Original Research

Effects of Gabapentin on Carrageenan–Induced Inflammation, Acute Phase Reactants and Gastric Mucus Secretion in Rats

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ABSTRACT

Objective: Gabapentin (GBP), which was first developed as an anticonvulsant agent, has recently gained great attention considering its pain relieving and anti-inflammatory effects. The aim of the present study was to investigate the anti-inflammatory effect of GBP on carrageenan (CAR)-induced paw edema and its gastric side effects by determining gastric mucus secretion in rats.

Methods: Paw edema was induced with 1% CAR immediately before intraperitoneal saline, GBP (10 or 30 mg/kg), and diclofenac (DIC) (5 mg/kg) injections. Paw thickness was measured hourly during 6 h. Serum cytokine levels were determined using enzyme-linked immunosorbent assay. For evaluation of gastric mucus secretion, saline, GBP, and DIC were administered orally for 10 days. On day 10, stomachs were removed, and gastric mucus of the glandular part was evaluated spectrophotometrically.

Results: Both doses of GBP reduced paw thickness at 6 h (p<0.05). GBP decreased interleukin (IL)-1β and tumor necrosis factor (TNF)-α levels similarly to DIC, whereas it increased IL-10 levels less than DIC (p<0.05). Both doses of GBP and DIC decreased gastric mucus secretion compared with control (p<0.05).

Conclusion: Our results suggest that GBP produces anti-inflammatory effect comparable with DIC. However, the effects of GBP on gastric mucus secretion were not better than those of DIC.

Keywords: Anti-inflammatory effect, carrageenan-induced inflammation, cytokine levels gabapentin, gastric mucus

INTRODUCTION

Gabapentin (GBP), a congener of gamma-aminobutyric acid, was first produced as an anticonvulsant agent. GBP has recently gained great attention considering its pain relieving and anti-inflammatory effects (1-3). In addition to its usage in epilepsy, it is also indicated for management of postherpetic neuralgia (4) and is found to be effective in clinical studies of inflammatory hyperalgesia or diabetic neuropathy and trigeminal neuralgia (1-3). Furthermore, the experimental antinociceptive effect of GBP on neuropathic or chronic pain was reported (5-8). GBP was proposed to present antinociceptive activity in rat formalin test (7) that involves the stimulation of inflammatory and sensitization processes (9, 10) and also to have analgesic effects in other inflammatory pain tests (11, 12).

Inflammation, a defensive mechanism of the body in response to tissue damage, includes complicated biochemical and cellular mechanisms evoked by inflammatory molecules, such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α, which stimulates the chemotaxis of neutrophils (12). The influence of proinflammatory cytokines in nociception is also reported (13, 14).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are frequently used in the treatment of various painful and inflammatory conditions. However, gastric adverse effects, such as ulcer formation, limit clinical use (15, 16). NSAIDs produce gastrointestinal damage via several mechanisms, such as reducing the secretion of cytoprotective components and causing injury on mucosal epithelium (15, 16). Gastric mucus secretion is one of the major mechanisms of mucosal protection (17).

Considering the antinociceptive effects of GBP and the association of pain and inflammation, it may be suggested that GBP may produce an anti-inflammatory effect. Therefore, we aimed to evaluate the anti-inflammatory activity of GBP in carrageenan (CAR)-induced paw edema in rats by measuring paw thickness and release of proinflammatory cytokines IL-1β and TNF-α and

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anti-inflammatory cytokine IL-10 and then to explore the gastric side effect of GBP on gastric mucus secretion and compare all these effects with an NSAID diclofenac (DIC).

METHODS

Animals
All experimental procedures were approved by the Eskişehir Osmangazi University local ethics committee for animal experimentation (no.: 502, date: 11.02.2016). Male Wistar rats (250–350 g, 14–16 weeks) were kept in standard conditions at 23°C±1°C and 12:12 h light: dark cycle.

Drugs
Drugs were dissolved in saline (0.9% NaCl). CAR (Alfa Aesar, Karlsruhe, Germany) was administered to rats by intradermal (i.d.) route at a dose of 100 µl. GBP (Sigma-Aldrich, St. Louis, MO, USA) 10 or 30 mg/kg was administered intraperitoneally (i.p.) for evaluation of anti-inflammatory effect and via gastric lavage for gastric mucus evaluation. An NSAID, DIC sodium (Sigma-Aldrich, St. Louis, MO, USA) 5 mg/kg was administered i.p. and via gastric lavage for gastric mucus evaluation.

