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No effect of water extract of *Scutellariae radix* on the anticonvulsant action of valproate, tiagabine and topiramate in two animal models of epilepsy

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Abstract: Water extract of *Scutellariae radix* (SR) was previously reported to display a significant anticonvulsant effect on maximal electroshock (MES)-induced seizures, and little anticonvulsant effect in pentylenetetrazole (PTZ)-induces clonic seizure model in mice. The aim of this study was to examine whether water extract of SR has any impact on anticonvulsant properties of valproate (VPA) and tiagabine (TGB) in PTZ-induced clonic seizure model in mice and VPA and topiramat (TPM) in the mouse MES test. Results indicated that water extract of SR did not significantly affect the anticonvulsant action of VPA and TPM against MES-induced tonic seizures. The experimentally derived median effective doses (ED_{50} values) for VPA administered alone and in combination with water extract of SR were 219 and 203 mg/kg and for TPM administered alone, and in combinations with water extract of SR were 41.6 and 40.8 mg/kg, respectively. Likewise, no effect of water extract of SR was observed on VPA and TGB in PTZ-induced clonic seizure models. The ED_{50} values for TGB administered alone and in combination with water extract of SR were 0.84 and 0.71 mg/kg, while those for VPA were 158 and 129 mg/kg, respectively. In conclusion, water extract of SR had no effect on the anticonvulsant activity of TPM and VPA in the mouse MES seizure model and on the antiseizure activity of VPA and TGB in PTZ-induced clonic seizures in mice.

Key word: water extract *Scutellariae radix*, valproate, topiramate, tiagabine, pentylenetetrazole, maximal electroshock

INTRODUCTION

Baicalin – a flavonoid isolated from the dried root of the Chinese herbal drug *Scutellariae radix* (SR) – has been widely used for centuries in traditional Chinese herbal medicine to treat allergic and inflammatory diseases [1]. It has been shown that baicalin has multiple biological activities, including anti-viral [2], anti-thrombotic [3], anti-oxidant [4] and anti-tumour activities [5, 6]. Baicalin was previously reported to induce anxiolytic-like effect devoid of sedation and myorelaxation in mice, acting through the γ-aminobutyric acid ($GABA_A$) receptor–benzodiazepine–chloride ionophor complex. In contrast to diazepam (a classical benzodiazepine), baicalin showed a significant affinity to α_2 and α_3 containing $GABA_A$ receptor subtypes compared to α_1 and α_5, which may suggest the selective anxiolytic profile of baicalin. It has been demonstrated that water extract of baicalin produced significant anticonvulsant effects in the maximal electroshock (MES)-induced tonic seizures, and little effect against pentylenetetrazole (PTZ)-induced clonic seizures in mice [7]. In contrast to the water extract of baicalin, the benzodiazepine agonist chlordiazepoxide had anticonvulsant activity in both models of epilepsy. These results suggest that baicalin might be active via the prevention of seizure spread [8].

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Due to its anticonvulsant effect in animal models of epilepsy, it was interesting to examine whether "*SR water extract*" affects the antiseizure properties of some selected AEDs in the PTZ-induced clonic seizures and MES-induced tonic seizures in mice. The MES test is thought to be an experimental animal model of generalized tonic-clonic seizures and, to a certain extent, of partial convulsions with or without secondary generalization in humans [9]. The PTZ model is considered to be an experimental pattern of epilepsy in which the antiepileptic drugs (AEDs) effective against myoclonic and, to a certain extent, against absence seizures in humans, also protect experimental animals against the clonic phase of PTZ-induced seizures [9, 10]. The AEDs chosen in these experiments have a wide spectrum of anticonvulsant activity: topiramate (TPM) is effective in MES-induced tonic seizures, tiagabine (TGB) in PTZ test, and valproate (VPA) has a very high anticonvulsant activity in both animal models of epilepsy.

In considering the anticonvulsant properties of "*SR water extract*" we expected to find its synergistic influence on AEDs used in the experiments. It is known that each additional AED necessary in refractory epilepsy has the risk of developing more side effects. Positive results with using "*SR water extract*" in combination with AEDs, such as lowering ED_{50} and the lack of side effects would be a great opportunity for all patients with refractory epilepsy

MATERIALS AND METHODS

Animals. Adult male Swiss mice weighting 22-26g were used in this study. They were kept in colony cages with free access to food and tap water under standardized conditions (natural light-dark cycle, temperature 21±ºC, relative humidity 55±%). After a week of acclimatisation to experimental conditions, the animals were randomly assigned to experimental groups of 8 mice each. Each mouse was used only once. All tests were performed between 09:00-14:00. Procedures involving animals and their care conformed to 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 listed below conformed to the *Guide for the Care and Use of Laboratory Animals* and approved by the II Local Ethics Committee at the University of Life Sciences in Lublin.

