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Pharmacological profile of parecoxib: a novel, potent injectable selective cyclooxygenase-2 inhibitor

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Abstract

The antinociceptive, anti-inflammatory, antipyretic effects along with gastric safety profile of parecoxib, a novel, potent selective cyclooxygenase-2 inhibiting prodrug, and those of ketorolac, a nonselective cyclooxygenase inhibitor, were evaluated in various animal models. Parecoxib (up to 20 mg/kg, i.v.) had no effect in two acute pain models, namely, the acetic acid-induced writhing (visceral pain) and the formalin test (tonic pain). However, ketorolac (up to 10 mg/kg, i.v.) showed marked antinociceptive effects in these models. In the models of carrageenan-provoked inflammatory hyperalgesia and inflammation, and in lipopolysaccharide-induced pyrexia, parecoxib significantly reversed all the behavioral changes and it was found to be more potent than ketorolac. Further, ketorolac (10 mg/kg, i.v.) produced visible gastric lesions with prominent petechiae and hemorrhagic streaks. However, parecoxib was without any effect on gastric mucosa. The present results showed that the cyclooxygenase-2 inhibitor, parecoxib, when administered parenterally, has potent antihyperalgesic, anti-inflammatory, antipyretic effects and has a better safety profile than with ketorolac, with sparing of cyclooxygenase-1 in the stomach in these animal models.

Keywords: Cyclooxygenase isoform; Nonsteroidal anti-inflammatory drug; Parecoxib; Ketorolac; Hyperalgesia

1. Introduction

Over the decades, nonsteroidal anti-inflammatory drugs (NSAIDs) have become the most commonly used analgesics in the management of acute and chronic pain. NSAIDs act by inhibiting prostaglandin synthesis, by inhibiting the cyclooxygenase enzyme. Two isoforms of cyclooxygenase have been identified, namely cyclooxygenase-1 and cyclooxygenase-2, where cyclooxygenase-1 is constitutively expressed in most cells for house-keeping functions and cyclooxygenase-2 is present in low levels under physiological conditions, but is rapidly induced by various stimuli (Garavito and DeWitt, 1999).

However, the use of NSAIDs is limited by ceiling effects and by adverse effects produced by inhibition of the cyclooxygenase-1 isoform (Allison et al., 1992; Murray and Brater, 1993). The discovery of cyclooxygenase-2 stimulated the search for agents that specifically inhibit cyclooxygenase-2, leading to the discovery and development of selective cyclooxygenase-2 inhibitors. Owing to the differential inhibitory activity on cyclooxygenase-1 and cyclooxygenase-2, the currently available NSAIDs are now classified as nonselective cyclooxygenase (e.g., ketorolac, diclofenac), preferential cyclooxygenase-2 (e.g., meloxicam, nimesulide), and selective cyclooxygenase-2 (e.g., celecoxib, rofecoxib) inhibitors (Kulkarni et al., 2000). Further, the selective cyclooxygenase-2 inhibitors showed similar or superior therapeutic efficacy in the modulation of pain and inflammation processes, while avoiding the severe side effects associated with nonselective cyclooxygenase inhibitors (Chan et al., 1995; Riendeau et al., 2001).

There are very few NSAIDs (e.g., diclofenac, ketorolac) that can be administered parenterally for the management of acute (post-surgical) and chronic (arthritic and cancer) pain. Further, their use is often associated with severe adverse effects, especially peptic ulcers, gastrointestinal haemorrhage, liver dysfunction, renal damage and inhibition of platelet function, possibly leading to increased post-operative bleeding (Allison et al., 1992; Murray and Brater, 1993; Strom et al., 1996). Thus, the critical clinical requirement in the recent past is the need

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for an injectable specific cyclooxygenase-2 inhibitor that possesses a superior safety profile over the existing parenteral nonsteroidal anti-inflammatory drugs. Moreover, the first-generation selective cyclooxygenase-2 inhibitors exhibit modest aqueous solubility, further restricting the dosing options. In order to over come solubility restrictions, Talley et al. (2000b) used a prodrug approach and designed parecoxib sodium, a highly water-soluble prodrug of a second-generation selective cyclooxygenase-2 inhibitor, valdecoxib, for parenteral administration. It is rapidly converted to valdecoxib, which has demonstrated potent analgesic and anti-inflammatory activities (Talley et al., 2000a).

Prostaglandins, synthesized in both the periphery and centrally, have long been thought to play key roles in inflammatory processes, sensitization of nociceptors and generation of pain, and to reflect the key target for cyclooxygenase inhibitors in providing analgesia (Garavito and DeWitt, 1999). Further, prostaglandins released following tissue injury and inflammation are involved in nociceptive processing and participated invariably in various animal models of pain and inflammation. The sensitivity of these models to drug treatments can differ as well, due to the variable inhibition of cyclooxygenase isoforms by nonsteroidal anti-inflammatory drugs. To date, there are no studies that examined the efficacy and safety of parenteral cyclooxygenase-2 inhibitors in comparison to that of classical nonsteroidal anti-inflammatory drugs. Thus, there are not only practical approaches to evaluate efficacy, but also mechanistic reasons to examine the possible superior efficacy of parecoxib over that of conventional NSAIDs after parenteral administration. In the present study, we compared the antinociceptive, anti-inflammatory, antipyretic activity and gastrointestinal tolerability of parecoxib sodium and ketorolac tromethamine, two parenteral nonsteroidal anti-inflammatory agents.

