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Selective inhibition of arachidonate 5-lipoxygenase by novel acetohydroxamic acids: effects on acute inflammatory responses

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1 Two selective inhibitors of arachidonate 5-lipoxygenase, BW A4C and BW A797C, have been studied for their effects on acute inflammatory responses following oral administration to rats and mice.

2 The concentrations of the lipoxygenase product leukotriene B_4 (LTB_4) in 6 h inflammatory exudates, induced in rats by the subcutaneous implantation of carrageenin-soaked polyester sponges, were reduced dose-dependently by BW A4C ($ED_{50} = 2.6\,mg\,kg^{-1}$) or BW A797C ($ED_{50} = 14.3\,mg\,kg^{-1}$).

3 BW A4C and BW A797C had little or no effect on prostaglandin E_2 (PGE_2) concentrations in inflammatory exudates ($ED_{50}s > 100\,mg\,kg^{-1}$).

4 Doses of up to $200\,mg\,kg^{-1}$ of either BW A4C or BW A797C had no effect on carrageenin-induced oedema in rat paws.

5 BW A4C and BW A797C had little or no effect on carrageenin-induced hyperalgesia in rats or phenyl-benzoquinone-induced writhing in mice.

6 Yeast-induced pyrexia in rats was reduced by both BW A4C ($ED_{50} = 32\,mg\,kg^{-1}$) and BW A797C ($ED_{50} = 23\,mg\,kg^{-1}$).

7 The accumulation of leucocytes in sponge exudates was reduced dose-dependently by BW A4C ($ED_{50} = 54\,mg\,kg^{-1}$) and BW A797C ($ED_{50} = 16.7\,mg\,kg^{-1}$).

8 The selective lipoxygenase inhibitors BW A4C and BW A797C do not suppress inflammatory oedema or pain although they are anti-pyretic and they do inhibit leucocyte migration. There is not, however, a close agreement between these *in vivo* activities and their potencies as lipoxygenase inhibitors.

Introduction

Some of the products of arachidonate cyclo-oxygenase, such as prostaglandin E_2 (PGE_2) and prostacyclin are important inflammatory mediators and inhibition of their synthesis explains the therapeutic effects of the broad class of non-steroid anti-inflammatory drugs (for review see Higgs *et al.*, 1984).

The elucidation of a second oxidative pathway of arachidonic acid metabolism (Hamberg & Samuelsson, 1974) led to the discovery of the leukotrienes which are products of arachidonate 5-lipoxygenase (Samuelsson *et al.*, 1980). Leukotrienes, together with prostaglandins, have been detected in a range of inflammatory responses in animals and man. Some leukotrienes have inflammatory properties, notably leukotriene B_4, (LTB_4) which is a potent chemotactic agent for leucocytes (Ford-Hutchinson *et al.*, 1980), induces neutrophil-dependent increases in vascular permeability (Wedmore & Williams, 1981) and causes hyperalgesia (Levine *et al.*, 1984). LTC_4 and LTD_4, as well as being powerful bronchoconstrictors, also increase vascular permeability and may contribute to inflammatory oedema (Dahlen *et al.*, 1981). These observations led to the proposal that dual inhibitors of arachidonate cyclo-oxygenase and lipoxygenase would have a more comprehensive anti-inflammatory effect than selective cyclo-oxygenase inhibitors (Higgs *et al.*, 1979). This hypothesis

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was supported by the evidence that BW 755C, a dual inhibitor of prostaglandin and leukotriene production, had qualitatively different anti-inflammatory effects from indomethacin, a selective inhibitor of cyclo-oxygenase (Higgs *et al.*, 1979).

The absence of selective inhibitors of lipoxygenase which are active *in vivo* has, however, prevented an adequate investigation of the role of leukotrienes in inflammation. A novel series of acetohydroxamic acids has now been described which selectively inhibits arachidonate 5-lipoxygenase *in vitro* (Tateson *et al.*, 1988). We have taken two compounds from this series, BW A4C and BW A797C, and investigated their effects on acute inflammatory responses in rats and mice.

Methods

Induction of inflammatory exudates

Inflammatory exudates were induced and collected following the subcutaneous implantation of polyester sponges soaked in carrageenin (Sigma; 1% w/v in sterile saline) in groups of 5 male Wistar rats (190–210 g) (Salmon *et al.*, 1983). Drugs or vehicle were administered orally at the time of sponge implantation and sponges were removed after 6 h. Inflammatory exudates were squeezed from the sponges and total leucocyte numbers were counted. The concentrations of PGE_2 and LTB_4 were determined by specific radioimmunoassay as previously described (Salmon *et al.*, 1983).

