Anticonvulsant Effect of Guaifenesin against Pentylenetetrazol-Induced Seizure in Mice

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Abstract

Background: There have been some reports about the possible N-methyl-D-aspartate (NMDA) antagonist activity of Guaifenesin. As drugs with a similar structure to Guaifenesin (i.e. Felbamate) and those with NMDA antagonist activity have been clinically used as anticonvulsants, the aim of this study was to determine whether Guaifenesin has an anticonvulsant effect in an animal model of seizure.

Methods: Anticonvulsant effect of Guaifenesin was assessed via Pentylenetetrazol (PTZ)-induced convulsion. Male albino mice received Guaifenesin (100, 200, 300, or 400 mg/kg; n=8-10) or 0.25% Tween (vehicle) intraperitoneally 30 minutes before the injection of PTZ (95 mg/kg). Diazepam (3 mg/kg; n=8) was used as a reference drug. The latency time before the onset of myoclonic, clonic, and tonic-clonic convulsions, percentage of animals exhibiting convulsion, and percentage of mortality were recorded. In addition, the effect of Guaifenesin on neuromuscular coordination was assessed using the Rotarod.

Results: Guaifenesin at all the studied doses significantly increased the latency to myoclonic and clonic convulsions in a dose-dependent manner. In addition, Guaifenesin at the dose of 300 mg/kg increased the latency to tonic-clonic seizure. The ED50s of Guaifenesin for protection against PTZ-induced clonic and tonic-clonic seizures and death were 744.88 (360-1540), 256 (178-363), and 328 (262-411) mg/kg, respectively. Guaifenesin at all the investigated doses significantly reduced neuromuscular coordination, compared to the vehicle-treated group.

Conclusion: These results suggest that Guaifenesin possesses muscle relaxant and anticonvulsant properties and may have a potential clinical use in absence seizure.

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Introduction

Epilepsy is the most common disabling chronic illness of the central nervous system,[1] and affects at least 50 million people worldwide. Although antiepileptic drugs are the mainstay of epilepsy treatment, less than 70% of those afflicted with epilepsy achieve satisfactory seizure control with the available antiepileptic drugs.[2] In addition, many of the current anticonvulsants have various complications and serious side effects such as hepatotoxicity and agranulocytosis,[1,3,4] which

necessitates new drugs with more suitable margins of safety and more tolerability.

In modern pharmacology, drug development and introduction of new drugs are mainly based on our understanding about the pathophysiology of the disease. The exact pathophysiological basis of epilepsy is unknown;[5] however, the excitatory glutamatergic system seems to play a key role in generating and spreading epileptic discharge.[6] Indeed, recent researches are focused on the development of drugs that counteract the activity of this system.

Guaifenesin is an expectorant used widely in cough preprations.[7] It is drawn upon as a skeletal muscle relaxant in some animal anaesthetic procedures as well.[8] Chemically, Guaifenesin is a Propanediol drug. Previous studies have shown the central nervous system effects of Propanediol drugs, mainly Mephenesin, and their relevant mechanisms. A study performed by Pralong et al.[9] reported that Mephenesin might be an antagonist of excitatory amino acids and might have NMDA-blocking activity and proposed that the NMDA-blocking activity of Mephenesin might be relevant to its muscle-relaxing activity. This notion was subsequently bolstered by some other studies, demonstrating the muscle-relaxing effect of Mephenesin in relation to an excitatory transmitter-blocking activity.[10] Interestingly, the chemical structure of Guaifenesin bears close resemblance to Mephenesin and both drugs have profound muscle-relaxing activity.[11] It can, therefore, be suggested that the muscle relaxant activity of Guaifenesin may be in consequence of NMDA-blocking activity.[12] It is also noteworthy that Guaifenesin has been utilized successfully in fibromyalgia.[13] The notion that increased levels of excitatory amino acids possibly participate in pain processes in fibromyalgia,[14] has led to the suggestion that the NMDA and glutamate-blocking activities of Guaifenesin are likely to contribute to its effectiveness in fibromyalgia.

