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Dose-related anti-inflammatory/analgesic effects of lornoxicam: A spinal c-Fos protein study in the rat

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Abstract. *Objective:* To evaluate the anti-inflammatory/analgesic effects of lornoxicam in the carrageenan model of inflammatory nociception.
Material and Methods: Three hours after intraplantar carrageenan (6 mg/150 μl of saline), we assessed the effects of pre-administered lornoxicam (0.1, 0.3, 1, 3 and 9 mg/kg i.v., n=10 rats for each group) on both the peripheral oedema and number of c-Fos-protein-like immunoreactive (c-Fos-LI) neurons in the lumbar L4-L5 segments, in the awake rat.
Results: Lornoxicam dose-relatedly reduced both the carrageenan evoked oedema (r=0.63 and r=0.53 for paw and ankle diameter respectively; p<0.001 for both) and total number of spinal c-Fos-LI neurons (r=0.79; p<0.001), with the strongest effect corresponding to a 75 ± 2% reduction of the number of c-Fos-LI neurons (p<0.001) for the highest dose (9 mg/kg), and a 45 ± 3% reduction (p<0.001) for the low dose of 0.3 mg/kg. Reductions of both the peripheral oedema and spinal c-Fos expression were correlated (r=0.74 and r=0.57 for the paw and ankle diameter respectively; p<0.001 for both).
Conclusions: Our results demonstrate that lornoxicam reduces in parallel both the carrageenan-evoked oedema and spinal c-Fos expression, with clear evidence for a potent effect of low doses of lornoxicam. Correlated reductions in c-Fos expression and paw oedema suggest a predominantly peripheral site of action of lornoxicam.

Key words: Carrageenan – c-Fos – Dorsal horn – Lornoxicam – Nociception

Introduction

Lornoxicam, previously known as chlortenoxicam, is a member of the oxicam group of non-steroidal anti-inflammatory drugs (NSAIDs); for review see [1]. Lornoxicam is a new compound combining the potency of the oxicams with a reduced risk of side effects. In comparison with other oxicams, lornoxicam has a relatively short elimination half-life (3 to 5 h [2]) and good gastrointestinal tolerability [3-5] which is an advantage in terms of less side effects than other oxicams. Like other NSAIDs, the anti-inflammatory/analgesic activity of lornoxicam is related to the inhibitory action on prostaglandin synthesis, via inhibition of cyclooxygenase (COX) activity in the COX pathway of arachidonic acid metabolism [6]. Recently, the varying anti-inflammatory/analgesic effects of different NSAIDs have been attributed to a different affinity for two isoforms of COX, COX-1 being expressed constitutively and COX-2 being expressed inducibly in response to inflammatory mediators [7–8] In a series of in vitro studies, lornoxicam was equipotent in the inhibition of both COX-1 and COX-2 (for review see [1]).

The potent anti-inflammatory and analgesic activity of lornoxicam in both animal models [2] and clinical trials [3, 5, 9] has been reported. Clinical trials have demonstrated the analgesic effect of lornoxicam in patients with acute pain (acute sciatica/lumbosciatica and low back pain [10]) or chronic inflammatory pain (osteoarthritis, rheumatoid arthritis [4, 5]), for review see [1]. In animal studies, lornoxicam had anti-inflammatory (carrageenan oedema) and analgesic (writhing test) activity about 10 times greater than that of tenoxicam [2].

In an attempt to study the anti-inflammatory/analgesic effects of lornoxicam we have used a combination of the measurement of the peripheral oedema and the method of c-Fos protein immunoreactivity in the carrageenan model of inflammatory nociception in the rat. There is considerable evidence that expression of the nuclear protein c-Fos encoded by the immediate-early gene c-*fos* (for review see [11–13]) reflects the longer term intracellular changes associated with sustained nociceptive processing at the spinal cord level [14, 15]. Numerous studies have demonstrated that at the level of the spinal cord, especially in the dorsal horn, c-Fos protein expression provides an indirect marker of neurones involved in spinal nociceptive transmission (for review see [16]). Furthermore, several studies demonstrated that systemic pre-administration of morphine, a classical analgesic, significantly reduced spinal c-Fos expression evoked by various types of peripheral

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nociceptive stimulation, such as intraplantar injection of formalin [17–19] or carrageenan [20], intraperitoneal injection of acetic acid [21], noxious heat [22–26], noxious cold [27] and noxious mechanical stimulation [28]. In addition, there is evidence for the effects of various NSAIDs on spinal c-Fos expression evoked in the condition of the inflammatory pain. In the Freund's adjuvant model of chronic inflammation, chronic treatment with aspirin, a classical NSAID, reduced both the development of inflammatory signs and spinal c-Fos expression during the development of polyarthritis [29]. Recently, we have performed the c-Fos protein studies to assess the anti-inflammatory/analgesic effects of an acute pre-administration of various NSAIDs such as aspirin [30], indomethacin [31], diclofenac [32], piroxicam [33], ketoprofen [34] and niflumic acid [35] in the intraplantar carrageenan model of acute inflammation. It is well known that an intraplantar injection of carrageenan induces inflammatory oedema [36, 37] associated with the development of mechanical allodynia [38], heat and mechanical hyperalgesia [39–41], and the expression of c-Fos protein at spinal cord level [31, 42–44].

