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Selective inhibition of arachidonate 5-lipoxygenase by novel acetohydroxamic acids: effects on bronchial anaphylaxis in anaesthetized guinea-pigs

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1 The effect of a novel series of orally-active acetohydroxamic acid inhibitors of arachidonate 5-lipoxygenase on 'leukotriene-dependent' anaphylactic bronchoconstriction has been investigated in anaesthetized, pump-ventilated guinea-pigs actively sensitized to ovalbumin (OA). In a complementary series of experiments, the pharmacokinetics of these compounds in the plasma compartment following oral administration to guinea-pigs has also been investigated.

2 In animals pretreated with mepyramine ($2\,mg\,kg^{-1}$, i.v.) and indomethacin ($10\,mg\,kg^{-1}$, i.v.) and challenged with antigen aerosol (OA $10\,mg\,ml^{-1}$; 5 s) compounds BW A4C, BW A137C and BW A797C ($10–200\,mg\,kg^{-1}$, p.o., 1 h pre-challenge) markedly reduced that component of anaphylactic bronchoconstriction shown to be 'leukotriene-dependent'.

3 The maximum degree of inhibition (up to 75%) of 'leukotriene-dependent' anaphylactic bronchoconstriction by these three compounds was equivalent to that seen with the leukotriene antagonist FPL 55712 ($10\,mg\,kg^{-1}$, i.v.).

4 The peak levels of unchanged acetohydroxamic acids in the plasma compartment occurred 0.5 h after their oral administration and were as follows: BW A4C: 11.3 ± 3.9; BW A137C: 7.6 ± 2.4; BW A797C: $3.9 \pm 1.3\,\mu g\,ml^{-1}$ plasma.

5 The inhibition by BW A4C and BW A137C ($50\,mg\,kg^{-1}$, p.o.) of 'leukotriene-dependent' anaphylactic bronchospasm persisted for up to 3 and 4 h respectively but did not extend to 6 h. The decline in inhibitory activity paralleled the fall in the concentration of unchanged drug in the plasma compartment over this time period.

6 The results of the present study are consistent with BW A4C, BW A137C and BW A797C attenuating 'leukotriene-dependent' bronchial anaphylaxis in anaesthetized guinea-pigs by selective inhibition of arachidonate 5-lipoxygenase.

Introduction

Products of arachidonate 5-lipoxygenase (5-LO), in particular the peptido-leukotrienes, leukotriene C_4 (LTC_4) and LTD_4, have been implicated as mediators in the pathogenesis of asthma (Lewis, 1985). Thus, the prevention of leukotriene biosynthesis by inhibition of 5-LO activity represents a possible target for therapeutic intervention in this disease (Higgs & Moncada, 1985). Compounds BW A4C, BW A137C and BW A797C are novel acetohydroxamic acids which selectively inhibit 5-LO activity in human polymorphonuclear leucocytes *in vitro* and inhibit the synthesis of LTB_4 in human and rat blood *ex vivo* (Tateson *et al.*, 1988). In view of their potential as anti-asthmatic agents we have investigated the effect of BW A4C, BW A137C and BW A797C on pulmonary 5-LO activity *in vivo* after oral administration. This was done by assessing their inhibition of the previously characterized 'leukotriene-dependent' component of anaphylactic brochospasm in anaesthetized guinea-pigs following challenge with antigen in an aerosol form (Daffonchio *et al.*, 1987). To aid interpretation of the results the pharmacokinetics of the three acetohydroxamic acids were also investigated in this species following oral dosing.

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Methods

Sensitization procedure

Male Dunkin Hartley guinea-pigs (250–300 g) were actively sensitized to ovalbumin (OA; Grade V) by intraperitoneal injection of 50 mg of this protein, together with a further 50 mg given subcutaneously (each in 1 ml of 0.9% saline).

