J. Pharm. Pharmacol. 1993, 45: 959–962
Received October 28, 1992

Effect of Fepradinol on Rat Hind Paw Oedema Induced by Several Inflammatory Agents

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Abstract—Fepradinol is an effective non-steroidal anti-inflammatory agent. The effect on rat paw oedema induced by various phlogistic agents was investigated. The inhibitory effect of fepradinol (25 mg kg^{-1}, p.o.) on dextran-induced oedema was nearly equal to that of cyproheptadine (10 mg kg^{-1}, p.o.). On oedema induced by platelet-activating factor only fepradinol (25 mg kg^{-1}, p.o.) and phenidone (100 mg kg^{-1}, p.o.) clearly inhibited the inflammatory process. Both the above induced oedemas are thought to be unrelated to prostaglandins in the rat system and therefore, the anti-inflammatory activity against them is not shared by selective cyclo-oxygenase inhibitors. Fepradinol (25 mg kg^{-1}, p.o.) displayed an inhibitory effect on the early and late stage of kaolin- and nystatin-induced oedemas in contrast with indomethacin (10 mg kg^{-1}, p.o.) and piroxicam (10 mg kg^{-1}, p.o.) which only inhibited the late stage. The results obtained in this study confirm that fepradinol is a potent anti-inflammatory agent and indicate that its mechanism of action is different from that of other anti-inflammatory compounds.

Fepradinol is a non-steroidal anti-inflammatory drug characterized by a remarkable activity in the acute inflammatory processes both after topical and systemic administration (Conde et al 1990; Massó et al 1990). It has also been shown that fepradinol has much lower gastric ulcerogenic effects in comparison with other active non-steroidal anti-inflammatory compounds tested. We have also shown the absence of systemic activity for this compound on the central and autonomic nervous systems (De la Fuente et al 1987).

The rat develops oedema easily in the loose subcutaneous connective tissue of its legs after local injection of some irritants, and the shape of its hind paws facilitates quantitative determination of the swelling. This model also allows the monitoring of the development of the process in time. It is probably for these reasons that the rat paw oedema is one of the most frequently used methods in anti-inflammatory drug research (Bonta 1969).

We have now examined the anti-inflammatory activity of fepradinol in the rat paw oedema test after the injections of several potent inflammatory agents which, when injected into the subplantar area of the rat hind paw, cause local oedema. However, the physiological or biochemical mechanisms of these oedemas and in consequence, their time course patterns, are not uniform. This indicates that their modes of action are dependent upon the synthesis or release of different inflammatory mediators. The results obtained in these experiments could help to elucidate the mechanism of action of fepradinol.

Materials and Methods

Materials

The following chemicals were used: fepradinol (ethyl-1-hydroxy-phenyl-2-(2-methyl-1-propanol)-amine) chlorhydrate was synthesized by Elmu S.A. Indomethacin (Impex Química), piroxicam (Chemo Ibérica), cyproheptadine chlorhydrate, phenidone and mepyramine maleate (Sigma). Dextran (Fluka), kaolin, nystatin and platelet-activating factor (1-α-phosphatidylcholine, β-acetyl-γ-*O*-alkyl, PAF, Sigma).

Animals

Male Wistar rats, 140–200 g, were housed in home cages with a 12 h light-dark cycle at 22 ± 1°C and 50 ± 10% humidity. They were fasted overnight (18 h) before use.

Induction and measurement of oedema

Groups of eight rats were used. Test compounds were administered orally as solutions or suspensions in an aqueous 5% Tween-80 vehicle, in a volume of 1 mL/400 g body weight, 30 min before the subplantar injections of irritants. Animals from control groups received the same volume of vehicle. Oedema was induced in the rats by subplantar injection of 0·1 mL of 5% dextran in 0·9% NaCl (saline), 20% kaolin, 15 000 units nystatin or 2 μg PAF, into the right hind paw. The volume of the injected paw was measured by means of a plethysmometer (Ugo Basile) before the oedema and at various time intervals after the injection. Hind-paw swelling was calculated as a percentage of the initial volume. The anti-inflammatory effect was expressed as the percentage inhibition caused by each compound in comparison with vehicle-treated animals.

Statistics

The values obtained were expressed as means ± s.e. Statistical significance of the differences between controls and treated groups was calculated using Student's *t*-test for unpaired samples.

Results

Dextran-induced oedema

Maximal swelling of the paw occurred about 30 min after the

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injection of dextran and then began to decline (Fig. 1). Indomethacin (10 mg kg^{-1}) did not inhibit significantly the dextran-induced oedema. In contrast, cyproheptadine (10 mg kg^{-1}) and fepradinol (25 mg kg^{-1}) were effective in inhibiting the inflammatory response elicited by dextran even at 30 min after injection (64·9 and 56·4% reduction, respectively), and this inhibition persisted until the end of the assay (Table 1). At the dose administered in this test the activity of fepradinol proved to be a little weaker than that of cyproheptadine. Mepyramine was only effective at 30 min after injection.

