

ORIGINAL RESEARCH

Medicine Science 2019;8(2):296-300

Effect of benidipine on experimental gastric ulcers in rats

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Received 15 October 2018; Accepted 26 October 2018

Available online 28.10.2018 with doi:10.5455/medscience.2018.07.8921

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Abstract

Peptic ulcer has been reported to increase acid secretion and lead to oxidative stress. Benidipine is an antihypertensive drug with antioxidant properties. This study aims to investigate the effects of benidipine on indomethacin-induced gastric ulcers in rats. Four groups were designed in this study. Gastric ulcer was occurred with indomethacin in the first three groups. These groups were treated with famotidine, benidipine and distilled water respectively 5 minutes before indomethacin administration. The fourth group rats didn't have gastric ulcer and they received only distilled water. Six hours after drug administration gastric tissue was extracted and macroscopic and biochemical examinations were performed. Damaged areas in the stomach of benidipine and famotidine receiving animal groups were smaller than the indomethacin received animals. Indomethacin elevated the levels of malondialdehyde and myeloperoxidase in the stomach tissue ($p < 0.0001$), and also decreased glutathione, glutathione related enzymes and superoxide dismutase ($p < 0.0001$). Benidipine may be useful in preventing the toxic effect of indomethacin on the stomach.

Keywords: Benidipine, oxidant, antioxidant, gastric ulcers, rats

Introduction

As is known, peptic ulcer is a common name used for stomach and duodenum ulcers. Incidence of peptic ulcer is 11-14% in male and 8-11% in female. However, stomach ulcers are seen equally in male and female [1]. Research has shown that 89-95% of peptic ulcers are associated with *Helicobacter pylori* and non-steroidal anti-inflammatory drugs (NSAIDs) usage [2]. Serious gastrointestinal complications have been clinically reported in 1-4% of patients taking NSAIDs [3]. The factors leading to peptic ulcer cause disruption of the permeability of stomach mucosa and the intracellular calcium accumulation [4]. Studies show that calcium stimulates gastric mucosal oxyntic cells and gastric acid secretion in in-vivo conditions [5]. It also known that intracellular calcium dysregulation is related with reactive oxygen species [6]. The increase of intracellular calcium was directly proportional to membrane lipid peroxidation increase and glutathione (GSH)

decrease [7]. Although the aggressive factors that make up the ulcer are different, the free oxygen radicals are one of the responsible mechanism of all ulcer types [8]. This information supports the fact that reactive oxygen species are closely related to ulcer pathogenesis [9]. It has reported that the amounts of malondialdehyde (MDA) and myeloperoxidase (MPO) increased, and the levels of glutathione and its enzymes, superoxide dismutase (SOD), catalase (CAT) decreased in damaged stomach tissue [10]. Indomethacin indole derivative is a NSAID that we utilized to create an experimental ulcer model in the present study. Indomethacin is the most preferred drug to create an experimental ulcer model because its ulcer making potential is more than other NSAIDs [11, 12]. It is argued that indomethacin causes damage to the stomach tissue by inhibiting the secretion of cytoprotective prostaglandin, mucus and bicarbonate, increasing the stomach acid secretion [12]. Cyclooxygenase-2 (COX-2) enzyme regarded as leading to the anti-inflammatory impact of indomethacin and Cyclooxygenase-1 (COX-1) enzyme inhibition for the gastrointestinal toxic effects [13]. Indomethacin have been reported to change the oxidant-antioxidant balance in stomach tissue including MDA, MPO, tGSH, GST, SOD, CAT and GPO in favor of oxidants [14,15,

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16]. All this information suggests that calcium channel blockage and antioxidant administration are treatments for gastric ulcer pathogenesis. Benidipine is an L-type calcium channel blocker drug used in hypertension [17]. Benidipine prevents the increase of oxidant parameters in tissues and decrease of antioxidants, and suppresses oxidative stress [18,19]. Calcium channel blockade and antioxidant properties of benidipine suggest that it may provide a treatment for the pathogenesis of gastric ulcer. Therefore, the study aims to investigate the effect of benidipine on indomethacin-induced gastric ulcers in rats.