Antigen challenge

Fourteen to twenty one days after sensitization, animals were assigned randomly to treatment groups. All animals were fasted for 12 h immediately before oral dosing with acetohydroxamic acids but were allowed drinking water *ad libitum*. Approximately 30 min before antigen challenge each animal was anaesthetized (sodium pentobarbitone 60 mg kg^{-1}, i.p.) and mechanically ventilated (54 × 1 ml per 100 g body wt min^{-1}) through a mid cervical tracheostomy. Both cervical vagi were transected at the level of the neck. For antigen challenge, aerosols of OA (10 mg ml^{-1}) were generated and administered for 5 s by use of an in-line ultrasonic nebuliser as described previously (Payne & Nucci, 1987). This method was slightly modified so that pulmonary inflation pressure (PIP; an index of intrathoracic airway calibre) was measured from a lateral port close to the insertion of the tracheal cannula rather than from a port in between the ventilator and the nebuliser. Arterial (carotid) blood pressure (BP) and heart rate (HR) were measured as previously described (Daffonchio *et al.*, 1987). Typical resting values were; PIP: 10–15 cmH_2O, BP: 60/40 mmHg (systolic/diastolic) and HR: 250–270 beats min^{-1}. Unless otherwise stated, all animals were routinely pretreated 10 min before antigen challenge with the histamine H_1-antagonist, mepyramine (2 mg kg^{-1}, i.v.) and the inhibitor of arachidonate cyclo-oxygenase, indomethacin (10 mg kg^{-1}, i.v.). This was to antagonize the bronchoconstrictor effect of histamine released on antigen challenge and to inhibit the concomitant biosynthesis of bronchoactive cyclo-oxygenase products, thus revealing a residual rise in PIP that is predominantly leukotriene-mediated (Daffonchio *et al.*, 1987).

Determination of acetohydroxamic acids in plasma

Separate groups of animals from those challenged with antigen were used in these studies. However, they were prepared (fasted and dosed) and subsequently anaesthetized and mechanically ventilated in the same way. A single 4 ml sample of arterial blood was withdrawn from each animal via the carotid catheter at the requisite time after dosing, each animal contributing data for only a single time point. Two 1.5 ml volumes of the sample were transferred immediately to 1.5 ml Eppendorf tubes containing heparin (10 u ml^{-1}). After centrifugation (12,000 *g*, 3 min) the plasma fraction from each tube was aspirated into another 1.5 ml Eppendorf tube, kept on ice until the completion of the experiment and then frozen to await assay. Details of the respective high performance liquid chromatographic (h.p.l.c.) assays used to determine levels of unchanged BW A4C, BW A137C and BW A797C in plasma have been described previously (Tateson *et al.*, 1988). The limits

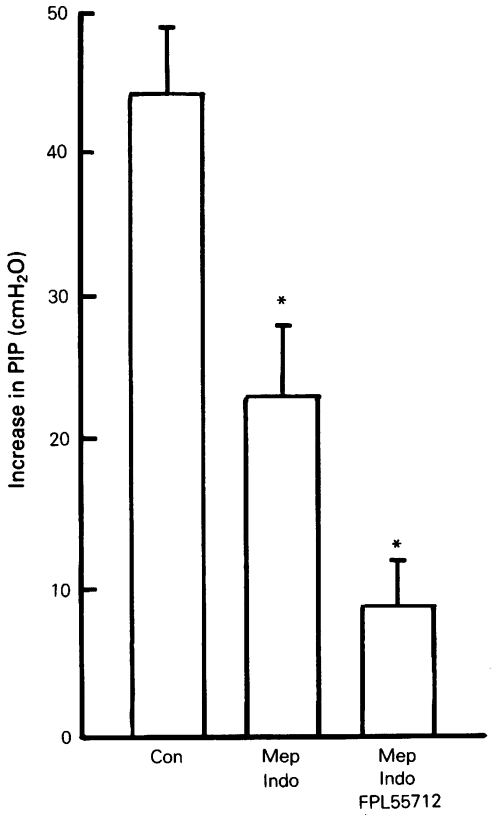

Figure 1 Pharmacological modulation of the peak instantaneous rise in pulmonary inflation pressure (PIP) measured in the first 10 min following a 5 s challenge of actively sensitized guinea-pigs with an inhaled aerosol of ovalbumin (10 mg ml^{-1}). Con = control, Mep = mepyramine, 2 mg kg^{-1}, i.v., Indo = indomethacin, 10 mg kg^{-1}, i.v.; FPL 55712 was used at a dose of 10 mg kg^{-1}, i.v. Each column represents the mean result from individual groups of 5 animals, vertical lines indicate s.e. mean. * $P < 0.05$ compared with the mean of the column to the immediate left.

of sensitivity for the assays for BW A4C and BW A797C were 0.1 $\mu g\,ml^{-1}$ plasma, and for BW A137C, 0.2 $\mu g\,ml^{-1}$.

