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The pharmacology of arachidonic acid-induced rat paw edema

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Abstract

Arachidonic acid (AA) injected into hindpaws of Lewis rats produces a severe edematous response. Treatment with corticosteroids (dexamethasone, prednisolone), dual inhibitors of arachidonate metabolism (phenidone, SK & F 86002), anti-histamine/serotonin agents (chlorpheniramine, cyproheptadine) and a gold compound (auranofin) inhibited AA-induced edema. In contrast, administration of high doses of cyclooxygenase inhibitors (indomethacin, piroxicam, naproxen, ibuprofen, meclofenamic acid and tiflamizole) did not affect AA-induced hind paw edema. The involvement of lipoxygenase products and mast cell mediators in the edematous response to arachidonic acid render this model potentially useful for studying antiinflammatory agents with a mechanism of action different from that of cyclooxygenase inhibitors.

Introduction

Both lipoxygenase and cyclooxygenase products of arachidonic acid metabolism have been implicated in acute and chronic inflammatory conditions including rheumatoid arthritis [1]. The recent description of the phlogistic activities of leukotrienes [2] have stimulated a search for agents that inhibit both the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism.

Although numerous animal models have been used in evaluating the antiinflammatory activity of cyclooxygenase inhibitors, there is a need for simple and reproducible *in vivo* assays which are sensitive to the antiinflammatory activity of lipoxygenase inhibitors. Since arachidonic acid-induced ear inflammation in mice has been reported to be sensitive in detecting the antiinflammatory activity of lipoxygenase inhibitors [3, 4], the present study was initiated to characterize the model of arachidonic acid-induced rat paw edema and evaluate its sensitivity to lipoxygenase inhibitors and other antiinflammatory agents.

The results of the present investigation demonstrate that the injection of arachidonic acid into the hind paw of rats induces a rapid, severe and consistent inflammation which is inhibited by dual inhibitors of arachidonate metabolism and corticosteroids but not by selective cyclooxygenase inhibitors.

Materials and methods

Paw edema

Paw edema was induced by a single subplantar injection of 0.10 ml of arachidonic acid in 0.2 *M* carbonate buffer (pH 8.43–8.56) into the right

hind paw of male Lewis rats (Charles River, Kingston, NY). The rats, weighing in the range of 144–241 g, were housed four per cage in a self-contained laminar flow room. Hind paw edema is defined as the difference in hind paw volume (ml), measured plethysmographically by water displacement prior to and after arachidonic acid injection.

Drug studies

Compounds were homogenized in aqueous 0.5% gum tragacanth and administered orally in a volume of 10 ml/kg b.wt. Unless noted otherwise, compounds and control vehicle (0.5% gum tragacanth) were administered 2 hours prior to arachidonic acid (0.50%) injection.

Statistical data analysis

The statistical significance of difference between drug-treated and control (vehicle-treated) animals was evaluated by the Dunnett's "t" test derived from 6 to 8 rats in drug-treated groups and 12 rats in vehicle-treated control groups.

Percent inhibition of edema was calculated by the following formula:

$$\% \text{ Inhibition} = 100 - \frac{\text{edema volume drug group} - \text{edema volume buffer control}}{\text{edema volume AA control} - \text{edema volume buffer control}} \times 100$$

Results and discussion

The subplantar injection of 0.5% arachidonic acid (AA) into the hind paw of Lewis rats produced significant edema within 5 minutes and reached peak response by 1 hour after injection (see Figure). One of the unique aspects of AA-induced rat paw edema was found to be its sensitivity to lipoxygenase inhibitors and resistance to selective cyclooxygenase inhibitors. Oral administration of indomethacin, piroxicam, naproxen, ibuprofen or meclofenamic acid did not significantly inhibit AA-induced edema (see Table) even when administered at dose levels several fold higher than

