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Influence of osthole on the anticonvulsant activity of phenytoin and valproate in the maximal electroshock-induced seizures in mice

Wpływ ostolu na przeciwdrgawkową aktywność fenytoiny i walproinianu w teście maksymalnego wstrząsu elektrycznego u myszy

INTRODUCTION

The application of plants and herbs in medicine has been well known for ages. At present, researchers are trying to isolate the active compounds from herbs and medicinal plants, which are responsible for their specific pharmacological activities [6]. Recently, there has appeared a trend to isolate the active compounds producing the anticonvulsant action [6].

Generally, the treatment of patients with pharmacoresistant epilepsy is based on the application of two or more drugs possessing anticonvulsant action [8, 9]. It is widely accepted that the new compounds with firmly confirmed anticonvulsant properties in preclinical studies are concomitantly administered with classical antiepileptic drugs (AEDs) [11].

Experimental evidence indicates that imperatorin [9-(3-methylbut-2-enyloxy)-7H-furo[3,2-g] chromen-7-one] – a natural coumarin derivative, possesses the anticonvulsant activity in preclinical studies by elevating the threshold for electroconvulsions [16], and enhancing the anticonvulsant action of several AEDs including: carbamazepine (CBZ), phenobarbital (PB), phenytoin (PHT), and lamotrigine (LTG) in the mouse maximal electroshock (MES)-induced seizure model [15,19]. In contrast, imperatorin did not potentiate the anticonvulsant action of valproate (VPA) in the mouse MES model [12]. It has been found that imperatorin and osthole [7-methoxy-8-(3-methyl-2-butenyl)-2H-1-benzopyran-2-one] – another natural coumarin derivative, exerted clear-cut anticonvulsant

activity against MES-induced seizures in mice [13,18]. Furthermore, experimental evidence indicates that some prenyloxy- and geranyloxy- coumarin derivatives were able to suppress seizures and produced clear-cut anticonvulsant activity in the mouse MES model [5].

Since osthole, imperatorin and some prenyloxy- and geranyloxy- coumarin derivatives exerted the anticonvulsant action in the animals when administered alone, and imperatorin potentiated the anticonvulsant action of some classical AEDs in the mouse MES model, it was of importance to determine whether osthole is also able to enhance the anticonvulsant action of PHT and VPA in this seizure model.

The aim of this study was to evaluate the effects of osthole upon the protective activity of two classical AEDs (PHT and VPA) against MES-induced seizures in mice. It is widely accepted that the MES test is considered as an experimental model of tonic-clonic seizures and, to a certain extent, of partial seizures with or without secondary generalization [11]. Moreover, this experimental model of epilepsy is widely used to investigate novel AEDs and to select the compounds possessing the anticonvulsant activity in preclinical studies [11]. In this model one can readily evaluate the anticonvulsant effects produced by AEDs in combination with osthole, therefore, it was appropriate to use this test in the present study.

Additionally, we investigated the combinations of osthole with PHT and VPA in relation to impairment of motor coordination, long-term memory and muscular strength by the use of the chimney test, step-through passive avoidance task and grip-strength test, respectively. Finally, to ascertain any pharmacokinetic contribution to the observed interactions between tested drugs, total brain concentrations of PHT and VPA were determined.

MATERIALS AND METHODS

A n i m a l s a n d e x p e r i m e n t a l c o n d i t i o n s. Adult male Swiss mice (weighing 22 – 26g) that were kept in colony cages with free access to food and tap water, under standardized housing conditions (natural light-dark cycle, temperature of 23 ± 1ºC, relative humidity of 55 ± 5%), were used. After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups comprising 8 mice. Each mouse was used only once and all tests were performed between 08.00 and 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 this manuscript were approved by the Second Local Ethics Committee at the University of Life Sciences in Lublin (License no. 78/2009) and complied with the European Community Council Directive of 24 November 1986 (86/609/EEC).

