J. Pharm. Pharmacol. 1989, 41: 757–761
Received March 3, 1989

On the Relevance of Non-steroidal Anti-inflammatory Drugs in the Prevention of Paralytic Ileus in Rodents

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Abstract—In the mouse, the gastrointestinal transit of a charcoal marker, halved following the intraperitoneal administration of acetic acid, was no longer inhibited after pretreatment with the non-steroidal anti-inflammatory drugs (NSAIDs), indomethacin, ketoprofen, piroxicam or ximoprofen (0·25–2·5 mg kg^{-1} orally). In the fasted rat, the migrating myoelectric complex pattern of the small intestine which was disrupted for about one hour by acetic acid was unaltered by pretreatment with indomethacin or ximoprofen (0·5 mg kg^{-1} i.p.). In the anaesthetized rat, the inhibition by about 50% of the gastrointestinal transit due to laparotomy, did not occur following treatment with NSAIDs. It is concluded that NSAIDs prevent the occurrence of chemically-induced and postoperative ileus in rodents, an effect probably related to the analgesic properties of NSAIDs.

Paralytic ileus is defined as a long lasting inhibition of gastrointestinal motility in response to nociception initiated at the abdominal level. A well-known example is the "adynamic" ileus which occurs following abdominal surgery in man (Furness & Costa 1974). Postoperative ileus has also been described in the rat (Dubois et al 1973; Bueno et al 1978a; Ruwart et al 1979), and as in the dog or the sheep, the ileus state is characterized by the disruption of the migrating motor complex (MMC) (Bueno et al 1978b). The inhibition of the gastrointestinal motility is mediated through a nervous reflex initiated by peritoneal incision or irritation. The afferent pathway is partly constituted by capsaicin-sensitive fibres (Holzer et al 1986) while the efferent limb is represented by an increased sympathetic outflow, especially via the splanchnic nerves (Bueno et al 1978b).

A currently used model of paralytic ileus in rodents is the nociceptive stimulation of the peritoneum by iodine or acetic acid intraperitoneally (Holzer et al 1986; Moore et al 1987). This procedure reflexly inhibits gastrointestinal motility and transit. This model is related to the test developed by Koster et al (1959) for the screening of analgesic drugs. In that test, acetic acid produces characteristic stretching movements that are abolished by analgesics, including non-steroidal anti-inflammatory drugs, which possess a peripheral analgesic action (Otterness & Gans 1987).

The aim of this study was to test the relevance of the analgesic effects of NSAIDs in the prevention of the acetic acid-induced inhibition of gastrointestinal transit and motility in the mouse and the rat, respectively. The role of nociception in postoperative ileus was assessed by the effects of NSAIDs in preventing the inhibition of gastrointestinal transit due to laparotomy in the rat.

Materials and Methods

Chemically-induced ileus

Male Swiss mice (strain OF1; Iffa Credo; France), 18–20 g, were used. Peritoneal irritation was obtained by the intraperitoneal administration of acetic acid (120 mM in saline) at a volume of 0·2 mL per mouse; control animals received saline alone. Gastrointestinal transit was expressed as the percentage of the length of the small intestine covered by the front of a charcoal marker. The marker (an aqueous suspension of 5% (w/v) gum arabic and 10% (w/v) carbon black) was given orally (0·4 mL per mouse), after a 20 h-fast, 30 min after the i.p. injection of acetic acid or saline. The mice were killed by cervical dislocation 20 min after the test meal, and the front of the charcoal suspension in the small intestine was detected visually. The tested drugs or their vehicles were given orally 5 min before peritoneal irritation.

