CHAPTER 1:

PHARMACOLOGICAL PROFILE OF PARECOXIB: A NOVEL, POTENT INJECTABLE SELECTIVE CYCLOOXYGENASE-2 INHIBITOR
1.1. INTRODUCTION

Over the decades, NSAIDs have become the most commonly used analgesics in the management of acute and chronic pain. NSAIDs act by inhibiting the COX enzyme thereby preventing PG synthesis. Two isoforms of COX have been identified, namely COX-1 and COX-2, where COX-1 is constitutively expressed in most cells and COX-2 is rapidly induced by various stimuli (Garavito and DeWitt, 1999). Recently, third isoform COX-3 has been identified in dogs, however, its existence in humans is in question (Chandrasekharan et al., 2002; Schwab et al., 2003).

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, the use of NSAIDs is limited by ceiling effects and often associated with severe adverse effects, especially peptic ulcers, gastrointestinal hemorrhage, 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). It is well reported that these adverse effects are produced by inhibition of the COX-1 isoform (Allison et al., 1992; Murray and Brater, 1993). The discovery of COX-2 stimulated the search for agents that specifically inhibit COX-2, leading to the discovery and development of selective COX-2 inhibitors. Further, the selective COX-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 COX inhibitors (Chan et al., 1995; Riendeau et al., 2001).

Thus, the critical clinical requirement in the recent past is the need for an injectable specific COX-2 inhibitor that possesses a superior safety profile over the existing parenteral NSAIDs. Moreover, the first-generation selective COX-2 inhibitors exhibited modest aqueous solubility, further restricting the dosing options. In order to overcome solubility restrictions, Talley et al. (2000b) used a prodrug approach and designed parecoxib sodium, a highly water-soluble prodrug of a second-generation selective COX-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; Gierse et al., 2005).

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 COX inhibitors in providing
analgesia (Garavito and DeWitt, 1999). Further, PGs 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 COX isoforms by NSAIDs. To date, there are no studies that examined the efficacy and safety of parenteral COX-2 inhibitors in comparison to that of classical NSAIDs. Thus there is a need are to evaluate efficacy of parecoxib over the conventional NSAIDs after its parenteral administration.

In the present study, the antinociceptive, anti-inflammatory, antipyretic activities and gastrointestinal tolerability of parecoxib sodium were compared with that of ketorolac tromethamine, a parenteral NSAID already available.

1.2. MATERIALS AND METHODS

1.2.1. Experimental animals

Albino Swiss mice (20 – 25 g) and Wistar rats (150 – 200 g) of either sex (bred in Central Animal House of Panacea Biotech Ltd., Punjab) were housed under standard conditions of light and dark cycle with food and water ad libitum. Animals were acclimatized to laboratory conditions before the test. All experiments were carried between 900 and 1500 h. The experimental protocols were approved by the Institutional Animal Ethics Committee and were carried out in accordance with the guidelines of the Indian National Science Academy for the use and care of experimental animals. Each animal was used for a single treatment and each group consisted of five or six animals, respectively.

1.2.2. Behavioral 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 activities of intravenously administered NSAIDs.

1.2.2.1. 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 (i.p.) 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 writhes was defined as contraction of the abdominal muscles accompanied by elongation of the
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body and hind limbs. Antinociceptive activity (reduction in writhes) is expressed as percent maximum possible effect, which was calculated with the following equation:

percent maximum possible effect = \[100 \times \left(\frac{\text{mean writhes in control group} - \text{mean writhes in drug(s)-treated group}}{\text{mean of writhes in control group}}\right)\]

1.2.2.2. 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 – 60 min. Two phases of spontaneous licking were observed after formalin injection. The interval from 0 – 10 min was defined as the early phase and the interval 10 – 60 min was defined as the late phase. 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 x \([\text{sum of early phase or late phase counts in control group} - \text{sum of early phase or late phase counts with drug}] / \text{sum of early phase or late phase counts in control group}\]

1.2.2.3a. Carrageenan-induced thermal 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. 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 sec was imposed to avoid injury to the paw. The mean paw withdrawal latency (L_{ab}) 4 h after carrageenan administration in vehicle and drug-treated animals was measured and the change in the paw withdrawal latency (L_{0h} – L_{ab}) 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.
1.2.2.3b. Carrageenan-induced mechanical hyperalgesia

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.

1.2.2.4. Carrageenan-induced paw edema

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 \( \lambda \)-carrageenan in normal saline into the plantar surface of the left hind paw of the rats (Jain et al., 2001b). The paw volumes were measured using a water displacement plethysmometer (Ugo Basile, Italy) 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. The % increase in edema at each time point for both the drugs was also calculated in comparison to vehicle treated control group. The area under curve for each dose of the drug was calculated using trapezoidal rule and ED\(_{50}\) was also calculated by taking the area under curve values in the control group as 0% inhibition.

