Br. J. Pharmacol. (1992), **107**, 1042–1047 © Macmillan Press Ltd, 1992

Pre-clinical pharmacology of ICI D2138, a potent orally-active non-redox inhibitor of 5-lipoxygenase

[1]R.M. McMillan, K.E. Spruce, G.C. Crawley, E.R.H. Walker & S.J. Foster

ICI Pharmaceuticals, Bioscience 1, Mereside, Alderley Park, Macclesfield, Cheshire, SK10 4TG

1 This paper describes the pre-clinical pharmacology of ICI D2138, a potent orally-active non-redox inhibitor of 5-lipoxygenase which is undergoing clinical evaluation.

2 ICI D2138 potently inhibited leukotriene synthesis in murine peritoneal macrophages (IC_{50} = 3 nM) and human blood (IC_{50} = 20 nM). In human and dog blood, ICI D2138 did not inhibit thromboxane B_2 synthesis at a concentration of 500 μM, thus the selectivity ratio (cyclo-oxygenase: 5-lipoxygenase) was greater than 20,000. In contrast, zileuton (a 5-lipoxygenase inhibitor also undergoing clinical evaluation) exhibited a selectivity ratio of 15–100.

3 ICI D2138 potently and dose-dependently inhibited *ex vivo* leukotriene B_4 (LTB_4) synthesis by rat blood with ED_{50} values of 0.9, 4.0 and 80.0 mg kg^{-1} p.o. at 3, 10 and 20 h respectively after dosing. Similar activity was observed for inhibition of LTB_4 production in a zymosan-inflamed rat air pouch model. Zileuton produced ED_{50} values of 5 and 20 mg kg^{-1} at 3 and 10 h respectively.

4 Oral administration of 1, 3 or 10 mg kg^{-1} ICI D2138 to dogs produced maximal inhibition of *ex vivo* LTB_4 synthesis by blood for 5, 9 and 31 h respectively. A dose of 5 mg kg^{-1} p.o. of zileuton caused maximal inhibition of LTB_4 for 24 h.

5 Oral administration of 10 mg kg^{-1} ICI D2138 caused total inhibition of LTB_4 production in zymosan-inflamed rabbit knee joint.

6 Topical administration of ICI D2138 to rabbit skin caused a dose-related inhibition of arachidonic acid-induced plasma extravasation with an ID_{30} of 1.08 nmol per site. Zileuton was approximately 40 times less potent.

7 Oral anti-inflammatory activity was assessed in an arachidonic acid-induced mouse ear oedema model in animals treated with indomethacin to block pro-inflammatory prostanoids. ICI D2138, given orally, caused dose-dependent inhibition of oedema with an approximate ID_{50} of 1.8 mg kg^{-1}. Zileuton was approximately 10 times less potent.

8 ICI D2138 caused a dose-dependent inhibition of antigen-induced broncho-constriction in guinea-pigs with an approximate ID_{50} of 0.1 mg kg^{-1}, i.v. Zileuton was approximately 10 times less potent.

9 In view of the pharmacological profile described here, ICI D2138 has the potential to provide improved clinical efficacy compared to existing lipoxygenase inhibitors such as zileuton.

Keywords: 5-Lipoxygenase inhibitor; leukotrienes; ICI D2138; zileuton; inflammation; anti-inflammatory; allergic bronchospasm; anti-asthmatic; inflammatory diseases

Introduction

Leukotrienes are a group of pro-inflammatory lipids that are derived from the metabolism of arachidonic acid by 5-lipoxygenase. Inhibitors of 5-lipoxygenase have therapeutic potential in a range of inflammatory diseases in which leukotrienes have been proposed to have a pathological role (for review see Salmon & Garland, 1991). These diseases include asthma, allergic rhinitis, rheumatoid arthritis, psoriasis and ulcerative colitis.

The mechanism of 5-lipoxygenase is thought to involve an iron-catalysed redox cycle and, of the large number of reported lipoxygenase inhibitors, the majority have the potential to ligand to iron or to participate in redox reactions. Use of such agents has provided support for the anti-inflammatory actions of lipoxygenase inhibitors in animal models (Foster *et al.*, 1990; Carter *et al.*, 1991) but drugs suitable for clinical evaluation have not been available. Recently, BW A4C, an acetohydroxamate which has the potential to chelate iron and possesses relatively weak redox properties, was shown to inhibit *ex vivo* leukotriene synthesis in man (Nicholls & Posner, 1991). A structurally related compound, zileuton, an N-hydroxy urea, inhibits *ex vivo* leukotriene synthesis and has been shown to produce clinical benefit in initial trials in ulcerative colitis, rheumatoid arthritis and pulmonary challenge studies (Collawn *et al.*, 1989; Israel *et al.*, 1990; Knapp, 1990; Weinblatt *et al.*, 1990).

