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Full paper

3,4,5-Trimethoxycinnamic acid, one of the constituents of Polygalae Radix exerts anti-seizure effects by modulating GABAAergic systems in mice

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ABSTRACT

Polygalae Radix is an important medicinal plant that is widely used in most of Africa. 3,4,5-Trimethoxycinnamic acid (TMCA) is one of the constituents of Polygalae Radix. Until now, the mechanisms involved in the anti-seizure property of TMCA are still unclear. We examined the anti-seizure effect of TMCA. TMCA administered at doses of 5, 10 and 20 mg/kg and evaluated anti-seizure effects by maximal electroshock (MES) and pentylenetetrazol (PTZ) models in mice. TMCA administered at doses of 10 and 20 mg/kg significantly reduced the incidence of MES-induced tonic hindlimb extension (THE). TMCA significantly delayed the onset of myoclonic jerks (MJ), and decreased the seizure severity and mortality compared with the vehicle-treated animals in PTZ seizure model. TMCA 10 and 20 mg/kg treated groups also did not determined generalized clonic seizures (GCS).

Pretreatment with a GABAA/benzodiazepine (BZ) receptor antagonist flumazenil blocked the anti-seizure effects of TMCA. These data support the further investigation of TMCA as a GABAA/BZ receptor agonist for anti-seizure therapy.

1. Introduction

Epilepsy is one of the most common and heterogeneous neurological disorders (1). It has been reported that more than 50 million people worldwide suffer from epilepsy, current anti-epileptic drugs are only effective in 60–70% of individuals (2). Anti-epileptic drugs used to treat epilepsy can cause severe, life threatening side effects. BZs are highly prescribed anti-epileptic drugs and of great clinical significance; however, the development of tolerance restricts their usefulness (3). A number of newer anti-epileptic drugs have been developed in the last few years to improve the treatment outcomes in epilepsy (4). But resistance to anti-epileptic drugs as well as intolerability led to serious demands for developing new drugs for epilepsy treatment (5).

In traditional Chinese medicine, Radix Polygalae (the root of *Polygala tenuifolia*) have been known to be an important herb that exhibits sedative effects in insomnia, and is widely used as an tranquillizer (6). 3,4,5-Trimethoxycinnamic acid (TMCA), one of the constituents of Polygalae Radix, has been reported prolonged sleeping time induced by pentobarbital or decreased by stress (7,8). Furthermore, TMCA also could decrease the locomotor activity in mice (7). The current study was conducted to investigate the anti-seizure properties of TMCA in the PTZ- and maximal electroshock-induced seizure models and to investigate mechanisms underlying the anti-seizure property of TMCA. The GABAA receptors are Cl^- channel selective ligand-gated ion channels that mediate fast inhibition in the CNS and are important targets for benzodiazepines (BZs) (9). BZ binding with GABAA receptor complex, enhancing Cl^- channel conductance primarily by increasing the frequency of receptor channel opening (10,11). The GABAA receptor γ subunit, in particular $\gamma 2$ subunit is essential for the formation of the binding site, binding efficacy for BZ (9,12,13). TMCA increased Cl^- channel influx and the expressions of γ-subunit of GABAA receptors in the cerebellar granule cells (7). These findings suggest that TMCA might have important effect on GABAergic system.

The current study was conducted to investigate the anti-seizure properties of TMCA in the PTZ and the maximal electroshock

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induced seizure models and to investigate mechanisms underlying the anti-seizure property of TMCA. We showed that TMCA exerted an anti-seizure activity. These effects were attenuated by pre-treatment with flumazenil, a BZ receptor antagonist. The above studies suggest that TMCA may exert anti-seizure activity by acting at the GABAA/BZ receptor complex.

2. Materials and methods

2.1. Animals

Ault male KunMing-strain mice (Experimental Animal Center, Fudan University, Shanghai, China) weighing 18–20 g were used. Animals were housed in cages at room temperature on a 12: 12 h day/night cycle and given ad libitum access to food and water. On days prior to seizure induction, animals were habituated to the test environment.

The experimental protocols were approved by the Committee on the Ethics of Animal Experiments of the University of Fudan, Shanghai Medical College (Permit Number: 20110307–049).

2.2. Drugs

TMCA, PTZ and flumazenil were purchased from Sigma–Aldrich Co. (St Louis, MO, USA), dissolved in saline containing 0.5% dimethylsulfoxide (DMSO), and administrated intraperitoneally (i.p.) in a constant volume of 10 ml/kg. The dosage selections, route of drug administration, and injection time of different compounds were based on preliminary experiments and pharmacokinetic considerations (7,14).

