2-Substituted indazolinones: orally active and selective 5-lipoxygenase inhibitors with anti-inflammatory activity

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- 1 This paper describes the pharmacological profile of ICI207968, a novel, orally-active and selective inhibitor of 5-lipoxygenase.
- 2 Inhibition of leukotriene B₄ (LTB₄) synthesis by 2-substituted indazolinones was not directly related to redox potential but was critically dependent on the nature of the N2 substituent. 2-(3-Pyridylmethyl)-indazolinone (ICI207968) combined selectivity and oral potency.
- 3 In several *in vitro* systems ICI207968 exhibited similar lipoxygenase inhibitory potency (IC₅₀ values from 1.5 μ M to 6.0 μ M) and was approximately 300 times less potent against cyclo-oxygenase, as measured by inhibition of prostaglandin E₂ (PGE₂) synthesis.
- 4 ICI207968 also produced selective lipoxygenase inhibition following oral administration in the rat. ED₅₀ values of 2.5, 10 and 25 mg kg⁻¹ p.o. for inhibition of LTB₄ release from A23187-stimulated blood were obtained 1, 3 and 5 h after dosing. The compound did not inhibit PGE₂ synthesis at oral doses up to 300 mg kg⁻¹.
- 5 Co-administration of ICI207968 with arachidonic acid, into rabbit dermis, potently inhibited both plasma extravasation and polymorphonuclear leucocyte (PMNL) infiltration induced by this inflammatory fatty acid. The anti-inflammatory potency of a number of intradermally administered indazolinones, with similar redox potentials, was related to their inhibitory potency against leukotriene generation in blood. Oral administration of ICI207968 (100 mg kg⁻¹) in the rabbit inhibited *ex vivo* leukotriene generation in blood and arachidonic acid-induced skin inflammation.
- 6 These data demonstrate that ICI207968 is an orally active and selective inhibitor of 5-lipoxygenase which has anti-inflammatory properties. ICI207968 will be a valuable agent for clarifying the biological roles of leukotrienes and the therapeutic potential of 5-lipoxygenase inhibitors.

Introduction

Leukotrienes are a family of biologically active molecules which are formed as a result of arachidonic acid metabolism by 5-lipoxygenase (Borgeat & Samuelsson, 1979). Leukotriene B₄ (LTB₄) is a potent chemotactic agent and induces inflammatory reactions in animals and in man (Bray, 1986; McMillan & Foster, 1988). Leukotrienes C₄, D₄ and E₄, which are known as peptidoleukotrienes, have powerful spasmogenic actions in vascular and bronchial tissue and they account for the biological properties previously described as 'slow reacting substance of anaphylaxis' (Samuelsson, 1983). In view of the biological actions of leukotrienes, drugs which block their biosynthesis, by inhibiting 5-lipoxygenase, are thought to have therapeutic potential in a variety of inflammatory conditions including asthma, psoriasis, inflammatory bowel diseases and inflammatory arthropathies (Bach, 1984).

Progress in elucidating the roles of leukotrienes in inflammatory diseases has been hampered by the lack of 5-lipoxygenase inhibitors with adequate selectivity and oral activity. Orally active inhibitors which have been described previously also inhibit arachidonic acid metabolism by cyclo-oxygenase (Higgs *et al.*, 1979; DiMartino *et al.*, 1987; Griswold *et al.*, 1987) and thus their pharmacological activity may be a consequence of inhibiting prostaglandin synthesis. More selective inhibitors have been described with no inhibitory activity against cyclo-oxygenase but such compounds have poor oral activity (Coutts *et al.*, 1985).

We have discovered a novel series of 5-lipoxygenase inhibitors, 2-substituted indazolinones, which combine high selectivity and oral activity. In this paper we describe the pharmacological profile of a member of this chemical series, 2-(3-pyridylmethyl)-indazolinone (ICI207968). ICI207968 is an orally-active, potent and selective 5-lipoxygenase inhibitor

which exhibits anti-inflammatory activity. We conclude that ICI207968 will be a valuable agent for clarifying the biological roles of leukotrienes and the therapeutic potential of 5-lipoxygenase inhibitors.