Redox-based inhibitors and iron ligands have relatively low selectivity for 5-lipoxygenase compared to cyclo-oxygenase and evidence is lacking for a specific interaction of such agents with 5-lipoxygenase. For example, no difference in lipoxygenase inhibitor potency has been observed between enantiomers of optically-active acetohydroxamates (Salmon *et al.*, 1989).

We have described a novel series of lipoxygenase inhibitors, methoxyalkyl thiazoles, which have neither iron-liganding nor redox properties and exhibit enantioselective inhibition of 5-lipoxygenase (McMillan *et al.*, 1990). Further development of this series has produced the compound, ICI D2138 (6-([3-fluoro-5-(4-methoxy-3,4,5,6-tetrahydro-2*H*-pyran-4-yl)phenoxy]methyl)-methyl-2-quinolone) (Crawley *et al.*, 1992) which is undergoing clinical evaluation. This paper describes the pre-clinical pharmacology of ICI D2138.

Methods

Leukotriene synthesis in vitro *and* ex vivo

Leukotriene synthesis by mouse peritoneal macrophages and human blood *in vitro* and by rat blood *in vitro* and *ex vivo*

[1] Author for correspondence.

was measured as previously described (Foster *et al.*, 1990; McMillan *et al.*, 1990). For measurement of leukotriene synthesis in dog, a blood sample was withdrawn from a vein in the foreleg and collected into heparin (5 iu ml^{-1}). The animals were then given a gelatin capsule containing either micronised ICI D2138 or zileuton mixed with lactose (50:50 w:w). Blood samples were collected at various times after dosing and challenged with A23187 as described for human blood (Foster *et al.*, 1990). All studies were on the same colony of 6 male beagle dogs (12–16 kg).

Leukotriene synthesis in inflamed rat air pouch

Male Alderley Park rats (180–220 g) were anaesthetized with Halothane and an air pouch was formed by injecting sterile air (20 ml) into the subcutaneous tissue of the back of each animal by use of a 0.22 μM millipore filter attached to a syringe. Three days later the air pouches were reinflated with a second injection of sterile air (10 ml). After a further 3 days, groups of 15 animals were dosed with vehicle and 5 were dosed orally with compound formulated by dissolving the compound in 0.3 ml dimethyl sulphoxide and mixing the solution with 15 ml of 0.5% hydroxypropyl methyl cellulose containing 0.1% polysorbate 80 (HPMC). At the same time a 1% suspension of zymosan in physiological saline (PS) (1 ml) was injected directly into each air pouch. Before administration the zymosan suspension was boiled for 30 min in PS, washed three times by centrifugation in PS at 2000 *g* for 5 min, resuspended in PS to 1% then autoclaved. The rats were killed at various times after zymosan injection using a rising concentration of carbon dioxide and the air pouches were lavaged with PS (1 ml) containing 20 iu of heparin. Lavage fluids were immediately placed on ice, centrifuged in an Eppendorf bench centrifuge and the supernatants analysed for leukotriene B_4 (LTB_4) by radioimmunoassay. The effect of compounds on LTB_4 production was expressed as percentage inhibition of the control values.

Inflamed rabbit knee

Groups of 12 female New Zealand White rabbits (2.5–3.5 kg: Ranch Rabbits, Crawley Down, Sussex) were dosed orally with 10 mg kg^{-1} of ICI D2138 polytroned in HPMC (dose volume 1 ml kg^{-1}) or vehicle alone. Immediately after dosing the fur from both knees was removed with electric clippers and the exposed skin was swabbed with 70% ethanol. This was followed immediately by intra-articular injections of PS (1 ml) into the left knee joint space and zymosan (1 ml of a 1% suspension in PS prepared as indicated above) into the right knee joint space. Animals were given a lethal dose of sodium pentobarbitone (Euthatal) 4 h later and the knee joints were lavaged with 2 ml of PS containing 20 iu ml^{-1} heparin. Following centrifugation the lavage supernatants were stored frozen at −20°C for subsequent analysis of LTB_4 levels by radioimmunoassay.

Arachidonic acid-induced inflammation

Arachidonic acid-induced inflammation in rabbit skin was measured as previously described (McMillan *et al.*, 1990).

Leukotriene-dependent inflammation in mouse ear was measured by a modification of the procedure of Opas *et al.* (1985). Groups of 10–20 male or female AP mice (25–30 g) were dosed orally with indomethacin (10 mg kg^{-1} in HPMC) and with ICI D2138 or zileuton in HPMC or HPMC alone 1 h before the application of arachidonic acid (1 mg in 10 μl of Analar acetone) to the inside of the right ear. The contralateral ear did not receive acetone alone since previous experiments have shown this to be unnecessary. Mice were killed 1 h later by cervical dislocation and 6 mm discs were punched from both ears.

