EFFECTS OF LORNOXICAM, PIROXICAM, AND MELOXICAM IN A MODEL OF THERMAL HINDPAW HYPERALGESIA INDUCED BY FORMALIN INJECTION IN RAT TAIL

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Non-steroidal anti-inflammatory drugs (NSAIDs) are frequently used as analgesics. Although the results of clinical studies indicate considerable disparity in the analgesic efficacy of NSAIDs, the pre-clinical models generally used for the study of nociception do not allow a clear distinction to be made between the analgesic properties of agents belonging to this family. As clinical pain is characterized by hyperalgesia, we evaluated the effects of NSAIDs with similar chemical structures but different selectivities for cyclo-oxygenase (COX)-1 and COX-2 in a new behavioural model of central hyperalgesia in rats. We assessed the effects of lornoxicam, piroxicam, and meloxicam on the reduction of hindpaw nociceptive thresholds to thermal stimulation produced by a 10% formaldehyde (formalin) injection into rat tail. Each drug was administered intraperitoneally (i.p.) at its ED50 for the anti-inflammatory effect (namely the inhibition of carrageenan-induced hindpaw oedema). At this dose (1.3 mg kg−1, 1.0 mg kg−1, and 5.8 mg kg−1, respectively), lornoxicam, piroxicam, and meloxicam produced the same anti-inflammatory effect, did not modify thermal nociceptive thresholds, and significantly reduced the hyperalgesia. However, only lornoxicam was fully effective for prevention of hyperalgesia. Our results indicate a dissociation between the anti-inflammatory and the anti-hyperalgesic activity of NSAIDs, where the latter seems to be more evident after the block of both COX-1 and COX-2. Finally, they suggest that our experimental model of thermal hindpaw hyperalgesia can be effectively utilized to assess the ability of different drugs to reduce central sensitization, and thus hyperalgesia.

KEY WORDS: carrageenan, formalin, hyperalgesia, NSAIDs.

INTRODUCTION

The results of clinical studies indicate considerable disparity in the analgesic efficacy of non-steroidal anti-inflammatory drugs (NSAIDs). However, the pre-clinical models generally used for the study of nociception do not allow a clear distinction to be made between the analgesic properties of agents belonging to this pharmacological family.

Traditionally, the analgesic action of NSAIDs has been explained on the basis of their inhibition of the enzymes that synthesize prostaglandins at peripheral cell-damage sites. More recently, it has been demonstrated that NSAIDs also have a direct action on spinal nociceptive processing that augments the peripheral mechanism, and correlates with their capacity as inhibitors of cyclo-oxygenase (COX) activity [1, 2]. In addition, it has been shown that prostaglandins (PGs), which are synthesized from arachidonic acid by COX, play an important role in the development of spinal hyperexcitability, and hyperalgesia [3, 4]. As is now generally accepted, COX exists as two distinct isoforms (COX-1 and COX-2), and both proteins are present in rat spinal cord [5, 6]. Several studies suggest that NSAIDs, through COX inhibition, may modulate the development of central hyperalgesia, and exert analgesic effects [1, 7, 8].

The different selectivities for COX-1 rather than COX-2 inhibition have been associated with gastrointestinal toxicity of NSAIDs [9]; however, the relevance of such selectivity to their analgesic effects has not been fully elucidated [10].

In attempting to study the analgesic drugs, it is important to distinguish between the pain we describe as physiological and pain which we describe as pathological. Unlike physiological pain, pathological pain does not represent a protective response to noxious stimuli; moreover, and most importantly, it is characterized by abnormal hypersensitivity, a substantial
component of which is generated within the spinal cord [11, 12]. For this reason, experimental models of hyperalgesia might be useful as indicators of the drug’s analgesic efficacy. We recently described a simple behavioural method of centrally-mediated hyperalgesia for the study of analgesic drugs in the rat [13–15].

For all these reasons, we considered it of interest to use our experimental model in an attempt to evaluate the anti-hyperalgesic activity of three NSAIDs of the oxicam class with similar structural characteristics but different selectivities for COX-1 and COX-2. All drugs (namely, lornoxicam, piroxicam and meloxicam) were administered in the same experimental conditions, at doses that produced the same anti-inflammatory effects.

**MATERIALS AND METHODS**

**Animals**
Male Sprague–Dawley CD rats (Charles River, Calco, Italy) were used in all experiments. The animals (225–250 g), were housed in groups of four per cage, allowed free access to water and food with a 14 light : 10 dark cycle. Rats were acclimatized to the laboratory at least 7 days prior to the experiments. The ethical guidelines of the International Association for the Study of Pain (IASP) were adhered to in these studies [16].