Male Sprague-Dawley rats (strain OFA; Iffa Credo; France), 250–300 g, were used. The myoelectrical activity of the small intestine was recorded through the use of chronically-implanted electrodes (Ruckebusch & Fioramonti 1975). Briefly, insulated nickel/chrome electrodes, 80 μm in diameter were implanted under pentobarbitone sodium anaesthesia (40–55 mg kg^{-1} i.p.) into the muscular coats of the intestine. Three groups of electrodes were thus implanted on the duodenum, 10 cm from the pylorus, and the jejunum, 10 and 20 cm from the ligament of Treitz. The rats were also fitted with a silicone tube (ID = 1 mm) in the abdomen, to allow injection in unrestrained animals. The free ends of the electrodes and of the tube were exteriorized on the back of the neck. Starting 5–7 days after surgery, the electrical activity was recorded continuously after a 16 h-fast, using a Physiograph recorder with a time constant of 0·1 s. Peritoneal irritation was obtained by the administration of 0·5 mL of acetic acid (120 mM in saline); control animals received 0·5 mL saline. The tested drugs or their vehicles were injected i.p. 30 min before peritoneal irritation.

Postoperative ileus

Male Sprague-Dawley rats (strain OFA; Iffa Credo; France) 200–220 g were anaesthetized by intravenous injection of pentobarbitone sodium (30 mg kg^{-1}). Surgical trauma was caused by laparotomy and removal of the caecum from the peritoneum for 10 min. After its replacement, the wound was

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closed. Fifteen minutes later, the test meal (an aqueous suspension of 5% gum arabic and 10% carbon black) was administered orally at a volume of 1 mL per animal, and the gastrointestinal transit, measured 30 min later, was expressed as the percentage of the length of the small intestine covered by the front of the charcoal marker. Control animals were treated in the same way except that they were not subjected to abdominal surgery. They received the test meal at the same time after induction of anaesthesia. The tested drugs or their vehicles were administered intraperitoneally 15 min before anaesthesia.

Drugs

Indomethacin (Indocid, Merck Sharp and Dohme) and ketoprofen (Profenid, Specia) were used. Piroxicam (Pfizer-Paris, France) was dissolved in 4% (w/v) dimethylsulfoxide. Ximoprofen (4-[3-oximino-cyclohexyl]hydratropic acid) was dissolved in sodium hydroxide (100 mM in distilled water) and phosphate buffer, to give a 0·5 mg kg^{-1} solution pH 7·2. When necessary, the prepared solutions were diluted in distilled water to administer 10 mL kg^{-1} orally in mice and 1 mL kg^{-1} intraperitoneally in rats.

Analysis of data

Values are expressed as mean ± s.d. Statistical analysis of the results was carried out using the Mann & Whitney and Student's *t*-tests for unpaired data and the Wilcoxon test for paired data.

Results

Chemically-induced ileus

Gastrointestinal transit did not differ among untreated, vehicle-treated and NSAIDs-treated mice. The effect of i.p. acetic acid was investigated in these three groups. Untreated and vehicle-treated mice all developed a paralytic ileus in response to i.p. acetic acid, gastrointestinal transit being lowered from 60 to 29% compared with mice receiving saline. Mice previously administered with either indomethacin, ketoprofen, piroxicam or ximoprofen orally, at doses as low as 0·25 mg kg^{-1} for indomethacin and ketoprofen, or 0·5 mg kg^{-1} for piroxicam and ximoprofen, did not show any significant inhibition of gastrointestinal transit in response to acetic acid (Fig. 1).

The myoelectrical activity of the rat small intestine was characterized during the fasted state by the appearance of migrating myoelectric complexes (MMCs) which propagated aborally (Fig. 2). The MMCs occurred approximately every 13 min on each recording site and were not significantly affected by the intraperitoneal injection of either saline, 0·5 mg kg^{-1} indomethacin or 0·5 mg kg^{-1} ximoprofen (Table 1). The injection of acetic acid disrupted the MMC pattern for