Carrageenin-induced oedema

Oedema was induced in the right hind paws of groups of 5 male Wistar rats (140–200 g) by intraplantar injection of 0.1 ml 1% carrageenin (Winter *et al.*, 1962). Drugs or vehicle were administered orally immediately prior to paw injection. The thickness of the right hind paw was measured across the widest point, with dial calipers, at 1 h and 4 h after injection. Oedema was expressed as the increase in foot thickness between 1 h and 4 h.

Yeast-induced hyperthermia

Hyperthermia was induced in groups of 6 male Wistar rats (140–200 g) by the subcutaneous injection of $10\,ml\,kg^{-1}$ of 15% brewer's yeast suspended in distilled water (Loux *et al.*, 1972). Control groups received a similar volume of distilled water alone. Rectal temperatures were measured 18 h after injection in all groups using a Light Laboratories electric thermometer fitted with a rectal probe. Drugs or vehicle were administered orally and rectal temperatures were recorded 0.5, 1, 1.5, 3 and 4.5 h after dosing. Pyrexia was expressed as the difference in temperature between yeast- and water-treated animals at each time point.

Phenyl-benzoquinone-induced writhing

Writhing was induced in groups of 5 female CD-1 mice (Charles River, 20–25 g) by intraperitonal injection of $2.5\,mg\,kg^{-1}$ phenyl-benzoquinone (PBQ) (Hendershot & Forsaith, 1959). Drugs or vehicle were administered orally 30 min before injection of PBQ and writhes were counted for individual animals 10 min after PBQ for a period of 2.5 min.

Carrageenin-induced hyperalgesia

Hyperalgesia was induced in rat paws and measured by a modification of the technique of Randall & Selitto (1957). Carrageenin (0.1 ml, 0.1% in saline) was injected into the right hind paws of groups of 5 male Wistar rats (140–200 g). Drugs or vehicle were administered orally immediately prior to paw injection. At 1 h and 4 h after injection the sensitivity of the paw was assessed by applying a constant pressure of 20 mmHg to each paw with a syringe plunger driven by compressed air. The time required to produce a characteristic shivering reaction was recorded and compared with the reaction time prior to carrageenin injection. A decrease in reaction time indicated an increase in hyperalgesia.

Drugs

BW 755C (3-amino-1-[*m*-(trifluoromethyl)phenyl]-2-pyrazoline) was synthesized by Dr F.C. Copp at the Wellcome Laboratories. BW A4C (N-(3-phenoxycinnamyl)-acetohydroxamic acid) and BW A797C (N-[3-(5,6,7,8-tetrahydro-2-naphthyl) prop-2-enyl]-acetohydroxamic acid) were synthesized as described by Jackson *et al.* (1988). Indomethacin was obtained from Merck Sharpe and Dohme. In all experiments, BW A4C and BW A797C were dissolved in polyethylene glycol 300 (BDH Chemicals) and administered orally; indomethacin and BW 755C were given in aqueous solutions.

Statistics

The activity of each drug was expressed as an ED_{50} value together with 95% confidence limits, calculated by linear regression analysis of data from drug-treated animals compared to vehicle-treated controls.

Results

Indomethacin ($1\,mg\,kg^{-1}$) selectively reduced the concentration of PGE_2 in 6 h inflammatory exudates

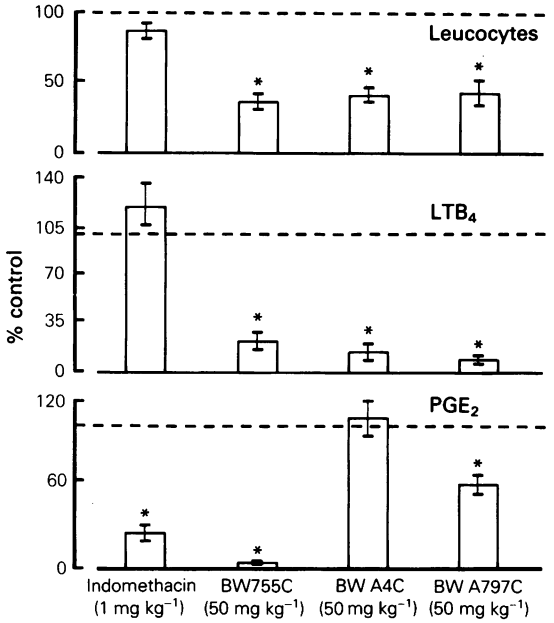

Figure 1 Effect of oral administration of drugs on total leucocyte numbers and concentrations of leukotriene B_4 (LTB_4) and prostaglandin E_2 (PGE_2) in 6 h inflammatory exudates. Each column represents the mean of 10–15 values obtained in 2–3 separate experiments and the bars represent ± 1 s.e. mean. * $P < 0.05$ compared to control values.