Taken together, glutamate and NMDA receptors have important roles in the pathophysiology of epilepsy, and there is evidence suggestive of the NMDA antagonistic activity of Guaifenesin. As a result, it is likely that Guaifenesin may possess an anticonvulsant effect. If it is proven to be true, Guaifenesin would be a suitable candidate as an antiepileptic courtesy of its wide margin of safety. Guaifenesin can also be used in pregnancy and breastfeeding,[15,16] in contrast to other antiepileptic drugs.

The aim of the present study was to determine whether Guaifenesin has an anticonvulsant effect in an animal model of seizure.

Materials and Methods

Chemicals

Guaifenesin was purchased from Exir Pharmaceutical Company, Iran and was dissolved in 0.25% Tween 20 in normal saline. Diazepam (10 mg/ml) was obtained from Darupakhsh Pharmaceutical Company, Iran, and Pentylenetetrazol (PTZ) was purchased from Sigma and was dissolved in normal saline.

Animals

Male albino mice (25-35 g) were obtained from the Animal House, Shiraz University of Medical Sciences. The animals were kept in plastic cages in groups of 4-5 with free access to food and water, 12h dark/12h light cycles, and at a temperature of 22ºC±2. The animals were treated in accordance with the guidelines of the Ethics Committee of Shiraz University of Medical Sciences.

PTZ-Induced Seizure

The anticonvulsant effect of Guaifenesin was assessed in an animal model of PTZ-induced seizure. Sixty mice were randomly assigned into 6 groups (n=10/group) and received Guaifenesin intraperitoneally at doses of 100, 200, 300, and 400 mg/kg 30 minutes before the injection of PTZ (95 mg/kg). The control group was administered 0.25% Tween in normal saline. A group of mice received Diazepam (3 mg/kg) as a reference drug. The doses of Guaifenesin and Diazepam were selected according to previous studies.[17,18] The latency time to the onset of myoclonic, clonic, and tonic-clonic convulsions was recorded after PTZ injection. PTZ initially produced myoclonic jerks, followed by facial and forelimb clonus, which then became sustained and led to generalized tonic-clonic jerking (loss of righting reflex and tonic forlimb flexion/extension, followed by whole body clonus).[19] In addition, the percentage of the animals exhibiting convulsion and the percentage of mortality were recorded for each studied group. The ED50s, median effective doses of Guaifenesin, protecting 50% of the mice against clonic and tonic-clonic convulsions and death were determined.

Neuromuscular Coordination–Rotarod

The effect of Guaifenesin on coordinated motor movements was assessed using the Rotarod test. A day before the test, the mice were trained to stay on the rotating wheel (3 cm in diameter, 20 rpm) for more than one minute. On the test day, the animals were tested on the Rotarod (model 7600, UGO Basile, Italy) before and 30 minutes

after the administration of 0.25% Tween20, Diazepam or different doses of Guaifenesin. The number of seconds each mouse remained on the rotating wheel was recorded for a maximum of 300 seconds.

Statistical Analysis

The data are presented as mean value±SEM. The data were deviated from normal distribution and analysed using the Kruskal-Wallis test, followed by the Mann-Whitney test. ED50s with 95% confidence limits were determined via the Litchfield and Wilcoxon method using PHARM/PCS version 4 software.[20] Linear regression was employed to evaluate the dose dependency of Guaifenesin effects. SPSS 11.5 was used for data analysis, and a P value <0.05 was considered a significant level.

