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The protective effect of nitroglycerin on gastrointestinal and renal side effects of lornoxicam in rats

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Abstract

The aim of this study is firstly, to determine the preventive effect of chronic usage of combination of nitroglycerin and lornoxicam on gastrointestinal and renal side effects and secondly, to investigate the oxidative and antioxidative effects of this combination in rats.
Methods: Thirty-seven Wistar male rats were divided into five groups for 15 days; isotonic group ($n=8$, sodium chloride 0.09, Group ISO), lornoxicam group ($n=8$, lornoxicam 1.3 mg/kg, Group L), nitroglycerin group ($n=6$, nitroglycerin 1 mg/kg, Group NTG), lornoxicam–nitroglycerin combination group ($n=8$, 1.3 mg/kg lornoxicam + 1 mg/kg nitroglycerin, Group L–NTG), and control group ($n=7$, no drug was administered, Group C). Nitric oxide, malondialdehyde, reduced glutathione (GSH), catalase, interleukin (IL)-6 and tumor necrosis factor (TNF)-alpha concentrations were measured before drug injection and on fifteenth day in all blood samples. Gastrointestinal and renal biopsies were performed on fifteenth day.
Results: Two rats died on tenth and twelfth days in Group L. There were significant differences in Group L compared to the other groups for the lesions of stomach and kidney ($p=0.01$, $p=0.028$ respectively). Gastric ulceration was occurred in a rat in Group L. Malondialdehyde, TNF-α, and IL-6 levels decreased in NTG and L–NTG groups, whereas catalase and glutathion levels increased in NTG, L and L–NTG groups compared to control group ($p<0.05$).
Conclusion: Lornoxicam may cause gastrointestinal and renal side effects without oxidative stress. Adding nitroglycerin to lornoxicam for chronic treatment may prevent these side effects and enhance antioxidative effect compared to the use of lornoxicam alone in rats.

Keywords: Lornoxicam; Nitroglycerin; Oxidative stress; Antioxidative effects; Side effects

1. Introduction

The non-steroidal anti-inflammatory drugs (NSAID) have been widely used for to relieve pain, fever and inflammation. Many NSAID may cause gastrointestinal, liver, bone marrow and renal toxicity which results in gastrointestinal bleeding, ulceration, fulminant hepatic failure, or acute tubuler necrosis (Galati et al., 2002).

It is widely accepted that both the beneficial and detrimental effects of NSAIDs are attributable to their ability to inhibit prostaglandin synthesis through a direct blockade of cyclooxygenase (COX) (Wallace, 2001). The anti-inflammatory properties of NSAIDs are mediated through the inhibition of COX-2, whereas the simultaneous inhibition of COX-1 is responsible for adverse gastrointestinal side-effects as a result of a reduction in the constitutive production of cytoprotective prostaglandins. Inhibition of gastric prostaglandins (particularly PGI_2 and PGE_2) synthesis promotes stomach acid secretion,

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reduces bicarbonate and mucus production, and restricts mucosal blood flow responses that counter gastric defense and could predispose stomach tissue to damage (Bandarage and Janero, 2001). COX-2 also plays an important role in gastric mucosal defense and ulcer healing (Galati et al., 2002).

The pathogenesis of NSAID-induced gastrointestinal damage may also depend on prostaglandin-independent mechanisms, such as uncoupling of oxidative phosphorylation, alterations of mucosal cell turnover, as well as neutrophil activation (cytokines released) followed by enhanced endothelial adhesion (Wallace, 2001). These mechanisms, in combination with those related to prostaglandin suppression lead to microvessel occlusion and subsequent hyperproduction of reactive oxygen metabolites. Such substances are then able to induce oxidative tissue injury which seems to play a prominent role in the development of mucosal ulceration caused by NSAIDs (Pohle et al., 2001; Gudis and Sakamoto, 2005).

The balance between generation of free radicals and antioxidants is of critical importance for functional integrity of the cells. The excessive production of free radicals or deficiency of antioxidants (such as glutathione, and catalase) may lead to cellular destruction and gastric mucosal damage (Yoshikawa, 2002).

