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Evaluation of the combined treatment with pregabalin and inhibitors of the renin-angiotensin system against maximal electroshock in mice

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Abstract

Introduction and objective. Hypertension is a common comorbid condition in patients with epilepsy. Combined treatment with antiepileptic drugs (AEDs) and antihypertensives may lead to pharmacokinetic and/or pharmacodynamic interactions. The purpose of the current study was to examine the effects of angiotensin-converting enzyme (ACE) inhibitors (captopril and perindopril) and angiotensin AT_1 receptor antagonists (losartan and candesartan) on the anticonvulsant activity of pregabalin in the maximal electroshock seizure (MES) test in mice.

Materials and method. The study was conducted on adult Swiss mice. Drugs were administered intraperitoneally. Electroconvulsions (50 Hz, 500 V, current intensity 25 mA) were produced by a Hugo Sachs generator. Additionally, adverse effects of the combined treatment were assessed in the step-through passive avoidance task and the chimney test.

Results. Captopril (50 mg/kg), perindopril (10 mg/kg), losartan (50 mg/kg) and candesartan (8 mg/kg) did not affect the anticonvulsant action of pregabalin in the MES test. In the chimney test, the combinations of pregabalin (315.7 mg/kg) with losartan (50 and 25 mg/kg) significantly impaired motor coordination in mice, $P<0.001$ and $P<0.05$, respectively. Combinations of pregabalin with other antihypertensives were ineffective in this test. In the passive avoidance task, co-administration of pregabalin with antihypertensives showed a strong tendency towards impaired retention.

Conclusions. It is suggested that the use of pregabalin with the examined antihypertensive drugs in patients with epilepsy is presumed neutral regarding anticonvulsant action of pregabalin. However, caution is advised during co-administration of pregabalin with losartan at high doses due to neurotoxicity in mice.

Key words

ACE inhibitors, AT_1 antagonists, pregabalin, seizures, motor coordination, memory

INTRODUCTION

Inhibitors of the renin-angiotensin system (RAS) and pregabalin are widely prescribed drugs for patients. The former comprise angiotensin-converting enzyme (ACE) inhibitors and angiotensin AT_1 receptor antagonists used in the treatment of hypertension, heart failure and diabetic nephropathy [1, 2]. Pregabalin is an antiepileptic drug (AED) used as an adjunctive treatment for focal seizures, neuropathic pain, fibromyalgia, and anxiety disorder in adult patients [3]. Hypertension and heart failure are common comorbid conditions in people with epilepsy [4]. Co-administration of antihypertensives with AEDs may lead to drug interactions in clinical practice. Recent experimental studies have shown that RAS inhibitors can affect anticonvulsant activity of AEDs against maximal electroshock (MES)-induced seizures in mice, considered as an experimental model of tonic-clonic seizures [5]. According to some authors, the MES test is also thought to be a model of partial convulsions with or without secondary generalization in humans [6]. In the MES test, losartan, an AT_1 receptor antagonist, enhanced the anticonvulsant action of valproate [7]. Captopril, an ACE inhibitor, increased the protective action of carbamazepine and lamotrigine [8], and perindopril (also an ACE inhibitor), potentiated the anticonvulsant activity of levetiracetam [9] against MES-induced convulsions.

Taking into consideration the above-mentioned data, the current study sought to assess the effects of losartan, captopril, perindopril and candesartan (another AT_1 receptor antagonist) with indicated neuroprotective activity in animals [10], on the anticonvulsant action of pregabalin in the MES test. Additionally, possible adverse effects of the drug combinations were examined in the passive avoidance task, which is considered a measure of long-term memory [11], and in the chimney test for evaluation of disturbances in motor coordination [12].

MATERIALS AND METHOD

Animals. Adult male Swiss mice (22–28 g weight) were used in the study. Animals were kept under standardized laboratory conditions (12-h light-dark cycle, room temperature 22±1 °C, humidity 50–60%) in colony cages with food pellets and tap water available *ad libitum*. They were randomly assigned to experimental groups consisting of 8 mice. All experimental procedures were approved by the Local Ethics Committee for

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Animal Experiments (License No.: 46/2008) and complied with Directive 86/609/EEC on the protection of animals used for scientific purposes.

Drugs. Angiotensin AT_1 receptor antagonists, losartan potassium (Xartan, Adamed, Poland) and candesartan cilexetil (Atacand, AstraZeneca AB, Sweden), and ACE inhibitors, captopril (Captopril Jelfa, Jelfa S.A., Poland) and perindopril arginine (Prestarium, Servier, France) as well as AED, pregabalin (Lyrica, Pfizer Ltd., UK) were employed in the study.

The antihypertensives and pregabalin were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water. All drugs were administered as single intraperitoneal (*i.p.*) injections in a volume of 5 ml/kg. Pretreatment times were as follows: 120 min (losartan and candesartan), 60 min (perindopril and pregabalin) and 45 min (captopril). Pretreatment times and doses of drugs were selected according to their biological activity reported previously in our and other papers [7, 8, 13].