The effect of the test agent on inflammatory oedema was assessed by determining the mean difference in weight of the discs from the arachidonic acid-treated and untreated ears from both control and drug-treated groups of mice. Inhibition was calculated as follows:

$$\% \text{ inhibition} = \frac{\text{Control} - \text{Drug-treated} \times 100}{\text{Control}}$$

Allergic bronchospasm in guinea-pig

Leukotriene-dependent bronchospasm in guinea-pigs was measured by a modification of the procedure of Anderson *et al.* (1983). Male Dunkin Hartley guinea-pigs, weighing 250–300 g on delivery were housed in groups of 5 and allowed food and water *ad libitum*. After 4 days acclimatatization, animals were sensitized by an i.p. injection of 1 mg of ovalbumin 5×10^9 *Bordetella pertussis* organisms in 0.5 ml pyrogen-free saline on days 1, 4 and 8. Guinea-pigs were used for testing between days 28 and 35. On the day of the test guinea-pigs were anaesthetized with 1.0 g kg^{-1} urethane (ethyl carbamate, 0.25 g ml^{-1}) and 20 mg kg^{-1} Sagatal (sodium pentobarbitone, 60 mg ml^{-1} solution) given i.p. and left approximately 20 min before surgery. To measure ventilatory pressure, a tracheotomy tube was surgically implanted in the trachea using polythene tubing. This was connected to a 'T' piece on one side to a small animal respirator set at stroke rate volume^{-1} of 37 min^{-1} and 1.0 cc 100 g^{-1} body weight and on the other to a recording device consisting of a pressure transducer, an amplifier and a flat bed recorder. The recorder was calibrated with a mercury manometer to give full scale deflection at approximately 180 mmHg pressure.

Guinea-pigs were pretreated with indomethacin (10 mg kg^{-1}, i.v.), succinylcholine (1.2 mg kg^{-1}, i.v.), pyrilamine (1 mg kg^{-1}, i.v.) and propranolol (0.1 mg kg^{-1}) before induction of bronchoconstriction with antigen (5 mg ovalbumin kg^{-1}); under these conditions the bronchospasm is primarily mediated by leukotrienes (Anderson *et al.*, 1983). All agents were administered via an i.v. cannula inserted in the jugular vein.

Statistics

Statistical significance was assessed by Student's paired *t* test, analysis of variance or Dunnett's test, as indicated in the appropriate figure legends, with $P<0.05$ regarded as significant.

Materials

Materials used were as previously described (Foster *et al.*, 1990 and references therein). Rev 5901 (α-pentyl-3-(2-quinolinylmethoxy)-benzene methanol), A64077 (zileuton) (N-(1-(benzo(b)thien-2-yl)ethyl)-N-hydroxy urea) and ICI D2138 were synthesized in Chemistry Department I, ICI Pharmaceuticals. WY-50295 (S-α-methyl-6-(2-quinolinylmethoxy)-2-napthalene acetic acid, tromethamine salt) was kindly supplied by Dr B.M. Weichmann of Wyeth-Ayerst Research, Princeton, NJ, U.S.A. Succinylcholine chloride, pyrilamine maleate, and ovalbumin were purchased from Sigma (Poole, U.K.) and propranolol was from ICI Pharma-ceuticals. Urethane (ethyl carbamate) and hydroxyethyl-cellulose were purchased from Fluka, Glossop, U.K. *Bordetella pertussis* organisms (Per Vac) as adjuvant was purchased from Wellcome, U.K.

Results

ICI D2138 potently inhibited leukotriene C_4 synthesis in a plasma-free preparation of murine peritoneal macrophages with an IC_{50} of 0.003 μM, Figure 1). A reduction in the potency of ICI D2138 was observed when leukotriene B_4 synthesis by blood was measured (IC_{50} in human blood =

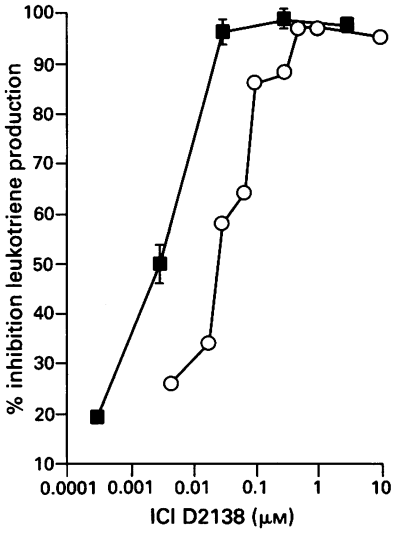

Figure 1 Inhibition of leukotriene production by ICI D2138. The calcium ionophore A23187 was used to stimulate leukotriene B_4 (LTB_4) production by human blood (○) and zymosan to stimulate LTC_4 production by mouse peritoneal macrophages (■) as previously described (Foster *et al.*, 1990). Values are the means of two experiments for human blood and the mean of 2 experiments (no error bars shown) or mean ± s.e.mean (error bars) of 3-5 experiments for mouse peritoneal macrophages.

0.02 μM, Figure 1).