Considering our previous studies of the effects of various NSAIDs (see references above), it was interesting to investigate the effects of lornoxicam, a new NSAID, in the same experimental conditions of inflammatory nociceptive processes. In the present study, we have evaluated the effect of systemic pre-administration of five doses of lornoxicam on both the carrageenan-evoked peripheral oedema and spinal c-Fos protein expression. A preliminary account of this study has been presented at the International Conference on Inflammopharmacology in San Francisco, March 1997 [45].

Materials and methods

Experiments were performed on 65 adult male albino Sprague-Dawley rats (Charles River, France; 60 carrageenan stimulated and 5 non-stimulated rats), weighing 225–250 g. The ethical guidelines of the International Association for the Study of Pain, for investigations of experimental pain in conscious animals were followed [46].

Lornoxicam (6-chloro-4-hydroxy-2-methyl-*N*-(2-pyridyl)-2*H*-thieno-[2,3-e][1,2]thiazine-3-carboxamide 1,1-dioxide; Hafslund Nycomed Pharma, Austria) was dissolved in bidistilled water (volume 0.25 ml) and injected into the tail vein of the conscious rat 25 min prior to intraplantar injection of carrageenan. The effects of five doses of lornoxicam (0.1, 0.3, 1, 3 and 9 mg/kg i.v.; n=10 rats for each group) were studied simultaneously in the two experimental series. Control carrageenan rats (n=10) received an equal volume of solvent under the same experimental conditions, i.e. an intravenous injection of 0.25 ml of bidistilled water 25 min prior to intraplantar carrageenan. Intravenous injections were made with a 25 gauge needle. The conscious rat was restrained using a cylindrical rodent restrainer (Harvard Apparatus, Ealing, France) for about 1 min during the injection. In this study, control rats receiving an intraplantar injection of saline were not included since we have previously shown negligible spinal c-Fos expression after intraplantar saline (< 5 c-Fos-LI neurons per section at L4-L5 segments level) which was not significantly different from spinal c-Fos expression in non-stimulated rats [31].

Carrageenan (λ-carrageenan, Sigma, Paris, France, 6 mg/150 ml of saline (0,9% NaCl)) was injected, intraplantarly, in the right hind paw of the non-anaesthetised rat. At 3 h after carrageenan, two indicators of the extent of the peripheral oedema (diameters of paw and ankle) were measured with calibrated callipers, under deep pentobarbital anaesthesia, immediately before perfusion (for more details see Methods in [47]). Enhanced paw and ankle diameters induced by carrageenan injection were measured in control group of rats (P_c, A_c, respectively) and drug treated rats (P_t, A_t, respectively). For comparison, paw and ankle diameters (P_n, A_n, respectively) of non-stimulated rats (n=5) were measured. The effects of drugs were determined as percentage decreases of the paw and ankle diameter of drug-treated rats (P_t - P_n, A_t - A_n, respectively) as compared to the ankle and paw diameter of control rats (P_c - P_n, A_c - A_n, respectively). The following formula for the paw diameter: $((P_t - P_n)/(P_c - P_n))\times100$ and for the ankle diameter: $((A_t - A_n)/(A_c - A_n))\times100$ was used. Studies of peripheral oedema and spinal c-Fos protein expression were performed in the same rats, thus possible correlations between the two parameters could be determined.

Experimental procedures have been described previously [31]. All carrageenan-stimulated rats were perfused 3 h after carrageenan, when the number of c-Fos-like immunoreactive (c-Fos-LI) neurons in the dorsal horn of the lumbar spinal cord is maximal [31]. Non-stimulated rats were perfused without carrageenan injection. Rats were deeply anaesthetised (Pentobarbital, Sanofi; 55 mg/kg i.p.) and perfused intracardially with 0.1 M phosphate buffered saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The spinal cord was removed, postfixed for 4 h and cryoprotected in 30% sucrose overnight. Frozen serial frontal sections (40 μm) of the lumbar spinal cord were cut. Immunohistochemistry of the free floating sections was performed with polyclonal antiserum, generated in rabbits and directed against the c-Fos protein (Oncogene Science Inc., Ab-2 solution 0.1 mg/ml diluted 1:4000), using the method of Hsu et al. [48]. The c-Fos protein-like immunoreactivity (c-Fos-LI) was visualised by 1-naphtol ammonium carbonate solution [49]. The sections were mounted on gelatin-subbed slides and intensified by 0.025% crystal violet in bidistilled water. After bidistilled water rinses to take off the excess stain, sections were differentiated in 70% alcohol (differentiation time was evaluated using control under the microscope). Finally, the slides were air-dried and coverslipped.

