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BRIEF ARTICLE

Mitigation of indomethacin-induced gastric mucosal lesions by a potent specific type Ⅴ phosphodiesterase inhibitor

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Abstract

AIM: To investigate the gastroprotective effect of vardenafil against indomethacin-induced gastric damage.

METHODS: Forty-eight female Wistar albino rats were randomly divided into 6 groups. Group 1 received saline only. Group 2 (indomethacin) received indomethacin. Rats in group 3 and 4 were pretreated with different doses of famotidine. Group 5 and 6 were pretreated with different doses of vardenafil. Rats in groups 3 to 6 received 25 mg/kg indomethacin 30 min after pretreatment. The animals were sacrificed 6 h later and their stomachs were opened. Gastric lesions were counted and measured. The stomach of each animal was divided in two parts for histopathological examinations and nitric oxide (NO) and malondialdehyde (MDA) assays, respectively.

RESULTS: There were no gastric mucosal lesion in the saline group but all rats in the indomethacin group had gastric mucosal ulcerations (ulcer count; 6.25 ± 3.49, and mean ulcer area; 21.00 ± 12.35). Ulcer counts were diminished with famotidine 5 mg/kg (4.12 ± 2.47, $P >$ 0.05), 20 mg/kg (2.37 ± 4.43, $P < 0.05$), vardenafil 2 mg/kg (4.37 ± 3.06), and vardenafil 10 mg/kg (1.25 ± 1.38, $P < 0.05$) compared to the indomethacin group. Gastric mucosal lesion areas were diminished with famotidine 5 mg/kg (8.62 ± 2.97, $P < 0.001$), famotidine 20 mg/kg (0.94 ± 2.06, $P < 0.001$), vardenafil 2 mg/kg (6.62 ± 5.87, $P < 0.001$), and vardenafil 10 mg/kg (0.75 ± 0.88, $P < 0.001$) compared to the indomethacin group. MDA levels were significantly higher in indomethacin group (28.48 ± 14.51), compared to the famotidine 5 mg/kg (6,21 ± 1.88, $P < 0.05$), famotidine 20 mg/kg (5.88 ± 1.60. $P < 0.05$), vardenafil 2 mg/kg (15.87 ± 3.93, $P < 0.05$), and vardenafil 10 mg/kg (10.97 ± 4.50, $P < 0.05$). NO concentration in gastric tissues of the famotidine groups were significantly increased ($P < 0.05$), but the NO increases in the vardenafil groups were not statistically significant. Histopathology revealed diminished gastric damage for pretreatment groups compared to the indomethacin group ($P < 0.05$).

CONCLUSION: Vardenafil affords a significant dose-dependent protection against indomethacin induced gastric mucosal lesions in rats.

Key words: Vardenafil; Gastric ulceration; Indomethacin; Gastroprotection; Rats

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INTRODUCTION

Gastric mucosal damage is a common disorder of the gastrointestinal system. The pathogenesis of gastric ulcers is based on complex interactions between aggressive and protective factors. Nonsteroidal anti-inflammatory drugs

(NSAID) are known to be aggressive agents for gastric ulcer development. People of advancing age need many drugs, including NSAIDs, for the treatment of pain and inflammation due to rheumatological disturbances.

Vardenafil is a phosphodiesterase (PDE) V inhibitor that has been used for the treatment of erectile dysfunction[1] and, more recently, for pulmonary hypertension[2,3]. Recent laboratory studies demonstrated successful effects of PDE V inhibitors on cardioprotection after ischemia reperfusion injury[4,5], as well as in ischemic injury of other organs, such as the colon, liver, and brain[6,7]. Deibert *et al*[8] revealed that vardenafil had increased portal flow and lowered portal pressure in patients with cirrhotic livers.

Diminished mucosal circulation has been blamed as one of the etiological factors in gastric ulcer formation. Like prostaglandins, the L-Arginine/nitric oxide (NO) pathway is a major protective system in gastric mucosa[9] *via* relaxation of the arterial smooth muscles. Inhibition of nitric oxide synthase aggravates the injury in animal models of gastric ulcers[10].

In this study, we have studied the effects of vardenafil on the acute gastric injury caused by administration of indomethacin.

