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Review Paper

Screening models for antiepileptic drugs: A Review

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Abstract

Considering the prevalence of epilepsy and the problems associated with currently available antiepileptic drugs like side effects, resistance, safety issue and high cost, herbal medicine with fewer complications could be very appropriate alternative. Therefore in the present study, we have examined the antiepileptic properties of ethanolic extract of leaves in mice using maximal electroshock seizures (MES) test, Pentylenetetrazole (PTZ), induced seizures, strychnine induced convulsion, Isoniazid-induced convulsions, Picrotoxin-induced convulsions, Kainic acid (KA) model etc. There is increased concern on agents for epilepsy disease modification and prevention. To solve these unmet needs, the research scientist must have a thorough knowledge of available animal models of epilepsy so that he can pick up the best model for his research. In this article, we are reviewing the diversity of animal models of epilepsy and their implications in antiepileptic drug discovery.

Keywords: Epilepsy, animal model, seizures,

1. INTRODUCTION:

Epilepsy is a serious neurological disorder with point prevalence of 6.38 per 1000 persons. Both conventional and newer antiepileptic drugs (AEDs) treat various types of seizures. The aim of treatment is to reduce seizure frequency within acceptable level of side effects. However, the currently used AEDs not only fail to control seizure in some patients, but it frequently causes side effects. The side effects are important reason of treatment failure with antiepileptic drugs. Cognitive impairment has been reported with the treatment of conventional as well as newer antiepileptic drugs. There is promising evidence focusing on the contribution of oxidative stress and mitochondrial dysfunction in causing epileptic seizures. Various animal studies show the role of free radical production and oxidative damage to cellular proteins in epileptic seizures. A number of studies have suggested the role of antioxidant from medicinal plants in protection of brain against seizures and improved seizures induce cognitive disorders. Thus, there is an increase attention in the prevention of seizures, oxidative damage and memory deficits associated with epilepsy. MES and PTZ test are the two primary tests employed for anticonvulsant screening of new drugs. The PTZ test represents a valid model for generalized tonic-clonic and absence seizures. ¹Seizures also may be a toxic manifestation of the action of central nervous system (CNS) stimulants and certain other drugs. Seizures often occur in hyperthermia (febrile seizures are very common in infants); sometimes in eclampsia, uremia, hypoglycemia, or pyridoxine deficiency; and frequently as a part of the

abstinence syndrome of individuals physically dependent on CNS depressants.²

1.1 Types of Epilepsy:

a. Generalised seizures:

1. Generalised tonic-clonic seizures (GTCS, major epilepsy, grand mal):

It is the commonest, lasts 1-2 min. The usual sequence is aura-cry-unconsciousness tonic spasm of all body muscles-clonic jerking followed by prolonged sleep and depression of all CNS functions.

2. Absence seizures (minor epilepsy, petit mal):

It is prevalent in children, lasts about 1/2 min. Momentary loss of consciousness, patient apparently freezes and stares in one direction, no muscular component or little bilateral jerking. EEG shows characteristic three cycles per second spike and wave pattern.

3. Atonic seizures (Akinetic epilepsy):

Unconsciousness with relaxation of all muscles due to excessive inhibitory discharges. Patient may fall.

4. Myoclonic seizures:

Shock-like momentary contraction of muscles of a limb or the whole body.

5. Infantile spasms (Hypsarrhythmia):

Seen in infants. Probably not a form of epilepsy. Intermittent muscle spasm and progressive mental deterioration. Diffuse changes in the inter-seizure EEG are noted.

b. Partial seizures

1. Simple-partial seizures (SPS, cortical focal epilepsy):

It is lasts 1/2-1 min. Often secondary. Convulsions are confined to a group of muscles or localized sensory disturbance depending on the area of cortex involved in the seizure, without loss of consciousness.

2. Complex partial seizures (CPS, temporal lobe epilepsy, psychomotor):

Attacks of bizarre and confused behaviour and purposeless movements, emotional changes lasting 1-2 min along with impairment of consciousness. An aura often precedes. The seizure focus is located in the temporal lobe.

