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Anticonvulsant Activity of Dimephosphonum

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Abstract
A novel Russian medicine dimephosphonum at its therapeutic doses of 50 mg/kg and 100 mg/kg showed anti-seizure activity on models of primary generalized seizures caused by corazolum or by maximal electroshock in rats and mice. Dimephosphonum prevented death of rats and mice in both seizure models like reference preparations phenazepam, valproic acid, and levetiracetam. The effects of dimephosphonum were less pronounced compared to phenazepam or valproic acid, but were comparable to those of levetiracetam.

Keywords Anticonvulsant activity · Dimephosphonum · Rats · Mice · Maximal electroshock test · Corazolum antagonism test · Anticonvulsant drugs

1 Introduction

Epilepsy is a chronic disease of the nervous system, which affects more than 0.5–1% of the world's population [1, 2]. According to the world's most recent statistics, the prevalence of epilepsy varies from 3 to 15.0 per 1000 people. Recently, epilepsy becomes more prevalent among neurological diseases, reaching the third place after stroke and dementia [3].

Animal models of primary generalized seizures, including convulsions caused by electrocution (maximal electroshock test) and chemical substances (corazole antagonism test), are used as the first step to identify potential anti-epileptic drugs in screening studies in mice and rats [4]. We chose these models because of the qualitative difference in the development and manifestation of these modeled convulsions. It is commonly believed that seizures caused by the MES are experimental equivalents of "large" "Grant mal" seizures, while convulsions caused by subcutaneous administration of corazolum—of "small, petit mal" seizures. The main component of seizures in MES model is tonic extensia of the hind limbs, and in the case of the corazole model, it is clonic convulsions [5, 6].

Dimephosphonum exerts a wide range of biological effects: antimicrobial, antiviral, anti-neoplastic, spasmolytic, psychotropic, and protective in case of poisoning with anticholinesterase agents [7]. Other low-toxic organo-phosphorus compounds (OPCs) with an inhibitory effect on the central nervous system exhibit anticonvulsant activity, as exemplified by phospholenes [8].

The objective of our study was to assess potential anticonvulsant activity of dimephosphonum (dimethyl ester1, 1-dimethyl-3-oxobutylphosphonic acid) at the range of therapeutic doses on models of primary generalized seizures caused by corazolum or maximum electric shock in animals. Previously, anticonvulsant properties of dimephosphonum were studied at a dose of 1000 mg/kg [9], and it was found that the drug exhibited the most powerful anticonvulsant and antidote effect against corazolum.

2 Material and Methods

The experiments were carried out on 36 mongrel rats of both sexes weighing 230 ± 9.2 g and aging 2–3 months and 36 mice weighing 30 ± 2.2 g and aging 1 month. The experimental animals were kept in vivarium conditions (with a natural light regime, at a temperature of 22–24 °C, relative air humidity of 40–50%) using a standard diet (GOST R 50258–92) [10]. The studies were conducted in accordance with the rules of good laboratory practice (GLP) in pre-clinical studies in the Russian Federation [11], as well as the rules and the International Recommendations of the European Convention for the Protection of Vertebrates used in experimental studies (1986) [12]. The study was approved by the local Ethics Committee.

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Table 1 Characteristics of anticonvulsant effects of dimephosphonum on the pentylenetetrazole model in rats as compared to those of reference anticonvulsants

Animal groups ($n = 6$)	Doses (mg/kg)	Latent period (min)	The number of convulsions per rat	The duration of convulsive attacks (min)	Death (number)
Control		10 ± 3.3	2 ± 0.01	17 ± 2.84	2
Dimephosphonum	50	15 ± 0.4*	2 ± 0.1	1.16 ± 0.01*	0
	100	10 ± 9.5	2 ± 0.1	4.3 ± 0.04*	0
Phenazepam	2	0	0	0	0
Acidum valproicum	400	0	0	0	0
Levetiracetam	54	14.7 ± 7	1 ± 0.02	0.18 ± 0.017	0

*$p < 0.05$, the significance of differences compared with the control group

We performed the pentylenetetrazole (corazolum) model in rats, and the maximum electric shock test in mice. For each convulsive model, experimental animals (36 mice and 36 rats) were divided into six groups of six animals in each. Group 1 was a control group; in groups 2 and 3, animals were treated with dimephosphonum once (50 mg/kg and 100 mg/kg); in groups 4, 5, and 6, animals received reference preparations (phenazepam (2 mg/kg), valproic acid (400 mg/kg), and levetiracetam (54 mg/kg)). Animals of control groups received equivalent amounts of distilled water. The drugs were administered intraperitoneally within 30 min of the beginning of the experiment.