Source and preparation of materials

The acetohydroxamic acids BW A4C (N-(3-phenoxy-cinnamyl)-acetohydroxamic acid), BW A137C (N-(4-benzyloxybenzyl)-acetohydroxamic acid) and BW A797C (N-[3-(5,6,7,8,-tetrahydro-2-naphthyl) prop-2-enyl]-acetohydroxamic acid) were prepared as described by Jackson *et al.* (1988), (see Tateson *et al.*, 1988 for structures). Indomethacin and OA were obtained from Sigma Chemical Co., Poole, Dorset. Mepyramine maleate and polyethylene glycol 300 (PEG 300) were obtained from BDH, Poole, Dorset. FPL 55712 (sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxy propoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxytate) was a gift from Dr P. Sheard, Fisons PLC, Loughborough.

BW A4C, BW A137C and BW A797C were each dissolved in 100% PEG 300 at drug concentrations of up to 80 $mg\,ml^{-1}$ in order to keep the dose volume administered constant at 2.5 $ml\,kg^{-1}$ p.o. Sodium pentobarbitone (60 $mg\,ml^{-1}$) was supplied as Sagatal (May and Baker, Dagenham, Essex) and thence diluted to 10 $mg\,ml^{-1}$ with 0.9% w/v NaCl solution (saline). Fresh stock solutions of other drugs were prepared daily as follows; mepyramine maleate 2 $mg\,ml^{-1}$ in saline; indomethacin 10 $mg\,ml^{-1}$ in 1 M Tris buffer pH 8.5 and FPL 55712 10 $mg\,ml^{-1}$ in twice distilled deionised H_2O. The dose volume used for intravenous administration was always 1 $ml\,kg^{-1}$.

Statistical analysis

Results are presented as mean ± standard error of the mean. Differences between means were analysed by Student's t test for unpaired data. A probability level of $P < 0.05$ was considered statistically significant.

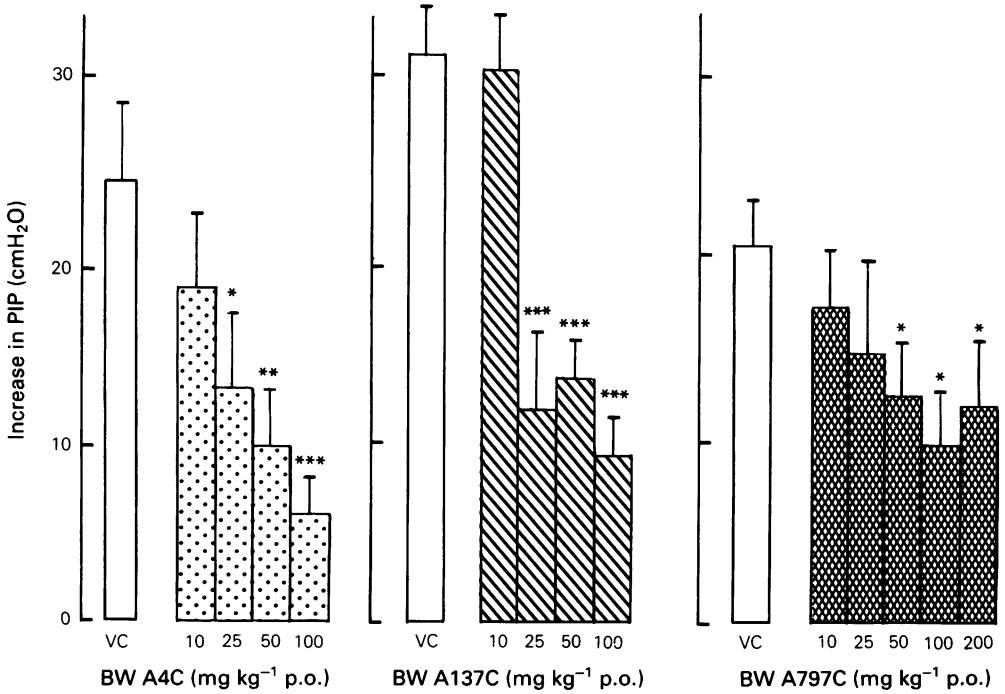

Figure 2 Effect of oral administration of increasing doses of BW A4C, BW A137C and BW A797C 1 h beforehand on 'leukotriene-dependent' anaphylactic bronchoconstriction in actively sensitized anaesthetized guinea-pigs following aerosol challenge (ovalbumin, 10 $mg\,ml^{-1}$, 5 s). All animals were additionally pretreated with mepyramine (2 $mg\,kg^{-1}$, i.v.) and indomethacin (10 $mg\,kg^{-1}$, i.v.). Each column represents the mean peak instantaneous rise in PIP measured in the first 10 min post-challenge in individual groups of 5–6 animals, vertical lines indicate s.e. mean. VC = vehicle (PEG 300) control, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$ versus VC in each case.

