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Dose-Dependent U-Shaped Effect of Losartan on Seizure Induced by Pentylenetetrazol in Rats

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ABSTRACT

Introduction: Some experimental data show that losartan can exert anticonvulsant activity; thereby it could create the potential therapy for treatment of epilepsy. However, there are limited and confusing data regarding the anticonvulsant action of losartan. The aim of this study was to answer the question why different studies found different effects of losartan on seizure and epilepsy.

Materials and methods: The Sub convulsive doses (37.5 mg/kg) of pentylenetetrazol (PTZ) were administered (at intervals of one another day), to induce chemical kindling in conscious, free-moving rats. Separate groups of full kindled rats were pretreated with 12.5, 25, 50, 100 and 200 mg/kg doses of losartan.

Results: The results showed although losartan had no significant effect on seizure stage, this drug induced a dose-dependent U-shaped effect on other seizure parameters.

Conclusion: These results may explain the discrepancy reported about the effect of losartan on kindling model of epilepsy.

key words: Seizure, Losatran, Pentylenetetrazol, Rat, Kindling

INTRODUCTION

Epilepsy is the common neurological disorders in humans, affecting about 1 to 3 percent of the people worldwide (1). Kindling is one of the most used models for the screening of anticonvulsant drugs (2). It refers to a phenomenon in which repeated injection of a conversant causes gradual seizure development culminating in generalized tonic–colonic seizures (2). Pentylenetetrazol (PTZ)-induced kindling is widely accepted as an experimental animal model for chronic temporal lobe epilepsy in rats (3).

Discovering a new antiepileptic drug needs much time and money, Continued endeavor has been made to find anti-epileptic effects among drugs that are already listed for the treatment of other diseases and repurpose them as potential anti-epileptic treatments the process that named

drug repositioning. Losartan is one of this drugs that repositioned for treatment of epilepsy.

Over the last 10 years, accumulated experimental and clinical evidence has supported the idea that angiotensin II receptor type 1(AT1) is involved in epilepsy (4-6). Recent experimental data showing that some ACE inhibitors and AT1 antagonists may possess anticonvulsant-like activity(5, 7-9). Therefore, it is likely that effective medications for cardiovascular system such as losartan may affect seizures.

Considerable research has been done on this case, with contradictory results. It has been reported that losartan have no anticonvulsant effects (10, 11), proconvulsant effects (12) and anticonvulsant effects (5, 8). Factors such as route of administration, gender, genetic differences, stimulus intensity and amount of kindling have been reported as being responsible for the lack of concordance in the results (10, 11, 13, 14). It seems that the discrepancy in the results of these studies may be due to the use of different doses of losartan, so in this study we aimed to investigate the effects of different doses of losartan on seizure induced by PTZ.

Materials and methods

Drugs and chemical

Pentylenetetrazol (PTZ) was purchased from Sigma, India and losartan was provided from Exir Pharmaceutical Company, Iran. PTZ and losartan were dissolved in saline. The control animals received saline.

Animals

Ninety male Wistar rats (Pastor Breeding Centre Tehran, Iran) weighing 200-250 g at the beginning of experiments, were used in this study. Animals divided into a control group (n= 10) and five losartan pretreatment groups (n= 16 in each group).The rats were housed in environmentally controlled conditions (12 h light/dark cycles, 7:00–19:00 light and 19:00–7:00 dark, temperature 22 °C ±2) at the Arak University of Medical Sciences animal facility. Food and water were supplied ad libitum. All procedures were carried out in accordance with EU Directive 2010/63/EU and the university ethics committee standards (Arak University of Medical Sciences Research Ethics Committee, ethical approval # 1398-241).

Kindling

To induce kindling, all animals received a sub convulsive dose of PTZ (37.5 mg/kg, ip injection) every other day for a period of 26 days (13 injections). After each injection, animals were kept in a plexiglas chamber (30 cm × 30 cm × 30 cm) and convulsive behavior was recorded for 30 minutes. Convulsive responses were classified by Racine's scale (15). Rats were considered fully kindled when seizure attacks (stage five) occurred after each injection for three consecutive injections. The recording parameters were as follows: seizure stage, latency to the onset of stage two (S2L) and five (S5L) seizures, and stage five duration (S5D).

Seizure scaling

The seizure responses observed over a cut off period of 30 minute and were classified as initially described by Racine zero: no response; one: ear and facial twitching; two: convulsive waves through the body; three: myoclonic jerks, rearing; four: tonic-clonic convulsions, turn over into side position; five: generalized tonic-clonic seizures, loss of postural control(16).

