11.6 ± 0.3 days, respectively.

3.2. Effect of LTG on the expression of a fully kindled behavioral seizure in vehicle-treated and LTG-treated amygdala kindled rats

Two days following the last kindling session, rats in both groups were challenged with an acute dose of LTG (15 mg/kg, i.p.). This dose of LTG blocked fully kindled seizures in vehicle-treated rats. The mean seizure score of the control group (1.2 ± 0.3) was significantly lower than that of the LTG-treated population (3.5 ± 0.7). Moreover, a lower percent of the population in the vehicle-kindled group was observed to display a generalized stage 4-5 seizure compared to the experimental group (i.e., those that received LTG during kindling acquisition). For example, 28.5% of the vehicle-kindled vs 62% of the LTG-kindled rats displayed a secondarily generalized seizure in response to the challenge dose of LTG (15 mg/kg). Furthermore, to verify if the LTG resistance is reproducible, we carried out several experiments in a subset of kindled animals and found a significant difference between LTG resistant and LTG-sensitive animals.

This group was considered to be LTG-resistant for all subsequent pharmacological evaluations. (Fig 2A)

3.3. Effect of acute CBZ pre-treatment on the expression of fully kindled behavioral seizures in vehicle-treated and “LTG-resistant” amygdala kindled rats

In an effort to assess whether “LTG-resistant” animals would respond to a second sodium channel blocker, rats in both groups were treated with increasing doses of CBZ. CBZ (10, 20 and 40 mg/kg, i.p.) displayed a dose-dependent protection against the fully kindled secondarily generalized behavioral seizures in vehicle-treated rats (Fig 2B). In contrast, CBZ was less effective in blocking the secondarily generalized seizure in LTG-resistant rats. For example, at a dose of 40 mg/kg dose of CBZ, 75% of rats in the “LTG-resistant” group exhibited a stage 4-5 seizure as compared to only 25% in the vehicle-treated group ($P < 0.05$). The estimated ED₅₀ and 95% CI for the control group was 25.1 (14.5 - 64.5) mg/kg. In contrast, the ED₅₀ in the “LTG-resistant” group was > 40 mg/kg. An actual ED₅₀ could not be calculated because of the marked toxicity observed at the highest dose tested; i.e., 40 mg/kg. CBZ (40 mg/kg) significantly reduced the mean seizure score to 1.8 ± 0.7 ($P < 0.05$; $n = 8$) in vehicle-treated rats; whereas, the same dose did not produce a significant reduction in seizure score (4.2 ± 0.31) in “LTG-resistant” rats. (Fig 2C). We did not attempt to further challenge the animals with higher doses of CBZ because of the marked toxicity observed at the highest dose tested; i.e., 40 mg/kg (Fig 2B). At 40 mg/kg, CBZ animals were hypoactive and lethargic. Animals also displayed an ataxic gait. However no mortality was observed at this dose of CBZ.

3.4. Effect of VPA on the expression of behavioral seizures in control and “LTG-resistant” amygdala kindled rats

VPA (300 mg/kg) effectively blocked the secondarily generalized behavioral seizure and reduced the ADD (see below) in both vehicle-treated and “LTG-resistant” kindled rats (Fig 2b). VPA (300 mg/kg) significantly reduced the mean seizure from 5 ± 0 score to 1.3 ± 0.7 ($P < 0.05$; $n = 8$) in vehicle-treated rats and completely blocked the seizures (seizure score 0) in “LTG-resistant rats. (Fig 2C).

3.5. Effect of LTG, CBZ, and VPA on the ADD in control and “LTG-resistant” amygdala kindled rats

On the last day of kindling, the mean ADD (mean $\pm$ SD) in the vehicle and LTG-treated groups was 41 ± 18 sec and 58 ± 18 respectively. Acute treatment with LTG (15 mg/kg) not only blocked the behavioral seizures but also reduced the ADD to 8 ± 12 in the control

group. This dose was found to be ineffective in reducing the ADD in the “LTG-resistant” population; e.g., the ADD in this group was 43 ± 30 (Table1).

CBZ (10, 20 and 40 mg/kg), did not significantly reduce the ADD in either the vehicle or “LTG-resistant” population. In contrast, VPA (300 mg/kg) significantly reduced the ADD in all animals tested. VPA reduced the ADD to 13 ± 11 in vehicle-treated group and 6 ± 5 in LTG and CBZ resistant group ($P < 0.05$; $n = 8$) (Table1).

