The role of sildenafil citrate in the protection of gastric mucosa from nonsteroidal anti-inflammatory drug-induced damage

Sildenafil sitrat'ın gastrik mukozayı non-steroid antienflamatuvar ilaçlara bağlı oluşan hasardan korumadaki rolü

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BACKGROUND

To investigate the protective effects of sildenafil citrate (SC) on indomethacin-induced gastric ulcer in a rat model.

METHODS

Gastric ulcers were induced by oral ingestion of indomethacin. Thirty rats were used in the study. The rats were divided into 3 groups, and given either SC (n=10) at a dose of 50 mg/kg or omeprazole (n=10), or no treatment (n=10, the control group). In addition to the measurements of ulceration areas, the sum of gastric tissue nitrite (NO_2^-) and nitrate (NO_3^-) were evaluated as an indicator of gastric tissue NO level. All the measurements were done at 6th hour of oral administration of indomethacin.

RESULTS

The mean values of ulceration areas were 4.0±2.31, 3.0±2.00, and 21.4±8.43 in the SC, omeprazole and control groups, respectively. The mean values of ulceration areas in the SC-treated group was lower than that of the control group. The contents of NO were 32.2±3.05, 24.8±3.23 and 21.0±0.82 (µmol/g protein) in gastric tissue in indomethacin, SC, omeprazole and control groups, respectively, The content of NO in the SC-treated groups was significantly higher than control group ($p<0.001$).

CONCLUSION

Sildenafil citrate may have a role in protecting gastric mucosa from the damage caused by indomethacin. This effect may be associated with the increased level of NO in gastric tissue.

Key Words: Gastric ulcers; nonsteroidal anti-inflammatory drug; rats, Sprague-Dawley; sildenafil citrate.

AMAÇ

İndometazinle oluşturulan sıçan mide ülseri modelinde sildenafil sitratın (SS) koruyucu etkileri araştırıldı.

GEREÇ VE YÖNTEM

Mide ülserleri indometazinin oral yoldan verilmesiyle oluşturuldu. Çalışmada 30 sıçan kullanıldı. Sıçanlar 3 gruba ayrıldı; 50 mg/kg dozunda SS (n=10) veya omeprazol (n=10) verildi ya da hiç tedavi verilmedi (n=10, kontrol grubu). Ülser alanlarının toplamına ilave olarak, mide dokusundaki NO düzeylerini saptamak amacıyla mide dokusunda nitrit (NO_2^-) ve nitrat (NO_3^-) düzeyleri değerlendirildi. Tüm ölçümler indometazinin oral uygulamasından 6 saat sonra yapıldı.

BULGULAR

Ülser alanlarının ortalaması SS, omeprazol ve kontrol grubunda sırasıyla 4,0±2,31, 3,0±2,00 ve 21,4±8,43 olarak ölçüldü. SS grubunda ortalama ülserasyon alanları kontrol grubuna göre daha düşük değerlerde saptandı. Mide dokusundaki NO içeriği SS, omeprazol ve kontrol grubunda sırasıyla 32,2±3,05, 24,8±3,23 ve 21,0±0,82 (µmol/g protein) şeklindeydi. NO muhtevası SS grubunda kontrol grubuna göre ciddi şekilde yüksekti ($p<0,001$).

SONUÇ

Sildenafil sitratın mide mukozasını indometazine bağlı oluşan hasardan koruyucu rolü olabilir. Bu etki NO'nun mide dokusundaki yükselmiş olan düzeylerine bağlı olabilir.

Anahtar Sözcükler: Gastrik ülser; nonsteroid antienflamatuvar ilaçlar; sıçanlar, Sprague-Dawley cinsi; sildenafil sitrat.

