

The effect of nonsteroidal anti-inflammatory drugs on rat gastric mucosa

The role of endothelin

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ABSTRACT

Objectives: To investigate the role of endothelin on nonsteroidal anti-inflammatory drugs (NSAIDs) and selective cyclooxygenase-2 (COX2) enzyme inhibitors-induced effects on the gastric mucosa.

Methods: This study was carried out in the Department of Pharmacology Laboratory, Faculty of Medicine, Dokuz Eylul University, Izmir, Turkey during the period January to December 2002. In the first group a cyclooxygenase-1 (COX1) and COX2 enzyme inhibitor, indomethacin (25 mg/kg, subcutaneous injection (s.c), n=7), a selective COX2 enzyme inhibitor, NS398 (10 mg/kg, s.c) and normal saline were administered. In the second group, endothelin-1 (ET1) was administered (200 pmol/kg) alone, in the presence of an endothelin receptor antagonist bosentan, (100 mg/kg) and PGE1 [40 µg/kg, orally] with submucosal injection. In the third group, NS398 and indomethacin were applied in the presence of bosentan. In the fourth group, NS398 were applied in the presence of N (G)-nitro-L-arginine methyl ester (L-NAME) (10 mg/kg, s.c).

Results: Indomethacin caused gastric mucosal injury. The effect of NS398 on gastric mucosa did not differ considerably from that of the control group. Submucosal injection of ET1 caused a gastric damage, which could not be prevented by intragastric administration of bosentan, while pretreatment with PGE1 prevented ET1-induced ulcer. Pretreatment with bosentan did not attenuate indomethacin-induced gastric mucosal damage but it increased NS398-induced damage by 1.5 fold. Pretreatment with L-NAME increased NS398-induced gastric mucosal damage as bosentan did.

Conclusion: These results suggest that neither endothelin-induced nor indomethacin-induced ulcer is completely receptor dependent. Cyclooxygenase-2 inhibitors caused ulcer in the presence of bosentan. Protective effects of gastric mucosal injury of COX2 inhibitors may be via endothelin receptor related nitric oxide release.

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are mostly used as anti rheumatoid agents. Gastroduodenal ulceration and bleeding are the major limitations of the use of NSAIDs. They can cause damage to the gastroduodenal mucosa via several mechanisms.¹ Especially inhibition of constitutively expressed cyclooxygenase-1 (COX1) by NSAIDs plays a predominant role in the gastrointestinal toxicity of NSAIDs.² Cyclooxygenase-2 (COX2) enzyme plays an important role in inflammation but does not contribute to the resistance of the gastrointestinal mucosa against injury.³ To minimize the gastric toxicity of NSAIDs; highly selective COX2 inhibitors have been developed. Whittle² reported note that the selective COX2 inhibitors are significantly better tolerated and do not produce toxic injuries of the healthy gastric mucosa. However, when COX2 inhibitors were administered in the presence of altered gastric mucosa, gastric injuries worsened.⁴

Endothelins (ET) are known as the most potent vasopressor agents.⁵ There are 2 subtypes of ET receptors. Endothelin-A (ETA) receptor is the most abundant receptor that causes smooth muscle contraction in all vascular regions; on the other hand, activation of endothelin-B (ETB) receptor is generally associated with vasodilatation through the stimulated release of endothelial nitric oxide (NO).⁶ Exogenous endothelin-1 (ET1) and endothelin-3 (ET3) are found to be ulcerogenic via ETA receptor.⁷⁻⁹ Furthermore, both ETA and ETB

receptors are involved in the reduction of gastric blood flow in the stomach.¹⁰ It is also suggested that an increase in ET1 levels enhances indomethacin-induced gastric mucosal injury.¹¹ However, whether the relationship between NSAIDs and ET is receptor-dependent or based on arachidonic acid inhibition is controversial. In spite of these studies, there is little knowledge regarding the role of COX2 enzyme inhibitors on the gastric mucosal integrity and there is no study investigating the relationship between selective COX2 enzyme inhibitors and ET1. Thus, the aim of this study was to determine the role of ET on NSAIDs and selective COX2 enzyme inhibitors-induced effects on the gastric mucosa.