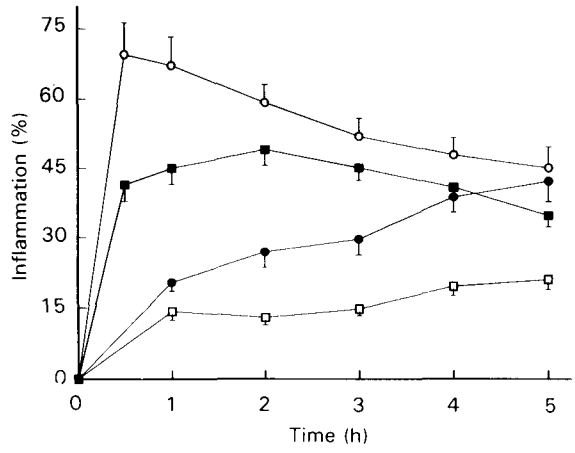

FIG. 1. Time course of oedemas produced by inflammatory substances after paw injection in male Wistar rats. ○ Dextran, ● kaolin, □ nystatin and ■ PAF. Each point represents the mean ± s.e. of eight rats.

Kaolin-induced oedema

Kaolin caused a swelling that developed slowly and peaked at 5 h (Fig. 1). The inhibitory effects of the drugs tested on kaolin-induced rat paw oedema are shown in Table 2. Fepradinol (25 mg kg^{-1}) was the most potent inhibitor of the early stage of the inflammatory response with an effect approaching 83% inhibition at 1 h. Indomethacin (10 mg kg^{-1}) and piroxicam (10 mg kg^{-1}) only inhibited significantly the inflammatory process from 3 to 5 h after the injection. This inhibition was of about 63 and 74% at 5 h, respectively. Cyproheptadine (10 mg kg^{-1}) had no effect in this model.

Nystatin-induced oedema

The intraplantar injection of 15000 units nystatin gave a slow rise in paw volume reaching a maximum after 5 h (Fig. 1). The anti-inflammatory effect of fepradinol (25 mg kg^{-1}) in this model was significant at 1 h after the injection of nystatin (72·6%), and persisted thereafter. In contrast, indomethacin (10 mg kg^{-1}), piroxicam (10 mg kg^{-1}) and cyproheptadine (10 mg kg^{-1}) began to show significant effects 3 h after nystatin injection which lasted until the end of the assay (Table 3).

PAF-induced oedema

Injection of 2 μg PAF induced an oedema of the rat hind paw that reached a maximum by 1 to 2 h and then gradually declined (Fig. 1). Fepradinol (25 mg kg^{-1}) was the most potent compound in inhibiting oedema formation in this test, with a percentage reduction of about 40% at all times tested. Among the other drug classes tested, only phenidone (100 mg kg^{-1}) was consistently effective against PAF-induced paw oedema. Indomethacin (10 mg kg^{-1}) and cyproheptadine (10 mg kg^{-1}) did not inhibit oedema formation. Piroxicam (10 mg kg^{-1}) showed an inhibitory effect from 3 to 5 h after injection (Table 4).

Discussion

Histamine, 5-hydroxytryptamine, bradykinin, lysosomal enzymes, PAF, in addition to prostaglandins, play an important role as chemical mediators in an inflammatory reaction. We investigated the effect of fepradinol on the oedema induced by several agents which develop inflammation by different mechanisms, to elucidate the anti-inflammatory activity and, if possible, the mode of action of this compound.

Fepradinol prevented or decreased signs of inflammation induced by all the inflammatory agents tested at an oral dose of 25 mg kg^{-1}, which is approximately the ED50 value obtained on the carrageenan-induced rat paw oedema model, as previously described (Conde et al 1990). Dextran is a polysaccharide of high molecular weight that induces an anaphylactoid reaction after intravenous injection into normal non-sensitized mice and rats, characterized by extravasation and oedema formation in the ears and extremities (Ankier & Neat 1972; Van Wauwe & Goossens 1989). Parratt & West (1958) showed that 5-hydroxytryptamine (5-HT) plays a major role in the anaphylactoid reaction produced in rats by the injection of dextran. The anti-5-HT compounds are effective against the dextran reaction even at

Table 1. Effect of fepradinol, indomethacin, cyproheptadine and mepyramine on dextran-induced paw oedema in rats.