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Peptic ulcers are multietiologic, frequently recurrent and widespread chronic disease.[1] Nonsteroidal anti-inflammatory drugs (NSAIDs) follow *Helicobacter pylori* in the ulcer etiopathogenesis. Indomethacin, a NSAID, causes damage in the gastric mucosa and impairs ulcer healing as an adverse effect. Although the inhibition of cyclooxygenase (COX), which leads to depletion of endogenous prostaglandins (PGs), is a major pathogenic factor, it is unlikely that PG deficiency alone is sufficient to initiate the process that ultimately results in gastric ulceration.[2] There are many antiulcerogenic drugs, among which the most effective is omeprazole. However, these drugs do not always provide an effective treatment of the ulcer.[1,3] Therefore, the treatment of ulcers is still an important problem, and new drugs are urgently needed for the treatment of gastroduodenal ulcer. Sildenafil citrate (SC) is currently used in the treatment of functional impotence; it increases the effect of the guanosine cyclic 3', 5'-monophosphate (cGMP), which displays an inhibitory effect on the smooth muscle cells of the arterioles supplying the human corpus cavernosum. The effect of SC is due to blockade of the phosphodiesterase-type 5, which inactivates the intracellular cGMP stimulated by nitric oxide (NO).[4,5] Accumulating evidence from both animal and human studies indicates that NO plays key roles in normal wound repair. The beneficial effects of NO on wound repair may be attributed to its functional influences on angiogenesis and inflammation.[6]

In the present study, we aimed to investigate whether the protective effect of SC on gastric mucosa was related to NO.

MATERIAL AND METHODS

Animals

The present study was carried out on 30 female Sprague-Dawley rats weighing 200-220 g from the Experimental Animal Laboratory of Medicine Faculty of Atatürk University. The rats were kept in separate cages at room temperature and deprived of food 24 h before oral administration of indomethacin but were allowed free access to water.

Induction of gastric ulcer

Gastric ulcers were inflicted by oral administration of indomethacin 24 h after starvation. Each of the rats was given 25 mg/kg indomethacin.[1]

Drugs treatments and determination of ulcer count and size

The animals were divided into 3 groups with 10 rats in each. While group I and group II received SC at a dose of 50 mg/kg (orogastric) and omeprazole at a dose of 20 mg/kg (orogastric), respectively, group III received saline at a dose of 0.1 ml/kg (orogastric) and served as the control group. Drugs were given 30 min before the oral administration of indomethacin. The rats were killed with 70 mg/kg i.p. of thiopental sodium 6 h after oral administration of indomethacin, and the stomach was removed. Macroscopic damage was assessed and the areas and number of ulcerative zones were determined.[1] For biochemical study, 1 g gastric tissue was removed, rinsed in ice-cold distilled water, and immediately placed in 5 ml of 1.15% KCL containing 0.2% Triton X-100 and homogenized. For the histopathological examination, all the samples were fixed in 10% neutral formalin solution.

NO assay

For NO measurement, homogenate was centrifuged at 8000 g for 10 min and the supernant was obtained. NO was measured by the method of Moshage et al.[7] The sum of serum nitrite (NO_2^-) and nitrate (NO_3^-) were evaluated as an indicator of serum NO level.

Histopathological examination

Gastric tissues were embedded in paraffin, and 5 mm thick sections were cut and stained with hematoxylin-eosin. The histological slides were examined by two pathologists for the evaluation of mucosal hemorrhage, ulcer, erosion, vascular congestion, edema, and necrosis.[8]

Statistical analysis

All the data were expressed as mean±SD, and the analysis was carried out using Mann-Whitney U test. All of the statistical analyses were performed using SPSS version 9.0 software for Windows (SPSS Inc., Chicago, IL). Values of $p<0.05$ were considered statistically significant.

RESULTS

Effects of SC on indomethacin-induced gastric lesions

The mean counts of ulceration areas were 4.0±2.3, 3.0±2.0, 21.4±8.4 in SC, omeprazole and

Table 1. Comparisons of the ulcer counts and ulcer areas for each group

Drugs	n	Ulcer count* (Mean±SD)	Ulcer area (mm^2)* (Mean±SD)
Sildenafil citrate	10	4.0±2.3	4.9±2.6
Omeprazole	10	3.0±2.0	3.7±2.3
Control	10	21.4±8.4	24.6±9.3

* The differences were significantly higher in both the SC and omeprazole groups compared with the control group ($p<0.01$).

control groups, respectively. The mean count of ulcerations in the SC-treated groups (50 mg/kg) was significantly lower than that of the control group ($p<0.01$) (Table 1).