**Drugs**
The following NSAIDs were used: lornoxicam (Prodotti Formenti S.r.l., Milano, Italy), piroxicam (Pfizer Italiana S.p.A., Borgo S. Michele, Italy), and meloxicam (Boehringer Ingelheim Italia S.p.A., Milano, Italy). All drugs were suspended in 0.5% methylcellulose and administered intraperitoneally (i.p.). Carrageenan (λ-carrageenan) was obtained from Sigma-Aldrich, Milano, Italy. Formaldehyde solution 37% was obtained from Merck-Bracco, Milano, Italy.

**Assessment of anti-inflammatory effects**
The inflammation was induced by the intraplantar injection of 0.1 ml of a 1% carrageenan solution (0.9% NaCl) in the left hindpaw. The intensity of oedema was assessed by measuring the paw volume (ml) by a 7150 Plethysmometer (Basile, Comerio, Italy). As previously described, the results were expressed as the algebraic difference between the volume of the treated and untreated hindpaw [17]. The observer was blind as to treatment allocation of the animals. All rats received carrageenan. All drugs tested were administered 15 min before carrageenan injection at three different doses. Control animals were injected i.p. with the same volume of vehicle (0.5% methylcellulose). Hindpaw oedema was measured 3 h after carrageenan injection. The ED$_{50}$ values for carrageenan-induced inflammation were estimated by computer-assisted linear regression analysis based on dose–response curves, plotting the percentage of inhibition for each tested dose.

In order to examine possible differences in pharmacokinetics, other groups of rats ($N=5$) were injected with the previously defined ED$_{50}$, and the drug effects over a period of time were evaluated by area under the curve (AUC) calculation. In this experimental set, the hindpaw oedema was measured every hour for the 6 h following the injection of carrageenan.

**Assessment of nociceptive latencies, and anti-hyperalgesic effect**
The method of Hargreaves was used to assess the hindpaw nociceptive thresholds to thermal stimuli [18]. We used a Plantar Test apparatus (Ugo Basile, Comerio, Italy). In brief, the rats (six in each experimental group) were placed in a clear plastic chamber and left to acclimatize for 5 min before testing. Light from a 8 V–50 W halogen bulb (64607 OSRAM) was delivered to the plantar surface of the rat’s hindpaw through the base of the plastic box. The beam was about 12 mm in diameter. The time taken for the animal to withdraw its left hindpaw was measured. Treatments were administered only to the rats in which basal latency had remained stable ($\pm 1.5$ s) for three subsequent measurements. The value obtained with the last measurement was considered as the basal latency. After basal evaluation, so as to avoid tissue damage and reduce animal adaptation, the animals were tested only once at each time point. Hyperalgesic state was assessed by delta reaction time (basal latency–test latency), positive results thereby indicating hyperalgesia.

The hindpaw hyperalgesia was induced by the intradermal injection of formalin (10% formaldehyde, 100 µl) in the distal part of the tail, as previously described [13]. In this experimental set, each drug was administered 30 min before formalin, at its previously defined ED$_{50}$ for the anti-inflammatory effect. Control animals did not receive any treatment. Thus, there were the following three experimental groups: (a) non-treated controls that received neither formalin nor drug; (b) vehicle-treated, formalin-injected controls; (c) drug-treated, formalin-injected experimental group.

**Statistical evaluation**
The data obtained by AUC calculation (anti-inflammatory effects) were analysed by one-way ANOVA followed by Bonferroni’s t-test. The statistical analysis of behavioural results (anti-nociceptive and anti-hyperalgesic effects) was performed using two-way ANOVA followed by Bonferroni’s t-test for multiple comparisons, considering as factors ‘time’, and ‘drug treatment’. An effect was determined to be significant if the $P$ value was less than 0.05.

**RESULTS**
The ED$_{50}$ of lornoxicam, piroxicam, and meloxicam for carrageenan-induced inflammation in our experimental conditions are shown in Table I. It is important to note...
that the assessment of the drug effects on paw oedema over a period of time confirmed that, at this dose, all drugs produced the same anti-inflammatory action (Fig. 1).

Table II shows that, when administered at these doses in normal animals, none of the tested drugs altered nociceptive thresholds in the Plantar Test. In conformity with our previous observations, the formalin injection in the tail induced a significant reduction of hindpaw withdrawal reflexes. The hyperalgesic condition was already apparent 30 min following formalin administration, was more evident 60 min after the injection of the irritant substance, and lasted at least 90 min (Figs 2 & 4).