As described previously [31], c-Fos-LI neurons were counted with a camera lucida attachment through 4 arbitrarily defined regions of the spinal grey matter of the L4-L5 segments, according to the cytoarchitectonic organisation of the spinal cord [50, 51]: superficial laminae (laminae I-II), nucleus proprius (laminae III-IV) and deep laminae (laminae V-VI; neck) of dorsal horn and, in addition, ventral horn (laminae VII-X) of the spinal cord. For each rat, two counts were made: (1) the total number of c-Fos-LI neurons in the grey matter for 10 sections through L4-L5 segments, and (2) in these 10 sections, the

Table 1. The number of carrageenan-evoked spinal c-Fos-LI neurons in the control carrageenan group and 5 groups with pre-administered lornoxicam (0.1, 0.3, 1, 3 and 9 mg/kg i.v., n=10 for each group) before the inflammatory stimulus (6 mg of carrageenan in 150 µl of saline). Results are expressed as mean number of c-Fos-LI neurons (± SEM), per L4-L5 segments (total), and per laminar region (laminae I-II, III-IV, V-VI, Ventral), 3 h after intraplantar injection of carrageenan.

Dose (mg/kg i.v.)	Number of carrageenan-evoked c-Fos-LI neurons/section L4-L5				
	total	laminae I-II	laminae III-IV	laminae V-VI	ventral
Control (vehicle)	122 ± 5	52 ± 3	7 ± 1	42 ± 2	18 ± 1
Lornoxicam 0.1	86 ± 5	39 ± 3	5 ± 1	31 ± 2	11 ± 1
Lornoxicam 0.3	67 ± 4	35 ± 2	3 ± 0.5	22 ± 2	8 ± 1
Lornoxicam 1.0	47 ± 2	25 ± 2	2 ± 1	16 ± 1	4 ± 0.5
Lornoxicam 3.0	42 ± 3	24 ± 2	2 ± 0.5	13 ± 1	3 ± 0.5
Lornoxicam 9.0	31 ± 3	19 ± 2	1 ± 0.5	9 ± 1	1 ± 0.5

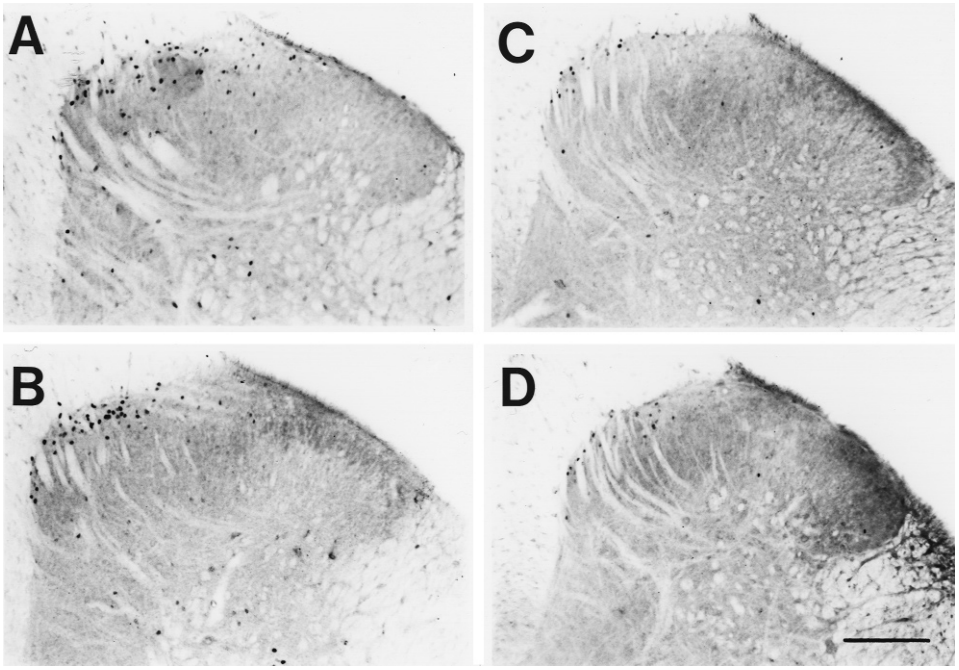

Fig. 1. Photomicrographs, of individual representative examples of 40-μm sections in segments L4-L5 of rat spinal cord, illustrating the effects of intravenous pre-administration of lornoxicam on the c-Fos protein-like immunoreactivity evoked by intraplantar injection of carrageenan. Each microphotograph includes nuclei of c-Fos-LI neurons (black dot) in laminae I-V of dorsal horn. Four experimental situations are represented: intraplantar injection of carrageenan plus prior administration of intravenous vehicle (A, control) or lornoxicam 0.3 mg/kg (B), 3 mg/kg (C) and 9 mg/kg (D). Scale bar = 200 μm.

number of c-Fos-LI neurons per 4 defined regions. Plotting and counting the c-Fos-LI neurons was performed blind. Statistical analysis was performed using analysis of variance (ANOVA) and the Fisher's protected least squares difference test for multiple comparisons. The dose-dependent effects of lornoxicam on both the number of spinal c-Fos-LI neurons and the peripheral oedema (paw and ankle diameter) and possible correlations between these parameters were determined using a simple regression and a correlation coefficient, respectively.