MATERIALS AND METHODS

The study was approved by the Zonguldak Karaelmas University (ZKU) Animal Experiments Local Ethic Committee. The study was carried out on 48 female Wistar albino rats weighing 200-250 g, obtained from the Experimental Animal Laboratory of Medical Faculty of ZKU. The rats were kept under standard conditions (temperature; 22-24℃, and 12:12 h light/dark). The experimental procedures were carried out in accordance with international guidelines for the use and care of laboratory animals. All animals were fed with pellet food produced especially for experimental animals. Water was available ad libitum. All experiments were performed at the same time of the day to avoid diurnal variations of putative regulators of gastric functions.

Famotidine and vardenafil were dissolved in distilled water. All drug solutions and suspensions were freshly prepared. Gastric ulcers were inflicted by oral administration of indomethacin 16-18 h after starvation.

Animals were randomly divided into six groups. In Group 1 (n:8) rats received only 8 mL/kg of saline by gavage. Rats in Group 2 (n:8) received 25 mg/kg indomethacin in a volume of 8 mL/kg of saline. The rats in group 3 and 4 were pretreated with famotidine, 5 mg/kg and 20 mg/kg, respectively. Rats in groups 5 and 6 were pretreated with 2 mg/kg and 10 mg/kg vardenafil, respectively. After 30 min, 25 mg/kg indomethacin in a volume of 8 mL/kg of saline were administered by gavage. Six hours after oral administration of indomethacin, all rat groups were anesthetized with an intramuscular injection of 100 mg/kg Ketamine (Ketalar®, Parke Davis-Eczacıbaşı, Istanbul, Turkey). A midline abdominal incision was then performed. All rat groups were sacrificed *via* cardiac puncture, and their stomachs rapidly removed, opened by an incision along the lesser curvature, and rinsed in ice-cold distilled water[11]. Gastric tissues were pinned out on a wax platform. Macroscopic damage to the gastric mucosa was assessed. Hemorrhagic and ulcerative lesions were counted and their lengths were measured on square millimeter paper. Gastric mucosal lesions were expressed as the sum of the lengths (mm) of all lesions for each stomach, which was used as the ulcer index (UI)[12,13]. Gastric lesions were judged by two independent researchers who were blinded to the protocol. The average score of the two independent observers were taken into account, and the sum of the total scores was divided by the number of animals to obtain the mean UI for each group.

The stomach of each animal was divided into two parts. One part of the stomach was excised, immersed in saline, and immediately stored at -40℃ for measurement of NO and MDA levels.

Gastric tissues were homogenized in ten volumes of 150 mmol/L ice-cold KCl using a glass teflon homogenizer (Ultra Turrax IKA T18 Basic) after cutting the tissues into small pieces with scissors (for 2 min at 5000 r/min). The homogenate was then centrifuged at 5000 × *g* for 15 min. The supernatant was used for analysis. High-performance liquid chromatographic analysis was performed using a Shimadzu HPLC system (Kyoto, Japan) with an MDA kit (Immundiagnostik AG, Bensheim, Germany). Spectrophotometric measurements of total antioxidant status (TAS) (Randox, Crumlin, UK) was performed using a Shimadzu UV-1601 (Kyoto, Japan) spectrophotometer. Serum nitric oxide levels (nitrite + nitrate) were measured, after conversion of nitrate to nitrite by copperized cadmium granules, by a spectrophotometer at 545 nm (Shimadzu, Tokyo, Japan)[14]. Protein assays were measured on an Advia 2400 chemistry analyzer (Bayer Healthcare Instruments, Tarrytown, NY, USA). Results were expressed as μmol/g protein for NO and nmol/g protein for MDA.

The other part of the stomach was fixed in 10% neutral formalin, embedded in paraffin, and cut into 5 μm sections. The sections were stained with hematoxylin eosin (HE) and examined under the light microscope by a blinded pathologist for histological changes.

The results obtained from vardenafil groups were evaluated by comparing them with those of sham, indomethacin, and famotidine groups.

Statistical analysis

The statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 12.0 for Windows. All data are expressed as the mean ± SD. Mann-Whitney *U* and χ^2 tests were used for statistical analysis of data among all groups. $P < 0.05$ was considered as statistically significant.

