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ORIGINAL ARTICLE

Effects of alizarin, betulin, curcumin, diosmin, linalool, menthofuran, α-terpineol, theobromine, β-thujaplicin and vanillin against maximal electroshock-induced seizures in mice

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Abstract

Introduction and objective: The study was aimed at performing the anticonvulsant screening test to select some naturally occurring substances isolated from herbs and medicinal plants that could suppress seizures in the maximal electroshock (MES)-induced tonic seizure model in mice.

Materials and methods: The screening test was performed for 10 natural substances (alizarin, betulin, curcumin, diosmin, linalool, menthofuran, α-terpineol, theobromine, β-thujaplicin and vanillin) administered intraperitoneally in a constant dose of 300 mg/kg at various pre-treatment times (15, 30, 60 and 120 min.) before the MES test.

Results: Only α-terpineol and vanillin produced a 12.5% protection against MES-induced tonic seizures in mice, when administered i.p. at 120 and 60 min., prior to the MES test, respectively. In contrast, the remaining substances (alizarin, betulin, curcumin, diosmin, linalool, menthofuran, theobromine and β-thujaplicin) produced no anti-convulsant activity after their i.p. administration to mice.

Conclusions: α-Terpineol and vanillin are worth considering as potentially favorable compounds in experimental epileptology.

Keywords

alizarin, betulin, curcumin, diosmin, linalool, menthofuran, α-terpineol, theobromine, β-thujaplicin and vanillin, maximal electroshock seizure test

INTRODUCTION

Traditional knowledge obtained from ancient medical literatures or from folkloric medicine plays an important role in developing new drugs. Particular attention is paid to medicinal plants and herbs that play an invaluable role in the drug discovery process [1, 2, 3, 4]. For instance, several plants that were reputed to possess anti-epileptic properties in different folklore cultures have been found to exhibit anti-convulsant activity in different animal models [5].

It has recently been documented that essential oils isolated from various medicinal plants and herbs, such as *Artemisia dracunculus*, *Eugenia caryophyllata*, *Pimpinella anisum*, *Ascorus tatarinowii*, *Cymbopogon citratus*, *Bunium persicum*, *Ferula gummosa* and *Zhumeria majdae* exerted anti-convulsant activity in the maximal electroshock (MES)-induced seizure model in mice [6, 7, 8, 9, 10, 11, 12, 13, 14]. There is no doubt that the screening test allowing identification of the active compounds naturally occurring in herbs and medicinal plants should be performed in experimental animal studies.

In our earlier studies we reported that imperatorin, osthole and xanthotoxin (three naturally occurring coumarins) produced a clear-cut anti-convulsant action in the mouse MES model [15, 16, 17, 18, 19]. Moreover, thymoquinone produced anti-convulsant action by supressing 25% of the animals tested against MES-induced seizures [20]. In contrast, bergapten, oxypeucedanin, arbutin, esculetin, esculin, ellagic acid, gallic acid, hesperidine, piperitol, piperonal, quercetin and ursolic acid had no impact on MES-induced tonic seizures in mice [19, 20].

Considering the above-mentioned facts, we endeavoured to perform the first anti-convulsant screening test to select some substances with anti-convulsant properties in the mouse MES model. This animal model allows the selection of agents possessing anti-convulsant activity, because in this test several classical, second- and third-generation AEDs suppress tonic seizures in mice [21]. Since these AEDs are clinically efficacious in patients with tonic-clonic seizures and partial convulsions with or without secondary generalization [22], and suppressed tonic seizures in the MES test in rodents, it was appropriate to use this experimental model as a first screening test allowing the selection of compounds with anti-convulsant properties. Of note, seizure models in laboratory animals are still the most important tools in the pre-clinical search for agents and compounds possessing anti-convulsant activity [21].

Therefore, in the presented study the anticonvulsant action of alizarin, betulin, curcumin, diosmin, linalool, menthofuran, α-terpineol, theobromine, β-thujaplicin and vanillin were

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tested against MES-induced tonic seizures in mice. All these naturally occurring substances were isolated from various herbs and medicinal plants growing in Poland, compounds belonging to various diverse chemical families, including, flavonoids, curcuminoids, monoterpenes and triterpenes.