Methods

Spectrophotometric analysis of 5-lipoxygenase

Inhibition of cell-free 5-lipoxygenase activity was determined spectrophotometrically by modification of the procedure of Aharony & Stein (1986), using the high speed supernatant from rat basophilic leukaemia (RBL-1) cells as the enzyme source (McMillan *et al.*, 1989).

Eicosanoid generation in whole blood

Male Alderley Park Wistar rats were killed in a carbon dioxide filled chamber. The abdominal wall was opened and blood was withdrawn from the inferior vena cava into heparin (5 units ml⁻¹). For *in vitro* studies, blood samples were pooled and stored on ice before use. Heparinised blood samples were incubated with drugs dissolved in dimethyl sulphoxide (DMSO; final concentration 1%) for 15 min at 37°C, then calcium ionophore A23187 (20 μ g ml⁻¹) was added and incubations were continued for a further 15 min. Incubations were terminated by addition of 0.1 volumes of a 'stop cocktail' containing indomethacin (1 mM), BW755C, (1 mM), EGTA (0.1 M) and sodium azide (0.15 M). The samples were centrifuged (12,000 g, 2 min) and the supernatant plasma was removed. Plasma samples were stored at -20°C before assay for eicosanoids by radio-immunoassay as previously described by Forder & Carey (1984) and Carey & Forder (1986).

For *ex vivo* studies in the rat, animals were dosed orally with compound in vehicle HPMC (10 ml kg⁻¹) or with vehicle alone; see Materials for composition of HPMC. At various

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times after dosing, rats were killed and bled and within 15 min individual blood samples were challenged with A23187 as described above.

For *ex vivo* studies in the rabbit, blood was withdrawn from a marginal ear vein into 10 units of heparin. Animals were then dosed 10 min later with compound in HPMC (1 ml kg⁻¹) by oral intubation. At subsequent times, blood samples were again withdrawn from a marginal ear vein into heparin. The *ex vivo* generation of LTB₄ and PGE₂ by A23187-stimulated blood was then determined as outlined above except that the ionophore concentration used was 10 µg ml⁻¹.

Eicosanoid generation in human blood was determined in samples collected by venepuncture from healthy adults who has taken no medication in the previous 7 days. Samples were anti-coagulated with heparin (5 units ml⁻¹) and drug effects on eicosanoid generation were studied as described above for rat blood.

Eicosanoid generation in mouse peritoneal macrophages

Resident cells were collected from the peritoneal cavity of Alderley Park mice by lavage with Minimal Essential Medium supplemented with foetal calf serum (10%), penicillin (100 units ml⁻¹), streptomycin (100 µg ml⁻¹), glutamine (300 µg ml⁻¹), polymyxin B sulphate (10 µg ml⁻¹) and heparin (20 units ml⁻¹). Cells were added to 24-well culture dishes (Falcon, 3047) and incubated for 2 h at 37°C to allow adherence. Non-adherent cells were removed by washing with phosphate buffered saline (PBS). After overnight incubation in serum- and heparin-free lavage medium, the predominantly macrophage cell population was washed with PBS and then incubated at 37°C in heparin-free lavage medium supplemented with 1 mM cysteine, zymosan (100 µg ml) and drug dissolved in DMSO (final concentration 0.1%). After 4 h the medium was removed and, following centrifugation, samples were stored at -20°C prior to measurement of eicosanoids by radio-immunoassay.