Results

Experimental model

In the absence of any drug pretreatment, aerosol challenge (OA, 10 mg ml^{-1}; 5 s) of actively-sensitized anaethetized guinea-pigs provoked rapidly developing (within 15 s), intense, bronchoconstriction. This was measured as an increase in PIP up to a maximum of 44 ± 4.0 cmH_2O; $n = 5$, occurring approximately 2 min post-challenge. Pretreatment with a combination of mepyramine (2 mg kg^{-1}, i.v.) and indomethacin (10 mg kg^{-1}, i.v.) increased the time to onset and slowed the subsequent rate of development of bronchospasm whilst reducing the maximum rise in PIP (occurring approximately 6 min post-challenge) to 23.5 ± 5.0 cmH_2O; $n = 5$ (Figure 1). This residual histamine and cyclo-oxygenase independent bronchoconstriction was markedly (>60%) reduced by additional pretreatment (1 min pre-challenge) with the leukotriene antagonist FPL 55712 (10 mg kg^{-1}, i.v.) following which the maximum rise in PIP was 8.0 ± 4.0 cmH_2O; $n = 5$ ($P < 0.05$). Cardiovascular effects (BP/HR) of aerosol challenge in the presence of mepyramine and indomethacin were usually minor in comparison with airway effects and for this reason will not be considered further.

Effects of BW A4C, BW A137C and BW A797C

When administered orally, 1 h before antigen challenge, BW A4C (10–100 mg kg^{-1}), BW A137C (10–100 mg kg^{-1}) and BW A797C (10–200 mg kg^{-1}) each inhibited the predominantly 'leukotriene-dependent' component of anaphylactic broncospasm in a dose-related manner (Figure 2) up to a maximum of 60–75%. This reduction was of the same order as that obtained with FPL 55712 (10 mg kg^{-1}, i.v.). The reduction in the maximum increase in PIP by the three inhibitors was associated with a slowing in the onset and subsequent rate of development of bronchoconstriction as shown in Figure 3, using the effect of BW A4C as a representative example. BW A137C (50 mg kg^{-1}, p.o.) did not modify histamine-induced bronchospasm whilst markedly reducing 'leukotriene-dependent' anaphylaxis provoked by subsequent antigen challenge in the same animals (results not shown). Neither BW A4C nor BW A797C was tested against histamine-induced bronchospasm.

Duration of action

In these studies a sub-maximal inhibitory dose (50 mg kg^{-1}, p.o.) of each 5-LO inhibitor was administered to separate animals at periods up to and including 6 h before challenge with antigen aerosol. Thus for each drug, single animals contributed data for one time point. As shown in Figure 4, the inhibitory effect of BW A4C and BW A137C at this dose on 'leukotriene-dependent' anaphylactic bronchoconstriction persisted for up to 3 and 4 h after dosing respectively. The inhibition seen with BW A797C at 1 h was not maintained to a statistically significant degree at 6 h. Data for intermediate time points with this compound were not obtained. The effect of BW A4C (50 mg kg^{-1}, p.o. 1–6 h) on the time course of the anaphylactic response is shown in Figure 5. Similar results (not shown) were obtained with BW A137C.