Experimental Design
Two sets of experiments were performed as follows:

In the first experiment, the anti-inflammatory effect of GBP was assessed by CAR-induced paw edema. The experimental groups were designed as follows (n=7 per group):

1. Control group: 100 µl saline (i.d.)+saline (i.p.),
2. CAR group: 100 µl CAR (i.d.)+saline (i.p.),
3. GBP 10 mg/kg group: 100 µl CAR (i.d.)+10 mg/kg GBP (i.p.),
4. GBP 30 mg/kg group: 100 µl CAR (i.d.)+30 mg/kg GBP (i.p.),
5. DIC 5 mg/kg group: 100 µl CAR (i.d.)+5 mg/kg DIC (i.p.).

In the second experiment, the effect of GBP on gastric mucus secretion was evaluated. Drugs were administered via gastric lavage during 10 days.
The experimental groups were designed as follows (n=7 per group):

1. Control group (saline),
2. GBP 10 mg/kg group,
3. GBP 30 mg/kg group,
4. DIC 5 mg/kg group.

Rats in groups used in the first and second experiments were different.

CAR-induced Paw Edema
Carrageenan (100 µl) was injected by i.d. route per rat into the right hind paw. The rats were injected with i.p. 10 or 30 mg/kg GBP immediately after CAR injection. Saline was given to the control group. A 5 mg/kg DIC was used as a reference anti-inflammatory agent. Paw thickness was measured using a vernier caliper just before (0 h) and after the injection of CAR at 1, 2, 3, 4, 5, and 6 h (18, 19).

Cytokine Assay
Intracardiac blood samples at 6 h were kept at −80°C until analysis. IL-1β, TNF-α, and IL-10 levels in serum were determined using eBioscience Enzyme-Linked ImmunoSorbent Assay (ELISA) kits (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instruction.

Determination of Gastric Mucus Secretion
Saline, GBP 10 or 30 mg/kg, or DIC 5 mg/kg was administered via oral gavage for 10 days. The animals were not given food, but water was accessible overnight before the animals were sacrificed on day 10. Their stomachs were removed immediately, and then their gastric lumens were rinsed with saline. The amount of the gastric mucus of the glandular part was evaluated spectrophotometrically at 605 nm by Alcian blue dye binding method (20). Briefly, the stomachs were removed, rinsed with saline, weighed, and incubated for 2 h in 20 mL of 0.1% of w/v Alcian blue in 0.16 mol/l sucrose solution buffered with 0.05 mol/l sodium acetate (pH 5.8). The gastric tissue was immersed in 0.5 mol/l $MgCl_2$ solution for 2 h to extract the mucus-bound dye. The blue extract was shaken with diethyl ether, and the resultant emulsion was centrifuged at 5000 g for 10 min. The supernatant was measured at 600 nm by spectrophotometry. Data are presented as µg Alcian blue/g tissue.