Arachidonic acid-inflamed rabbit skin

New Zealand White rabbits (3.0–4.0 kg; Ranch Rabbits, Crawley Down, Sussex), were used to evaluate the effects of indazolinones, dosed intradermally or orally, on plasma extravasation and leukocyte recruitment assessed as previously described by Aked *et al.* (1986) and Rampart & Williams (1988) respectively. Fur from the rabbit's back was removed with clippers 24 h before an intravenous injection of ¹²⁵I-human serum albumin, in a 0.5% solution of Evans blue in physiological saline, and a suspension of ¹¹¹In-labelled autologous PMNL (approx. 5 × 10⁷). This was followed 5 min later by six replicate intradermal injections of arachidonic acid suspension or arachidonic acid combined with test compound. Concentrated solutions of test compounds were made in DMSO then combined with a suspension of arachidonic acid, in pyrogen-free saline, such that intradermal injections of 0.1 ml of the suspension contained 100 µg of arachidonic acid and the appropriate drug concentration. The DMSO concentration of all injected suspensions was 5.0%. Animals were killed 60 min later with a lethal intravenous dose of sodium pentobarbitone (Euthatal; May and Baker, Dagenham). The skin was removed and the injection sites separated with a 15 mm diameter metal punch. ¹²⁵I counts were determined in the skin sites and aliquots of plasma with an LKB ultragamma counter and the plasma volume at each injection site calculated in µl. ¹¹¹In counts were also determined in the skin sites and autologous labelled-PMNL preparation with an LKB ultragamma counter.

For oral studies, groups of rabbits were dosed with ICI207968 in HPMC (1 ml kg⁻¹) or vehicle alone. One hour later inflammatory reactions were induced in several skin sites on the backs of the animals by the intradermal injection of 100 µg of arachidonic acid suspended in 0.1 ml physiological

saline. This was followed immediately by an intravenous injection of 5 µCi ¹²⁵I human serum albumin in a 5.0% solution of Evans blue in physiological saline to monitor plasma extravasation. After a further 1 h the animals were killed and the plasma extravasation in the inflamed skin sites was determined as described above.

Statistical analysis

Differences between means were assessed either by Student's paired *t* test or by use of a one tail Student's *t* test with *P* < 0.05 regarded as significant in both cases.

Materials

The materials used were as previously described (Foster *et al.*, 1986; Aked *et al.*, 1986) with the following additions. Polymyxin B sulphate, zymosan, streptomycin, phenidone and cysteine were obtained from Sigma (Poole, Dorset). Minimal essential medium, foetal calf serum and glutamine were obtained from Gibco (Paisley). Penicillin G was obtained from Glaxo (Greenford). The aqueous suspending vehicle used for oral dose of ICI207968 is referred to as HPMC and consists of 0.5% hydroxypropylmethylcellulose (BDH, Speake, Liverpool) in 0.1% polysorbate 80 (ICI Speciality Chemicals, Letherhead, Surrey). [1-¹⁴C]-arachidonic acid and [5,6,8,9,11,12,14,15-³H]-thromboxane B₂ were supplied by Amersham International, Amersham, UK. BW 755C, BWA4C, A-64077, Rev 590 and AA861 were synthesized in Chemistry Department 1, ICI Pharmaceuticals.

Results

Selective inhibition of leukotriene synthesis by 2-substituted indazolinones

The potency and selectivity of lipoxygenase inhibition by indazolinones was determined using A23187-stimulated eicosanoid synthesis in whole blood which allows *in vitro* and *ex*

Table 1 Influence of the redox potential of indazolinones on their potency as inhibitors of leukotriene B₄ (LTB₄) generation by A23187-stimulated rat blood

Compound	IC ₅₀ (µM)	Redox potential (V)
1	0.2	0.73
2	0.5	0.73
3	0.9	0.72
4	1.6	0.72
5	3.1	0.72
6	6.3	0.73
7	14.0	0.70
BW755C	20.0	0.31