The mean areas of ulceration were 4.9±2.6 mm^2 in SC group, 3.7±2.3mm^2 in the omeprazole group, and 24.6±9.3 mm^2 in the control group. The mean areas of ulceration in the SC-treated groups (50 mg/kg) were significantly lower than that of the control group ($p<0.01$).

The content of nitric oxide in gastric tissue

The contents of NO in gastric tissue were 32.2±3.0 µmol/g protein, 24.8±3.2 µmol/g protein and 21.0±0.8 µmol/g protein in the SC, omeprazole and control groups, respectively. The content of NO in the SC group was significantly higher than that in the control group (Fig. 1) ($p<0.001$).

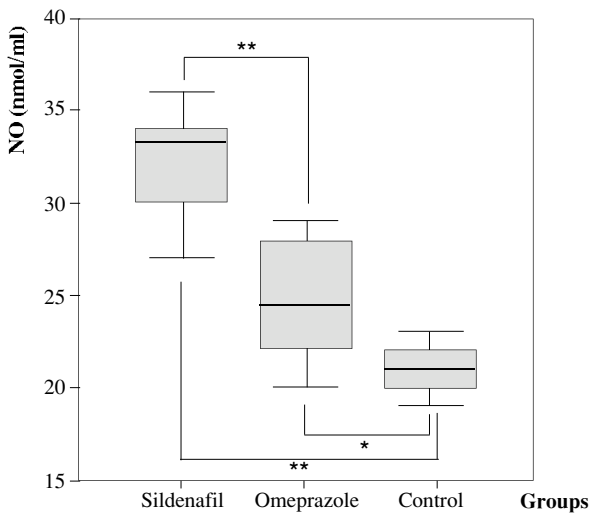

Fig. 1. While NO value was significantly higher in SC group compared with those of the omeprazole and control groups, this was also significantly higher in omeprazole group compared with that of the control group (*=$p<0.01$, ** $p<0.001$).

Histopathological examination

Histopathologically, minimal hemorrhage and focal necrosis of superficial cells were determined in the SC group (Fig. 2a, b), minimal hemorrhage and minimal necrosis of superficial cells in the omeprazole group (Fig. 3a, b), and drop out and necrosis of epithelial cells, massive hemorrhage and submucosal edema in the multiple areas of the control group (Fig. 4a, b).

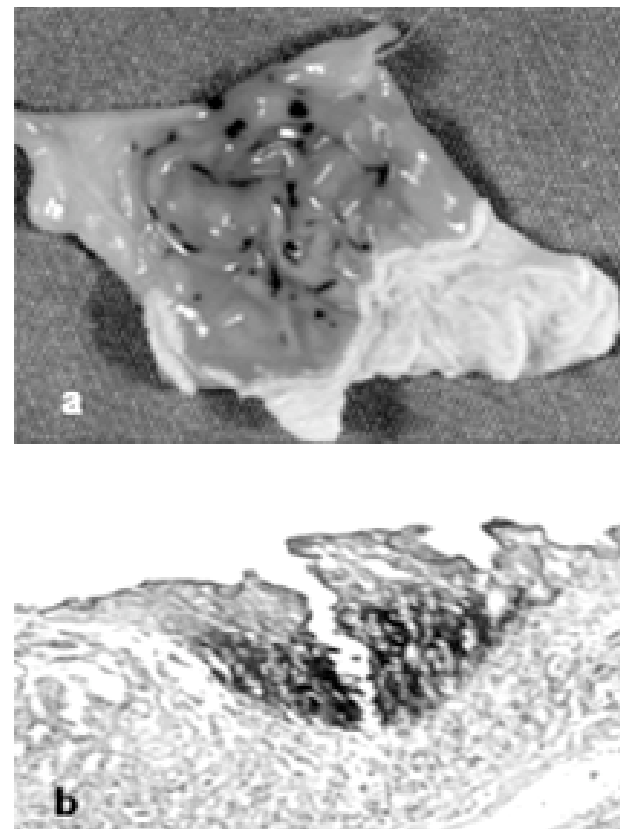

Fig. 2. **(a, b)** The figure shows multiple ulcers and mucosal hemorrhagia macroscopically and the ulcer extending from mucosa to submucosa microscopically.