Comparative data for inhibition of leukotriene synthesis in blood by ICI D2138 and 3 other lipoxygenase inhibitors are shown in Table 1. ICI D2138 was 25–100 times more potent than zileuton, depending on the species and also exhibited a higher level of selectivity. In dog and human blood, no inhibition of thromboxane B_2 (TxB_2) synthesis was observed with ICI D2138 at the highest concentration tested (500 μM) and the selectivity ratio (IC_{50} cyclo-oxygenase:IC_{50} 5-lipoxygenase) for ICI D2138 was greater than 20,000. In rat blood, significant inhibition of TxB_2 synthesis was apparent at 500 μM and the selectivity ratio was therefore reduced to 4000. In contrast, zileuton inhibited formation of TxB_2 synthesis at concentrations of 15–100 times those that inhibited LTB_4 synthesis.

Also shown in Table 1 are the effects of two previously described non-redox inhibitors, Rev 5901 and WY 50,295. Both compounds selectively inhibited LTB_4 synthesis in rat blood without inhibiting TxB_2 synthesis at concentrations up to at least 30 μM for Rev 5901 and 100 μM for WY 50,295. Rev 5901 and WY 50,295 were respectively 150 and 900 times less potent than ICI D2138 at inhibiting LTB_4 synthesis in rat blood and failed to inhibit LTB_4 synthesis in human blood at concentrations up to 40 and 100 μM respectively.

Figure 2a shows the effect of ICI D2138 on *ex vivo* LTB_4 synthesis by rat blood. Dose-dependent inhibition was observed at 3, 10 and 20 h with ID_{50} values of 0.9, 4.0 and 80.0 mg kg^{-1} p.o. respectively. In comparative studies (data

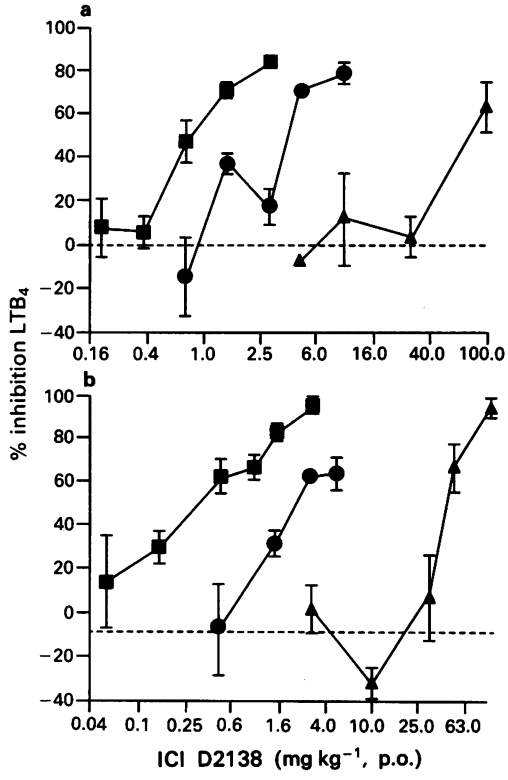

Figure 2 Dose-dependent inhibition of ICI D2138 on *ex vivo* leukotriene B_4 (LTB_4) synthesis by A23187-stimulated rat blood (a) as described in Foster *et al.* (1990) and LTB_4 synthesis in zymosan-inflamed rat air pouch (b) at 3 h (■), 10 h (●) and 20 h (▲) following oral administration. Data are the mean of two experiments (no error bars shown) or mean ± s.e.mean (error bars, 3 or 4 experiments).

Table 1 Inhibition of eicosanoid generation in blood

	Human		*Rat*		*Dog*	
	LTB_4	TxB_2	LTB_4	TxB_2	LTB_4	TxB_2
ICI D2138	0.024 (0.012-0.030) $n=4$	>500 $n=2$	0.033 (0.02-0.04) $n=3$	156 (150-162) $n=2$	0.020 $n=2$	>500 $n=2$
Zileuton	2.60 (2.4-2.8) $n=2$	40 – $n=1$	2.30 $n=1$	>100 $n=1$	0.56 (0.46–0.66) $n=2$	51 (27–75) $n=2$
Rev 5901	>40 ($n=2$)	>40 ($n=2$)	3.0 (2–5) ($n=5$)	>30[1] ($n=6$)	ND	ND
WY 50295	>100 ($n=2$)	>100 ($n=2$)	30 ($n=2$)	>100 ($n=2$)	ND	ND

Results are mean IC_{50} (μM) values with the range of individual values shown in parentheses.
[1]PGE_2 synthesis measured instead of TxB_2. The percentage inhibition caused by a concentration of 30 μM ranged from – 14 to 30 in 6 experiments.

not shown), zileuton produced ID_{50} values at 3 and 10 h of 5 mg kg^{-1} (mean of 2 experiments) and 20 mg kg^{-1} (mean of 3 experiments) respectively. Synthesis of LTB_4 in zymosan-inflamed rat air pouch was inhibited by comparable doses of ICI D2138: ID_{50} values of 0.3, 2.0 and 40.0 mg kg^{-1} p.o. were obtained at 3, 10 and 20 h after dosing (Figure 2b).