2. Materials and methods

2.1. Experimental animals

Albino Swiss mice (20–25 g) and Wistar rats (150–200 g) of either sex (bred in Central Animal House of Panacea Biotec, Punjab) were housed under standard conditions of light and dark cycle with food and water ad libitum. The protocol was approved by the Institutional Animal Ethics Committee and was carried out in accordance with the guidelines of the Indian National Science Academy. Each animal was used for a single treatment and each group consisted of five or six animals.

2.2. Behavioural assays for efficacy

All animals were acclimatized to the laboratory environment for at least 2 h before testing. Five or six animals were used at each of at least four doses, to determine a dose–response curve. In all assays, normal saline was used as vehicle. Dose–response curves were constructed to assess the analgesic, antihyperalgesic, anti-inflammatory and antipyretic activity of intravenously administered NSAIDs.

2.2.1. Acetic acid-induced writhing assay

This algesiometric assay was carried out as described in a previous study (Jain et al., 2001a). In brief, mice were injected intraperitoneally with 1 ml/100 g of 1% acetic acid in normal saline and the number of writhes was counted for 20 min, starting 3 min after the administration of the acetic acid solution. A writhe was defined as contraction of the abdominal muscles accompanied by elongation of the body and hind limbs. Parecoxib (1–20 mg/kg) and ketorolac (1–10 mg/kg) were administered intravenously 30 min before acetic acid challenge. Antinociceptive activity (reduction in writhes) is expressed as percent maximum possible effect, which was calculated with the following equation: percent maximum possible effect=[100 × (mean writhes in control group − mean writhes in drug(s)-treated group)]/mean of writhes in control group.

2.2.2. Formalin-induced tonic pain

The mouse paw formalin test was carried out as described by Murray et al. (1988). In brief, mice were injected with 20 μl of 5% formalin solution in normal saline subcutaneously into the plantar surface of the left paw with a 26-guage needle fitted to a microsyringe. Pain behavior was quantified by counting the time spent in licking and biting the injected paw for 5-min periods from 0 to 60 min. Two phases of spontaneous licking were observed after formalin injection. The interval from 0 to 10 min was defined as the early phase and the interval 10–60 min was defined as the late phase. Parecoxib (1–20 mg/kg) and ketorolac (1–10 mg/kg) were administered intravenously 30 min before formalin challenge. Antinociceptive activity (reduction in time spent for licking) is expressed as percent maximum possible effect, which was calculated with the following equation: percent maximum possible effect = 100 × [(sum of early phase or late phase counts in control group − sum of early phase or late phase counts with drug)/sum of early phase or late phase counts in control group].

2.2.3. Carrageenan-induced thermal and mechanical hyperalgesia

Hyperalgesia was induced by injecting 100 μl of a 1% solution of λ-carrageenan (Sigma, USA) in normal saline into the plantar surface of the left hind paw of two groups of rats. Carrageenan was administered immediately after intravenous administration of parecoxib (0.5–20 mg/kg) and ketorolac (1–10 mg/kg). In one group of animals, thermal hyperalgesia was measured using the

procedure described by Jain et al. (2001b). The mean paw withdrawal latency of the carrageenan-injected paw when dipped in water bath maintained at 47 ± 0.5 °C was measured. The baseline latency of paw withdrawal from thermal source was established three times, 5 min apart and averaged. A cut-off time of 15 s was imposed to avoid injury to the paw. The mean paw withdrawal latency (L_{4h}) 4 h after carrageenan administration in vehicle and drug-treated animals was measured and the change in the paw withdrawal latency ($L_{0h}-L_{4h}$) was calculated as a measure of hyperalgesia. Antihyperalgesic activity is expressed as percent inhibition of hyperalgesia and was calculated by taking the values in the control group as 0% inhibition.

The other group of animals was used to measure the nociceptive mechanical threshold, expressed in grams, using an Analgesymeter (Ugo Basile, Italy) as described by Randall and Selitto (1957). The test was performed by applying noxious pressure to the inflamed paw. By pressing a pedal that activated a motor, the force was increased at a constant rate in a linear fashion. When the animal displayed pain by withdrawal of the paw or vocalization, the pedal was immediately released, and the nociceptive pain threshold was read on the scale. A cut-off of 500 g was used to avoid potential tissue injury. The mean paw withdrawal threshold (T_{4h}) 4 h after carrageenan administration in vehicle and drug-treated animals was measured and the change in the paw withdrawal latency ($T_{0h}-T_{4h}$) was calculated as a measure of hyperalgesia. The antihyperalgesic activity is expressed as percent inhibition of hyperalgesia and was calculated by taking the values in the control group as 0% inhibition.