MES test. Electroconvulsions (50 Hz, 500 V, stimulus duration of 0.2 s) were produced by a generator (Rodent Shocker, Type 221, Hugo Sachs Elektronik, Freiburg, Germany) and delivered via auricular electrodes. A fixed current intensity of 25 mA was applied in the MES test. Full tonic extension of both hind limbs was the endpoint. The protective activity of pregabalin was determined as its median effective dose (ED_{50} value in mg/kg) against MES. To evaluate ED_{50} values for pregabalin alone and in combinations with antihypertensive drugs, at least 3 groups of mice (8 animals per group), after receiving progressive doses of pregabalin, were challenged with MES. A dose-response curve for pregabalin was subsequently constructed on the basis of a percentage of animals protected against the convulsions.

Step-through passive avoidance task. On the first day, mice pretreated with drugs were individually placed in an illuminated box (12 × 20 × 15 cm) connected to a dark box (24 × 20 × 15 cm) which was equipped with an electric grid floor. A guillotine door (4 × 7 cm), located at floor level in the center of the connecting wall, was opened after an accommodation period of 15 s. Entrance into the dark box was punished by an electric foot shock (0.6 mA for 2 s). During the training trial, mice that did not enter the dark box within 90 s were excluded from further testing. Twenty-four hours later, the retention test was conducted in which the same animals with no treatment were put into the illuminated box, and the latency (time) to enter the dark box was noted. The mice that avoided the dark box for 180 s were considered as remembering the task.

Chimney test. In the chimney test, mice had to climb backwards up a plastic tube (3 cm inner diameter, 25 cm length). Motor impairment was defined as the inability of mice to climb backward up the tube within 60 s.

Statistics. ED_{50} values and their statistical comparisons were calculated by computer probit analysis, according to Litchfield and Wilcoxon [14]. A Kruskal-Wallis non-parametric ANOVA followed by Dunn's test, was applied to calculate data from the passive avoidance test. Analysis with Fisher's exact probability test was employed for the chimney test. Group differences were considered statistically significant at $P < 0.05$.

RESULTS

Losartan (50 mg/kg), candesartan (8 mg/kg), captopril (50 mg/kg) and perindopril (10 mg/kg) were used at doses that did not effectively change the threshold for electroconvulsions [7, 8, 9] and do not cause adverse effects in the passive avoidance and chimney tests [9]. Both AT_1 receptor antagonists and ACE inhibitors used at these subthreshold doses did not affect the anticonvulsant action of pregabalin in the MES test (Fig. 1). However, in the chimney test, the combinations of pregabalin (315.7 mg/kg) with losartan at doses of 25 and 50 mg/kg significantly impaired motor coordination in mice ($P < 0.05$ and $P < 0.001$, respectively). Combinations of pregabalin with losartan (12.5 mg/kg), candesartan (8 mg/kg), captopril (50 mg/kg) or perindopril (10 mg/kg) were ineffective in this test (Tab. 1). Regarding data from the passive avoidance test, analysis with Kruskal-Wallis test revealed a significant overall effect ($H = 15.274$; $P = 0.0093$). However, further analysis with Dunn's test showed no significant differences between control and other groups ($P > 0.05$) (Tab. 2).

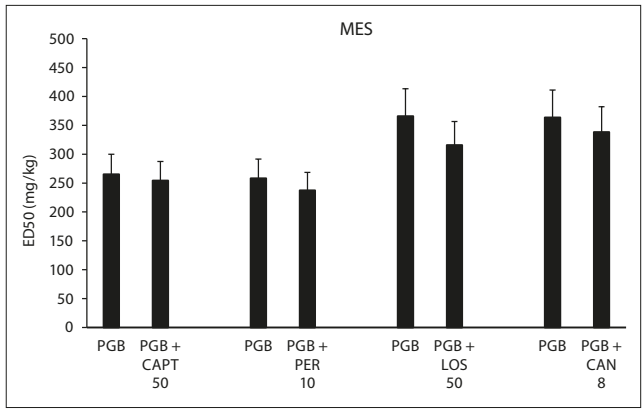

Figure 1. Effect of ACE inhibitors and AT_1 antagonists on the anticonvulsant activity of pregabalin in the MES test. This effect of antihypertensive drugs administration (mg/kg) was evaluated on different days.
Data are expressed as median effective doses (ED_{50} in mg/kg) with S.E.M. values. In order to calculate each ED_{50} value for pregabalin, 24 or 32 mice were used. PGB – pregabalin, CAPT – captopril, PER – perindopril, LOS – losartan, CAN – candesartan. $P > 0.05$ *vs.* respective control groups (Litchfield and Wilcoxon method)

Table 1. Effect of combined treatment with antihypertensives and pregabalin on motor coordination in the chimney test

