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Effects of nimesulide and pentoxifylline on decreased contractile responses in rat ileum with peritonitis

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Abstract

The aim of this study was to determine the effects of nimesulide and pentoxifylline on the contractile effects of KCl, carbachol and substance P in the longitudinal muscle of rat ileum during peritonitis. Peritonitis was induced in rat ileum by cecal ligation and puncture. Thirty rats were operated on to induce peritonitis, 10 of which received nimesulide (5 mg/kg, subcutaneously) and 10 of which received pentoxifylline (25 mg/kg, subcutaneously) before the operation; 10 other rats underwent a sham operation and acted as controls. Twenty-four hours after the operation, ileum segments were transferred to isolated organ baths and responses to KCl, carbachol and substance P were recorded. E_{max} values of KCl, carbachol and substance P were markedly lower ($P < 0.05$), with no change in the pD_2 values, in the peritonitis group than in the controls. Peritonitis-induced changes in the KCl, carbachol and substance P responses of ileum were significantly restored by nimesulide ($P < 0.05$), but not by pentoxifylline. The improved contractile responses following nimesulide treatment indicate that products of cyclooxygenase-II may be, at least in part, responsible for the decreased contractile responses to KCl, carbachol and substance P in peritonitis.

Keywords: Nimesulide; Pentoxifylline; Contractile response; Ileum; (Rat)

1. Introduction

Peritonitis (sepsis) causes severe local damage to intra-abdominal organs due to overproduction of various proinflammatory mediators such as tumor necrosis factor, interleukin-1β, interleukin-6 (Thijs and Hack, 1995) and cyclooxygenase products (Pinsky and Matuschak, 1990). It is now considered that these mediators bring about systemic microcirculatory injury which is thought to be the main mechanism responsible for damage in sepsis (Judges et al., 1986; Sibbald et al., 1981; Avila et al., 1985; Hersch et al., 1990). The ileum is especially sensitive to this process in terms of contractility (Lodato et al., 1999). Pentoxifylline, a methylxanthine derivative, is a phosphodiesterase inhibitor and also down-regulates the production of proinflammatory cytokines such as tumor necrosis factor-α (Doherty et al., 1991; Badger et al., 1994), interleukin-6 (Holzheimer et al., 1995), and interleukin-1 (Hadjiminas et al., 1994). Many anti-inflammatory effects of pentoxifylline are probably due to the inhibition of phosphodiesterase IV (Liang et al., 1998; Badger et al., 1994).

Cyclooxygenases are the enzymes responsible for the conversion of arachidonic acid to prostaglandins. There are two types of cyclooxygenase described, cyclooxygenase-I and -II. Cyclooxygenase-I is constitutively expressed and is thought to be responsible for the synthesis of physiological prostaglandins that protect the gastric mucosa and which are involved in renal tubular function. Cyclooxygenase-II, which is an inducible form, was first described in 1991 (Vane et al., 1998) and is induced during tissue damage by several factors, including endotoxin, interleukin-1, hypoxia (Fosslien, 1998). Nimesulide is an anti-inflammatory drug that acts by selectively inhibiting a cyclooxygenase-II enzyme (Famaey, 1997).

The aim of this study was to investigate whether pentoxifylline or nimesulide protects ileal longitudinal smooth muscle responses to KCl, substance P and carbachol in an

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experimental cecal ligation and puncture model of peritonitis in rats.

2. Materials and methods

Forty male Wistar albino rats each weighing 230 to 330 g were maintained in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals and the experiments were approved by the Cumhuriyet University-Medical Faculty, Animal Care Committee. The experimental peritonitis model used in this study was based on the findings of a report by Martin et al. (1993). This model has previously been shown to induce hyperdynamic normotensive sepsis within 24 h.