D r u g s. The following AEDs were used in this study: PHT (Polfa, Warszawa, Poland) and VPA (magnesium salt; kindly donated by ICN-Polfa S.A., Rzeszow, Poland). PHT was suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water, while VPA was directly dissolved in distilled water. All drugs were administered intraperitoneally (i.p.) as a single injection, in

a volume of 5 ml/kg body wt. Fresh drug solutions were prepared on each day of experimentation and administered as follows: PHT was administered 120 min and VPA – 30 min before electroconvulsions, motor coordination, grip-strength and long-term memory tests, as well as before brain sampling for the measurement of AED concentrations. The pretreatment times before testing of PHT and VPA in the mouse MES model and all behavioral tests were based upon information about their biological activity from the literature and our previous experiments [13, 15, 18]. Moreover, these pretreatment times were considered as the times to the peak of maximum anticonvulsant effects for the studied AEDs. Osthole ([7-methoxy-8-(3-methyl-2-butenyl)-2H-1-benzopyran-2-one]; C15H16O3; 244.29 MW; chemical purity >97%) was extracted from roots of Peucedanum ostruthium (L.) Koch, which were collected from plants in September 2007, in Karpacz Gorny (Sudetes, Poland). The air-dried and powdered roots (920 g) were extracted exhaustively (~120 h) in the Soxhlet extractor with petroleum ether. After extraction and cooling procedure osthole was crystallized and drained off. The petroleum ether extract was concentrated in a rotary vacuum evaporator. The remains were dissolved in methanol and left for final osthole precipitation (40 g of remains were dissolved in 200 ml of boiling methanol). Osthole obtained from the methanol extract was added to that from petroleum ether and recrystallized by obtaining 10 g of pure osthole. The identity of osthole was confirmed by TLC and 1H-NMR analyses. Osthole was suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water and administered i.p. at 30 min before the initiation of electroconvulsions and all the behavioural tests.

M a x i m a l e l e c t r o s h o c k s e i z u r e t e s t. Electroconvulsions were produced by means of an alternating current (0.2 s stimulus duration, 50 Hz, maximum stimulation voltage of 500 V, fixed current intensity of 25 mA) 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 the tonic hind limb extension (i.e. the hind limbs of animals outstretched 180° to the plane of the body axis). The protective activity of PHT and VPA was determined as their median effective doses (ED50 values in mg/kg) against MES-induced seizures. The animals were administered with different drug doses so as to obtain a variable percentage of protection against MES, allowing the construction of a dose-response relationship curve for each AED administered alone, according to Litchfield and Wilcoxon [10]. Each ED50 value represents the dose of a drug required to protect half of the animals tested against maximal electroshock seizures. Similarly, the anticonvulsant activity of a mixture of an AED with osthole was evaluated and expressed as ED50 corresponding to the dose of an AED necessary to protect 50% of mice against tonic hindlimb extension in the MES test. In the present study, to determine the ED50 values of PHT and VPA, the AEDs were administered i.p. at the following dose ranges: PHT, 6–14 mg/kg and VPA, 175–300 mg/kg.

C h i m n e y t e s t. The chimney test of Boissier et al. [1] was used to quantify the adverse effect potential of two classical AEDs administered in combination with osthole on motor performance in mice. In this test, the animals had to climb backwards up a plastic tube (3 cm

inner diameter, 25 cm length), and motor performance impairment was indicated by the inability of the mice to climb backward up the transparent tube within 60 s. The acute adverse effect potentials of two classical AEDs administered in combination with osthole were determined for AEDs administered at doses corresponding to their ED50 values from the MES test when combined with osthole at a dose of 150 mg/kg.

Grip–strength test. The effects of combinations of osthole (at a dose of 150 mg/kg) with two classical AEDs at their ED50 values from the MES test, on skeletal muscular strength in mice were quantified by the grip-strength test of Meyer et al. [20]. The time before the commencement of the grip-strength test (after drug administration) was identical to that for the MES test. The grip-strength apparatus (BioSeb, Chaville, France) comprised a wire grid (8 x 8 cm) connected to an isometric force transducer (dynamometer). The mice were lifted by the tails so that their forepaws could grasp the grid. The mice were then gently pulled backward by the tail until the grid was released. The maximal force exerted by the mouse before losing grip was recorded. The mean of 3 measurements for each animal was calculated and subsequently, the mean maximal force of 8 animals per group was determined. The neuromuscular strength in mice was expressed in Newtons (N) as means ± standard error (S.E.) of at least 8 determinations.