Plant material and water extract. Dry extract of the root of *Radix Scutellariae baicalensis* (SR) was obtained from the Medical University in Wroclaw, Poland. Ground up root was boiled twice for 10 min in 6-fold volume of distilled water and then centrifuged at 12,000 × g for 5 min. Supernatant was filled with distilled water to obtain a solution containing 5 g/kg dry weight of SR. The supernatant referred to as "*SR water extract*" (BAI) was used in this study.

Drugs. The following AEDs were used in this study: valproate (VPA – magnesium salt (donated by ICN-Polfa Rzeszów SA, Poland), topiramate (TPM; Topamax®, Cilag AG, Schaffhausen, Switzerland), tiagabine (TGB; Gabitril®, Sanofi Winthrop, Gentilly, France). All drugs, except for VPA, were suspended in a 1% aqueous solution of Tween 80 (Sigma Aldrich, St. Louis, MO, USA) in saline, whereas VPA was dissolved in 0.9% NaCl. Drugs were administered intraperitoneally (i.p.) in a volume of 5ml/kg of body weight. Fresh drug solutions were prepared on each day before experiments and administered as follows: TPM–60 min, VPA–30 min, and TGB–15 min before experiments. These pretreatment times were based on the biologic activity of the AEDs from the literature and confirmed in our previous experiments [11, 12, 13]. The "*SR water extrac"t* was administered i.p. at 60 min before the seizure initiation in the MES and PTZ tests.

Maximal electroshock seizure test. Electroconvulsions were produced by means of an alternating current (0.2s; 25mA; 500V; 50Hz) delivered via ear-clip electrodes by a generator (Rodent Shocker, Type 221; Hugo Sachs, Freiburg, Germany). The criterion for the occurrence of seizure activity was the tonic hindlimb extension. The protective activities of VPA and TPM administered alone and in combination with *"SR water extract"* against MES-induced seizures were evaluated as their median effective doses (ED_{50} values in mg/kg) according to the log-probit method by Litchfield and Wilcoxon [14]. The ED_{50} value reflects the dose of an AED which protects 50% of animals against tonic hindlimb extension in the mouse MES model. At least 4 groups of animals (8 mice per group) were used to estimate each ED_{50} value for the AEDs. The *"SR water extract"* was administered at a constant dose of 5g/kg b.w.

Pentylenetetrazole seizure test. The anticonvulsant effect of TGB and VPA administered alone and in combination with *"SR water extract"* against PTZ-induced clonic seizures were determined after s.c. administration of PTZ at its CD_{97} (90.09 mg/kg). The animals were treated with increasing doses of the AEDs and the anticonvulsant activity of each drug was separately evaluated as its ED_{50} value (i.e., the dose of an AED protecting 50% of mice against PTZ-induce clonic convulsions). At least 4 groups of animals (8 mice per group) were used to estimate each ED_{50} value for AEDs, calculated from the respective dose-response curves, according to the log-probit method of Litchfield and Wilcoxon [14].

Statistics. ED_{50} values with their 95% confidence limits were calculated by computer log-probit analysis according to Litchfield and Wilcoxon (1949). Differences among values were considered statistically significant if $P<0.05$.

RESULTS

Influence of *"SR water extract"* on the anticonvulsant effect of VPA and TPM in the mouse MES model. TPM and VPA administered singly were associated with effective anticonvulsant activity in the MES test; their ED_{50} values are presented in Table 1. The combination of *"SR water extract"* with TPM and VPA did not significantly alter the ED_{50} values of the studied AEDs (Table 1). The experimentally derived ED_{50} value for TPM in combination with *"SR water extract"* was 40.8 (32.8 - 50.8) mg/kg, and for VPA in combination with *"SR water extract"* – 203 (165 - 250) mg/kg (Table 1).

Table 1 Influence of *"SR water extract"* on the anticonvulsant effect of VPA and TPM in mouse MES test.

Treatment	ED_{50}	n	SE
VPA + vehicle	219 (181 - 265)	32	21.11
VPA + *SR water extract*	203 (165 - 250)	32	21.64
TPM + vehicle	41.6 (31.2 - 55.5)	24	6.11
TPM + *SR water extract*	40.8 (32.8 - 50.8)	24	4.57

Results are shown as median effective doses (ED_{50} in mg/kg; with 95% confidence limits in parentheses) of VPA and TPM that protected 50% of animals tested against MES-induced seizures. VPA was administered i.p. at 30 min and TPM at 60 min prior to the MES test. *"SR water extract"* was administered i.p. at a constant dose of 5g/kg b.w. Statistical evaluation data was performed with log-probit method according to Litchfield and Wilcoxon [14].
n – total number of animals used at doses where anticonvulsant effects ranged between 4-6 probits;
SE – standard error of ED_{50} values.

