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Effects of IZP-94005 (contignasterol) on antigen-induced bronchial responsiveness in ovalbumin-sensitized guinea-pigs

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1 We have investigated the novel naturally occurring marine compound, IZP-94005 (contignasterol), as a potential anti-asthma agent, using both *in vivo* and *in vitro* models of allergen-induced bronchoconstriction and airway smooth muscle contraction.

2 Tracheal rings from ovalbumin (OA)-sensitized guinea-pigs were treated with various concentrations of IZP-94005 for 20 min prior to challenge with ovalbumin. IZP-94005 (3-30 μM) inhibited responses of sensitized tracheal rings stimulated with OA in a concentration-dependent manner, with an IC_{50} of 10 μM.

3 IZP-94005 (10 μM) had no effect on carbachol-induced contractions of sensitized guinea-pig tracheal rings, although it did inhibit histamine-induced responses of OA sensitized guinea-pig tracheal rings.

4 The effects of IZP-94005 *in vivo* were examined using OA-sensitized guinea-pigs which were tracheotomized under anaesthesia and placed in a body plethysmograph. Measurements of lung resistance and compliance were performed by isovolumetric analysis of volume and trans-pulmonary pressure.

5 IZP-94005 (50 and 200 $\mu g\ kg^{-1}$), by inhalation 20 min prior to OA challenge caused significant inhibition of the increase in lung resistance induced by OA in sensitized guinea-pigs, compared to vehicle-treated animals. Nedocromil sodium (20 $mg\ kg^{-1}$), with a similar protocol, also inhibited OA-induced responses in this model.

6 We therefore suggest that IZP-94005 is a good candidate for further investigation as a possible anti-asthma agent.

Keywords: IZP-94005; natural product; responsiveness; sensitized; tracheal smooth muscle; steroid

Introduction

In the search for novel anti-inflammatory and anti-asthma agents attention has been focused on the marine environment as a potential source of new compounds. To date many marine organisms have been found to be a source of numerous unusual compounds, some of which have been explored both chemically and pharmacologically. For example, the Caribbean coral *Pseudopterogorgia elisabathae* has been found to contain a number of compounds called pseudopterosins, which have anti-inflammatory activity (Leudke, 1990). Another marine-derived product, fucoside, isolated from the sponge *Eunicea fusca*, has been found to possess inhibitory activity against phorbol ester-induced oedema in the mouse ear model of inflammation (Jacobsen & Jacobs, 1992a). Furthermore, fucoside inhibits 5-lipoxygenase enzymes in leukoctyes (Jacobsen & Jacobs, 1992b).

Two other compounds with an unusual sterol-based configuration have also been isolated from the marine sponge *Xestospongeria berquistia*, called xestobergsterols A and B (Noboru *et al.*, 1992; Takei *et al.*, 1993). Both xestobergsterol A and B have powerful inhibitory activity against IgE-mediated histamine release from rat mast cells, although only xestobergsterol A shows inhibitory activity against phospholipase C and inositol trisphosphate (IP_3) generation (Takei *et al.*, 1993). Thus, it has been suggested that at least a component of the inhibitory activity of xestobergsterol A against histamine release may be mediated by inhibition of phospholipase C, although this mechanism does not explain the inhibitory actions of the other compound, xestobergsterol B. IZP-94005 (contignasterol) is the first of a group of naturally occurring compounds which have been isolated from another marine sponge *Petrosia sp.* (Burgoyne *et al.*, 1992). The compound has a sterol configuration which is highly oxygenated and possesses an unusual side-chain (Figure 1). The present study evaluates the pharmacological activity of IZP-94005 using both *in vitro* and *in vivo* guinea-pig models of acute antigen-induced bronchoconstriction in which animals have been sensitized to ovalbumin (OA).

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Methods

Cam/Hartley guinea pigs 350-400 g were used in this study, obtained from Charles-River, Montreal. The animals were housed in climate-controlled animal quarters and were given food and water *ad libitum*. All protocols described were approved by the University of British Columbia Animal Care Ethics Committee, and were in accordance with the Canadian Council for Animal Care.

Effects of IZP-94005 on antigen-induced contraction of tracheal smooth muscle in vitro

Guinea pigs were sensitized by a modified version of an existing protocol (Salari *et al.*, 1992) by injection of 100 mg ovalbumin (OA) (i.p.) and 100 mg OA (i.m.) in 0.9% saline. This was followed by a repeat injection of 50 mg OA (i.m.) 24 h later. After 21 days the animals were acutely sensitized i.e. they would respond with anaphylactic bronchospasm to a further antigen challenge. Two groups of guinea-pigs were used, one group was sensitized to OA, and the second sham-sensitized with saline, which served as the non-sensitized group.