2.1. Peritonitis model (cecal ligation and puncture)

Each rat was anesthetized with 3 mg/kg xylazine and 90 mg/kg ketamine intramuscularly. Once adequate anesthesia was attained, the animal was placed in a supine position. A 2-cm abdominal incision was made to expose the cecum. Then, the cecum was ligated just below the ileocecal valve with 4-0 silk ligature, so that intestinal continuity was maintained. Using an 18-gauge needle, the cecum was punctured in three locations, 1 cm apart, on the antimesenteric surface of the cecum, and cecum was gently compressed until the feces were extruded. The cecum was replaced in the peritoneal cavity, and the incision was closed in two layers. Afterwards, the rats were observed in a recovery cage for 24 h. The rats had free access to food and water after the operation.

The control group (sham group) underwent laparotomy, and the cecum was manipulated but not ligated or punctured.

2.2. Experimental design

The day before the surgical procedures, the animals were fasted overnight but allowed ad libitum access to water. Nimesulide and pentoxifylline were administered in a blinded fashion. Rats were assigned randomly to four groups: (1) The control group (sham operated); rats were anesthetized and their cecum was manipulated but not ligated or punctured. They received 1 ml distilled water subcutaneously as placebo ($n=10$). (2) Peritonitis group; rats were made septic by cecal ligation and puncture. They received equivalent volumes of distilled water as placebo ($n=10$). (3) Nimesulide-peritonitis group; rats received a subcutaneous dose of 5 mg/kg nimesulide (Wakita et al., 1999; Tardieu et al., 2000) dissolved in polyethylene glycol 400 before cecal ligation and puncture ($n=10$). (4) Pentoxifylline-peritonitis group; rats received a subcutaneous dose of 25 mg/kg pentoxifylline (Yada-Langui et al., 2000; Koyluoglu et al., 2001) dissolved in 1 ml of distilled water before cecal ligation and puncture ($n=10$).

2.3. Tissue preparation

Twenty-four hours later, the rats were killed by stunning and cervical dislocation. The abdomen was immediately opened and the ileum was removed and placed in previously aerated (95% O_2 and 5% CO_2) Krebs' solution (composition in mM = 115.48 NaCl, 4.61 KCl, 2.5 $CaCl_2$, 1.16 $MgSO_4$, 1.14 NaH_2PO_4, 21.9 $NaHCO_3$, and 10.09 glucose).Whole full thickness segments of the ileum in Krebs' solution were allowed to equilibrate for 4 h at 4 °C in a refrigerator. This procedure decreases spontaneous ileal contractions and stabilizes subsequent contractile responses to carbachol and substance P. After this procedure, whole full thickness segments of the ileum were placed in a longitudinal direction in a 10-ml muscle bath, filled with pre-aerated Krebs' solution at 37 °C. The upper end of the preparation was tied to an isometric transducer (Grass FT 03, Quincy, MA, USA) and preloaded with 1–1.5 g. Tissue was allowed to equilibrate for 30 min until a stable baseline was attained.

2.4. Isometric measurements

At the start of each experiment, 30 mM KCl was added to the bath and the contraction was considered as a reference response. At the end of the experiment, the response to 30 mM KCl was measured again. After the KCl response, the contractile responses to carbachol (10^{-9}–10^{-4} mol/l) and substance P (10^{-9}–10^{-5} mol/l) were obtained cumulatively. The amplitude of contraction is expressed as a percentage of the initial KCl reference response. Isometric tension was recorded on a Grass model 79 E polygraph. The number of experiments performed with tissue samples taken from different animals is given (n). All experiments were performed in a paired way.