Light–dark, step–through passive avoidance task. Each animal was administered an AED either singly or in combination with osthole on the first day before training. The acute adverse effect potentials of two classical AEDs administered in combination with osthole were determined for AEDs administered at doses corresponding to their ED50 values from the MES test when combined with osthole at a dose of 150 mg/kg. The time before the commencement of the training session (after drug administration) was identical to that for the MES test. Subsequently, animals were placed in an illuminated box (10 x 13 x 15 cm) connected to a larger dark box (25 x 20 x 15) equipped with an electric grid floor. Entrance of animals to the dark box was punished by an adequate electric footshock (0.6 mA for 2 s). The animals that did not enter the dark compartment were excluded from subsequent experimentation. On the following day (24 h later), the pre-trained animals were placed again into the illuminated box and observed up to 180 s. Mice that avoided the dark compartment for 180 s were considered to remember the task. The time that the mice took to enter the dark box, was noted and the median latencies (retention times) with 25th and 75th percentiles were calculated. The step-through passive avoidance task gives information about ability to acquire the task (learning) and to recall the task (retrieval). Therefore, it may be regarded as a measure of long-term memory [21].

Measurement of total brain antiepileptic drug concentrations. The measurement of total brain concentrations of PHT and VPA was undertaken at doses of the AEDs, which corresponded to an interaction of PHT and VPA with osthole (150 mg/kg) in the MES test. Mice were killed by decapitation at times chosen to coincide with that scheduled for the maximal electroshock test and the whole brains of mice were removed from skulls, weighed, and homogenized using Abbott buffer (1:2 weight/volumen) in an Ultra-Turrax T8 homogenizer (Staufen,

Germany). The homogenates were centrifuged at 10,000 g for 10 min. The supernatant samples (75 µl), appropriately diluted so as to fall within the linear concentration range, were analyzed for PHT or VPA content by fluorescence polarization immunoassay (FPIA) using a TDx analyzer and reagents exactly as described by the manufacturer (Abbott Laboratories, North Chicago, IL, USA). The total brain concentrations of PHT and VPA were expressed in µg/ml of brain supernatant as means ± S.E. of at least 8 determinations (separate brain preparations).

S t a t i s t i c s. The ED50 values with their 95% confidence limits were calculated by computer log-probit analysis according to Litchfield and Wilcoxon [10]. Subsequently, the respective 95% confidence limits were transformed to standard errors (S.E.) as described previously [14]. Statistical analysis of data from the MES test was performed with one-way analysis of variance (ANOVA) followed by the post-hoc Tukey-Kramer test for multiple comparisons. Qualitative variables from the chimney test were compared by use of the Fisher's exact probability test, whereas, the results obtained in the passive avoidance task were statistically evaluated using Kruskal-Wallis nonparametric ANOVA. The results from the grip-strength test were verified with one-way ANOVA. The total brain concentrations of PHT and VPA administered alone or in combination with osthole were statistically analyzed using the unpaired Student's t-test. All statistical tests were performed using GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA). Differences among values were considered statistically significant if $P<0.05$.

RESULTS

Effects of osthole on the protective action of phenytoin and valproate in the mouse maximal electroshock-induced seizure model

The investigated classical AEDs (PHT and VPA) administered alone exhibited a clear-cut anticonvulsant activity in the MES test in mice (Figures 1 and 2). When osthole at doses of 50, 100 and 150 mg/kg was co-administered with PHT, it did not significantly enhance the anticonvulsant effect of the latter drug against MES-induced seizures in mice (Figure 1). Similarly, osthole at doses of 50, 100 and 150 mg/kg had no impact on the protective action of VPA against MES-induced seizures in mice (Figure 2). In all cases, the osthole-induced reduction in ED50 values of two classical AEDs did not attain statistical significance with one-way ANOVA (Figures 1 and 2).

