

Effects of omeprazole in caffeine and phentyletetrazole-induced generalized seizures in mice

Farelerde kafein ve pentilentetrazol ile oluşturulan generalize nöbetlere omeprazolün etkileri

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This study was presented in the V. National Neuroscience Congress, April, 10-14, 2006, Zonguldak, Turkey.

This manuscript can be downloaded from the webpage:
[http://tipdergisi.erciyes.edu.tr/download/2007;29\(3\)184-188.pdf](http://tipdergisi.erciyes.edu.tr/download/2007;29(3)184-188.pdf)

Submitted : July 28, 2006
Revised : March 2, 2007
Accepted : April 24, 2007

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Abstract

Purpose: Because omeprazole has a carbonic anhydrase inhibitor activity, it was aimed to investigate whether omeprazole has anticonvulsant effect on caffeine and phentyletetrazole (PTZ)-induced generalize seizures in mice.

Material and Methods: Omeprazole (0.25- 0.5- 1- 2 mg/kg), diazepam (0.5 mg/kg for PTZ model and 5 mg/kg for caffeine model) and distilled water were administrated, 30 min later caffeine (300 mg/kg) or PTZ (100 mg/kg) were injected to all groups, intraperitoneally. Following the caffeine or PTZ injections, the time taken for the onset of the animals' first generalize tonic-clonic convulsion was measured in second, was accepted as the latency period. Tolerance potential of omeprazole were done with 0.5 mg/kg (upon repeated administrated) in caffeine-induced convulsion model.

Results: Following the caffeine and PTZ injections, omeprazole prolonged the latency periods in comparison with caffeine and PTZ groups. The longest latency was observed by omeprazole 0.5 mg/kg dose in the caffeine model (307.47 %, $p < 0.05$), and omeprazole had showed more protective effect in caffeine seizures than in PTZ seizures. In the tolerance study, latency periods were shortened by omeprazole on following days.

Conclusion: Low doses of omeprazole, in especially caffeine-induced seizures, presented an anticonvulsant activity, but tolerance across to this action developed as the other carbonic anhydrase inhibitors.

Key Words: **Caffeine; Carbonic anhydrase inhibitors; Convulsive seizures; Omeprazole; Pentylene tetrazole.**

Özet

Amaç: Karbonik anhidraz inhibitör özelliği bilinen omeprazolün, farelerde kafein ve pentilentetrazol ile oluşturulan generalize nöbetlerde anti-konvulsan etkilerinin araştırılması amaçlandı.

Gereç ve Yöntemler: Omeprazol(0,25- 0,5- 1- 2 mg/kg), diazepam (PTZ modeli için 0,5 mg/kg ve kafein modeli için 5 mg/kg) ve distile su uygulandıktan 30 dakika sonra gruplara kafein (300 mg/kg) veya PTZ (100 mg/kg) intraperitoneal olarak uygulandı. Kafein veya PTZ enjeksiyonlarını takiben, hayvanlarda ilk generalize tonik klonik konvülsiyonların başlamasına kadar geçen süre saniye olarak ölçüldü ve latent periyot olarak kabul edildi. Omeprazolün tolerans potansiyeli 0,5 mg/kg dozla (tekrarlayan uygulamalarda) kafein ile oluşturulan konvülsiyon modelinde çalışıldı.

Bulgular: Kafein ve PTZ enjeksiyonlarını takiben, omeprazol latent periyotları uzattı. En uzun latent periyot 0,5 mg/kg dozla kafein modelinde gözlemlendi (% 307,47; $p < 0,05$), ve omeprazol kafein nöbetlerinde PTZ nöbetlerine göre daha koruyucu idi. Tolerans çalışmasında ise takip eden günlerde omeprazol latent periyotları kısalttı.

Sonuç: Omeprazol düşük dozlarda, özellikle kafein ile oluşturulan nöbetlerde, anti-konvulsan aktivite göstermiştir, fakat bu etkisine karşı diğer karbonik anhidraz inhibitörleri gibi tolerans gelişmiştir.