2.2.4. Carrageenan-induced paw oedema

In order to measure paw volume, animals were marked with a permanent marker at the ankle of their left hind paws to define the area of the paw to be monitored. Paw edema was induced by injecting 100 μl of a 1% solution of λ-carrageenan (Sigma, USA) in normal saline into the plantar surface of the left hind paw of the rats (Jain et al., 2001b). The carrageenan was administered immediately after intravenous administration of parecoxib (0.5–20 mg/kg) and ketorolac (1–10 mg/kg). The paw volumes were measured using a water displacement plethysmometer (Ugo Basile) at 1, 2, 3, 4 and 6 h after carrageenan administration, and the change in paw volume ($V_{4h}-V_{0h}$) 4 h after carrageenan administration in vehicle and drug-treated animals was calculated. The anti-inflammatory activity is expressed as percent inhibition of paw edema and was calculated by taking the values in the control group as 0% inhibition.

2.2.5. Endotoxin-induced pyrexia

Endotoxin-induced pyrexia in rats was studied as described by Chan et al. (1995). In brief, rats were fasted for 16–18 h before the day of experimentation and the resting rectal temperature was recorded using a probe connected to a telethermometer (Yellow Springs, OH, USA). At time zero, the rats were administered intraperitoneally either saline or *Salmonella typhosa* lipopolysaccharide (0.36 mg/kg), and the rectal temperature was measured at 5, 6 and 7 h after lipopolysaccharide injection. An equivalent volume of normal saline or drugs (1, 2 and 5 mg/kg, i.v.) was administered to lipopolysaccharide-injected rats after the rise in temperature had reached a plateau (5 h), and the increase in rectal temperature ($T_{7h}-T_{5h}$) in drug-treated animals was calculated. Antipyretic activity is expressed as percent reversal of the rise in rectal temperature, calculated by taking the values obtained at 7 h as 0% reversal.

2.3. Visible gastric lesions in rats

NSAID-induced gastric damage in rats was evaluated following the procedure described by Chan et al. (1995). In fasted (16–18 h) rats, parecoxib sodium (20 mg/kg), ketorolac tromethamine (10 mg/kg) or vehicle was administered intravenously. Four hours later, the rats were killed and the stomach was excised along its greater curvature, rinsed with normal saline, and the mucosa was examined for the presence of petechiae or frank hemorrhagic lesions. Petechiae were assigned a score of 1, and lesions were scored according to their length (a score of 5 for lesions with a length between 1 and 3 mm; a score of 10 for lesions greater than 3 mm). The sum of total scores was used for comparison.

2.4. Materials

Parecoxib sodium (Panacea Biotec, India) and ketorolac tromethamine (Ketanov® 15 mg/ml) for intravenous injection (Ranbaxy, India) were used. Parecoxib sodium was freshly prepared by dissolving it in normal saline. All the drugs were administered in a dose volume of 1 ml/100 g body weight of mice and 2 ml/kg body weight of rats at the times mentioned above. Carrageenan-λ (type IV) and *S. typhosa* lipopolysaccharide (Sigma) were dissolved in normal saline to a suitable concentration.

2.5. Statistical analysis

All the values are expressed as means ± S.E.M. ED_{50} values with 95% confidence intervals were calculated by standard linear regression analysis of log dose–response curves for all assays except for NSAIDs-induced visible gastric lesions. The ED_{50} was the dose estimated to produce 50% maximum possible effect in the acetic acid-induced writhing assay and the formalin test, 50% inhibition of carrageenan-induced hyperalgesia and inflammation or 50% reversal of lipopolysaccharide-induced pyrexia. ED_{50} values for anti-inflammatory activity were also calculated based on the area under the curve for each treatment by the trapezoidal rule. In this case, ED_{50} means the effective dose estimated to produce a 50% decrease in

area under the curve for vehicle-treated animals. The data were analyzed by one-way analysis of variance with Dunnett's test ($P<0.05$) for multiple comparisons. The mean of the sum of visible gastric lesion scores was analyzed by *t*-test between the two groups. $P<0.05$ was considered as statistically significant.

3. Results

The ED_{50} values with 95% confidence intervals are summarized in Table 1.

3.1. Acetic acid-induced writhing

Intraperitoneal administration of acetic acid resulted in the characteristic writhing response in control animals. Ketorolac (1–10 mg/kg, i.v.) produced a dose-dependent antinociceptive effect with an ED_{50} value of 2.58 mg/kg. In contrast, parecoxib (1–20 mg/kg, i.v.) did not show antinociceptive activity in this assay (Fig. 1A and B).