4. Discussion

Development of pharmacoresistance to available AED's during the course of epilepsy treatment is a major concern for patients with epilepsy, clinicians, and basic scientists. Although 15 new drugs (in the United States) for the treatment of epilepsy have been introduced since 1993, a large percentage of the patient population is still considered intractable to the available therapies. The present findings suggest that chronic treatment with a low dose of LTG during kindling does not affect the development of kindling and leads to subsequent resistance to higher doses of LTG. These findings confirm our previous results in the PTZ-kindled rat (Unpublished observations of Srivastava et al) and are in agreement with those of Postma et.al 2000. In Postma's original study, they defined an interesting contingent tolerance phenomenon with LTG in the amygdala kindled rat model of complex partial seizures. In another study, Krupp et al observed that repeated treatment with LTG in fully kindled rats will lead to tolerance to LTG and CBZ but not to VPA and diazepam. A striking difference between these two studies was the difference in the LTG treatment paradigm and kindling stimulation paradigms employed. In the study by Krupp et al, LTG displayed efficacy against fully kindled seizures but rats became tolerant with time when LTG was administered repeatedly. In contrast, in Postma's study tolerance to LTG developed when an ineffective dose of LTG was administered during kindling acquisition. However, Postma and colleagues did not define the cross-tolerance or resistance phenomenon with other AEDS in the same model. We explored Postma's findings in order to develop an animal model of drug resistant epilepsy. In the current study we tested whether CBZ and VPA would display resistance in the LTG-resistant kindled rat, as defined by Postma et al., (2001), in order to further establish this as a useful model of drug-resistant epilepsy. It is also worth mentioning here that unlike our kindling paradigm where we used 200 microamp supramaximal current for kindling stimulation and also for subsequent drug testing, Krupp et al, utilized a higher stimulation intensity (a greater pathological drive) of 800 and 500 microamp to measure the anticonvulsant response of AEDs. This would certainly have an important impact on drug efficacy.

As suggested by Postma and colleagues, and supported by our current findings, we hypothesized that early exposure to a subeffective dose exposure may lead to subsequent failure when a drug would ordinarily be highly effective. This phenomenon may be exemplified by post traumatic epilepsy, where PHT and CBZ are effective in the treatment of early symptomatic seizures but ineffective in preventing the development of post traumatic epilepsy (Temkin et al., 1999). These clinical data suggest that PHT and CBZ are ineffective in modifying the epileptogenic process (e.g; transition from early post traumatic seizures to late post traumatic seizures). We believe that a process of epileptogenesis exist between acute early seizures and late onset seizures which may reflect a process of kindling. Data presented in the present study is consistent with this conclusion; i.e., LTG administered during kindling acquisition did not modify kindling acquisition. Perhaps even more important is that LTG-treatment during kindling acquisition did lead to a subsequent state of resistance to LTG.

Drug resistance is a complex phenomenon and patients with refractory epilepsy are often resistant to several anticonvulsant drugs including, but not limited to, sodium channel blockers. In our model we have so far found that LTG-resistance extends mostly to the sodium channel blockers but not to valproate and other drugs with different mechanism of action such as levetiracetam and the potassium channel opener retigabine (ezogabine).

We compared the pharmacology of AEDs in LTG-resistant rats with that of another widely studied animal model of pharmacoresistance i.e the PHT-resistant kindled rat. There are many similarities and some interesting differences between these two models. As far as the pharmacology is concerned, CBZ significantly increased the afterdischarge threshold (ADT) in both PHT-responders and nonresponders (Loscher 1991). However, in the current study carbamazepine displayed only a weak anti-seizure effect in LTG-resistant animals as compared to its marked anti-seizure effect against both behavioral and electrographic seizures in LTG-sensitive animals. A reduction in the effectiveness of CBZ in LTG-resistant rats indicates that resistance to LTG extends to other drugs with a similar mechanism of action.

Felbamate and levetiracetam were both effective in the PHT-resistant and LTG-resistant kindled rats (Ebert U 2000, Loscher 2000, Srivastava 2006, 2007 AES meeting abstract). Valproic acid was effective in both LTG-resistant and LTG-sensitive kindled rats. In contrast, it was only effective in PHT-sensitive, but not the PHT-resistant, rats. Interestingly, LTG was effective in both PHT-responders and nonresponders. The difference in the pharmacology of valproate, and LTG in PHT-resistant and LTG-resistant rats might be associated with differences in the kindling and drug-treatment paradigm. Also, the outcome measure utilized in the PHT-resistant model was the afterdischarge threshold (ADT) whereas in the LTG-resistant animals we determined whether a particular drug would modify the secondarily generalized seizure or afterdischarge duration. Further, rat strain differences could account for the differences in AED responsiveness observed between these two models. For example, Wistar rats were more likely to display PHT-resistance than Sprague Dawley rats (Loscher et al., 1998).