Methods. Animals. This study was carried out in the Department of Pharmacology Laboratory, Faculty of Medicine, Dokuz Eylul University, Izmir, Turkey during the period January to December 2002 and was approved by the Medical Faculty, Dokuz Eylul University local ethics committee. Male Wistar rats weighing 150-200 g (177 ± 4 g) were supplied by the Dokuz Eylul University Animal Research Center. All rats were kept in a room with controlled temperature, humidity and a 12-hour light/dark cycle. Animals were housed with 4 per cage and had free access to standard rat chow and water before the study. They were deprived of food 12 hours before the experiment but were allowed access to tapwater ad libitum. To prevent bias in all groups, syringes were coded, and researchers were blinded. Rats were randomly divided into 4 groups, which had 0-4 subgroups ($n=7$ in each subgroup). In group I ($n=21$) there were 3 subgroups. A COX1 and COX2 enzyme inhibitor, indomethacin [25 mg/kg, subcutaneous injection (s.c) $n=7$] or a selective COX2 enzyme inhibitor, NS-398, (10 mg/kg, s.c, $n=7$) or normal saline as placebo (s.c, $n=7$) were administered. Four hours after the administration of the drugs or placebo, rats were sacrificed. In group II ($n=28$), there were 4 subgroups. Endothelin-1 (200 pmol/kg) was given through submucosal route 2 hours after the administration of nonspecific endothelin receptor antagonist bosentan, [100 mg/kg, taken orally, $n=7$] or 30 minutes after the administration of PGE1 (40 µg/kg, p.o, $n=7$) or normal saline, (p.o, $n=7$). For gastric submucosal injection of ET1, rats were anesthetized with ether and stabilized on a surgical stand. After a midline incision in the abdomen, the stomach was inspected and ET1 was injected into the submucosal layer of the anterior wall of the corpus of the stomach. The fourth subgroup of rats received peroral saline followed by gastric submucosal saline. Twenty-four hours after the administration of drugs or saline, rats were sacrificed. In group III ($n=14$), 2 subgroups ($n=7$ each) were formed.

One group received indomethacin (25 mg/kg, s.c) and the second group was given NS-398 (10 mg/kg, s.c) 2 hours after pretreatment with bosentan (100 mg/kg, p.o). Four hours after the administration of drugs, rats were sacrificed. In group IV ($n=7$), 30 minutes after the administration of nitric oxide synthase inhibitor, N (G)-nitro-L-arginine methyl ester (L-NAME), (20 mg/kg, s.c), NS-398 (10 mg/kg, s.c) were given. Four hours after the administration of the drugs, rats were sacrificed. The stomachs of all groups were dissected and assessed for macroscopic and microscopic damages. The stomachs were dissected along the major curvature and cleared with water. The specimens were then fixed in 10% formalin solution. Three samples of the corpus were excised from comparable regions in each stomach and were processed by applying routine techniques before embedding in paraffin. Sections (5 µm) were stained with hematoxylin and eosin and examined under a light microscope. The 1 cm length of each section was divided into 3 fields. The presence of gastric erosions was assessed histologically. Each field was scored on a 0-4 scale according to the following criteria: 0=normal; 1=epithelial cell damage; 2=glandular disruption, vasocongestion or edema in the upper mucosa; 3=mucosal disruption, vasocongestion or edema in the mid-lower mucosa; 4=extensive mucosal disruption involving the full thickness of the mucosa.¹² The overall mean value of the scores for each of the 3 fields was taken as the histologic ulcer index (HUI) from that section. All assessments were performed in a randomized manner and histologic sections were coded to eliminate observer bias.

Statistical analysis. All data are expressed as the mean \pm standard error of mean. Comparisons of groups by nonparametric Mann-Whitney U-test with a p value of 0.05 was considered statistically significant.

Results. The effects of COX2 inhibitor (NS-398) and indomethacin alone. The HUI of indomethacin (Figure 1) was significantly higher than the HUI of NS-398 (Figure 2) and control group (Table 1).

The effects of endothelin-1. Local submucosal injection of ET1 caused damage in the gastric mucosa (Figure 3). In the control group, where peroral saline was followed by intramucosal injected saline caused epithelial cell damage but the HUI was significantly lower than that of ET injected group. Oral pretreatment with bosentan reduced the ET1-induced gastric damage but it was not significantly different from the HUI of endothelin group. Pretreatment with PGE1 30 minutes before ET administration decreased the gastric mucosal damage significantly when compared with ET administered group (Table 2).