Anahtar Kelimeler: **Kafein; Karbonik anhidraz inhibitörleri; Konvülsif nöbet; Omeprazol; Pentilentetrazol**

Introduction

Carbonic anhydrase (CA) enzyme is quite abundant in the brain, mainly as the cytosolic isozymes CA II, CA VII, and the membrane-bound isoform CA XIV. CA inhibitors (acetazolamide, topiramate and zonisamide) are used in treatment of epilepsy. It has been postulated that the anticonvulsant effects of these are due to CO₂ retention secondary to inhibition of red blood cells and brain CA (1), and to a mild intra neuronal acidosis; in addition, there is a decrease in depolarizing bicarbonate efflux from neurons via GABA receptor ion channels, which occurs secondary to CA inhibition (2). It has been reported that inhibition of this enzyme significantly increased the latency of onset of seizures and that alterations of the extracellular and intracellular pH by acetazolamide decreased the efficacy of synaptic transmission in several areas of the brain (3). However, although these drugs have anticonvulsant activity, the rapid appearance of drug tolerance limits the value of these drugs as an anticonvulsant.

Omeprazole has long been used as an antiulcerogenic drug in the treatment of peptic ulcer. It has a dual mechanism of action: it inhibits both H⁺-K⁺ ATPase enzyme in the gastric mucosa and CA types I, II and IV (4).

In the literature, only two studies were found related with the anticonvulsant activity of omeprazole. In both studies, anticonvulsant activity was examined in maximal electroshock-induced seizure model (5,6). While Balakrishnan et al.(5) reported that this drug showed anticonvulsant activity, Swiader et al.(6) suggested that it did not.

The aim of the present study was to evaluate whether omeprazole has protective effect against generalized seizures induced with two different chemical agents (caffeine and phentyletetrazole), and whether repeated omeprazole administration has caused the tolerance, in mice.

Material and Methods

Animals. In the present study, 156 male Swiss albino mice (25-35g) were used (Pharmacology Department, Medical Faculty, Atatürk University, Erzurum). Lighting operated on 12h dark 12h light cycle (lights on a.m.) and the temperature was maintained at 20-23°C. The animals were left for 24h to become accustomed to laboratory conditions and were maintained on a standard pellet diet

and water ad libitum. The study was performed according to the international, national and institutional rules considering animal experiments rights. Caffeine and PTZ-induced seizures in the mice were performed between 09:00 and 12:00 h to minimize the effect of circadian rhythm.

Drugs. Omeprazole (Losec, Astra Zeneca-Turkey), Caffeine (Sigma-USA), PTZ (Sigma-USA) and diazepam (Diazem, Deva-Turkey) used in the study were dissolved in distilled water and were injected in a volume of 0.1ml/10gr intraperitoneally (ip).

PTZ-induced convulsion model. Animals were separated as control (distilled water), diazepam and omeprazole groups. Omeprazole (0.25- 0.5- 1- 2 mg/kg), diazepam (0.5 mg/kg) and distilled water were administrated ip, 30 minutes before PTZ (100 mg/kg, ip) injections (7).

Caffeine-induced convulsion model. Animals were separated as control (distilled water), diazepam and omeprazole groups. Omeprazole (0.25- 0.5- 1- 2 mg/kg), diazepam (5 mg/kg) and distilled water were administrated ip, 30 minutes before caffeine (300 mg/kg, ip) injections (7).

Tolerance study. This section of the study was evaluated in caffeine model. On first day, the most effective anticonvulsant dose of omeprazole had been determined as 0.5 mg/kg, and it was administrated once/day for following six days (5). On the following days, six mice were distinguished from the group on each day. Omeprazole (0.5 mg/kg) had been injected, and 30 minutes later caffeine (300 mg/kg) was administrated.

Following caffeine or PTZ injections, the animals were observed for 30 minutes, and the time taken for the onset of the animals' first generalize tonic-clonic convulsion was measured in second (s), was accepted as the latency period.

Statistical analysis. The latency periods of each group were presented as Mean SEM. Following one-way ANOVA analysis, results were compared by using post-hoc LSD test. The significance level was accepted as $p < 0.05$.

