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Effect of Combined Treatment with AT_1 Receptor Antagonists and Tiagabine on Seizures, Memory and Motor Coordination in Mice*

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Losartan and telmisartan, angiotensin AT_1 receptor antagonists, are widely used antihypertensive drugs in patients. It is also known that arterial hypertension is often present in people with epilepsy, therefore, drug interactions between AT_1 receptor antagonists and antiepileptic drugs can occur in clinical practice.
Objectives. The aim of the current study was to assess the effect of losartan and telmisartan on the anticonvulsant activity of tiagabine, a second-generation antiepileptic drug, in mice. Additionally, the effect of the combined treatment with AT_1 receptor antagonists and TGB on long-term memory and motor coordination has been assessed in animals.
Material and Methods. The study was performed on male Swiss mice. Convulsions were examined in the maximal electroshock seizure threshold test. Long-term memory was measured in the passive-avoidance task and motor coordination was evaluated in the chimney test. AT_1 receptor antagonists and TGB were administered intraperitoneally.
Results. Losartan (50 mg/kg) or telmisartan (30 mg/kg) did not influence the anticonvulsant activity of TGB applied at doses of 2, 4 and 6 mg/kg. However, both AT_1 receptor antagonists in combinations with TGB (6 mg/kg) impaired motor coordination in the chimney test. The concomitant treatment of the drugs did not decrease retention in the passive avoidance task.
Conclusions. It is suggested that losartan and telmisartan should not affect the anticonvulsant action of TGB in people with epilepsy. Because the combined treatment with AT_1 receptor antagonists and TGB led to neurotoxic effects in animals, caution is advised during concomitant use of these drugs in patients (**Adv Clin Exp Med 2015, 24, 4, 565–570**).

Key words: losartan, telmisartan, tiagabine, electroconvulsions, memory, locomotor activity.

Losartan and telmisartan, angiotensin AT_1 receptor antagonists, are routinely used drugs in the treatment of hypertension and heart failure [1], which are common comorbid conditions in people suffering from epilepsy [2, 3]. Epidemiological studies have revealed an even higher prevalence of heart failure in patients with epilepsy [3]. A higher risk of hypertension in epileptic patients is also postulated in some studies [2]. Experimental data shows that losartan and telmisartan can modulate the renin–angiotensin system (RAS) in the brain. It is now well established that RAS is systemically and locally present. The brain RAS is associated with the regulation of body water balance and thirst, blood pressure maintenance and endocrine functions [4]. Moreover, it plays a role in the regulation of cerebral

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blood flow and cerebroprotection, stress, depression, alcohol consumption and memory consolidation [4]. Some of these functions and behaviors have been affected by peripheral administration of losartan or telmisartan in animals [5–7]. Additionally, there is evidence that the brain RAS can mediate seizure susceptibility. Angiotensin peptides such as ang II, III and IV have shown anticonvulsant properties in different animal seizure models [8]. On the other hand, AT_1 antagonists, losartan and telmisartan, have also been demonstrated to affect convulsions. In a rat audiogenic model of epilepsy, losartan impaired the triggering and maintenance of seizures [9]. Losartan reduced the intensity of pentylenetetrazol-kindled seizures in mice [10]. Further, losartan and telmisartan enhanced the anticonvulsive action of valproate against maximal electroshock (MES)-induced seizures in mice [11]. In the maximal electroshock seizure threshold test (MEST), losartan significantly increased the convulsive threshold raised by gabapentin [12]. In turn, telmisartan, through pharmacokinetic mechanisms, enhanced the anticonvulsant action of topiramate in the MES test [13].

In the current study, the effects of losartan and telmisartan on the anticonvulsant activity of tiagabine, a second-generation antiepileptic drug, in the MEST test have been evaluated. TGB with anticonvulsant action related to the GABAergic system, is licensed for the adjunctive treatment of partial seizures with secondary generalization [14]. The MEST test is considered as an experimental model of tonic-clonic seizures [15] and its choice was based upon the TGB efficacy in it. According to our knowledge, AT_1 receptor antagonists have not been tested in combination with TGB in animal models of seizures. Additionally, the passive avoidance task [16] and chimney test [17] have been used for the assessment of long-term memory and motor coordination, respectively, in mice. The occurrence of adverse effects of the combinations of AT_1 antagonists with TGB used in the MEST test, such as memory and/or motor impairments would be undesirable effects and could decrease their therapeutic value.