Nitroglycerin can also produce antioxidative effect (Gewaltig and Kojda, 2002; Kwiecien et al., 2002). In this respect, the antioxidative effects of the drug might be particularly important for preventing gastrointestinal side effects. Transdermal nitroglycerin patch may, in fact, reduce gastric damage induced by paranteral administration of indomethacin (Barrachina et al., 1995). It was also demonstrated that nitric oxide-releasing NSAIDs (NO-NSAID) can prevent gastrointestinal side effects in acute (Davies et al., 1997) and chronic administration in animals (Cuzzolin et al., 1995).

Lornoxicam (chlorotenoxicam) is a NSAID which has a relatively short plasma half-life (3 to 5 h). Lornoxicam leads to inhibition of both COX-1 and COX-2 without a clear selectivity. Anti-inflammatory and analgesic properties of lornoxicam have greater potency (Bianchi and Panerai, 2002), and it also prevents gastrointestinal side effects as compared to other NSAIDs (Radhofer-Welte and Rabasseda, 2000).

Although side effects of lornoxicam (oral route) have been well documented in previous studies (Pohlmeyer-Esch et al., 1997), intravenous administration of a mixture of lornoxicam and nitroglycerin has not been investigated to the best of our knowledge. The aim of this study is firstly, to determine the preventive effect of chronic usage of combination of nitroglycerin and lornoxicam on gastrointestinal and renal side effects and secondly, to investigate the oxidative and antioxidative effects of this combination in rats.

2. Material and methods

2.1. Animals

Male Wistar rats weighting approximately 300–450 g were obtained from Adnan Menderes University Medical Faculty Animal Research Laboratory Center (Aydin, Turkey). They were housed in polypropylene cages in groups of five per cage, and received standard laboratory chow and tap water ad libitum with 12/12 h light/dark cycles. All experimental protocols were approved by Animal Ethics Committee of Adnan Menderes University Medical Faculty.

2.2. Drugs

Lornoxicam (Xefo®, Abdi Ibrahim, Istanbul, Turkey), and nitroglycerin (Perlinganit®, Adeka, Istanbul, Turkey) were used. Drug solutions were prepared so that the desired dose, expressed in terms of saline, was contained in a volume 10 ml kg^{-1} of body weight for intraperitoneal (i.p.) injection. Thirty-seven rats were divided into five groups. No drug was given in control group (Group C, $n=7$). The drugs were injected by intraperitoneal route for each group, Group ISO ($n=8$, 0.09% NaCl, Group isotonic), Group L ($n=8$, lornoxicam 1.3 mg/kg), Group L–NTG ($n=8$, nitroglycerin and lornoxicam, 1 mg/kg + 1.3 mg/kg), Group NTG ($n=6$, nitroglycerin, 1 mg/kg) throughout 15 days.

2.3. Biochemical analysis

Blood samples were prepared for measurement of serum nitric oxide (NO), malondialdehyde, reduced glutathione (GSH), catalase, tumor necrosis factor-alpha (TNF-alpha) and interleukin 6 (IL-6) levels. Sera were obtained by centrifugation at room temperature and the samples were stored at −80 °C until analysis.

2.3.1. Measurement of nitric oxide (nitrite + nitrate) level

Nitric oxide has a very short half-life in sera. When NO is carried in the bloodstream, it is oxidized by erythrocytes and forms the stable end-products, nitrate (NO_3^-) and nitrite (NO_2^-), which provide an indirect measure of NO. The sum of nitrite and nitrate ($NO_2^- + NO_3^-$) has been confirmed to be a good indicator of NO production. In this study, NO (nitrite + nitrate) levels were analyzed using a modification of the cadmium-reduction method as described by Cortas and Wakid (1990). Nitrate (NO_3^-) was reduced to nitrite (NO_2^-) with cadmium granules and the nitrite concentration was then measured with the Griess reagent.

2.3.2. Catalase enzyme activity

Catalase level was measured in a fresh suspension of hemolysates. 1:1000 dilution of this concentrated homolysate was prepared with phosphate buffer immediately before the assay catalase activity was determined by the method of Aebi (1984).