In the pentylenetetrazole (corazolum) model, the anticonvulsant activity of dimephosphonum was determined by preventing seizures with subcutaneous administration of corazolum at a dose of 80 mg/kg [4]. Intensity of anticonvulsant activity was assessed according to the following indices: latent period (min), number of seizures per animal, duration of seizures (min), and death rate of animals in a group (%). We considered a decrease in number of seizures and of death rate as an anticonvulsant effect.

Anticonvulsant activity of dimephosphonum (JSC «TATCHEMPHARMPREPARATY», RF) was also determined by preventing the tonic-extensor phase of convulsive seizure of maximal electroshock (electrical stimulation by applying corneal electrodes to the eyeballs of mice). MES parameters: 50 mA current with constant frequency of 60 Hz; duration—0.2 s. The animal received an electrical stimulus, causing tonic extension of the hind limbs in mice.

For statistical processing of the results, we used Statistica 511 software and calculated arithmetic means M and their standard deviations (M ± SD). To assess the reliability of the differences in means of samples with normal distribution (as confirmed by Shapiro-Wilk test for normality), we used the two-sample t test (also known as Student's t test or independent t test). To double-check, we also ran the nonparametric Mann-Whitney U Test, which confirmed our statistical evaluations. The difference was judged to be statistically significant at a probability level of 95% or more ($p \leq 0.05$).

3 Results and Discussion

Pentylenetetrazole (Corazolum) Model We found that in groups of reference preparations (phenazepam 2 mg/kg and valproic acid 400 mg/kg), there were no convulsive seizures observed. In the dimephosphonum group (at doses of 50 mg/kg and 100 mg/kg), the latent period increased by 50% compared with the control (see Table 1), which was comparable to the effect of levetiracetam for the latent period.

Table 2 Characteristics of anticonvulsant effects of dimephosphonum on the maximum electroshock model (MES) in mice as compared to those of reference anticonvulsants

Animal groups ($n = 6$)	Doses (mg/kg)	Latent period (min)	The number of convulsions per mouse	The duration of convulsive attacks (min)	Death (number)
Control		10 ± 3.3	2 ± 0.01	17 ± 2.84	2
Dimephosphonum	50	15 ± 0.4*	2 ± 0.1	1.16 ± 0.01*	0
	100	10 ± 9.5	2 ± 0.1	4.3 ± 0.04*	0
Phenazepam	2	0	0	0	0
Acidum valproicum	400	0	0	0	0
Levetiracetam	54	14.7 ± 7	1 ± 0.02	0.18 ± 0.017	0

*$p < 0.05$, the significance of differences compared with the control group

Administration of dimephosphonum at the doses of 50 and 100 mg/kg did not affect the number of seizures, but at a dose of 50 mg/kg dimephosphonum significantly reduced the duration of convulsive attack by 17 times (1.16 ± 0.01 min), compared with the control group (17 ± 2, 84) ($p < 0.05$), and at a dose of 100 mg/kg—by nearly four times of control values (4.3 ± 0.04 and 17 ± 2.84 respectively) ($p < 0.05$). Lethal outcomes were observed only in the control group and amounted to 33.3%, confirming literature data [2].

Maximal Electroshock Test (MES) Dimephosphonum at the doses of 50 mg/kg and 100 mg/kg exhibited an anticonvulsant effect, as well as reference preparations (fenazepam, valproic acid, and levetiracetam): Dimephosphonum completely prevented convulsions caused by MES. Accordingly, parameters such as the latent period, the number of seizures, the duration of seizures, and the death rate were equal to zero; unlike in the control group (the latent period was 10.1 ± 3.3, the number of seizures per animal was 15.7 ± 1, 5, duration of seizures was 1 ± 0.01 min). There were no deaths in all groups (Table 2).

Thus, these results provide a solid basis for further research of dimephosphonum as potential anticonvulsant agent to assess mechanisms of its anticonvulsant activity, and the possible effectiveness of its use in the complex therapy of epilepsy.

4 Conclusions

The results indicate to potential anticonvulsive activity of dimephosphonum (at the doses of 50 mg/kg and 100 mg/kg) on the models of primary generalized seizures: caused by electrical (test of maximal electric shock) and chemical (antagonism test with corazolum) triggers.

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