3.2. Formalin-induced tonic pain

The administration of formalin into the hind paw of mice induced a typical biphasic licking and biting response with an early and a late phase. Parecoxib (up to 20 mg/kg, i.v.) failed to have any effect on either phase of the formalin assay after intravenous administration. Ketorolac did not show protection against the early phase of the formalin response but it dose-dependently (1–10 mg/kg, i.v.) blocked the development of the late phase with an ED_{50} value of 4.84 mg/kg (Fig. 2A, B and C).

Table 1
Summary of ED_{50} values with 95% confidence intervals for parecoxib and ketorolac in various animal models of pain, inflammation and endotoxin-induced pyrexia

Animal model	ED_{50} (mg/kg, i.v.)	
	Parecoxib	Ketorolac
Acetic acid-induced writhing in mice	NA	2.58 (1.49–4.35)
Formalin-induced tonic pain in mice	NA	4.84 (3.71–6.4)
Carrageenan-induced thermal hyperalgesia in rats	2.45 (2.05–2.92)	3.69 (3.03–4.56)
Carrageenan-induced mechanical hyperalgesia in rats	1.97 (1.42–2.84)	3.27 (2.59–3.86)
Carrageenan-induced paw edema in rats	3.03[a] (2.41–3.80)	4.54[a] (3.91–5.56)
	3.09[b] (2.51–3.80)	4.94[b] (4.02–6.19)
Endotoxin-induced pyrexia in rats	0.91 (0.37–1.34)	1.69 (1.06–2.82)

NA, not achieved.
[a] ED_{50} value calculated 4 h after carrageenan injection.
[b] ED_{50} value calculated from area under curve.

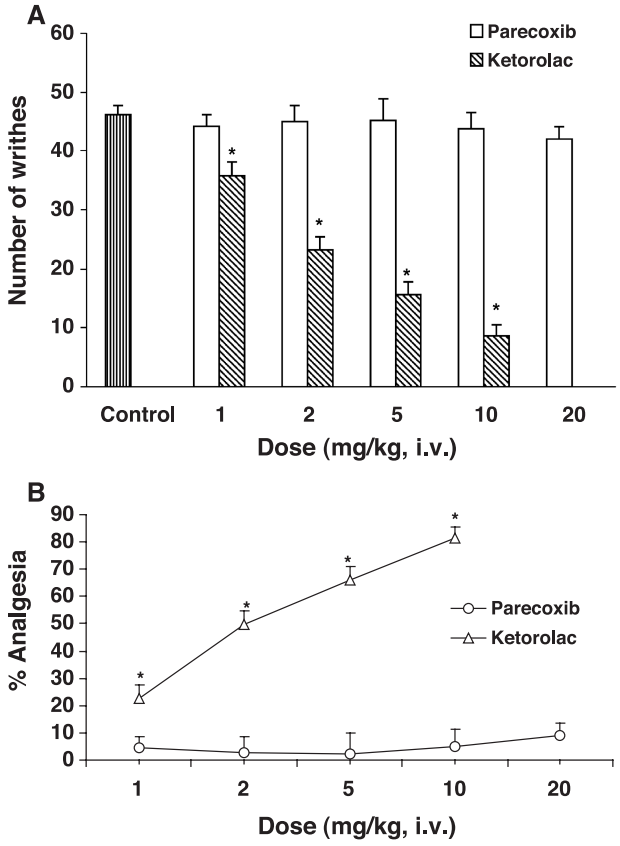

Fig. 1. (A) Dose-dependent inhibition of acetic acid-induced writhes by parecoxib (1–20 mg/kg) and ketorolac (1–10 mg/kg) in mice. Acetic acid (1%; 1 ml/100 g) solution was administered intraperitoneally 30 min after intravenous drug administration, and the writhes were recorded for 20 min after acetic acid administration. (B) Percent analgesic effect observed with various doses of parecoxib and ketorolac in writhing assay in mice. Values are means ± S.E.M. *$P<$ 0.05 vs. vehicle control.

3.3. Carrageenan-induced thermal and mechanical hyperalgesia

Carrageenan administration into the hind paw produced significant edema associated with hyperalgesia, as shown by a decreased paw withdrawal latency in response to a thermal stimulus and the paw withdrawal threshold in response to mechanical pressure 4 h after injection. Both parecoxib (0.5–20 mg/kg, i.v.) and ketorolac (1.0–10 mg/kg, i.v.) showed dose-dependent inhibition of carrageenan-induced thermal and mechanical hyperalgesia with almost 80% and 100% inhibition observed at 20 mg/kg of parecoxib, respectively (Fig. 3A and B). In both assays, parecoxib was approximately 1.5 times more potent than ketorolac (ED_{50} values of 2.45 and 3.69 mg/kg, i.v. in thermal hyperalgesia and ED_{50} values of 1.97 and 3.27 mg/kg, i.v. in mechanical hyperalgesia, respectively).