Blood was collected into tubes containing citrate as anticoagulant. Blood samples were centrifuged at 3000 rpm 10 min at +4 °C. The buffy coat on the erythrocyte sediment was separated carefully after the plasma was removed. The erythrocyte sediment was washed three times with 0.9% NaCl solution to remove leftover leukocytes and plasma components. After each procedure, erythrocyte-saline mixture was centrifuged at 3000 rpm 10 min at +4 °C. Erythrocyte sediments were treated with 4-fold ice-cold deionized water to obtain stock hemolysate containing ~5 g hemoglobin/100 ml.

Hemoglobin content of hemolysates was measured using GEN-S coulter haemotology analyser.

2.3.3. Glutathion (GSH) determination

Blood was collected into tubes containing EDTA as anticoagulant. Reduced glutathione (GSH) level was estimated by monitoring the reduction of DTNB (dithiobis-2-nitrobenzoic acid) forming a yellow coloured anion at 412 nm (Beutler et al., 1963).

2.3.4. Malondialdehyde determination

Serum malondialdehyde concentration was measured as an indirect marker of oxidative stress in terms of thiobarbituric acid reactive substances, spectrophotometrically (Yoshioka et al., 1979).

Serum samples (0.125 ml) were mixed with 20% trichloroacetic acid (1.25 ml) and 0.67% thiobarbituric acid (0.5 ml). Mixture was then boiled at 95 °C for 30 min, immediately followed by cooling on ice. Reaction mixture was then vortexed, following the addition of *n*-Butanol (2 ml). All vials were then centrifuged at 3000 rpm for 10 min. Absorbance of the supernatant was then measured at 535 nm. Concentration of lipid peroxidation products was calculated as malondialdehyde concentration using the extinction coefficient for malondialdehyde-thiobarbituric acid complex of 1.56×10^5/mol/cm.

2.3.5. Interleukin (IL)-6 and Tumor Necrosis Factor (TNF)-alpha analysis

Serum TNF-alpha and IL-6 concentrations were measured with Biosource enzyme-linked immunosorbent assay (ELISA) kit (for TNF-alpha Cat no: KRC3011; for IL-6, Cat no: KRC0061; BioSource Europe S.A.; Nivelles, Belgium).

2.4. Histomorphologic evaluation

2.4.1. Histomorphologic evaluation of gastric mucosal damage

Each stomach was dissected in greater curvature and macroscopic examination made. If there was a macroscopic change in the mucosa, dissection was made in this area and antrum region. Tissues from these areas were processed after overnight fixation in 10% neutral-buffered formalin than embedded to paraffin blocks. Five micrometers thick paraffin sections were cut using a microtome and stained with hematoxylin and eosin. Sections were examined by light microscopy. Histological features of gastroduedonal lesions were examined 1+ to 4 scores: Normal, quiescent chronic gastritis (1+ to 3+) and active chronic gastritis as 4 (Correa and Yardley, 1992).

2.4.2. Histomorphologic evaluation of renal histology

Kidney was fixed in a 10% neutral buffered formalin solution, embedded in paraffin and used for histopathological examination. Five-micrometer thick sections were cut, deparaffinized, hydrated and stained with hematoxylin and eosin. A minimum of 10 fields for each kidney slide were examined and assigned semiquantitatively for severity of changes using scores on a scale of none (−), mild (+), moderate (++) and severe (+++) damage.

