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Short communication

Anticonvulsant effects of four linear furanocoumarins, bergapten, imperatorin, oxypeucedanin, and xanthotoxin, in the mouse maximal electroshock-induced seizure model: a comparative study

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Abstract:
The aim of this study was to determine and compare the anticonvulsant activities of four natural furanocoumarins [bergapten (5-methoxypsoralen), imperatorin (8-isopentenyloxypsoralen), oxypeucedanin (5-epoxy-isopentenyloxypsoralen) and xanthotoxin (8-methoxypsoralen)] in the maximal electroshock-induced seizure test in mice. The anticonvulsant effects of bergapten, imperatorin, oxypeucedanin, and xanthotoxin were evaluated at 15, 30, 60 and 120 min after their systemic (intraperitoneal) administration. Tonic hind limb extension (seizure activity) was evoked in adult albino Swiss mice by a current (sine-wave, 25 mA, 500 V, 50 Hz, 0.2 s stimulus duration) delivered *via* auricular electrodes. The time courses of protection by bergapten, imperatorin, oxypeucedanin and xanthotoxin against maximal electroshock-induced seizures revealed that 300 mg/kg imperatorin and xanthotoxin (C-8 substituted derivatives of psoralen) exerted strong anticonvulsant activity, whereas 300 mg/kg bergapten and oxypeucedanin (C-5 substituted derivatives of psoralen) did not produce any anticonvulsant activity in this model. In conclusion, imperatorin and xanthotoxin protected the animals against maximal electroshock-induced seizures, whereas bergapten and oxypeucedanin, despite their chemical and structural similarities to xanthotoxin and imperatorin, exerted no anticonvulsant activity in this seizure test.

Key words:
bergapten, imperatorin, oxypeucedanin, xanthotoxin, maximal electroshock seizure test

Introduction

For centuries, herbs have been used in traditional medicine to treat a wide range of ailments, including central nervous system disorders such as epilepsy [3, 5, 6, 22–26]. Compelling evidence in preclinical *in vivo* studies has indicated that some naturally occurring compounds isolated from plants possess anticonvulsant properties [2, 3, 5–9, 11, 13, 14, 17–22]. The presence of flavonoids, steroids, triterpenoids and essential oils may be responsible for the anticonvulsant activity of plants [2, 3, 5–9, 11, 13, 14, 17–23, 27].

It has recently been reported that two coumarin derivates (imperatorin and osthole) exerted clear-cut anticonvulsant activity in the maximal electroshock-induced seizure test in mice [17–21]. Because imperatorin and osthole are coumarin derivates with anticonvulsant activity, we wanted to determine whether other coumarin derivatives (such as bergapten, oxypeucedanin and xanthotoxin) exerted anticonvulsant activity in the mouse maximal electroshock-induced seizure test when administered intraperitoneally at various times (i.e., 15, 30, 60, and 120 min) before the initiation of the electroconvulsions. The times used for the administration of the coumarin derivatives in this study were identical to those reported earlier for imperatorin and osthole [17–21]. These times were partly based on information from the NIH Anticonvulsant Drug Development (ADD) Program, which evaluates the anticonvulsant action of novel compounds or agents in preclinical studies in animals [26].

This study was designed to compare the anticonvulsant activities of bergapten, oxypeucedanin and xanthotoxin (belonging to linear furanocoumarins) to imperatorin, which also belongs to a group of linear furanocoumarins and has a firmly established anticonvulsant effect in the mouse maximal electroshock-induced seizure model. Generally, the maximal electroshock-induced seizure test in rodents has been used to detect the anticonvulsant actions of drugs that are clinically used in suppression of tonic-clonic seizures and partial convulsions with or without secondary generalization in humans [15, 16]. It is important to note that seizure models in laboratory animals are still the most important tool in the preclinical search for agents and compounds possessing anticonvulsant activity.

Materials and Methods

Animals and experimental conditions

Six-week-old adult male Swiss albino mice (weighing 22–26 g) were purchased from a licensed breeder (Dr. T. Gorzkowska, Warszawa, Poland). The mice were kept in colony cages (20 mice per cage) with free access to food and tap water under standardized housing conditions (12-h light-dark cycle with a temperature of 23 ± 1°C and a relative humidity of 55 ± 5%). After 7 days of adaptation to laboratory conditions, the mice were randomly assigned to experimental groups, and each group contained 8 mice. Each mouse was only used once, and all tests were performed between 08:00 and 15:00 hours. The total number of mice in this study was 128. Procedures involving animals and their care were conducted in accordance with current European Community and Polish legislation on animal experimentation. In addition, all efforts were made to minimize animal suffering and use only the number of animals necessary to produce reliable scientific data. The experimental protocols and procedures described in this manuscript were approved by the First Local Ethics Committee at the Medical University of Lublin (Licenses nos.: 14/2006; 37/2006; 27/2008) and the Second Local Ethics Committee at the University of Life Sciences in Lublin (License no.: 85/2009). In addition, these procedures complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drugs