Results

Carrageenan-evoked c-Fos protein expression in the lumbar spinal cord

In accordance with our previous studies (see [34] and refs. therein) the intraplantar injection of carrageenan evoked a peripheral oedema and an associated c-Fos protein expression in the spinal cord ipsilateral to the carrageenan hind paw inflammation. Three hours after intraplantar injection of carrageenan, the c-Fos-LI neurons were numerous in lumbar segments L4-L5 (Fig. 1A). For the control carrageenan groups of two experimental series, the total number of c-Fos-LI neurons was extremely similar and not significantly different (118 ± 5 and 125 ± 8 c-Fos-LI neurons per section in segments L4-L5 respectively, n=5 rats in each experimental series). These results allow the statistical analyses of results from two experimental series together. Thus for n=10 rats, the total number of c-Fos-LI neurons was 122 ± 5 c-Fos-LI neurons per section in segments L4-L5 of control carrageenan-stimulated rats (Table 1). c-Fos-LI neurons were essentially located in the dorsal horn of the spinal cord, with a predominant and similar distribution in both the superficial (I-II) and deep (V-VI) laminae (43 ± 2 and 35 ± 2 % of the total number of c-Fos-LI neurons, respectively), see Table 1 and Fig. 2 (control). The number of c-Fos-LI neurons in the ventral horn (laminae VII-X) was moderate and very few c-Fos-LI neurons were present in nucleus proprius (laminae III-IV; <8 c-Fos-LI neurons per L4-L5 section, Table 1), see also Fig. 2 (control). c-Fos-LI neurons are virtually absent in the contralateral lumbar spinal cord (<3 c-Fos-LI neurons per section in segments L4-L5).

Effects of lornoxicam on carrageenan-evoked spinal c-Fos protein expression

Pre-administered lornoxicam (i.v.) reduced the number of spinal c-Fos-LI neurons, 3 h after intraplantar carrageenan (Fig. 1B-D and Fig. 2). The effects of lornoxicam on carrageenan-evoked spinal c-Fos protein expression were significant when considering both the total number of c-Fos-LI neurons and their laminar distribution in L4-L5 segments (ANOVA-test $F_{5,54}$=75.19 and $F_{5,216}$=120.12, respectively, $p<0.001$ for both), see Fig. 3 and Table 1. Lornoxicam (0.1, 0.3, 1, 3 and 9 mg/kg i.v.) strongly reduced the total number c-Fos-LI neurons in L4-L5 segments (29 ± 4, 45 ± 3, 61 ± 2, 66 ± 3 and 75 ± 2% reduction, respectively, $p<0.001$ for all doses) as compared to the control carrageenan group (Fig. 4), with these effects being dose-related (regression coefficient r=0.79, $p<0.001$). Lornoxicam greatly reduced the number of c-Fos-LI neurons in the superficial (I-II) and deep (V-VI) laminae, with the effect of the highest dose (9 mg/kg) being 63 ± 4 and 79 ± 2% reduction, respectively ($p<0.001$ for both, Fig. 4). Laminar analyses revealed that the effects of