3.4. Carrageenan-induced paw edema

Subcutaneous administration of carrageenan into the hind paw produced significant edema, with a maximum

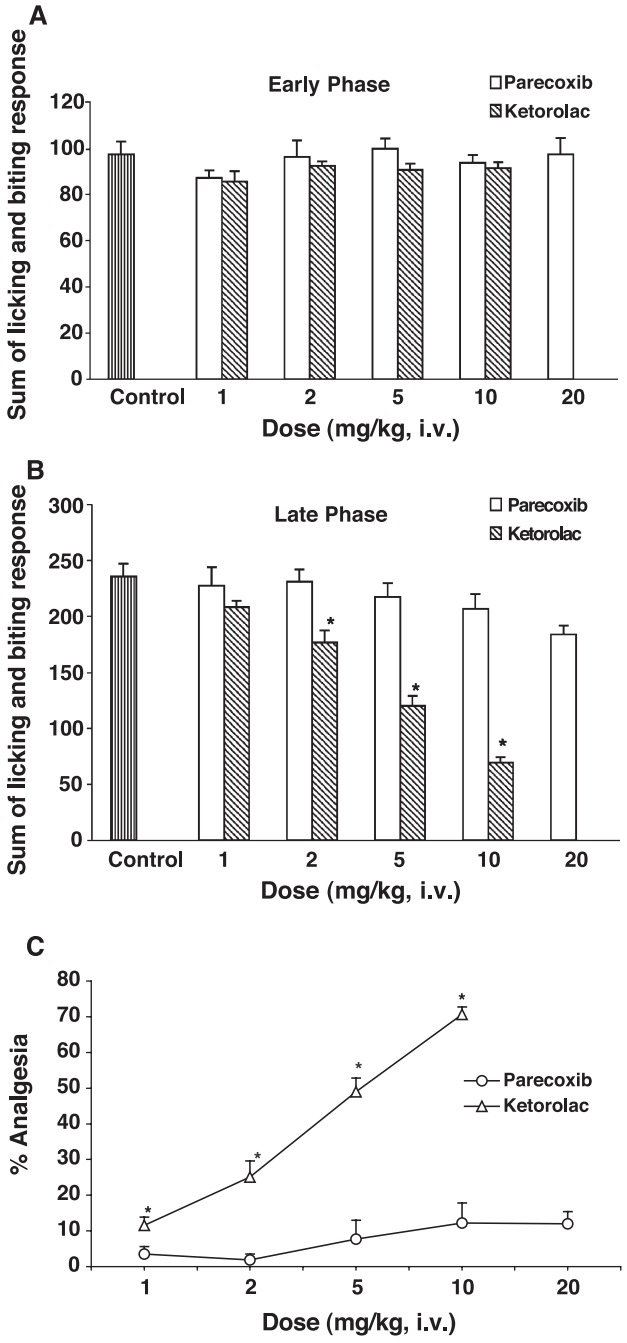

Fig. 2. Dose-dependent inhibition of the licking and biting response (indicated as nociceptive response) by parecoxib (1–20 mg/kg) and ketorolac (1–10 mg/kg) in (A) the early phase and (B) the late phase of the formalin test in mice. Formalin (5%; 20 μl/paw) was administered intraperitoneally 30 min after intravenous drug administration, and the nociceptive response was recorded for every 5-min periods from 0 to 60 min after formalin injection. (C) Percent analgesic effect observed with various doses of parecoxib and ketorolac against the formalin-induced licking and biting response in the late phase in mice. Values are means ± S.E.M. *$P<0.05$ vs. vehicle control.

response being observed 4 h after injection. Parecoxib, when administered intravenously immediately after carrageenan, inhibited paw edema in a dose-dependent