Compound	Dose (mg kg^{-1})	% Increase in foot volume after dextran injection (% inhibition) 30 min	3 h	5 h
Control	—	69·5 ± 6·8	52·0 ± 3·9	45·3 ± 4·5
Fepradinol	25	30·3 ± 4·1 (56·4)**	33·8 ± 4·4 (35·0)*	31·3 ± 3·7 (30·9)*
Indomethacin	10	61·0 ± 5·9 (12·2)	50·2 ± 1·9 (3·5)	43·6 ± 3·3 (3·8)
Cyproheptadine	10	24·4 ± 4·6 (64·9)**	25·0 ± 3·3 (51·9)**	26·2 ± 3·3 (42·2)**
Mepyramine	30	42·3 ± 4·8 (39·1)**	45·2 ± 2·7 (13·1)	40·8 ± 2·1 (9·9)

Each value represents the mean ± s.e. of eight rats. Compounds were administered orally 30 min before dextran. Significant differences from control group determined by Student's *t*-test: * $P < 0·05$, ** $P < 0·01$.

Table 2. Effect of fepradinol, indomethacin, piroxicam and cyproheptadine on kaolin-induced paw oedema in rats.

Compound	Dose (mg kg^{-1})	% Increase in foot volume after kaolin injection (% inhibition) 1 h	3 h	5 h
Control	—	20·4 ± 1·8	29·7 ± 3·4	42·4 ± 4·4
Fepradinol	25	3·4 ± 1·2 (82·8)**	9·5 ± 2·2 (73·9)**	26·0 ± 2·9 (43·7)*
Indomethacin	10	18·0 ± 1·2 (12·2)	15·4 ± 2·5 (57·9)**	15·9 ± 5·8 (63·4)**
Piroxicam	10	15·7 ± 2·9 (25·5)	10·0 ± 1·7 (73·3)**	11·3 ± 1·6 (74·1)**
Cyproheptadine	10	19·3 ± 2·4 (6·9)	30·1 ± 2·6 (16·0)	42·0 ± 4·6 (1·2)

Each value represents the mean ± s.e. of eight rats. Compounds were administered orally 30 min before dextran. Significant differences from control group determined by Student's *t*-test: * $P < 0.05$, ** $P < 0.01$.

Table 3. Effect of fepradinol, indomethacin, piroxicam and cyproheptadine on nystatin-induced paw oedema in rats.

Compound	Dose (mg kg^{-1})	% Increase in foot volume after nystatin injection (% inhibition) 1 h	3 h	5 h
Control	—	14·3 ± 1·9	14·8 ± 1·4	21·2 ± 2·1
Fepradinol	25	3·6 ± 1·5 (72·6)**	5·1 ± 1·5 (64·2)**	7·7 ± 2·0 (63·2)**
Indomethacin	10	11·9 ± 2·3 (7·8)	5·7 ± 1·9 (56·9)**	7·6 ± 1·3 (64·7)**
Piroxicam	10	9·5 ± 1·2 (26·6)	4·7 ± 1·1 (65·7)**	6·5 ± 0·9 (68·6)**
Cyproheptadine	10	8·3 ± 2·3 (33·6)	7·3 ± 0·5 (43·1)**	12·2 ± 1·0 (51·2)**

Each value represents the mean ± s.e. of eight rats. Compounds were administered orally 30 min before dextran. Significant differences from control group determined by Student's *t*-test: * $P < 0.05$, ** $P < 0.01$.

Table 4. Effect of fepradinol, indomethacin, piroxicam, cyproheptadine and phenidone on PAF-induced paw oedema in rats.

Compound	Dose (mg kg^{-1})	% Increase in foot volume after PAF injection (% inhibition) 30 min	3 h	5 h
Control	—	41·5 ± 3·6	45·2 ± 2·7	34·9 ± 2·4
Fepradinol	25	22·0 ± 2·4 (47·0)**	28·1 ± 4·3 (37·7)**	21·0 ± 4·0 (39·8)**
Indomethacin	10	40·0 ± 2·1 (3·6)	44·3 ± 1·8 (1·9)	38·5 ± 2·2 (−10·3)
Piroxicam	10	35·6 ± 2·1 (14·2)	33·2 ± 1·3 (26·5)*	25·0 ± 1·6 (28·4)*
Cyproheptadine	10	39·5 ± 3·0 (4·8)	44·2 ± 2·5 (2·3)	37·8 ± 2·8 (−8·4)
Phenidone	100	25·9 ± 2·7 (37·6)**	35·4 ± 2·3 (21·6)*	26·2 ± 2·4 (28·9)*

Each value represents the mean ± s.e. of eight rats. Compounds were administered orally 30 min before dextran. Significant differences from control group determined by Student's *t*-test: * $P < 0.05$, ** $P < 0.01$.