Losartan pretreatment

Eighty full kindled rats pretreated by losartan in five different doses; 12.5; 25; 50; 100 and 200 mg/kg (all doses injected intraperitoneally, ip). After an hour, PTZ (37.5 mg/kg, ip) injected and seizure behavior was recorded for 30 minutes.

Statistical analyses

Statistical analyses were performed using graphpad prism (Version 6). A one-way analysis of variance was conducted, followed by a Tukey's test for multiple comparisons. An unpaired Student's t-test (two-tailed) was used to compare two different groups of animals. Seizure stage data was analyzed by nonparametric Kruskal-Wallis with post-hoc Mann Whitney U tests. The criterion for statistical significance was $p < 0.05$. Results are

reported as mean ± standard deviation of the mean (SD).

Results

Seizure parameters

The results of this study showed that although different doses of losartan did not have significant effects on the seizure stage (Fig. 1A, P= 0.73), this drug had significant effects on other seizure parameters such as S5D ((F4,75)= 8.824, P= 0.0004), S2L ((F4,75)= 4.067, P= 0.0049) and S5L ((F4,75)= 8.694, P= 0.0005). The results showed that although low (12.5mg/kg, P= 0.0001) and high doses (100 and 200 mg/kg, P= 0.0072) of losartan significantly reduced S5D, middle doses (25 and 50 mg/kg, Fig. 1B) had no significant effects on this parameter, that means unlike high and low doses of losartan, middle doses of losartan had no inhibitory effects on seizure duration. Statistical analysis of the effect of losartan on stage two latency showed that losartan were significantly increased S2L in higher (200 mg/kg, P= 0.0096) and lower (12.5 mg/kg, P= 0.0128) doses as compare to medium dose (50mg/kg) (Fig 1C). The effect of different doses of losartan on S5L had same pattern of response as S2L, and higher (200 mg/kg, P= 0.0065) and lower doses (12.5 mg/kg, P= 0.00.021) of losartan prolonged S5L as compared to middle dose 50 mg/kg (Fig. 1D). Thus it seems that the effect of losartan on S2L, S5L and S5D were dose-dependent and dose of 50 mg/kg has lowest inhibitory effects in PTZ seizures.