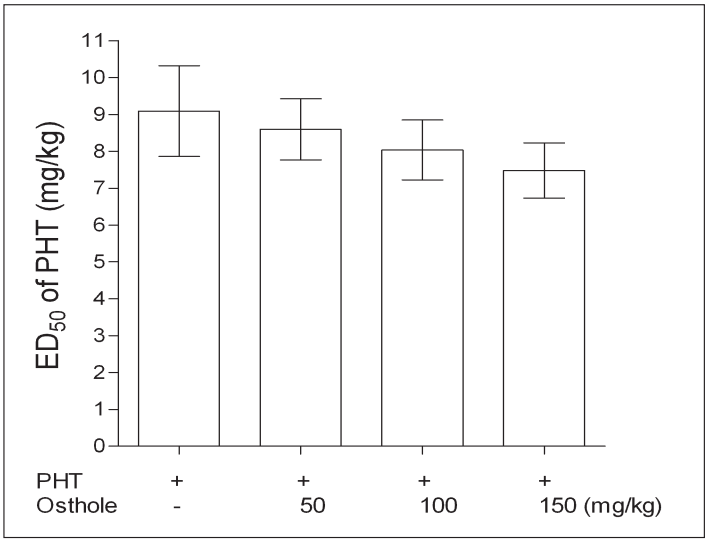

Fig. 1. Effect of osthole on the anticonvulsant action of phenytoin (PHT) in the mouse maximal electroshock (MES)-induced seizure model

Results are presented as median effective doses (ED50 in mg/kg ± S.E.) of PHT, protecting 50% of animals tested against MES-induced hindlimb extension. The drugs were administered i.p.: PHT at 120 min. and osthole at 30 min. prior to the MES test. Statistical analysis of data was performed with one-way ANOVA followed by the post-hoc Tukey-Kramer test for multiple comparisons.

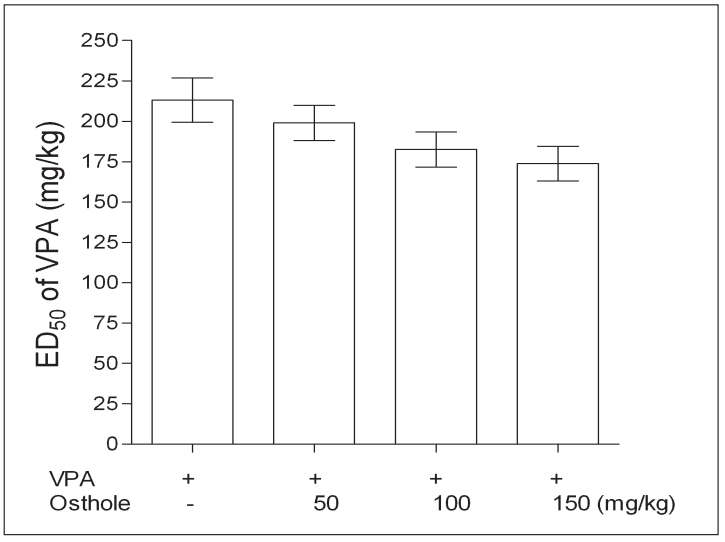

Fig. 2. Effect of osthole on the anticonvulsant action of valproate (VPA) in the mouse maximal electroshock (MES)-induced seizure model

Results are presented as median effective doses (ED50 in mg/kg ± S.E.) of VPA, protecting 50% of animals tested against MES-induced hindlimb extension. The drugs were administered i.p.: VPA and osthole at 30 min. prior to the MES test. Statistical analysis of data was performed with one-way ANOVA followed by the post-hoc Tukey-Kramer test for multiple comparisons.

Effects of osthole in combination with phenytoin and valproate on motor performance, long-term memory, and muscular strength of animals in the chimney, step-through passive avoidance and grip-strength tests

When osthole (150 mg/kg) was administered in combination with PHT and VPA at doses corresponding to their ED50s from the MES test, motor performance as assessed by the chimney test was unaffected (Table 1). Furthermore, none of the combinations studied significantly impaired long-term memory as determined in the passive avoidance test (Table 1). Likewise, osthole (150 mg/kg) combined with PHT and VPA had no significant impact on muscular strength of animals as assessed by the grip-strength test (Table 1).