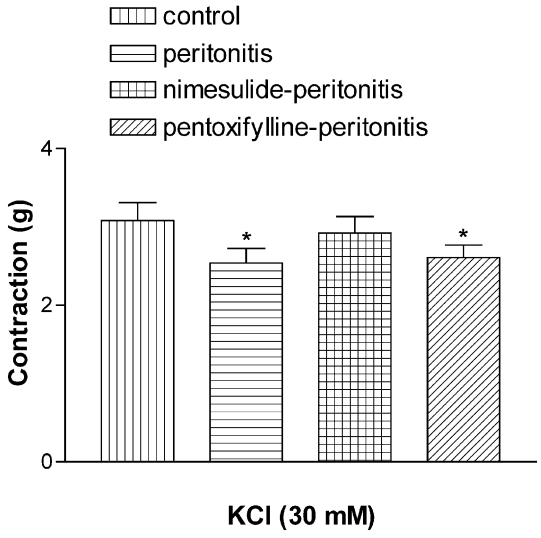

Fig. 1. Mean contraction elicited by 30 mM KCl of longitudinal ileum muscle isolated from control, peritonitis, nimesulide-peritonitis and pentoxifylline-peritonitis rats. Data are expressed as the means ± SEM of 10 experiments. * $P<0.05$ denotes significant difference from control group.

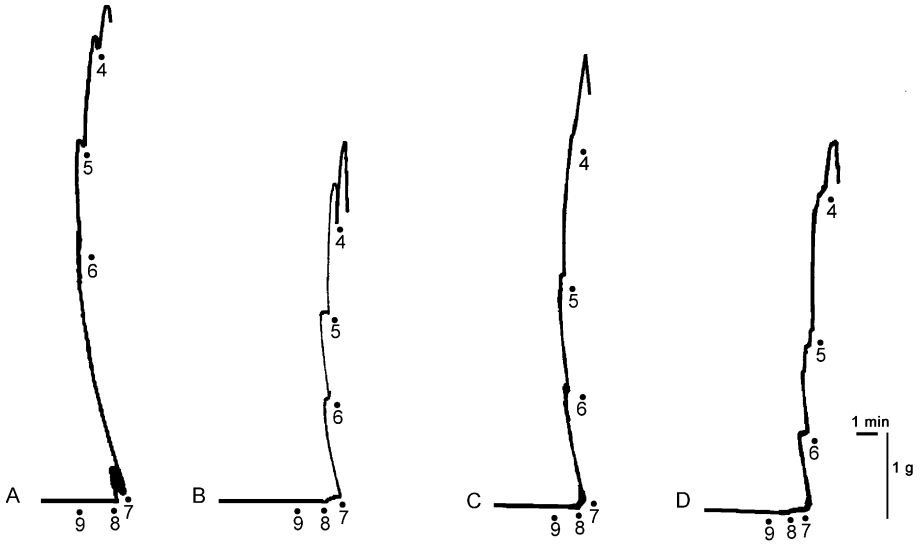

Fig. 2. Original tracings showing the responses elicited by different concentrations of carbachol in longitudinal ileum muscle isolated from control (A), peritonitis (B), nimesulide-peritonitis (C), and pentoxifylline-peritonitis (D) rats.

Four sets of experiments were performed with ileal longitudinal smooth muscle obtained from rats. In the first set of studies, we evaluated the contractile effect of carbachol and substance P on ileal longitudinal smooth muscle isolated from control (sham-operated) rats ($n = 10$). In the second set of studies, we evaluated the contractile effect of carbachol and substance P on ileal longitudinal smooth muscle isolated from rats with peritonitis ($n = 10$). In the third set of studies, we evaluated the contractile effect of carbachol and substance P on ileal longitudinal smooth muscle isolated from rats with peritonitis and pretreated with nimesulide ($n = 10$). In the fourth set of studies, we evaluated the contractile effect of carbachol and substance P on ileal longitudinal smooth muscle isolated from rats with peritonitis and pretreated with pentoxifylline ($n = 10$).