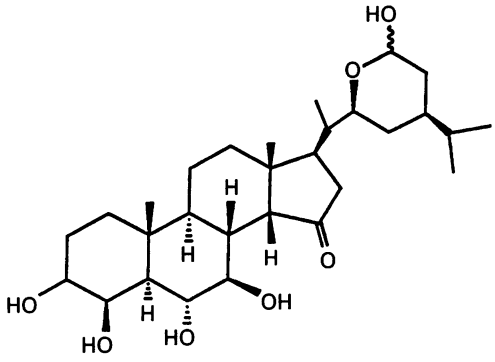

Figure 1 Structure of IZP-94005.

Twenty one days following sensitization, animals were killed and the trachea removed and placed in cold Krebs-Henseleit solution (KHS) of the following composition (mM): NaCl 118, KCl 4.7, $MgSO_4$ 7 H_2O 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, D-glucose 11.7 and $CaCl_2$ 2 H_2O 2.5 at pH 7.4. Intact tracheal rings (2–3 cartilage rings wide) were suspended under 2 g tension in 5 ml tissue baths containing KHS at 37°C and aerated with 5% CO_2 in O_2. Isometric contractions were measured with force-displacement transducers (FT03.C; Grass Instruments, Quincy, MA, U.S.A.) coupled to a Grass 7D polygraph. Tissues were equilibrated for 60 min during which time the buffer was changed three times.

At the beginning of each experiment, using one tracheal ring from the isolated trachea, a standard contractile response to carbachol was obtained to establish the viability of the tissue. Following this, cumulative concentration-response curves to OA were obtained in the presence of IZP-94005 or the vehicle alone (0.1% dimethylsulphoxide). A separate tracheal ring was used for each concentration-response curve. At the end of each experiment, the tissues were washed and contracted with a standard concentration of carbachol (100 μM). This was repeated in tracheal rings from both sensitized and non-sensitized guinea-pigs. The magnitude of contraction induced by OA in the presence and absence of the IZP-94005 was standardized as a percentage of the maximum contraction obtained in the presence of carbachol (100 μM).

In a separate group of experiments full cumulative concentration-response curves to both carbachol and histamine (10^{-8}–10^{-4} M) were obtained in the same tissue bath system, and the effects of IZP-94005 evaluated. In these experiments IZP-94005 (10 μM) was added to the tissue bath 20 min before addition of carbachol or histamine.

Effects of IZP-94005 and nedocromil sodium on antigen-induced bronchoconstriction in vivo

Two groups of guinea-pigs were used. One group was sensitized by the above injection sensitization protocol. The second group was sensitized by inhalation of 1% OA in saline, according to the method of Ishida *et al.* (1990). A number of guinea-pigs were also sham-sensitized with saline inhalation (non-sensitized). Using this protocol, the animals were challenged 10–12 days following sensitization. The animals were anaesthetized with 1% halothane by inhalation, followed by ketamine (50 mg kg^{-1}, i.p.) and xylazine (10 mg kg^{-1}, i.p.) as maintenance anaesthesia. A tracheostomy was performed and an oesophageal catheter inserted before the animal was positioned in a body plethysmograph and attached to a fixed tracheal tube on the box. Cardiac function was monitored by electrocardiograph (ECG). The guinea-pig was paralyzed with succinylcholine (2 mg kg^{-1}, i.p.) and then ventilated with 3 ml tidal breaths using a Harvard small animal ventilator (Harvard, Instruments, MA, U.S.A.), at a frequency of 60 breaths min^{-1}. Pulmonary resistance and dynamic compliance data were obtained from the volume flow and pressure signals, according to the method of Von Neerguard & Wirtz (1927), using a modified multipoint regression model for analysis (Ludwig *et al.*, 1991) and calculated as either absolute change in lung resistance (R_L; cmH_2O ml^{-1} s^{-1}) or % change in lung resistance from baseline (Ishida *et al.*, 1990).

A baseline resistance trace was obtained, and then a saline treatment administered directly into the airway by a Hudson disposable nebulizer (six breaths). Subsequent to this, IZP-94005 (50 and 200 μg kg^{-1}), nedocromil sodium (20 mg kg^{-1}) or vehicle (0.9% saline) alone were given over 30 s at a rate of one breath s^{-1}. Following 20 min incubation, concentrations of acetylcholine (ACh) 50 and 150 mg kg^{-1} or OA (3%) were administered as an aerosol by the nebulizer.