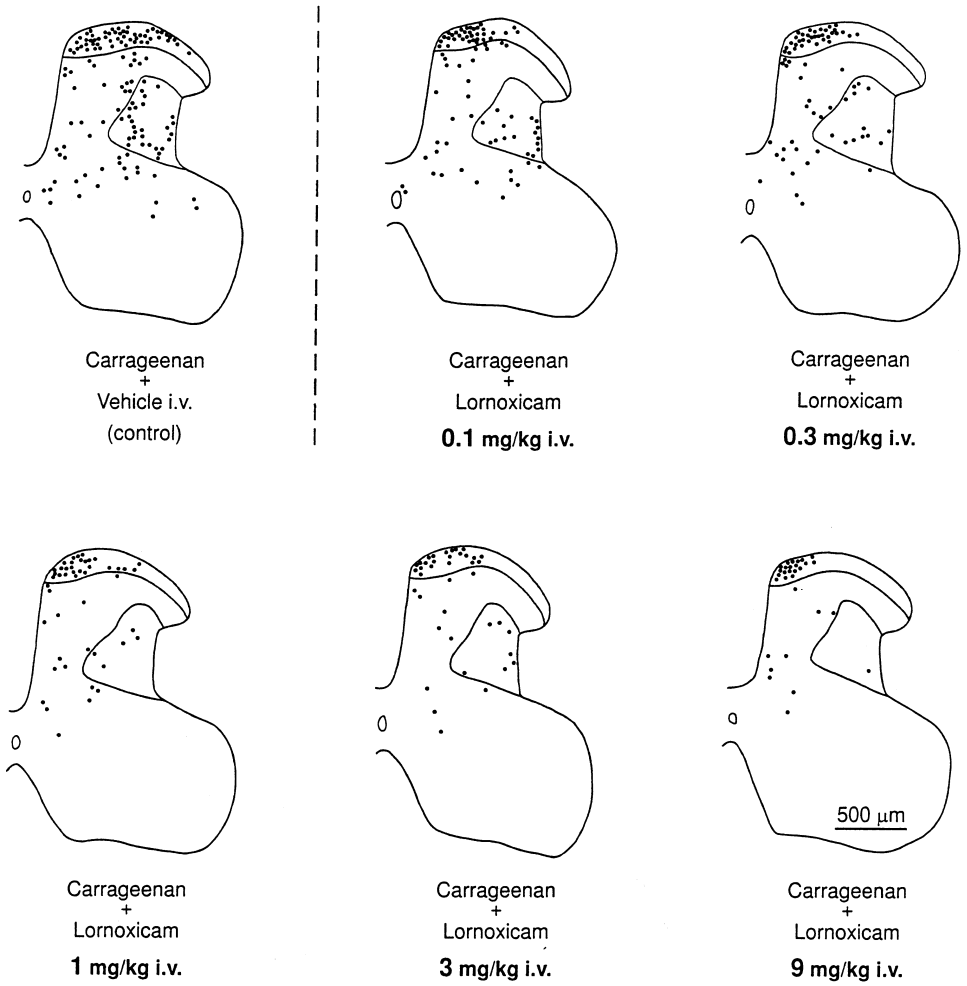

Fig. 2. Camera lucida drawings, of individual representative examples of 40-μm sections of segments L4-L5 of rat spinal cord, illustrating the c-Fos protein-like immunoreactivity evoked by intraplantar carrageenan (control) and the effects of intravenous pre-administration of lornoxicam (0.1, 0.3, 1, 3 and 9 mg/kg) on the number of c-Fos-LI neurons. Each drawing includes all c-Fos-LI neurons in one section of segments L4-L5, each dot represents one c-Fos-LI neuron. The boundaries of the superficial laminae and of the reticular part of the neck of the dorsal horn are outlined.

lornoxicam were dose-related in both the superficial (I-II) and deep (V-VI) laminae (regression coefficient r=0.66 and r=0.80 respectively, $p<0.001$ for both). Low dose of lornoxicam (0.1 mg/kg i.v.) had a similar effect in both superficial and deep laminae, whereas the four higher doses (0.3, 1, 3 and 9 mg/kg i.v.) had a significantly stronger effect on the number of c-Fos-LI neurons in deep laminae as compared to that in superficial laminae (Fig. 4). Interestingly, the relatively low dose of 0.3 mg/kg of lornoxicam is sufficient to produced marked effects (48 ± 4 and $33 \pm 5\%$ reduction of the number of c-Fos-LI neurons in deep and superficial laminae respectively, $p<0.001$ for both), Fig. 4.

Effects of lornoxicam on the carrageenan-evoked peripheral oedema

Three hours after intraplantar carrageenan, ipsilateral oedema at both the paw and ankle levels was observed. In the control carrageenan group, diameters of paw (1.1 ± 0.1 cm) and ankle (1.1 ± 0.1 cm) were significantly increased as compared to non-stimulated rats (0.5 ± 0.1 and 0.7 ± 0.1 cm for paw and ankle diameters, respectively). The carrageenan-evoked swelling of paw and ankle represented 133 ± 2 and $48 \pm 2\%$ increase in diameter respectively, as compared to non-stimulated rats ($p<0.001$ for both). Contralateral oedema was not observed.

The effects of intravenously pre-administered lornoxicam (0.1, 0.3, 1, 3 and 9 mg/kg) on carrageenan evoked oedema were significant when considering both the paw and ankle diameters (ANOVA-test $F_{6,58}=61.64$ and $F_{6,58}=21.96$ respectively, $p<0.001$ for both). As shown in Fig. 5, lornoxicam (0.1, 0.3, 1, 3 and 9 mg/kg i.v.) blocked the swelling of both paw and ankle diameters, with a stronger effect on the ankle oedema (34 ± 8, 61 ± 9, 66 ± 8, 80 ± 6 and $83 \pm 5\%$ reduction of the control carrageenan enhanced ankle diameter, respectively, $p<0.001$ for all doses). The effects of lornoxicam on the oedema of paw and ankle were dose-related (regression coefficient r=0.63 and r=0.53 respectively, $p<0.001$ for both). Furthermore the effects of lornoxicam on the carrageenan evoked paw and ankle oedema and the total number of spinal c-Fos-LI neurons were positively correlated (correlation coefficient r=0.74;

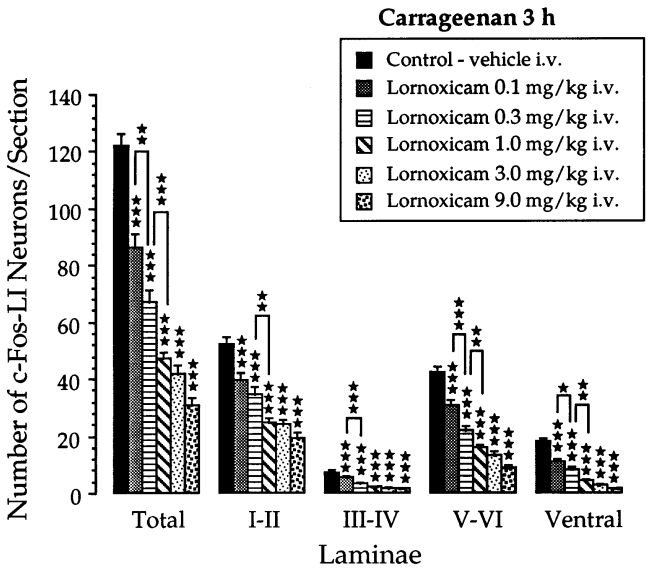

Fig. 3. Effects of intravenous pre-administration of lornoxicam (0.1, 0.3, 1, 3 and 9 mg/kg, n=10 for each group) on the number of c-Fos-LI neurons in the L4-L5 segments of the rat spinal cord, 3 h after intraplantar carrageenan. Results are expressed as mean number of c-Fos-LI neurons (± SEM), per L4-L5 segments (Total), and per laminar region (laminae I-II, III-IV, V-VI, ventral). Significance compared with control carrageenan group was performed using ANOVA and Fisher's PLSD-test (*p<0.05, **p<0.01, ***p<0.001).

