

The Effects of Pregabalin on Gastric Ulcer Formation and Antioxidant Parameters

Pregabalinin Gastrik Ülser Oluşumu ve Antioksidan Parametreler Üzerine Etkileri

¹Bilgin Kaygisiz, ¹Sule Aydın, ¹Cafer Yildirim, ²Hadi Karimkhani, ³Setenay Oner, ¹Fatma Sultan Kilic

¹Eskisehir Osmangazi University Medical School Department of Pharmacology, Eskisehir, Turkey

²Istanbul Medipol University, Medical School, Department of Biochemistry, Istanbul, Turkey

³Eskisehir Osmangazi University Medical School Department of Biostatistics, Eskisehir, Turkey

Abstract: Pregabalin, a drug used in epilepsy, anxiety, neuropathic pain is reported to have analgesic effects in inflammatory pain. We aimed to investigate whether pregabalin have gastric side effects and to compare with a non steroidal antiinflammatory drug (NSAID) in rats. The effects of pregabalin on antioxidant levels, which are suggested to protect against gastric mucosal damage were also studied. Pregabalin 30, 50, 100 mg/kg, indomethacin 5 mg/kg (reference-NSAID), saline (control group) were administered orally for 10 days. At the end of 10 day treatment, rats were sacrificed, gastric tissues were removed out, mucus secretion was determined spectrophotometrically, ulcer index was scored from score 0:(no-petechia) to score 3:(petechia>5mm). Also, to evaluate the antioxidant effects of pregabalin, malondialdehyde (MDA) levels, catalase and superoxide dismutase (SOD) activities in gastric tissue were studied. Pregabalin 50 mg/kg and 100 mg/kg similar to indomethacin significantly reduced mucus secretion and increased ulcer index compared to control while pregabalin 30 mg/kg did not. Pregabalin 30 mg/kg and 100 mg/kg decreased SOD and catalase levels. Pregabalin 100 mg/kg dose increased MDA levels. 50 mg/kg and 100 mg/kg pregabalin showed gastric side effects as reduced mucus secretion and ulcer formation similar to indomethacin and 30 mg/kg pregabalin may be reasonable dose without showing gastric side effects. Pregabalin 50 mg/kg seems to have enhancing effects on antioxidant levels

Keywords: pregabalin, malondialdehyde, superoxide dismutase, catalase, gastric mucus secretion, gastric ulcer index

Özet: Pregabalin epilepsi, anksiyete ve nöropatik ağrıda kullanılan analjezik etkiye sahip antiinflamatuvar bir ajandır. Biz çalışmamızda pregabalinin sıçanlarda mide üzerine yan etkilerinin olup olmadığını ve bir non-steroidal antiinflamatuvar bir ajan ile etkisini karşılaştırmayı amaçladık. Pregabalinin gastrik mukozal hasara karşı koruyucu olduğu düşünülen antioksidan seviyeleri üzerine olan etkilerini de araştırdık. Pregabalin 30, 50, 100 mg/kg, indometasin 5 mg/kg (referans-NSAID), salin (kontrol grubu) 10 gün boyunca oral olarak uygulandı. 10 günlük tedavinin sonunda, sıçanlar kurban edildi, mide dokuları çıkarıldı, mukus salgılanması spektrofotometrik olarak belirlendi, ülser indeksi 0'dan (no-peteşi) skor 3'e (peteşi > 5mm) kadar skorlandı. Ayrıca pregabalinin mide dokusundaki antioksidan etkilerini değerlendirmek üzere malondialdehid (MDA), katalaz ve süperoksit dismutaz (SOD) aktiviteleri çalışıldı. Pregabalin 50 mg/kg ve 100 mg/kg dozları kontrol grubu ile karşılaştırıldığında indometazine benzer şekilde anlamlı olarak mukus salgılanmasını azalttı ve ülser indeksini artırdı. Pregabalin 30 mg/kg dozunda bu etki görülmedi. Pregabalin 30 mg/kg ve 100 mg/kg SOD ve katalaz seviyelerini düşürürken pregabalin 100 mg/kg dozunda MDA seviyeleri artmıştır. 50 mg/kg and 100 mg/kg pregabalin mukus salgılanmasını ve ülser oluşumu indometazin 30 mg/kg dozuna benzer şekilde azaltarak gastrik yan etkiler göstermiştir. Pregabalin 30 mg/kg dozu ise gastrik yan etkilerin ortaya çıkmadığı uygun doz olabilir. Pregabalin 50 mg/kg dozunun ise antioksidan seviyelerini artırıcı etkiye sahip olduğu görülmüştür.