Contractile responses to carbachol and substance P were calculated as a percentage of the contraction caused by KCl (30 mM). To evaluate the effects of agonists, the maximum response (E_{max}) and pD_2 values (i.e. the negative logarithm of the concentration for the half-maximal response, ED_{50}) were calculated. The concentration–response data obtained in each experiment were plotted as the response/concentra-

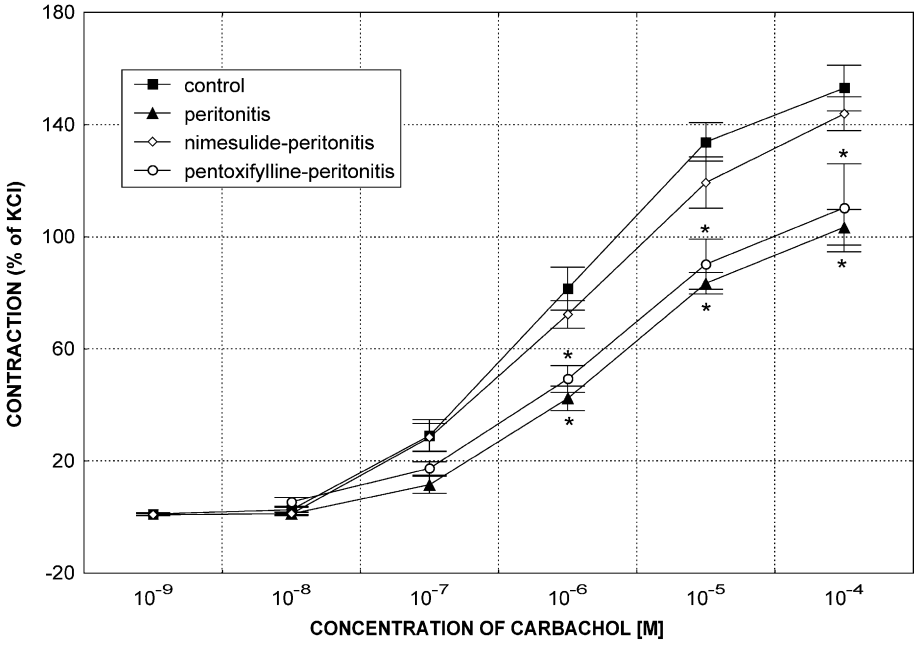

Fig. 3. Concentration–response curves of carbachol in longitudinal ileum muscle isolated from rats. Data are expressed as the means ± SEM of 10 experiments. * $P < 0.05$ denotes significant difference between the peritonitis and pentoxifylline-peritonitis groups and the control and nimesulide-peritonitis groups.

Table 1
E_{max} (% of KCl) and pD_2 values of carbachol and substance P. Data expressed as means ± S.E.M.

	Control	Peritonitis	Nimesulide-peritonitis	Pentoxifylline-peritonitis
Carbachol				
E_{max}	153.04 ± 18.14	103.38 ± 14.30[a]	143.88 ± 14.76	110.36 ± 35.05[a]
pD_2	6.70 ± 0.37	6.34 ± 0.32	6.91 ± 0.22	6.40 ± 0.24
Substance P				
E_{max}	143.30 ± 19.5	71.78 ± 20.32[a]	135.34 ± 32.77	79.72 ± 20.43[a]
pD_2	6.87 ± 0.10	6.54 ± 0.26	7.16 ± 0.38	6.66 ± 0.25

[a] $P<0.05$, statistically different from control group.

tion against the response, producing a sigmoid curve in each experiment, as predicted from the Scatchard equation for the drug-receptor interaction. pD_2 values (apparent agonist affinity constants) were calculated from each agonist concentration–response curve by linear regression of the linear median part of the sigmoid curve and taken as a measure of the sensitivity of the tissues to each agonist.

2.5. Drugs

Carbachol and substance P were purchased from Sigma (St. Louis, MO, USA). Nimesulide and pentoxifylline were purchased from ICN (Costa Mesa, CA, USA). Drugs were dissolved in distilled water, except for nimesulide and dilutions which were made up in distilled water. Nimesulide was dissolved in polyethylene glycol 400 (300 μl) and diluted to 1 ml with distilled water. The volume added to the muscle bath never exceeded 5% of the carbachol and substance P total volume. All solutions were prepared just before usage.