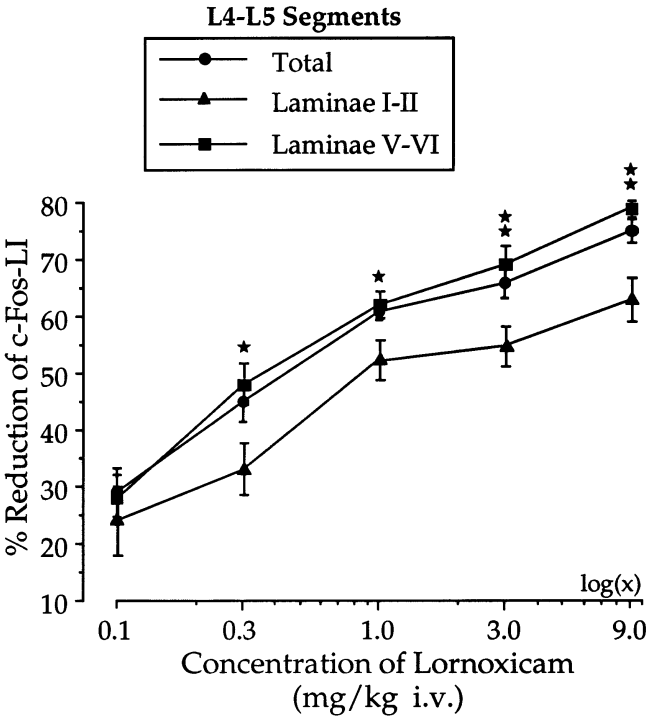

Fig. 4. Effects of pre-administered lornoxicam (0.1, 0.3, 1, 3 and 9 mg/kg i.v., n=10 for each group) on the total number c-Fos-LI neurons (total, circles), the number of superficial (laminae I-II, triangles) and deep (laminae V-VI, squares) c-Fos-LI neurons in the L4-L5 segments, 3 h after intraplantar carrageenan. Results are expressed as % reduction of the control value for the total number of c-Fos-LI neurons and their number in each region (± SEM). Statistical comparisons between the effects of lornoxicam on the number of c-Fos-LI neurons in the superficial versus deep laminae were performed using ANOVA and Fisher's PLSD-test (*p<0.05, **p<0.01).

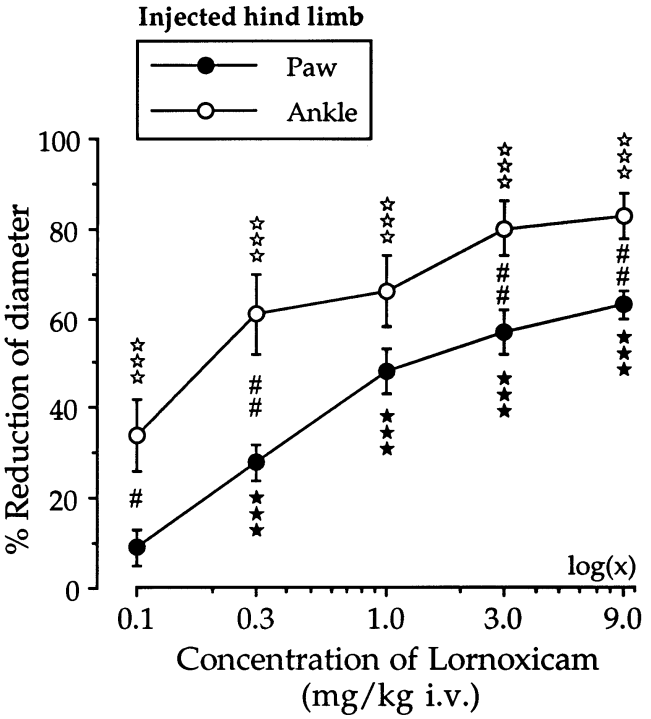

Fig. 5. Effects of pre-administered lornoxicam (0.1, 0.3, 1, 3 and 9 mg/kg i.v., n=10 for each group) on the carrageenan-enhanced paw (black circles) and ankle (open circles) diameters, 3 h after intraplantar carrageenan. Results are expressed as % reduction of the control carrageenan-evoked oedema for both the paw and ankle diameter (± SEM). Significance as compared with the control value was performed using ANOVA and Fisher's PLSD-test (***p<0.001). Statistical comparisons between the effects of lornoxicam on the carrageenan-evoked paw versus ankle oedema were performed using ANOVA and Fisher's PLSD-test ($^{\#}$p<0.05, $^{\#\,\#}$p<0.01).