MATERIALS AND METHOD

Animals and experimental conditions. Adult male Swiss mice (weighing 22–26 g) were used, kept in colony cages with free access to food and tap water, under standardized housing conditions (natural light-dark cycle, temperature – 23 ± 1 °C, relative humidity – 55 ± 5%). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups, each group comprising 8 mice. Each mouse was used only once and all tests were performed between 08.00–15.00 hours. Procedures involving animals and their care were conducted in accordance with current European Community and Polish legislation on animal experimentation. Additionally, all efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. The experimental protocols and procedures described in the presented study were approved by the Second Local Ethics Committee at the University of Life Sciences under License No. 85/2009 in Lublin, Poland, and complied with European Communities Council Directive 86/609/EEC of 24 November 1986

Substances. The following substances were used in this study: alizarin [1,2-dihydroxy-9,10-anthracenedione (POCH, Poland); betulin [Lup-20(29)-ene-3β,28-diol (Sigma-Aldrich, USA); curcumin [(1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (Carl Roth, Germany); diosmin [5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-[[(2*R*,3*R*,4*R*,5*R*,6*S*) -3,4,5-trihydroxy-6-methyloxan-2-yl] oxymethyl]oxan-2-yl]oxychromen-4-one (Extrasynthese, France); linalool [3,7-dimethylocta-1,6-dien-3-ol (Merck, Germany); menthofuran [3,6-dimethyl-4,5,6,7-tetrahydro-1-benzofuran (Fluka, Switzerland); α-terpineol [4-methyl-1-(1-methylethyl)-1,3-cyclohexadienol (Fluka, Switzerland); theobromine [3,7-dimethyl-1*H*-purine-2,6-dione (Fluka, Switzerland); β-thujaplicin [2-hydroxy-6-propan-2-yl-cyclohepta-2,4,6-trien-1-one (Sigma-Aldrich, USA), and vanillin [4-hydroxy-3-methoxybenzaldehyde (Sigma-Aldrich, USA). All the tested substances were isolated from herbs and medicinal plants provided by Dr. M. Gleńsk and Dr. M. Włodarczyk at the Department of Pharmacognosy of the Medical University in Wroclaw, Poland. All substances were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water and administered intraperitoneally (i.p.) as a single injection in a volume of 5 ml/kg body wt. In the screening test, the studied compounds were administered i.p. at a constant dose of 300 mg/kg at four pre-treatment times (15, 30, 60 and 120 min) before the MES test.

Maximal electroshock-induced seizures. Electroconvulsions were produced by means of an alternating current (sine-wave, 0.2 s stimulus duration, 50 Hz, 25 mA, 500 V) delivered via ear-clip electrodes by a Rodent Shocker generator (Type 221, Hugo Sachs Elektronik, Freiburg, Germany). The electrical system of the stimulator was self-adjustable so that changes in impedance did not result in alterations of current intensity (i.e. the system provides constant current stimulation). The criterion for the occurrence of seizure activity was tonic hind limb extension (i.e. the hind limbs of animals outstretched 180° to the plane of the body axis). The protective activity of the various compounds in the screening test was determined as the percentage of protection of animals against MES-induced tonic seizures. In the screening test, the studied compounds were administered i.p. at a constant dose of 300 mg/kg at four pre-treatment times (15, 30, 60 and 120 min) before the MES test.

RESULTS

Effects of alizarin, betulin, curcumin, diosmin, linalool, menthofuran, α-terpineol, theobromine, β-thujaplicin and vanillin against maximal electroshock-induced seizures in mice in the screening test. None of the investigated substances administered i.p. at a constant dose of 300 mg/kg produced a potent anti-convulsant effect against MES-induced tonic seizures in mice at the respective pretreatment times (Tab. 1). In the case of α-terpineol, the substance administered i.p. at 120 min. before the MES-induced tonic seizures, protected one out of 8 mice – 12.5% of the animals tested. Similarly, vanillin when administered systemically (i.p.) at 60 min. before the MES test also protected 12.5% of the animals tested (Tab. 1).

