Relation between plasma concentration and therapeutic efficacy of a new anti-inflammatory compound, benoxaprofen (LRCL 3794) in rats with adjuvant-induced arthritis

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Benoxaprofen (2-(4-chlorophenyl)-α-methyl-5-benzoxazoleacetic acid, LRCL 3794) one of a series of 2-arylbenzoxazolealkanoic acids (Dunwell, Evans & others, 1975), has notable anti-inflammatory, analgesic and antipyretic activity in animal models (Cashin, Dawson & Kitchen, 1977). In an attempt to define the plasma concentration of the drug likely to be associated with therapeutic efficacy in rheumatoid patients, we have determined its plasma concentrations after repeated dosing in rats with chronic inflammation.

Developing adjuvant-induced arthritis in female Sprague Dawley rats was used as the animal model of inflammation (Newbould, 1963). Plasma concentrations were measured by ultraviolet spectrophotometry after extraction of the plasma with chloroform at pH 1. The specificity of the method was confirmed by g.l.c. using a flame ionization detector. Groups of nine rats received 16 daily oral doses of benoxaprofen at 1, 3, 10 and 33 mg kg^{-1}, as a suspension in 0·5% sodium carboxymethylcellulose, on the day before adjuvant injection and for 14 days after. Three rats at each dose were killed 2 h after the final dose and bled by cardiac puncture. The remaining six rats in each group were kept until the 18th day after adjuvant injection without further treatment with benoxaprofen. The therapeutic response has been expressed as the percentage inhibition of the integrated increase in volume of the hind feet of untreated rats, measured from day 10 to day 18 of the test. This period covered the development of secondary lesions of adjuvant arthritis.

The mean plasma concentrations in the rats 2 h after the final dose of benoxaprofen were proportional to the dose in the range 1–10 mg kg^{-1} day^{-1}. At 33 mg kg^{-1} day^{-1}, the concentrations were less than expected (169 ± 59 μg ml^{-1}).

An apparent linear relation was also observed between the logarithm of the 2 h plasma concentration measured on day 14 and the therapeutic response (Fig. 1). Previous studies (Chatfield & Green, unpublished observations) had shown that on multiple oral dosing at 10 mg kg^{-1} day^{-1} the 2 h plasma concentrations were closely similar to the 24 h trough values found immediately before the next dose. On this basis, Fig. 1 demonstrates that a 50% inhibition of secondary lesions was achieved at a trough plasma concentration of 12·5 μg ml^{-1}. A 30% and 70% inhibition was associated with trough concentrations of 4·6 and 35 μg ml^{-1} respectively. These results suggest that plasma concentrations of between 5 and 35 μg ml^{-1} should be suitable for the drug's clinical efficacy.

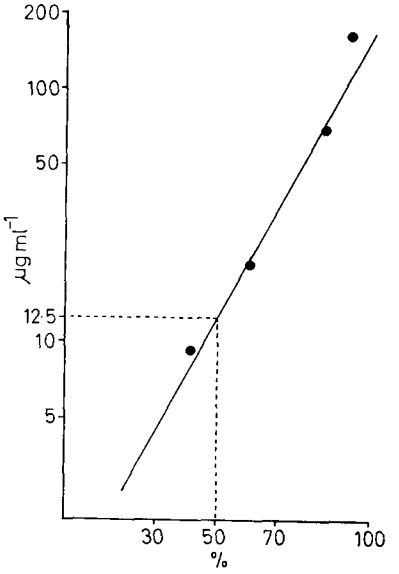

FIG. 1. Relationship of plasma concentrations of benoxaprofen (μg ml^{-1}) and therapeutic response in rats with adjuvant arthritis. Abscissa—% inhibition of increase in foot volume.