Statistical Analysis
Data were analyzed using statistical Package for the Social Sciences (SPSS) statistical package software version 21.0 (SPSS IBM Corp.; Armonk, NY, USA). Results of paw edema and gastric mucus concentration were expressed as mean±standard error (SEM). Data of paw edema were analyzed using one-way repeated measures ANOVA, and gastric mucus secretion was analyzed using one-way ANOVA. Data of cytokine levels were analyzed using independent Kruskal–Wallis test. One-way ANOVA or Kruskal–Wallis tests were used based on the normal or non-normal distribution of data, respectively. Dunnett's C or Tukey's tests were used as post hoc tests. Cytokine levels were expressed as median and percentiles (25%–75%). A p-value <0.05 was considered as statistically significant.
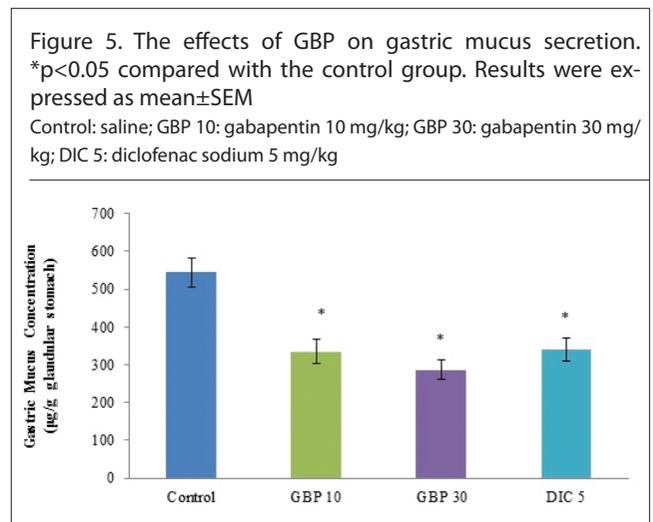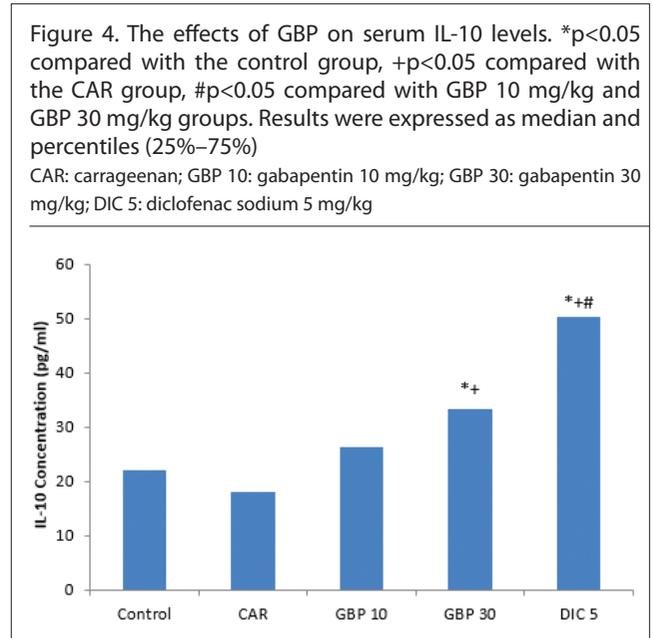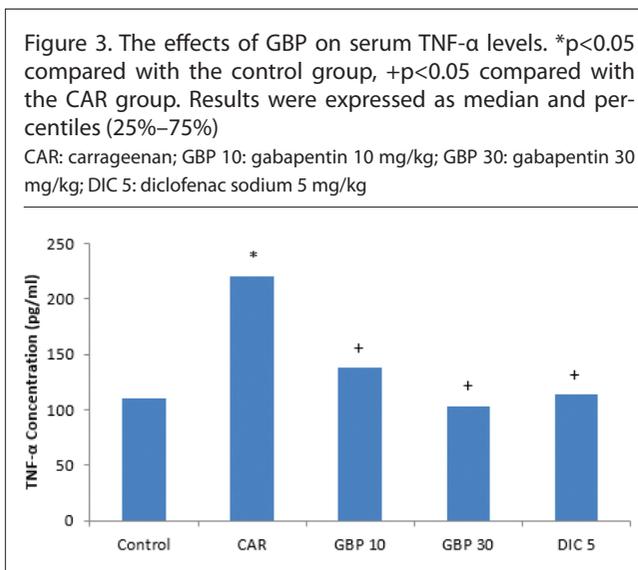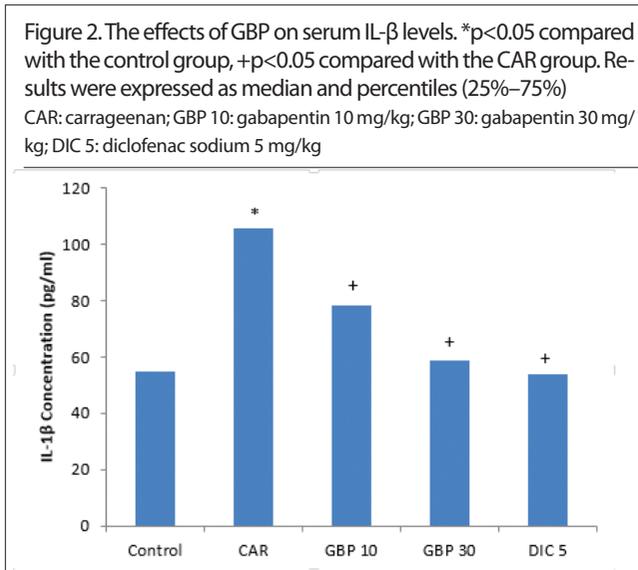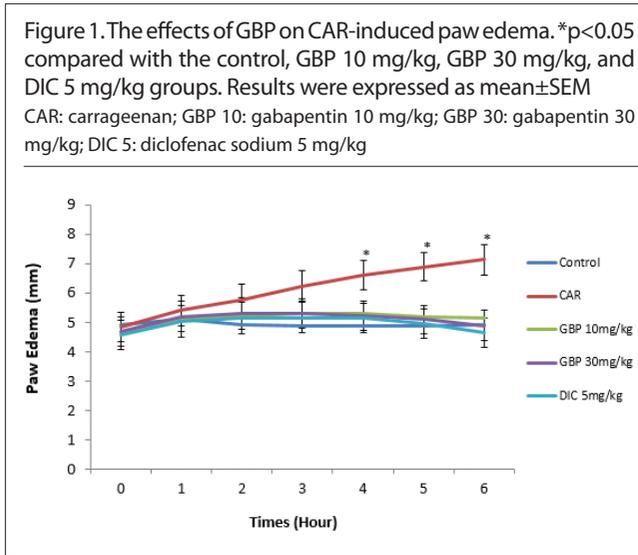
RESULTS