Oral administration of 1 mg kg^{-1} ICI D2138 to dogs produced transient inhibition of LTB_4 synthesis with maximal effects at 3–5 h. At 3 or 10 mg kg^{-1}, maximal inhibition was evident at the earliest time point studied (1 and 5 h respectively). Maximal inhibition was maintained for 9 h and at least 31 h following single oral doses of 3 and 10 mg kg^{-1} respectively (Figure 3). Zileuton also produced prolonged inhibition of LTB_4 synthesis in dog: inhibition following a dose of 5 mg kg^{-1} p.o. was maintained for 24 h (data not shown).

Figure 4 shows the effect of oral administration of ICI D2138 on LTB_4 synthesis in zymosan-inflamed rabbit knee joint. Intra-articular injection of zymosan stimulated LTB_4 concentrations in lavage fluid by approximately 6 fold to 3.0 ± 0.55 ng ml^{-1}. Prior administration of ICI D2138 reduced LTB_4 concentrations in lavage fluid from zymosan-inflamed knee to the baseline levels detected in fluid from control, saline-injected knee joint.

Topical administration of ICI D2138 to rabbit skin caused dose-related inhibition of plasma extravasation induced by arachidonic acid with an ID_{30} of 1.08 ± 0.33 nmol per site (mean ± s.d.; $n = 6$). Zileuton was approximately 40 times less potent with an ID_{30} of 42 nmol per site (mean of 2 experiments).

Oral anti-inflammatory activity was measured using arachidonic acid-induced mouse ear oedema. In the presence of indomethacin to block synthesis of pro-inflammatory prostanoids, ICI D2138 produced dose-dependent inhibition of oedema (Figure 5). In this model ICI D2138 was approximately 10 times more potent than zileuton: respective ID_{50} values 1.8 mg kg^{-1} p.o. and 18 mg kg^{-1} p.o. were obtained.

Figure 6 shows the effects of ICI D2138 and zileuton on allergic bronchospasm in the guinea-pig. In the presence of indomethacin, pyrillamine and propranolol, intravenous administration of ICI D2138 caused a dose-dependent inhibition of the antigen-induced increase in ventilatory pressure. In this system zileuton (ID_{50} approximately 1.0 mg kg^{-1}) was approximately 10 times less potent than ICI D2138 (ID_{50} approximately 0.1 mg kg^{-1}) (Figure 6).

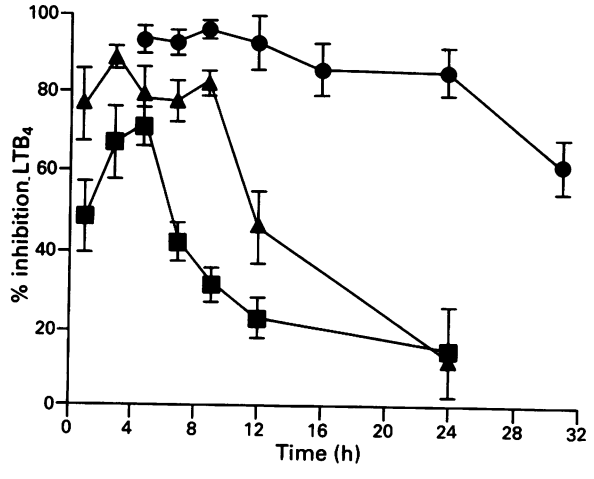

Figure 3 Time-dependent effect of ICI D2138 on *ex vivo* leukotriene B_4 (LTB_4) synthesis by A23187-stimulated dog blood as previously described in Foster *et al.* (1990) for human blood. Values are the means ± s.e.mean (vertical bars) % inhibition of LTB_4 production in 6 dogs treated with 1 mg kg^{-1} (■), 3 mg kg^{-1} (▲) or 10 mg kg^{-1} (●) of ICI D2138.

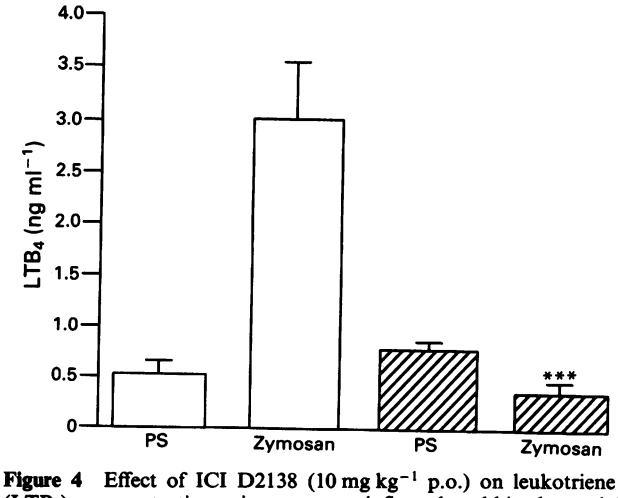

Figure 4 Effect of ICI D2138 (10 mg kg^{-1} p.o.) on leukotriene B_4 (LTB_4) concentrations in zymosan-inflamed rabbit knee joint. Zymosan suspension was injected into the right and PS into the left knee joint space respectively. Four hours later the animals were killed and the joints were lavaged with PS. Data are the means ± s.e.mean (vertical bars) ng LTB_4 ml^{-1} of lavage fluid from 11 vehicle-dosed (open columns) and 12 ICI D2138-dosed (cross-hatched columns) animals. ***Indicates $P < 0.001$ with respect to the zymosan-inflamed knee joint in the vehicle-dosed group calculated by Student's t test.