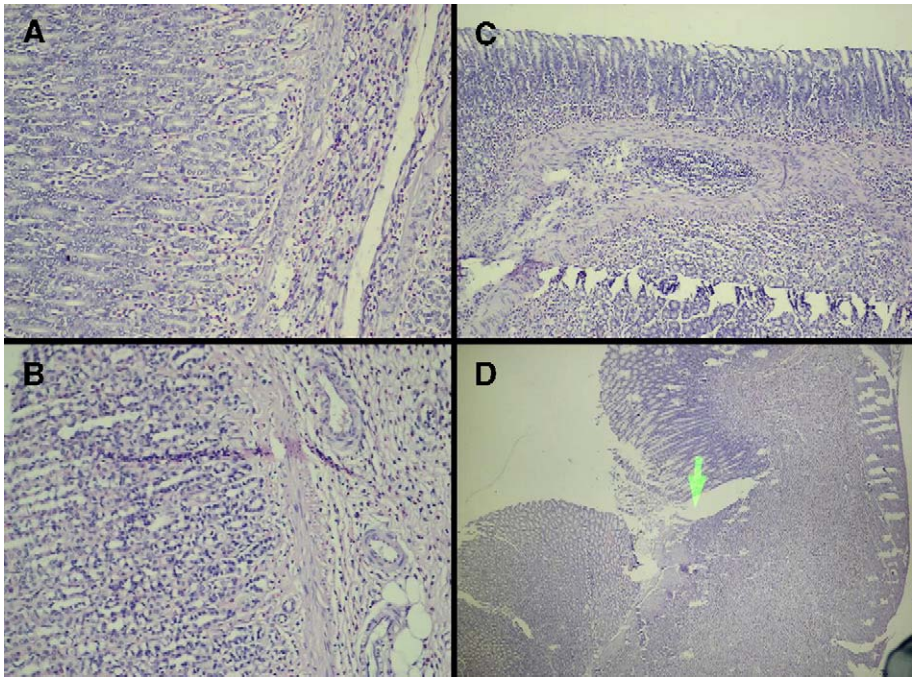

Fig. 1. H and E-stained sections of rat gastric mucosa (×200). Rats were treated with isotonic (0.09, Group ISO), lornoxicam (1.3 mg/kg, Group L), nitroglycerin (1 mg/kg, Group NTG) and lornoxicam plus nitroglycerin (lornoxicam 1.3 mg/kg and nitroglycerin 1 mg/kg, Group L–NTG). No drug was administered in control group ($n=7$, Group C). (A) Rats treated with nitroglycerin 1 mg/kg plus lornoxicam 1.3 mg/kg (Group L–NTG); there is little inflammation in the gastric mucosa. (B), (C), (D), treatment with lornoxicam 1.3 mg/kg (Group L); (B) moderate inflammation (Polymorphonuclear cells can be evidenced). (C) Severe inflammation which were associated with destruction of glandular architecture, submucosal edema, and extensive infiltration by polymorphonuclear cells; (D) severe lesion with ulcer of gastric mucosa (arrow).

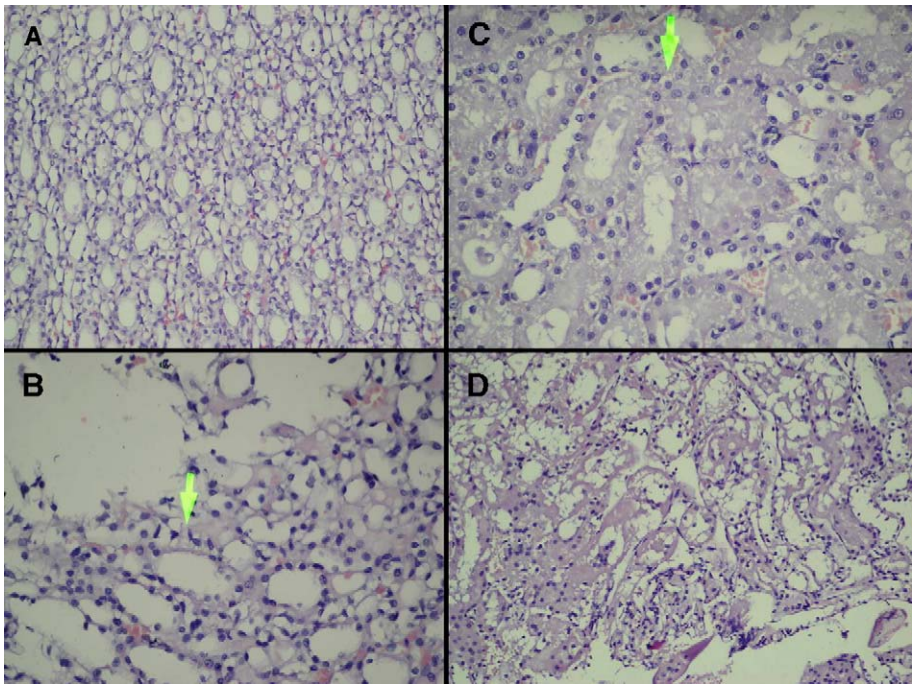

Fig. 2. Effects of lornoxicam and nitroglycerin plus lornoxicam on renal histology (×200). Rats were treated with isotonic (0.09, Group ISO), lornoxicam (1.3 mg/kg, Group L), nitroglycerin (1 mg/kg, Group NTG) and lornoxicam plus nitroglycerin (lornoxicam 1.3 mg/kg and nitroglycerin 1 mg/kg, Group L–NTG). No drug was administered in control group ($n=7$, Group C). (A) Normal renal histology (Group C). (B) Less damage with swelling of epithelial cell (stage 1) in Group L–NTG (arrow). (C) Moderate damage with vacuolization and single cell necrosis of epithelial cells (stage 2) in Group L (arrow). (D) Severe damage with desquamation and necrosis of epithelial cell (stage 3) in Group L.