IC₅₀ values were determined as described in methods and the values show the means of two experiments. Compound 1 is 1,2-dihydro-2-phenyl mercaptomethyl-3H-indazol-3-one; compound 2 is 1,2-dihydro-5-chloro-2-phenylmethyl-3H-indazol-3-one. In compounds 3 to 8 the phenyl mercaptomethyl group of compound 1 is replaced by the following groups: (3), (1-phenyl ethyl); (4), (2-quinolinyl methyl); (5), (2-pyridyl methyl); (6), (4-isopropoxyphenyl); (7), (2,4,6-trimethoxybenzyl). Redox potentials were determined by cyclic voltammetry with a Thompson Electrochem Ltd instrument connected to a 3 electrode cell comprising a saturated calomel electrode as the reference. A solution of DMSO containing 0.1 M of (nBu)₄N⁺. BF₄⁻ (tetrabutylammonium tetrafluoroborate; Fluka, purum quality, Bucks, Switzerland) was used as the solvent and supporting electrolyte. Each sample was measured at room temperature under nitrogen at a scan rate of 0.1 V s⁻¹. The reported redox potentials were read from the recorded voltammograms as the potential versus standard calomel electrode of the half of the cathodic peak. BW755C is shown for comparison.

Table 2 Inhibition of eicosanoid generation by indazolinones.

R	<i>in vitro</i> IC ₅₀ (μM)		Selectivity ratio	<i>ex vivo</i> ED ₅₀ (mg kg ⁻¹ p.o.) LTB ₄
	LTB ₄	PGE ₂
0.34 ± 0.07 (3)	2.2 ± 0.1 (3)	6.5	> 100
CH ₃	1.4 ± 0.5 (3)	18 ± 8 (3)	12.9	1.0 (2)
0.38 ± 0.2 (6)	19.1 ± 8.8 (7)	50	16 ± 4 (4)
1.5 ± 0.6 (6)	470 ± 70 (3)	313	3.8 ± 1.8 (9)

Rat blood was stimulated with A23187 following incubation of drugs for 15 min *in vitro* or, for the *ex vivo* studies, 1 h after oral dosing. Eicosanoids were measured by radioimmunoassay. Results are mean values ± s.d. The number of separate experiments is shown in parentheses. Redox potentials for the phenyl, methyl, benzyl and pyridyl methyl 2-substituted indazolinones were 0.78, 0.66, 0.69 and 0.71 V respectively.

vivo characterization in a range of species (McMillan *et al.*, 1986; Tateson *et al.*, 1988). Indazolinones are redox active compounds. However, the potency of 2-substituted indazolinones as inhibitors of leukotriene synthesis in rat blood was not directly related to redox potential (Table 1). The majority of compounds in this series have redox potentials in the range 0.7–0.8 V and are therefore considerably weaker inhibitors of oxidative processes than other inhibitors such as BW755C (0.31 V). Despite this narrow band of redox potentials, the IC₅₀ values for inhibition of LTB₄ release ranged from 0.2–14.0 μM (Table 1).

The nature of the N2 substituent was critical for the selectivity and oral activity of indazolinones (Table 2). The 2-phenyl analogue inhibited LTB₄ synthesis at submicromolar concentrations *in vitro* but exhibited only weak oral activity (47 ± 10% inhibition at 100 mg kg⁻¹; *P* < 0.001). The 2-methyl compound was a less potent inhibitor of both LTB₄ and PGE₂ synthesis *in vitro* and exhibited comparable selectivity to the phenyl analogue. However, the methyl analogue was a much more potent inhibitor *ex vivo*. The benzyl derivative retained the desirable properties of the methyl and phenyl analogues. Thus, it combined the higher LTB₄ inhibitory potency of the phenyl compound with the lower PGE₂ inhibitory potency of the methyl analogue and consequently showed an increased selectivity ratio. The *ex vivo* potency of the benzyl compound was intermediate between that of the methyl and phenyl compounds. Further increases in selectivity were obtained with other aromatic substituents (data not shown) and in the case of the 3-pyridyl substituent on N2 (ICI207968) this was accompanied by potent *ex vivo* activity (Table 2).