Bergapten (5-methoxypsoralen; 4-methoxy-7*H*-furo-[3,2-g]chromen-7-one; $C_{12}H_8O_4$; 216.19 MW; chemical purity > 99%) was extracted from the fruits of *Pastinaca sativa (Apiaceae)*, which were collected in the Medical Plant Garden of the Department of Pharmacognosy, Medical University of Lublin (Poland). The air-dried and powdered fruits were exhaustively extracted with petroleum ether in a Soxhlet apparatus. Next, the petroleum ether extract was concentrated in a rotary vacuum evaporator. A semi-crystalline sediment of furanocoumarins was dissolved in hot dichloromethane before being crystallized with cold *n*-hexane. The obtained sediment was re-crystallized twice in methanol. The identity and purity of bergapten was confirmed by HPLC and ^{1}H-NMR analyses.

Imperatorin (8-isopentenyloxypsoralen; 9-(3-methylbut-2-enyloxy)-7*H*-furo[3,2-g]chromen-7-one; $C_{16}H_{14}O_4$; MW: 270.29; chemical purity > 99%) was extracted from the fruits of *Angelicae archangelica*, which were collected in the second year of vegetation from plants cultivated in the Medical Plant garden of the Department of Pharmacognosy, Medical University of Lublin (Poland). The air-dried and powdered fruits were exhaustively extracted with petroleum ether in a Soxhlet extractor. Afterwards, the petroleum ether extract was concentrated in a rotary vacuum evaporator. A semi-crystalline fraction of furanocoumarins was obtained. Separation of pure imperatorin was achieved by column chromatography using hexane and dichloromethane as eluents. The identity of imperatorin was confirmed by TLC and ^{1}H-NMR analyses.

Oxypeucedanin (5-epoxy-isopentenyloxypsoralen; 4-(3,3-dimethyloxiranyl)methoxy-7*H*-furo[3,2-g]-chromen-7-one; $C_{16}H_{14}O_5$; MW: 286.28; chemical purity > 99%) was extracted from the roots of *Peucedanum ostruthium* (L.) Koch, which were collected from plants in September 2007 in Karpacz Górny (Sudetes, Poland). The air-dried and powdered roots (800 g) were exhaustively extracted (~120 h) with petroleum ether and chloroform in a Soxhlet extractor. Afterwards, the chloroform extract was separated by column chromatography using chloroform/ethyl acetate (95:5, v/v) as the eluent and Silica gel 60 (0.063–0.200 mm; Merck, Darmstadt, Germany) as the sorbent. The eluates were dissolved in methanol and left for final oxypeucedanin precipitation. The precipitated oxypeucedanin was recrystallized, and we obtained 0.6 g of pure oxypeucedanin. The identity of oxypeucedanin was confirmed by TLC and ^{1}H-NMR analyses.

Xanthotoxin (8-methoxypsoralen; 9-methoxy-7*H*-furo[3,2-g]chromen-7-one; $C_{12}H_8O_4$; MW: 216.19, grade RD) was purchased from ChromaDex (Irvine, CA, USA).

Bergapten, imperatorin, oxypeucedanin, and xanthotoxin were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water and administered intraperitoneally as a single injection in a volume of 10 ml/kg body weight at various times (15, 30, 60 and 120 min) before the initiation of electroconvulsions.

Maximal electroshock seizure test

Electroconvulsions were produced by an alternating current stimulation (sine-wave, 25 mA, 50 Hz, 500 V, 0.2 s stimulus duration) delivered *via* ear-clip electrodes by a Rodent Shocker generator (Type 221, Hugo Sachs Elektronik, Freiburg, Germany). The criterion for the occurrence of seizure activity was tonic hind limb extension. The anticonvulsant activity of bergapten, imperatorin, oxypeucedanin, and xanthotoxin (administered at 15, 30, 60 and 120 min) was determined as percent protection against maximal electroshock-induced seizures in mice. The animals were administered a constant dose of 300 mg/kg of each furanocoumarin at different time points, and the percent protection against maximal electroshock-induced seizures was noted for each compound. This experimental procedure was based on the NIH ADD Program, which tests all compounds in the mouse maximal electroshock-induced seizure model at a maximal dose of 300 mg/kg [26]. Thus, all compounds in the present study (bergapten, imperatorin, oxypeucedanin and xanthotoxin) were administered at a fixed dose of 300 mg/kg. Moreover, the treatment times for the tested linear furanocoumarins (i.e., 15, 30, 60 and 120 min) were based upon our previous studies [17–21].