2.5. Statistical analyses

Statistical analyses were performed using the one-way analysis of variance (ANOVA) with post-hoc Dunnett's multiple comparison test for comparing means from different treatment groups. Statistical differences of $P<0.05$ were considered to be significant.

3. Results

Treatment-related moderate and severe gastric erosion lesions of the gastrointestinal tract were found in five of 6 rats and an ulcer occurred in the sixth rat in Group L. Gastric and renal lesions are shown in Figs. 1 and 2. The scores of gastric and renal histological lesions were significantly higher in Group L ($P<0.0001$ and $P=0.003$) compared to Group C. There were minor gastric erosions in Group L–NTG. The scores of gastric and renal histological lesions were significantly higher in Group L ($P=0.001$ and $P=0.042$) compared to Group L–NTG. The scores of gastric and renal histological lesions are presented in Table 1.

Hemoglobin levels, and body weight gain were significantly decreased in Group L compared to control group on the fifteenth day ($P<0.05$). No significant difference was found in other groups on the fifteenth day (Table 2).

3.1. Biochemical results

Nitric oxide levels significantly increased in both NTG and L–NTG groups (97.3±14.4 and 69.2±5.8) compared to control group (24.3±5.5) ($P<0.001$) on the fifteenth day. Malondialdehyde levels decreased in both NTG and L–NTG groups (0.31±0.03 and 0.29±0.02) compared to Group C (0.45±0.01) ($P<0.05$) on the fifteenth day. There was no difference in Group L, Group ISO and Group C for NO and malondialdehyde levels on the fifteenth day. The results of NO and malondialdehyde levels are summarized in Table 3.

Although TNF-alpha and IL-6 levels decreased in both NTG and L–NTG groups, compared to control group ($P<0.05$), there was no difference in Group L, Group ISO and Group C on the fifteenth day. The results of TNF-alpha and IL-6 levels are summarized in Table 4.

Table 1
The scores of gastric and renal histological lesions on the fifteenth day

	For gastric lesions	For renal lesions
	Range	Range
Group C	0–1	0–1
Group ISO	0–1	0–1
Group L	2–3[a]	1–3[b]
Group NTG	0–1	0–1
Group L–NTG	1–2[c]	1–2[d]

Rats were treated with isotonic (0.09, Group ISO), lornoxicam (1.37 mg/kg, Group L), nitroglycerin (1 mg/kg, Group NTG) and lornoxicam plus nitroglycerin (lornoxicam 1.3 mg/kg and nitroglycerin 1 mg/kg, Group L–NTG). No drug was administered in control group ($n=7$, Group C).
The scores of gastric and renal histological lesions were significantly higher in Group L ([a]$P<0.0001$ and [b]$P=0.003$) compared to Group C.
The scores of gastric and renal histological lesions were significantly lower in Group L–NTG ([c]$P=0.001$ and [d]$P=0.042$) compared to Group L.

Table 2
Changes in hemoglobin and body weight gain of the rat groups before drugs administration and on the fifteenth day

	Hemoglobin (g/dl)		Body weight gain (grams)	
	Basic value	Fifteenth day	Basic value	Fifteenth day
Group C	13.58±0.96	12.38±1.12	330±47	333±40
Group ISO	13.98±1.25	13.47±0.83	334±31	337±30
Group L	14.30±1.23	10.53±0.50[a]	320±22	280±28[b]
Group NTG	14.06±2.9	13.65±1.43	335±57	330±42
Group L–NTG	14.12±2.7	12.65±1.89	326±43	318±39

Hemoglobin and body weight levels decreased in Group L ([a]$P<0.0001$ and [b]$P=0.023$) compared to Group C group on the fifteenth day.