Pulmonary function was continually monitored throughout these experiments and measurements made at various time-points (0, 1, 3, 10, 20 and 30 min) following antigen challenge or administration of acetylcholine. Data were collected on a computer-linked physiological measurement system using DIREC Physiological recording software (Raytech Inst., Vancouver, Canada) and analyzed with ANADAT (RHT. Infodat Inc, Montreal, Canada).

Determination of histamine release from lung tissue

Lungs from guinea-pigs sensitized to OA were perfused *in situ* with Ca^{2+}-free HEPES buffer (composition, mM: NaCl 140, KCl 4.5, glucose 10, HEPES 5, $MgCl_2$ 1, pH 7.4) at 37°C containing 1000 u ml^{-1} heparin. The lungs were then excised and sub-pleural strips prepared and washed in HEPES buffer containing 2.5 mM Ca^{2+}. Segments approximately 2–3 mm^3 were incubated in 1 ml of buffer at 37°C for 1 h, and every 30 min the buffer was replaced. Tissues were treated with DMSO alone (vehicle), OA (0.1 μg ml^{-1}), IZP-94005 (30 μM) or IZP-94005 (30 μM) followed by OA (0.1 μg ml^{-1}). Tissues were treated with IZP-94005 20 min before stimulation. Following 5 min incubation, the tissues were removed, flash frozen and stored for determination of total histamine content. The incubation media and tissues were stored at −80°C until assayed for histamine content. Histamine content was measured with a radio-immunoassay kit (Amgen Inc, M.E.), and histamine release was expressed as the concentration of histamine in the medium per mg of each tissue.

Statistical analysis

Results are expressed as the mean ± s.e.mean (standard error of the mean) for n number of animals. All statistical analysis was performed using Sigmastat (Jandel Scientific, CA, U.S.A.). For the *in vivo* studies, the data were analyzed by repeated measures ANOVA with correction for multiple comparisons, by the Bonferroni method. *In vitro* data were analyzed by Student's t test for unpaired data with correction for multiple comparisons, for the antigen studies, while a one way ANOVA was used for analysis of the effects of the drugs on histamine-induced contractions. The IC_{50} for IZP-94005 was calculated by analysis of separate concentration-response curves and expressed as the geometric mean. The IC_{50} value was determined as the concentration of IZP-94005 required to inhibit a submaximal dose of OA (EC_{70}, 1 μg ml^{-1}) by 50%. Histamine release studies were analyzed by a non-parametric Mann-Whitney test. Changes were considered significant when $P<0.05$.

Materials

Acetylcholine, carbachol, histamine and ovalbumin were obtained from Sigma Chemical Co., MO, U.S. DMSO (dimethylsulphoxide) was from Fisher Scientific, Vancouver Canada. IZP-94005 (contignasterol) was obtained from Inflazyme Pharmaceuticals Ltd, Vancouver Canada. For both *in vitro* and *in vivo* studies IZP-94005 was dissolved in DMSO and subsequent dilutions were made in Krebs buffer.

Results

Effects of IZP-94005 on antigen-induced contractions of airway smooth muscle in vitro

IZP-94005 produced significant inhibition of contractions of tracheal rings from sensitized guinea-pigs in response to cumulative administration of OA ($0.001-10\ \mu g\ ml^{-1}$). The threshold of activity for IZP-94005 *in vitro* was approximately $1-3\ \mu M$, and effects of IZP-94005 were found to be concentration-dependent between 3 and 30 μM (Figure 2). The IC_{50} for IZP-94005 in this system was 10 μM i.e. at a concentration of 10 μM IZP-94005 there was $53.08 \pm 0.59\%$ inhibition of the OA response.

IZP-94005 (10 μM) significantly inhibited the maximum response induced by histamine ($10^{-8}-10^{-3}$ M), from 100% of histamine control in the absence of IZP-94005 to $73 \pm 4\%$ of histamine control in the presence of IZP-94005, but there was no significant change in EC_{50} in tracheal rings from sensitized guinea-pigs, (Figure 3). However, no significant difference was obtained with IZP-94005 (10 μM) on carbachol-induced responses in tracheal rings from sensitized guinea-pigs. Maximum responses in g tension for carbachol responses were 1.45 ± 0.26 g in the absence and 1.37 ± 0.15 g ($95 \pm 9.6\%$ of carbachol control) in the presence of IZP-94005. EC_{50} values were also no different, being $0.3 \pm 0.05\ \mu M$ in control and $0.5 \pm 0.15\ \mu M$ in the IZP-94005-treated group. In tracheal rings from non-sensitized guinea-pigs, IZP-94005 (10 μM) also had no significant effect on carbachol responses. Maximum responses in IZP-94005-treated tissues were $103 \pm 9.8\%$ of control, and the EC_{50} values were $2.3 \pm 0.7\ \mu M$ in control and $1.8 \pm 0.8\ \mu M$ in the IZP-94005-treated tissues.