ICI207968 showed comparable potency and selectivity in other *in vitro* assays. Using a cytosolic fraction from RBL-1 cells, the initial rate of 5-lipoxygenase was inhibited by ICI207968 with an IC₅₀ value of 3.3 μM. The compound inhibited zymosan-stimulated LTC₄ and PGE₂ release from mouse peritoneal macrophages with IC₅₀ value of 3.2 μM and >1000 μM respectively (selectivity ratio >300). LTB₄ release from human blood stimulated with A23187 was inhibited with an IC₅₀ value of 6.0 ± 0.9 μM (*n* = 3) whilst no significant inhibition of thromboxane B₂ (TXB₂) synthesis was observed at doses up to 100 μM.

ICI207968 retained the high levels of selectivity in *ex vivo* systems. In a representative experiment the compound potently inhibited LTB₄ synthesis (ED₅₀ value 4 mg kg⁻¹ p.o.)

but did not significantly inhibit PGE₂ synthesis at 300 mg kg⁻¹ p.o. (Figure 1a). The duration of action of ICI207968 is shown in Figure 1b: the compound produced ED₅₀ values for inhibition of LTB₄ synthesis of 2.5, 10 and 25 mg kg⁻¹ p.o. at 1, 3 and 5 h after dosing. In a separate experiment (data not shown) ED₅₀ values of 4.5, 20 and 40 mg kg⁻¹ p.o. were obtained at 1, 3 and 5 h respectively.

Figure 1 Inhibition of leukotriene synthesis *ex vivo* by ICI207968. (a) ICI207968 in HPMC was given orally to groups of rats and 1 h later blood was withdrawn and stimulated with A23187. Leukotriene B₄ (LTB₄: ●) and prostaglandin E₂ (PGE₂: ■) generation were measured by radio-immunoassay. Results are mean values with s.d. shown by vertical bars. (*n* = 5) ****P* < 0.001. (b) ICI207968 in HPMC was given orally to groups of rats and at 1 h (●), 3 h (■) and 5 h (▲) after dosing blood was withdrawn and stimulated with A23187. LTB₄ generation was measured by radio-immunoassay. Results are mean values (*n* = 5) with s.d. shown by vertical bars. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Table 3 Comparison of lipoxygenase inhibitors

Lipoxygenase inhibitor	In vitro (IC ₅₀ μ M)			Ex vivo LTB ₄ ED ₅₀ (mg kg ⁻¹ , p.o.)
	LTB ₄	PGE ₂	Ratio	
BW755C	20	5	0.25	30
Phenidone	10	3	0.3	5
AA861	0.3	15	50	>100
Rev 5901	3	>300	>100	>100
BWA4C	0.35	7	20	10
A-64077	2	25	12.5	5
ICI 207968	1.5	600	400	3

Rat blood was stimulated with A23187 following incubation of drugs for 15 min *in vitro* or, for the *ex vivo* studies, 1 h after dosing.

Figure 2 Inhibition of arachidonic acid-induced polymorphonuclear leucocyte (PMNL) infiltration and plasma extravasation in rabbit skin by intradermally administered ICI207968. Arachidonic acid (100 μ g) alone or combined with various concentrations of ICI207968 was injected intradermally into six separate skin sites and the plasma extravasation and PMNL infiltration accumulated in the skin sites was assessed, as described in the methods, 1 h later. After subtracting the mean value for background of the saline-injected sites, mean values for plasma extravasation (μ l) and PMNL infiltration (¹¹¹In counts) in the skin sites were calculated for each animal and the percentage inhibition of control values for both parameters determined. Typical control values for plasma extravasation were $78.0 \pm 5.2 \mu$ l and for ¹¹¹In-PMNL infiltration were $11,370 \pm 944$ c.p.m. The values shown are the mean % inhibition of 4 or 5 rabbits with s.e.mean shown by vertical bars; **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Table 3 shows a comparison of ICI207968 with other reported lipoxygenase inhibitors. BW755C and phenidone were orally active but inhibited synthesis of 5-lipoxygenase and cyclo-oxygenase products. AA861 and Rev 5901 were selective for 5-lipoxygenase although neither compound was orally effective at the highest dose tested (100 mg kg⁻¹). ICI207968 had comparable *in vitro* potency to A-64077 but was less potent than BWA4C. However, the *ex vivo* potency of the three compounds was comparable and ICI207968 exhibited greater selectivity for lipoxygenase inhibition than either BWA4C or A-64077.

