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Anti-inflammatory, Analgesic and Antipyretic Activities of Loxoprofen Sodium Given Intramuscularly in Animals

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The evaluation of the anti-inflammatory, analgesic and antipyretic activities of loxoprofen sodium given in intramuscular route was investigated as compared to oral application in rats and mice. The intramuscular ED_{50} values of loxoprofen sodium in carrageenan edema and vascular permeability tests are 1.15 and 7.8 mg/kg, respectively, which represent more potent than in case of oral application. Its therapeutic effects in adjuvant arthritis were shown at 6 mg/kg i.m. and 3mg/kg p.o. Analgesic effect was shown to be more potent as given intramuscularly. Similar potency of antipyretic effects was shown in both administration routes. Considerably weak gastric damages were observed in intramuscular application.

Key words: Loxoprofen sodium, Anti-inflammatory, Analgesic, Antipyretic action, Gastric irritation, i.m. injection

INTRODUCTION

Loxoprofen sodium is clinically used as an anti-inflammatory, analgesic and antipyretic agent. The chemical structure is sodium 2-[4-(2-oxocyclopentan-1-ylmethyl) phenyl] propionate dihydrate which was first synthesized in the Central Research Laboratory of Sankyo Co. Ltd., Japan. It is characterized in potent analgesic action compared to the anti-inflammatory and antipyretic actions (Misaka *et al.*, 1981).

For alleviation or inhibition of the pain symptoms regardless of referred or superficial pain, the fast absorption of a drug and its immediate bioavailability might be generally required for its formulation or for development of any other new drugs.

Thus, this study was performed to evaluate the analgesic, anti-inflammatory and antipyretic effects of loxoprofen sodium as given in intramuscular and oral administration in rats and mice. The occurrence of gastric damages which is common in nonsteroidal anti-inflammatory drugs was also observed.

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MATERIALS AND METHODS

Drugs

Loxoprofen sodium was kindly supplied from Shinpoong Pharmaceutical Co. Ltd. The drug was dissolved in saline, and administered in a volume of 0.2 ml/100g (b.w.) to rats and 0.05 ml/10g to mice in i.m. administration, and 0.5 ml/100g to rats and 0.1 ml/10g to mice in p.o. administration. The complete Freund's adjuvant (1 mg/ml of *Mycobacterium tuberculosis* H37 Ra in a mixture of paraffin oil and an emulsifying agent) was the product of Difco Laboratories (Detroit, USA). Carrageenan (Type IV), Brewer's yeast and lipopolysaccharide (LPS: from *Escherichia coli.* serotype O26:B6) were purchased from Sigma Chem. Co. (St. Louis, USA). Acetic acid and formalin were the products of Duksan Chem. Co. (Korea), and other drugs used were either pharmaceutical or reagent grade.

Animals

Male Sprague-Dawley rats weighing 150-180 g and male ICR mice weighing 18-25 g were supplied from breeding facilities of Natural Products Research Institute, Seoul National University. Solid food (Samyang Yuji Co. Ltd.) and tap water were provided *ad libitum*. All animals were housed for 1 week in a controlled 12 h light-dark

environment at 2 ± 21°C.

Anti-inflammatory effect

Carrageenan-induced paw edema in rats

Each groups of 10 rats was used. The test was conducted according to the method of Winter *et al.* (1962). In brief, the rats were fasted for 16 h before the test and then edema was induced by s.c. injection of 0.1 ml of 1% carrageenan solution in the right hindpaw. The paw volume was measured using a plethysmometer (Ugo Basile, Varese, Italy) before, 0.5, 1, 2 and 3 h after carrageenan injection. The drug was administered 30 min prior to carrageenan injection.

Vascular permeability test in mice

Each groups of 6 mice was used.. The test was carried out according to the method of Wittle (1964). In brief, the mice were fasted for 16 h before the test. At 20 min after drug administration, 0.1 ml/10 g of 1% Evan's blue solution was injected i.v. and immediately 0.1 ml/10 g of 0.6% acetic acid solution was injected i.p. Twenty min later, the mice were sacrificed by cervical dislocation and the peritoneal cavity was washed out with i.p. injection of 0.9% saline to make 10 ml. The washings were centrifuged and the optical density of the fluid was read at 610 nm in a spectrophotometer (Molecular Devices, USA).