Discussion

The studies described here demonstrate that ICI D2138 is a potent, selective and orally-active inhibitor of 5-lipoxygenase. The compound has certain structural features in common with a series of methoxyalkyl thiazoles, which have been shown previously to produce enantioselective inhibition of 5-lipoxygenase (McMillan *et al.*, 1990).

The majority of previously reported 5-lipoxygenase inhibitors have the potential to participate in redox reactions or to ligand to iron. Such compounds usually exhibit only limited selectivity for 5-lipoxygenase compared to the related

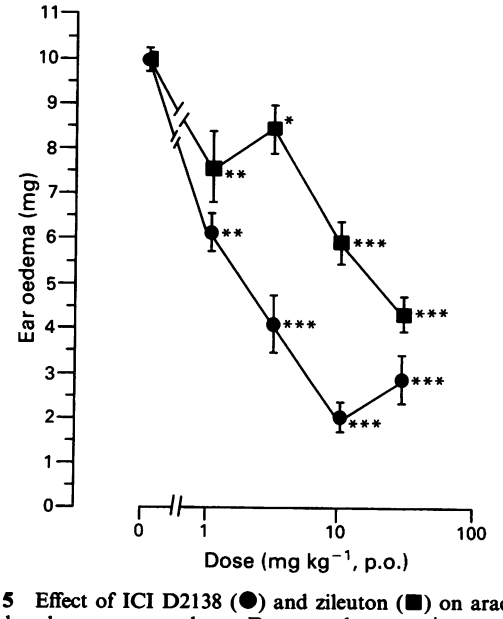

Figure 5 Effect of ICI D2138 (●) and zileuton (■) on arachidonic acid-induced mouse ear oedema. Data are the mean ± s.e.mean (vertical bars) mg oedema calculated from 8 separate experiments where the total number of control animals was 159 and the drug-treated group sizes were either 20 or 40. Statistical significance was calculated by analysis of variance. *$P < 0.05$; **$P < 0.01$ and ***$P < 0.001$ with respect to controls.

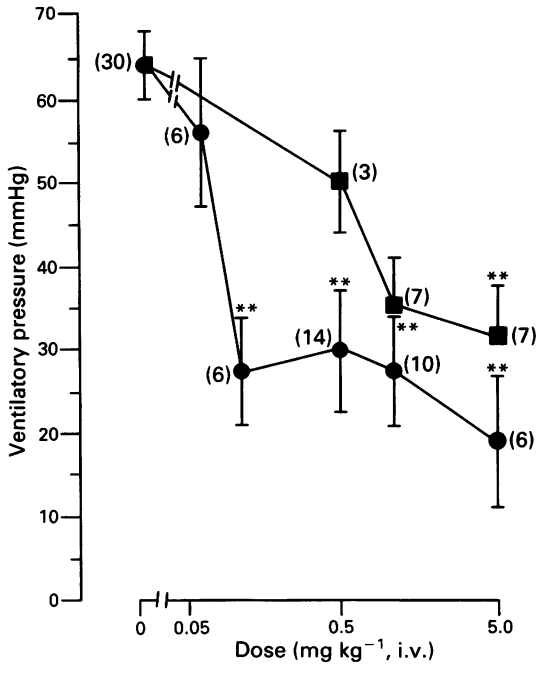

Figure 6 Effect of ICI D2138 (●) and zileuton (■) on antigen-induced bronchoconstriction in guinea-pigs. Values are the mean ± s.e.mean (vertical bars) ventilatory pressure (mmHg) with number of animals for each group shown in parentheses. Statistical significance was calculated by Dunnett's test to compare each dose group to control. $^{**}P<0.01$.

enzyme cyclo-oxygenase. For example, in these studies, zileuton, an N-hydroxy urea with iron liganding and weak redox properties, exhibits 15–100 fold selectivity depending on the *in vitro* assay employed. In contrast, ICI D2138 produced selectivity ratios (cyclo-oxygenase:5-lipoxygenase) of up to 25,000 fold. The effects of ICI D2138 on arachidonic acid metabolism are essentially the mirror image of those of potent non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin or flurbiprofen. In view of their potency and selectivity, ICI D2138 and related compounds can be considered to be the NSAIDs of the 5-lipoxygenase pathway.