Efficacy of ICI207968 in arachidonic acid-induced rabbit skin inflammation

The anti-inflammatory activity of ICI207968 was evaluated using arachidonic acid-induced inflammation in rabbit skin, which depends in part on local generation of LTB₄ (Aked & Foster, 1987). Figure 2 shows that intradermally administered ICI207968 caused a parallel and dose-dependent inhibition of both PMNL infiltration and plasma extravasation into arachidonic acid inflamed skin sites. The doses of ICI207968 causing 30% inhibition (IC₃₀) of PMNL infiltration and plasma extravasation were 6.7 and 40.0 nmol per site respec-

Figure 3 Bioavailability of ICI207968 following oral administration in rabbit: effect on eicosanoid generation by A23187-stimulated blood *ex vivo*. Heparinised blood samples drawn from a marginal ear vein was stimulated *ex vivo* with A23187 and the amount of leutotriene B₄ (LTB₄) (●) and prostaglandin E₂ (PGE₂) (▲) generated was determined. The rabbits were then dosed orally 10 min later with 30 or 100 mg kg⁻¹ ICI207968 as indicated. At subsequent times, as indicated, further heparinised blood samples were taken and the generation of eicosanoids *ex vivo* determined as above. Values show the mean % inhibition of the control *ex vivo* stimulated eicosanoid levels; vertical bars show s.e.mean. Control levels were 69.0 ± 7.1 and 11.1 ± 1.3 ng ml⁻¹ for LTB₄ and PGE₂ respectively. Values shown are from 3 rabbits (100 mg kg⁻¹) and 2 rabbits (30 mg kg⁻¹); ***P* < 0.01; ****P* < 0.001.

tively. The anti-inflammatory activity of other intradermally administered indazolinones, assessed by plasma extravasation, was related to their potency as inhibitors of LTB₄ generation in rat blood as shown in Table 4. The table also shows that the anti-inflammatory activity of these indazolinones was not closely related to their redox potentials.

Prior to evaluating the potential oral anti-inflammatory efficacy of ICI207968 it was considered important to establish the bioavailability of the compound in rabbit blood. Figure 3

Figure 4 Inhibitory effect of ICI207968 on arachidonic acid-induced plasma extravasation in inflamed rabbit skin. Groups of 10 rabbits were given doses of ICI207968, 100 mg kg⁻¹ in HPMC, or vehicle alone, by oral intubation. Fifty min later 1 ml of blood was withdrawn into 10 units of heparin and used to determine the *ex vivo* generation of leukotriene B₄ (LTB₄) by stimulation with A23187. Ten min later various concentrations of arachidonic acid emulsion in 100 μ l physiological saline, and saline alone, were each injected intradermally into 6 separate skin sites and plasma extravasation was assessed after a further 60 min. Data show the mean skin plasma volume for the control and drug-treated animals; vertical bars show s.e.mean ****P* < 0.01; **P* < 0.05.