Effects of IZP-94005 on antigen-induced bronchoconstriction in vivo

IZP-94005 (50 $\mu g\ kg^{-1}$), administered by inhalation directly into the trachea 20 min prior to OA challenge, produced significant inhibition of OA-induced bronchoconstriction in anaesthetized guinea-pigs. This result was achieved in guinea-pigs sensitized by both injection and inhalation protocols (Figure 4a, b, c). This was measured as an inhibition in the increase in lung resistance (Figure 4a and 4b) and protection against the decrease in lung compliance (Figure 4c) which were observed following an acute OA challenge. IZP-94005 protected against increases in lung resistance for up to 10 min post allergen challenge (Figure 4a) and caused a significant inhibition of the peak response to OA challenge which occurred at 1-3 min (Figure 4a and 4b). A higher concentration of IZP-94005 (200 $\mu g\ kg^{-1}$) provided even greater protection against OA-induced increases in lung resistance (Figure 5a) and decreases in lung compliance (Figure 5b). Approximately 70% inhibition of the increase in resistance following OA challenge was obtained, indicating the protective effects of IZP-94005 appear to be dose-dependent for these two doses.

In a separate study IZP-94005 (200 $\mu g\ kg^{-1}$) was compared to nedocromil (20 $mg\ kg^{-1}$), both IZP and nedocromil protected against acute allergen challenge (Figure 6). In this study the protective effects of nedocromil were not statistically significant compared to control, probably due to the large variation in control values in this particular experiment. However, although the effects of IZP-94005 were significant compared to control, there was no significant difference between the effects of IZP-94005 and nedocromil *per se*. IZP-94005 200 $\mu g\ kg^{-1}$ also significantly inhibited bronchoconstriction in response to acetylcholine (50 and 150 $mg\ ml^{-1}$) in ovalbumin-sensitized guinea-pigs (Figure 7). However IZP-94005 had no effect on ACh responses in sham (non-sensitized) guinea-pigs (Figure 7b).

Effects of IZP-94005 on histamine release from guinea-pig lung tissue

Basal histamine release from sensitized lung tissue following incubation of tissue with DMSO (0.1%) for 20 min was found to be $31.9 \pm 1.7\%$ of total histamine content. During this period the concentration of histamine in the bathing media was 2.89 ± 0.1 nmol mg^{-1} tissue. During stimulation with OA, the concentration was increased approximately two fold. IZP-94005 (30 μM) did not affect the level of histamine released under basal conditions but significantly reduced the level of histamine released in the presence of OA (Figure 8).

Discussion

In this study we have demonstrated that IZP-94005 significantly inhibits the response of airways from sensitized

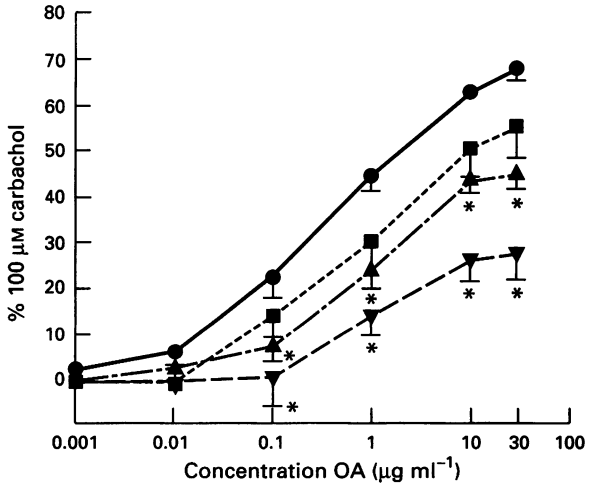

Figure 2 Effect of 3 μM, 10 μM and 30 μM, IZP-94005 on ovalbumin (OA)-induced contraction of tracheal rings from OA-sensitized guinea-pigs. IZP-94005 was administered 20 min before the OA challenge. (●) OA and vehicle-treated; IZP-94005-treated: (■) 3 μM, (▲) 10 μM, (▼) 30 μM ($n=7$). Values represent the mean ± s.e.mean of 7 determinations; *indicates significant difference from control response to OA, $P<0.05$, Student's t test.