Material and Methods

Experimental animals

In total, 24 male albino Wistar rats, each weighed 270–287g, were used in these experimental research. The rats were provided from Medical Experimental Research and Application Center of Ataturk University. The animals were housed and fed for a week (7 days) in the normal laboratory environment (22°C) in groups before the experiment. This experiment was confirmed by the local animal experimentation committee of ethics (Ataturk University animal experiments local ethics committee, Date: 30.03.2017, Meeting no: 3, Decision: 33).

Chemical substances

Thiopental sodium was obtained from IE Ulagay (Turkey) and its commercial form benidipin from Deva (Turkey).

Experimental groups

The animals used in the experiment were grouped as Indomethacin (IND), Indomethacin + Benidipine (IBN), Indomethacin + Famotidine (IFN) and healthy (HG) groups.

Indomethacin ulcer test

Benidipine was given orally at 2 mg/kg dose for the IBN (n-6) group and famotidine 20 mg/kg for the IFN (n-6) group to 24 h fasted rats. IND (n-6) and HG (n-6) groups were applied distilled water of an equal volume of (0.5 ml) as a solvent [20,21]. Five minutes after the application of the drugs, all rats were administered orally 25 mg/kg of indomethacin (excluding HG) [21]. Six hours after the administration of indomethacin, the animals were sacrificed by administration of high dose of anesthetic (thiopental sodium 50 mg/kg). The stomach of the killed animals was removed, and the ulcers on the stomach surface were macroscopically evaluated. The ulcer area on the stomach surface was measured on the paper rulers with the scale of mm². Then, MDA, MPO, total glutathione (tGSH), glutathione peroxidase (GPO), glutathione S transferase (GST), GSHRd and SOD levels were measured in all stomach tissues.

Biochemical processes

Preparation of samples

The phosphate buffer containing 0.5% HDTMAB (0.5% hexadecyltrimethyl ammonium bromide) pH=6 for the determination of MPO, the potassium chloride solution of 1.15% for the determination of MDA were used, and for the other measurements, it was adjusted to 2 mL in phosphate buffer (pH 7.5) and homogenized on ice. Then, it was centrifuged at 10000

rpm at +4 °C for 15 minutes. The supernatant was used as an analysis sample.

Malondialdehyde analysis

The barbituric acid test was used by assessment MDA to define the amount of lipid peroxidation in gastric tissue [22]. MDA levels are expressed as µmol/g protein.

Myeloperoxidase analysis

MPO activity was measured according to the method of Bradley et al. [23]. MPO activity are expressed as U/g protein.

Total glutathione analysis

The amount of tGSH in the stomach tissue was performed according to the method defined by Sedlak and Lindsay [24]. The tGSH levels in the gastric tissue are expressed as nmol/g protein.

Glutathione peroxidase analysis

GPO activity was measured according to the method of Lawrence and Burk [25]. Results were expressed as U/g protein.

Glutathione reductase analysis

GSHRd activity was determined spectrophotometrically by measuring the rate of NADPH oxidation at 340 nm according to Carlberg and Mannervik method [26]. Results were expressed as U/g protein.

Glutathione s-transferases analysis

GST activity was measured by using 1-chloro-2, 4-dinitrobenzene (CDNB) and GSH as described in Habig et al. [27]. Results were expressed as U/g protein.

Superoxide dismutase analysis

SOD activity was measured according to Sun et al. [28]. Estimates were based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with nitroblue tetrazolium (NBT) to form formazan dye. SOD activity is expressed as U/g protein.

Statistical analysis

The results were shown as “mean±standart error of the mean” ($\bar{x} \pm \text{SEM}$). The differences between the groups were defined using one-way ANOVA and followed it Fisher’s post-hoc LSD test. “SPSS for Windows 18.0” software used for data analysis, and $p < 0.05$ was considered statistically significant.

Results

Indomethacin ulcer test

Macroscopic examination results

Macroscopically black-colored damage was observed in the stomach tissue of indomethacin, benidipine and famotidine treated groups. The damage foci on various shapes and sizes scattered across the entire stomach surface. The damage foci were round, oval and irregular mucosal defects at different diameter and depth. The boundaries of the damages were clear. The number and area of damage foci in the stomachs of benidipine and famotidine receiving animal groups were less than the control group. Severe hyperemia was observed in the control group receiving only indomethacin (Figure 1).