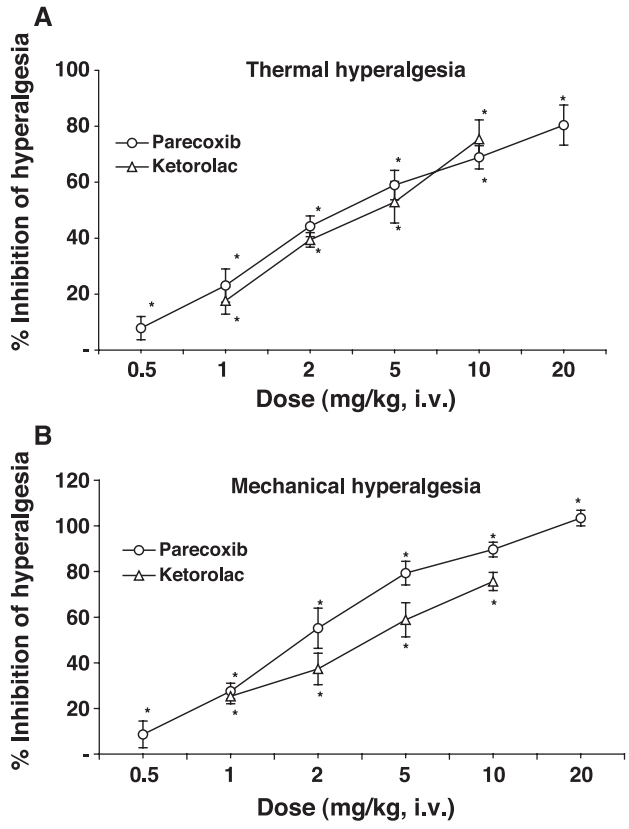

Fig. 3. Dose-dependent inhibitory effect of intravenously administered parecoxib (0.5–20 mg/kg) and ketorolac (1–10 mg/kg) against carrageenan-induced (A) thermal and (B) mechanical hyperalgesia in rats. Carrageenan (100 μg/paw) was injected subplantarly immediately after intravenous drug administration, and percent inhibition of hyperalgesia was calculated 4 h after carrageenan administration. Values are means ± S.E.M. *$P<0.05$ vs. vehicle control.

manner with an ED_{50} value of 3.03 mg/kg at 4 h. This anti-inflammatory activity was similar to that of ketorolac with an ED_{50} of 4.54 mg/kg (Fig. 4). In this

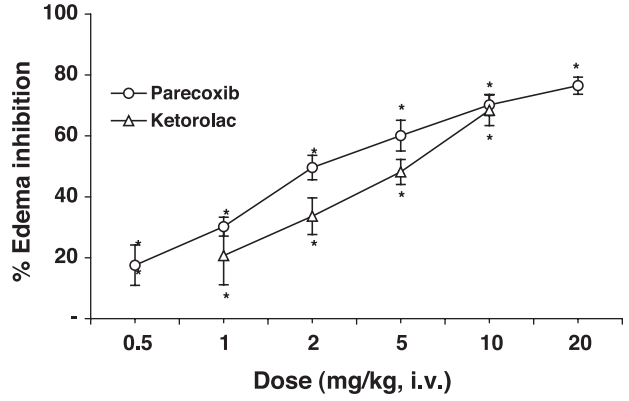

Fig. 4. Dose-dependent inhibitory effect of intravenously administered parecoxib (0.5–20 mg/kg) and ketorolac (1–10 mg/kg) against carrageenan-induced paw edema in rats. Carrageenan (100 μg/paw) was injected subplantarly immediately after intravenous drug administration and the percent inhibition of edema was calculated 4 h after carrageenan administration. Values are means ± S.E.M. *$P<0.05$ vs. vehicle control.

assay, parecoxib was 1.5 times more potent than ketorolac.

3.5. Endotoxin-induced pyresis

Lipopolysaccharide induced hyperthermia (2.18 ± 0.12 °C increase in rectal temperature) 7 h post-injection as compared with the effect of saline. The administration of parecoxib or ketorolac (1, 2 and 5 mg/kg, i.v.) at the plateau of temperature elevation (5 h) reversed the lipopolysaccharide-induced pyresis in a dose-dependent manner with more than 85% reversal observed at 5 mg/kg of parecoxib (ED_{50} = 0.91 mg/kg, Fig. 5A and B). Parecoxib was about twice as potent as ketorolac (ED_{50} = 1.69 mg/kg) in this assay.

3.6. Visible gastric lesions in rats

Intravenous administration of 10 mg/kg of ketorolac produced marked, visible, hemorrhagic gastric lesions with more petechiae 4 h after its administration. In contrast, parecoxib was without any effect at the highest tested dose (20 mg/kg. i.v.) (Fig. 6).