Anahtar Kelimeler: pregabalin, malondialdehid, süperoksit dismutaz, katalaz, gastrik mukus sekresyonu, gastrik ülser indeksi

ORCID ID of the authors: B.K. 0000-0001-5910-9914, S.A. 0000-0003-2498-8378, C.Y. 0000-0002-1565-9217, H.K.0000-0002-4966-1745, S.O. 0000-0002-4759-4913, F.S.K. 0000-0002-5356-696X

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Correspondence: Bilgin KAYGISIZ- Eskisehir Osmangazi University Medical School Department of Pharmacology, Eskisehir, Turkey
e-mail-bilginkay@yahoo.com

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1. Introduction

Pregabalin (PGB) (S-(+)-3- isobutylgaba), a gabapentin derivative, is an anti-convulsant agent used in the treatment of epilepsy (1). In addition, PGB is used in the treatment of central and peripheral neuropathic pain (2). PGB has a similar mechanism of action to gabapentin that acts via GABAergic neurotransmission, voltage-dependent potassium channels and calcium channels (3).

PGB is reported to have antinociceptive effect in neuropathic pain as well as inflammatory pain (4). PGB was recommended as major therapeutic drug in the treatment of neuropathic pain although it presents weight gain as a side effect (5). In addition, PGB showed antinociceptive effect in experimental studies (6). It is suggested that PGB exerted antinociceptive effect in inflammatory pain via inhibiting the release of neuropeptides on sensory neurons (7). Furthermore, it was reported that gabapentin which has structural and functional similarities to PGB, showed anti-inflammatory effect in rats (8).

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used in the treatment of acute and chronic pains, rheumatoid arthritis, osteoarthritis, neuromuscular diseases, muscle-skeleton diseases. However, major and common gastrointestinal side effects such as ulcer developments restricted their clinical usage (9, 10).

NSAIDs cause tissue damage and free oxygen oscillation and relatedly oxidative stress. The molecules occurring by oxidative stress are known as reactive oxygen metabolites and they damage cell components such as lipid and DNA. The increase in malondialdehyde (MDA) levels is an indicator of lipid peroxidation. Lipid peroxidases increase especially during inflammation. Important indicators of antioxidant enzymes preventing oxidative stress are superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px) (11, 12).

As mentioned above, PGB might have anti-inflammatory effect and may be a potential use as an alternative anti-inflammatory agent

to NSAIDs. Thus, it is important to investigate if pregabalin also exerts gastric side effects which restrict its clinical use. In this study, we aimed to investigate the effects of PGB on gastric mucus secretion and gastric ulcer index which are considered as gastric side effects, and antioxidant parameters which are reported to protect against gastric damage in rats and compare its effects with a reference NSAID, indomethacin.

2. Material and Methods

Animals: Adult female Wistar albino rats (250-300 g) were used in this study. Rats were housed in standard conditions under controlled temperature ($23\pm 2^{\circ}\text{C}$) and lighting (12/12 h light/dark cycle), with food and water available ad libitum. All experiments were approved by the Local Ethical Committee of Experimental Animal Research of Eskisehir Osmangazi University (429/2015).

Drugs: Pregabalin (Lyrica, Pfizer, New York, USA) and indomethacin (Fluka, BioChemika, New York, USA) were dissolved in saline and administered via oral gavage for 10 days. Indomethacin, a NSAID, was used as a reference drug.