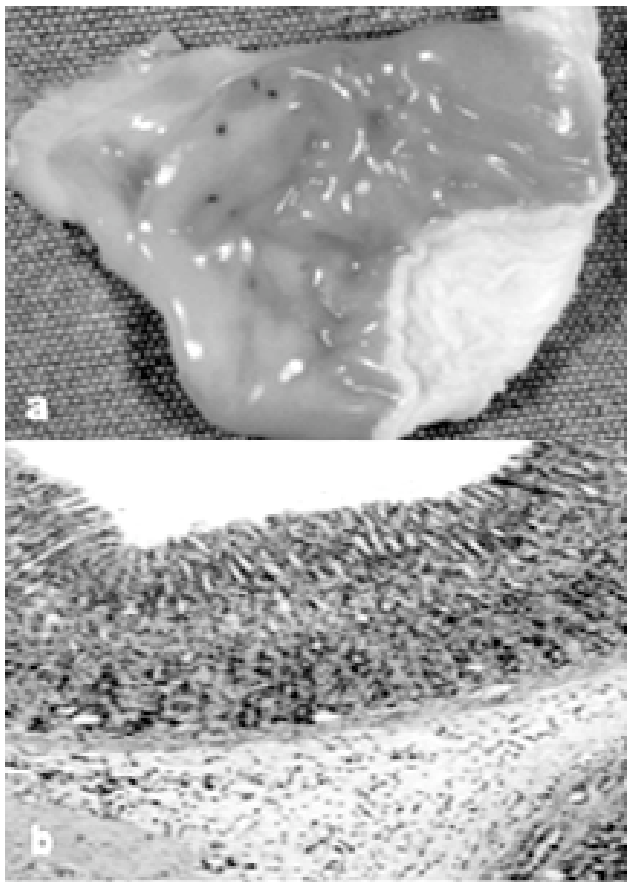

Fig. 3. **(a, b)** These figures exhibit few macroscopic ulcers and no microscopic ulcers.

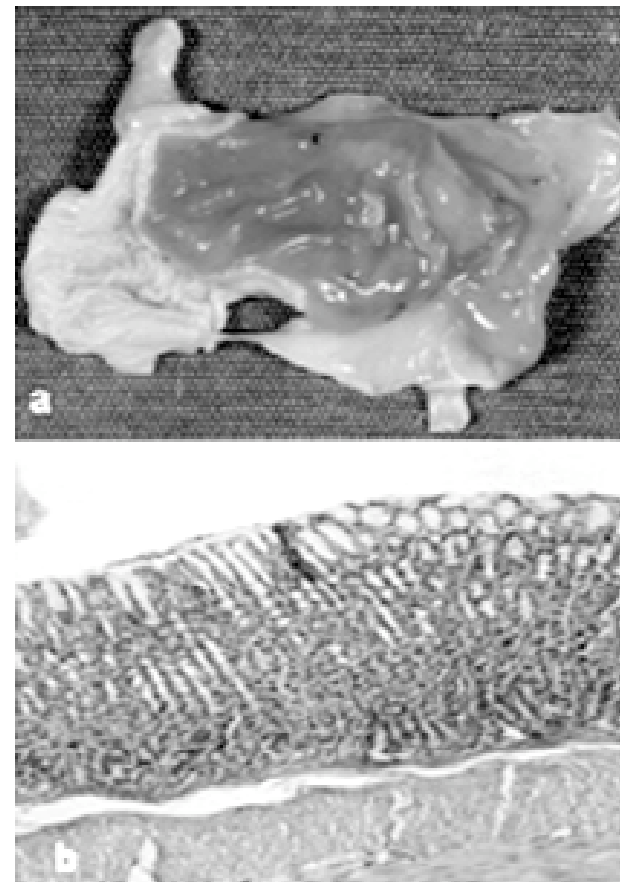

Fig. 4. **(a, b)** Few macroscopic ulcers and no microscopic ulcers.