Results

Effects of Guaifenesin on PTZ-Induced Seizures

Guaifenesin at doses of 100, 200, 300, and 400 mg/kg significantly increased time to the onset of clonic seizure by 141.8, 124.2, 473, and 1721%, respectively, compared to the control group (P<0.05, figure 1). The effect of Guaifenesin in delaying clonic seizure was

dose-dependent (r=0.748, P<0.001). Guaifenesin also increased latency to the onset of myoclonic seizure in a dose-dependent manner (r=0.728, P<0.001). Guaifenesin at all the studied doses, i.e. 100, 200, 300, and 400 mg/kg, delayed the onset of myoclonic seizure by 141.8, 124.2, 167, and 232.9%, respectively, in comparison to the control group (P<0.01, figure 1). Moreover, 300 mg/kg of Guaifenesin increased time to the onset of tonic-clonic seizure by 133%, compared with the control group (P<0.01, figure 1). Only one mouse showed tonic-clonic seizure in the 400 mg/kg Guaifenesin-treated group (figure 1).

Guaifenesin protected the studied animals against PTZ-induced death with an ED50 of 328 mg/kg (table 1). Guaifenesin at the doses of 300 and 400 mg/kg protected 50 and 90% of the animals against PTZ-induced tonic-clonic seizure and 37.5 and 90% against PTZ-induced death, respectively (table 1). Diazepam (3 mg/kg) protected all the animals from PTZ-induced seizure and death.

Effect of Guaifenesin on Neuromuscular Coordination

To evaluate the effect of the different doses of Guaifenesin on neuromuscular coordination, the percent change of stay time on the Rotarod from baseline was analyzed using the Kruskal-Wallis
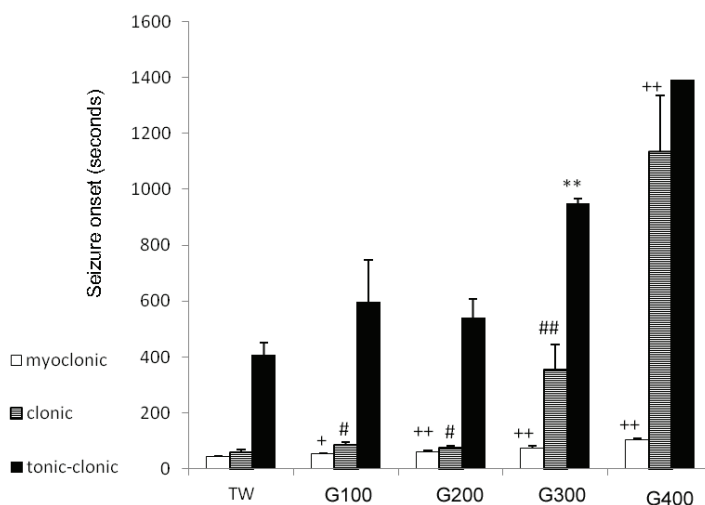

Figure 1: Effects of different doses of Guaifenesin (G100-G400 mg/kg) on the onset of PTZ-induced myoclonic, clonic, and tonic-clonic seizures in mice (N=8-10/group). **, ++, ## P<0.01; +, # P<0.05 significantly different from the control group (TW)

Table 1: Effects of different doses of Guaifenesin on protection against PTZ-induced clonic and tonic-clonic seizures and death (number of animals protected/number of animals per group). ED50s of Guaifenesin (95% confidence interval; 95%CI) for protection against each type of seizures and death have been shown.

Treatment groups	Mortality protection	Clonic seizure protection	Tonic-clonic seizure protection
TW	0.10	0.10	0.10
Guaifenesin 100mg/kg	0.10	0.9	2.10
Guaifenesin 200mg/kg	0.10	1.10	1.10
Guaifenesin 300mg/kg	3.8	0.10	5.10
Guaifenesin 400mg/kg	9.10	3.10	9.10
ED50 (95% CI) (mg/kg)	328 (262-411)	744.88 (360-1540)	256 (178-363)

test. Diazepam (3 mg/kg) and Guaifenesin at all the studied doses, i.e. 100-400 mg/kg, significantly reduced the number of seconds spent on the Rotarod, compared to the control group (P<0.05) (figure 2). However, the effect of Guaifenesin at higher doses, i.e. 300 and 400 mg/kg, on neuromuscular coordination was more pronounced and was significantly different from that of 100 mg/kg of Guaifenesin (P<0.01). Linear regression analysis also showed the dose-dependent effect of Guaifenesin on motor coordination (slope=−0.28, P<0.01). In other words, increasing the dose of Guaifenesin enhanced its effect on motor coordination (figure 2).