Pharmacokinetics

After oral administration of BW A4C, BW A137C, and BW A797C (50 mg kg^{-1}, p.o.) the peak level of

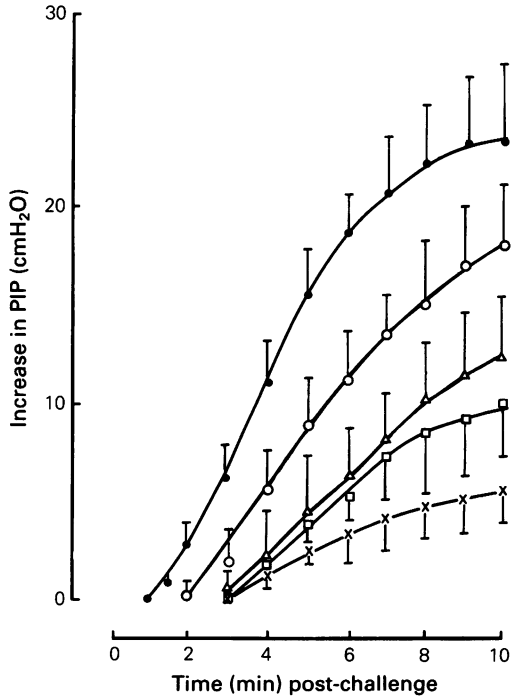

Figure 3 Effect of oral administration of increasing doses of BW A4C 1 h beforehand on the time course of the rise in PIP provoked by aerosol challenge (ovalbumin, 10 mg ml^{-1}, 5 s) of actively sensitized anaesthetized guinea-pigs pretreated with mepyramine (2 mg kg^{-1}, i.v.) and indomethacin (10 mg kg^{-1}, i.v.) (●) = Vehicle control; (○) = BW A4C, 10 mg kg^{-1}, p.o; (△) = BW A4C 25 mg kg^{-1}, p.o; (□) = BW A4C 50 mg kg^{-1}, p.o.; (×) = BW A4C 100 mg kg^{-1}, p.o. Each point represents the mean result from 5 animals per treatment group, vertical lines indicate s.e. mean.

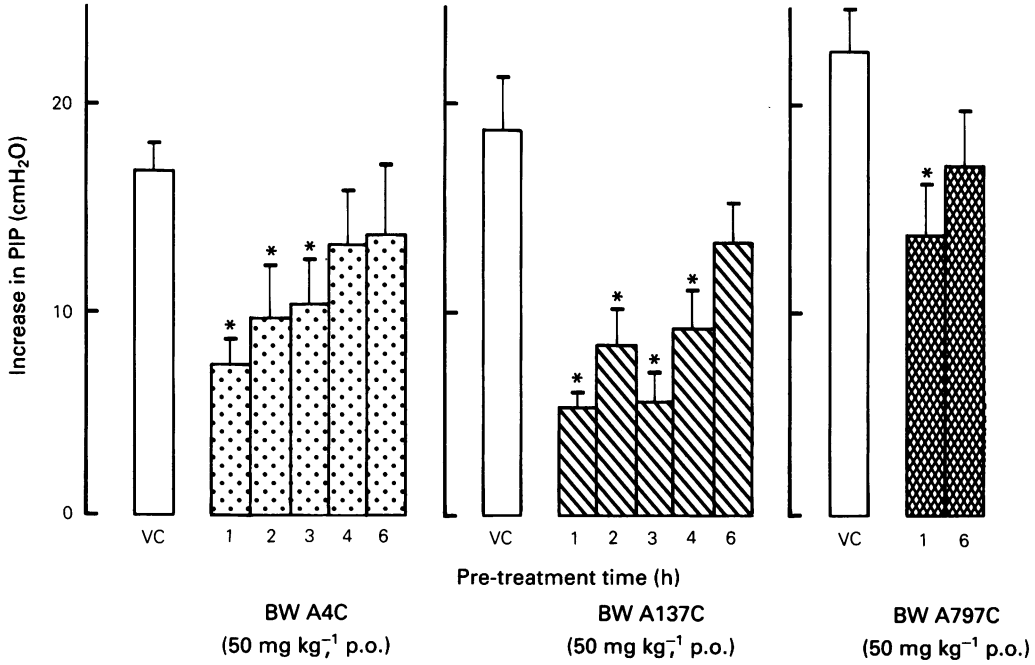

Figure 4 Effect of increasing the time of pretreatment from 1 to 6 h on the inhibition by BW A4C, BW A137C and BW A797C (each at 50 mg kg^{-1}, p.o. at time zero) of the peak instantaneous increase in PIP measured in the first 10 min following aerosol challenge (ovalbumin, 10 mg ml^{-1}, 5 s) of actively sensitized anaesthetized guinea-pigs. All animals received mepyramine (2 mg kg^{-1}, i.v.) and indomethacin (10 mg kg^{-1}, i.v.), 10 min before challenge. Each column represents the mean result from individual groups of 6 to 8 animals. Vertical lines indicate s.e. mean; VC = vehicle (PEG 300) control, * $P < 0.05$ versus VC.