Adjuvant induced arthritis in rats

Each groups of 8 rats was used. The test was carried out according to the method of Claude *et al.* (1969). Arthritis was induced by subplantar injection of 0.1 ml of Complete Freund's Adjuvant into the right hind paw. The drugs were given i.m. or p.o. twice a day from the 18th day to 25th day after adjuvant injection for determination therapeutic effect. Arthritis was assessed by measuring the paw swelling with a plethysmometer (Ugo Basile, Varese, Italy) throughout a period of 25 days. Percent inhibition of swelling was calculated from the following formula.

$$\text{Percent inhibition} = \left(1 - \frac{\text{Volume of hindpaw at the 18th day}}{\text{Volume of hindpaw at the 25th day}}\right) \times 100$$

Analgesic effect

Acetic acid-induced writhing syndrome in mice

Each groups of 8 rats was used. The test was conducted according to the method of Koster *et al.* (1959). In brief, 30min after drug administration, 0.1 ml/10 g of 0.7% acetic acid-saline was injected i.p. Ten min later, the number of writhes for 10 min was counted.

Randall-Selitto assay in rats

Each groups of 7 rats was used. The test was conducted according to the method of Randall and Selitto (1957) by blind procedure. The rats were injected s.c. with 0.1ml of 20% yeast suspension into the hind paw 30 min after drug administration. The pressure was given with an analgesymeter (Ugo Basile) on the paw with increasing pressure, and the weight that induced pain reaction to the rat was recorded as a pain threshold. Pain threshold was measured before, 0.5, 1, 2 and 3 hr after yeast injection. According to a description by Swingle *et al.* (1971), the rats in the drug-treated groups were designated as "protected", if the individual reaction threshold to the pressure exceeded the control group mean threshold by two standard deviations of that mean.

Antipyretic effect in rats

Each groups of 9 rats was used. According to the method of Marshall *et al.* (1994), the rats were given the drug and 1 h later, 50 g/kg of LPS was injected i.v. to induce fever. The rectal temperature was measured before, 1, 2 and 3 h after febrile treatment.

Gastric lesion in rats

Each groups of 7 rats was used. According to the method of Melarange *et al.* (1994), rats were deprived of food but allowed free access to water 16hr before the experiment and 3.5 h after drug administration, the rats were sacrificed by ether anesthesia. The stomach of each rat was removed and cut along the greater curvature, the length (mm) of gastric lesion was measured under a magnifying glass (×5). The sum of lengths of all lesions in each stomach was expressed in terms of lesion index. The dose-response of lesion indices showed linearity and 50% lesion-inducing dose (median irritative dose, IrD_{50}) was calculated by the method of Litchfield-Wilcoxon (1949).

Statistical analysis

All data represent means ± S.E. Statistical analyses of the data were performed using analysis of variance followed by Student's *t*-test. All data were evaluated at the $p<0.05$ level of significance.

RESULTS

Anti-inflammatory effect

Carrageenan-induced paw edema

Table I shows the changes in paw volume at 0.5, 1, 2, 3 h after carrageenan injection. The inhibitory effect of the drug was the strongest at 2 h after carrageenan injection, with the inhibition of 44.7, 52.1 and 68.1% at 0.5, 1.5 and 4.5 mg/kg i.m., respectively. From the dose response line of the inhibition percents at 2 h after edema induction, its median inhibition dose (ID_{50}) was 1.15 mg/kg i.m. (Fig. 1), and at 1.8 mg/kg p.o., inhibition was 48.6 %.

Table I. Effect of loxoprofen sodium on carrageenan-induced paw edema

Treatment	Dose (mg/kg)	No. of rats	Increase percent of paw volume (mean ± S.E.)			
			0.5	1	2	3 h
Control	-	10	27.6 ± 3.5	41.0 ± 4.2	89.0 ± 8.6	99.6 ± 7.6
Loxoprofen sodium (*im*)	0.5	10	20.0 ± 3.1(27.4)	30.2 ± 4.6 (26.3)	49.3 ± 6.2** (44.7)	85.0 ± 6.3 (14.6)
	1.5	10	18.1 ± 2.7* (34.3)	27.3 ± 3.2* (33.5)	42.6 ± 4.8** (52.1)	70.1 ± 8.7* (29.6)
	4.5	10	16.6 ± 2.2* (39.8)	21.7 ± 1.7** (47.2)	28.4 ± 2.3** (68.1)	41.3 ± 4.3** (58.6)
Loxoprofen sodium (*po*)	1.8	10	21.62.3 (21.5)	29.4 ± 2.5* (28.2)	45.8 ± 7.0* (48.6)	76.9 ± 10.7 (22.7)

The values in parentheses indicate percent inhibition. *p<0.05, **p<0.01; Significantly different from control group.

Vascular permeability test

Table II shows the effect on the vascular permeability in mice. The ID_{50} at i.m. and p.o. dose of loxoprofen sodium was 7.8 mg/kg and 8.1 mg/kg, respectively, which indicates that the vascular permeability of the drug by inhibitory of i.m. administration was a little stronger than that of p.o. administration.

Adjuvant arthritis

Table III shows the therapeutic effect on adjuvant-induced arthritis in rats. At the doses of 0.7, 2, 6 mg/kg i.m., the inhibition rates were -9.1, 29.7, 44.5%, respectively, and at a dose of 3 mg/kg p.o., the inhibition was 55.2%. Indomethacin showed 48.1% of inhibition rate at a dose of 5 mg/kg p.o.