Antilucer activity of benidipine and famotidine

As shown in Table 1, the average of ulcer area in the stomach of rats in the control group of indomethacin was 44.5 ± 1.6 , and the average of the ulcer area in rats receiving benidipine at the 2 mg/kg doses and famotidine in 20 mg/kg doses were 0.5 ± 0.2 and 0.3 ± 0.2 mm². In the indomethacin group, the ulcer area was significantly greater than the group of benidipine and famotidine. However, there was no statistically significant difference for the mean ulcer area between benidipine and famotidine groups.

Biochemical findings

Oxidant parameters

As our results show, indomethacin increased MDA and MPO levels in stomach tissue of indomethacin group rats significantly compared to the HG group ($p < 0.0001$). MDA and MPO levels decreased in benidipine and famotidine groups compared to the IND group ($p < 0.0001$). The difference of the MDA and MPO levels between IBN and IFN group was found to be statistically insignificant ($p > 0.05$) (Figure 2).

Antioxidant parameters

Indomethacin decreased the levels of tGSH, GPO, GST, GSHRd, and SOD in stomach tissue of indomethacin group rats significantly compared to the HG group ($p < 0.0001$). The levels tGSH, GPO, GST, GSHRd, and SOD increased in the IBN and IFN groups ($p < 0.0001$). The difference of tGSH, GPO, GST, GSHRd and SOD levels was found to be insignificant between IBN and IFN groups ($p > 0.05$) (Figure 3).

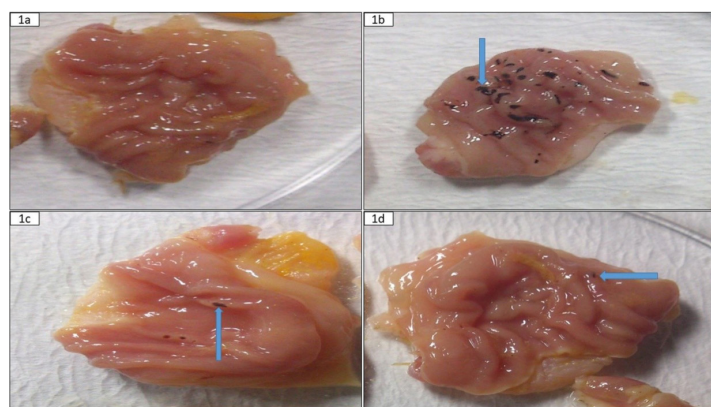


Figure 1. Ulcer areas (Arrow) of stomach tissue in the HG (1a), IND (1b), IBN (1c) and IFN (1d) groups.

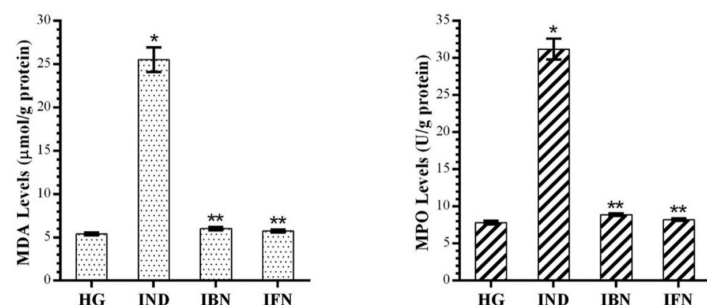


Figure 2. MDA and MPO levels in the stomach tissue of study groups.

*= $p < 0.0001$ according to HG group.

**= $p < 0.0001$ according to IND group.

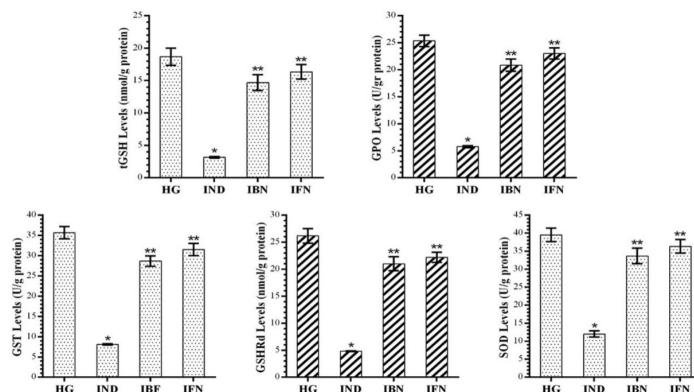


Figure 3. tGSH, GPO, GST, GSHRd and SOD levels in the stomach tissue of study groups.