drug found in the plasma compartment occurred at the 0.5 h time point (Figure 6). Actual values at this time were; BW A4C: $11.3 \pm 3.9\,\mu g\,ml^{-1}$; BW A137C: $7.6 \pm 2.4\,\mu g\,ml^{-1}$ and BW A797C $3.9 \pm 1.3\,\mu g\,ml^{-1}$ plasma. These values were only slightly reduced at 1 h but fell rapidly thereafter. By 6 h the concentration of all three drugs in the plasma compartment had fallen to between 0.1 and 0.4 of the level at 0.5 h. This finding is consistent with their reduced biological activity in our test model of anaphylaxis at the 6 h time point.

Discussion

There is experimental evidence to suggest that products of 5-LO may have a role in the pathogenesis of asthma (Lewis, 1985). For example both LTC_4 and LTD_4 are potent bronchoconstrictors in man (Holroyde *et al.*, 1981) and LTB_4, may contribute to airway hyperreactivity (O'Byrne *et al.*, 1985). However, the involvement of lipoxygenase products in human asthma can only be assessed by the action of compounds that either block their synthesis or specifically antagonize the receptors that mediate their actions. Such compounds must be not only safe and potent but also must have persistent action in the lungs, preferably after oral administration. BW A4C, BW A137C and BW A797C are potent, selective inhibitors of 5-LO and, after oral administration, have relatively persistent actions in the blood (Tateson *et al.*, 1988). The present series of experiments was carried out to investigate the effect of these acetohydroxamic acids in the lungs. For this reason we selected an animal model of anaphylactic bronchospasm previously characterized as being predominantly leukotriene-mediated and provoked by bronchial antigen challenge rather than systemic (intravenous) challenge (Daffonchio *et al.*, 1987). It is important to note, however, that the attenuation of bronchospasm by the leukotriene antagonist FPL 55712 (at a high dose) was incomplete. Thus in this experimental model, other anaphylactic mediators, e.g. platelet-activating factor (Darius *et al.*, 1986; Fitzgerald *et al.*, 1986), may play a lesser role. This is

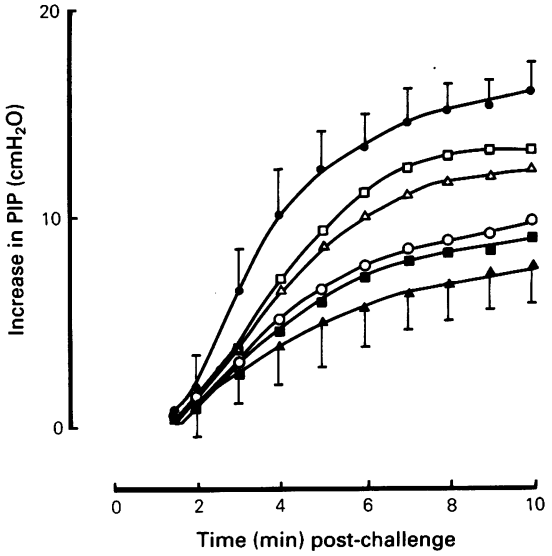

Figure 5 Effect of increasing the time of pretreatment from 1 to 6 h on the effect of BW A4C (50 mg kg^{-1}, p.o. at time zero) on the time course of the rise in PIP provoked by aerosol challenge (ovalbumin, 10 mg ml^{-1}, 5 s) in anaesthetized actively sensitized guinea-pigs. All animals received mepyramine (2 mg kg^{-1}, i.v.) and indomethacin (10 mg kg^{-1}, i.v.) 10 min before challenge. (●) = Vehicle (PEG 300) control; (▲) = BW A4C 1 h; (■) = BW A4C, 2 h; (○) = BW A4C, 3 h: (△) = BW A4C, 4 h; (□) = BW A4C, 6 h. Each point represents the mean result from 6 animals at each time interval, vertical lines indicate s.e. mean.