Benoxaprofen has a long plasma half-life in both rat (about 28 h) and man (about 33 h) and is strongly bound to plasma proteins in both species. The proportion bound is higher in man than in normal rats (respectively 99·8% bound at 40 μg ml^{-1} and 99·3% bound at 60 μg ml^{-1}; Tarrant & Green, unpublished observations). This species difference in protein binding may be significant if it is the free concentration of drug that is responsible for the biological activity. A higher total concentration of benoxaprofen will be required in human plasma than in rat plasma to achieve equal concentration of free drug. Species differences in binding sites (Witiak & Whitehouse, 1969) and changes in plasma proteins between normal and arthritic subjects (Weiner, Wood & Pearson, 1968; Clarke, Freeman & Pryse-Phillips, 1970) are other factors which may influence the activity of acidic anti-inflammatory compounds in different species. However, Mizushima (1968) has suggested that the strong non-specific protein binding properties of this class of compounds is an important factor for activity.

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Separate anti-inflammatory effects of indomethacin, flurbiprofen and benoxaprofen

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An important query about non-steroidal anti-inflammatory drugs is whether they have either a common single mode of action or different multiple interactions with various aspects of inflammatory responses. An inhibitory action on prostaglandin biosynthesis has been advanced as a mechanism for the first possibility (Vane, 1973). More recently evidence has accumulated that the drugs possess anti-inflammatory effects which appear to be independent of prostaglandin systems (Bonta, Bult & others, 1977; Crook, Collins & others, 1976). One of the most relevant is an interference with the emigration of leucocytes into inflammatory sites (Walker, Smith & Ford-Hutchinson, 1976a). In the present work the effects of three acidic non-steroidal drugs, indomethacin, flurbiprofen and benoxaprofen, on the production of prostaglandins and the migration of leucocytes into an inflammatory exudate *in vivo*, have been studied. All three drugs possess a similar spectrum of experimental anti-inflammatory activity (Glenn, Rohloff & others, 1973; Adams, McCullough & Nicholson, 1975; Cashin, Dawson & Kitchen, 1977) but differ in that the first two are potent inhibitors of prostaglandin synthetase activity *in vitro* (Crook & Collins, 1975) whereas benoxaprofen is only a weak inhibitor of the enzyme system (Cashin & others, 1977).

Indomethacin was obtained from Merck Sharp and Dohme. Benoxaprofen (2-[4-chlorophenyl]-α-methyl-5-benzoxazone acetic acid) was obtained from Dr W. Dawson, Lilly Research Centre, Ltd, Windlesham, Surrey, England, and flurbiprofen (2-[fluoro-4-biphenylyl] propionic acid) was obtained from Dr S. Adams, Boots Drug Co. Ltd, Nottingham, England. The 9 h sponge implantation technique, the estimation of leucocyte migration and prostaglandin-like activity in the sponges were as described previously (Walker & others, 1976a). Drugs were administered orally as a suspension in Tween 80 to groups, each of 5 rats, 1 h before sponge implantation.

The effects of the three drugs on prostaglandin accumulation and leucocyte migration into the 9 h sponge exudates are shown in Table 1. Indomethacin and flurbiprofen, which are potent inhibitors of prostaglandin synthetase *in vitro*, strongly inhibited the accumulation of prostaglandin activity *in vivo* in the sponges. This inhibition occurred at lower dosages than those required to inhibit white cell migration in the system and well below those required to inhibit other experimental models of inflammation (Glenn & others, 1973; Adams & others, 1975). Benoxaprofen only showed inhibition of prostaglandin production *in vivo* at doses equivalent to those required to inhibit white cell migration in the model and similar to those needed to affect other experimental models (Cashin & others, 1977). These findings demonstrate a lack of correlation

Table 1. *Effects of systemic administration of indomethacin, benoxaprofen and flurbiprofen in prostaglandin content (PG) and leucocyte migration (LM) in 9 h sponge exudates in the rat.*

Dosage mg kg^{-1}	Mean inhibition (% of control values†) Indomethacin		Benoxaprofen		Flurbiprofen	
	PG	LM	PG	LM	PG	LM
0·1	35	0	—	—	69*	28
0·3	53*	0	—	—	81*	52*
1·0	93*	18*	36*	26	85*	72*
3·0	97*	50*	68*	40*	96*	54*
9·0	98*	73*	82*	68*	—	—

* $P < 0.05$ from results of corresponding control group.
† In the sponge exudates of the corresponding control group, the mean prostaglandin-like content was 22 ± 8 ng ml^{-1} and the total leucocyte count was 658 ± 163 × 10^4 ml^{-1} (15 rats).

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