Table 4 Comparison of the anti-inflammatory activity of several indazolinones with their potency as inhibitors of leukotriene biosynthesis

Compound structure	Plasma extravasation IC ₃₀ (nmol per site)	In vitro blood IC ₅₀ (μM)	Redox potential (V)
15.6 ± 6.3 (4)	0.4 ± 0.2 (6)	0.69
40.0 ± 5.3 (4)	1.5 ± 0.6 (6)	0.71
> 170.0 (3)	> 50.0 (3)	0.81
> 125 (3)	> 100 (3)	0.78

Arachidonic acid (100 μg) alone or combined with various concentrations of indazolinones (2 to 200 nmol) in 100 μl of pyrogen-free saline was injected intradermally in the rabbit. Plasma extravasation into the skin sites was assessed 60 min later. Mean percentage inhibition over the control values was calculated for each indazolinone. Graphs of percentage inhibition of plasma extravasation against log drug concentration in nmol per site were plotted from which IC₃₀ values were determined. The data show the mean IC₃₀ ± s.e.mean values from 3 to 4 rabbits. IC₅₀ values for inhibition of LTB₄ generation in A23187-stimulated rat blood were determined as described in the methods. Number of experiments is shown in parentheses.

shows that an oral dose of 100 mg kg⁻¹ of ICI207968 caused almost total inhibition of the generation of LTB₄ by A23187-stimulated blood for at least 2 h without inhibiting PGE₂ generation. Although the *ex vivo* PGE₂ generation was enhanced 1 h post-dose the effect was not statistically significant. However, an oral dose of ICI207968 (30 mg kg⁻¹) was much less effective in inhibiting *ex vivo* LTB₄ generation over 2 h as shown in Figure 3. Therefore, an oral dose of ICI207968 (100 mg kg⁻¹) was chosen to evaluate its anti-inflammatory efficacy. As shown in Figure 4, an oral dose of ICI207968 (100 mg kg⁻¹) caused statistically significant inhibition (approximately 40%) in the local plasma extravasation induced by the intradermal injection of a range of arachidonic acid concentrations into rabbit skin. *Ex vivo* generation of LTB₄, in this study, was inhibited by 72% (*P* < 0.001) assessed 50 min after dosing.

Discussion

Since elucidation of the structures of LTB₄ and the peptido-leukotrienes and demonstration of their potent spasmogenic and pro-inflammatory properties, it has been speculated that inhibitors of their biosynthesis would have therapeutic potential in diseases such as asthma, arthritis and psoriasis. Until recently agents with appropriate selectivity and oral efficacy have not been available to investigate this hypothesis. The discovery of ICI207968, a potent selective and orally active 5-lipoxygenase inhibitor, has now provided a suitable agent to explore the consequences of inhibiting leukotriene biosynthesis.

Studies in several *in vitro* systems demonstrate that ICI207968 is at least 200 fold more potent as an inhibitor of synthesis of leukotrienes than of PGE₂. Moreover, this selective biochemical effect was also observed *in vivo*: following oral administration of ICI207968 to rats no inhibition of *ex vivo* PGE₂ generation in blood was evident at a dose 100 times that required to inhibit LTB₄ generation. In rat blood, ICI207968 was a more potent inhibitor of TXB₂ synthesis

than of PGE₂ (data not shown) and this was observed at concentrations approximately 10 times higher than the lipoxygenase inhibitory doses. This can be attributed to inhibition of thromboxane synthase due to the presence in the molecule of a pyridine substituent which has previously been shown to inhibit this enzyme (Tai *et al.*, 1980).

We do not know the precise mechanism of action of ICI207968. Since 5-lipoxygenase is believed to be an iron-containing enzyme, which is rendered inactive by reduction from the ferric to ferrous form, a variety of compounds may inhibit the enzyme by redox mechanisms. 5-Lipoxygenase exhibits bizarre kinetics and mechanistic studies require complex mathematical analyses. Unfortunately the best available model of the kinetics of 5-lipoxygenase (Aharony & Stein, 1986) does not permit mechanistic studies with drugs which have redox properties. Although 2-substituted indazolinones have the potential to participate in redox reactions our data suggest that their efficacy as lipoxygenase inhibitors is not solely related to this property. Firstly, ICI207968 and other indazolinones have weaker redox properties than previously reported inhibitors such as BW755C. Secondly, there is no direct relationship between the redox potentials of 2-substituted indazolinones and their potency as inhibitors of leukotriene synthesis. Thirdly, the selectivity and oral activity of indazolinones are profoundly influenced by the nature of the substituent on the N-2 nitrogen in a manner which is independent of redox potential. Another possible mechanism of ICI207968 is iron chelation and since we have not measured iron binding with ICI207968 or related indazolinones, we cannot exclude the possibility that this contributes to their activity.