Discussion

Guaifenesin, a Propanediol drug used as an expectorant, showed an anticonvulsant effect in our animal model of seizure induced by PTZ. PTZ produces tonic–clonic convulsions in rats or mice and is commonly employed as a reliable animal model for screening new anti-epileptic drugs for absence seizure.[21,22] We evaluated the anticonvulsant effects of Guaifenesin using PTZ-induced seizure in the present study, our results demonstrated that Guaifenesin could not only decrease the susceptibility of mice to PTZ-induced myoclonic, clonic, and especially tonic-clonic seizures but also protect the mice against PTZ-induced death. These results are in agreement with previous studies indicating that Propanediol drugs can exert anticonvulsant activity.[23,24] Felbamate and Meprobamate are among Propanediol drugs previously shown to have anticonvulsant effects. Indeed, Felbamate is currently used as an anti-epileptic drug in clinical practice. Nonetheless, these drugs have serious side effects, including aplastic anaemia. This side effect is less likely to occur with Guaifenesin, which makes it a good candidate as an anticonvulsant drug. Additionally, Guaifenesin can be used during pregnancy and breastfeeding; this further underscores the desirability of this drug as a potential anticonvulsant in clinical practice. Be that as it may, future clinical trials should address its usefulness in absence seizure in humans.

The mechanism by which Guaifenesin may exert anticonvulsant activity is not clear. However, animal models of epilepsy could partly predict the mechanism of action of some antiepileptic drugs.[25] In a model of PTZ-induced seizure, the glutamatergic system, especially NMDA receptors, has been shown to play an important role. Thus, microdialysate, collected from hippocampal regions during seizures induced by PTZ, has revealed a rise in the concentration of glutamate.[26] It has also been demonstrated that the administration of PTZ could up-regulate NMDA receptors in several regions of the rat brain.[27] Therefore, it can be suggested that the NMDA antagonist activity of Guaifenesin may contribute to its anticonvulsant activity seen in this study. This notion requires further elucidation in future studies.

In concordance with previous studies,[11] Guaifenesin at all the studied doses in the present investigation exhibited muscle relaxant activity as indicated by the findings of the Rotarod test, which raises the possibility that the effects of Guaifenesin against PTZ-induced seizure may be due to its muscle relaxant activity. In support of this concept, several experiments have shown similarities between the pathology of muscle spasm and seizure but in different areas of the central nervous system.[28] Considering that the regulation of muscle tone depends on
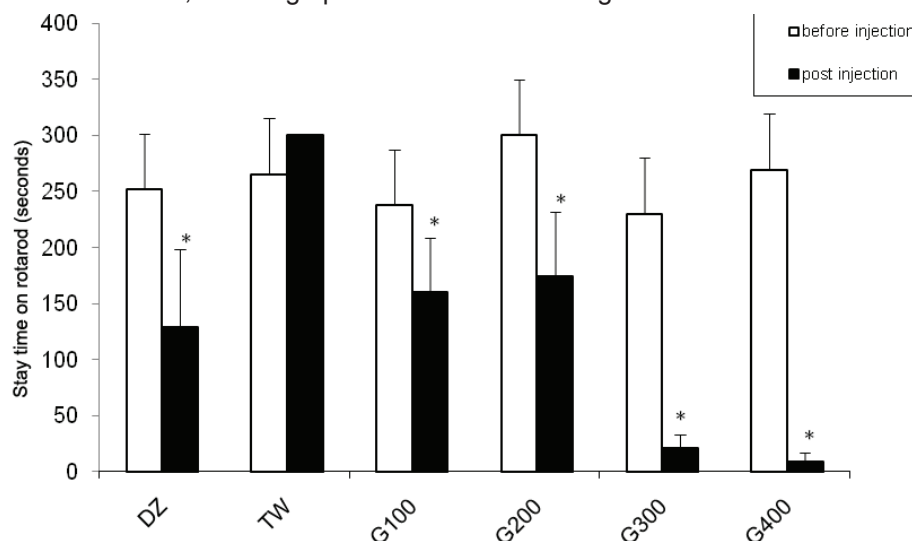