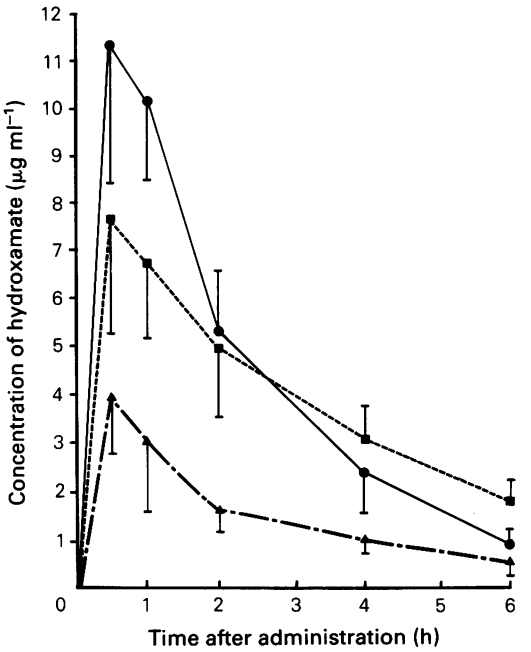

Figure 6 Concentrations of unchanged acetohydroxamic acids ((●) = BW A4C; (■) = BW A137C; (▲) = BW A797C) detected in the plasma of anaesthetized guinea-pigs at various times after their oral administration (50 mg kg^{-1} at time zero). Each point represents the mean ± s.e. mean (vertical lines) result obtained from individual groups of at least 4 animals at each time interval.

an essential consideration when assessing the maximum inhibition of 'leukotriene-dependent' anaphylactic bronchoconstriction found with BW A4C, BW A137C and BW A797C.

After oral administration (1 h beforehand) BW A4C, BW A137C and BW A797C (10–200 mg kg^{-1}) reduced 'leukotriene-dependent' anaphylactic bronchospasm to at least the same degree as the leukotriene antagonist FPL 55712. In view of the previously documented property of BW A4C, BW A137C and BW A797C as selective 5-LO inhibitors, both *in vitro* and *ex vivo*, it is reasonable to assume that they inhibit 'leukotriene-dependent' anaphylactic bronchospasm by this mechanism. This interpretation is supported by the related findings that one of this series of compounds (BW A137C; 10^{-4} M), neither antagonized LTD_4-induced contraction of guinea-pig isolated trachea nor did it affect binding of [^{3}H]-LTC_4 or [^{3}H]-LTD_4 to guinea-pig airway tissue (unpublished data). Thus LTC_4/LTD_4 receptor antagonism as an alternative mode of action of these compounds can be excluded.

The order of potency among the acetohydroxamic acids for inhibition of anaphylactic bronchospasm was BW A4C = BW A137C > BW A797C (approx. ED_{50} values: 27, 25 and 100 mg kg^{-1} respectively). The lower potency of BW A797C compared with either BW A4C or BW A137C is in agreement with the activities of these compounds *in vitro* (Tateson *et al.*, 1988) and the lower concentration of unchanged BW A797C attained in plasma compared with levels of BW A4C and BW A137C achieved after a similar dosing regime. The latter observation suggests either decreased bio-availability or increased metabolism of BW A797C. Interestingly, a different pattern of relative absorption and a different rank order of potency following oral dosing with these acetohydroxamic acids occurred in rats (Tateson *et al.*, 1988). This finding implies differences in absorption and metabolism of these compounds between species and possibly also between tissues (blood and lungs). The persistence of action of BW A4C and BW A137C (3–4 h) in guinea-pigs after a single administration of a sub-maximal inhibitory dose (50 mg kg^{-1}, p.o.) was

in parallel with the fall in concentration of unchanged drug in the plasma compartment over this time period. At 6 h after dosing there was no statistically significant reduction of 'leukotriene-dependent' bronchospasm although unchanged compound was still detectable in the plasma. The same was true for BW A797C.

In conclusion, BW A4C, BW A137C and BW A797C have oral activity in an *in vivo* model of pulmonary 5-LO activity. This activity is consistent with their previously reported *in vitro* and *ex vivo* properties of being selective 5-LO inhibitors. Present results show BW A4C and BW A137C to be more potent than BW A797C *in vivo* and that this activity persists for at least 3 h after dosing. It remains to be established whether the selective and persistent effect of these compounds so far described can be exploited for therapeutic purposes in asthma or other inflammatory conditions where 5-LO products are thought to play a role. In this respect the effects of BW A4C and BW A797C on acute inflammatory responses are described in an accompanying paper (Higgs *et al.*, 1988).

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