THE EFFECT OF TROXERUTIN ON THE DEVELOPMENT OF PENTYLENETETRAZOLE KINDLING IN MICE

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ABSTRACT

Various synthetic derivatives of natural flavonoids are known to have neuroactive properties. The aim of the present study was to investigate the anticonvulsant effects of troxerutin which is a flavonoid and the derivative of rutin, which is an important dietary constituent of food and plant-based beverages. To this end, we assessed the anticonvulsant effects of troxerutin in mice treated with subconvulsive dose of pentylenetetrazole (PTZ) (35 mg/kg, i.p.) and observed for the kindling stages. Oral administration of troxerutin (50, 75 and 100 mg/kg) shows no significant effect on stages of convulsions induced by PTZ kindling.

KEY WORDS: Anticonvulsant; Troxerutin; Flavonoids; GABAA receptor; Rutin

INTRODUCTION

Epilepsy is a serious and common neurological condition. In contemporary society, the frequency and importance of epilepsy can hardly be overstated from epidemiologic studies. However, in most studies, the overall incidence of epilepsy in developed societies has been found to be around 50 cases per 100,000 persons per year, and rises steeply with age.1,2 Seizures are controlled in nearly 70% of patients with epilepsy, mostly by pharmacologically modulating membrane ion channels or GABAergic or glutamatergic transmission.3 γ-aminobutyric acid type A (GABAA) receptors are targets for neuroactive drugs, such as benzodiazepines, and this interaction mediates their anxiolytic, hypnotic and anticonvulsant effects at the benzodiazepine site on the receptors.4 Classical benzodiazepines, the most widely prescribed drugs, exert their therapeutic effects by binding to the benzodiazepine site of GABAA receptors, and allosterically modulate chloride flux through the ion channel complex.5 They have multiple actions and a broad range of side effects.5,6 On the other hand, natural products from folk remedies have contributed significantly in the discovery of modern drugs and can be an alternative source for antiepileptic drugs with novel structures and better safety and efficacy profiles.7 Several studies have shown that flavonoids have neuroactive properties.4,8,9 It was shown that many of these compounds are ligands for GABAA receptors in the central nervous system (CNS).8,10 Furthermore, it was found that they act as benzodiazepine-like molecules.11,12,13 These findings are supported by their behavioral effects in animal models of anxiety, sedation and convulsion.8,10,14 Troxerutin is a derivative of the natural bioflavonoid rutin. Troxerutin is found in many plants, and can be easily extracted from Sophora japonica (Japanese pagoda tree). Troxerutin is an antioxidant and has beneficial effects on venous health. Troxerutin is used in the treatment of varicose veins and haemorrhoids. It has abortifacient, antibacterial, anticholesterolemic, anti-inflammatory, antispasmodic, diuretic, emetic, emollient, febrifuge, hypotensive, purgative, styptic, and tonic properties. It was reported that rutin has several pharmacological properties including antioxidiant, anticancerogenic, cytoprotective, anti-platelets, antithrombic and vasoprotective activities.16-22 Moreover, rutin was found to be a neuroprotective agent, and can ameliorate ischemic reperfusion injury in the heart,23,24 brain25 and skeletal muscle.25 Pretreatment with rutin at 50 and 100 mg/kg attenuated seizure severity from the beginning of the kindling procedure by lowering the mean seizure stage. Moreover, it appears that the effects of rutin at higher doses are more significant. Thus, in this study we examined the anticonvulsant effects of the oral administration of troxerutin by using the PTZ kindling model in mice. It was predicted that troxerutin will show anticonvulsive effects in this
model, because it is derivative of rutin which has anticonvulsant effect in PTZ kindling.

2. Material and methods:

2.1. Animals
Male Swiss albino mice (25-30 g) were obtained from the Serum Institute of India Limited, Pune, India and housed in groups of four per cage under standard laboratory conditions. They were kept at a constant room temperature (21±2 °C) under a normal 12 L: 12 D regimen with free access to food and water. Behavioral observations and evaluations were performed by experimenters who were unaware of the pharmacological treatment. All experiments were approved by the Institutional Animal Ethics Committee (IAEC) of Sinhgad College of Pharmacy, Vadgaon (Bk.), Pune.

2.2. Drugs
Troxerutin and PTZ were purchased from Sigma and were dissolved in physiological saline prior to the experiments. Troxerutin is administered orally and PTZ by intraperitoneal route.