Figure 2: Effects of different doses of Guaifenesin on neuromuscular coordination in mice (N=6/group). Bar graphs represent mean±SEM of time spent on the Rotarod for each group before and after the administration of Guaifenesin (G100-G400 mg/kg), 0.25% Tween 20 (TW) or Diazepam (DZ) 3mg/kg. *P<0.05 significantly different from the control group (TW)

the equilibrium between excitatory and inhibitory neurotransmission within the spinal cord and supra-spinal motor centers, it has been proposed that a pathologically increased muscle tone can be ameliorated by the antagonists of excitatory amino acids.[29] Consequently, the blockade of NMDA-mediated events results in a myorelaxant effect, comparable in efficacy to that of some drugs in clinical use.[30,31] Analogously, studies have indicated that glutamate plays crucial roles in the initiation, spread, and maintenance of epileptic activity,[32,33] and NMDA receptor antagonists have anticonvulsant activity.[34] In this line,Turski et al.[35] found a potent NMDA blocker, which had muscle relaxation and anticonvulsant activity simultaneously. Therefore, Guaifenesin, via a similar mechanism, could produce both muscle relaxation and anticonvulsant effects. On the other hand, a comparison of the muscle relaxant and anticonvulsant effects between Diazepam and Guaifenesin in the current study showed that although Guaifenesin at doses of 300 and 400 mg/kg had more profound effects on muscle relaxation than Diazepam, the effects of Guaifenesin at similar doses in preventing myoclonic and clonic seizures were less marked than those of Diazepam. Therefore, the mechanism whereby Guaifenesin exerts its anticonvulsant effects might be, at least partly, different from that of muscle relaxant activity.

Conclusion

Guaifenesin has anticonvulsant and muscle relaxant properties. As PTZ-induced seizure is a model of absence seizure, it can be suggested that Guaifenesin may be useful in the treatment of absence seizure in humans.

Conflict of Interest: None declared.

References

1   Perucca P, Gilliam FG, Schmitz B. Epilepsy treatment as a predeterminant of psychosocial ill health. Epilepsy Behav. 2009;15:S46-50. doi: 10.1016/j.yebeh.2009.03.016. PubMed PMID: 19303947.

2   Schmidt D. Drug treatment of epilepsy: Options and limitations. Epilepsy Behav. 2009;15:56-65. doi: 10.1016/j.yebeh.2009.02.030. PubMed PMID: 19236951.

3   Cramer JA, Fisher R, Ben-Menachem E, French J, Mattson RH. New antiepileptic drugs: comparison of key clinical trials. Epilepsia. 1999;40:590-600. PubMed PMID: 10386528.

4   Harbord MG. Significant anticonvulsant side-effects in children and adolescents. J Clin Neurosci. 2000;7:213-6. doi: 10.1054/jocn.1999.0190. PubMed PMID: 10833618.

5   Bianchi MT, Pathmanathan J, Cash SS. From ion channels to complex networks: magic bullet versus magic shotgun approaches to anticonvulsant pharmacotherapy. Med Hypotheses. 2009;72:297-305. doi: 10.1016/j.mehy.2008.09.049. PubMed PMID: 19046822.

6   Chapman AG. Glutamate and Epilepsy. J Nutri. 2000;130:1043S-5S. PubMed PMID: 10736378.

7   Kuhn JJ, Hendley JO, Adams KF, Clark JW, Gwaltney JM. Antitussive effect of guaifenesin in young adults with natural colds. Objective and subjective assessment. Chest. 1982;82:713-8. PubMed PMID: 6754274.