Table 1. Time-course of anti-convulsant effects of the various naturally occurring compounds against maximal electroshock (MES)-induced seizures in mice

Pre-treatment time (min.)	15	30	60	120	
Substance	p/t (%)	p/t(%)	p/t (%)	p/t (%)	**Plants**
Alizarin	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)	*Rubia tinctorum*
Betulin	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)	*Betula pubescens*
Curcumin	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)	*Curcuma longa*
Diosmin	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)	*Citrus limon*
Linalool	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)	*Lavandula offcinalis*
Menthofuran	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)	*Mentha piperita*
α-Terpineol	0/8 (0)	0/8 (0)	0/8 (0)	1/8 (12.5)	*Melaleuca alternifolia*
Theobromine	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)	*Theobroma cacao*
β-Thujaplicin	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)	*Thuja plicata*
Vanillin	0/8 (0)	0/8 (0)	1/8 (12.5)	0/8 (0)	*Vanilla planifolia*

Data are presented as the number of animals protected against maximal electroshock (MES)-induced seizures out of 8 animals per group (and as % in parentheses). MES test was performed at various pre-treatment times (15, 30, 60 and 120 min.) after systemic (i.p.) administration of the investigated compounds in a constant dose of 300 mg/kg.
p – number of animals protected against MES-induced seizures;
t – number of animals tested per group.

DISCUSSION

Results from this screening test of 10 naturally occurring substances isolated from herbs and medicinal plants revealed that the tested agents have no clear-cut anti-convulsant activity in the mouse MES model, at the respective pretreatment times. As shown in Table 1, the tested compounds suspended in a 1% solution of Tween 80 in sterile saline in a constant dose of 300 mg/kg did not protect the animals against MES-

induced tonic seizures, with the exception of α-terpineol and vanillin, which protected maximally 12.5% of animals tested (1 out of 8 mice) when administered i.p. at 120 and 60 min. after their systemic administration, respectively (Tab. 1). Generally, it is widely accepted that the anti-convulsant effects are considered of pivotal importance if at least 50% of the animals tested are protected against experimentally evoked seizures in the screening test.

It is worth mentioning that the screening procedure conducted in the presented study was almost identical to that accepted by the National Institutes of Health (USA) and Anti-epileptic Drug Development Program for searching for novel AEDs [23, 24]. There is no doubt that herbs and medicinal plants contain a series of compounds that could be clinically favourable, especially, in epileptic patients, and could thus become a very important source in the search for compounds possessing anti-convulsant properties in preclinical studies.

As mentioned in the Introduction, we have documented that imperatorin, osthole and xanthotoxin (three naturally occurring coumarins isolated from various medicinal plants) exerted a definite anti-convulsant action in the mouse MES model [19, 20]. In contrast, bergapten and oxypeucedanin (two other naturally occurring coumarins) produced no or negligible anti-convulsant action against MES-induced tonic seizures in mice [19]. It must be mentioned that the anti-convulsant potency of imperatorin, osthole and xanthotoxin have also been evaluated in a screening test identical to that applied in the presented study.

Bearing in mind the fact that the screening procedure used in our previous studies was sensitive enough to detect the anti-convulsant action of xanthotoxin, osthole and imperatorin, we attempted to perform the screening test in order to detect the anticonvulsant action of substances isolated from various herbs and medicinal plants. There is no doubt that herbs and plants are the perfect source of active agents that could be used in patients with epilepsy [6, 7, 8, 9, 10, 11, 12, 13, 14]. On the other hand, pharmaceutical companies and industries are able to create novel compounds more effective than their natural counterparts due to the selective and rational modification of chemical structure of the investigated compounds. Of note, in the presented study, various compounds belonging to several diverse chemical families were tested, including, flavonoids, curcuminoids, monoterpenes and triterpenes.

Generally, all the tested compounds administered systemically (i.p.) at a constant dose of 300 mg/kg produced no or negligible adverse effects in mice. Additionally, no signs of ataxia or motor coordination impairment were observed in the presented study in animals receiving alizarin, betulin, curcumin, diosmin, linalool, menthofuran, α-terpineol, theobromine, β-thujaplicin and vanillin.

Summing up, the pre-clinical screening test of 10 various compounds isolated from herbs and medicinal plants provides evidence that alizarin, betulin, curcumin, diosmin, linalool, menthofuran, theobromine and β-thujaplicin do not possess anti-convulsant properties in the mouse MES model. Only α-terpineol and vanillin are worth considering as potentially favourable therapeutic agents in experimental epileptology.

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