Experimental Design: Animals were divided into 5 groups (n=7 per group):

- 1-Control group
- 2- PGB 30 mg/kg group
- 3- PGB 50 mg/kg group
- 4- PGB 100 mg/kg group
- 5- Indomethacin (INDO) 5 mg/kg group

Pregabalin doses were determined based on our previous published studies (6).

a-Determination of gastric mucus secretion: Rats were deprived of food 16 hours before the experiment. Animals were sacrificed on the 10th day of the drug treatment procedure. Stomachs were removed out and gastric lumens were washed with saline. Gastric mucosal amount at glandular area was measured spectrophotometrically at 605 nm wave length by *alcian blue* method. Results were obtained as $\mu\text{g alcian blue} / \text{gram tissue}$ (13).

b- Determination of gastric ulcer index: Rats were anesthetized on the 10th day of the drug treatment procedure. Gastric tissues were removed out and petechiae were counted. 5 petechia was accepted as 1mm. ulcer and ulcer index was scored between 0 and 3 as follows: 0: No petechia, 1: Minor lesion (0,5 mm ulcer); 2: medium level lesion (3-4 mm ulcer); 3: major lesion (ulcer> 5mm) (14).

c- Determination of MDA levels in gastric tissue: MDA levels in gastric tissue was determined as it indicated in the literature (15). It is based on the reaction of tiobarbituric acid with malondialdehyde, a product of lipid peroxidation. Briefly, diethylacetate was used as standard in the study. 100 ml saline, 40 ml 8.1% sodium dodecyl sulfate (SDS), 300 ml 20% acetic acid (pH 3,5) and 300 ml 0.8% tiobarbituric acid were mixed and the mixture was completed to 800 ml with water and warmed at 95,8°C in water bath for 60 mins. After cooled, 200 ml water and 1 ml N-butanoyl/pyridine was added and shaken. After 10 minutes of centrifuge at 4000 rpm, organic layer was abstracted and its absorbance was measured at 532 nm wave length. Lipid peroxidation level was stated as tiobarbituric acid/mg protein.

d- Determination of Catalase Activity in gastric tissue: Catalase activity in gastric tissue was determined spectrophotometrically as it indicated in the literature (16). Briefly, samples were added to 1 M Tris- HCl /5mM dissolved in 50% EDTA H₂O₂, pH 8,0 and the mixture was incubated at 37 °C for 10

minutes. Change in absorbance was recorded at 230 nm wave length. Decrease per min at optic intensity was determined and enzyme activity was stated as U/ml.

e- Determination of SOD Activity in gastric tissue: SOD activity in gastric tissue was determined spectrophotometrically as it indicated in the literature (17). Briefly, the enzyme activity that reduce the amount of nitro blue tetrazolium by 50% was determined.

Statistical Analysis: Statistical analysis were performed using SPSS version 15.0 statistical pack software 15.0 (SPSS Inc., Chicago, IL, USA). The data of gastric mucus secretion was analyzed statistically with One-Way ANOVA, while the data of gastric ulcer, Catalase, Superoxide dismutase (SOD) and Lipid Peroxidase (Malondialdehyde: MDA) were evaluated by Kruskal-Wallis test. P<0,05 value was accepted as statistically significant.

3. Results

a-The effects of pregabalin on gastric mucus secretion

PGB 50 mg/kg, 100 mg/kg and INDO groups decreased gastric mucus secretion compared to control group (p<0,05), but there was no significant difference between PGB 50, 100 mg/kg and INDO groups (Fig. 1). Furthermore, PGB 30 mg/kg did not change gastric mucus secretion compared to control group (p<0,05).

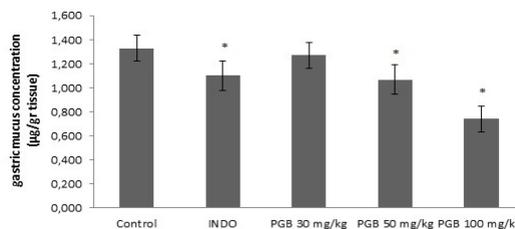


Figure 1. The effects of pregabalin on gastric mucus secretion. Results are given as mean \pm SEM. * p<0,05 compared to control group. PGB: pregabalin, INDO: indomethacin.

b-The effects of pregabalin on gastric ulcer index

PGB 50 mg/kg, 100 mg/kg and INDO groups increased gastric ulcer index compared to control group ($p < 0,05$) while PGB 30 mg/kg did not increase gastric ulcer index (Fig. 2).