Glutathion levels increased in both NTG and L–NTG groups compared to control group on the fifteenth day ($P<0.05$). Even though glutathion levels increased in Group L, compared to control group ($P=0.043$), it was lower than in NTG and L–NTG groups ($P=0.02$, and $P=0.031$). There was no change in glutathion levels in Group ISO and Group C compared to basic value on the fifteenth day. The results of GSH levels are summarized in Table 5.

Catalase levels increased in both NTG and L–NTG groups compared to control group ($P<0.05$). Catalase levels also increased in Group L, compared to control group ($P=0.024$) but it was lower than in NTG and L–NTG groups ($P=0.0001$, and $P=0.0042$). No change was observed in catalase levels in Group ISO and Group C compared to basic values on the fifteenth day. The results of catalase levels are summarized in Table 5.

4. Discussion

The main result of our study was that the chronic co-administration of lornoxicam and nitroglycerin prevents gastrointestinal and renal side effects compared to that of lornoxicam alone in rats. We also found that malondialdehyde (as oxidative stress marker) and cytokines (such as TNF-α, IL-6) decreased, while the antioxidative markers (catalase and glutathion) increased using these two drugs in combination.

Lornoxicam has high therapeutic potency and less gastrointestinal side effect when compared to naproxen (Radhofer-Welte and Rabasseda, 2000). Pohlmeyer-Esch et al. (1997) investigated different doses of lornoxicam (such as 0.06, 0.16 or 0.40 mg/kg/day) for chronic toxicity in rats. In their study, drug-related and dose dependent toxicity of lornoxicam mainly included mortality, reduced body weight gain, some clinico-pathological changes (such as anaemia resulting from blood loss), renal damage (renal papillary necrosis) and gastrointestinal mucosal lesions but none of these changes was present after the recovery period. Our study differs from Pohlmeyer-Esch et al.'s (1997) study by administration of higher doses of lornoxicam by intraperitoneal route but not orally. We preferred the dose of 1.3 mg/kg for lornoxicam since it was found to be fully effective to prevent hyperalgesia in rats (Bianchi and Panerai, 2002).

Table 3
Changes in NO and malondialdehyde levels before drugs administration and on the fifteenth day

	NO levels (μmol/L)		Malondialdehyde levels (nmol/mL)	
	Basic value	After fifteenth day	Basic value	Fifteenth day
Group C	22.5±5.4	24.3±5.5	0.44±0.02	0.45±0.01
Group ISO	22.4±5.2	22.1±7.9	0.43±0.03	0.46±0.02
Group L	22.3±5.6	21.5±7.4	0.42±0.02	0.43±0.04
Group NTG	24.2±6.4	97.3±14.4[a]	0.43±0.01	0.31±0.03[c]
Group L–NTG	23.4±5.3	69.2±5.8[b]	0.40±0.03	0.29±0.02[d]

Rats were treated with isotonic (0.09, Group ISO), lornoxicam (1.3 mg/kg, Group L), nitroglycerin (1 mg/kg, Group NTG) and lornoxicam plus nitroglycerin (lornoxicam 1.3 mg/kg and nitroglycerin 1 mg/kg, Group L–NTG). No drug was administered in control group ($n=7$, Group C).
NO levels decreased in Group NTG ([a]$P<0.0001$) and Group L–NTG ([b]$P<0.01$) compared with the other groups on the fifteenth day.
Malondialdehyde levels decreased in Group NTG ([c]$P=0.034$) and in Group L–NTG ([d]$P=0.001$) compared to Group C the on fifteenth day.

Table 4
Changes in TNF-alpha and IL-6 levels before drugs administration and on the fifteenth day

	TNF-alpha levels (pg/mL)		IL-6 levels (pg/mL)	
	Basic value	Fifteenth day	Basic value	Fifteenth day
Group C	36.7±7.3	38.8±5.8	43.3±5.2	46.6±5.1
Group ISO	38.3±7.5	40.6±6.9	44.6±4.8	45.7±7.8
Group L	36.5±7.4	37.3±6.4	44.5±6.2	46.3±5.3
Group NTG	35.4±8.8	28.3±6.8[a]	45.3±8.4	32.3±4.6[b]
Group L–NTG	38.3±8.3	29.1±5.7[c]	44.4±5.3	33.2±4.8[d]