1.2.2.5. 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
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intraperitoneally either saline or *Salmonella typhimurium* lipopolysaccharide (LPS) (0.36 mg/kg), and the rectal temperature was measured at 5, 6, and 7 h after LPS injection. The increase in rectal temperature ($T_{7h} - T_{5h}$) in LPS- and 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.

1.2.3. 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 lesions i.e. petechiae (erosion) or frank hemorrhagic lesions (ulcers). 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.

1.2.4. Drugs and treatment schedule

Parecoxib sodium (Panacea Biotec Ltd., India) and ketorolac tromethamine (Ketanov® 15 mg/ml) for intravenous injection (Ranbaxy Ltd., India), acetic acid (SD Fine Chemicals, India) and formalin (37% formaldehyde) (SD Fine Chemicals, India) were used in this study. 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 *Salmonella typhimurium* LPS (Sigma, USA) were dissolved in normal saline to a suitable concentration.

Parecoxib (1, 2, 5, 10 or 20 mg/kg) and ketorolac (1, 2, 5 or 10 mg/kg) were administered intravenously 30 min before acetic acid or formalin challenge. In the case of carrageenan-induced edema and hyperalgesia, carrageenan was administered immediately after intravenous administration of parecoxib (0.5, 1, 2, 5, 10 or 20 mg/kg) and ketorolac (1, 2, 5 or 10 mg/kg). In order to evaluate antipyretic effect of drugs, an equivalent volume of normal saline or drugs (1, 2 or 5 mg/kg, i.v.) was administered to LPS-injected rats after the rise in temperature had reached a plateau (5 h).
1.2.5. Statistical analysis
All the values are expressed as mean ± S.E.M. ED50 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 ED50 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 LPS-induced pyrexia. ED50 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, ED50 mean 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.

1.3. RESULTS
The ED50 values with 95% confidence intervals for all the assays of behavioral efficacy are summarized in table 1.1.

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 ED50 value of 2.58 mg/kg. In contrast, parecoxib (1 – 20 mg/kg, i.v.) did not show antinociceptive activity in this assay (Fig. 1.1A and 1.1B).

1.3.2. 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 ED50 value of 4.84 mg/kg (Fig. 1.2A, 1.2B, and 1.3).

1.3.3. Carrageenan-induced thermal and mechanical hyperalgesia
Carrageenan administration into the hind paw produced significant edema associated with hyperalgesia, as shown by decreased paw withdrawal latency in response to a
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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. 1.4 & 1.5). In both assays, parecoxib was approximately 1.5 times more potent than ketorolac (ED50 values of 2.45 and 3.69 mg/kg, i.v. in thermal hyperalgesia and ED50 values of 1.97 and 3.27 mg/kg, i.v. in mechanical hyperalgesia, respectively).

1.3.4. Carrageenan-induced paw edema

Subcutaneous administration of carrageenan into the hind paw produced significant edema, with a maximum response being observed 4 h after injection. Parecoxib (0.5 – 20 mg/kg), when administered intravenously immediately after carrageenan, inhibited paw edema in a dose-dependent manner with an ED50 value of 3.03 mg/kg at 4 h (Fig. 1.6). This anti-inflammatory activity was similar to that of ketorolac (1.0 – 10 mg/kg, i.v.) with an ED50 of 4.54 mg/kg (Fig. 1.7 and 1.8). The % inhibition of area under curve as a measure of anti-inflammatory activity was also resulted in ED50 values of 3.09 and 4.94 mg/kg for parecoxib and ketorolac, respectively (Fig. 1.9). In this assay, parecoxib was 1.5 times more potent than ketorolac from both the calculations.

1.3.5. Endotoxin-induced pyresis

LPS induced hyperthermia (2.18 ± 0.12 °C increase in rectal temperature) 7 h post-injection as compared with the effect of saline (Fig. 1.10). The administration of parecoxib or ketorolac (1, 2, and 5 mg/kg, i.v.) at the plateau of temperature elevation (5 h) reversed the LPS-induced pyresis in a dose-dependent manner with more than 85% reversal observed at 5 mg/kg of parecoxib (ED50 = 0.91 mg/kg, Fig. 1.10 and 1.11). Parecoxib was about twice as potent as ketorolac (ED50 = 1.69 mg/kg) in this assay.