RESULTS

Macroscopic analysis showed that there was no gastric mucosal lesion in the sham group. There were gastric mucosal lesions in all stomachs of the indomethacin 25 mg/kg treated group. The mean ulcer area was 21.00 ± 12.35 in the indomethacin group. Gastric mucosal damage was significantly reduced by famotidine 20 mg/kg and vardenafil 10 mg/kg pretreatments. In both groups, the mean count of ulceration and the mean count of

Table 1 Macroscopic evaluation of gastric mucosa

Groups	*n*	Weight (gr)	GML count	GML area mm^2
Sham	8	223.50 ± 18.40 (200-249)	0	0
Indomethacin	8	225.00 ± 13.77 (201-248)	6.25 ± 3.49 (1-11)[a]	21.00 ± 12.35 (1-36)[b]
Famotidine 5 (F5)	8	223.25 ± 13.13 (203-236)	4.12 ± 2.47 (2-8)	8.62 ± 2.97 (3-12)
Famotidine 20 (F20)	8	224.75 ± 14.78 (200-247)	2.37 ± 4.43 (0-13)	0.94 ± 2,06 (0-6)[d]
Vardenafil 2 (V2)	8	221.12 ± 13.27 (204-242)	4.37 ± 3.06 (0-8)	6.62 ± 5.87 (0-16)[e]
Vardenafil 10 (V10)	8	224.12 ± 15.16 (202-250)	1.25 ± 1.38 (0-3)[c,g]	0.75 ± 0.88 (0-2)[d,g]

GML: Gastric mucosal lesion. The values are presented as mean ± SD, (min-max). [a]$P < 0.05$, [b]$P < 0.001$ *vs* all other groups; [c]$P < 0.05$, [d]$P < 0.001$ *vs* group F5; [e]$P < 0.05$ *vs* group F20; [g]$P < 0.05$ *vs* group V2.

Table 2 MDA and NO levels in gastric tissues in each group

Group	*n*	MDA (nmol/g protein)	NO (μmol/g protein)
Sham	8	9.4 ± 4.47 (3.6-14.3)	35.67 ± 5.69 (30.21-46.63)
Indomethacin	8	28.48 ± 14.51 (7.1-45)[a]	27.20 ± 6.25 (20.0-38.78)
Famotidine 5 (F5)	8	6.21 ± 1.88 (3.4-9.5)	40.82 ± 9.42 (31.08-59.92)[c]
Famotidine20 (F20)	8	5.88 ± 1.60 (3.3-7.7)	51.22 ± 15.27 (34.24-77.50)[c]
Vardenafil 2 (V2)	8	15.87 ± 3.93 (11.6-23.8)[e]	31.01 ± 20.27 (21-55.15)[e]
Vardenafil 10 (V10)	8	10.97 ± 4.50 (5.4-19.9)[g,h]	33.55 ± 9.29 (22.16-48.51)[g]

The values are presented as mean ± SD, (min-max). [a]$P < 0.05$ *vs* the other group; [c]$P < 0.05$ *vs* indomethacin group; [e]$P < 0.05$ *vs* famotidine groups (F5 and F20); [g]$P < 0.05$ *vs* group F20; [h]$P < 0.01$ *vs* group F20.

ulcer area were significantly lower than the control group. Gastric mucosal lesion areas were significantly diminished in rats pretreated with famotidine 5 mg/kg and vardenafil 2 mg/kg, when compared with the control group, but this did not reach statistical significance in respect to ulcer count. The mean ulcer area in the vardenafil 2 mg/kg group and vardenafil 10 mg/kg group were 6.62 ± 5.87 and 0.75 ± 0.88, respectively. Macroscopic evaluation of gastric mucosal lesion counts and gastric mucosal lesion areas for each group are presented in Table 1. In damaged stomachs, mucosal lesions of various sizes and forms were dispersed to all stomach surfaces. Those lesions consisted of elongated bands parallel to the long axis of the stomach. Lesions of the gastric mucosa in each group are shown in Figure 1. Tissue MDA and NO levels are presented in Table 2 for each group.

