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The topical anti-inflammatory effects of a topical preparation of meclofenamic acid on carrageenan-induced footpad swelling in mice

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A topical preparation of meclofenamic acid (Meclomen) was tested for anti-inflammatory activity in a murine model of carrageenan footpad oedema. The preparation significantly inhibited swelling when applied to the carrageenan-injected paw. Maximum inhibition was observed 4–5 h after carrageenan injection. The topical effects could not be attributed to systemic absorption because the preparation was more inhibitory when applied topically to the carrageenan-injected paw than to a distant site or orally.

Meclofenamic acid is a potent cyclooxygenase inhibitor which is used orally for a variety of inflammatory conditions (Wilkins 1978; Eberl & Dunky 1983; Petrick & Black 1983). Recent reports suggest that it may also be effective when administered topically in the treatment of psoriasis (Winthrop 1982; Ellis & Voorhees 1983). To investigate further the value of the drug, we prepared it as a topical preparation and tested it in murine carrageenan footpad oedema, a model of acute inflammation.

Materials and methods

Carrageenan paw oedema was induced in female C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME) as described by Levy (1969). Briefly, mice were injected intradermally in the left rear footpad with 50 μL of a 1% solution of carrageenan (FMC-Marine Colloids Division, Springfield, NJ) in 0·9% NaCl (saline), and in the contralateral footpad with 50 μL of saline. Meclofenamic acid (Meclomen, Warner-Lambert/Parke-Davis, Ann Arbor, MI) and indomethacin (Sigma, St Louis, MO) were dissolved in a vehicle containing 50% polyethylene glycol monolaurate, 10% H_2O, and 40% isopropanol. One hour after carrageenan injection, 50 μL of drug solution (or vehicle) was administered orally or topically by rubbing into the appropriate hindpaw for 15 s. Hindpaw swelling was assessed by a mercury plethysmograph (Buxco Electronics, Sharon, CN) at various times thereafter. Differences in volume between carrageenan- and saline-injected footpads were calculated for each experimental group (at least 8 mice per group). Each study was repeated at least once. Results are expressed as percent inhibition of swelling in drug-treated compared with vehicle-treated mice. Statistical differences between the experimental groups were determined by Student's *t*-test analysis.

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Results and discussion

Topical administration of meclofenamic acid or indomethacin to the carrageenan-injected footpad induced a dose-dependent reduction in swelling (Fig. 1). For these experiments, paw volume was measured 5 h after carrageenan injection. The effects of the meclofenamic acid preparation were maximal at the 3% concentration (49·7% inhibition). Peak inhibition was also observed for indomethacin at this dose (39·0% inhibition), although similar effects were also observed for indomethacin at the 0·3 and 1% concentrations. The topical effects of indomethacin were comparable with published results for rat carrageenan paw oedema (Wada et al 1982).

The duration of action of the topical preparation was determined by studying the effects of the 3 and 5% solutions of the drug on swelling over 10 h during which the inflammatory response remained relatively constant in the vehicle-treated mice. A 3% solution of indome-

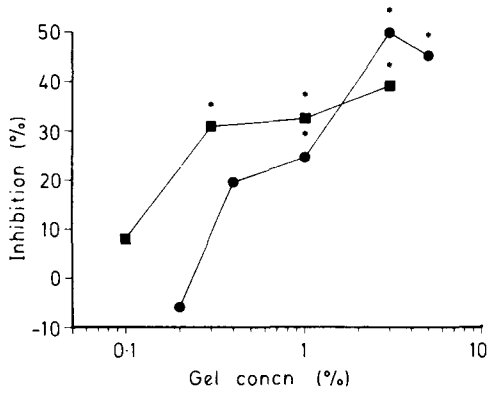

FIG. 1. Dose-response of topically-applied (●) meclofenamic acid and (■) indomethacin in mice with carrageenan footpad oedema. Results are expressed as the mean percent inhibition of oedema compared with vehicle-treated controls (* significant inhibition of oedema compared with vehicle-treated control mice, $P < 0·05$).