Influence of *"SR water extract"* on the anticonvulsant effect of VPA and TGB in the mouse PTZ test. TGB and VPA administered alone displayed a clear-cut anticonvulsant effect against PTZ-induced clonic seizures in mice; their ED_{50} values are presented in Table 2. The *"SR water extract"* administered at a constant dose of 5g/kg b.w. in combination with TGB and VPA did not significantly alter the antiseizure activity of the studied AEDs (Table 2). The ED_{50} value for VPA in combination with BAI was 129 (109 - 153) mg/kg, and for TGB with *"SR water extract"* - 0.77 (0.59-0.99) mg/kg (Table 2).

Table 2 Influence of *"SR water extract"* on the anticonvulsant effect of VPA and TGB against PTZ-induced clonic seizures in mice.

Treatment	ED_{50}	n	SE
VPA + vehicle	158 (138 - 181)	32	10.95
VPA + *SR water extract*	129 (109 - 153)	32	11.20
TGB + vehicle	0.84 (0.64-1.10)	16	0.117
TGB + *SR water extract*	0.77 (0.59-0.99)	16	0.102

Results are shown as median effective doses (ED_{50} in mg/kg with 95% confidence limits in parentheses) of VPA and TGB that protected 50% of animals against PTZ-induced clonic seizures in mice. VPA was administered i.p. at 30 min and TGB at 15 min prior to the PTZ test. The PTZ-induced seizures were produced by s.c. injection of PTZ at its CD_{97} value (90.09 mg/kg). *"SR water extract"* was administered i.p. at a constant dose of 5g/kg b.w. Statistical evaluation data was performed with log-probit method according to Litchfield and Wilcoxon [14].
n – total number of animals used at doses where anticonvulsant effects ranged between 4 - 6 probits;
SE – standard error of ED_{50} values.

DISCUSSION

The results presented indicate that *"SR water extract"* administered i.p. at a dose of 5g/kg did not significantly alter the anticonvulsant effect of VPA and TGB in PTZ test as well as that of VPA and TPM in the MES test in mice. These results are in contrast to those obtained previously by Wang et al. [7], who found that *"SR water extract"* significantly prevented MES-induced tonic seizures and death, but had little effect on PTZ-induced clonic seizures in mice. It is known from previous studies that *"SR water extract"* shows a high affinity to the benzodiazepine binding site within the $GABA_A$ receptor-benzodiazepine-chloride ionophor complex [15]. As mentioned in the Introduction, the anticonvulsant effect of baicalin is different in comparison to chlordiazepoxide (a benzodiazepine agonist). It has been reported that chlordiazepoxide has anticonvulsant activity in both the MES and PTZ seizure models [7]. In $^{36}Cl^-$ uptake assay, *"SR water extract'* had no significant effect on GABA stimulated $^{36}Cl^-$ uptake, but chlordiazepoxide increased by 2.25-fold the $^{36}Cl^-$ uptake, compared to the control. This suggests that the antiseizure effect of *"SR water extract'* in the MES test might be not related to the activation of the benzodiazepine binding site of the $GABA_A$ receptor-benzodiazepine-chloride ionophor complex, but probably via the prevention of seizure spread. Similarly, no anticonvulsant effect of baicalin was shown in picrotoxin-induced seizure test in mice [8]. The antiseizure activity of diazepam and baicalin was compared in the picrotoxin-induced seizure test in mice. It was found that diazepam significantly increased the latency of the first seizure activity, and decreased the percentage of death to 0%, whereas baicalin did not significantly change the latency of the first seizure activity nor did it reduce the percentage of animals deaths [8].

It is noteworthy that there are many other natural substances whch display anticonvulsant properties in preclinical studies. It has recently been found that osthole, a natural coumarin derivative, produces a clear-cut antielectroshock activity in mice and the experimentally-derived ED_{50} values for osthole ranged from 259-631 mg/kg in the mouse MES model [16]. Moreover, imperatorin (another coumarin derivative) also exerted anticonvulsant effects in the mouse MES model with ED_{50} values ranged from 167-290 mg/kg [17]. Additionally, it has been documented that imperatorin enhances the antiseizure effects of carbamazepine, phenobarbital and phenytoin (classical AEDs) in the mouse MES model [18]. The results obtained by Łuszczki et al. [17] showed that the protective indices (as a ratio of TD_{50} and ED_{50} values) for imperatorin and osthole are quite similar to the protective index for VPA. Thus, one can ascertain that both natural coumarin derivatives, osthole and imperatorin, have some potentially favourable activities in terms of seizure suppression, similar to those reported for valproate.

In conclusion, *"SR water extract"* administered in combination with VPA and TGB in the PTZ test, as well as with VPA and TPM in the MES test,exhibited no effect on the anticonvulsant activities of the studied AEDs. More advanced studies are needed using a pure baicalin to verify the anticonvulsant properties of this flavonoid in preclinical studies.

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