Material and Methods

Animals

Experiments were performed on adult male Swiss mice. The body weight of the mice used in the experiments was 20–26 g. The mice were housed in colony cages in the animal room at a room temperature of 21 ± 1°C, humidity of 50–60% and 12-h light-dark cycle. The animals were given free access to food and tap water *ad libitum*. The mice were subjected to one week of adaptation to laboratory conditions before the experiments. Next, they were randomly assigned to experimental groups consisting of 8 mice. All procedures employed in this study were approved by the Local Ethics Committee for Animal Experiments (University of Life Sciences, Lublin). They followed the European Communities Council Directive of 24 November 1986 (86/609/EEC). The experiments were performed between 9:00 a.m. and 3:00 p.m. and each mouse was tested only one time.

Drugs

AT_1 receptor antagonists such as losartan potassium (Xartan, Adamed, Poland) and telmisartan (Micardis, Boehringer Ingelheim, Germany), and antiepileptic tiagabine (TGB, Gabitril, Cephalon, France) were used in the experiments. Both AT_1 antagonists and TGB were suspended in a 1% aqueous solution of Tween 80 (Sigma, St. Louis, MO, USA). The mice were subjected to single intraperitoneal (*i.p.*) injections of the drugs that were administered in a volume of 5 mL/kg body weight. The vehicle was given to the control animals. TGB was administered 15 min prior to tests whereas AT_1 antagonists were pretreated 120 min before them. Treatment times and doses of the drugs were selected according to their biological activity reported in earlier studies [6, 7, 11, 18].

Maximal Electroshock Seizure Threshold Test

Seizures were evoked by transauricular application of an alternating current (50 Hz, stimulus duration of 0.2 s) by means of electrodes and delivered by a Hugo Sachs generator (Rodent Shocker, Type 221, Freiburg, Germany). The endpoint was considered as the full tonic extension of both hind limbs. In this test, the mice were subjected to electroshocks of different intensities. CS_{50} (the convulsive threshold) was defined as the current strength (in mA) necessary to produce tonic hindlimb extension in 50% of the animals tested. At least 3 groups of mice (consisting of 8 animals per group) were applied to calculate the CS_{50} value. On the basis of a percentage of animals having seizures in the experimental groups, an intensity-response curve was constructed with a computer.

Step-Through Passive Avoidance Task

The apparatus consisted of an illuminated box (12 × 20 × 15 cm) adjacent to a dark box (24 × 20 × 15 cm). The grid floor of the dark box was connected to generator and between the boxes

in the middle at floor level, there was a doorway (4 × 7 cm). During a training trial, the pretreated mice were separately put into an illuminated box. The mice were immediately punished by an electric foot shock (0.6 mA for 2 s) after entering the dark box. The next day (24 h after the training trial) a retention test was performed. The same mice with no treatment were placed in the illuminated compartment. The time the mice spent in the illuminated box until entering the dark box was calculated. If a mouse avoided the dark box for 180 s, it was considered remembering the task.

Chimney Test

In this test, the animals were climbing backwards up a plastic tube (25 cm in length, 3 cm inner diameter). Animals showing inability to climb backwards up the tube within 60 s, were considered with impaired motor coordination.

Statistics

Computer log-probit analysis based on a method by Litchfield and Wilcoxon [19] was used to calculate median current strengths (CS_{50}s values in mA) along with their 95% confidence limits. Next, standard errors of the mean (SEM) were obtained from the confidence limits according to a method described previously [20]. Statistical comparison of the data from the MEST test was performed either by the log-probit method [19] or one-way ANOVA (analysis of variance) and the *post hoc* Dunnett's test for multiple comparisons. The passive avoidance results were analyzed with a Kruskal-Wallis test (non-parametric ANOVA) and Dunn's multiple comparisons test. Fisher's exact-probability test was applied to analyze the data from the chimney test. A significance level $p < 0.05$ was considered for group differences. GraphPad Prism 5 (v. 5.01) software was employed for statistical analysis.

Results

MEST Test

Losartan (50 mg/kg) and telmisartan (30 mg/kg) alone did not influence the threshold for electroconvulsions (CS_{50}) in mice, which is in agreement with earlier studies [11]. TGB alone at doses of 4 and 6 mg/kg significantly elevated the convulsive threshold ($p < 0.01$, Dunnett's test). TGB (4 and 6 mg/kg) administered with losartan (50 mg/kg) increased the convulsive thresholds ($p < 0.05$ and $p < 0.01$) as well as when combined with telmisartan (30 mg/kg) ($p < 0.01$, Dunnett's test). However, the thresholds for combinations of AT_1 receptor antagonists and TGB did not significantly differ from the thresholds for TGB alone groups ($p > 0.05$, Litchfield and Wilcoxon method) (Table 1).