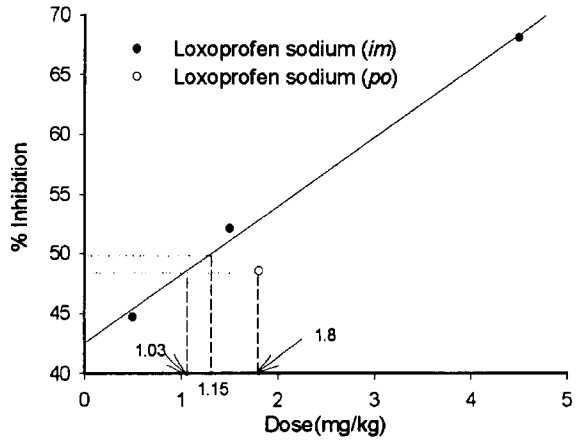

Fig. 1. Inhibitory effect of loxoprofen sodium on carrageenan-induced paw edema in rats (n=10).

Analgesic effect

Acetic acid-induced writhing syndrome

Table IV shows the effect on writhing syndrome in mice. At the doses of 1, 3, 10 mg/kg i.m., the inhibition were 53.8, 64.2, 88.1%, and at the same p.o. doses, the inhibition was 46.7, 57.1, 79.6%, respectively. The ID_{60} was used for comparing effectiveness between i.m. and p.o., and each ID_{60} value was 2.3 mg/kg i.m. and 8.1mg/kg p.o., indicating that the potency in i.m. administration was 1.8 times stronger than that of p.o. route.

Randall-Selitto assay

The result is shown in Table V. An increase in pain threshold was the highest at 1 h after drug administration, and the analgesic effects which are expressed "protected" as described by Swingle *et al.* (1967) were 42.9, 57.1, 85.7% at 0.1, 0.5, 1.0 mg/kg i.m., and 14.3, 42.9, 57.1% at the same p.o. doses, respectively. In a separate three point assay, the ED_{50} values and 95% confidence limits for the drug were 0.17(0.06-0.49)mg/kg i.m. and 0.63 (0.13-3.02)mg/kg p.o., of which the potency in i.m. was 2.9 times stronger than that of p.o. route.

Antipyretic effect

The result is shown in Table VII. The inhibition rates of

Table II. Effect of loxoprofen sodium on vascular permeability

Treatment	Dose (mg/kg)	No. of mice	Evan's blue (/mouse; mean ± S.E.)	% Inhibition	ID_{50} (mg/kg)
Control	-	6	138.61 ± 7.8	-	-
Loxoprofen sodium (*im*)	1	6	105.21 ± 6.2	24.1	
	3	6	78.12 ± 1.9	43.7	7.8
	10	6	62.8 ± 6.3*	54.7	
Loxoprofen sodium (*po*)	1	6	99.31 ± 1.9	28.4	
	3	6	86.8 ± 7.8*	37.4	8.1
	10	6	62.5 ± 11.9*	54.9	

*p<0.05; Significantly different from control group.

Table III. Therapeutic effect of loxoprofen sodium on adjuvant-induced arthritis

Treatment	Daily dose (mg/kg)	No. of rats	Increased percent of paw volume (%, mean ± S.E.)	
			Day 18	Day 25
Control	0	8	47.1 ± 5.2	46.4 ± 5.5 (1.4)
Loxoprofen sodium (*im*)	0.7	8	40.7 ± 4.3	44.4 ± 6.2 (-9.1)
	2	8	38.6 ± 3.9	27.1 ± 4.6 (29.7)
	6	8	52.4 ± 5.2	29.1 ± 4.5* (44.5)
Loxoprofen sodium (*po*)	3	8	52.4 ± 7.5	23.5 ± 6.0* (55.2)
Indomethacin (*po*)	5	8	31.2 ± 7.3	16.2 ± 9.2* (48.1)

The values in parentheses indicate percent inhibition.
*p<0.05; Significantly different from pretreatment data in the same group.

Table IV. Effect of loxoprofen sodium on writhing syndrome

Treatment	Dose (mg/kg)	No. of mice	No. of writhing syndrome (mean ± S.E.)	%Inhibition	ID_{60} (mg/kg)
Control	-	8	24.0 ± 0.6	-	-
Loxoprofen sodium (*im*)	1	8	11.1 ± 4.7*	53.8	2.3
	3	8	8.6 ± 4.9*	64.2	
	10	8	2.9 ± 1.8**	88.1	
Loxoprofen sodium (*po*)	1	8	12.8 ± 3.4*	46.7	4.16
	3	8	10.3 ± 3.4**	57.1	
	10	8	4.9 ± 3.0**	79.6	

*p<0.05, **p<0.01; Significantly different from control group.