Results

Anticonvulsant actions of bergapten, imperatorin, oxypeucedanin and xanthotoxin against maximal electroshock-induced seizures in mice

Imperatorin and xanthotoxin (300 mg/kg, *ip*) produced a clear-cut anticonvulsant activity in mice at all time points tested (Tab. 1). In contrast, bergapten (300 mg/kg, *ip*) did not produce any anticonvulsant activity in mice, and oxypeucedanin (300 mg/kg, *ip*) only resulted in anticonvulsant activity when it was administered 30 min before the maximal electroshock-induced seizure test (Tab. 1).

Discussion

The present results suggested that the anticonvulsant activity of xanthotoxin and imperatorin in the mouse maximal electroshock-induced seizure model was related to the conformational structure of the compounds. Similarly, the lack of anticonvulsant activity

Tab. 1. Anticonvulsant effects of bergapten, imperatorin, oxypeucedanin, and xanthotoxin in the mouse maximal electroshock-induced seizure model

Time (min)	Bergapten	Imperatorin	Oxypeucedanin	Xanthotoxin
	P/T (%)	P/T (%)	P/T (%)	P/T (%)
15	0/8 (0)	8/8 (100)	0/8 (0)	6/8 (75)
30	0/8 (0)	8/8 (100)	1/8 (12.5)	6/8 (75)
60	0/8 (0)	8/8 (100)	0/8 (0)	8/8 (100)
120	0/8 (0)	7/8 (87.5)	0/8 (0)	5/8 (62.5)

Data are presented as percentage of protection against maximal electroshock-induced seizures (in parentheses). Bergapten, imperatorin, oxypeucedanin and xanthotoxin were administered systemically (intraperitoneally) at a dose of 300 mg/kg at various times (15, 30, 60 and 120 min) prior to the maximal electroshock-induced seizure test. P – number of animals protected against maximal electroshock-induced seizures; T – total of number of animals tested per group

by bergapten and oxypeucedanin confirmed that the conformational structure plays an important role during the evaluation of the antiseizure activity of natural compounds in preclinical studies (Fig. 1). The results also indirectly indicated that both xanthotoxin and imperatorin may act through a specific binding site that allows xanthotoxin and imperatorin to exert their anticonvulsant action in the mouse maximal electroshock-induced seizure model. It is interesting to note that bergapten and xanthotoxin have an identical molecular weight. Indeed, the only difference between the compounds is the position of the methoxy group substituted on the psoralen ring. Interestingly, bergapten and oxypeucedanin, which did not have anticonvulsant activity in the mouse maximal electroshock-induced seizure test, contained substitutions at the C-5 position of the psoralen ring. In contrast, imperatorin and xanthotoxin, which exerted strong anticonvulsant effects in the mouse maximal electroshock-induced seizure model, contained substitutions at the C-8 position of the psoralen ring (Fig. 1). The experimentally derived median effective dose (ED_{50}) for imperatorin was 206 mg/kg, 60 min after drug administration [20]. Similarly, the ED_{50} for xanthotoxin was 243 mg/kg, 60 min after drug administration (results not shown). Because bergapten and oxypeucedanin did not protect the animals against maximal electroshock-induced seizures, it was impossible to determine their ED_{50} values. It is noteworthy that this comparative study was performed at the same time with the same laboratory conditions; therefore, it may be suggested that the specific structural conformation of the tested furanocoumarins played an important role in their anticonvulsant activity. For instance, xanthotoxin (8-methoxypsoralen) produced clear-cut anticonvulsant activity whereas bergapten (5-methoxypsoralen) did not exert any anticonvulsant activity in the mouse maximal electroshock-induced seizure test, although both compounds were administered at identical times prior to the electroshock.