*= $p < 0.0001$ according to HG group.

**= $p < 0.0001$ according to IND group.

Table 1. Ulcer areas of stomach tissue in the Healthy (HG), Indomethacin (IND), Indomethacin + Benidipine (IBN) and Indomethacin + Famotidine (IFN) groups.

Groups	HG	IND	IBN	IFN
Ulcer area (mm ²)	0	44.5±1.6	0.5±0.2	0.3±0.2
P	-	<0.0001	>0.05	>0.05

Discussion

In this study, effects of benidipine on indomethacin-induced gastric ulcers in rats was investigated and compared with famotidine. This study was carried out based on the effect mechanism of benidipine and the literature information on the pathogenesis of gastric ulcer. Our results showed that indomethacin damaged the stomach tissue of animals and that benidipine protected the stomach tissue from indomethacin damage. The stomach tissue of the animals that indomethacin was applied alone developed affected areas with different diameter and depth in various shapes and sizes macroscopically in black color. These macroscopic symptoms are considered to be gastric ulcers in literature [16]. In many previous studies, antiulcer activity was evaluated with the area width of the damage to stomach tissue [29,30]. The area of ulcer foci in the indomethacin group was found to be larger than the group of benidipine and famotidine. This suggests that benidipine and famotidine have an antiulcer effect.

Oxidant and antioxidant parameters are used to evaluate the biochemical effect of indomethacin on the stomach [15,31]. As we can see from our experimental results, oxidant parameter levels such as MDA and MPO in the indomethacin group with larger ulceration area showed a significant increase compared to the healthy, benidipine and famotidine group. It has reported that the MDA level increases in the damaged stomach tissue due to indomethacin [32]. Polat et al. [33] reported increased MPO activity in the stomach tissue of the indomethacin group, where MDA also increased. There were no studies showing the effects of benidipine on MDA and MPO induced by indomethacin in stomach tissue, but it was reported that benidipine inhibited MDA increase in damage tissue induced by over ischemia-reperfusion [20]. Also, the findings about benidipine reduced MPO activity in heart tissue was reported [34].

The levels of tGSH, GPO, GST, GSHRd and SOD in the stomach tissue of indomethacin group were significantly decreased compared to the healthy, benidipine and famotidine groups. These results show that indomethacin changes oxidant-antioxidant balance in stomach tissue in favor of oxidants. There are many studies reporting that antioxidant parameters decreases in the experimental damaged stomach tissue, and oxidized parameters increases. Especially MPO and MDA levels increased in damaged stomach tissue and GSH, GST, SOD and GPO levels decreased [35]. This literature information demonstrates the importance of maintaining oxidant-antioxidant balance with the superiority of antioxidants so that drugs can have an anti-inflammatory effect. It also reveals that the relationship between antioxidant activity and anti-ulcer effect is important. Famotidine has been reported to inhibit the decrease of antioxidant levels in stomach tissue and oxidants to increase in the dose of anti-inflammatory effect [15, 33, 36]. Benidipine has also been shown experimentally to prevent the increase of MDA and the decrease of tGSH in damaged tissue [20]. These results suggest that the protective effect of benidipine on the stomach is due to antioxidant activity, same as famotidine. In literature, we can see whether tissue damage occurs is evaluated with oxidant/antioxidant balance [37]. There was no study in literature about the effects of benidipine on gastric ulcers. However, a L-type calcium channel blockers belonging to the same group have been documented to suppress the formation of indomethacin ulcers; also, lacidipine has been determined to decrease the oxidant parameters and increase the antioxidant parameters, same as benidipine [21]. As a result, indomethacin caused significant damage to the stomach tissues. Oxidant-antioxidant balance in the stomach tissue of the animal groups receiving indomethacin changed in favor of oxidants. Benidipine and famotidine significantly reduced the gastric damage induced by indomethacin. Oxidant-antioxidant balance in stomach tissues of benidipine and famotidine group resulted in the superiority of antioxidants.

Conclusion

Our results show that benidipine has as much anti-inflammatory efficacy as famotidine. Therefore, benidipine may be used in place of famotidine to eliminate the gastrototoxic effect of indomethacin.

Competing interests

The authors declare that they have no competing interest

Financial Disclosure

The financial support for this study was provided by the investigators themselves.

Ethical approval

Before the study, permissions were obtained from local ethical committee.

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