The Effects of GBP on CAR-induced Paw Thickness
Paw thickness did not differ between the groups at all time points, except the CAR group (Figure 1). CAR significantly increased paw thickness at 4, 5, and 6 h compared with other groups (p<0.05). Both doses of GBP significantly decreased paw thickness, and this effect was comparable with DIC (Figure 1). Results were expressed as mean±SEM.

The Effects of GBP on Serum Cytokine Levels
Carrageenan significantly increased IL-1β and TNF-α levels compared with control (p<0.05). GBP decreased IL-1β and TNF-α levels compared with the CAR group (p<0.05). These effects of GBP were similar to DIC (Figures 2, 3). GBP increased IL-10 levels compared with the CAR group and control (p<0.05). DIC increased IL-10 levels more than the GBP groups (p<0.05) (Figure 4). Results were expressed as median and percentiles (25%–75%).

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Figure 1. The effects of GBP on CAR-induced paw edema. *p<0.05 compared with the control, GBP 10 mg/kg, GBP 30 mg/kg, and DIC 5 mg/kg groups. Results were expressed as mean±SEM
CAR: carrageenan; GBP 10: gabapentin 10 mg/kg; GBP 30: gabapentin 30 mg/kg; DIC 5: diclofenac sodium 5 mg/kg

Figure 2. The effects of GBP on serum IL-β levels. *p<0.05 compared with the control group, +p<0.05 compared with the CAR group. Results were expressed as median and percentiles (25%–75%)
CAR: carrageenan; GBP 10: gabapentin 10 mg/kg; GBP 30: gabapentin 30 mg/kg; DIC 5: diclofenac sodium 5 mg/kg

Figure 3. The effects of GBP on serum TNF-α levels. *p<0.05 compared with the control group, +p<0.05 compared with the CAR group. Results were expressed as median and percentiles (25%–75%)
CAR: carrageenan; GBP 10: gabapentin 10 mg/kg; GBP 30: gabapentin 30 mg/kg; DIC 5: diclofenac sodium 5 mg/kg

Figure 4. The effects of GBP on serum IL-10 levels. *p<0.05 compared with the control group, +p<0.05 compared with the CAR group, #p<0.05 compared with GBP 10 mg/kg and GBP 30 mg/kg groups. Results were expressed as median and percentiles (25%–75%)
CAR: carrageenan; GBP 10: gabapentin 10 mg/kg; GBP 30: gabapentin 30 mg/kg; DIC 5: diclofenac sodium 5 mg/kg

Figure 5. The effects of GBP on gastric mucus secretion. *p<0.05 compared with the control group. Results were expressed as mean±SEM
Control: saline; GBP 10: gabapentin 10 mg/kg; GBP 30: gabapentin 30 mg/kg; DIC 5: diclofenac sodium 5 mg/kg

The effects of GBP on Gastric Mucus Secretion
Both doses of GBP decreased gastric mucus secretion compared with control (p<0.05). DIC also reduced gastric mucus secretion compared with control (p<0.05) (Figure 5). However, GBP and DIC did not differ according to the effects on gastric mucus secretion (Figure 5). Results were expressed as mean±SEM.

DISCUSSION
Gabapentin, an antiepileptic agent, has been indicated for relief of neuropathic pain. It was reported that GBP exhibited central and peripheral antinociceptive effects via nitrergic and serotonergic pathways (21). Considering its antinociceptive effects, the present study was designed to investigate if GBP induced gastropathy that is seen with the use of analgesic NSAIDs. DIC, which is an NSAID, was used as a reference drug to compare its effects on inflammation and gastric mucus secretion with GBP.