0.58<r<0.84 and r=0.57; 0.35<r<0.73 for the paw and ankle diameters respectively, p<0.001 for both), see Fig. 6. As shown in Fig. 6, after pre-administration of lornoxicam, the number of c-Fos-LI neurons followed the decrease of the carrageenan-evoked oedema at both the paw (Fig. 6A) and ankle (Fig. 6B) levels.

Discussion

The present study, using the method of c-Fos protein-like immunoreactivity, demonstrated the potent effects of lornoxicam in the carrageenan model of inflammatory nociception. Lornoxicam decreased dose-relatedly both the carrageenan-evoked spinal c-Fos protein expression and peripheral oedema. Furthermore, there was a positive correlation between the effects of lornoxicam on these two parameters.

The choice of the method of c-Fos protein immunoreactivity to study the effect of pre-administration of lornoxicam upon inflammatory stimuli has been made with the consideration of our previous studies. Recently, we performed a study of the development of the inflammatory signs and spinal c-Fos protein expression in the model of acute inflammation induced by carrageenan [31]. In other studies performed in this model, we have demonstrated the

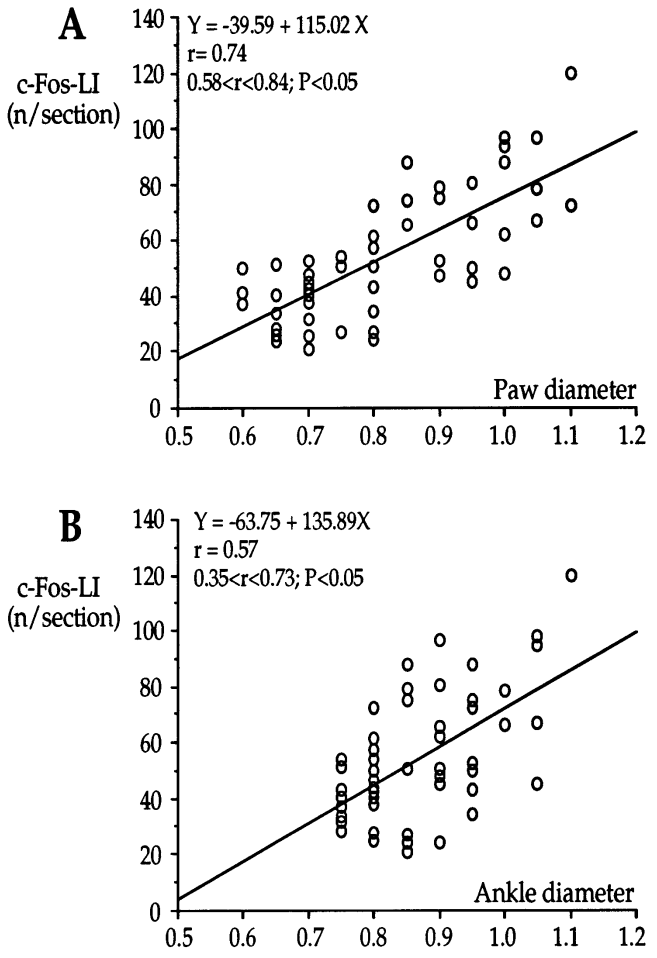

Fig. 6. Correlation between the effects of lornoxicam (0.1, 0.3, 1, 3 and 9 mg/kg i.v., n=10 for each group) on the carrageenan-evoked peripheral oedema at paw (paw diameter, A) and ankle (ankle diameter, B) level and total number of c-Fos-LI neurons per section of L4-L5 segments (c-Fos-LI (n/section)).

correlation between the effect of different NSAIDs on the carrageenan-induced peripheral oedema and spinal c-Fos protein expression (see [34] and references therein). These correlations suggest that the combination of the measure of peripheral oedema with the evaluation of the spinal c-Fos protein expression represent a suitable and appropriate approach to gauge the effectiveness of different NSAIDs in the model of inflammatory nociception. The fact that various substances, such as morphine [28] or aspirin and acetaminophen [29], were able to prevent spinal c-Fos protein expression induced by inflammatory stimulus, but not to influence already existing c-Fos protein expression, determined our decision for a single pre-administration of NSAIDs before the carrageenan inflammatory stimulus.

As previously shown, intraplantar carrageenan induced a peripheral oedema and an associated c-Fos protein expression at ipsilateral spinal cord level in non-anaesthetised freely moving rats [31, 42–44]. In agreement with our previous studies (see [34] and refs. therein), 3 h after carrageenan, the number of c-Fos-LI neurons was maximal in the L4-L5 segments of lumbar spinal cord, corresponding to the projection from hind limb dermatomes [52]. Carrageenan-induced c-Fos-LI neurons were essentially localised in the superficial (I-II) and deep (V-VI) laminae of the dorsal horn of the spinal cord. This laminar pattern is in good keeping with the findings of electrophysiological studies, which have demonstrated that a high proportion of superficial and deep laminae dorsal horn neurons are driven by noxious input (see references in [53]). Furthermore, noxious (Aδ- and C-fibre intensity) but not innocuous (Aα/β-fibre intensity) electrical stimulation of the sciatic nerve has been shown to induce c-Fos protein expression in superficial and deep neurons of the dorsal horn at lumbar spinal cord level [54]. Overall, the laminar pattern of carrageenan-induced spinal c-Fos protein expression in the present study is in concordance with previous studies of the noxious stimulus-induced c-Fos protein expression at the level of the spinal cord (see Introduction).