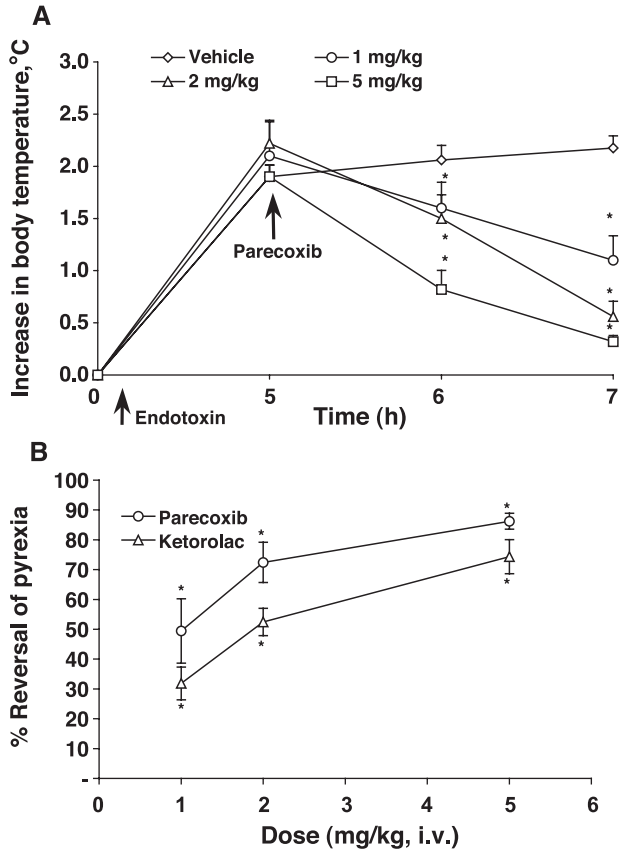

Fig. 5. (A) Dose-dependent inhibitory effect of parecoxib (1–10 mg/kg) against lipopolysaccharide-induced pyrexia in rats. Lipopolysaccharide (0.36 mg/kg) was injected intraperitoneally at time 0. (B) Percent antipyretic effect observed with various doses of parecoxib and ketorolac against lipopolysaccharide-induced pyrexia in rats. Parecoxib and ketorolac at the dose indicated were administered intravenously 5 h after injection of lipopolysaccharide. Values are means ± S.E.M. *P < 0.05 vs. vehicle control.

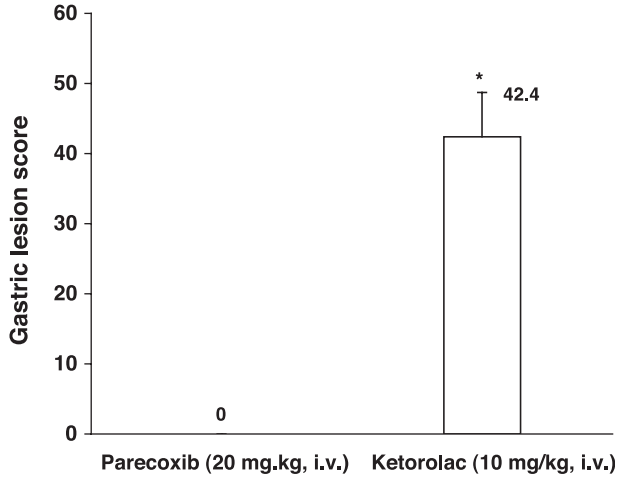

Fig. 6. Nonsteroidal anti-inflammatory drug-induced gastric lesions in rats. Parecoxib (20 mg/kg) and ketorolac (10 mg/kg) were administered intravenously 4 h before rats were killed. Visible gastric lesions were scored and the sum score was determined. Values are means ± S.E.M. *P < 0.05 vs. parecoxib group.

4. Discussion

In the present study, we systematically investigated selective cyclooxygenase-2 inhibitors for parenteral administration. We compared the analgesic, anti-inflammatory, antipyretic effects and gastric tolerability of two cyclooxygenase inhibitors following intravenous administration. It has been previously reported that systemic administration of NSAIDs in animals can markedly attenuate pain and related behaviors (Chan et al., 1995; Jett et al., 1999; Riendeau et al., 2001). We have extended this observation to show that parecoxib, a prodrug of valdecoxib, which is a second-generation selective cyclooxygenase-2 inhibitor, can also attenuate the pain and associated behaviors.

Intravenous administration of ketorolac resulted in dose-dependent antinociception against acetic acid-induced writhing and formalin-induced licking and biting. However, parecoxib lacked analgesic efficacy in these tests. Our present results are consistent with previous reports in which systemically as well as spinally administered non-selective cyclooxygenase inhibitors were effective, whereas selective cyclooxygenase-2 inhibitors failed to alter nociceptive behavior in these tests (Malmberg and Yaksh, 1992; Dirig et al., 1997; Jain et al., 2001a). Further, cyclooxygenase-2 mRNA and protein in spinal cord increased 3–6 h after injection of carrageenan in the hind paw (Beiche et al., 1996), but acetic acid-induced writhing for 20 min and formalin-induced licking and biting for 60 min would not be sufficient to activate cyclooxygenase-2 mRNA and the generation of cyclooxygenase-2. Recently,

it has been reported that the number of writhing responses induced by acetic acid in cyclooxygenase-1 knockout mice, but not in cyclooxygenase-2 knockout mice, was less than that in wild-type mice emphasizing the role of prostaglandins derived from cyclooxygenase-1 rather than from cyclooxygenase-2 in acetic acid-induced writhing (Ballou et al., 2000). These results imply that prostaglandins derived from the cyclooxygenase-1 pathway but not from the cyclooxygenase-2 pathway play a role, whereby cyclooxygenase-1 inhibition is involved in decreasing nociceptive inputs. This supports the lack of efficacy of parecoxib in these tests.