8   Braun C, Trim CM, Eggleston RB. Effects of changing body position on oxygenation and arterial blood pressures in foals anesthetized with guaifenesin, ketamine, and xylazine. Vet Anaesth Analg. 2009; 36:18-24. doi: 10.1111/j.1467-2995.2008.00423.x. PubMed PMID: 19121155.

9   Pralong E, Millar JD, Lodge D. Specificity and potency of N-methyl-d-aspartate glycine site antagonists and of mephenesin on the rat spinal cord in vitro. Neurosci Lett. 1992;136:56-8. doi: 10.1016/0304-3940(92)90646-O. PubMed PMID: 1378953.

10 Shuker MA, Bowser-Riley F, Davies SN. Possible NMDA antagonist properties of drugs that affect high pressure neurological syndrome. Br J Pharmacol. 1994; 111:951-5. PubMed PMID: 7912632; PubMed Central PMCID: PMC1910069.

11 Souri E, Sharifzadeh M, Farsam H, Gharavi N. Muscle relaxant activity of methocarbamol enantiomers in mice. J Pharm Pharmacol. 1999;51:853-5. PubMed PMID: 10467962.

12 Peoples RW, Ren H. Inhibition of N-methyl-D-aspartate receptors by straight-chain diols: implications for the mechanism of the alcohol cutoff effect. Mol Pharmacol. 2002;61:169-76. PubMed PMID: 11752218.

13 Zhang Z, Cherryholmes G, Mao A, Marek C, Longmate J, Kalos M, et al. High plasma levels of MCP-1 and eotaxin provide evidence for an immunological basis of fibromyalgia. Exp Biol Med (Maywood). 2008;233:1171-80. doi: 10.3181/0712-RM-328. PubMed PMID: 18535166.

14 Larson AA, Giovengo SL, Russell IJ, Michalek JE. Changes in the concentrations of amino acids in the cerebrospinal fluid that correlate with pain in patients with fibromyalgia: implications for nitric oxide pathways. Pain. 2000;87:201-11. doi: 10.1016/S0304-3959(00)00284-0. PubMed PMID: 10924813

15 Aselton P, Jick H, Milunsky A, Hunter JR, Stergachis A. First-trimester drug use and

congenital disorders. Obstet Gynecol. 1985;65:451-5. PubMed PMID: 3982720.

16 Morrow J, Russell A, Guthrie E, Parsons L, Robertson I, Waddell R, et al. malformation risks of antiepileptic drugs in pregnancy: a prospective study from the UK Epilepy and Pregnancy Register. J Neurol Neurosurg Psychiatry. 2006;77:193-8. PubMed PMID: 16157661; PubMed Central PMCID: PMC2077578.

17 de Sousa DP, Gonçalves JCR, Quintans-Júnior L, Cruz JS, Araújo DAM, de Almeida RN. Study of anticonvulsant effect of citronellol, a monoterpene alcohol, in rodents. Neurosci Lett. 2006;401:231-5. doi: 10.1016/j.neulet.2006.03.030. PubMed PMID: 16650577.

18 Doležal TD, Kršiak MK. Guaifenesin enhances the analgesic potency of paracetamol in mice. Naunyn Schmiedebergs Arch Pharmacol. 2002; 366:551-4. PubMed PMID: 12444496.

19 Rhodes ME, Frye CA. Progestins in the hippocampus of female rats have antiseizure effects in a pentylenetetrazole seizure model. Epilepsia. 2004;45:1531-8. PubMed PMID: 15571511.

20 Hosseinzadeh H, Nassiri Asl M. Anticonvulsant, sedative and muscle relaxant effects of carbenoxolone in mice. BMC Pharmacol. 2003;3:3. PubMed PMID: 12720572; PubMed Central PMCID: PMC156636.

21 Löscher W, Hönack D, Fassbender CP, Nolting B. The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. III. Pentylenetetrazole seizure models. Epilepsy Res. 1991;8:171-89. doi: 10.1016/0920-1211(91)90062-K. PubMed PMID: 1907909.