Results

Effects of omeprazole in PTZ-induced seizures. Omeprazole dose dependently prolonged the latency periods, while compared to control groups. But, it was not as effective as diazepam on latency periods (Table I).

Effects of omeprazole in caffeine-induced seizures. Low doses of omeprazole (0.25 - 0.50 mg/kg) prolonged the latency periods ($p < 0.05$), when compared to control groups (Table II). At a dose of 0.5 mg/kg omeprazole was found to be more effective than diazepam on latency periods ($p < 0.01$).

Influence of omeprazole in the tolerance study. This section of the study was carried out with omeprazole 0.5 mg/kg, in caffeine model. While the latency time was 527.00 ± 21.87 s on the first day, the latency times were especially shortened on day 3, 4, 5 and 7 (Table III).

Discussion

In the study, we found that omeprazole (0.25 and 0.5 mg/kg), in a single-dose, showed significant protection against especially caffeine-induced generalized seizures. In the PTZ model, antiepileptic activity was also observed, although this activity was found less than that of diazepam.

Several CA inhibitors (acetazolamide, methazolamide, topiramate, and zonisamid) are still used as antiepileptic drugs. The anticonvulsant effects of these are probably due to CO₂ retention, secondary to inhibition of red cell and brain enzymes. However, other mechanisms of activity, such as blockade of sodium channels and kainite/AMPA receptors, as well as enhancement of GABA-ergic transmission, were also hypothesized /proved for some of these drugs (1). It was found that there were only two studies related with the anticonvulsant activity of omeprazole in the literature, and in both studies, the anticonvulsant activity of omeprazole was evaluated in maximal electroshock-induced seizure model (5,6). While Balakrishnan et al. showed that it afforded significant protection against seizures in a single-dose study (dose-dependently) (5), Swiader et al. reported that it did not alter the threshold and did not affect the anticonvulsant activity of conventional antiepileptic drugs (6). In the present study, epileptic seizures were induced by caffeine or PTZ. Although convulsive activity of PTZ is not fully understood, it has been reported that PTZ-induced seizures are accompanied by the increases in intracellular calcium and extracellular potassium ion concentrations (8,9,10). In PTZ-induced seizures, although omeprazole was not as effective as diazepam, it showed anti-epileptic activity (dose-dependently); the anti-epileptic activity of omeprazole in PTZ-induced seizures may be due to a blockage of the ion channels (especially voltage-dependent calcium channels). Okabe et al. reported that the

vasoinhibitor effect of leminoprazole on aorta vessels might be due to inhibitor effect on voltage-dependent calcium channels (11). Therefore, in PTZ-induced seizures, omeprazole may act as calcium antagonist. In caffeine-induced seizures, the low doses of omeprazol prolonged the latency periods. Caffeine is a methylxanthine and has some direct and indirect effects on intracellular calcium concentration via cell membrane hyper-polarisation, and it also antagonises adenosine receptors (12). The high doses of caffeine induce convulsions by blocking adenosine (A1) receptors or by interfering with GABA-benzodiazepine receptor complex (13), and may decrease the cerebral blood flow in situations such as hypoxia, ischemia and seizures (14,15). In the caffeine model, the anti-convulsive effect of omeprazole may be due to the following: (a) blocking of caffeine-induced cerebral blood flow decrease, (b) blocking of caffeine-caused antagonist effect on adenosine A1 receptors, and (c) acting as adenosine. As adenosine is a powerful endogenous anti-convulsant (16,17), it causes a rapid and marked cerebral vasodilatation with increased cerebral blood flow, and also has anti-thrombotic activity and neuroprotective effect in cerebral ischemia (18). In addition, omeprazole, as other CA inhibitors, dependent on the CA inhibitor effect may cause the cerebral vasodilation (19,18) and/or it may decrease cerebrospinal fluid production and intracranial pressure, because its inhibitor effect on K⁺,H⁺-ATPase enzyme (20, 21). In the caffeine model of the present study, high doses of omeprazole were found to shorten the latency periods. Inhibition of CA enzyme causes the metabolic acidosis; the high doses of this drug may facilitate the caffeine caused acidosis. In the literature, it was also reported that omeprazole at high doses induced generalized convulsive crises (22).