Rats were treated with isotonic (0.09, Group ISO), lornoxicam (1.3 mg/kg, Group L), nitroglycerin (1 mg/kg, Group NTG) and lornoxicam plus nitroglycerin (lornoxicam 1.3 mg/kg and nitroglycerin 1 mg/kg, Group L–NTG). No drug was administered in control group ($n=7$, Group C).
TNF-alpha levels decreased in Group NTG ([a]$P=0.026$) and Group L–NTG ([b]$P=0.024$) compared to Group C on the fifteenth day.
IL-6 levels decreased in Group NTG ([c]$P=0.034$) and Group L–NTG ([d]$P=0.044$) compared to Group C on the fifteenth day.

Table 5
Changes in glutathione and catalase levels before drugs administration and on the fifteenth day

	GSH levels (μmol/g Hb)		Catalase levels (k/g Hb)	
	Basic value	Fifteenth day	Basic value	Fifteenth day
Group C	3.3±0.47	3.28±0.68	418.43±51.23	406.6±54.15
Group ISO	3.2±0.56	3.3±0.49	424.32±63.91	412.73±72.8
Group L	3.4±0.35	3.8±0.24[a]	420.2±87.05	492.43±53.35[b]
Group NTG	3.2±0.34	4.7±0.38[c]	416.7±48.6	589.34±48.6[d]
Group L–NTG	3.3±0.45	5.5±0.47[e]	421.4±75.96	687.22±74.8[f]

Rats were treated with isotonic (0.09, Group ISO), lornoxicam (1.3 mg/kg, Group L), nitroglycerin (1 mg/kg, Group NTG) and lornoxicam plus nitroglycerin (lornoxicam 1.3 mg/kg and nitroglycerin 1 mg/kg, Group L–NTG). No drug was administered in control group ($n=7$, Group C).
Lornoxicam significantly increased the serum levels of GSH ([a]$P=0.034$) and catalase ([b]$P=0.036$) compared to Group C, though less potently as compared with NTG and L–NTG groups on the fifteenth day.
Glutathione levels increased in Group NTG ([c]$P=0.0019$) and Group L–NTG ([d]$P<0.0001$) compared to Group C on the fifteenth day.
Catalase levels also increased in Group NTG ([e]$P=0.0028$) and Group L–NTG ([f]$P<0.0001$) compared with Group C on the fifteenth day.

Oxicams such as piroxicam and meloxicam can cause severe inflammation which was associated with destruction of glandular architecture, submucosal edema, and extensive infiltration by polymorphonuclear cells (Villegas et al., 2004). In our study, the use of lornoxicam alone caused similar effects on gastric and renal systems on the fifteenth day where as the administration of lornoxicam and nitroglycerin together prevented these side effects.

Many NSAIDs can cause gastric mucosal injury by proinflamatory cytokines; however lornoxicam has a moderate effect on the formation of cytokines (Radhofer-Welte and Rabasseda, 2000). It does not cause shunting of arachidonic acid to the lipoxygenase cascade, and therefore does not increase production of leukotrienes (Skjodt and Davies, 1998). It was also determined that many idiosyncratic NSAIDs produce prooxidative effects which contribute gastrointestinal side effects (Galati et al., 2002). Malondialdehyde represents a suitable index of oxidative tissue injury which is an end-product resulting from peroxidation of polyunsaturated fatty acids (Kwiecien et al., 2002). Blandizzi et al. (2005), showed that gastric damage of NSAID was characterized by a marked increase in mucosal malondialdehyde content and a decrease in GSH concentration. In contrast to these studies, in our study, there was no change in cytokines (TNF-alpha and IL-6) and malondialdehyde levels, when lornoxicam was used alone. We also determined that catalase and glutathion levels increased in Group L compared to those in control group. Similar to our results, it was demonstrated that oxicams are more reactive against reactive oxygen species than nimesulide and ibuprofen in rats (Van Antwerpen and Neve, 2004) and moreover lornoxicam appears to be a significantly better antioxidant than tenoxicam. It was stated that the antioxidant properties of lornoxicam might be related to its chemical structure. Bulbuloglu et al. (2005) concluded that the use of lornoxicam was effective in decreasing the oxidative stress of tissue during peritonitis. Based on these data, it is assumed that although lornoxicam may enhance antioxidative effects, the cytokines and malondialdehyde are not actively involved in the pathogenic mechanism of the gastric and renal adverse reactions of lornoxicam.