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The present study demonstrated that GBP reduced inflammation induced by CAR injection in rats supported with the reduced IL-1β and TNF-α with GBP 10 and 30 mg/kg doses. Moreover, there was a significant enhancement in the levels of anti-inflammatory cytokine IL-10 with only GBP 30 mg/kg compared with the CAR and control groups.

Therefore, our results point out that GBP is an anti-inflammatory drug against inflammatory painful stimulus. Our results agree with previous studies showing that GBP exhibited anti-inflammatory activity on CAR-induced paw edema (12, 22). We also found that the anti-inflammatory effect of GBP was independent from dose and comparable with DIC. These results were comparable with previous studies showing that low doses of GBP, 1 mg/kg i.p. administration of GBP in mice and 25 and 50 mg/kg subcutaneous administration of GBP in rats, reduced the inflammatory response in CAR-induced paw edema that was also less than those of one of the NSAIDs indomethacin (12, 22). Generally, data suggest that GBP is not better than NSAIDs for reducing inflammation.

Carrageenan-induced paw edema is a widespread model to study the anti-inflammatory effects of drugs. Local injection of CAR causes the release of various proinflammatory and inflammatory (i.e., prostaglandins (PGs), leukotrienes, histamine, bradykinin, and TNF-α) cytokines (23). Recently, this model is used for localized inflammatory pain.

The inflammatory response to CAR consists of three phases. The first phase is intervened by histamine and 5-hydroxytryptamine, and then second, the kinin-mediated phase occurs. The third phase is associated with PGs, especially those of the E series. Cyclooxygenase (COX) enzymes catalyze the production of PGs and thromboxanes from arachidonic acid. NSAIDs inhibit COX enzymes that are associated with their clinical benefit in the management of pain and inflammation (4, 24). Thus, CAR-induced paw edema has been a fundamental method in the development of NSAIDs and new COX inhibitors (23).

Both doses of GBP decreased gastric mucus secretion compared with control. DIC also reduced gastric mucus secretion compared with control. However, there was no significant difference between GBP 10 and 30 mg/kg and DIC 5 mg/kg with regard to the effects on gastric mucus secretion.

In addition, in the literature, GBP was found to prevent gastric mucosal lesions induced by an NSAID drug indomethacin, and it was suggested that GBP showed a gastroprotective effect (22). This finding partly disagrees with the results of our study. We observed that GBP reduced gastric mucus secretion, indicating a probability of gastric mucosal damage formation. However, this effect was comparable with NSAID DIC. On the other hand, we did not investigate the effect of GBP on gastric mucosal lesions as gastric ulcer index. NSAIDs are commonly used in the treatment of inflammatory diseases, rheumatoid arthritis, osteoarthritis, dysmenorrhea for relief of pain, inflammation, and fever. Long-term use of NSAIDs is accompanied with severe gastrointestinal adverse effects, such as mucosal erosions, bleeding, ulcer formation, perforations, and intestinal obstructions (9).

There are three main mechanisms of NSAID-induced gastrointestinal complications: inhibition of COX-1 enzyme and gastroprotective PGs, increase of membrane permeabilization, and proinflammatory mediators (10). COX enzymes consist of two isoforms with different functional properties (12). COX-1 is constitutively resident and contributes to the maintenance of physiological functions of gastric mucosa by catalyzing PG synthesis that protects gastric tissue against gastric acid and produces HCO_3 and sustains gastric mucosal blood flow (13, 14). NSAID-induced gastropathy mainly occurs with the inhibition of COX-1. COX-2, an inducible isoform, is triggered by cell damage, proinflammatory cytokines, and tumoral factors (20).

In the present study, we observed that both doses of GBP reduced gastric mucus secretion. On the other hand, this reduction was similar to the gastric mucus reduction as seen with DIC.

CONCLUSION
We observed that the anti-inflammatory effect of GBP was supported by decreased serum levels of IL-1β and TNF-α. In addition, especially GBP 30 mg/kg increased anti-inflammatory cytokine IL-10 levels in serum. Therefore, our results exerted that GBP is an anti-inflammatory drug against inflammation. GBP may provide at least some benefit, but it might not be recommended over NSAIDs. We suggest that GBP reduces gastric mucus secretion; however, this was not worse than DIC.

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