The tolerance study was evaluated in caffeine-induced seizures. The tolerance developing to the anticonvulsant effect of the drug (0.5mg/kg) was observed on days 2, 3, 4 and 5, although it appeared that the tolerance could be overcome on day 6 (but on this day, SEM value was very high) and again the latency time was shortened on day 7. While Balakrishnan et al. stated in their study that tolerance to this drug developed rapidly upon repeated administration of the drug (5), Swiader et al. reported that omeprazole administered for three days or seven days did not alter the electroconvulsive threshold (6). The findings of the present study are similar to those of Balakrishnan et al (5). The rapid tolerance to CA inhibitors, which develops limits their clinic usefulness. Whereas, acetazolamide

used in the treatment of epilepsy has adverse effects including kidney stones, metabolic acidosis, lethargy, appetite suppression, paresthesias and rare blood dyscrasias (23), omeprazole has few side effects and has an excellent tolerability even upon long-term usage, and this property of omeprazole may be an advantage.

Omeprazole showed the protective effect on both caffeine- and PTZ-induced seizures, but its low doses were more effective especially in caffeine model. This study is a preliminary study and omeprazole should be examined in different seizures models and in further preclinical studies.

Table I. Effects of omeprazole on latency periods in PTZ-induced seizures (for one-way ANOVA analysis, p value is 0.000).

Groups	n	Convulsion rate	Latency periods (s) (Mean \pm SEM)	Prolongation %	p
Control (PTZ 100mg/kg)	10	10 / 10	66.60 \pm 7.44	-	-
Diazepam (0.5 mg/kg)	10	8 / 10	353.75 \pm 23.39	531.16	0.000
Omeprazole (0.25mg/kg)	10	10 / 10	74.68 \pm 8.02	112.01	> 0.05
Omeprazole (0.5 mg/kg)	10	10 / 10	88.17 \pm 8.60	132.39	> 0.05
Omeprazole (1 mg/kg)	10	10 / 10	93.50 \pm 8.13	140.39	> 0.05
Omeprazole (2 mg/kg)	10	10 / 10	110.33 \pm 15.45	165.66	0.015

p: levels as compared to control group (Post-hoc LSD test).

n: represents number of animals in each group

Table II. Effects of omeprazole on latency periods in caffeine-induced seizures (for one-way ANOVA analysis, p value is 0.000).

Groups	n	Convulsion rate	Latency periods (s) (Mean \pm SEM)	Prolongation %	p
Control (caffeine300mg/kg)	10	10 / 10	171.40 \pm 19.44	-	-
Diazepam (5 mg/kg)	10	10 / 10	284.00 \pm 16.81	165.69	0.000
Omeprazole(0.25mg/kg)	10	9 / 10	232.50 \pm 21.47	135.65	0.021
Omeprazole (0.5 mg/kg)	10	9 / 10	527.00 \pm 21.86	307.47	0.000
Omeprazole (1 mg/kg)	10	10 / 10	129.16 \pm 12.01	-	> 0.05
Omeprazole (2 mg/kg)	10	10 / 10	125.66 \pm 12.48	-	> 0.05

p: levels as compared to control group (Post-hoc LSD test).

n: represents number of animals in each group.

Table III. In caffeine model, tolerance study with omeprazole (for one-way ANOVA analysis, p value is 0.000).

Days	n	Latency periods(s) (Mean ± SEM)	p
1 st day	10	527.00 ± 21.86	-
2 nd day	6	492.17 ± 50.94	> 0.05
3 rd day	6	187.00 ± 27.62	0.000
4 th day	6	120.50 ± 7.47	0.000
5 th day	6	161.83 ± 11.22	0.000
6 th day	6	485.83 ± 76.78	> 0.05
7 th day	6	233.67 ± 43.67	0.000

p: levels as compared to control group (Post-hoc LSD test).

n: represents number of animals in each group

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