In our study, nitric oxide level raise was higher in nitroglycerin group than the combination of nitroglycerin and lornoxicam group. This might be related to the pharmacokinetic interaction between nitroglycerin and lornoxicam. Similarly, administration of a single dose of NO-NSAID in the rat leads plasma levels of NSAID to be greatly reduced, i.e. 40±50%, compared with those observed after injecting equimolar doses of the parent compounds. This evidence also supports the possibility that lower plasma NSAID concentrations following NO-NSAID administration have a bearing on their enhanced gastrointestinal tolerability (Fiorucci et al., 2001). Furthermore lornoxicam can inhibit endogenous NO formation in experimental models (Berg et al., 1999).

Nitric oxide which produces nitric oxide donors can act as a multifunctional gastroprotective mediator by influencing several aspects of gastric physiology, including mucus and bicarbonate secretion, blood flow in the gastrointestinal wall and tissue inflammatory responses (Barrachina et al., 1995).

Kwiecien et al. (2002) found that NO-donors reduced gastric lesions and that this protection was accompanied by the fall in oxidative stress parameters, decrease of malondialdehyde levels and increase of superoxide dismutase (antioxidant enzyme) activity to the level observed in the intact mucosa. It was also demonstrated that nitric oxide-releasing non-steroidal anti-inflammatory drugs (NO-NSAID) inhibited cytokine generation such as TNF-alpha and caspase activity (Fiorucci et al., 2001).

On the other hand, Husain (2003) suggested that nitroglycerin treatment significantly enhanced plasma catalase, and glutathione activities while decreasing malondialdehyde levels in rats. Sokolowska et al. (2004) also determined that the usage of 2.5 mg/kg nitroglycerin administered by intraperitoneal route in rats for 5, 10 and 17 days, participates in antioxidant reactions without any hypotensive effects. These studies support our findings, including a decrease of malondialdehyde and cytokines, and an increase of antioxidant enzymes in NTG and L–NTG groups.

While the gastrointestinal toxicity of NSAIDs is well known, it is becoming increasingly apparent that the kidney is also an important target for untoward clinical events (Gambaro and Perazella, 2003). In the kidney, effects of NSAIDs include electrolyte imbalance, acute renal failure, and nephrotic syndrome associated with interstitial nephropathy and papillary necrosis. Such changes have been held to be a consequence of pharmacologic depression of prostaglandin synthesis produced by the inhibitory effect of NSAIDs on cyclooxygenase (COX), thereby causing decreased blood supply to the kidneys (Basivireddy et al., 2005). Another possible mechanism that may be operational in the pathogenesis of NSAID-induced renal injury is inhibition of oxidative phosphorylation by NSAIDs, an event that may depress renal function (Mingatto et al., 1996). The protective role of NO may lie in its abilities to ameliorate vasoconstriction and improve organ blood flow to the kidney (Rahman et al., 1999; Tokuyama et al., 2002), to act as a scavenger of oxygen free radicals (Majid et al., 2004) and to stimulate COX activity, resulting in increased levels of reparative prostaglandins (Salvemini et al., 1993). Thus, multiple mechanisms may underlie the beneficial effects of NO. Interestingly, NO-NSAIDs have been shown to accelerate the recovery of renal function and structure in rats subjected to renal ablation (Muscara and Wallace, 1999). These results may illuminate why the renal side effects were less observed in the use of nitroglycerin and lornoxicam together compared to the use of lornoxicam alone in our study.

Mechanisms which were mentioned above or their combinations might be contributed to protective effects of nitroglycerine when added to lornoxicam for chronic use on gastrointestinal and renal side effects.

We concluded that chronic co-administration of nitroglycerin and lornoxicam might prevent gastrointestinal and renal side effects compared to lornoxicam alone. Nitroglycerin enhances the antioxidative effects of lornoxicam and different mechanisms might also play role to prevent side effects. Further studies

must be carried out with experimental models and different drug doses to reach an ultimate conclusion before its routine use.

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