The efficacy of CBZ, one of the most commonly used AEDs for human TLE were determined in both LTG-sensitive the LTG-resistant amygdala kindled rats. In vehicle-treated rats, CBZ displayed a dose-dependent block of behavioral but not electrographic seizures. This effect is consistent with CBZ's ability to block the secondarily generalized but not the focal seizure. In contrast, CBZ was ineffective in blocking the expression of behavioral seizures in "LTG-resistant rats". One possible explanation for the observed ineffectiveness of CBZ in this population of rats is the emergence of cross tolerance between LTG and CBZ. Given that these two AEDs share a common mechanism of action i.e. inhibition of voltage-gated sodium channels (Krupp et al., 2000), the present findings suggest that the presence of LTG during kindling acquisition leads to the development of an altered molecular target and subsequent resistance to CBZ and perhaps other sodium channel blockers. In contrast to CBZ, no resistance was observed with the broad-spectrum AED VPA. i.e., VPA effectively blocked the expression of both the behavioral seizure and the ADD in both "LTG-resistant" and vehicle-treated animals. VPA has been demonstrated to possess both antiseizure and disease-modifying properties in the amygdala-kindled model as well as in low Mg bursting slice (Silver et al., 1991; Dreier and Heinemann, 1990). Lack of resistance to VPA in the LTG-resistant animals may, in part, be associated with VPA's different mechanism of action. VPA exerts its antiseizure effect through a number of postulated mechanisms; e.g., in addition to its ability to block the voltage-dependent sodium channels (Macdonald and Kelly, 1994), VPA has also been found to exert other neurochemical and neurophysiological properties that include an ability to potentiate GABA-mediated inhibition, inhibit T-type voltage-activated Ca^{2+} currents, and attenuate

glutamate-mediated excitation (Loscher, 2002). These additional mechanisms distinguish VPA from LTG and CBZ and may account for its sustained efficacy in the LTG-resistant kindled rat.

Conceivably, the pharmacoresistance to LTG observed in this study may be associated with pharmacokinetic and pharmacodynamic changes either in the brain or in the periphery. For example, it is possible that drug metabolizing enzymes present in the liver increase the metabolism of LTG during the course of LTG treatment. However, we did not observe any difference in the plasma levels in either LTG-sensitive (0.418 ± 0.33 nmoles/100 microliter; CI = 0.338 – 0.496., n=8) or LTG-resistant amygdala kindled (0.412 ± 0.53 nmoles/100 microliters; CI = 0.307 - 0.516., n= 8) rats when challenged with a high dose of LTG. This finding suggests that the observed pharmacoresistance to LTG is not likely accounted for by a reduction in plasma LTG levels.

Restricted entry of a drug into the parenchyma of the brain and the epileptic focus may be another important mechanism underlying pharmacoresistance. Numerous reports have reported that increased expression of drug transporter efflux protein, such as P-glycoprotein (PGP) and multidrug resistance-related protein (MRP), in the blood-brain barrier and in the epileptic focus leads to drug resistance (Aronica et al., 2004; Sisodiya et al., 2002; Volk and Loscher, 2005). However, it is not clear whether the kindling process, per se, or drug treatment during kindling acquisition, would lead to over-expression of these proteins. Regardless, studies reported that CBZ is not a substrate for PGP have (Owen et al., 2001, Maines 2005). Thus, it is likely that the resistance to CBZ is mediated by yet another mechanism.

Modulation or alteration in ion channel pharmacology, particularly sodium channels, may also contribute to refractory seizures. For example Remy *et al.* reported that use-dependent block of voltage-dependent sodium channels by CBZ is completely lost in CBZ-resistant patients (Remy et al., 2003b). In addition, use-dependent block of sodium channels by CBZ was found to be decreased in tissues obtained from chronic experimental epilepsy models, i.e., chronic spontaneous seizures that evolve post-pilocarpine status epilepticus (SE). It has been proposed that these changes might be associated with transcriptional or post-transcriptional changes of the sodium channel. Thus, it appears that pharmacoresistance to CBZ may involve a complex set of mechanisms.