2.3. Seizure models

2.3.1. Maximal electroshock seizure (MES) method

The MES method produces reproducible tonic convulsion characterized by tonic hindlimb extension (THE). THE is the hindlimbs of animals outstretched 180°to the plane of the body (15). Electroconvulsions were produced by a current (fixed current intensity of 70 mA, 0.4 s stimulus duration), delivered by an electric stimulator (2-MU-2; Shanghai Medical College, China) (16,17). For the control (vehicle) group, animals received vehicle. For the positive control group, animals received diazepam (2 mg/kg, i.p.). For the test groups, mice were respectively injected with TMCA at 5, 10, 20 mg/kg. Thirty minutes later all the mice were treated with electrical stimulation. Animals were considered protected if they did not exhibit THE. The protective efficacy of TMCA was determined as ability to protect 50% of animals against the maximal electroshock-induced THE and expressed as respective values of the median effective dose (ED50) (18).

2.4. PTZ-induced convulsion

Animals were placed individually in Plexiglas boxes and seizure behaviors were observed for 60 min after PTZ injection (60 mg/kg, i.p.). The seizure behavior was evaluated as follows (14,17,19,20): Stage 0, no response; Stage 1, ear and facial twitching; Stage 2, myoclonic jerks (MJ); Stage 3, clonic forelimb convulsions; Stage 4, generalized clonic seizures (GCS), with turning to a side position; and Stage 5, generalized clonic-tonic seizures (GTCS) or death within 60 min. Latency to the onset of MJ and GCS, the seizure stage were measured. The mice were divided into 5 groups and were injected i.p. with vehicle, TMCA at a dose of 5, 10, 20 mg/kg and diazepam 2 mg/kg; and 30 min later were injected with PTZ (60 mg/kg, i.p.).

2.5. Study on receptor mechanism involved in anti-seizure effects of TMCA

Flumazenil was chosen to probe the role of the GABAA/BZ receptors in the anti-seizure effects of TMCA. Flumazenil 1 and 5 mg/kg was given 60 min before TMCA administration. The mice were given TMCA 20 mg/kg 30 min before PTZ injection or exposition for MES procedure.

2.6. Statistical analysis

All data were expressed as the mean ± S.E.M. (n = 5–10). Seizure stages were evaluated by using the Kruskal–Wallis test followed by Nemenyi. For parametric data, single comparisons were tested using the t-test, whereas multiple comparisons among groups were analyzed using one-way or two-way ANOVA followed by LSD's test. Fisher's exact test followed by bonferroni was used to determine the overall differences in the incidence of THE due to convulsions. All statistical analyses were carried out by using SPSS 17.0 for Windows. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Anti-seizure property of TMCA

The protective effect of TMCA against maximal electroshock seizure (MES)-induced THE was observed 30 min after TMCA administration. In the control group, 100% of the mice exhibited THE. The tonic flexion of the limbs occurred immediately after the shock and progressed into tonic extension of the hind limbs followed by stupor and recovery. TMCA given at 10 and 20 mg/kg significantly decreased the incidence of MES-induced THE to 50% and 20% of the value of the vehicle controls (n = 10, Fig. 1). However, TMCA given at 5 mg/kg decreased the incidence of MES-induced THE to only 80%. The ED_{50} for the anti-convulsion effect of TMCA was 10 mg/kg. No mice died in both vehicle- and TMCA-treated groups. Diazepam as a positive control significantly decreased the incidence of MES-induced THE to 10%. However, we did not observed any prolongation on duration of THE induced by TMCA. These results indicate that TMCA given at 10 and 20 mg/kg doses has potent anti-seizure effects against MES-induced seizures.

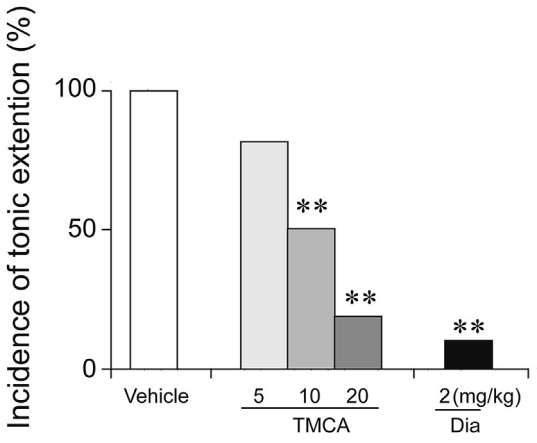

Fig. 1. Incidence of tonic extension (%) after treatment with TMCA and diazepam in the MES model. Each value represents the incidence of tonic hindlimb extension (THE) as a percentage (n = 10). $^{**}P < 0.01$, compared with the control group (Fisher's exact test followed by bonferroni for correction).