The effects of NS-398 and indomethacin with bosentan. Indomethacin caused a gastric mucosal damage. Pretreatment with bosentan did not change the HUI of indomethacin-induced gastric mucosal damage. However, in the presence of bosentan, NS-398 caused a gastric mucosal damage while a nonsignificant ulcerogenic effect was observed when the drug was administered alone (Table 3).

The effects of NS-398 with nitric oxide synthase inhibitor (L-NAME) on gastric mucosa. Pretreatment with L-NAME caused a mucosal disruption, vasocongestion or edema in the mid-lower mucosa in NS-398 group (Figure 4). The HUI was calculated and found significantly different from the group that was administered with NS-398 alone (Table 4).

Discussion. As NSAIDs are the most important group of self-prescribed pharmaceuticals, some side effects such as irritation and damage to the gastric mucosa limit their use. Some NSAIDs cause more gastro-intestinal side-effects than others.¹³ Selective inhibition of COX2 has been shown to be associated with a significant less gastric erosion formation than that which was seen with antiinflammatory doses of conventional NSAIDs.¹⁴ Our study showed similar results, however, a few epithelial cell damage were seen in our control group.

In general, the properties of NSAIDs that contribute to ulcerogenesis are topical irritancy, suppression of prostaglandin synthesis activity or some effects on microcirculation.¹⁵ Endothelin-1 causes contraction of the gastrointestinal smooth muscle.⁸ Duggan et al¹⁴

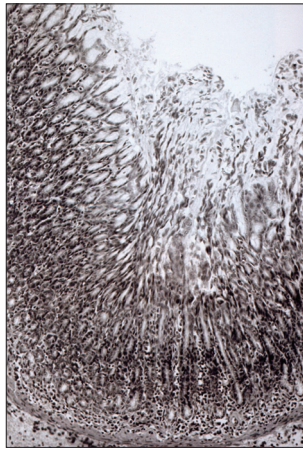


Figure 1 - Indomethacin-induced vasocongestion, edema and glandular disruption in the upper mucosa (Hematoxylin and eosin x100).

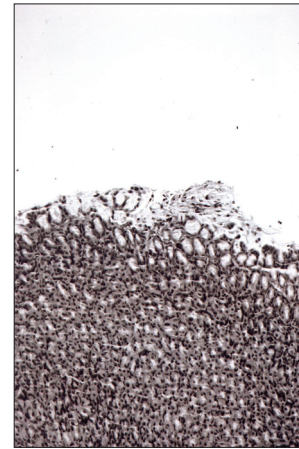


Figure 3 - Development of endothelin-1 induced gastric ulcer. (Hematoxylin and eosin x100).

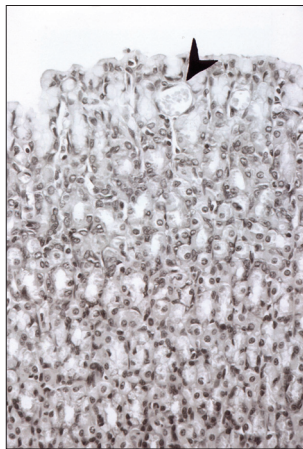


Figure 2 - The effect of NS-398 on rat gastric mucosa (Hematoxylin and eosin x100).

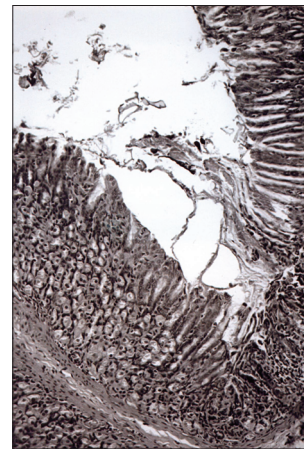


Figure 4 - The NS-398 caused, mucosal disruption, vasocongestion or edema were seen in the mid-lower mucosa in the presence of nitric oxide synthase inhibitor, L-NAME - N (G)-nitro-l-arginine methyl ester (Hematoxylin and eosin x100).

Table 1 - Comparison of the histological ulcer index (HUI) of NS398 and indomethacin versus control.

Group	Control	NS398	Indomethacin
HUI	1.43 ± 0.2*	1.71 ± 0.2	2.14 ± 0.1

*Indomethacin versus NS398 and control, $p < 0.05$

Table 2 - Comparison of ulcerogenic effect of endothelin-1 (ET1) on rat gastric mucosa. Values are expressed as mean ± standard error of mean of 7 rats for each group.