Two other non-redox compounds, Rev 5901 and WY 50,295 also show high selectivity for inhibition of leukotriene synthesis. However, several features distinguish ICI D2138 from those compounds. First, WY 50,295 does not produce enantioselective inhibition of leukotriene synthesis (Musser & Kreft, 1990) whilst no evidence for enantioselective inhibition has been reported for Rev 5901. Second, ICI D2138 is considerably more potent than the other compounds: in rat blood it was 150 and 900 times more potent than Rev 5901 and WY 50,295 respectively. Finally, ICI D2138 exhibited comparable potency in rat, dog and man whereas the other compounds inhibited leukotriene synthesis in rat blood but failed to inhibit in human blood. The reduced potency of Rev 5901 is probably a consequence of insensitivity of human leucocytes to the compound (Coutts *et al.*, 1985). In the case of WY 50,295, the discrepancy between man and rat is at least partly due to differences in binding of the compound to human and rat plasma proteins (Carlsson, R.P., Personal communication).

A problem with previous lipoxygenase inhibitors lacking redox or iron liganding properties, including Rev 5901 and methoxyalkyl thiazoles, was that oral activity was either weak or absent (McMillan *et al.*, 1991). In contrast, ICI D2138 has potent oral activity in rat and dog with comparable ED_{50} values as inhibitors of leukotriene synthesis in blood of approximately 1 mg kg^{-1} 3 h after dosing. However there was a marked difference in duration of action in the two species. In dog, inhibition of leukotriene synthesis following a single oral dose of 10 mg kg^{-1} persisted for at least 32 h. In rat, inhibition by oral doses up to 30 mg kg^{-1} had reversed by 24 h. These data are consistent with differences in the half life of ICI D2138 in rat and dog of 2.0 and 6.0 h respectively (E. Pywell and M. Hutchinson, unpublished). ICI D2138 also inhibited LTB_4 synthesis at an inflammatory site in the rat with potency comparable to that demonstrated in blood *ex vivo*. Inhibition of LTB_4 synthesis in an inflamed rabbit knee joint was also demonstrated. Thus, ICI D2138 exhibits biochemical efficacy in both peripheral blood and inflammatory exudates.

Anti-inflammatory activity of ICI D2138 has been demonstrated by use of arachidonic acid-induced skin oedema. Inhibition of arachidonic acid-induced inflammation in rabbit skin has been shown to be related to 5-lipoxygenase inhibitor potency (Foster *et al.*, 1990) and topical administration of ICI D2138 in this model produced dose-related anti-inflammatory activity. In order to investigate oral anti-inflammatory activity, a smaller species was desirable and therefore arachidonic acid-induced oedema in mouse ear was adopted. In our experience, there is a variable contribution of pro-inflammatory prostaglandins in this model and this complicates evaluation of lipoxygenase inhibitors which also show variations in potency. To overcome this problem, anti-inflammatory activity of lipoxygenase inhibitors was evaluated in animals treated with indomethacin to block prostanoid synthesis. Under these conditions, the model was predominantly dependent on leukotrienes and ICI D2138 produced consistent anti-inflammatory activity. In both rabbit and mouse models ICI D2138 was at least 10 times more potent than zileuton.

The pulmonary actions of ICI D2138 were studied on a model of allergic bronchospasm in the guinea-pig. In animals pretreated with indomethacin, propranolol and pyrillamine, the bronchoconstriction is mediated primarily by leukotrienes (Anderson *et al.*, 1983). Under these conditions, ICI D2138 produced potent inhibition of the antigen-induced increase in ventilatory pressure and was approximately 10 times more potent than zileuton. A detailed evaluation of the effect of ICI D2138 on pulmonary mechanics changes in this model will be given elsewhere (Buckner and Kusner, unpublished).

Based on the biological profile described here, ICI D2138 was selected for clinical development. The compound was well tolerated in human volunteers and inhibited *ex vivo* leukotriene synthesis (Yates *et al.*, 1992). The beneficial clinical effects observed with zileuton in ulcerative colitis and rheumatoid arthritis and in challenge models of asthma and allergic rhinitis (Collawn *et al.*, 1989; Israel *et al.*, 1990; Knapp, 1990; Weinblatt *et al.*, 1990) support the therapeutic potential of lipoxygenase inhibitors in inflammatory diseases. In view of the pharmacological profile described here, ICI D2138 has the potential to provide improved clinical efficacy compared to existing lipoxygenase inhibitors such as zileuton.

The authors wish to thank Christine Potts, Margaret McCormick and Steve Moores for carrying out the experimental work and Mavis Bloor for secretarial assistance.

References

ANDERSON, W.H., O'DONNELL, M., SIMKO, B.A. & WELTON, A.F. (1983). An *in vivo* model for measuring antigen-induced SRS-A-mediated bronchoconstriction and plasma levels in the guinea pig. *Br. J. Pharmacol.*, **78**, 67–74.

CARTER, G.W., YOUNG, P.R., ALBERT, D.H., BOUSKA, J., DYER, R., BELL, R.L., SUMMERS, J.B. & BROOKS, D.W. (1991). 5-Lipoxygenase inhibitory activity of zileuton. *J. Pharmacol. Exp. Ther.*, **256**, 929–937.