On histopathological examination, erosion, inflammation, hemorrhage, and necrosis were abundant in the indomethacin group. Those lesions were encountered with increasing frequency in the Famotidine 20 mg/kg, vardenafil 10 mg/kg, famotidine 5 mg/kg, and vardenafil 2 mg/kg groups. There were statistically significant ($P < 0.05$) differences between the indomethacin group and pretreatment groups. Famotidine 20 mg/kg pretreatment had the most efficient protective effect against indomethacin-induced gastric mucosal lesions. Minimal hemorrhage, minimal focal necrosis, and superficial erosions were observed in 25% of the rats given 2 mg/kg vardenafil. A high dose (10 mg/kg) of vardenafil had a potent protective effect against indomethacin-induced gastric mucosal lesions, but vardenafil in low doses (2 mg/kg) did protect the gastric mucosa against the harmful effects of indomethacin, similarly famotidine 5 mg/kg did. Microscopic views of the normal and damaged gastric mucosa are shown in Figure 2. In our study, vardenafil has gastroprotective effects against indomethacin-induced gastric mucosal damage. The potency of this effect is stronger in high doses than low doses.

DISCUSSION

Despite the great progress in the treatment protocols, peptic ulcers are still a major ongoing heath problem. The gastric barrier protects the mucosa against damage of its deeper structures by noxious substances. Mucosal microcirculation of the stomach has an important role in gastric mucosal protection[15]. Prostaglandins and NO are the main factors that regulate gastric blood flow. NSAIDs cause gastric mucosal damage by inhibiting endogenous prostaglandins due to inhibition of COX-1 and COX-2[16,17]. Prospective data from the Arthritis, Rheumatism, and Aging Medical Information System (ARAMIS) states that 13 of every 100 patients with rheumatoid arthritis treated with NSAID for one year suffer from serious gastrointestinal complications related to the NSAIDs[18]. Indomethacin administration increases aggressive factors but decreases protective factors[19,20].

There is no doubt on the protective effect of H2 blockers. The gastroprotective effects of H2 blockers are significantly greater when given in high doses than in low doses. The results in our experiment, either in low or in high doses of H2 blockers, were in accordance with the literature. Widespread use of H2 blockers could not prevent peptic ulcer related disorders. Therefore, the search for new alternatives with novel mechanisms of action is ongoing.

PDE type-5 inhibitors, which were developed as cardioprotective drugs, are commonly used in the treatment of erectile dysfunction. Sildenafil citrate

