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Taurine supplementation to anti-seizure drugs as the promising approach to treat pharmacoresistant epilepsy: A pre-clinical study

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ABSTRACT

Background: Pharmacoresistance leads to severe, irreversible disabilities and premature death in ~30% cases of epilepsy despite adequate and appropriate treatment with available anti-seizure drugs (ASDs) without any underlying cause. In light of the large body of evidence which suggests the anti-seizure action of taurine in experimental animals and its wide safety margins in human, supplementation of this inhibitory amino-sulfonic acid to available ASDs seems promising to treat pharmacoresistant epilepsy.
Methods: We examined the anti-seizure effect of lamotrigine (15 mg/kg), levetiracetam (40 mg/kg), carbamazepine (40 mg/kg), phenytoin (35 mg/kg) & taurine (50, 100 & 200 mg/kg) in lamotrigine pre-treated pentylenetetrazole-kindled mice (LPK) which mimic core features of pharmacoresistant epilepsy, either alone ASDs or in combinations whereby three different doses of taurine were supplemented with tested ASDs.
Results: Both, the ASDs and the taurine were failed to suppress generalized tonic-clonic seizures in LPK mice. However, taurine supplementation clearly restored the anti-seizure effect of tested ASDs. Further neurochemical studies revealed that higher levels of taurine in the hippocampus and cerebral cortex restored the imbalance between major excitatory neurotransmitters glutamate & its inhibitory counterpart GABA.
Conclusions: These findings emphasize that supplementation of taurine with ASDs may be useful to treat pharmacoresistant epilepsy. Thus, further clinical validation is encouraged.

1. Introduction

Persistent seizures is a characteristic feature of pharmacoresistant epilepsy as clear from ~30% of 65 million total worldwide cases of epilepsy, despite adequate and appropriate treatment with available ASDs.[1–3] It leads to developmental delay, severe irreversible disabilities and premature deaths, suggesting that seizure control is important.[4]

In order to limit severity and frequency of persistent disabling seizures, ASD combinations possessing multiple mechanisms of actions can be considered[1] however; serious adverse effects and drug interactions often limit their usage.[5] Moreover, chronic polytherapy further aggravates epilepsy associated comorbidities such as depression and memory impairment.[6] On the other side, available non-pharmacological alternatives such as neurosurgery, central & peripheral neurostimulation are clinically underutilized, either due to ineffectiveness or inappropriateness.[7–10]

Thus, epilepsy field suffers from pharmacoresistance despite intensive epilepsy research over the years and clinical availability of more than ten leading ASDs. Thus, much interest is in the development of safe and effective treatment approaches, with an emphasis to restore drug response in pharmacoresistant epilepsy.

The precise pathological mechanisms underlying pharmacoresistance in epilepsy stay elusive.[3] However, disturbance in regulatory roles of excitatory and inhibitory amino acids are thought to lead neuronal hyperexcitability in epilepsy.[11–13] In this context, uses of neuroactive amino acids have been recently drawn in for treatments of pharmacoresistant epilepsy.[14,15] Another believed disturbance is the failure of neuronal regulation by taurine, an inhibitory amino sulfonic acid.[13]

In parallel to this, supplementation of taurine to available ASDs seems an promising approach to treat pharmacoresistant epilepsy, considering favourable effects of this inhibitory amino sulfonic acid such as neuroprotection from glutamate induced excitetoxicity,[16,17] direct agonistic action at $GABA_A$ receptor complex,[18,19] enhancement of GAD activity and GAD-positive neurons[20,21] as well proven anti-convulsant effect[22,23] with wide margin of clinical safety[24] made the choice convincing.

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The Epilepsy Therapy Screening Program of National Institute of Neurological Disorder & Stroke (NINDS) offer a battery of well-established rodent seizure models to screen promising molecules. Among these, lamotrigine resistant seizures in Swiss albino mice mimics core features of pharmacoresistant epilepsy.[25–28] Therefore, this study was aimed to evaluate taurine supplementation to known ASDs, as promising approach to treat pharmacoresistance on LPK mice model of pharmacoresistant epilepsy.

2. Materials and methods

2.1. Animals

Experiments were performed on total forty-two adult male Swiss albino mice (obtained from a breeder, Lala Lajpat Rai University of Veterinary and Animal Science, Hisar, Haryana, India). Mice were kept in plastic cages (6 mice/cage) in the animal house condition, at controlled room temperature (22 ± 3 °C), humidity (50 ± 5%) and light-dark cycle (12 h light: 12 h dark, lights on at 8:00 am) with free access to food (standard pellets for rodents) and water (ad libitum), except during experimental schedules. The experimental protocol was duly approved by the Institutional Animal Ethics Committee (protocol approval no. 107/99/CPCSEA/2014-08). Experiments were carried out as per guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India. The cages were cleaned regularly. Mice were acclimatized to the laboratory for a week, before these experiments. For pentylenetetrazole kindling model, n = 10 mice were used, termed as PK mice. For lamotrigine pre-treated pentylenetetrazole kindling model, n = 32 mice were used, termed as LPK mice. For both the models, mice were of twelve-week-age and individual body weight 25–28 g at the time of seizure induction.