Treatment (mg/kg)	n	Percentage of mice impaired (%)
control	8	0
losartan (50)	8	0
PGB (338.3)	8	0
PGB (315.7)	8	0
PGB (315.7) + losartan (50)	8	87.5 ***
PGB (315.7) + losartan (25)	8	62.5 *
PGB (315.7) + losartan (12.5)	8	25
PGB (338.3) + candesartan (8)	8	25
PGB (237.5) + perindopril (10)	8	0
PGB (254.3) + captopril (50)	8	0

Results are shown as percentage of animals that failed to perform in the chimney test. n – number of mice. ***$P < 0.001$, *$P < 0.05$ *vs.* control group (Fisher's exact probability test)

Table 2. Effect of combined treatment with antihypertensives and pregabalin on memory retention in the passive avoidance test

Treatment (mg/kg)	n	Retention time (s)
control	8	180 (134, 180)
PGB (338.3)	8	77 (25, 135)
PGB (338.3) + candesartan (8)	8	35 (23, 72)
PGB (315.7) + losartan (50)	8	45 (36, 86)
PGB (237.5) + perindopril (10)	8	180 (84, 180)
PGB (254.3) + captopril (50)	8	35 (24, 56)

Results are presented as median values (in s) along with 25th and 75th percentiles. n – number of mice. H = 15.274, P = 0.0093 (Kruskal-Wallis non-parametric ANOVA). P > 0.05 *vs.* control group (Dunn's test)

DISCUSSION

In the current study, RAS inhibitors did not affect the anticonvulsant activity of pregabalin in the MES test, but losartan, AT_1 receptor antagonist, significantly impaired motor coordination in pregabalin-treated mice. At the moment, the mechanism responsible for the phenomenon is unknown.

Losartan and pregabalin individually at used doses did not influence motor performance in the chimney test. This is consistent with others studies showing that pregabalin (170 mg/kg *i.p.*) [15] and losartan (50 mg/kg *i.p.*) [7] did not exert such an effect in this test. There are results showing that losartan, when co-administered with other AEDs, can induce motor coordination deficits. This effect was observed for losartan (50 mg/kg *i.p.*) combined with valproate [7], gabapentin [16] and tiagabine [17]. Furthermore, a combination of losartan with vigabatrin caused motor coordination impairment in 37.5% mice of a tested group (statistically not significant) [18]. All these AEDs are suggested to increase GABA transmission [19] which is known to be involved in motor coordination impairment [20]. According to some authors, losartan can reduce locomotor activity due to its interaction with the central dopaminergic system [21]. Based on these findings, it has been suggested that co-administration of the mentioned AEDs with losartan may affect motor coordination in mice through modulation of GABAergic and/or dopaminergic systems [7, 16, 17]. Regarding pregabalin, motor coordination impairment and ataxia belong to the most common side-effects in patients treated with this drug [22]. Pregabalin is a potent ligand of $\alpha_2\delta$ type 1 and 2 subunits of voltage-gated calcium channels in the central nervous system, and through binding to the $\alpha_2\delta$ site it decreases calcium inward currents with a consequential modulation in excitatory neurotransmitter release [23]. The highest level of expression of these channels has been found in the cerebellum and in the hippocampus [22]. It is suggested that their dysfunction/decreased activity may affect the vestibulocerebellar/brainstem structures and higher cortical functions leading to dizziness, blurred vision, ataxia and cognitive impairment [22]. It should be added that pregabalin does not interact with GABA receptors, uptake or metabolism [24]; therefore, an involvement of this system in pregabalin-induced motor coordination deficits is rather impossible.

The exact mechanism through which losartan affected performance of pregabalin-treated mice in the chimney test remains to be elucidated. Apart from pregabalin, the inhibitory effect on voltage-gated calcium channels containing the $\alpha_2\delta$ subunit has also been attributed to gabapentin, which is a compound structurally related to pregabalin [25], and its activity in the chimney test was affected by losartan, as mentioned above [16]. Therefore, it would be of interest to perform electrophysiological studies on the effect of losartan on the open probability and the conductance of these calcium channels.

In the passive avoidance task, co-administration of pregabalin with antihypertensives showed a strong tendency towards impaired retention. It should be noted that pregabalin alone also exhibited retention deficits (statistically not significant; Dunn's test). The examined drugs were administered before exposure to an electric foot shock (negative learning factor). It is suggested that the observed effect of the combined treatment with pregabalin and antihypertensives in the passive avoidance task may be related, at least in part, to an antinociceptive effect of pregabalin. This AED is well known for its antinociceptive effects in animal models [26]. Drugs with antinociceptive effects may attenuate aversive (punitive) stimulus in the passive avoidance test and, therefore, animals subjected to them cannot properly learn the task [27].

CONCLUSIONS

From the current study, it is suggested that the use of captopril, perindopril, losartan and candesartan in patients with epilepsy receiving pregabalin is presumed neutral regarding its anticonvulsant activity. However, because the combined treatment with losartan at high doses and pregabalin caused the neurotoxic effect in the chimney test in mice, caution is advised during concomitant use of these drugs in patients. The obtained results should be considered in respect of experimental studies suggesting that much larger doses of losartan, several times above the currently approved maximum doses, can better prevent the progression of renal disease [28].

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