Further, prostaglandins play an important role in promoting the signs and symptoms of inflammation (Vane, 1971) and they sensitize terminal afferent C fibers in the periphery and enhance the response of C fibers to algesic stimuli resulting in hyperalgesia (Martin et al., 1987; Cohen and Perl, 1990). One of the defining features of inflammatory pain is a pronounced hypersensitivity to noxious mechanical and thermal stimulation of the skin. Thus, carrageenan-induced paw edema is the most commonly used test for studying anti-inflammatory activity and hyperalgesia in animals (Dirig et al., 1998; Jain et al., 2001b). In the present study, prophylactic administration of parecoxib and ketorolac resulted in the dose-dependent inhibition of inflammation and hyperalgesia during the experimental period. Consistent with previous studies where maximum inflammation and hyperalgesia developed 3–4 h after carrageenan administration (Chan et al., 1995; Jain et al., 2001b; Jett et al., 1999), the peak anti-inflammatory and antihyperalgesic effect was observed 4 h after carrageenan administration. Further, approximately 70% inhibition of inflammation was observed with 10 mg/kg of both the drugs. In contrast to the similar inhibition of inflammation, 10 mg/kg of parecoxib caused almost 70% and 90% inhibition of thermal and mechanical hyperalgesia, respectively, whereas the same dose of ketorolac caused only 75% inhibition in both the tests.

The difference in the efficacy and potency of these drugs in these nociceptive models could be due to participation of prostaglandins generated by both cyclooxygenase isoforms. Although cyclooxygenase-2 expression increased after carrageenan injection, prostaglandins generated by both cyclooxygenase isoforms participate equally in the mediation of edema and hyperalgesia (Dirig et al., 1998; Martinez et al., 2002). This agrees with previous reports in which nonselective cyclooxygenase, selective cyclooxygenase-1 and cyclooxygenase-2 inhibitors showed antihyperalgesic effects with variable potency (Chan et al., 1995; Martinez et al., 2001; Riendeau et al., 2001). Thus, the results demonstrated that parecoxib had marked and comparatively similar anti-inflammatory and more potent antihyperalgesic activity than ketorolac.

Lipopolysaccharide injected into animals causes severe hyperthermia, hyperalgesia and loss of appetite due to the enhanced formation of cytokines, such as interleukin-1β, interleukin-6, interferon α and β, and tumor necrosis factor-α (Wachulec et al., 1997). These cytokines increase the synthesis of prostaglandin E_2 in the circumventricular organs and near to the preoptic hypothalamic area (Oka et al., 1997). Further, cyclooxygenase-1 is constitutively present in vagal afferents, and cyclooxygenase-2 expression is induced in brain endothelial cells following lipopolysaccharide challenge, which results in increased levels of prostaglandin E_2 in cerebrospinal fluid (Matsumura et al., 2000). Thus, the enhanced release of prostaglandin E_2 is involved in immune-brain signalling by increasing cyclic AMP levels, which trigger hypothalamic area to elevate body temperature. It is well known that NSAIDs suppress hyperthermia by inhibiting the synthesis of prostaglandin E_2. In the present study, both the drugs dose-dependently attenuated the lipopolysaccharide-induced increase in body temperature, with parecoxib being more potent than ketorolac. Unlike nonselective cyclooxygenase inhibitors, which are highly polar and cross the blood–brain barrier with difficulty, selective cyclooxygenase-2 inhibitors are less polar and readily cross the blood–brain barrier, and central effects have been observed after systemic administration in animals and humans (Chan et al., 1995; Riendeau et al., 2001; Schwarz et al., 1999). A similar explanation may account for the relatively better efficacy of parecoxib than ketorolac against lipopolysaccharide-induced pyrexia in the current study.

Despite the increasing use of nonselective cyclooxygenase inhibitors in pain and inflammatory conditions, their immense therapeutic potential is severely hampered by associated adverse effects, paramount among which is gastropathy manifested as peptic ulcers, gastrointestinal hemorrhage and alterations in gut motility (Allison et al., 1992; Jain et al., 2002). This is mainly caused by the inhibition of cyclooxygenase-1 in the gastrointestinal tract, thereby inhibiting the release of useful prostaglandins that regulate gastric mucosal secretion. In view of the absence of cyclooxygenase-2 in the stomach together with the cyclooxygenase-1-sparing effect and marked antinociceptive effect of selective cyclooxygenase-2 inhibitors (O'Neill and Ford-Hutchinson, 1993), it is likely that their use would result in a beneficial and better safety profile. In the current study, parecoxib was without any effect on gastrointestinal mucosa, whereas ketorolac produced significant gastric damage. This is advantageous because incremental doses of parenteral NSAIDs administered over an extended period of time are needed in human patients to achieve a maximum analgesic effect. Therefore, the use of selective cyclooxygenase-2 inhibitors seems to be appropriate because these do not alter the normal physiological functions of cyclooxygenase-1-derived prostaglandins in the stomach, blood and kidney.

In summary, the present study has shown that parecoxib is effective at relieving inflammatory pain, inflammation and endotoxin-induced pyrexia. Overall, the pharmacological effects of ketorolac and parecoxib in animal models were broadly similar, although parecoxib was markedly more potent and efficacious in all the models investigated. The emerging role of cyclooxygenase-2 in various diseases

and the present results further support the potential of parenteral selective cyclooxygenase-2 inhibitors with a better safety profile as future therapeutics.

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