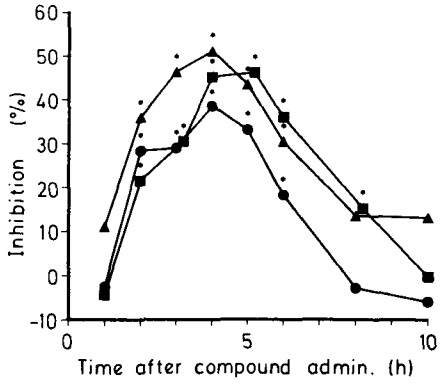

FIG. 2. Kinetic response of topically applied meclofenamic acid and indomethacin in mice with carrageenan footpad oedema. Results are expressed as the mean percent inhibition of oedema compared with vehicle-treated controls (*significant inhibition of oedema compared with vehicle-treated control mice, $P < 0.05$). Key: (●) 3% and (▲) 5% meclofenamic acid; (■) 3% indomethacin.

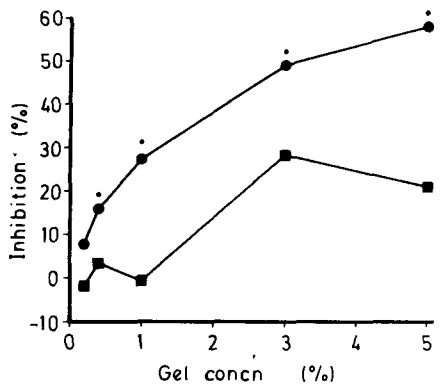

FIG. 4. A comparison of the anti-inflammatory effects of (■) orally and (●) topically applied meclofenamic acid to the carrageenan-injected paw. Results are expressed as the mean percent inhibition of oedema compared with vehicle-treated controls (*significantly greater inhibition when applied topically to the carrageenan-injected paw than orally at the same dose of the drug $P < 0.05$).

thacin was used for comparison. Both concentrations of meclofenamic acid inhibited swelling for 4–6 h beginning at 2 h (Fig. 2). A similar kinetic response was observed for indomethacin.

To determine whether the observed anti-inflammatory effect of the topical preparation was due to systemic absorption, two control studies were performed. In the first, the meclofenamic acid preparation was applied either to the carrageenan- or saline-injected paw and the effects on swelling were determined 5 h after carrageenan injection. Significantly greater inhibition was observed with the 1, 3, and 5% concentrations when these were applied to the carrageenan-injected compared with the saline-injected paw (Fig. 3), suggesting that the drug has significant local anti-inflammatory activity. To confirm these results, a second study was done in which the same protocol was used, except that the drug was administered either topically to the carrageenan-injected paw or orally. Inhibition of swelling in the topical treatment groups was significantly greater than that in the oral treatment groups at the 0·4, 1, 3, and 5% concentrations, which suggests that the topical effect cannot be explained entirely on the basis of oral absorption (as a result of preening) (Fig. 4). However, in several models of inflammation, including UV erythema and yeast pyresis, meclofenamic acid has been found to be extremely potent when administered orally 1 h before the inflammatory stimulus (Wax 1978). It is possible that prostaglandin-dependent events occur relatively early in the inflammatory response to carrageenan in mice. Therefore, if a cyclooxygenase inhibitor is given orally 1 h after carrageenan injection, prostaglandin-mediated inflammatory events may already have occurred. It is also conceivable, however, that the topical vehicle prevented optimal oral uptake.

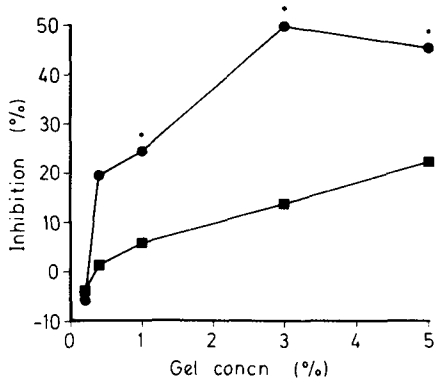

FIG. 3. A comparison of the topical anti-inflammatory effects of meclofenamic acid applied to the (●) carrageenan- or (■) saline-injected paw. Results are expressed as the percent inhibition of oedema compared with vehicle-treated controls (*significantly greater inhibition when applied to the carrageenan-injected than to the saline-injected paw at the same dose of drug, $P < 0.05$).