Table 1. Effect of AT_1 receptor antagonists and tiagabine (TGB) on the convulsive threshold

	CS_{50} [mA] ± SEM				
Drug (mg/kg)		**n**		**n**	**Litchfield and Wilcoxon method**
	losartan (0)		losartan (50)		
Vehicle	6.3 ± 0.51	24	7.4 ± 0.42	16	$p > 0.05$
TGB (2)	6.9 ± 0.76	16	8.1 ± 0.76	8	$p > 0.05$
TGB (4)	9.3 ± 0.25**	16	9.7 ± 0.47*	16	$p > 0.05$
TGB (6)	10.7 ± 0.47**	16	9.7 ± 0.57**	24	$p > 0.05$
ANOVA	F (3,68) = 14.934, $p < 0.0001$		F (3,60) = 4.372, $p = 0.0075$		
	telmisartan (0)		telmisartan (30)		
Vehicle	6.3 ± 0.51	24	6.3 ± 0.24	16	$p > 0.05$
TGB (2)	6.9 ± 0.76	16	7.9 ± 0.43	8	$p > 0.05$
TGB (4)	9.3 ± 0.25**	16	9.9 ± 0.43**	8	$p > 0.05$
TGB (6)	10.7 ± 0.47**	16	10.6 ± 0.41**	24	$p > 0.05$
ANOVA	F (3.68) = 14.934, $p < 0.0001$		F (3,52) = 26.733, $p < 0.0001$		

Results are presented as the median current strengths (in mA) with SEM values. Twenty four mice were used to calculate each CS_{50} value, except for the group: losartan (50) + TGB (2), where 32 animals were employed. n – the number of animals subjected to current strengths for which the convulsant effect ranged between 16% and 84% for the examined group.
** $p < 0.01$; * $p < 0.05$ as compared with respective control groups (Dunnett's test).
$p > 0.05$ as compared with respective control values (Litchfield and Wilcoxon method).

Passive Avoidance and Chimney Tests

Memory retention was not significantly impaired by any of the studied drug combinations in the passive avoidance task (Table 2). Combined treatment with TGB (6 mg/kg) and losartan (50 mg/kg) or telmisartan (30 mg/kg) impaired motor coordination in mice in the chimney test. Lower doses of losartan (30 mg/kg) and telmisartan (20 mg/kg) in combination with TGB (6 mg/kg) were ineffective in this test (Table 3). TGB (6 mg/kg) alone was not effective in any of the behavioral tests. Losartan (50 mg/kg) and telmisartan (30 mg/kg) alone were tested in mice in the passive avoidance and chimney tests in our previous study (they did not impair motor coordination or memory retention) [11].

Discussion

The present study shows that combined treatment with TGB and AT_1 receptor antagonist does not lead to the enhancement of protection against electroconvulsions but may cause signs of neurotoxicity such as impaired motor coordination in mice.

TGB is a derivative of nipecotic acid, which increases the level of GABA in the synaptic cleft *via* inhibition of its neuronal and glial uptake [21]. Actually, TGB is a direct and potent inhibitor of the GABA GAT-1 transporter [22]. TGB inhibited tonic seizures induced by pentylenetetrazol or 6,7-dimethoxy-4-ethyl-b-carboline-3-carboxylate (DMCM) in mice and was also effective against amygdala-kindled seizures in rats, reducing both the kindling process (an antiepileptogenic effect) and the expression of the fully kindled seizure [21]. TGB antagonized sound-induced seizures in DBA/2 mice [23]. TGB is thought to be ineffective against MES [23], however, TGB elevates the electroconvulsive threshold in the MEST test [24]. The present data is in agreement with those of Łuszczki and Czuczwar [18] showing that TGB at doses higher than 2 mg/kg *i.p.* elevates the convulsive threshold. The MEST test has already been used to examine the pharmacological interactions between TGB and other drugs or substances [18, 25]. In the current study, combined treatment with TGB (6 mg/kg) and losartan (50 mg/kg) or telmisartan (30 mg/kg) did not affect seizures but significantly impaired motor coordination in mice. The mechanism(s) of this phenomenon remains to be elucidated. Both specific and unspecific mechanisms could be taken under consideration. Losartan and telmisartan were tested at doses at which a decrease in blood pressure should be assumed [5, 6]. However, the decrease in mean arterial blood pressure as a factor interfering with mice performance in the chimney test seems rather unlikely since losartan or telmisartan alone did not impair motor coordination in mice. TGB is known to disturb the motor performance of mice in a dose-dependent manner and the toxic dose causing motor impairment in 50% of the animals tested (TD_{50}) for TGB was found as 13.6 mg/kg [18]. Thus, it is not surprising that TGB (6 mg/kg) alone was ineffective in the chimney test. On the other hand, there are reports showing that TGB at doses even lower than 6 mg/kg in combination with some drugs produces neurotoxic effects in this test. Łuszczki et al. [24] reported that TGB at a dose of 2 mg/kg simultaneously injected with gabapentin (37.5 mg/kg) disturbed motor coordination. TGB (6 mg/kg) combined with captopril (50 mg/kg), an angiotensin-converting enzyme (ACE) inhibitor, caused motor impairment in 37.5% animals of the tested group (statistically not significant) [26]. There is also data showing that AT_1 receptor antagonists when co-administered with some antiepileptic drugs can impair motor coordination. Actually,