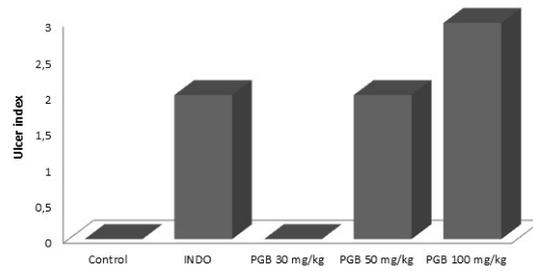


Figure 2. The effects of pregabalin on gastric ulcer index. Results are given as median. * $p < 0,05$ compared to control group. PGB: pregabalin, INDO: indomethacin.

c-The effects of pregabalin on superoxide dismutase (SOD) levels

PGB 30 mg/kg, 100 mg/kg and INDO groups decreased SOD levels compared to control ($p < 0,05$) while PGB 50 mg/kg did not alter SOD levels compared to control ($p > 0,05$) (Fig. 3).

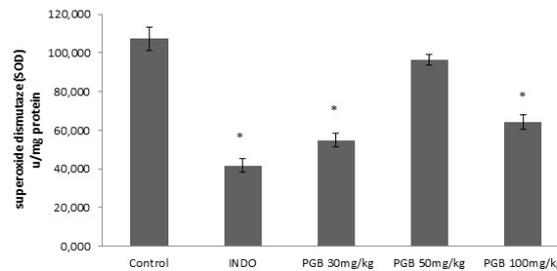


Figure 3. The effects of pregabalin on superoxide dismutase (SOD) levels. Results are given as mean ± SEM. * $p < 0,05$ compared to control group. PGB: pregabalin, INDO: indomethacin.

d-The effects of pregabalin on Catalase levels

PGB 30 mg/kg and 100 mg/kg groups decreased catalase levels compared to control ($p < 0,05$) while PGB 50 mg/kg did not alter catalase levels compared to control ($p > 0,05$) (Fig. 4).

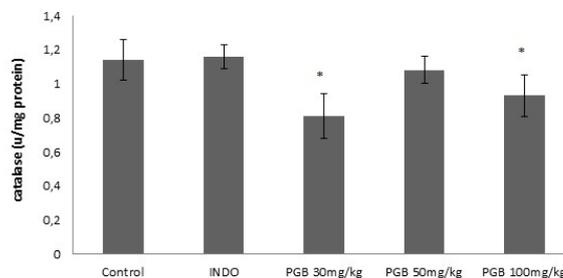


Figure 4. The effects of pregabalin on catalase levels. Results are given as mean ± SEM. * $p < 0,05$ compared to control group. PGB: pregabalin, INDO: indomethacin.

e-The effects of pregabalin on lipid peroxidase (MDA) levels

PGB 30mg/kg, 50 mg/kg doses and indomethacin groups did not alter MDA levels compared to control ($p>0.05$), but PGB 100mg/kg dose increased MDA levels compared to control ($p<0.05$) (Fig. 5).

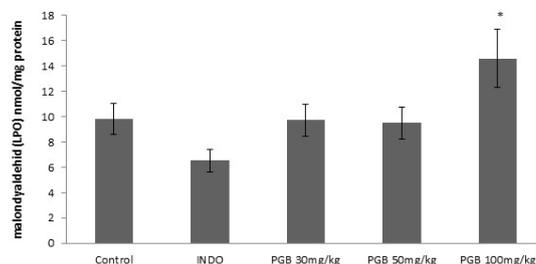


Figure 5. The effects of pregabalin on MDA levels. Results are given as mean \pm SEM.
* $p<0,05$ compared to control group. PGB: pregabalin, INDO: indomethacin.

4. Discussion

PGB is reported to have antinociceptive effects especially in neuropathic and also inflammatory pain (4). Considering PGB might have antiinflammatory effect, we investigated the effects of PGB on gastric mucus secretion and gastric ulcer index as well as on the antioxidant parameters in rats and compared its effects with INDO, a reference NSAID. We observed that PGB 50 mg/kg and 100 mg/kg decreased gastric mucus secretion and increased gastric ulcer index which may be suggested as a gastric side effect that is also seen with the use of NSAIDs. However, the effects of PGB on gastric mucus secretion and ulcer index were similar to INDO, the reference NSAID. Furthermore, PGB 30 mg/kg neither decreased gastric mucus secretion nor increased gastric ulcer index. In addition, PGB 30 mg/kg and 100 mg/kg decreased catalase and SOD levels which are functioning as antioxidant enzymes while PGB 50 mg/kg did not alter catalase and SOD levels. These results point out a biphasic effect of pregabalin on SOD and catalase levels. On the other hand, PGB 30 mg/kg and 50 mg/kg doses did not increase but also PGB 100 mg/kg dose increased MDA levels.