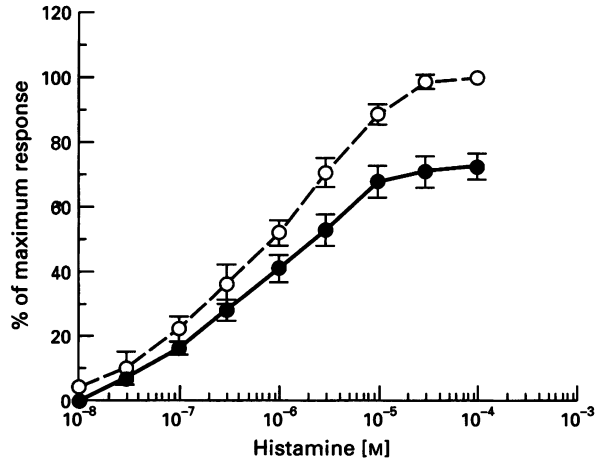

Figure 3 Effect of IZP-94005 (10 μM) on histamine-induced contractions in tracheal rings from sensitized guinea-pigs. IZP was administered 20 min before stimulation with cumulative additions of histamine (0.01 μM - 1 mM). Vehicle-treated (○), IZP-94005-treated (●). Values represent the mean ± s.e.mean of 5 determinations/experiments. *Indicates significant differences from control (vehicle-treated), $P<0.05$.

guinea-pigs challenged with antigen in both *in vitro* and *in vivo* model systems. The effects of a 20 min pretreatment with IZP-94005 were found to be both concentration and dose-dependent, with a rapid onset of action, protective effects being observed up to 10 min following acute OA challenge. In addition when compared to an established anti-asthma agent, nedocromil sodium, IZP-94005 was found to be at least as potent in protecting against acute bronchoconstriction. In this model although the protective effects of nedocromil were not significant, this was probably due to the large variation in control values. However, nedocromil has been shown to give significant protection in a very similar guinea-pig allergen model (Schellenberg *et al.*, 1991). We felt however, the degree of protection afforded by nedocromil in our study is partly reflective of the concentration of OA challenge solution. In the study by Schellenberg *et al.* (1991) a 1% OA challenge solution was used which gave a 2–3 fold increase in lung resistance. In the present study we obtained at least a 10–15 fold increase in lung resistance over baseline using a 3% challenge solution.

At the present time the exact mode of action of IZP-94005 is not known. Although IZP-94005 has a basic sterol structure, it is not likely that the drug is acting through a classical glucocorticosteroid mechanism to inhibit phospholipase A_2 (Lan *et al.*, 1984), due to the structural configuration of the molecule and since the onset of action is very rapid. Classical glucocorticosteroid action involves a delayed effect which includes