COLLAWN, C., RUBIN, P., PEREZ, H., REYES, E., BOBADILLA, J., CABRERA, G., MORAN, M.A. & KERSHENOBICH, D. (1989). Experimental use of a 5-lipoxygenase inhibitor (Abbott-64077) in human ulcerative colitis (UC). *Am. J. Gastroenterol.*, **84**, 1178.

COUTTS, S.M., KHANDWALA, A., VAN INWEGAN, R., CHAKRABORTY, U., MUSSER, J., BRUENS, J., JARIWALA, N., DALLY-MEADE, V., INGRAM, R., PRUSS, T., JONES, H., NEISS, E. & WEINRYB, I. (1985). Arylmethyl phenyl ethers: a new class of specific inhibitors of 5-lipoxygenase. In *Prostaglandins, Leukotrienes and Lipoxins*. ed. Baily, M.J. pp. 627–637. New York: Plenum Press.

CRAWLEY, G.C., DOWELL, R.I., EDWARDS, P.N., FOSTER, S.J., MCMILLAN, R.M., WALKER, E.R.H. & WATERSON, D. (1992). Methoxytetrahydropyrans: A new series of selective and orally potent 5-lipoxygenase inhibitors. *J. Med. Chem.*, (in press).

FOSTER, S.J., BRUNEAU, P., WALKER, E.R.H. & MCMILLAN, R.M. (1990). 2-Substituted indazolinones: orally active and selective 5-lipoxygenase inhibitors with anti-inflammatory activity. *Br. J. Pharmacol.*, **99**, 113–118.

ISRAEL, E., DERMARKARIAN, R.M., ROSENBERG, M.A., SPERLING, R., TAYLOR, G., RUBIN, P. & DRAZEN, J.M. (1990). The effects of a 5-lipoxygenase inhibitor on asthma induced by cold, dry air. *New Engl. J. Med.*, **323**, 1740–1744.

KNAPP, H.R. (1990). Reduced allergen-induced nasal congestion and leukotriene synthesis with an orally active 5-lipoxygenase inhibitor. *N. Engl. J. Med.*, **323**, 1745–1748.

MCMILLAN, R.M., BIRD, T.G.C., CRAWLEY, G.C., EDWARDS, M.P., GIRODEAU, J-M., KINGSTON, J.F. & FOSTER, S.J. (1991). Methoxyalkyl thiazoles: A novel series of potent, orally active and enantioselective inhibitors of 5-lipoxygenase. *Agents Action*, **34**, 110–112.

MCMILLAN, R.M., GIRODEAU, J-M. & FOSTER, S.J. (1990). Selective chiral inhibitors of 5-lipoxygenase with anti-inflammatory activity. *Br. J. Pharmacol.*, **101**, 501–503.

MUSSER, J.H. & KREFT, A.F. (1990). Substituted-[2-quinolinyl-(bridged)aryl] compounds: modulators of eicosanoid biosynthesis and action. *Drugs of the Future*, **15**, 73–80.

NICHOLLS, A.J. & POSNER, J. (1991). Investigation of single doses of BWA4C, a selective 5-lipoxygenase inhibitor, in healthy volunteers. *Br. J. Clin. Pharmacol.*, **31**, 577P.

OPAS, E.E., BONNEY, R.J. & HUMES, J.L. (1985). Prostaglandin and leukotriene synthesis in mouse ears inflamed by arachidonic acid. *J. Invest. Dermatol.*, **84**, 253–256.

SALMON, J.A. & GARLAND, L.G. (1991). Leukotriene antagonists and inhibitors of leukotriene biosynthesis as potential therapeutic agents. *Prog. Drug Res.*, **37**, 9–90.

SALMON, J.A., JACKSON, W.P. & GARLAND, L.G. (1989). Development of 5-lipoxygenase inhibitors: potential drugs for asthma and inflammation. In *Therapeutic Approaches to Inflammatory Disease*. ed. Lewis, A.J., Doherty, N.S. & Ackerman, N.R. pp.137–146. New York: Elsevier.

WEINBLATT, M., KREMER, J., HELFGOTT, S., COBLYN, J., MAIER, A., SPERLING, R., PETRILLO, G., KESTERSAN, J., DUBE, L., HANSON, B., TEAH, H. & DUBIN, P. (1990). A 5-lipoxygenase inhibitor in rheumatoid arthritis (RA). *Arthritis Rheum.*, **33**, S152.

YATES, R.A., HUTCHINSON, M., SPRUCE, K., MCMILLAN, R.M., GILMORE, E. & WILLIAMS, A.J. (1992). Tolerability, pharmacokinetics and effects on blood 5-lipoxygenase of ICI D2138, a new lipoxygenase inhibitor. *Br. J. Clin. Pharmacol.*, (in press).

(Received April 24, 1992
Revised July 1, 1992
Accepted August 4, 1992)