2.3. Experimental procedure for kindled seizures:
The animals were placed in a plastic cage (32×18×24 cm). To induce kindling, 35 mg/kg of PTZ was injected i.p. once every 48 h. After pentylenetetrazol administration, the behavioral seizures were monitored for 20 min. Convulsive behavior was assessed according to a modified Racine's scale as follows27, 28:

- stage 0, no response;
- stage 1, ear and facial twitching;
- stage 2, myoclonic body jerks;
- stage 3, forelimb clonus, rearing;
- stage 4, clonic convulsions, turn onto the side; and
- stage 5, generalized clonic convulsions, turn onto the back. After the animals developed the final stage of generalized seizures (seizure stage 4 or 5), pentylenetetrazol administration was repeated 3 more times to establish fully kindled mice. One week after the last pentylenetetrazol-kindling injection, these mice received a final challenge dose of pentylenetetrazol (35 mg/kg) to check the persistence of enhanced susceptibility to the convulsant. In the treatment group 50, 75 and 100 mg/kg of troxerutin was administered 1 hr before PTZ every other day (35 mg/kg, ip, total 11 injections).

2.4. Statistical analysis:
All data were analyzed using two way ANOVA followed by Bonferroni post hoc test.

3. Result:

Effects of troxerutin pretreatment on kindling development:
As shown in Fig. 1, the repeated application of 35 mg/kg PTZ induced behavioral seizures of increasing severity, culminating in clonic convulsions with loss of posture during stage 4 at the end of the kindling procedure. Pretreatment with troxerutin (50, 75 and 100 mg/kg) before each kindling injection did not modify the development of kindling. Pretreatment with troxerutin even at 100 mg/kg did not reduce the mean seizure stage during the 11 kindling injections as compared with the control. (Fig. 1).

![Fig. 1. Effects of repeated administration of troxerutin (50, 75, 100 mg/kg) on the development of PTZ-induced kindling (35 mg/kg, ip, 11 injections total), compared with control. Data represent mean seizure stages ±SEM, n=6.](image)

4. Discussion:
Troxerutin is a derivative of rutin which is reported extensively for its anticonvulsant activity. In many papers it is reported that rutin has neuroprotective effect. Moreover it also possesses strong antioxidant actions shown in D- galactose-induced oxidative DNA damage.29 With its antioxidant properties, it has protective effect in diabetic retinopathy.30 Also it has free radical scavenging property and it elevates the
antioxidant enzyme SOD and decreases the MDA level which is a marker of lipid peroxidation. \(^{29}\) Rutin is a ligand for benzodiazepine receptors. Furthermore, it was shown that not only do some flavonoids have moderate binding affinities for the benzodiazepine site, but that they are also partial agonists of GABAA receptors. Rutin possesses agonistic activity on the GABAergic system, and this effect can be reversed by a benzodiazepine receptor antagonist. \(^{32}\) Rutin has inhibitory effects on (GABA transaminase) GABA-T and succinic semialdehyde dehydrogenase (SSADH) through an in vitro study. Inhibition of both enzymes in brain tissue increases the GABA level and may have therapeutic applications in neurological diseases. GABA-T has been validated as an important target for neuroactive drugs. Therefore, the inhibitory action of flavonoids on these enzymes apparently contributes to their neuropharmacological effects. \(^{33}\) Due to this mechanism rutin shows anticonvulsant action in PTZ kindled rats. Therefore we focused on the anticonvulsant effect of troxerutin on PTZ kindled mice as it is the derivative of rutin. But the neuroprotective and antioxidant effects of troxerutin were not sufficient to protect against the excessive neuronal firing and behavioral seizures produced in PTZ-kindled mice. Our results demonstrated that oral administration of troxerutin doesn't affects epileptic seizures induced by PTZ kindling. Troxerutin has no significant effect on PTZ kindling. Also, the anticonvulsant effects of various synthetic components of herbal medicines have been studied. Several flavone derivatives may provide important leads for the development of potent and selective benzodiazepine receptor ligands. Studies on neuroactive flavonoids from herbal medicines could lead to their establishment as potential therapeutics for GABAA receptor mediated disorders. Thus, finding novel ligands for the benzodiazepine site with therapeutic effectiveness and minimal side effects are needed.

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