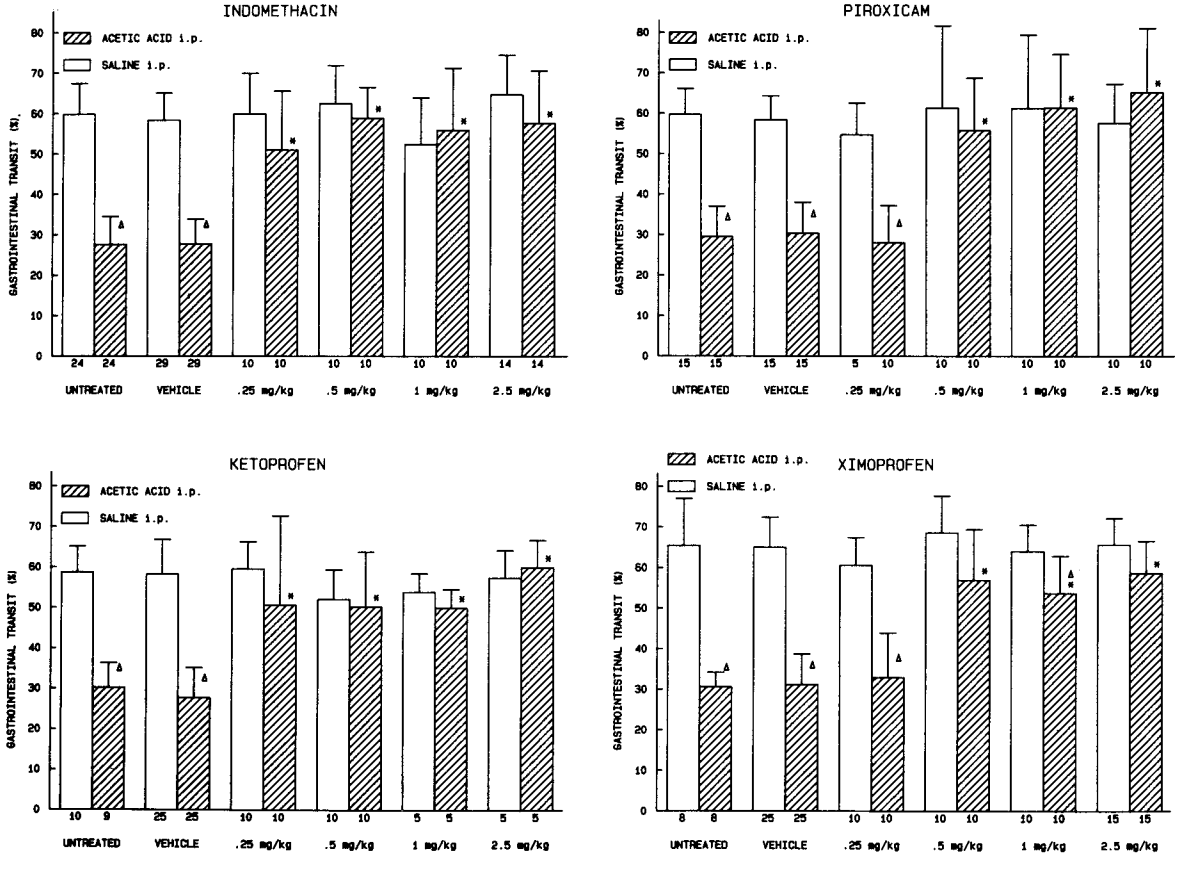

FIG. 1. Protective effects of indomethacin, ketoprofen, piroxicam and ximoprofen on the inhibition of gastrointestinal transit due to acetic acid in mice. Transit is expressed as the percentage of the length of the small intestine covered by the charcoal marker within 20 min following its oral administration. Values are given as mean ± s.d., n as indicated. $\Delta P < 0{\cdot}01$ acetic acid versus saline; * $P < 0{\cdot}01$ drug versus vehicle.