Table 1. Effects of osthole and its combination with phenytoin (PHT) and valproate (VPA) on long-term memory, muscular strength and motor performance in mice

Treatment (mg/kg)	Retention time (s)	Grip-strength (N)	Impairment of motor coordination (%)
Control	180 (180; 180)	102.54 ± 5.90	0
Osthole (150) + vehicle	180 (180; 180)	97.54 ± 5.27	0
PHT (7.48) + osthole (150)	180 (180; 180)	98.49 ± 5.54	0
VPA (173.8) + osthole (150)	175 (155.3; 180)	98.33 ± 5.32	0

Results are presented as: 1) median retention times (in seconds; with 25th and 75th percentiles in parentheses) from the passive avoidance task, assessing long-term memory in mice; 2) mean grip-strengths (in Newtons ± S.E.) from the grip-strength test, assessing muscular strength in mice; and 3) percentage of animals showing motor coordination impairment in the chimney test in mice. Statistical analysis of data from the passive avoidance task was performed with nonparametric Kruskal-Wallis ANOVA test, whereas those from the grip-strength test were analyzed with one-way ANOVA. The Fisher's exact probability test was used to analyze the results from the chimney test. All drugs were administered i.p. at times scheduled from the MES test and at doses corresponding to the ED50 values of AEDs from the MES test. For more details see the legends to Figures 1 and 2.

Influence of osthole on total brain concentrations of phenytoin and valproate

The administration of osthole (150 mg/kg) and PHT (7.48 mg/kg) was associated with no significant change in total brain PHT concentrations when compared to animals administered PHT alone (Table 2). Similarly, total brain concentrations of VPA administered at a dose of 173.8 mg/kg were unaffected following the i.p. administration of osthole at 150 mg/kg (Table 2).

Table 2. Total brain concentrations of phenytoin (PHT) and valproate (VPA) administered singly or in combination with osthole.

Treatment (mg/kg)	Brain concentration (μg/ml)
PHT (7.48) + vehicle	0.51 ± 0.18
PHT (7.48) + osthole (150)	0.65 ± 0.17
VPA (173.8) + vehicle	108.2 ± 13.1
VPA (173.8) + osthole (150)	119.8 ± 15.5

Results are presented as means (in μg/ml ± S.E. of 8 determinations) of total brain PHT and VPA concentrations. Statistical evaluation of data was performed using the unpaired Student's t-test. Brain tissue samples were taken at times scheduled for the MES test and the total brain AED concentrations were quantified using fluorescence polarization immunoassay. For more details see the legends to Figures 1 and 2.

DISCUSSION

Results presented in this study indicate that osthole administered systemically (i.p.) at doses up to 150 mg/kg had no significant impact on the anticonvulsant action of two classical AEDs (PHT and VPA) in the mouse MES model. It was found that osthole reduced the ED50 values of two classical AEDs, although statistical analysis of data with one-way ANOVA followed by the post-hoc Tukey-Kramer test revealed no significance between these ED50 values. Previously, it has been documented that osthole administered i.p. exerted a clear-cut anticonvulsant action in the mouse MES model [13,18]. Moreover, it has been reported that imperatorin (a naturally occurring coumarin) enhanced the anticonvulsant action of CBZ, PHT, PB and LTG, but not that of VPA in the mouse MES model [15,19]. Considering the above-mentioned facts, one can ascertain that osthole has a narrower anticonvulsant spectrum of action than imperatorin, because osthole did not affect the protective action of PHT, whereas imperatorin significantly enhanced the anticonvulsant action of PHT in the mouse MES model [15].

Experimental studies have revealed that osthole inhibited L-type calcium channels [23], and positively modulated N-type and P/Q-type calcium channels in rat hippocampal nerve terminals [22]. Moreover, in in vitro studies, it has been found that osthole is a low affinity GABAA receptor ligand [24]. It seems that osthole produces the anticonvulsant action by modulating calcium channels and affecting the inhibitory GABA neurotransmission in the brain.