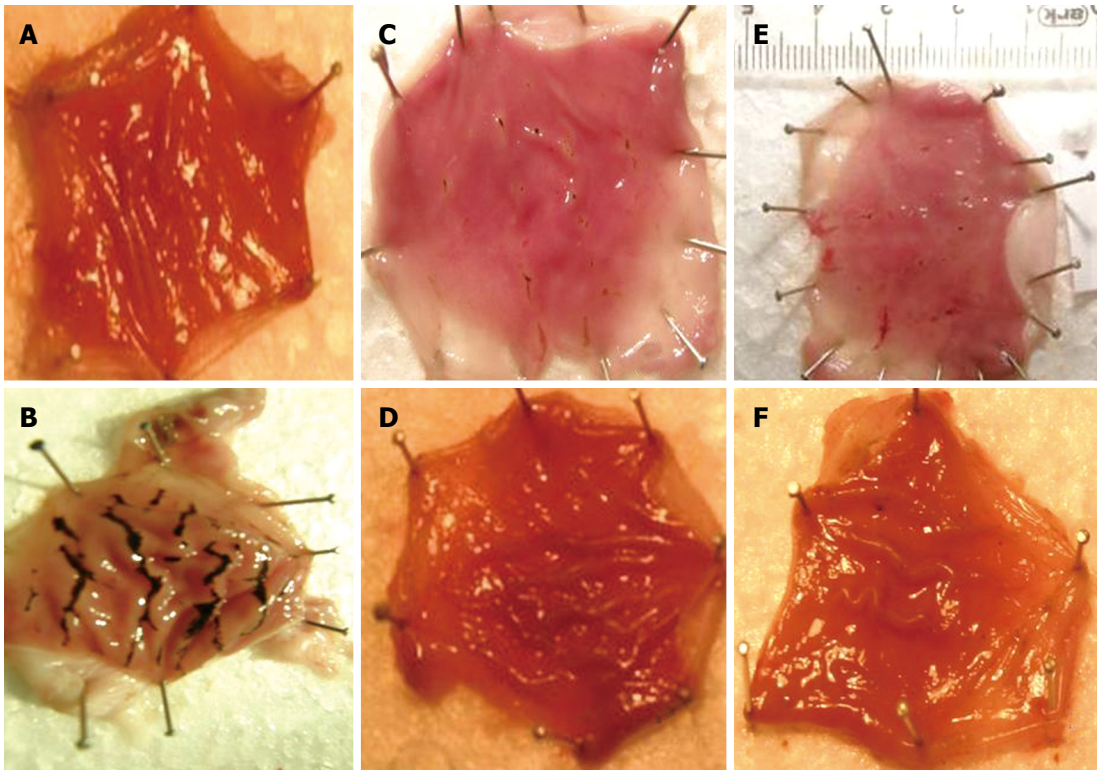

Figure 1 Gross appearance of the opened stomachs in the experimental groups. A: Appearance of normal mucosa of the stomach (Saline); B: Severe mucosal injury (Indomethacin); C: Diminished mucosal injury (Group F5); D: Gastric mucosa without any lesion (Group F20); E: Partially protected gastric mucosa against the harmful effect of indomethacin (Group V2); F: Lesion free gastric mucosa (Group V10).

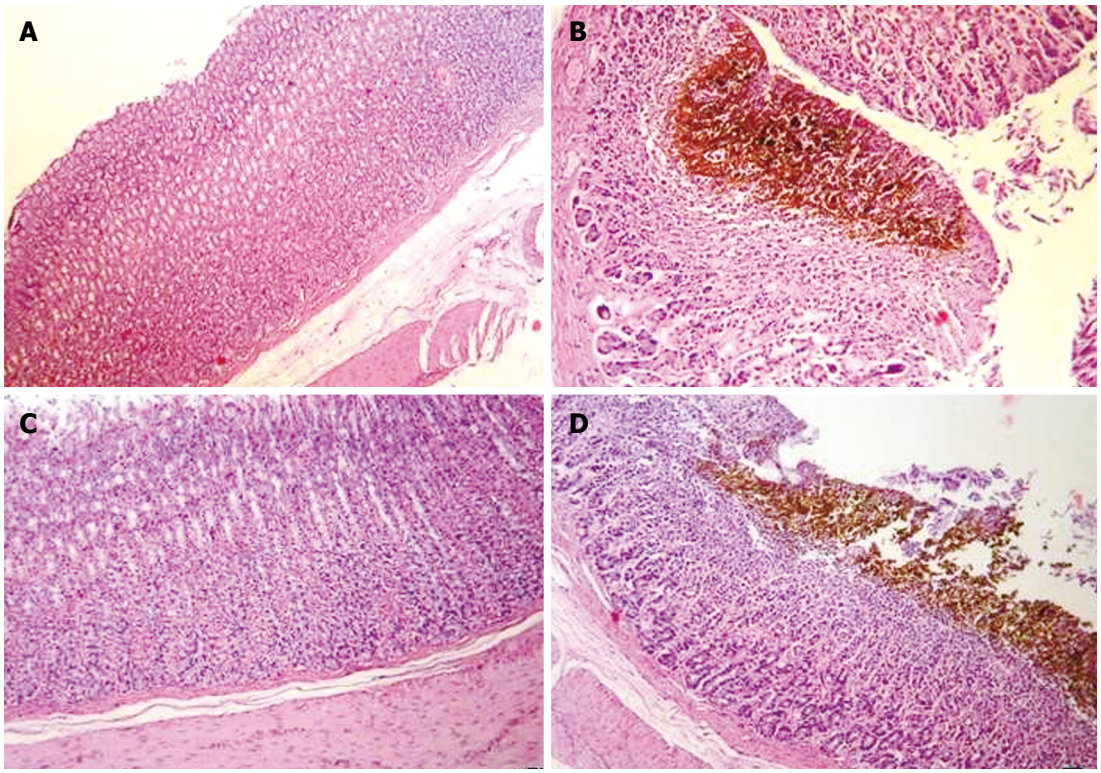

Figure 2 Normal rat gastric mucosa in the saline group and Group V10 (A and C, HE; × 100); gastric mucosal hemorrhage and necrosis in indomethacin group and Group V2 (B and D, HE; × 200, × 100) are shown.