Our data suggest that sodium channel blockers, which are not capable of blocking acquisition of kindling, if given during the kindling acquisition process, will be ineffective against fully kindled seizures. These findings suggest that exposure of LTG during a critical period of kindling development might lead to abnormal pathological changes in the brain so that subsequent LTG treatment is rendered less effective, or even ineffective. Thus, in the present study, we hypothesize that modulation of kindling in the presence of a sodium channel blocker would lead to an altered neuronal substrate and neurobiological or plasticity changes in ion channel properties including gating and activation-inactivation kinetics. However, further experiments are required to validate this hypothesis.

In the present investigation CBZ was found to be ineffective in blocking the ADD in “LTG-resistant rats, which suggest that CBZ is not an effective candidate for controlling the focal seizures and their spread (generalized seizure) at least in LTG-resistant amygdala kindled rats. These results support further evaluation of CBZ's role in refractory focal epilepsy. Since animals are showing resistant to a class of drugs (CBZ, PHT) which are clinically prescribed as the first line drugs for both partial seizures and tonic clonic seizures, we considered it as a novel model of drug resistant epilepsy. Although it is true that in order to be considered as an ideal model of pharmacoresistance, animals should display resistance to

other mechanistically distinct AEDs. We feel, the current model is important as the VGSC blockers represent the most commonly prescribed first line drugs.

Collectively the present findings support the “LTG-resistant” kindled rat as a model of pharmacoresistant epilepsy for the early identification and differentiation of novel AEDs.

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Fig 1.

Two separate groups of male Sprague-Dawley rats (n 8-10) were treated with vehicle (0.5% methylcellulose) or LTG (5 mg/kg) 60 min prior to each amygdala kindling stimulation for a period of 3 weeks. Kindling was induced by delivering a daily suprathreshold train of 200 microamp current for 2 sec to the basolateral amygdala. A stable kindled state was established in both vehicle- and LTG-treated rats showing that pretreatment with LTG (5 mg/kg) does not affect kindling development.

Fig 2.

Fig 2A: Two days after the last kindling session, rats in both the vehicle-treated and the LTG (5 mg/kg)-treated group received a single acute dose of LTG (15 mg/kg) prior to the kindling stimulus. This acute dose of LTG was found to block the expression of fully kindled seizures in the vehicle-treated rats but was ineffective in the LTG-kindled rats. These rats were considered LTG-sensitive and LTG-resistant, respectively for subsequent pharmacological screening. (n =8 in both groups)

Fig 2B: Increasing doses of CBZ (10, 20 and 40 mg/kg., n =8) and a single dose of VPA (300mg/kg) were tested in vehicle- and LTG- resistant rats. CBZ was effective in blocking the generalized behavioral seizure in vehicle-treated and less-effective in LTG-resistant rats. VPA, on the other hand blocked the expression of the fully kindled secondarily generalized seizures in both treatment groups ($P < 0.05$., n =8).

Fig 2C: Increasing doses of CBZ (10, 20 and 40 mg/kg) and a single dose of VPA (300mg/kg) were evaluated in vehicle- and LTG-resistant rats. CBZ dose-dependently reduced the seizure score (seizure severity) in vehicle-treated, but not in LTG-resistant, rats. VPA, on the other hand reduced the seizure score in both treatment groups.

Table 1

Acute LTG treatment (15 mg/kg i.p.) decreased the afterdischarge duration in vehicle kindled, but not in LTG-kindled, animals. Increasing doses of CBZ (10, 20 and 40 mg/kg) and a single dose of VPA (300 mg/kg) were evaluated in vehicle and LTG-resistant groups. CBZ at the doses tested did not decrease the duration of the afterdischarge in either the vehicle- or the LTG-treated animals. VPA, on the other hand blocked the expression of the electrographic afterdischarge in both vehicle and LTG-resistant groups.

	Afterdischarge Duration (sec) + SD	
	Control	LTG treated
Last day of kindling	41 ±18	58 ±18
LTG (15mg/kg)	8 ± 12 *	43 ±30
CBZ (10 mg/kg)	79 ± 19	82 ±28
CBZ (20mg/Kg)	72 ±38	75 ±43
CBZ (40mg/kg)	49 ± 18	78 ±27
VPA (300 mg/kg)	13 ±11*	6±5*