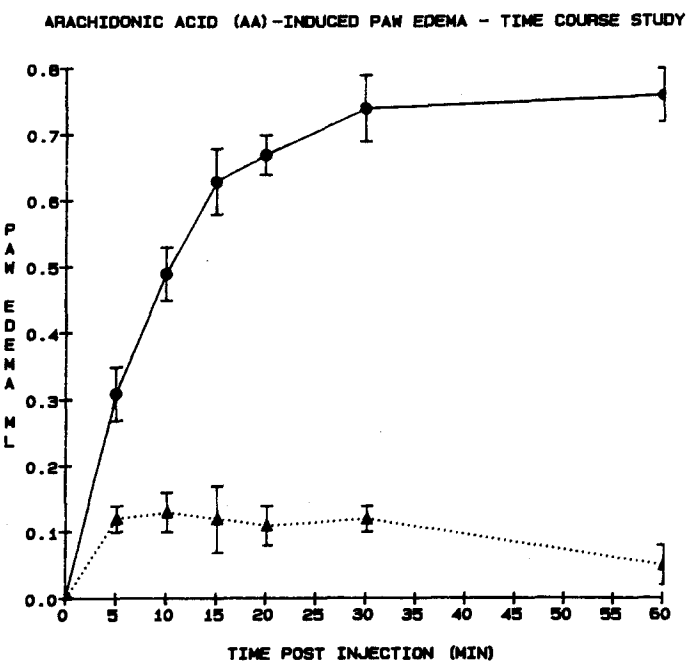

Figure
Temporal development of hindpaw edema induced by 0.5% arachidonic acid (–●–) or carbonate buffer (···▲···). Hind paw edema is expressed as mean ± S.D. of 8 rats in arachidonic acid group and 4 rats in carbonate buffer control group. Pre-injected paw volume (mean ± S.D.) = 0.96 ± 0.03 ml.

Table
Pharmacology of arachidonic acid-induced rat paw edema.

Compound	Dose (mg/kg, p.o.)	% Inhibition
I. Cyclooxygenase inhibitors		
Tiflamizole	1	10
Indomethacin	10	5
Piroxicam	20	12
Naproxen	40	12
Ibuprofen	120	12
Meclofenamic Acid	5	1
II. Corticosteroids and dual inhibitors of arachidonic acid metabolism		
Dexamethasone	0.1	38**
Prednisolone	17	40**
Phenidone[a]	40	32**
SK&F 86002	40	53**
III. "Disease modifying" antiarthritic agents		
Chloroquine	300	17*
D-Penicillamine	300	0
Auranofin	17	43**
IV. Anti-histamine and anti-serotonin agents		
Chlorpheniramine	25	38**
Cyproheptadine	25	37**

* $p<0.05$; ** $p<0.01$.
[a] 0.5 hour pretreat.

doses reported to be effective in carrageenan-induced rat paw edema [5]. In contrast, corticosteroids and the dual inhibitors of arachidonic acid metabolism, phenidone [6] and SK&F 86002 [6-(4-fluorophenyl)-2,3-dihydro-5-(4-pyridinyl) imidazo (2,1-b)thiazole] [7], markedly inhibited AA-induced paw edema.

The suppressive effects of chlorpheniramine and cyproheptadine (anti-histamine/serotonin agents) suggest that mast cell mediator release may contribute, at least in part, to AA-induced paw edema. Thus, the inhibitory effects of auranofin on AA-induced edema may be due to inhibition of mast cell mediator release since this compound has been reported to inhibit mast cell degranulation *in vitro* and *in vivo* [8]. Inhibition of mast cell release may also contribute to the antiedematous activity of dual inhibitors of arachidonate metabolism and corticosteroids in this model.

In conclusion, the arachidonic acid-induced paw edema in the rat is highly sensitive to inhibition by dual inhibitors of arachidonic acid metabolism and corticosteroids but is insensitive to selective cyclooxygenase inhibitors. Therefore, this model may provide a valuable tool for evaluating the *in vivo* antiinflammatory activity of lipoxygenase inhibitors and other agents with a mechanism of action different than cyclooxygenase inhibition.

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