2.6. Statistical analysis

Data were analyzed by two-way of analysis of variance (ANOVA) with repeated measures ANOVA, and groups were compared statistically using general linear models of ANOVA followed by Newman–Keuls test. Differences were considered to be significant when $P<0.05$. All data are expressed as means ± standard error of the mean (S.E.M.).

3. Results

At the start of each experiment, 30 mM KCl was added to the isolated organ bath and the KCl-induced contraction was considered as a reference response of the ileal longitudinal muscle. The contractions elicited by 30 mM KCl were significantly decreased in the peritonitis (2.54 ± 0.18 g) and pentoxifylline-peritonitis (2.61 ± 0.16 g) groups compared with the control (3.08 ± 0.23 g) and nimesulide-peritonitis groups (2.92 ± 0.21 g) ($n=10$ in all groups) (Fig. 1).

Carbachol (10^{-9}–10^{-4} M) elicited a concentration-dependent contraction in the ileal longitudinal muscle isolated from rats in all groups (Fig. 2). Carbachol-induced contractions reached statistical significance at 10^{-7} M. The E_{max} value for the carbachol response was significantly lower in the group with peritonitis than in the control group ($P<0.05$), but there was no change in the corresponding pD_2 value (Fig. 3; Table 1). The peritonitis-induced changes in contractile responsiveness to carbachol were significantly restored in group 3, but not in group 4 (Fig. 3). No significant difference was found in terms of pD_2 values for the carbachol-induced contractile response of ileal longitudinal muscles isolated from rats in all groups ($P>0.05$) (Table 1).

Substance P (10^{-9}–10^{-5} M) elicited a concentration-dependent contraction in ileal longitudinal muscle isolated from rats in all groups (Fig. 4). The E_{max} value for substance P was significantly lower in the group with peritonitis than in the control group ($P<0.05$), but there was no change in the corresponding pD_2 value (Fig. 5; Table 1). The peritonitis-induced changes in contractile responsiveness to substance P were significantly restored in group 3, but not in group 4 (Fig. 5). There were no significant changes in terms of pD_2 values for substance P in all groups (Table 1).

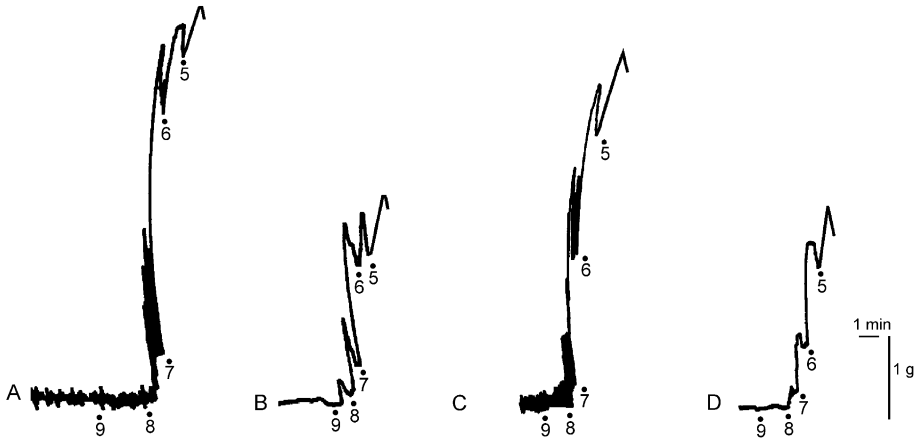

Fig. 4. Original tracings showing the responses elicited by different concentrations of substance P in the longitudinal ileum muscle isolated from control (A), peritonitis (B), nimesulide-peritonitis (C), and pentoxifylline-peritonitis (D) rats.