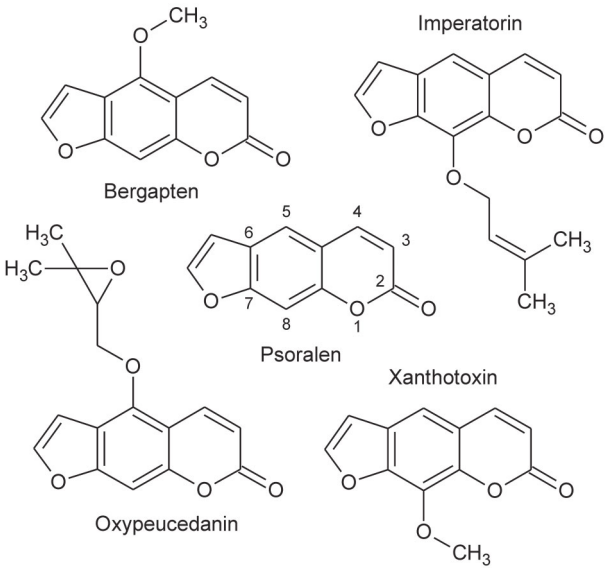

Fig. 1. Structural formulas of bergapten, imperatorin, oxypeucedanin, psoralen and xanthotoxin

Considering the effects produced by imperatorin and xanthotoxin, one could hypothesize that some modifications and/or manipulations of the chemical structures of both compounds at the C-8 position of the psoralen ring might be profitable from a preclinical standpoint. Indeed, these modifications could contribute to new anticonvulsant agents that are much more effective in suppressing tonic-clonic seizures. Interestingly, both imperatorin and xanthotoxin could be readily modified or transformed to novel agents possessing stronger anticonvulsant activity than both natural coumarin derivatives. Nevertheless, more advanced neurochemical and electrophysiological studies are required to confirm our hypothesis that a substitution of the psoralen ring at the C-8 position by an alkyl group creates a compound that could readily exert anticonvulsant activity against maximal electroshock-induced seizures in mice.

It is important to note that none of the tested compounds produced significant changes in the behavior of the mice. In other words, no acute adverse effects (such as ataxia or motor coordination impairment) were associated with administration of the tested compounds. On the other hand, experimental and clinical studies have suggested that some coumarin derivatives possess anti-coagulant effects. For instance, coumarin is a parent molecule of warfarin (a vitamin K antagonist), which is clinically used as an anticoagulant and widely employed as a rodenticide [4]. In addition, bishydroxycoumarin (dicoumarol), which is formed from 4-hydroxycoumarin, is clinically useful as an anticoagulant [4]. With respect to imperatorin, a chronic toxicological study revealed that imperatorin (up to 150 mg/kg) administered orally once daily for 4 consecutive days did not affect blood clotting and had little or no systemic (renal, hepatic or pulmonary) toxicity in mice [10]. Both bergapten and xanthotoxin have been shown to exhibit antiplatelet aggregation activity, but oxypeucedanin had no impact in *in vitro* arachidonic acid- and collagen-induced platelet aggregation models [1, 12].

In conclusion, this study suggested that imperatorin and xanthotoxin, which both contain a substitution at the C-8 position of the psoralen ring, warrant further evaluation as potential therapeutic agents against tonic-clonic seizures. In addition, other furanocoumarins substituted at the C-8 position of the psoralen ring may also have anticonvulsant actions. Future studies of compounds with C-8 substitutions on the psoralen ring could lead to new anticonvulsant therapeutics.

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References:

1. Chen IS, Chang CT, Sheen WS, Teng CM, Tsai IL, Duh CY, Ko FN: Coumarins and antiplatelet aggregation constituents from formosan *Peucedanum japonicum*. Phytochemistry, 1996, 41, 525–530.
2. Elisabetsky E, Brum LF, Souza DO: Anticonvulsant properties of linalool in glutamate-related seizure models. Phytomedicine, 1999, 6, 107–113.
3. Grynkiewicz G, Gadzikowska M: Tropane alkaloids as medicinally useful natural products and their synthetic derivatives as new drugs. Pharmacol Rep, 2008, 60, 439–463.
4. Hoult JR, Paya M: Pharmacological and biochemical actions of simple coumarins: natural products with therapeutic potential. Gen Pharmacol, 1996, 27, 713–722.
5. Huen MS, Leung JW, Ng W, Lui WS, Chan MN, Wong JT, Xue H: 5,7-Dihydroxy-6-methoxyflavone, a benzodiazepine site ligand isolated from *Scutellaria baicalensis* Georgi, with selective antagonistic properties. Biochem Pharmacol, 2003, 66, 125–132.
6. Jastrzębska-Więsek M, Librowski T, Czarnecki R, Marona H, Nowak G: Central activity of new xanthone derivatives with chiral center in some pharmacological tests in mice. Pol J Pharmacol, 2003, 55, 461–465.
7. Kasture VS, Chopde CT, Deshmukh VK: Anticonvulsive activity of *Albizzia lebbeck*, *Hibiscus rosa sinesis* and *Butea monosperma* in experimental animals. J Ethnopharmacol, 2000, 71, 65–75.
8. Kasture VS, Deshmukh VK, Chopde CT: Anxiolytic and anticonvulsive activity of *Sesbania grandiflora* leaves in experimental animals. Phytother Res, 2002, 16, 455–460.
9. Kasture VS, Kasture SB, Chopde CT: Anticonvulsive activity of *Butea monosperma* flowers in laboratory animals. Pharmacol Biochem Behav, 2002, 72, 965–972.
10. Kleiner HE, Vulimiri SV, Starost MF, Reed MJ, DiGiovanni J: Oral administration of the citrus coumarin, isopimpinellin, blocks DNA adduct formation and skin tumor initiation by 7,12-dimethylbenz[a]anthracene in SENCAR mice. Carcinogenesis, 2002, 23, 1667–1675.
11. Kubacka M, Librowski T, Czarnecki R, Frąckowiak B, Lochyński S: Anticonvulsant and antidepressant activity of the selected terpene GABA derivatives in experimental tests in mice. Pharmacol Rep, 2006, 58, 936–943.
12. Lee YY, Lee S, Jin JL, Yun-Choi HS: Platelet anti-aggregatory effects of coumarins from the roots of *Angelica genuflexa* and *Angelica gigas*. Arch Pharm Res, 2003, 26, 723–726.
13. Librowski T, Czarnecki R, Mendyk A, Jastrzębska M: Influence of new monoterpene homologues of GABA on the central nervous system activity in mice. Pol J Pharmacol, 2000, 52, 317–321.
14. Librowski T, Kubacka M, Meusel M, Scolari S, Müller CE, Gütschow M: Evaluation of anticonvulsant and analgesic effects of benzyl- and benzhydryl ureides. Eur J Pharmacol, 2007, 559, 138–149.
15. Löscher W: Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. Epilepsy Res, 2002, 50, 105–123.
16. Löscher W, Fassbender CP, Nolting B: The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. II. Maximal electroshock seizure models. Epilepsy Res, 1991, 8, 79–94.
17. Łuszczki JJ, Andres-Mach M, Cisowski W, Mazol I, Głowniak K, Czuczwar SJ: Osthole suppresses seizures in the mouse maximal electroshock seizure model. Eur J Pharmacol, 2009, 607, 107–109.
18. Łuszczki JJ, Głowniak K, Czuczwar SJ: Imperatorin enhances the protective activity of conventional antiepilep-

tic drugs against maximal electroshock-induced seizures in mice. Eur J Pharmacol, 2007, 574, 133–139.
19. Łuszczki JJ, Głowniak K, Czuczwar SJ: Time-course and dose-response relationships of imperatorin in the mouse maximal electroshock seizure threshold model. Neurosci Res, 2007, 59, 18–22.
20. Łuszczki JJ, Wojda E, Andres-Mach M, Cisowski W, Gleńsk M, Głowniak K, Czuczwar SJ: Anticonvulsant and acute neurotoxic effects of imperatorin, osthole and valproate in the maximal electroshock seizure and chimney tests in mice: A comparative study. Epilepsy Res, 2009, 85, 293–299.
21. Łuszczki JJ, Wojda E, Raszewski G, Głowniak K, Czuczwar SJ: Influence of imperatorin on the anticonvulsant and acute adverse-effect potential of lamotrigine in maximal electroshock-induced seizures and chimney test in mice. Pharmacol Rep, 2008, 60, 566–573.
22. Mendyk A, Salat K, Librowski T, Czarnecki R, Malawska B: Influence of new γ-aminobutyric acid amide derivatives and its phthalimide precursors on the central nervous system activity in mice. Pol J Pharmacol, 2001, 53, 689–693.
23. Minami E, Shibata H, Nomoto M, Fukuda T: Effect of shitei-to, a traditional Chinese medicine formulation, on pentylenetetrazol-induced kindling in mice. Phytomedicine, 2000, 7, 69–72.
24. Samuels N, Finkelstein Y, Singer SR, Oberbaum M: Herbal medicine and epilepsy: proconvulsive effects and interactions with antiepileptic drugs. Epilepsia, 2008, 49, 373–380.
25. Schachter SC: Botanicals and herbs: a traditional approach to treating epilepsy. Neurotherapeutics, 2009, 6, 415–420.
26. Stables JP, Kupferberg HJ: Chapter 16 – The NIH Anticonvulsant Drug Development (ADD) Program: preclinical anticonvulsant screening project. In: Molecular and cellular targets for anti-epileptic drugs. Eds. Avanzini G, Regesta G, Tanganelli P, Avoli M, John Libbey, London, 1997, 191–198.

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