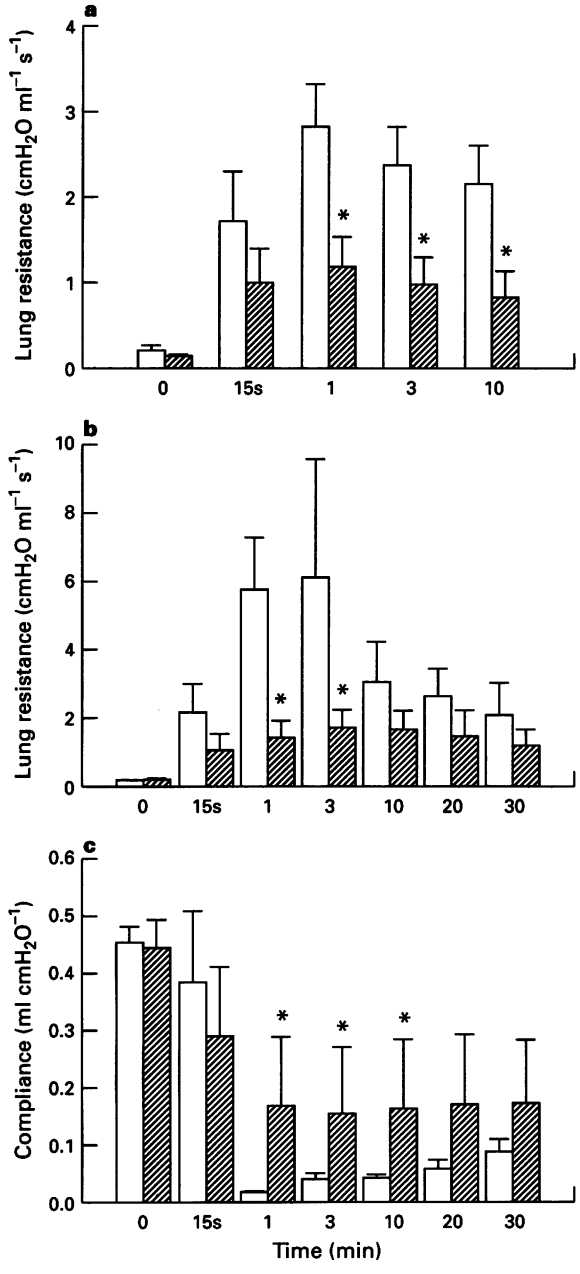

Figure 4 (a) The effect of IZP-94005 (50 $\mu g\,kg^{-1}$) on the increase in lung resistance and induced by OA challenge in guinea-pigs sensitized by the injection protocol. IZP-94005 (hatched columns) or vehicle control (open columns) were administered by inhalation 20 min before antigen challenge. All data points indicate the mean ± s.e.mean of 6 experiments. *Indicates significant difference from control (vehicle-treated) response, $P<0.05$. (b) The effect of IZP-94005 (50 $\mu g\,kg^{-1}$) on the increase in lung resistance induced by OA challenge in sensitized guinea-pigs. IZP-94005 (hatched columns) or vehicle control (open columns) were administered by inhalation 20 min before antigen challenge. All data points indicate the mean ± s.e.mean of 6 experiments. *Indicates significant difference from control (vehicle-treated) response, $P<0.05$. (c) The effect of IZP-94005 (50 $\mu g\,kg^{-1}$) on the decrease in dynamic lung compliance induced by OA challenge in sensitized guinea-pigs. IZP-94005 (hatched columns) or vehicle control (open columns) were administered by inhalation 20 min before antigen challenge. All data points indicate the mean ± s.e.mean of 6 experiments. *Indicates significant difference from control (vehicle-treated) response, $P<0.05$.

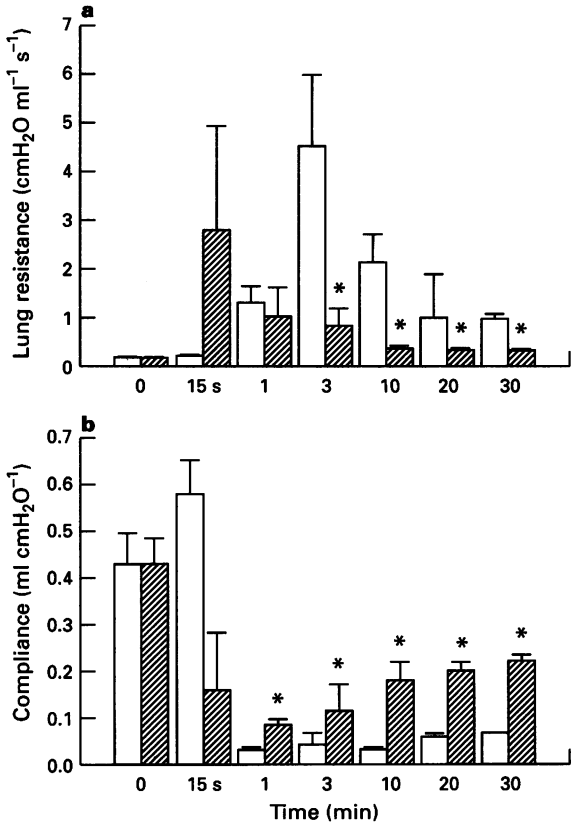

Figure 5 (a) Effect of IZP-94005 (200 $\mu g\,kg^{-1}$) on OA-induced bronchoconstriction in sensitized guinea-pigs. IZP-94005 was administered by the inhaled route 20 min before antigen challenge. Open columns represent vehicle-treated, hatched columns IZP-94005-treated. All data points indicate the mean ± s.e.mean of 6 experiments. *Indicates significant difference from control (vehicle treated) response, $P<0.05$. (b) The effect of IZP-94005 (200 $\mu g\,kg^{-1}$) on the decrease in dynamic lung compliance induced by OA challenge in sensitized guinea-pigs. IZP-94005 (hatched columns) or vehicle control (open columns) were administered by inhalation 20 min before antigen challenge. All data points indicate the mean ± s.e.mean of 6 experiments. *Indicates significant difference from control (vehicle-treated) response, $P<0.05$.