Group	Control	ET1	Bosentan+ET1	PGE1+ET1
HUI	1.42 ± 0.2	2.71 ± 0.2*	2.57 ± 0.2 [†]	1.14 ± 0.2 [§]

*ET1 versus control $p < 0.01$, [†]Bosentan+ET1 versus ET1 $p > 0.05$,
[§]PGE1+ET1 versus ET1 $p < 0.01$

Table 3 - Comparison of the effects of indomethacin, NS-398, in the absence and presence of the endothelin receptor antagonist bosentan on histological ulcer index (HUI). Values are expressed as mean ± standard error of mean of 7 rats for each group.

Group	Indomethacin	Bos+ indomethacin	NS398	Bos+NS398
HUI	2.14 ± 0.1	2.29 ± 0.2	1.71 ± 0.2	2.57 ± 0.2*

Bos+indomethacin versus indomethacin $p > 0.05$
 *Bos+NS398 versus NS398 $p < 0.05$, Bos - bosentan

Table 4 - Comparison of NS389 in the presence of L-NAME versus NS-398 alone ($*p < 0.01$). Values are expressed as mean ± standard error mean of 7 rats for each group.

Group	NS398	L-NAME+NS398
HUI	1.71 ± 0.2	3.14 ± 0.3*

HUI - histological ulcer index,
 L-NAME - N (G)-nitro-L-arginine methyl ester
 *L-NAME+NS398 versus NS-398 $p < 0.01$

have showed that submucosal injection of ET1 into the rat gastric body causes gastric damage and this can be effectively prevented by treatment with bosentan, an ET receptor antagonist.⁷ Furthermore, Reynolds et al¹⁶ reported that endothelin stimulates the release of arachidonic acid in the vascular smooth muscle cells. Thus, the metabolites of arachidonic acid, eicosanoid, may be responsible for the contractile effects of ET.¹⁶ Several factors seem to be involved in the mechanisms of the ET1-induced gastric mucosal damage. In our study, submucosal injection of ET1 caused gastric damage but

bosentan did not reduce the damage significantly. On the other hand, administration of PGE1 prevented the ulcerogenic effects of ET1 significantly. According to our results, ET1-induced gastric mucosal damage may be related to arachidonic acid metabolites. There are contradictory findings regarding indomethacin-induced ET1 release and gastric damage. Matsumaru et al¹⁷ reported that indomethacin-induced endogenous ET1 release diminishes gastric mucosal blood flow and causes gastric damage and bosentan prevents this gastric damage. On the contrary, it also reported the role of ET-converting enzyme-1 in indomethacin-induced gastric mucosal damage.^{18,19} In our study, pretreatment with bosentan did not change indomethacin-induced gastric mucosal damage. An increase in the ET-1 level combined with a decrease of regulatory cytokine may be responsible for the gastric mucosal injury caused by indomethacin. Further studies are needed to clarify the relationship between endothelin and NSAIDs. Our results showed that while NS-398 alone did not cause any statistically significant mucosal damage when compared to that of the control group, pretreatment with bosentan enhanced the NS-398-induced gastric mucosal damage. Pretreatment with an ETA receptor antagonist, BQ-123 reduced the gastric mucosal damage and ETA receptor transduces the vasopressor effects, while ETB receptors mediate the vasodilator actions of ET via NO.⁹ The NO plays a role in the maintenance of gastro-intestinal microvascular integrity under the experimental conditions.²⁰ Preventing NO release by blocking the ET receptors with nonspecific receptor antagonist, bosentan may be one of the explanations of the increase of the ulcer index in bosentan pretreated NS-398 group. Additionally, in our study, pretreatment L-NAME increased NS-398-induced gastric ulcer as bosentan did. Prevention of nitric oxide synthesis by L-NAME may enhance the COX2 inhibitor-induced gastric mucosal damage.

In conclusion, selective COX2 inhibitors did not cause any gastric damage when compared with conventional NSAIDs in rat gastric mucosa. Bosentan did not prevent ET-induced mucosal damage in our study; this effect seems not to be completely receptor dependent. Indomethacin-induced ulcer may not be mediated via ET receptors. The inhibition of ET receptors resulted in an enhancement of COX2 inhibitor-induced ulcer. Protective effects of gastric mucosal injury of COX2 inhibitors may be via ET receptor related NO release. Further studies are needed to clarify the role of the protective effects of ET receptor related NO release in gastric mucosa related to COX2 inhibitors.

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