Bearing in mind the fact that osthole possesses some L-type calcium channel blocking properties, we compared the interactions of three well-known L-type calcium channel blockers (i.e. amlodipine, diltiazem and verapamil) with the two studied AEDs (PHT and VPA) in the mouse MES model. It was found that amlodipine administered i.p. at a dose of 10 mg/kg significantly potentiated the anticonvulsant action of VPA, but not that of PHT in the mouse MES model [7]. In case of diltiazem, the calcium channel blocker administered i.p. at a dose of 2.5 mg/kg significantly enhanced the antiseizure activity of PHT and VPA in the mouse MES model [3]. In contrast, verapamil administered i.p. up to 10 mg/kg had no impact on the anticonvulsant action of PHT and VPA against MES-induced seizures in mice [3]. Thus, comparing the interaction profiles of osthole with well-known L-type calcium channel blockers, one can ascertain that osthole possesses a similar profile as verapamil in the mouse MES model.

On the other hand, considering the fact that osthole modulates N-type and P/Q-type calcium channels, one should compare the interaction profile of PHT and VPA with gabapentin (GBP) and pregabalin (PGB) – two AEDs, whose anticonvulsant action is related with the blockade of $\alpha 2\delta$ subunit of N-type and P/Q-type calcium channels [4]. Experimental studies have revealed that GBP interacted synergistically with PHT and VPA in the mouse MES model [2]. With respect to PGB, the AED in the mouse MES model interacted additively with PHT [12] and VPA [unpublished data]. Thus, one can suggest that the anticonvulsant action of osthole is quite similar to that of PGB in the mouse MES model. However, it should be mentioned that the evaluation of interactions between PGB and GBP with PHT and VPA were performed with isobolographic analysis of interaction, whereas the interactions of osthole with PHT and VPA in this study were determined with a subthreshold method.

Moreover, in our previous study, we reported that imperatorin administered i.p. at a dose of 40 mg/kg significantly potentiated the anticonvulsant action of PHT, but not that of VPA in the mouse MES model [15]. It seems that the interaction profile of osthole considerably differs from that of imperatorin in the mouse MES model.

Pharmacokinetic evaluation of total brain concentrations of PHT and VPA after administration of osthole revealed that the naturally occurring coumarin had no significant effect on concomitantly administrated AEDs in this study. Thus, we documented that osthole produced pharmacodynamic interactions with PHT and VPA in the mouse MES model. Previously, it has been reported that only total brain AED concentrations adequately characterize interaction profile between AEDs [15,17]. In other words, the concentrations of AEDs should be measured only in the brain tissue, i.e. in the place where the AEDs exert their anticonvulsant activity. It seems that the evaluation of total brain AED concentrations in this study perfectly characterized pharmacological interactions that occurred between PHT, VPA and osthole in the mouse MES model.

As regards the acute adverse effects produced by osthole in combination with classical AEDs, it should be stressed that only acute adverse effects produced by the combinations were evaluated in this study. Thus, the effects of the drug combinations on long-term memory, motor performance and muscular strength in mice were determined in the passive avoidance, chimney and grip-strength tests, respectively. Other effects were not examined in this study, although the combinations might theoretically affect various systems of living organisms, not only the central nervous system. The evaluation of acute adverse-effect potentials of osthole in combination with two classical AEDs (PHT and VPA) revealed that osthole, similarly to imperatorin, did not affect long-term memory, motor performance or muscular strength in mice. Thus, one can suggest that the combined therapy of osthole with classical AEDs is devoid of any acute side effects.

It is important to note that osthole was used in our study at doses that per se exerted neither the anticonvulsant action, nor acute adverse effects. Previously, we determined the median effective dose (ED50 value) for osthole in the mouse MES model, which was 253 mg/kg [18]. On the other hand, the median toxic dose (TD50 value) for osthole was 583 mg/kg in the chimney test [18]. Thus, it is impossible that the naturally occurring coumarin administered at doses up to 150 mg/kg produced neurotoxic effects in mice.

Considering the fact that osthole administered i.p. at doses up to 150 mg/kg did not affect the anticonvulsant action of PHT and VPA against MES-induced seizures, one can ascertain that osthole produced neutral pharmacodynamic interaction when combined with PHT and VPA in the mouse MES model. It is highly likely that osthole could potentiate the anticonvulsant action of some classical AEDs in other experimental models of epilepsy, especially, in limbic psychomotor (6Hz) or PTZ-induced clonic seizure models. To confirm this hypothesis more advanced studies are required.