DISCUSSION

It has become clear in the past decade that three different etiologies underlie virtually all ulcers: *H. pylori* infection, use of NSAIDs, and massive acid hypersecretion secondary to gastrinoma.[9]

According to prospective data from the Arthritis, Rheumatism, and Aging Medical Information system (ARAMIS), 13 of every 100 patients with rheumatoid arthritis who take NSAID for one year suffer from a serious gastrointestinal complication. It has been estimated conservatively that 16.500 NSAID-related deaths occur among patients with rheumatoid arthritis or osteoarthritis every year in the United States.[10]

NSAIDs inhibit COX in the gastrointestinal mucosa. COX inhibitions decrease the synthesis of cytoprotective prostaglandins (PG). Indomethacin produces the greatest ulcer area due to its pronounced inhibition of COX-1.[11] Süleyman et al.[12] reported that indomethacin inhibited COX-1, reduced synthesis of PGE2, and increased gastric acid secretion, gastric mucosal and myeloperoxidase activity and gastric motility.

Many agents have been used in the prevention of NSAID-associated gastro-duodenal ulcers such as mucosal protective agents (sucralfate), H2 receptor antagonist, prostaglandins (misoprostol), and proton-pump inhibitors.[10]

Sildenafil citrate is used in the treatment of functional impotence; it increases the effect of the guanosine cyclic 3', 5'-monophosphate (cGMP), which displays an inhibitory effect on the smooth muscle cells of the arterioles supplying the human corpus cavernosum. The effect of SC is due to blockade of the phosphodiesterase-type 5, which inactivates the intracellular cGMP stimulated by nitric oxide.[4,5]

NO plays an important role in the host defense and inflammatory response. It also plays an impor-

tant role in the mechanism of gastric mucosal protection.[6]

NO interacts with neuropeptides and PG to maintain mucosal integrity in basal conditions. Thus, although inhibition of NO synthesis alone does not cause gastric damage, lesions appear if this treatment is combined with ablation of sensory neurons following treatment with capsaicin or with a non-ulcerogen dose of indomethacin. It is clearly indicated that mucosal integrity depends on the co-ordinated action of these three systems.[13-15]

NO modulates epithelial barrier function,[16] increases mucus and bicarbonate secretion,[17] and mucosal blood flow,[18] decreases leukocyte-endothelium interactions,[19] decreases gastric acid secretion by inhibiting HCL producing cells,[20] and inhibition of apoptosis.[21]

In addition, NO acts as the endogenous mediator for the gastroprotective actions of different anti-ulcer agents,[22] several hormones[23] and modulators of neural activity.[18]

Chemical addition of a NO-releasing moiety to classical NSAID, significantly reduces the gastric detrimental actions of the parent drugs while maintaining anti-inflammatory, analgesic, and antipyretic activites.[24] On the other hand, the classical NO donors such as nitroglycerin by transdermal patches have gastro-protective effects opposite to indomethacin induced gastric damage.[25]

The results of the present study have shown that the NO content of the tissue from the SC-treated group was significantly higher than that of the control group. Therefore, it might be concluded that SC can increase NO production. Nevertheless Buvinic et al.[26] demonstrated that sildenafil increases tissue cGMP without modifying NO production, and it antagonizes the noradrealine induced vasoconstriction in rat mesenteric bad. It is highly possible that SC increases either NO itself or tissue cGMP without modifying NO, and thus it may prevent indomethacin induced gastric damage.

Sildenafil citrate, which was developed as a cardiovascular drug, is usually used in the treatment of erectile dysfunction.[5] Also, there are some trials focusing on new areas of use of this drug including primary pulmonary hypertension,[27] the enhancement of retinal blood flow[28] and hypercontractile eosophageal motility disorders.[29]

It may be used to protect the gastrointestinal mucosa from the side effects of NSAID, or at least may be considered for future studies in the development of new drugs. However, further studies are needed to understand clearly the protective effect of SC on mucosa and determine its certain clinical benefits.

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