1.3.6. 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 did not produce any gastric lesions even at the highest tested dose (20 mg/kg, i.v.) (Fig. 1.12).
Fig. 1.1. (A) Dose-dependent inhibition of acetic acid-induced writhes by parecoxib (1 – 20 mg/kg) or ketorolac (1 – 10 mg/kg) in mice. Acetic acid (1%; 1 ml/100g) 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 mean ± S.E.M. *P < 0.05 vs vehicle control.
Fig. 1.2. Dose-dependent inhibition of the licking and biting response (indicated as nociceptive response) by parecoxib (1 – 20 mg/kg) or 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 per paw) was administered intraperitoneally 30 min after intravenous drug administration, and the nociceptive response was recorded for every 5 min periods from 0 – 60 min after formalin injection. Values are mean ± S.E.M. *P < 0.05 vs vehicle control.
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Thermal hyperalgesia

Fig. 1.3. Percent analgesic effect observed with various doses of parecoxib (1 – 20 mg/kg, i.v.) or ketorolac (1 – 10 mg/kg, i.v.) against the formalin-induced licking and biting response in the late phase in mice. Values are mean ± S.E.M. *P < 0.05 vs vehicle control.

Fig. 1.4. Dose-dependent inhibitory effect of intravenously administered parecoxib (0.5 – 20 mg/kg) or ketorolac (1 – 10 mg/kg) against carrageenan-induced thermal hyperalgesia in rats. Carrageenan (100 µg per paw) was injected subplantarly immediately after intravenous drug administration, and percent inhibition of hyperalgesia was calculated 4 h after carrageenan administration. Values are mean ± S.E.M. *P < 0.05 vs vehicle control.
Fig. 1.5. Dose-dependent inhibitory effect of intravenously administered parecoxib (0.5 – 20 mg/kg) or ketorolac (1 – 10 mg/kg) against carrageenan-induced mechanical hyperalgesia in rats. Values are mean ± S.E.M. *P < 0.05 vs vehicle control.

Fig. 1.6. Dose-dependent inhibitory effect of intravenously administered parecoxib (0.5 – 20 mg/kg) against carrageenan-induced paw edema in rats. Carrageenan (100 µg per paw) was injected subplantarily immediately after intravenous drug administration. Values are mean ± S.E.M.
Fig. 1.7. Dose-dependent inhibitory effect of intravenously administered ketorolac (1 – 10 mg/kg) against carrageenan-induced paw edema in rats. Carrageenan (100 μg per paw) was injected subplantarily immediately after intravenous drug administration. Values are mean ± S.E.M.

Fig. 1.8. Dose-dependent inhibitory effect of intravenously administered parecoxib (0.5 – 20 mg/kg) or ketorolac (1 – 10 mg/kg) against carrageenan-induced paw edema in rats. The percent inhibition of edema was calculated 4 h after carrageenan administration by taking the values in the control group as 0% inhibition. Values are mean ± S.E.M. *P < 0.05 vs vehicle control.
Fig. 1.9. Dose-dependent inhibitory effect of intravenously administered parecoxib (0.5 – 20 mg/kg) or ketorolac (1 – 10 mg/kg) on area under curve of time-course of carrageenan-induced paw edema in rats. The percent inhibition of area under curve as a measure of anti-inflammatory activity was calculated by taking the values in the control group as 0% inhibition. Values are mean ± S.E.M. *P < 0.05 vs vehicle control.

Fig. 1.10. Dose-dependent inhibitory effect of parecoxib (1 – 10 mg/kg) against lipopolysaccharide (LPS)-induced pyrexia in rats. LPS (0.36 mg/kg) was injected intraperitoneally at time 0 h. Parecoxib or ketorolac at the dose indicated were administered intravenously 5 h after injection of LPS. Values are mean ± S.E.M. *P < 0.05 vs vehicle control.
Fig. 1.11. Dose-dependent reversal of increase in body temperature caused by lipopolysaccharide (LPS) by intravenously administered parecoxib (0.5 - 20 mg/kg) or ketorolac (1 - 10 mg/kg) in rats. The percent reversal of the rise in rectal temperature calculated by taking the values obtained at 7 h as 0% reversal. Values are mean ± S.E.M. *P < 0.05 vs vehicle control.

Fig. 1.12. Non-steroidal anti-inflammatory drug-induced gastric lesions in rats. Parecoxib (20 mg/kg) or ketorolac (10 mg/kg) were administered intravenously 4 h before rats were sacrificed. Visible gastric lesions were scored and the sum score was determined. Values are mean ± S.E.M. *P < 0.05 vs parecoxib group.
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