small doses, most probably by the specific inhibition of locally released 5-HT (Ankier & Neat 1972). However, large doses of antihistamine substances are generally required, and even the relative effectiveness of such compounds appears to depend not so much on their antihistamine potency as on their ability to antagonize the actions of 5-HT. Thus, cyproheptadine in the present study was much more effective than mepyramine in preventing the anaphylactoid reaction, since the former possesses greater anti-5-HT activity than does the latter. Indomethacin did not exhibit significant activity in the dextran-induced oedema, in accordance with the results obtained by other authors who demonstrated that cyclo-oxygenase inhibitors were unable to attenuate the dextran response (Rovati et al 1979; Ito et al 1982). Fepradinol was a potent substance in the protection of rats against dextran oedema, a model of inflammation that is insensitive to cyclo-oxygenase inhibitors such as indomethacin.

Fepradinol was also active in a second model of the inflammatory process, kaolin-induced paw oedema. In this case, the incipient pattern of oedema is characterized by the participation of kinins. Fujiyoshi et al (1990) demonstrated that 1 min after the intraperitoneal injection of kaolin, kinins were produced in the peritoneal cavity of the mouse, and this production could continue even 60 min after the kaolin injection. Furthermore, the inhibition of kaolin-induced rat paw oedema by depletion of kinins and the detection of kinin activity in kaolin-injected rat air blebs, suggest a kinin involvement in the kaolin-induced response (Lewis et al 1976). Prostaglandins are likely mediators of the inflammatory response at the late stage of kaolin-induced oedema, since indomethacin inhibited this rat paw oedema and the accumulation of pleural fluid and white cell migration at 3 h in the rat pleurisy induced by kaolin (Lewis et al 1976; Kawamura & Oh-Ishi 1985). Neither the early nor the late phases of kaolin paw oedema were affected by either histamine and 5-HT depletion or antagonism, so that an involvement for these amines in this oedema is improbable (Lewis et al 1976). Using this test model, while the anti-inflammatory action of indomethacin and piroxicam was observed 3 h after kaolin challenge, the effect of fepradinol had already appeared within 1 h, suggesting that the inhibition of the kinin phase might be essentially responsible for such an effect. In agreement with other investigations,

cyproheptadine was not effective against the kaolin-induced swelling.

Nystatin is a polyene antibiotic that induces an oedema by its membrane labilizing action, thereby releasing hydrolytic enzymes which play an important role in promoting inflammation. It has been demonstrated that prostaglandins are also involved in this oedema (Niemegeers et al 1975). In this test, indomethacin and piroxicam prevented the oedema development, 3 h after injection and this effect lasted until the end of the assay, presumably blocking the action of prostaglandins. The significant inhibitory action of cyproheptadine 3 h after nystatin injection suggested the involvement of mast cell mediators in this model at that time. In contrast, the onset of the anti-inflammatory action of fepradinol took place within the first 60 min after injection and remained sustained thereafter, suggesting an inhibitory activity on all mediators involved.

PAF is a lipid mediator that has been considered to be involved in allergic and inflammatory reactions. Injection of PAF into the rat paw elicited an oedema characterized by a rapid onset with maximum effect by 1 to 2 h, and then gradually declined. Fepradinol, at an oral dose of 25 mg kg^{-1}, was clearly the most potent inhibitor of PAF-induced oedema. The onset of its anti-inflammatory action took place within the first 30 min after injection and remained sustained thereafter. We found indomethacin to be ineffective at doses higher than those required to inhibit carrageenan-induced oedema. These results agree with those found by other authors in rat and mouse paw oedema (Di Martino et al 1987; Calhoun et al 1987; Swingle & Reiter 1986) and in rabbit skin oedema (Peers & Flower 1987). Lipoxygenase derivatives seem to play a role in the inflammatory reaction triggered by PAF as demonstrated by Castro-Faria-Neto et al (1990) and Calhoun et al (1987) testing the lipoxygenase inhibitors, nordihydroguaiaretic acid and phenidone, respectively. In contrast, Swingle & Reiter (1986) found ineffective antihistamines and anti-5-HT compounds against PAF-induced rat paw oedema. In agreement with these investigations, we observed that cyproheptadine failed to inhibit the oedema, whereas phenidone significantly reduced the inflammatory process, demonstrating the involvement of lipoxygenase metabolites in this phenomenon. Nevertheless, a different result was obtained by Calhoun et al (1987) with PAF-induced mouse oedema which was sensitive to cyproheptadine.

In the inflammatory tests carried out in the present study, fepradinol exhibited a spectrum of activities different from the anti-inflammatory drugs used as references. In conclusion, although other studies have to be developed in order to confirm and establish the mechanism of the anti-inflammatory effect of fepradinol, the results obtained in these experiments point out that its mechanism of action is considerably different from classical non-steroidal anti-inflammatory drugs.

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