In conclusion, the results from the present study indicate that the topically applied meclofenamic acid has significant local anti-inflammatory activity. These effects cannot be explained on the basis of systemic absorption. Results from this study also indicate that murine carrageenan footpad oedema may be a useful model for development of novel topical anti-inflammatory agents.

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Effects of microiontophoretic pentobarbitone on conditioned inhibitions mediated by GABA-A receptors in the cuneate nucleus of the rat in-vivo

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We have studied the effects of microiontophoretic sodium pentobarbitone on the conditioned inhibition of the negative potential (N-wave) evoked in the cuneate nucleus of the rat by electrical stimulation (5 V, 0·2 ms, 0·5 Hz) of the ipsilateral forepaw. Five- or seven-barelled micropipettes were used, the tip being placed at a depth of 600–900 μm below the dorsal surface of the medulla oblongata. The conditioned inhibition was elicited by a previous identical stimulus. When the interval between the stimuli is shorter than about 40 ms (short duration) the inhibition is thought to be mediated by γ-aminobutyric acid (GABA), acting on GABA-A receptors. When it is longer (long duration conditioned inhibition) GABA-A receptors are not thought to be involved. Microiontophoretic sodium pentobarbitone potentiated both short (15 ms) and long (45 ms) duration conditioned inhibitions. The effect was current-dependent and appeared whether or not the first N-wave was depressed. Microiontophoretic application of (−)-bicuculline methiodide (a GABA-A antagonist) reduced the potentiation by pentobarbitone up to the basal inhibition when the interval between the stimuli was 45 ms or longer and to a greater extent when it was 30 ms or shorter. It seems likely that pentobarbitone prolongs the GABA-ergic mechanism which produces the short duration inhibition, making it visible with long stimulus intervals, superimposed upon the normal long duration conditioned inhibition which is not potentiated by local pentobarbitone.

Barbiturates potentiate inhibitions mediated by endogenous γ-aminobutyric acid (GABA) in the mammalian central nervous system, both in-vivo and in-vitro. Their mechanism of action appears to be the prolongation of the open time of the chloride channels associated with GABA-A receptors (Barker & Mathers 1981; Johnston & Willow 1982; Simmonds 1981). Ligand studies show that barbiturates inhibit the binding of [^{3}H]dihydropicrotoxinin, a non-competitive antagonist of GABA, to synaptic membranes (Olsen 1982; Ticku et al 1978). However, the actions of barbiturates in-vivo seem to be complex, judging from the variety of effects they cause (hypnotic, anaesthetic, anticonvulsant) (Enna 1981; Snodgrass 1983).

We describe here the effects of microiontophoretic sodium pentobarbitone, a hypnotic barbiturate, on a characterized and reproducible model of endogenous inhibition in-vivo: the inhibition by a previous stimulus (*conditioned* inhibition) of the negative potential (N-wave) evoked in the cuneate nucleus of the rat by electrical stimulation of the ipsilateral forepaw (Andersen et al 1970; Andres-Trelles et al 1976). When the interval between both stimuli is smaller than about 40 ms (*short duration* conditioned inhibition), the inhibition is due to GABA, since it is reduced by microiontophoretic GABA-A antagonists. When it is longer than 40 ms (*long duration*) the inhibition is resistant to GABA-A antagonists (Andres-Trelles et al 1976), as well as to antagonists of glycine, 5-hydroxytryptamine and histamine (unpublished observations). Nevertheless, the involvement of GABA in this long duration conditioned inhibition cannot be excluded, for it is now known that GABA receptors insensitive to (−)-bicuculline methiodide (GABA-B) are present in the cuneate nucleus of the rat (Orviz et al 1986).

Materials and methods

Male wistar rats (200–250 g) were anaesthetized with urethane (1·8 g kg^{-1} i.p.) or halothane (1–1·5% in 30% O_2 and 70% N_2O) and fixed in a stereotaxic frame. Two stainless steel electrodes placed in the centre pad of the paw and under the skin of the forelimb stimulated the forepaw at a rate of 0·5 Hz with supramaximal electric shocks (5 V), 0·2 ms wide. To study the conditioned

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