2.2. Pentylenetetrazole kindling model

The pentylenetetrazole kindling is a well-established rodent seizure model that mimics core features of pharmacoresponsive epilepsy and widely employed in the screening of potential molecules.[25] It involves a progressive increase in seizure susceptibility of rodents due to repeated pentylenetetrazole treatments.[29–31] For this, n = 10 mice were treated with a sub-convulsive dose of pentylenetetrazole (40 mg/kg) on alternate days. Pentylenetetrazole (Sigma-Aldrich, USA) was dissolved in normal saline and administered via i.p route at every 48 ± 2 h intervals. After every injection, mice were placed individually in transparent plexiglass cages (20 × 20 × 30 cm) and convulsive seizures were recorded visually for a time period of 30 mins, as per modified Racine's scale which is mentioned as: Stage 0: no response; Stage 1: hyperactivity, restlessness and vibrissae twitching; Stage 2: head nodding, head clonus and myoclonic jerks; Stage 3: unilateral or bilateral limb clonus; Stage 4: forelimb clonic seizures; Stage 5: generalized tonic–clonic seizures with falling; Stage 6: hind limb extension. Pentylenetetrazole treatments continue for each mouse until it has achieved the criterion of 3 consecutive stage 5 seizures, whereby it is considered in a "stable kindled state".[29–31] Mortality was observed in 2 out of 10 mice.

2.3. Lamotrigine pre-treated pentylenetetrazole kindling model

The lamotrigine pre-treated pentylenetetrazole kindled mice mimic core features of pharmacoresistant epilepsy and described in detail.[25,26] Briefly, this model has modified the traditional kindling protocol with the addition of lamotrigine pre-treatments during kindling acquisition phases, which does not inhibit kindling acquisition, but leads to the subsequent development of pharmacoresistant seizures in kindled animals. For this study, n = 32 mice received lamotrigine pre-treatments (5 mg/kg suspended in 0.5% methylcellulose, i.p) at 45 min before every pentylenetetrazole

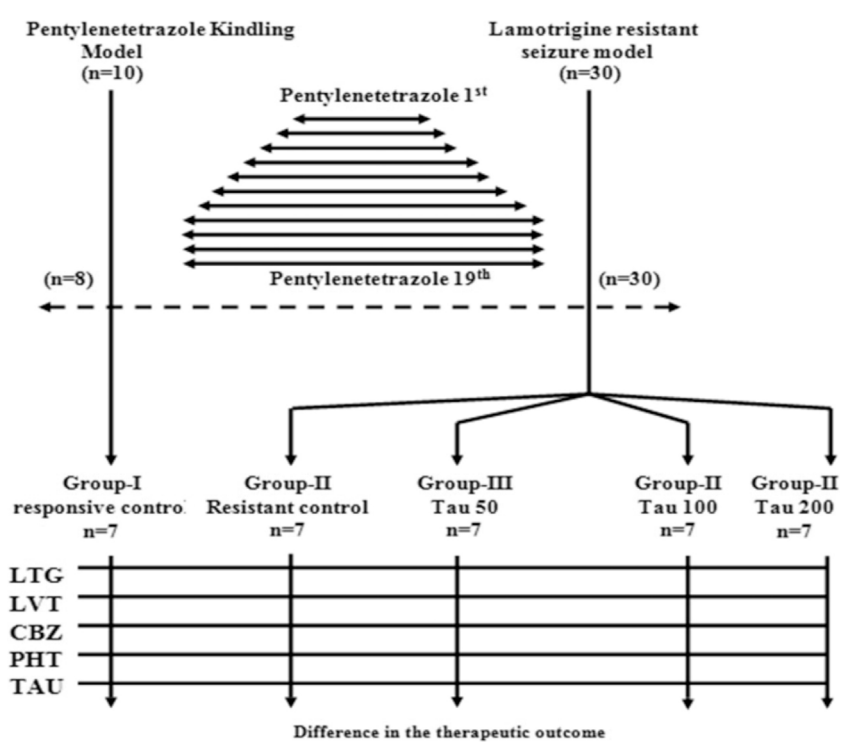

Fig. 1. Schematic illustration of the study design.

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