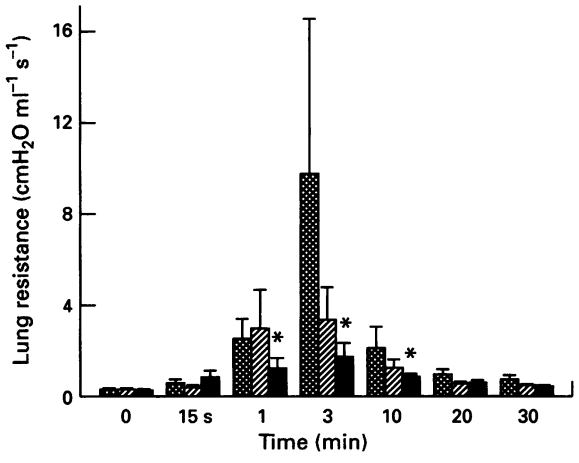

Figure 6 Effect of IZP-94005 (200 $\mu g\,kg^{-1}$) and nedocromil (20 $mg\,kg^{-1}$) on OA-induced bronchoconstriction in sensitized guinea-pigs. IZP-94005 was administered by the inhaled route 20 min before antigen challenge and nedocromil by the same route 10 min before OA challenge. Stippled columns represent vehicle-treated, solid columns IZP-94005-treated, and hatched columns, nedocromil-treated. All data points indicate the mean ± s.e.mean of 3–4 experiments. *Indicates significant difference from control (vehicle-treated) response, $P<0.05$.

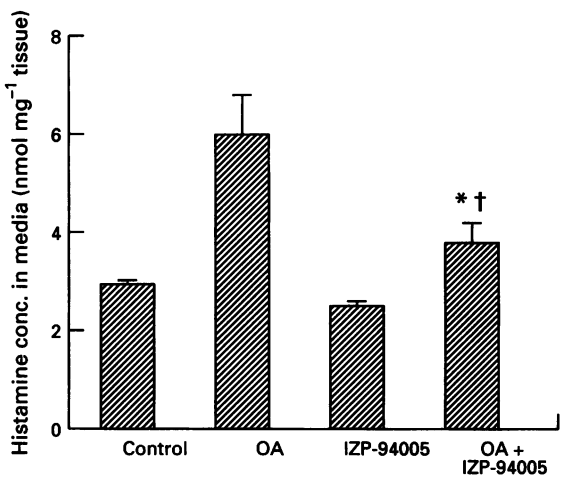

Figure 8 The effect of IZP-94005 (30 μM) on OA-induced histamine-release from lung parenchymal tissue of sensitized guinea-pigs. Histamine levels were determined in the bathing media (1 ml) and expressed as a concentration in the media per mg tissue ($nmol\,mg^{-1}$ tissue). The data represent the mean ± s.e.mean (mean of 5 determinations). *Indicates significant from basal (control); † indicates significant difference from OA-treated, $P<0.05$ (Mann Whitney test).

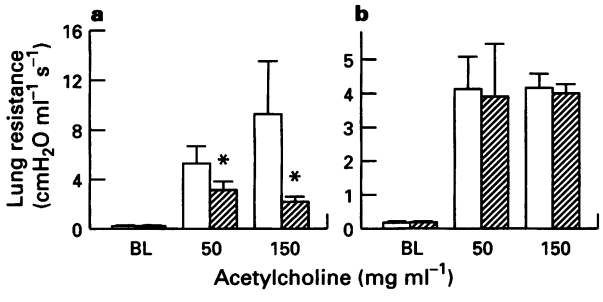

Figure 7 (a) Effect of IZP-94005 (50 $\mu g\,kg^{-1}$) on bronchoconstriction induced by acetylcholine (ACh, 50 and 150 $mg\,ml^{-1}$) in sensitized guinea-pigs. IZP-94005 was administered by inhalation 20 min before ACh challenge. Open columns represent the vehicle-treated animals and the hatched columns represent IZP-94005-treated animals. Values represent the mean ± s.e.mean of 5 experiments. *Indicates significant difference from control (vehicle-treated), $P<0.05$. (b) Effect of IZP-94005 (50 $\mu g\,kg^{-1}$) on bronchoconstriction induced by ACh (50–150 $mg\,ml^{-1}$) in non-sensitized guinea-pigs. IZP-94005 was administered by inhalation 20 min before ACh challenge. Open columns represent the vehicle-treated animals and the hatched columns represent IZP-94005-treated animals. Values represent the mean ± s.e.mean of 5 experiments.

the generation of newly synthesized protein (lipocortin) which inhibits phospholipase A_2 (Flower & Blackwell, 1979; Flower, 1988).

In addition to the protective action of IZP-94005 on acute OA-induced bronchoconstriction, IZP-94005 also inhibited the release of histamine from lung tissue slices from sensitized guinea-pigs, suggesting an interaction with inflammatory cells involved in mediator release, such as mast cells. In addition IZP-94005 has also been shown to inhibit histamine release from rat peritoneal mast cells (Takei *et al.*, 1994). These findings suggest that IZP-94005 could be having a component of its inhibitory effect on acute bronchoconstriction via an action similar to that elicited by disodium cromoglycate or nedocromil sodium, although nedocromil has additional modes of action. The suggestion is supported by our *in vivo* data with nedocromil for this study and other preliminary observations with disodium cromoglycate inhibiting acute OA-induced contraction of tracheal rings from sensitized guinea-pigs (data not shown).