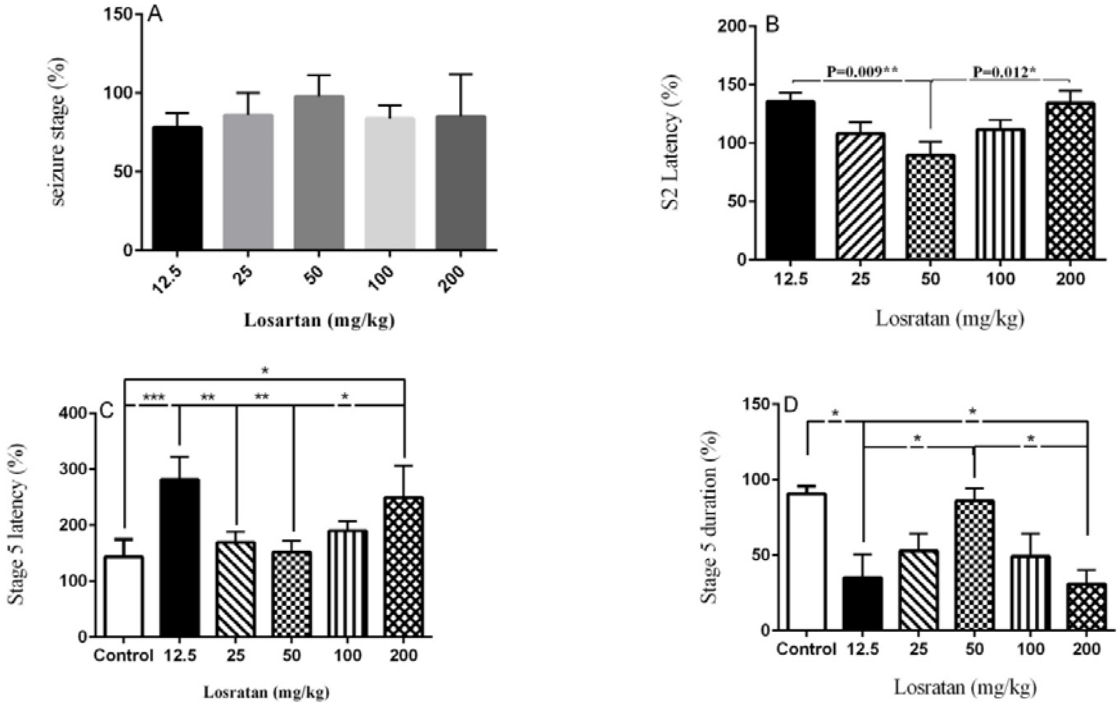

*Figure 1. Effects of different doses of losartan on seizure stage (A), stage 2 latency (B), stage 5 latency (C) and stage 5 duration (D), in penteylenetetrazol induced kindled rats. While the varying doses of losartan had no significant effect on seizure stage. Losartan at the 12.5 and 200 mg/kg doses significantly increases latency to stage 2 (S2L) and stage 5(S5L) seizure. Also, losartan at the 12.5 and 200 mg doses significantly reduced the stage 5 duration (S5D). *P < 0.05, **P < 0.01, ***P < 0.001*

Discussion

In this study, we investigated the effectiveness of different doses of losartan on epileptogenesis induced by PTZ kindling. We found that although intra peritoneal administration of losartan had no effect on seizure stage, this drug has U-shaped effect on the other seizure parameters such as S2L, S5L and S5D. These results may help to explain the contradictory

effects of losartan on seizure that was reported by different laboratories.

Although considerable research has been done on the effects of losartan on seizure and epilepsy, the results reported from these researches have not been unanimous. One of the possible reasons for these contradictory results could be due to the fact that only the seizure stage (but not other seizure parameters) was reported in these investigations. Our data showed that different doses of losartan had no significant effect on seizure stage. in accordance to this point previous research reported that losartan was not protective against seizure stage (11, 17). On the other hand our results showed that this drug had a significant effects on other seizure parameters. Recently Jadhav et al(18), showed that losartan only can increase S2 latency in PTZ induced seizure and it hasn't any anticonvulsant effects on other seizure parameters. However, our results revealed that losartan in addition to increase S2l and S5l; decreased S5D.This discrepancy may be due to differences in the doses of losartan used in the two studies (50 mg/kg vs. 12.5, 25, 50 100 and 200 mg/kg used in our research).

Another point about our results is the U-shaped effects of different doses of losartan on seizure parameters. Our results showed that although dose of 50 mg/kg of losartan was no effective on seizure parameters, higher and lower doses of losartan have significant effects on these parameters. In accordance with our data some studies revealed that, losartan although suppressed the seizure parameters in dose10 mg/kg (9, 19-21) or 100 mg/kg(22, 23), it didn't have any antiepileptic effects in 50 mg/kg(10, 11).

Jialong Zhuo et al(24) revealed meaningful increasing and dose dependent antiepileptic effect of losartan in doses 1 to 10 mg/kg. However, we evaluated antiepileptic effects of losartan from 12.5 to 200mg/kg and found that, in contrast to medium dose (50mg/kg), losartan can show meaningful anti-epileptic effect in lower (12.5mg/kg) and high (200mgkg) doses and there is a U-shaped dose dependent antiepileptic effect. Results of Zhuoet study confirmed our results and if they continued evaluation of losartan in higher doses probably could found U-shaped effects of this drug.

Although, there have been no studies comparing the effects of different doses of losartan on epilepsy, Jun Ming Wang et al(25) showed that inhibitory effect of losartan increased dose dependently from 10 to 30mg/kg and 100 mg/kg. But in this work, the effect of 50 mg dose has not been evaluated. Probably if a dose of 50 mg/kg of losartan was evaluated in this research they would revealed the U-shaped dose dependent effect of losartan.

The results suggest that the losartan that, commonly used as a strategy for prevention of high blood pressure, may be useful (higher and lower doses than 50 mg/kg) as an adjunctive treatment to reduce seizure severity.

Conclusion

The relationship between losartan with seizure parameters remains unclear. The published data in this area are highly inconsistent. It seems that the underlying causes of the inconsistence include small sample size, heterogeneity in. Another possible explanation for the contradictory results is that losartan may have U-shaped effects on some seizure parameters. It would be interesting to investigate the effect of losartan in inbred animals and in other animal models of epilepsy.

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Ethical approval

Ethical approval for the study was provided by the Arak University of Medical Sciences Research Ethics Committee # 1398-241.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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