The hindpaw hyperalgesia was completely prevented by the administration of a dose of lornoxicam (1.3 mg kg⁻¹ i.p.) which in itself did not produce any effect on thermal nociceptive thresholds (Fig. 2 and Table II). Indeed, statistical analysis showed that the values obtained in the lornoxicam-treated animals were significantly different from those of formalin-treated rats, but not from those of control animals \[F(2, 71) = 18.20, P < 0.0001; post hoc \textit{comparisons}, t = 5.70, P < 0.05, \textit{and} t = 1.13, \textit{N.S., respectively}].

Piroxicam and meloxicam induced a significant reduction of hindpaw hyperalgesia \[F(2, 71) = 41.7, P < 0.0001, \text{ and } F(2, 71) = 50.7, P < 0.0001, \text{ respectively}]. However, the effects induced by these drugs were not complete (Figs 3 and 4). Indeed, the \textit{post hoc} \textit{comparisons} showed that the values obtained in the drug-treated animals were significantly different from both formaldehyde-treated \[t = 5.62, P < 0.05, \textit{and} t = 7.11, P < 0.05, \textit{respectively}]; and control rats \[t = -3.43, P < 0.05, \textit{and} t = -2.63, P < 0.05, \textit{respectively}].

**DISCUSSION**

In this study we used a new and simple behavioural method to investigate the anti-hyperalgesic properties of NSAIDs with similar chemical structures but different selectivity for COX-1 and COX-2. We have included an
The evaluation was performed by the Plantar Test. Data are expressed as mean ± SEM of latencies (s) for the baseline (BL) and over a period of time following drug administration.

agent such as piroxicam, which has been described as a better inhibitor of COX-1 than COX-2 [19, 20], an agent such as meloxicam, which shows a preferential activity towards COX-2 [20, 21], and lornoxicam, a compound that produces inhibition of both COX-1 and COX-2 without a clear selectivity [22, 23].

When administered at comparable anti-inflammatory doses, all these drugs were able to reduce hyperalgesia. Moreover, we observed that lornoxicam exerted a more evident anti-hyperalgesic action than piroxicam and meloxicam. So the use of this experimental model enabled us to show a statistically relevant distinction between the anti-hyperalgesic effects of these NSAIDs.

Our results are therefore in agreement with data indicating a dissociation between the anti-inflammatory and analgesic effects of NSAIDs [24, 25]. Moreover, our present behavioural observations are consistent with electrophysiological data demonstrating that spinal cord neurones receiving nociceptive input are unresponsive to NSAIDs, unless the spinal cord has been rendered hypersensitive by repetitive stimulation of peripheral afferents [5]. In this respect, we observed that all the NSAIDs tested are ineffective in enhancing nociceptive thresholds (that is to say when the spinal cord neurons are not sensitized), but they are able to prevent the development of the hyperalgesia related to a facilitation of spinal neuron activity [15].

Peripheral inflammation has been shown to increase the spinal levels of PGs [26]. Several studies have revealed PGE$_2$ as the major prostaglandin involved in the spinal nociceptive processing [27], and the injection of 10% formaldehyde into rat tail is associated with an increase of PGE$_2$ concentrations in the CSF that correlates with hyperalgesic behaviour [28]. Thus, it is reasonable to argue that the anti-hyperalgesic activity of lornoxicam, piroxicam, and meloxicam is related, at least in part, to their ability to inhibit PG formation in the spinal cord. The relative contributions from both COX-1 and COX-2 in the development of spinally-mediated hyperalgesia remains to be elucidated. Anyway, the marked effects of lornoxicam (a NSAID equally effective against COX-1 and COX-2) in our experimental model are in keeping with previous animal studies showing a role for both COX-1 and COX-2 in mediating hyperalgesia [29–31], and suggest that the inhibition of both COX isoenzymes might be necessary in order to achieve maximal analgesic effects. However, it is important to keep in mind that NSAID centrally mediated analgesia may be achieved by mechanisms which are independent of prostaglandin synthesis inhibition [24, 25, 32].

Among the drugs considered in this study, lornoxicam (chlorotentoxicam) is the most recently proposed compound of NSAID oxicam class. The potent anti-inflammatory activity of this agent in animal models has been reported [33] as well as its analgesic efficacy in humans [22, 34, 35]. Our present data further suggest that lornoxicam might offer a valid choice for the treatment of inflammatory pain.

REFERENCES


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