Table V. Analgesic effect of loxoprofen sodium in Randall-Selitto assay

Treatment	Dose (mg/kg)	No. of rats	Increase percent of pain threshold (mean ± S.E.)				Protected[a]
			0.5	1	2	3 h	
Control	-	7	8.4 ± 1.4	8.7 ± 1.5	8.0 ± 1.5	8.1 ± 1.5	-
Loxoprofen sodium (*im*)	0.1	7	16.9 ± 4.5	16.9 ± 2.0**	11.7 ± 1.6	12.8 ± 3.6	42.9
	0.5	7	14.4 ± 1.8*	22.2 ± 4.0**	12.9 ± 1.5*	12.6 ± 1.9	57.1
	1.0	7	20.2 ± 3.2**	24.4 ± 2.8**	13.5 ± 1.6*	12.7 ± 1.3	85.7
Loxoprofen sodium (*po*)	0.1	7	15.5 ± 2.6*	17.1 ± 2.2**	12.2 ± 2.1	11.2 ± 1.3	14.3
	0.5	7	15.3 ± 2.4*	21.4 ± 2.7**	17.4 ± 2.9*	12.3 ± 2.3	42.9
	1.0	7	19.4 ± 2.2**	23.9 ± 2.7**	17.4 ± 2.4**	13.5 ± 1.8*	57.1

a)The incidence of "protected" was estimated 1 h after drug administration.
*p<0.05, **p<0.01; Significantly different from control group.

Table VI. Antipyretic effect of loxoprofen sodium on LPS-induced fever

Treatment	Dose (mg/kg)	No. of rats	Increased body temp.[a] (°C) (mean ± S.E.)	% Inhibition
Control	-	12	0.66 ± 0.1	-
Loxoprofen sodium (*im*)	0.3	9	0.6 ± 0.1	9.0
	0.9	9	0.29 ± 0.1*	56.1
	2.7	9	0.25 ± 0.04*	62.1
Loxoprofen sodium (*po*)	1.0	9	0.27 ± 0.04*	59.1

a) Body temperature measured at 2 h after LPS-injection which showed highest body temperature in control group.
*p<0.01: Significantly different from control group.

the drug were 9.0, 56.1, 62.1% at 0.3, 0.9, 2.4 mg/kg i.m., respectively. At 1.0 mg/kg p.o., the inhibition rate was 59.1%.

Gastric damage

Table VI shows gastric lesion induction of the drug in rats. The incidences of erosion were 0, 28.5, 71.4, 85.7% at 3, 6, 12, 24mg/kg i.m., and 28.5, 57.1, 71.4, 85.7% at the same p.o. doses, respectively. Each IrD_{50} was 11 mg/kg i.m. and 9 mg/kg p.o.

Table VII. Irritative effect of loxoprofen sodium on gastric lesion

Treatment	Dose (mg/kg)	No. of rats	No. of rats with erosion	Lesion index	Incidence of erosion	IrD_{50} (mg/kg)
Control	-	7	0/7	-	-	-
Loxoprofen sodium (*im*)	3	7	0/7	0	0	11
	6	7	2/7	0.07 ± 0.04	28.5	(7.09-17.05)
	12	7	5/7	1.04 ± 0.2	71.4	
	24	7	6/7	1.23 ± 0.47	85.7	
Loxoprofen sodium (*po*)	3	7	2/7	0.61 ± 0.3	28.5	9 (5.14-15.75)
	6	7	4/7	1.64 ± 0.4	57.1	
	12	7	5/7	1.69 ± 0.2	71.4	
	24	7	6/7	3.99 ± 0.7	85.7	

DISCUSSION

Loxoprofen is one of the potent NSAIDs which has been used orally in clinic. However, no reports have been available in parenteral uses. Therefore, the anti-inflammatory, analgesic and antipyretic effects in i.m. application were evaluated in animals as compared with p.o. application.

It is revealed that the anti-inflammatory effects as evaluated by carrageenan edema, vascular permeability and adjuvant arthritis tests were shown to be more potent as given in intramuscular injection than in oral application. The analgesic effect as evaluated by a test of writhing syndromes in mice and Randall-Selitto test in rats were also confirmed to be more potent in intramuscular injection than in oral application. These results could be assumed that loxoprofen sodium might have better bioavailability in case of injection. However, its antipyretic effect did not show considerable differences between administration routes, even if the reason could not be explained at present.

Gastric irritation as side effect of loxoprofen was also slightly less in case of parenteral administration than oral application. The fact that its gastric irritation occurred in this study at a dose up to about 10 times the ED_{50} in edema test indicates loxoprofen sodium to have weak gastric damages. This is well coincided with the result by Misaka *et al.* (1981) in case of oral doses.

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