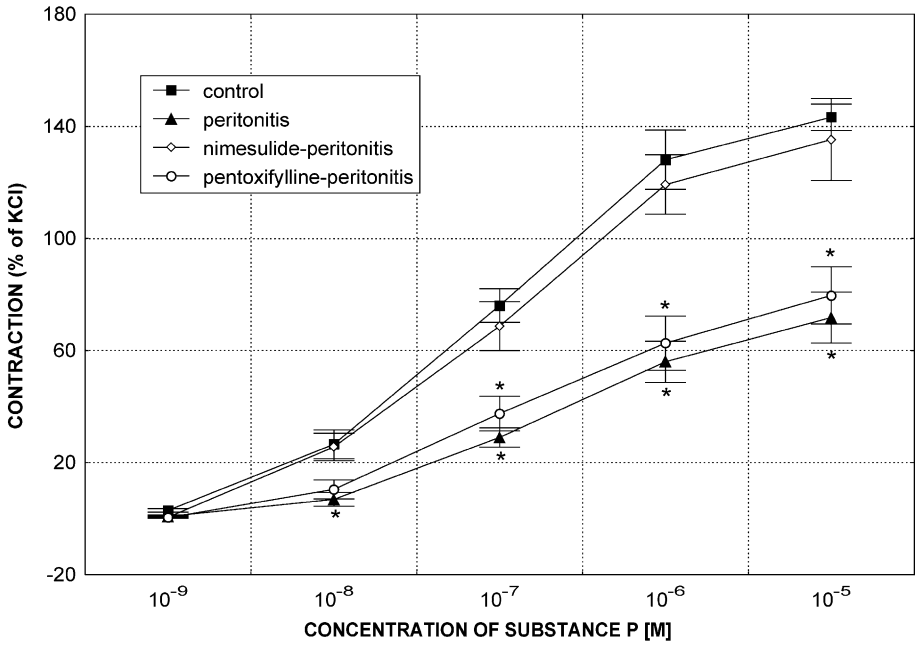

Fig. 5. Concentration–response curves of substance P in longitudinal ileum muscle isolated from rats. Data are expressed as the means ± SEM of 10 experiments. * $P < 0.05$ denotes significant difference between the peritonitis and pentoxifylline-peritonitis groups and the control and nimesulide-peritonitis groups.

Vehicle (polyethleneglycol 400) in which nimesulide was dissolved did not have a considerable effect on carbachol- and substance P-induced contractile responses in ileal longitudinal smooth muscle obtained from vehicle-control and vehicle-peritonitis groups compared to control and peritonitis groups, respectively (data not shown).

4. Discussion

The results of our study show that KCl-induced non-receptor-mediated and carbachol- and substance P-induced receptor-mediated ileal contractions are significantly reduced in an experimental cecal ligation and puncture model of peritonitis in rats. Pretreatment of rats with nimesulide (5 mg/kg) improved the reduced responses and returned them to control values, whereas pretreatment with pentoxifylline (25 mg/kg) did not.

It is well known that the KCl-induced contraction in smooth muscle is due to an increase in Ca^{2+} influx through voltage-operated Ca^{2+} channels. Therefore, peritonitis appears to affect the activity of voltage-operated Ca^{2+} channels. We hypothesized that if a receptor-mediated mechanism were involved, the peritonitis-induced changes would differ for different agonists. Therefore, we selected two contractile agonists, carbachol and substance P. Carbachol and substance P are the classic excitatory neurotransmitters in the small intestine. It is accepted that carbachol- and substance P-induced contractile response following receptor activation requires an increase in intracellular Ca^{2+} which is provided by both calcium influx through L-type Ca^{2+} channels and Ca^{2+} release from intracellular calcium stores (Tanovic et al., 2000). In our study, the decrease in response to carbachol and substance P paralleled the decreased response to KCl. The unchanged pD_2 values for the contractile agents studied suggests that peritonitis induced by cecal ligation and puncture does not affect specific receptor-dependent mechanisms. The decreased E_{max} values for carbachol and substance P in peritonitis may be related, at least in part, to an alteration in the regulation of postreceptor excitation–contraction coupling. In addition, responses to substance P were depressed to a larger degree than those to carbachol. This finding suggests that substance P-mediated postreceptor mechanisms are more sensitive to peritonitis-induced alterations in the ileum.