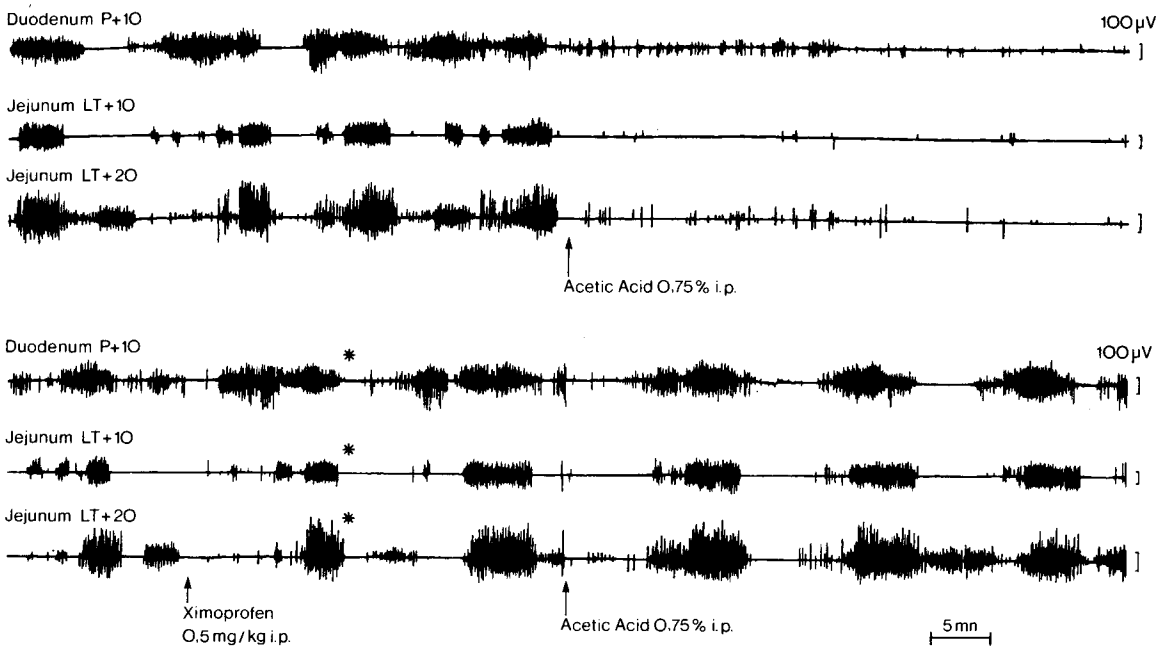

FIG. 2. Electrical activity of the duodenum at 10 cm from the pylorus (P), and the jejunum, at 10 and 20 cm from the ligament of Treitz (LT) in the fasted rat and its inhibition following the injection of acetic acid (upper panel). The pretreatment with ximoprofen was sufficient to relieve the effect of acetic acid (lower panel). (*) migrating myoelectric complex propagating along the small intestine.

Table 1. Effect of pretreatment with ximoprofen and indomethacin on chemically-induced inhibition of small intestinal MMC pattern in the fasted rat. Mean MMC intervals were calculated during the control state and the 30 min following NSAID treatment. The acetic acid or saline injections were performed after the end of an MMC, the interval between this MMC and the following was measured.

MMC interval (min)	Control	Saline	Acetic acid	Drug 0·5 mg kg^{-1}	Drug +acetic acid
Ximoprofen (n=12)					
Duodenum P+10	12·0±3·8	12·6±3·8	75·0±23·0 (*)	13·1±3·7	14·8±4·4 (†)
Jejunum LT+10	12·5±5·6	13·5±3·7	76·8±23·8 (*)	12·8±6·0	15·7±6·2 (†)
Jejunum LT+20	13·9±4·9	14·7±5·7	85·9±28·4 (*)	13·8±4·0	15·1±5·3 (†)
Indomethacin (n=7)					
Duodenum P+10	14·4±2·3	15·0±3·5	68·1±23·2 (*)	15·7±3·3	15·9±3·8 (†)
Jejunum LT+10	13·9±3·7	16·6±5·6	55·9±26·3 (*)	15·9±5·4	17·4±5·9 (†)
Jejunum LT+20	13·3±3·7	16·0±5·3	58·7±28·9 (*)	17·3±4·6	17·7±6·4 (†)

Values are given as mean±s.d., n as indicated. (*) $P<0·01$ acetic acid versus saline, (†) $P<0·01$ drug+acetic acid versus acetic acid alone.

approximately 1 h, an effect suppressed by indomethacin or ximoprofen pretreatment.