MDA levels that is considered as a marker of lipid peroxidation.

NSAIDs are widely use in the treatment of

pain and inflammatory diseases such as rheumatoid arthritis, osteoarthritis (18). These agents act by inhibiting the cyclooxygenase (COX) enzymes which results in the inhibition of prostaglandin synthesis (18). On the other hand, long term use of NSAIDs is associated with serious gastrointestinal complications such as mucosal lesions, bleedings, peptic ulcer formation (18). Decrease in the gastric mucus secretion which is a protective factor for the gastric mucosa may aggravate the NSAID-induced gastrointestinal complications. Considering this complications limiting the use of NSAIDs, there is a need for new antinociceptive and anti-inflammatory agents which do not induce or cause minor gastrointestinal complications. 3 different mechanisms were defined for NSAID-induced gastrointestinal complications: Inhibition of COX-1 enzyme and gastroprotective prostaglandins, membrane permeabilisation, production of proinflammatory cytokines (19).

Two isoforms of COX enzymes are defined: COX-1 and COX-2. One of the mechanisms suggested for the gastrointestinal complications induced by NSAIDs is the inhibition of cyclooxygenase-1 (COX-1) enzyme (18,20). COX-1 is formed constitutively and protects the gastric tissue

from the gastric acid damage, maintains the gastric mucosal blood flow, contributes to the production of HCO₃ secretion (21). COX-2 is induced by cell damage, the release of proinflammatory cytokines and tumoral factors (18). In this study, we suggested that PGB might have an anti-inflammatory effect resembling a NSAID. Since, it is reported that NSAIDs act by inhibiting both of the COX isoforms, exert their anti-inflammatory effect by inhibiting COX-2 while their inhibition on COX-1 contributes to the gastropathy. Thus, we aimed to investigate the gastric side effects of PGB by observing its effects on gastric ulcer formation and gastric mucus secretion which is a barrier for the gastric ulcer formation. We observed that PGB 50 mg/kg and 100 mg/kg doses reduced gastric mucus secretion and increased gastric ulcer index. However, these effects were similar to the reference NSAID, INDO. In addition, low dose of PGB 30 mg/kg neither reduced gastric mucus secretion nor increased gastric ulcer index.

Inflammation is a defence mechanism that protects the body from the damage caused by the endogenous or exogenous stimuli (22). Reactive oxygen species (ROS) are reported to be centrally involved in the progression of many inflammatory diseases and present functions in signaling and mediation of the inflammation (23). Free radicals are reactive molecules that occur during energy transformation of nutrition (11). These molecules, also known as reactive oxygen species, damage the cell components such as lipids, proteins and DNA (11). The marker of lipid peroxidation is the increase in malondyaldehyd (MDA) levels (24). Significant increases occur in lipid

peroxidation especially during the inflammatory processes. There are also enzymes such as superoxide dismutase (SOD) and catalase which have antioxidant features and prevent the oxidative stress (11,12). SOD is found in all aerobic cells and protects the cells against the damaging effects of superoxide radicals (12). Catalase is an enzyme found in peroxisomes and catalyses the formation of H₂O and molecular oxygen from H₂O₂ (12). In our study, we observed that PGB 30 mg/kg and 50mg/kg doses, did not alter MDA levels in gastric tissue. But PGB 100 mg/kg dose increased MDA levels. PGB 30 mg/kg and 50 mg/kg doses suggests an antioxidant property exerted by PGB. On the other hand PGB 30 mg/kg and 100 mg/kg doses decreased SOD and catalase levels which may be interpreted as an biphasic oxidant effect. However, PGB 50 mg/kg did not alter SOD and catalase levels compared to control. It seems that PGB 50 mg/kg is the optimal dose contributing to the antioxidant effect.

As a conclusion, we suggest that PGB 50 mg/kg and 100 mg/kg doses decreased gastric mucus secretion and increased gastric ulcer index indicating a gastropathy. However, this effect was similar to INDO, a NSAID, that is in use in clinical practice. PGB 30 mg/kg did not show antioxidant features, however it did not induce gastropathy. We suggest that PGB 30 mg/kg and 50 mg/kg may be used as anti-inflammatory agent.

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