The cecal ligation and puncture model closely reproduces the clinically recognized sepsis syndrome (Members of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee, 1992). The effects of pentoxifylline treatment on survival in experimental sepsis are conflicting. Some studies have found improved survival (Judges et al., 1986; Sibbald et al., 1981; Ishizaka et al., 1988; Langenfeld et al., 1991), but others have not (Avila et al., 1985; Hersch et al., 1990). Pentoxifylline reduces the production of free oxygen radicals (Thiel et al., 1991), neutrophil adhesiveness (Tighe et al., 1990), TNF release (Doherty et al., 1991; Ishizaka et al., 1988) and attenuates endothelial and epithelial damage (Tighe et al., 1989). Several reports have claimed that a single dose of pentoxifylline (25 mg/kg and acts for 24 h) is beneficial in experimental models of sepsis by reducing bacterial translocation (Yada-Langui et al., 2000; Koyluoglu et al., 2001).

In our study, pentoxifylline did not improve the reduced smooth muscle responses. More continuous exposure to bacterial products and bacteria in our model may account for the failure of pentoxifylline, or the beneficial effect of pentoxifylline may be dose dependent. However, Flammand et al. (1995) have shown that treatment with the same or higher dosages in other experiments where benefit was shown did not ameliorate local injury to intra-abdominal organs in a rat model of sepsis. Therefore, the use of a single dosage in our study does not seem to be responsible for the lack of beneficial effect of pentoxifylline on ileal contractions, but it is effective in reducing bacterial translocation, as shown by Koyluoglu et al. (2001).

The pharmacological activity of nimesulide is mediated through various mechanisms. The selective inhibition of cyclooxygenase-II is the most important mechanism (Famaey, 1997; Taniguchi et al., 1995). Cyclooxygenase-II is induced in inflamed tissues by a variety of stimuli including cytokines, endotoxin, hormones, growth factors and mitogens. Inhibition of cyclooxygenase-II reduces the production of pro-inflammatory prostaglandins. Although we did not measure the amount of prostaglandins produced in this study, Shemi et al. (2000) have found that cyclooxygenase inhibitors inhibit the lipopolysaccharide-induced elevation of prostaglandin E_2. Another mechanism of nimesulide action is the inhibition of phosphodiesterase IV in leukocytes (Bevilacqua et al., 1994), like pentoxifylline. Since pentoxifylline was ineffective, the improved contractility obtained with nimesulide in our study does not seem to involve the inhibition of phosphodiesterase IV. Therefore, it is highly likely that the improved ileal longitudinal smooth muscle contractility is related to a reduced production of prostaglandins. In addition, nimesulide also prevents the oxidative and proteolytic inactivation of α_1-proteinase inhibitor, which inhibits elastase activation and connective tissue degradation (Ottonello et al., 1993; Dallegri et al., 1992a,b). This mechanism could also be responsible for the improved ileal smooth muscle responses in our study as well as for the inhibition of prostaglandin production.

One of the common complications of sepsis is prolonged gastrointestinal stasis, or ileus. The delay in transit causes overgrowth of bacteria within the lumen of the small intestine and eventual translocation of bacteria from the gut to the regional lymph nodes (Lodato et al., 1999).These observations show that gut stasis may be a result of sepsis but may also cause sepsis, particularly persistent or recurrent sepsis, by virtue of a "vicious cycle". Therefore, nimesulide treatment may be a logical strategy in the treatment of gastrointestinal stasis in septic patients. In conclusion, this is the first report showing that nimesulide prevents the reduction of ileal longitudinal smooth muscle responses to contractile substances occurring in the cecal ligation and puncture model of peritonitis. Further studies, such as measurement of the amount of prostaglandins produced and the effect of other nonsteroidal anti-inflammatory drugs and cyclooxygenase-II inhibitors in this model, are required for a better understanding of the exact mechanism responsible for the protective effect of nimesulide.

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