Although several compounds with comparable *in vitro* potency and selectivity have been described previously, they have lacked demonstrable biochemical efficacy following oral administration (Foster *et al.*, 1986; McMillan *et al.* 1986). Furthermore, those compounds which have been demonstrated to inhibit leukotriene generation following oral dosing also inhibit prostaglandin synthesis which makes interpretation of their pharmacological actions difficult (Higgs *et al.*, 1979;

DiMartino *et al.*, 1987; Griswold *et al.*, 1987). Only the aceto-hydroxamic acid, BWA4C (Tateson *et al.*, 1988) and the N-hydroxy urea analogue, A-64077 (Carter *et al.*, 1989) fulfil similar criteria. In comparative studies, we observed that, although ICI207968 has lower *in vitro* potency than BWA4C, it has comparable *ex vivo* potency to BWA4C and A-64077 and has greater selectivity than either compound. To our knowledge ICI207968 is the most selective of the orally-active 5-lipoxygenase yet described.

One of the key issues surrounding selective 5-lipoxygenase inhibitors whether such agents would have anti-inflammatory properties. Since LTB₄ is a potent chemotactic agent (Bray *et al.*, 1981; Rollins *et al.*, 1983; Camp *et al.*, 1983) and peptido-leukotrienes can induce plasma extravasation in animals (Ueno *et al.*, 1981) and wheal and flare reactions in man (Camp *et al.*, 1983; Soter *et al.*, 1983), it can be argued that agents which inhibit their biosynthesis might be anti-inflammatory. Until recently, there has been no suitable model in which to evaluate the anti-inflammatory efficacy of selective 5-lipoxygenase inhibitors. Intradermal injection of arachidonic acid in the rabbit causes a PMNL-dependent inflammatory reaction (Aked *et al.*, 1986) which depends, in part, on the local generation of LTB₄ (Aked & Foster, 1987). This appeared, therefore, to be an appropriate model in which to evaluate the anti-inflammatory efficacy of ICI207968. In this model ICI207968 was anti-inflammatory when administered by both local or oral routes. Intradermal studies demonstrated that the compound inhibited both plasma extrava-

sation and PMNL infiltration which is consistent with previous studies on the contribution of LTB₄ in this model (Aked *et al.*, 1986). Parallel *ex vivo* analyses in rabbit blood demonstrated that ICI207968 caused marked inhibition of LTB₄ generation without inhibiting PGE₂ synthesis. These results suggest that the anti-inflammatory action of ICI207968 is a consequence of its inhibitory effect on leukotriene biosynthesis and support the view that selective 5-lipoxygenase inhibitors will have anti-inflammatory properties. An anti-inflammatory effect due to a non-specific redox mechanism of ICI207968 is inconsistent with our results since indazolinones having similar redox potential to ICI207968 showed widely different anti-inflammatory activity in this model. In fact, the anti-inflammatory activity of a range of indazolinones was found to be related to their potency as 5-lipoxygenase inhibitors and not redox potential.

In conclusion, ICI207968 is an orally active selective 5-lipoxygenase inhibitor which has anti-inflammatory properties. The pharmacology described here supports the view that selective 5-lipoxygenase inhibitors may be useful therapeutic agents for the treatment of inflammatory disease. Moreover, ICI207968 will be a useful agent to probe the role of leukotrienes in biological systems.

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