In the present study, the total number of c-Fos-LI neurons was extremely similar for the control carrageenan groups of the two experimental series. Furthermore, in both series, the control carrageenan groups had a similar localisation of c-Fos-LI neurons, predominantly in the superficial (I-II) and deep (V-VI) laminae of the dorsal horn of the spinal cord. This homogeneity of data allowed the statistical analysis of the drug effects. The intravenous pre-administration of lornoxicam reduced the total number of carrageenan-induced c−Fos−LI neurons in dose-related manner. The effect of lornoxicam was dose-related as considering the number of c−Fos−LI neurons in both superficial and deep laminae of dorsal horn. Furthermore, the effects of lornoxicam on both the number of spinal c-Fos-LI neurons and peripheral oedema were correlated. This correlation suggests a predominantly peripheral site of action of lornoxicam with a subsequent reduction of nociceptive inputs to the spinal cord and thus an attenuation of c-Fos protein expression in the spinal neurons. Nevertheless, considering the spinal role of prostaglandins [55], a central site of action of NSAIDs, including lornoxicam, cannot be excluded [56–59].

Our results obtained with lornoxicam are in good agreement with, and extend previous studies showing a potent anti-inflammatory effect of lornoxicam on carrageenan- or Freund's adjuvant-induced oedema in the rat [2]. Furthermore, in clinical trials, lornoxicam has been shown to produce dose-related analgesia after dental surgery ([9], for review see [1]). Overall, our study provides further evidence for the potency of lornoxicam as an anti-inflammatory/analgesic compound, for review see [1]. Considering the undesirable side effects of NSAIDs (see [60] and references therein), the clinical use of these agents in the treatment of inflammation remains limited. However, in our study, the relatively low dose (0.3 mg/kg) of lornoxicam produced a marked reduction of both the carrageenan-induced peripheral oedema (28 ± 4 and 61 ± 9% reduction of the diameter of paw and ankle, respectively), and of the number of spinal c-Fos-LI neurons (45 ± 3% reduction of the total number of c-Fos-LI neurons, with 48 ± 4 and 33 ± 5% reduction of the number of c-Fos-LI neurons in deep and superficial laminae, respectively). Thus, it seems likely that the low dose of lornoxicam could be an advantageous NSAIDs therapy associated with less risk of adverse side effects.

Considering our previous studies, the effects of lornoxicam are in good keeping with effects of various NSAIDs on spinal c-Fos protein expression under the same experimental

conditions, the carrageenan model of inflammatory nociception [30–35, 61]. In the present study, the efficacy of a relatively low dose of lornoxicam was greater than that previously obtained with other NSAIDs in the same experimental paradigm (see references above). For example a 61 ± 2% reduction in the total number of carrageenan-evoked c-Fos-LI neurons was observed with 1 mg/kg of lornoxicam. In contrast, the effects of other intravenously pre-administered NSAIDs were: 47 ± 5% reduction for 1 mg/kg of ketoprofen [34]; 27 ± 3% reduction for 1 mg/kg of indomethacin [31]; 21 ± 6% reduction for 1.5 mg/kg of diclofenac [32] and no effect for 1 mg/kg of niflumic acid [35] under the same experimental conditions. However, for higher doses, the effects of lornoxicam were comparable to those obtained previously with other intravenously pre-administered NSAIDs, a 50–70% reduction in the number of c-Fos-LI neurons. The marked effects of lornoxicam in the carrageenan model of inflammatory nociception are in keeping with previous animal studies showing lornoxicam to be 10-fold more active than tenoxicam as an inhibitor of carrageenan- or Freund's adjuvant-induced peripheral oedema [2]. In addition, lornoxicam has been shown to have a marked analgesic activity in the acetylcholine-induced writhing test in the mouse, with activity approximately 10-fold greater than tenoxicam [2]. Furthermore, the clinical trials demonstrated that lornoxicam (a single dose of 8 mg p.o.) tended to give better pain relief than aspirin (a single dose of 650 mg p.o.) in patients after dental surgery [3].

In conclusion, spinal c-Fos protein expression was used as an indicator of spinal nociceptive transmission during carrageenan-induced inflammation. From our study, it appears that pre-administration of lornoxicam, a new NSAID, results in a strong reduction in nociceptive inflammatory processing, as shown by the correlation between the reduction in both the carrageenan-induced peripheral oedema and the number of spinal c-Fos-LI neurones. The relatively low doses of lornoxicam produced a marked reduction of both the peripheral oedema and the number of spinal c-Fos-LI neurons, with these effects more pronounced in comparison to those of other previously studied NSAIDs. It is likely that low-dose therapy with lornoxicam could produce profound anti-inflammatory/analgesic effects with less risk of the side effects of classical NSAIDs.

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