3. Simple partial or complex partial seizures secondarily generalized:

The partial seizure occurs first and evolves into generalized tonic-clonic seizures with loss of consciousness. Antiepileptic drugs (AEDs) often cause problems by dampening neuronal excitability and by altering underlying systems, which can lead to impairment of cognitive functioning within various neuronal subsystems. AED effects can be drug-specific and dose-dependent and may be (supra-) additive in drug combinations. Natural products from folk remedies have contributed significantly in the discovery of modern drugs and can be alternative source for the discovery of antiepileptic drugs with novel structures and better safety and efficacy profiles. Now, various phytochemical and pharmacological studies have been carried out on this antiepileptic plants.²

2. SCREENING METHODS

There are various methods used for screening of anti-epileptic drugs and some methods are discussed here.

a. Effect on leptazole-induced convulsions in rats:

All the animals were injected subcutaneously with 80 mg/kg of leptazole in the loose skin over the back, 1 h after the administration of the extracts and the standard drug diazepam (2 mg/kg, i.p.). The animals were observed for a further 1 h and the presence or absence of convulsions was recorded. The occurrence of facial or forelimb clonus for more than 5 s was taken as the convulsion threshold.

b. Picrotoxin-induced convulsions:

Groups of 10 mice of either sex with a weight between 18 and 22 g are treated either orally or i.p. with the test compound or the standard (e.g. 10 mg/kg diazepam i.p.). Thirty min after i.p. treatment or 60 min after oral administration the animals are injected with 3.5 mg/kg s.c. picrotoxin and are observed for the following symptoms during the next 30 min: clonic seizures, tonic seizures, death.³

c. Isoniazid-induced convulsion:

10 mice of either sex with a weight of 18 to 22 g are treated with the test compound or the standard (e.g. diazepam 10 mg/kg i.p.) by oral or intraperitoneal administration. Controls receive the vehicle only. 30 min after i.p. or 60 min after p.o. treatment the animals are injected with a subcutaneous dose of 300 mg/kg isoniazid (isonicotinic acid

hydrazide). During the next 120 min the occurrence of clonic seizures, tonic seizures and death is record.³

d. Subcutaneous pentylenetetrazole (PTZ) method:

This model identifies compounds that raise seizure threshold [9]. Drugs effective against this seizure model are potential therapies for non-convulsive seizures (absence seizure). The subcutaneous convulsive dose of PTZ (produces clonic seizure in 97% animals lasting for at least 5 second duration i.e. CD97) for mouse is 85 mg/kg and in rat 70 mg/kg. Animals are observed for 30 minutes. The animal shows altered behavioural responses like vibrissae twitching, myoclonic jerk may be with associated vocalisation and straub's tail, loss of righting reflex but regaining the same after few seconds, freezing movements, breathing is increased, jumping and proceed to clonic seizure and lastly hind limb tonic extensor phase. Absence of clonic phase in the observed period indicates that the compound under investigation increases seizure threshold. MES and Sc. PTZ model are use in the primary screen while evaluating antiepileptic drugs as per NIH anticonvulsant drug development programme.⁴

e. Penicillin model of absence seizure:

Penicillin G when given by intramuscular route (≥ 3 lac unit /kg) to cat, epileptiform activity begins after 1 hour which is characterised by repeated arrested activity, myoclonus, staring and occasionally progressing to GTCS. This model shows spike wave discharge with normal background activity in EEG as seen in human absence seizure¹³. Another method of inducing absence seizure is subcutaneous injection of pentylenetetrazole (85 mg/kg). EEG of the treated animals shows spike wave discharges. Drugs effective in preventing PTZ induced seizure are effective in managing human absence seizure.⁴

f. Maximal Electroshock (MES) Induced Convulsions:

The animals were divided into five groups, each group comprised six rats. Different groups were treated with distilled water (10 mL/kg), diazepam (5 mg/kg), and ScMeOH at doses of 125, 250, and 500 mg/kg, BW. Thirty minutes later, convulsions were induced in all the groups of animals using electroconvulsometer. A 60 Hz alternating current of 150 mA for 2 s was delivered through the ear electrodes. The animal was observed for the occurrence of tonic hind limb extension.⁵

g. Pentylenetetrazole (PTZ) Induced Convulsions in Mice:

Swiss albino mice of 4-6 weeks of either sex weighing to 20-30 g were randomly selected and marked to permit individual identification, and further grouped into four groups each group comprising 6 animals. Control: Distilled water (5 ml/kg, p.o.) + PTZ (80 mg/kg, i.p.) Standard: Diazepam (4 mg/kg, i.p.) + PTZ (80 mg/kg, i.p.) Test I: Ethanolic leaves extract (100 mg/kg, p.o.) + PTZ (80 mg/kg, i.p.) Test II: Ethanolic leaves extract (200 mg/kg, p.o.) + PTZ (80 mg/kg, i.p.) The test drug was administered continuously for the period of 7 days. On 7th day convulsions were induced by PTZ. All animal groups were injected with PTZ, along with after administering respective treatment prior to experiment.⁶

h. Lithium pilocarpine model:

Epilepticus was induced by administration of pilocarpine at a dose of 350 mg/kg, i.p. Atropine 1 mg/kg i.p. was administered 30 min prior to pilocarpine to reduce the peripheral cholinergic effects of pilocarpine. Diazepam (5 mg/kg) was used as standard. The test drug was given orally 1 h before injection of pilocarpine nitrate.¹⁵ The severity of

status epilepticus was observed every 15 min till 90 min and thereafter every 30 min till 180 min using the following scoring system such as Stage 0 - no response, Stage 1- fictive scratching, Stage 2- tremor, Stage 3-head nodding, Stage 4- Forelimb clonus and Stage 5-Rearing and falling back.¹⁶ Martin ED (2006) described the alternative method on lithium pretreatment, followed by one or several low doses of pilocarpine, produces status epilepticus (SE) and chronic epilepsy with much lower mortality rates than a single dose of pilocarpine. Pretreatment of lithium chloride (3mEq/ kg, i.p) between 2-24 hours prior to pilocarpine injection potentiates the epileptogenic action of pilocarpine and allows a 10-fold reduction in the drug dose.⁷

i. Yohimbine model:

Antagonism against yohimbine-induced seizures in mice is considered to be a model predictive of potential GABA-mimetic agents. In mice the test compounds were administered intraperitoneally. 30 min prior to 45 mg/kg Subcutaneous route of yohimbine HCl. The animals are observed for the onset and number of clonic seizures for 60 min.⁷

j. Kainic acid (KA) model:

Systemic administration of the appropriate dose of KA induces 'wet dog shakes', generalized tonic-clonic convulsions, teeth chattering and altered motor activity including an initial hypo-activity which transforms to a hyperactivity at later stage. Neurodegenerative occurs in the pyramidal layer of CA3 area of hippocampus and in the piriform cortex as early as 3 hours following injection. At this time point, a positive correlation exists between the dose of KA and the extent of the acute neurochemical changes including increases of 3, 4- dihydroxyphenylacetic acid and decrease in noradrenaline levels in all brain regions

investigated. By 13 hours to 2 weeks, neuronal somata degenerate and disappear in areas such as the olfactory cortex and parts of the amygdaloid complex, hippocampal formation, thalamus and neocortex.⁷

k. Bicuculline model:

Bicuculline has been applied focally and systemically. It has been used to induce acute simple focal epilepsy after topical application in the sensorimotor cortex in rats. Another model using bicuculline with induction of chronic simple partial seizures was developed by Remler and Coworker. This model mixes features of focal and generalized epilepsy and is referred to as systemic focal epileptogenesis. In this model, rats receive radiation to a limited volume (0.25 ml) of cerebrum. Three to six months later, when the blood-brain barrier is injected systemically, inducing an epileptic focus with recurrent EEG spikes and focal seizures enduring for several weeks after a single injection. The spikes are suppressed by phenytoin, Phenobarbital, chlorodiazepoxide and valproic acid.¹² Bicuculline is believed to exert its epileptogenic effect through blocking GABAergic neurotransmission by competing with GABA for its binding sites.⁷

l. Strychnine Induced Convulsion:

Albino mice of either sex were divided into 4 groups (6 animals each): Control (0.9% Saline), standard (Diazepam 5 mg/kg, i.p.), group III (EERA 200 mg/kg, p.o.) and group IV (EERA 400 mg/kg, p.o.). EERA was administered once daily to group III and IV for three weeks. On the 21st day, after 30 min. of i.p injection of Diazepam and 60 min post oral administration of extract, strychnine nitrate (2mg/kg, i.p.) was given. The time until occurrence of tonic extensor convolution and death were noted during 1 h period.⁸

Table no. 1 Common methods used to induce convulsion in animal models

Animal models	Methods to induce convulsion	Types of seizures
<i>In-vivo</i> model	Electrical stimulation : Maximal electroshock (MES) Kindling Chemoconvulsants : Pentylenetetrazole (PTZ) Strychnine PicROTOxin Isoniazid Lithium pilocarpine Yohimbine Bicuculline 4-aminopyridine n-methyl d- aspartate Penicillin	Generalised tonic - clonic seizures Myoclonic and absence seizures Acute simple partial seizures Clonic-tonic seizures Status epilepticus Clonic seizures Generalised tonic-clonic and absence seizures
<i>In-vitro</i> Model	Hippocampal slices GABA _A receptor binding Assay	Complex partial seizures
Genetic Models	Photosensitive baboons Audiogenic seizures mice Totterer mice and seizures -prone mouse strains Genetically epilepsy - prone rats	Generalised tonic - clonic seizures

3. CONCLUSION:

Pharmaceutical industry primarily uses mechanism specific approach as primary screening tool and mechanism independent models are used to verify different mechanism based hypothesis. Seizure type models are used for secondary evaluation. Different models of epilepsy significantly improved our understandings about epileptogenesis and ictogenesis. We need new and more validated animal models which can mimic different types of human epilepsy more closely and improve our understandings about epilepsy far better.

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