Postoperative ileus

The effect of laparotomy and exteriorization of the caecum on gastrointestinal transit of a test meal was examined in untreated, vehicle-treated and NSAIDs-treated rats. These experiments were performed under pentobarbitone sodium anaesthesia (30 mg kg^{-1} i.v.) which, as tested in untreated rats, by itself lowered gastrointestinal transit from 72·1%±15·0 (n=9) to 41·8%±8·9 (n=9) compared with conscious animals. Surgery caused a further decrease in gastrointestinal transit, from 46·5%±9·4 (n=36) to 26·5%±8·5 (n=38). This postoperative ileus was not observed after pretreatment with either indomethacin, ketoprofen or ximoprofen, at doses as low as 0·1 to 0·3 mg kg^{-1} i.p. (Fig. 3).

Discussion

These results are in accordance with the prevention of chemically-induced ileus and postoperative ileus by NSAIDs. Doses in the range of 0·25–0·5 mg kg^{-1}, given orally or intraperitoneally, were active in chemically-induced

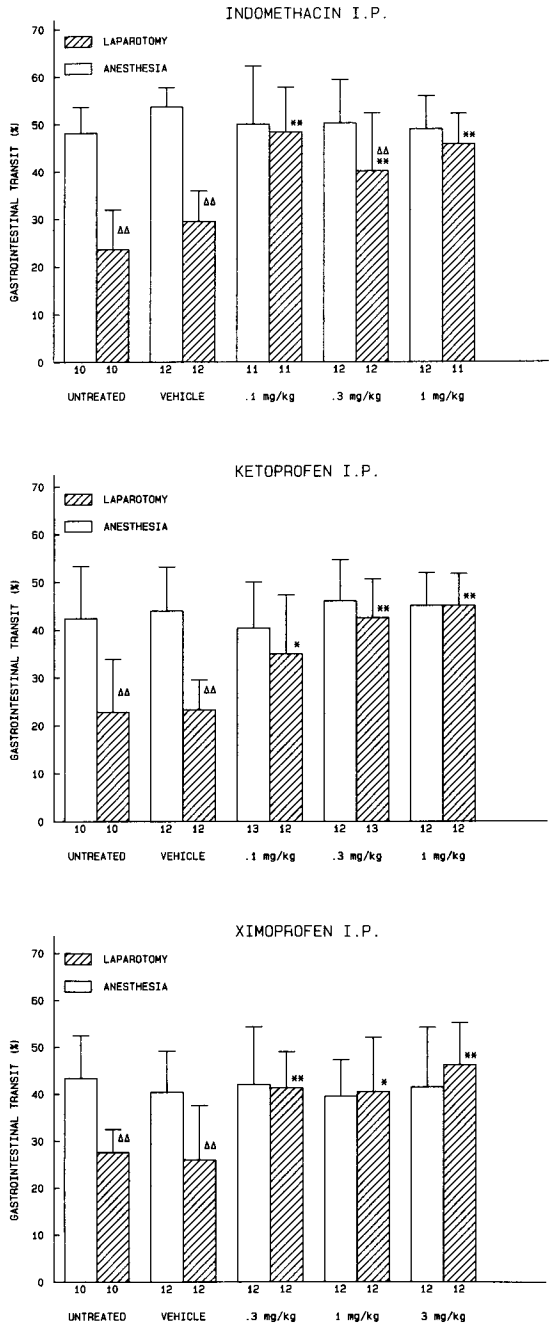

FIG. 3. Inhibition of the gastrointestinal transit in rats following a laparotomy with exteriorization of the caecum for 10 minutes and protective effects of indomethacin, ketoprofen and ximoprofen, given 15 min before anaesthesia. Values are given as mean ± s.d., n as indicated. $\Delta P < 0.05$ and $\Delta\Delta P < 0.01$ laparotomy versus anaesthesia, * $P < 0.05$ and ** $P < 0.01$ drug versus vehicle.

ileus, and still lower doses were effective in post-operative ileus (0·1 to 0·3 mg kg^{-1} i.p.).