<table>
<thead>
<tr>
<th>Animal model</th>
<th>ED$_{50}$ (mg/kg, i.v.)</th>
<th>Parecoxib</th>
<th>Ketorolac</th>
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<tr>
<td></td>
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<td></td>
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<tr>
<td>Acetic acid-induced writhing in mice</td>
<td>-</td>
<td>2.58</td>
<td>4.84</td>
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<tr>
<td></td>
<td></td>
<td>(1.49 - 4.35)</td>
<td>(3.71 - 6.4)</td>
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<tr>
<td>Formalin-induced tonic pain in mice</td>
<td>NA</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>4.84</td>
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<td>(3.71 - 6.4)</td>
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<td>Carrageenan-induced thermal hyperalgesia in rats</td>
<td>2.45</td>
<td>3.69</td>
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<td></td>
<td>(2.05 - 2.92)</td>
<td>(3.03 - 4.56)</td>
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<tr>
<td>Carrageenan-induced mechanical hyperalgesia in rats</td>
<td>1.97</td>
<td>3.27</td>
<td></td>
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<tr>
<td></td>
<td>(1.42 - 2.84)</td>
<td>(2.59 - 3.86)</td>
<td></td>
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<tr>
<td>Carrageenan-induced paw edema in rats</td>
<td>3.03 $^a$ (2.41 - 3.80)</td>
<td>4.54 $^a$ (3.91 - 5.56)</td>
<td></td>
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<tr>
<td></td>
<td>3.09 $^b$ (2.51 - 3.80)</td>
<td>4.94 $^b$ (4.02 - 6.19)</td>
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<tr>
<td>Endotoxin-induced pyrexia in rats</td>
<td>0.91</td>
<td>1.69</td>
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<td></td>
<td>(0.37 - 1.34)</td>
<td>(1.06 - 2.82)</td>
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NA, Not achieved; $^a$, ED$_{50}$ value calculated 4 h after carrageenan injection; $^b$, ED$_{50}$ value calculated from area under curve

1.4. DISCUSSION
The present study systematically investigated the antinociceptive and anti-inflammatory effects of selective COX-2 inhibitor on parenteral administration. The analgesic, anti-inflammatory, antipyretic effects and gastric tolerability of two COX inhibitors following intravenous administration were compared. 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 COX-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 nonselective COX inhibitors were effective, whereas selective COX-2 inhibitors failed to alter nociceptive behavior in these tests (Malmberg et al., 1992; Dirig et al., 1997; Jain et al., 2001a). Further, COX-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 COX-2 mRNA and the generation of COX-2. Recently, it has been reported that the number of writhing responses induced by acetic acid in COX-1 knockout mice, but not in COX-2 knockout mice, was less than that in wild-type mice emphasizing the role of PGs derived from COX-1 rather than from COX-2 in acetic acid-induced writhing (Ballou et al., 2000). These results imply that PGs derived from the COX-1 pathway but not from the COX-2 pathway play a role, whereby COX-1 inhibition is involved in decreasing nociceptive inputs. This supports the lack of efficacy of parecoxib in these tests.

Further, PGs 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; Jett et al., 1999; Jain et al., 2001b), the peak anti-inflammatory and anti-hyperalgesic 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 PGs generated by both COX isoforms. Although COX-2 expression increased after carrageenan injection, PGs generated by both COX 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 COX, selective COX-1, and COX-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.

LPS when injected into animals causes severe hyperthermia, hyperalgesia, and loss of appetite due to the enhanced formation of cytokines, such as IL-1β, IL-6, interferon (INF) -α and-β, and TNF-α (Wachulec et al., 1997). These cytokines increase the synthesis of PGE2 in the circumventricular organs and near to the preoptic hypothalamic area (Oka et al., 1997). Further, COX-1 is constitutively present in vagal afferents, and COX-2 expression is induced in brain endothelial cells following LPS challenge, which results in increased levels of PGE2 in cerebrospinal fluid (Matsumura et al., 2000). Thus, the enhanced release of PGE2 is involved in immune-brain signaling 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 PGE2. In the present study, both the drugs dose dependently attenuated the LPS-induced increase in body temperature, with parecoxib being more potent than ketorolac. Unlike non-selective COX inhibitors, which are highly polar and cross the blood-brain barrier with difficulty, selective COX-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; Schwartz et al., 1999; Riendeau et al., 2001). A similar explanation may account for the relatively better efficacy of parecoxib than ketorolac against LPS-induced pyrexia in the current study.
Despite the increasing use of nonselective COX 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 COX-1 in the gastrointestinal tract, thereby inhibiting the release of useful PGs that regulate gastric mucosal secretion. In view of the absence of COX-2 in the stomach together with the COX-1-sparing effect and marked antinociceptive effect of selective COX-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 COX-2 inhibitors seems to be appropriate because these do not alter the normal physiological functions of COX-1-derived PGs in the stomach, blood, and kidney.

In summary, the present study has shown that intravenously administered 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 and is without any gastric side effects. The emerging role of COX-2 in various diseases and the present results further support the potential of parenteral use of selective COX-2 inhibitors with a better safety profile in the management of pain and inflammatory disorders.