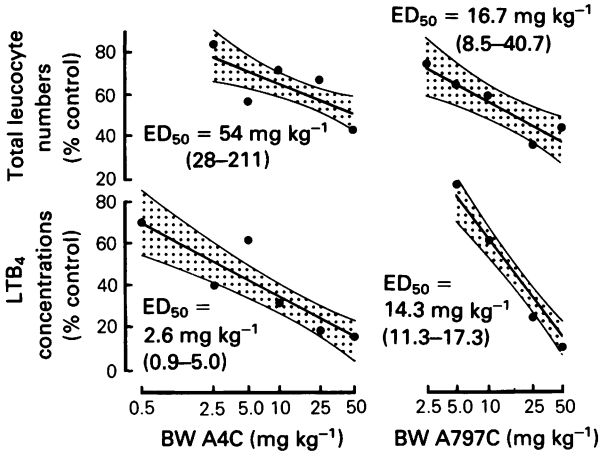

Figure 2 Effect of oral administration of BW A4C or BW A797C on total leucocyte numbers and leukotriene B_4 (LTB_4) concentrations in 6 h inflammatory exudates. Each point represents the mean of 5–15 values obtained in 1–3 separate experiments. The lines were fitted by linear regression analysis and the shaded areas represent the 95% confidence limits.

but did not significantly change LTB_4 concentrations or total leucocyte numbers (Figure 1). BW 755C (50 mg kg^{-1}), however, caused a reduction in the synthesis of both eicosanoids and suppressed the accumulation of leucocytes (Figure 1). In contrast, BW A4C and BW A797C reduced LTB_4 concentrations and leucocyte numbers but had little or no effect on PGE_2 concentrations (Figure 1). The effects of BW A4C and BW A797C on leucocyte migration were dose-dependent (Figure 2) but did not correlate closely with inhibition of LTB_4 production. LTB_4 was reduced by up to 95% by both compounds but reduction of leucocyte migration did not exceed 65%.

Indomethacin and BW 755C caused a dose-dependent reduction in carrageenin-induced oedema (Table 1). BW A4C and BW A797C, however, had no effect on oedema at doses of up to 200 mg kg^{-1}. Furthermore, combinations of BW A4C (50 mg kg^{-1}) and indomethacin (0.5–5.0 mg kg^{-1}) did not significantly reduce the ED_{50} value for indomethacin alone.

Writhing in mice, induced by intraperitoneal injections of PBQ, was partially inhibited by BW 755C

Table 1 Effect of drugs on acute inflammatory responses

Inflammatory response	ED_{50} (mg kg^{-1}, p.o.) (95% confidence limits)			
	Indomethacin	*BW 755C*	*BW A4C*	*BW A797C*
Carrageenin-induced oedema (rat)	5.7 (3.4–15.0)	17.0 (6.7–28.0)	>200	>200
Carrageenin-induced hyperalgesia (rat)	3.5 (0.38–6.7)	31.1 (10.3–89.9)	233 (no limits)	>200
PBQ-induced writhing (mouse)	1.3 (0.7–1.8)	39.8 (32.4–47.6)	>200	224.5 (199.4–255.5)
Yeast-induced hyperthermia (rat, 1 h)	1.56 (no limits)	4.15 (1.92–6.31)	37.0 (26.8–47.1)	21.6 (11.7–29.7)

PBQ = phenyl-benzoquinone

(50 mg kg^{-1}) and BW A797C (200 mg kg^{-1}) but BW A4C (200 mg kg^{-1}) had no significant effect (Table 1). Carrageenin-induced hyperalgesia was reduced by indomethacin and BW 755C but BW A4C and BW A797C were inactive at doses up to 200 mg kg^{-1} (Table 1).

All the drugs tested reduced yeast-induced hyperthermia in a dose-dependent manner (Table 1). The onset of action of indomethacin appeared to be slower than that of the lipoxygenase inhibitors.

Discussion

These results demonstrate that BW A4C and BW A797C selectively inhibit the synthesis of LTB_4 at a peripheral site of inflammation following oral administration. The ED_{50} values are comparable with those obtained for the same two compounds against LTB_4 production in whole blood *ex vivo* (Tateson *et al.*, 1988). In this study BW A4C is also more potent as a lipoxygenase inhibitor than BW A797C.