Chemically-induced ileus is related to a model developed for the screening of analgesic drugs. In that test, i.p. acetic acid produces characteristic stretching movements which are abolished by analgesics, including NSAIDs (Koster et al 1959). Accordingly, it is tempting to speculate that NSAIDs block the occurrence of acetic acid-induced ileus via their analgesic properties. The mean anti-inflammatory ED50, as determined from the carrageenan oedema test in the rat, was 9, 33 and 4·5 mg kg^{-1} orally, for indomethacin, ketoprofen and piroxicam, respectively, whereas the mean analgesic ED50, as determined from the phenylbenzoquinone or acetic acid stretching test in mice, was 0·39, 1·5 and 1 mg kg^{-1} for the same drugs (Otterness & Gans 1987). Thus, the analgesic doses are far lower than the anti-inflammatory doses and in the same order as those we found to prevent ileus (0·25–0·5 mg kg^{-1} either in mice or rats). This supports the role of analgesia in the prevention of ileus by NSAIDs.

The anti-inflammatory and analgesic effects of NSAIDs are explained on the basis of their inhibitory effects on prostaglandin synthesis through the blockade of a cyclo-oxygenase (Vane 1971). Peritoneal irritation with acetic acid was shown to stimulate the local release of prostaglandins (Berkenkopf & Weichman 1988; Deraerdt et al 1980). Furthermore, arachidonic acid and prostaglandins E_1 and I_2 produce similar repeated stretching movements as those described with acetic acid (Collier & Schneider 1972; Doherty et al 1987; Helfer & Jaques 1968). Thus peritoneal irritation with acetic acid may locally stimulate the breakdown of arachidonic acid into prostaglandins, which in turn sensitize afferent fibres, leading to a reflex inhibition of gastrointestinal motility and transit. Accordingly, NSAIDs may prevent chemically-induced ileus by a mechanism related to their analgesic action, which may be related to their inhibitory effects on prostaglandin synthesis.

The pathogeneses of chemically-induced and postoperative ileus have similarities: (i) both are mediated through a nervous reflex, and (ii) the afferent pathways are constituted by capsaicin-sensitive fibres and the efferent limbs involve sympathetic noradrenergic neurons in both types of ileus (Holzer et al 1986). Thus NSAIDs may prevent the occurrence of postoperative as well as chemically-induced ileus via their analgesic and cyclo-oxygenase blocking actions. Further studies are needed to test this hypothesis.

Finally, both in-vitro and in-vivo studies demonstrated that prostaglandins act as local regulatory agents in the control of digestive motility (Sanders 1984; Thor et al 1985). Another mechanism of NSAIDs could thus be stimulation of the digestive motility through the blockade of a tonic inhibitory effect of endogenous prostaglandins. However, in our experimental conditions, NSAIDs did not stimulate control gastrointestinal motility and transit whereas post-operative ileus and ileus caused by peritoneal irritation with acetic acid were completely blocked. This is in agreement with the role of analgesia in the protective effects of NSAIDs.

Many substances have been tested in order to prevent postoperative ileus. These include α_1, α_2- and β- adrenoceptor antagonists (Glise & Hallerbäck 1983; Sagrada et al 1987), cholinergic agents (Ruwart et al 1979), drugs known to stimulate the release of acetylcholine by enteric neurons, such as metoclopramide and cisapride (Davidson et al 1979; Von Ritter et al 1987), prostaglandins $F_{2\alpha}$ (Fiedler 1980) as well as cholecystokinin (Frisell et al 1985). Most of these substances were ineffective or were weakly active only at doses that stimulate control gastrointestinal motility. Adrenergic antagonists, particularly α_2-antagonists, are the only drugs that seem to act specifically by counteracting the increased sympathetic outflow in response to nociceptive stimulation but their effects are weak (Holzer et al 1986).

Thus NSAIDs seem to be the first therapeutic agents that totally prevent chemically-induced and postoperative ileus in rodents at doses that do not stimulate digestive motility under control conditions.

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