One of the more important mechanisms of action of disodium cromoglycate is thought to be via inhibition of IgE-dependent histamine release from mast cells (Altounyan, 1967; Cox, 1967). This is now thought to involve specifically bronchoalveolar lavage (BAL) mast cells (Flint *et al.*, 1985), and disodium cromoglycate may also prevent IgE receptor binding to other inflammatory cells including macrophages and eosinophils. Mast cells play a pivotal role in many of the events leading to regulation of airway smooth muscle contraction, by releasing mediators such as histamine, leukotrienes and prostanoids. The most important mechanism for the release of histamine from mast cells involves antibodies of the IgE class attached to the surface of the cell. During exposure to antigen, the antigen molecule combines with at least two adjacent antibody molecules bound to the cell surface, and the mast cell releases its granule contents containing histamine. The released histamine then interacts with smooth muscle cells to cause muscle contraction, and initiate inflammatory responses and mucus secretion (Langlands *et al.*, 1993). In addition to inhibiting the release of histamine from lung tissue strips the observation that IZP-94005 attenuated the maximum contractile response to histamine in sensitized tissue suggests that a component of the contractile response of sensitized tissue stimulated with histamine is indirect via the release of other mediators. The exact mechanism whereby IZP-94005 exerts this effect still remains unclear. It is attractive to suggest, however, that IZP-94005 may indirectly interact with cellular signalling systems involving inositol phospholipid metabolism.

Phospholipase C has been shown to be involved in the generation of putative second messengers such as inositol trisphosphate and diacylglycerol, which are both believed to play an important role in the initiation and maintenance of the contractile response in airway smooth muscle (Hall *et al.*, 1990; Langlands *et al.*, 1990). Furthermore Salari *et al.* (1992) observed that IP_3 generation was elevated in tracheal smooth muscle from sensitized guinea-pigs compared to non-sensitized animals, indicating an increase in phospholipase C activity. Previous studies have also demonstrated that the aminosteroid, U73122, inhibited phospholipase C (PLC) activity *in vitro* and reduced contraction of guinea-pig tracheal tissue. This supports the idea that increased inositol phospholipid meta-

bolism in the inositol phospholipid signal transduction pathway could be an important factor in hyperresponsive of guinea-pig airways (Salari *et al.*, 1993). However this effect would have to be mediated through an indirect mechanism since in these studies IZP-94005 did not inhibit carbachol-induced *in vitro* responses, and the main signalling system for carbachol is via a PLC mechanism.

The observation that IZP-94005 inhibited ACh-induced bronchoconstrictor responses of sensitized guinea-pigs *in vivo*, but not of carbachol-induced responses of sensitized tracheal smooth muscle *in vitro*, also suggests that IZP-94005 is working via a different inhibitory mechanism of action evident *in vivo* only. However the effects of IZP-94005 blocking contraction of histamine *in vitro* and bronchoconstriction by ACh *in vivo* are unlikely to be non-specific, as IZP-94005 had no inhibitory effect in non-sensitized guinea-pigs *in vivo* or *in vitro* with either ACh, histamine or carbachol. We feel the reason protection against bronchoconstriction occurs only in sensitized guinea-pigs *in vivo* is because the mechanism of action of IZP-94005 is in some way dependent on targeting the abnormal changes which occur in sensitized guinea-pigs. If, for example, IZP-94005 has a component of its action through indirect inhibition of mast cell mediator release, then it is likely the effects would be observed only where enhanced recruitment and priming of mast cells occurs as in sensitized and challenged guinea-pigs. Also any inhibitory effect of IZP-94005 on the IP_3 signal transduction pathway is more likely to occur when there has been enhancement of PLC activity and IP_3 production in sensitized hyperresponsive guinea-pigs.

It has previously been shown that inhibition of histamine release by another marine product xestobergsterol A is accompanied by inhibition of phospholipase C activity (Takei *et al.*, 1993). The possibility exists therefore that IZP-94005 and xestobergsterol A could be acting via a similar mechanism, and this theory is made attractive by the striking similarity in the structures of these two compounds (Takei *et al.*, 1993; Noboru *et al.*, 1992).

In conclusion, we have demonstrated that IZP-94005 inhibits antigen-induced contractions of tracheal tissue from sensitized guinea-pigs, and protects against the bronchoconstriction produced during acute antigen challenge *in vivo*.

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