The lipoxygenase inhibitors suppress the accumulation of leucocytes in inflammatory exudates and this may be due to the inhibition of the synthesis of LTB_4, which is a potent chemotactic factor (Ford-Hutchinson *et al.*, 1980). The inhibition of leucocyte migration cannot, however, be directly correlated with the inhibition of LTB_4 (Figure 2). In common with BW 755C (Salmon *et al.*, 1983), the selective lipoxygenase inhibitors BW A4C and BW A797C had a greater effect on LTB_4 synthesis than on leucocyte migration. Doses which prevented LTB_4 synthesis by 80–90%, only suppressed leucocyte migration by 50–65% (Figure 2). The lack of a direct correlation between inhibition of LTB_4 synthesis and reduction of leucocyte migration is difficult to interpret. It is possible that different acetohydroxamic acids penetrate from the plasma to the inflamed tissue at different rates during the 6 h time course of the experiment. If LTB_4 is important in the initiation of cell migration and the drugs are not available in the relevant tissues at the beginning of the response, this may explain lack of drug activity. Alternatively, it has been suggested that LTB_4 is not a major chemotactic mediator in the rat (Foster *et al.*, 1986), which could account for the failure of lipoxygenase inhibitors to suppress leucocyte accumulation at doses which reduce LTB_4 synthesis.

The lipoxygenase inhibitors did not inhibit carrageenin-induced oedema in rat paws where the selective cyclo-oxygenase inhibitor indomethacin and the dual cyclo-oxygenase and lipoxygenase inhibitor BW 755C did reduce swelling in the period 1–4 h after injection of carrageenin. This reflects the importance of cyclo-oxygenase products such as PGE_2 and prostacyclin in the later phase of the response. Cyclo-oxygenase inhibitors do not, however, inhibit swelling in the first hour and their maximal effects on total paw oedema formed from 0–4 h do not exceed 55% inhibition even at doses which reduce prostaglandin synthesis by more than 95% (Winter *et al.*, 1962; Higgs *et al.*, 1976).

Carrageenin-induced swelling in the rat paw depends upon the presence of mediators other than prostaglandins, but also on the presence and activation of circulating leucocytes. Oedema is reduced by up to 80% in leucopenic animals (Higgs, 1984; García-Leme *et al.*, 1985). The failure of selective lipoxygenase inhibitors to reduce oedema in this model suggests, therefore, that leukotrienes do not contribute to increased vascular permeability either through a direct action on the vasculature or through the activation of leucocytes.

It is possible that BW A4C and BW A797C may reduce oedema in other models such as arachidonic acid-induced oedema in mouse ears. This response is reduced by lipoxygenase inhibitors and leukotriene antagonists but is also susceptible to a wide range of unrelated drugs (Carlson *et al.*, 1985). For this reason it is difficult to interpret results from the mouse ear model in terms of specific mechanisms of action.

Carrageenin-induced hyperalgesia and PBQ-induced writhing are reduced by cyclo-oxygenase inhibitors, again indicating that these responses are mediated by PGE_2 and prostacyclin. The lack of activity of the lipoxygenase inhibitors in these models also argues against a direct involvement of leukotrienes in inflammatory pain. It has been shown, however, that LTB_4 causes a leucocyte-dependent hyperalgesia in rat paws (Levine *et al.*, 1984). From the results reported in the present study, it would appear that in carrageenin-induced hyperalgesia there is a sufficient production of other hyperalgesic mediators to maintain the response in the absence of LTB_4 synthesis.

It is interesting that both BW A4C and BW A797C were effective against yeast-induced hyperthermia. In the case of BW A797C, the ED_{50} for inhibition of LTB_4 synthesis is not significantly different from the ED_{50} against pyrexia. With BW A4C, however, the dose required to inhibit LTB_4 synthesis was approximately 10 times less than that having an anti-pyretic effect. It cannot be discounted, therefore, that the anti-pyretic activity may be due to a property of these compounds other than lipoxygenase inhibition. Nonetheless, the possibility that lipoxygenase products may be pyretic agents is interesting and will be the subject of further investigation.

In summary, we have shown that selective inhibition of LTB_4 synthesis at a site of inflammation is

accompanied by reduced accumulation of leucocytes, although there is not a direct correlation between these parameters. With the exception of pyrexia, there was no reduction in other signs of acute inflammation. It would appear, therefore, that selective lipoxygenase inhibitors, would have little value as acute anti-inflammatory drugs.

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