22 Sarkisian MR. Overview of the Current Animal Models for Human Seizure and Epileptic Disorders. Epilepsy Behav. 2001;2:201-16. doi: 10.1006/ebeh.2001.0193. PubMed PMID: 12609365.

23 Giorgi O, Carboni G, Frau V, Orlandi M, Valentini V, Feldman A, et al. Anticonvulsant effect of felbamate in the pentylenetetrazole kindling model of epilepsy in the rat. Naunyn Schmiedebergs Arch Pharmacol. 1996; 354:173-8. PubMed PMID: 8857594.

24 Swinyard EA, Sofia RD, Kupferberg HJ. Comparative Anticonvulsant Activity and Neurotoxicity of Felbamate and Four Prototype Antiepileptic Drugs in Mice and Rats. Epilepsia. 1986;27:27-34. PubMed PMID: 3004930.

25 White HS. Clinical significance of animal seizure models and mechanism of action studies of potential antiepileptic drugs. Epilepsia. 1997;38:S9-17. PubMed PMID: 9092952.

26 Li ZP, Zhang XY, Lu X, Zhong MK, Ji YH.

Dynamic release of amino acid transmitters induced by valproate in PTZ-kindled epileptic rat hippocampus. Neurochem Int. 2004;44:263-70. PubMed PMID: 14602089.

27 Ekonomou A, Angelatou F. Upregulation of NMDA receptors in hippocampus and cortex in the pentylenetetrazol-induced "kindling" model of epilepsy. Neurochem Res. 1999;24:1515-22. PubMed PMID: 10591400.

28 Meldrum BS, Croucher MJ, Czuczwar SJ, Collins JF, Curry K, Joseph M, et al. A comparison of the anticonvulsant potency of (±) 2-amino-5-phosphono-pentanoic acid and (±) 2-amino-7-phosphonoheptanoic acid. Neuroscience. 1983;9:925-30. doi: 10.1016/0306-4522(83)90281-6.

29 Turski L, Schwarz M, Turski WA, Klockgether T, Sontag KH, Collins JF. Muscle relaxant action of excitatory amino acid antagonists. Neurosci Lett. 1985; 53:321-6. doi: 10.1016/0304-3940(85)90558-0. PubMed PMID: 2858836.

30 Schwarz M, Turski L, Sontag K-H. Reversal of the muscle relaxant effect of diazepam but not of progabide by a specific benzodiazepine antagonist: Ro 15-1788. Eur J Pharmacol. 1983;90:139-42. doi: 10.1016/0014-2999(83)90226-1. PubMed PMID: 6409655.

31 Turski WA, Schwartz M, Turski L, Sontag K-H. A specific benzodiazepine antagonist CGS 8216 reverses the muscle relaxant effect of diazepam but not that of phenobarbitone. Eur J Pharmacol. 1984;98:441-4. doi: 10.1016/0014-2999(84)90296-6. PubMed PMID: 6723782.

32 Doherty J, Dingledine R. The roles of metabotropic glutamate receptors in seizures and epilepsy. Curr Drug Targets CNS Neurol Disord. 2002;1:251-60. PubMed PMID: 12769618.

33 Bradford HF. Glutamate, GABA and epilepsy. Prog Neurobiol. 1995;47:477-511. doi: 10.1016/0301-0082(95)00030-5. PubMed PMID: 8787032.

34 Löscher W, Nolting B, Hönack D. Evaluation of CPP, a selective NMDA antagonist, in various rodent models of epilepsy. Comparison with other NMDA antagonists, and with diazepam and phenobarbital. Eur J Pharmacol. 1988;152:9-17. doi: 10.1016/0014-2999(88)90830-8. PubMed PMID: 3061831.

35 Turski L, Klockgether T, Sontag K-H, Herrling PL, Watkins JC. Muscle relaxant and anticonvulsant activity of 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid, a novel N-methyl-d-aspartate antagonist, in rodents. Neurosci Lett. 1987;73:143-8. doi: 10.1016/0304-3940(87)90008-5. PubMed PMID: 3029636.