Table 2. Effect of AT_1 receptor antagonists and tiagabine (TGB) on motor coordination in the chimney test

Drug (mg/kg)	n	% of mice impaired
Control	8	0
TGB (6)	8	12.5
Losartan (50) + TGB (6)	8	75*
Losartan (30) + TGB (6)	8	37.5
Telmisartan (30) + TGB (6)	8	50*
Telmisartan (20) + TGB (6)	8	37.5

Data is expressed as the percentage of animals with impaired motor coordination in the chimney test.
n – the number of animals.
* $p < 0.05$ as compared with the control group (Fisher's exact test).

Table 3. Effect of AT_1 receptor antagonists and tiagabine (TGB) on memory retention in the passive avoidance test

Drug (mg/kg)	n	Retention (s)
Control	8	180 (180, 180)
TGB (6)	8	180 (131, 180)
Losartan (50) + TGB (6)	8	125 (71, 180)
Telmisartan (30) + TGB (6)	8	125 (76, 161)

Results are shown as the median values (in s) along with the 25^{th} and 75^{th} percentiles.
n – the number of animals.
$p > 0.05$, for each of the tested group vs. the control group (Dunn's test).

this effect was observed for losartan (50 mg/kg) in combined treatment with valproate (194.6 mg/kg) [11] and gabapentin (50 mg/kg) [12].

In the current study, AT_1 antagonists in combination with TGB, a GABA enhancer, produced motor impairment. A broad range of data indicates that angiotensin II can modulate the inhibitory responses to GABA and vice versa. For example, pressor, tachycardic, and renal sympathoexcitatory responses to acute blockade of $GABA_A$ receptors in the hypothalamic paraventricular nucleus depend on the activation of local angiotensin II AT_1 receptors [27]. Angiotensin II attenuates synaptic GABA release and excites paraventricular-rostral ventrolateral medulla output neurons [28]. On the other hand, for example, GABAergic stimulation inhibits the central actions of angiotensin II including pressor responses, drinking and release of vasopressin [29]. Further, it has been reported that intracerebroventricular injection of losartan inhibits angiotensin II-sensitive neurons *via* GABA inputs in the anterior hypothalamic area [30]. It is noteworthy that moderate levels of AT_1 receptors have been found in the cerebellum [4], the brain structure that is concerned primarily with the coordinated execution of ongoing movements [31] and whose cortex and nuclei include different types of GABAergic inhibitory neurons [32]. Since GABAergic neurotransmission is involved in motor coordination impairments [33], this system may play a role in the observed phenomenon. It is suggested that a blockade of angiotensin II action in the brain by AT_1 receptor antagonists may modulate GABAergic transmission and potentiate TGB inhibitory influence on the motor performance of mice in the chimney. However, if this is enhancement of the GABAergic neurotransmission to be responsible for the significant impairment of motor coordination in the chimney test, then a positive interaction in the seizure test might also be expected. Anyway, a pharmacokinetic mechanism does not seem to contribute to the observed motor impairment because the evaluated combinations were ineffective in other tests.

In conclusion, from the preclinical point of view, the concomitant use of AT_1 receptor antagonists and TGB is presumed neutral as regards its anticonvulsant action in patients with epilepsy. Although the combined treatment with TGB and AT_1 antagonists impaired motor coordination in mice, it is evidently premature to announce that such treatment can cause neurotoxic effects in humans. Clinical studies are needed on this subject. However, caution is advised when combining TGB and losartan or telmisartan due to the appearance of motor impairment in animals.

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