CONCLUSIONS

The results presented herein indicate that osthole exerts neutral pharmacodynamic interactions with two classical AEDs (PHT and VPA) in the mouse MES model. Osthole produces no acute adverse effects when combined with the classical AEDs in the mice and has no impact on total brain AED concentrations. It is likely that the combinations of osthole with PHT and VPA could be advantageous in other epilepsy models.

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SUMMARY

Accumulating evidence indicates that some naturally occurring substances extracted from plants and herbs possess anticonvulsant properties. One of these substances is osthole isolated from Peucedanum ostruthium (L.) Koch. Therefore, the aim of this study was to determine the effect of osthole on the anticonvulsant activity of two classical antiepileptic drugs (AEDs: phenytoin [PHT] and valproate [VPA]) in the mouse maximal electroshock seizure (MES) model. Electroconvulsions (tonic-clonic seizures) were evoked in adult Albino Swiss mice by a current (25mA, 50Hz, 500V, 0.2s stimulus duration,) delivered via auricular electrodes. Adverse-effect profiles of the combination of osthole with PHT and VPA with respect to motor performance, long-term memory and skeletal muscular strength were measured. Total brain concentrations of PHT and VPA were estimated to characterize the interaction profile between osthole and classical AEDs. Results indicate that osthole administered intraperitoneally (i.p.) at doses of 50, 100 and 150 mg/kg did not significantly affect the

protective action of PHT and VPA in the MES test in mice. Moreover, osthole in combination with PHT and VPA did not alter motor performance, long-term memory or skeletal muscular strength in experimental animals. Additionally, osthole had no significant impact on total brain concentrations of PHT and VPA in mice. The present study demonstrates that osthole had no significant effect on the anticonvulsant action of PHT and VPA in the mouse MES model. If the results from this study could be extrapolated into clinical settings, osthole combined with PHT and VPA would offer neutral pharmacodynamic interaction.

Keywords: Osthole, phenytoin, valproate, maximal electroshock seizure test

STRESZCZENIE

Liczne dane naukowe wskazują, że niektóre naturalnie występujące substancje wyizolowane z roślin i ziół posiadają właściwości przeciwdrgawkowe. Jedną z tych substancji jest ostol izolowany z Peucedanum ostruthium (L.) Koch. Dlatego też, celem pracy była ocena wpływu ostolu na przeciwdrgawkowe działanie dwóch klasycznych leków przeciwpadaczkowych (LPP: fenytoiny [PHT] i walproinianu [VPA]) w modelu maksymalnego wstrząsu elektrycznego (MES) u myszy. Drgawki elektryczne (toniczno-kloniczne) były wywoływane u dorosłych samców myszy Albino Swiss prądem (25mA, 50Hz, 500V, 0,2s czas trwania impulsu) przez elektrody uszne. Profil ostrych działań niepożądanych kombinacji ostolu z PHT i VPA oceniono w odniesieniu do koordynacji ruchowej, pamięci długotrwałej i siły mięśni szkieletowych u myszy. Całkowite stężenia PHT i VPA oceniano, aby scharakteryzować profil interakcji pomiędzy ostolem a klasycznymi LPP. Wyniki wskazują, że ostol podany dootrzewnowo (i.p.) w dawkach 50, 100 i 150 mg/kg nie wpływał istotnie na ochronne działanie PHT i VPA w teście MES u myszy. Ponadto, ostol w kombinacji z PHT i VPA nie zmieniał koordynacji ruchowej, pamięci długotrwałej i siły mięśni szkieletowych u badanych zwierząt. Dodatkowo ostol nie miał istotnego wpływu na całkowite stężenia mózgowe PHT i VPA u myszy. Bieżące badanie wykazało, że ostol nie miał istotnego wpływu na przeciwdrgawkowe działanie PHT i VPA w modelu MES u myszy. Jeśli wyniki z tego badania mogłyby być ekstrapolowane do warunków badań klinicznych, ostol w kombinacji z PHT i VPA mógłby oferować neutralną interakcję farmakodynamiczną.

Słowa kluczowe: Ostol, fenytoina, walproinian, maksymalny wstrząs elektryczny