has shown gastroprotective effects in experimental studies[21-23], and its gastroprotective effect was dose-dependent. Vardenafil is more potent than sildenafil. The gastroprotective effect of vardenafil has not yet been

studied. In our study, the antiulcer activity of vardenafil was investigated against indomethacin-induced gastric mucosal damage. Vardenafil decreased indomethacin-induced gastric mucosal lesions significantly at high doses (10 mg/kg). Macroscopically, vardenafil at a dose of 2 mg/kg has a protective effect on the gastric mucosa similar to famotidine at 5 mg/kg.

Macroscopic evaluations of gastric tissues revealed that vardenafil given in 2 mg/kg protects gastric mucosa better than famotidine at a dose of 5 mg/kg. Vardenafil has clinically important gastroprotective effects at high doses (10 mg/kg). Thus, the gastroprotective effect of vardenafil was dose dependent. In the stomach tissue of rats given indomethacin, the level of the lipid peroxidation product, MDA, increased significantly compared to the sham operated group. Tissues exposed to oxidative stress include large amounts of toxic oxygen radicals, which induce lipid peroxidation leading to MDA formation[24,25]. The lowest MDA values were detected in the famotidine groups. The mean MDA values in the vardenafil groups were similar to the sham group (Table 2). Thus, vardenafil pretreatment has inhibited MDA production in indomethacin treated rats.

Possible mechanisms of gastroprotection of PDE V inhibitors are increased production of tissue NO[23,26-28] or increased tissue cGMP level without modifying NO content[22,25,29,30]. The NO levels are slightly elevated in vardenafil pretreated rats in our study; however, the level of NO in either of vardenafil groups did not surpass the level of NO determined in the sham group. Some studies revealed gastroprotective effects of some agents without significant alterations in NO or MDA levels[31]. Determination of tissue cGMP level was not included in our study design. This is a short armcoming of our study design. PDE V inhibitors might prevent indomethacin-induced gastric mucosal damage in either mechanism.

In conclusion, vardenafil reduced gastric mucosal damage significantly at a high dose. Patients treated with PDE type-5 inhibitors might benefit from the additional gastroprotective advantages of these drugs, especially in high doses.

COMMENTS

Background

Peptic ulcer is a common disorder of the gastrointestinal system. The increase in non-steroid anti inflammatory drug (NSAID) ingestion in the treatment of inflammation, fever, and pain is one of the major etiological factors for peptic ulcers. Despite the many drug treatment protocols used to date, peptic ulcer still remains a major public health problem.

Research frontiers

Vardenafil has being used in the treatment of functional impotence; however, its effects on gastric mucosa have not yet been investigated. The research's aim was to determine its effectiveness in gastroprotection against NSAID-induced gastric lesions in comparison with famotidine.

Innovations and breakthroughs

Multiple agents have been used to prevent NSAID-induced peptic ulcer. This work is the first experimental study that show the beneficial effects of Vardenafil (a phosphodiesterase type V inhibitor) on NSAID-induced gastric ulcer. The gastroprotective effect of vardenafil against NSAID-induced peptic ulcer is dose dependent.

Applications

The study results suggest that vardenafil might be used as a potential therapeutic drug to prevent NSAID-induced gastric ulcer formation.

Peer review

This work might provide the first experimental data that directly shows the beneficial effect of a phosphodiesterase V inhibitor on gastric ulcers. In this manuscript, Karakaya et al report that administration of a phosphodiesterase V inhibitor (Vardenafil), dose-dependently suppresses indomethacin-induced gastric ulcers in rats. For comparison purposes, famotidine was used. There is only limited information suggesting the potential beneficial effect of vardenafil on ulcer healing, the data presented would be considered to provide attractive clinical information, although a clear mechanistic insight is not provided.

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