

IPL576,092, a novel anti-inflammatory compound, inhibits leukocyte infiltration and changes in lung function in response to allergen challenge

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Received 5 February 2004; revised 26 June 2004; accepted 14 July 2004

Abstract

IPL576,092, a lead compound from a novel class of polyhydroxylated sterols, was tested in models of allergen-induced bronchoconstriction and airway inflammation. In a rat ovalbumin lung inflammation model, orally administered IPL576,092 significantly inhibited the challenge-mediated increase in total bronchoalveolar lavage leukocyte numbers, and macrophage and lymphocyte infiltration (1–10 mg/kg/day). There was a similar trend towards inhibition of eosinophil and neutrophil accumulation. Sheep were treated with IPL576,092 by inhalation (400 µg/kg/day), and lung resistance and airway hyper-responsiveness (AHR) were determined after *Ascaris suum* challenge. IPL576,092 significantly reduced the early and late phase bronchoconstrictor responses by 63 ± 4.6 and $84 \pm 4.6\%$, respectively. IPL576,092 also blocked AHR ($2.2 \pm 5.7\%$ change from pre-challenge PC400), whereas control animals showed a $62.2 \pm 2.6\%$ decrease in the PC400 ($p < 0.05$). Oral IPL576,092 (5 mg/kg/day) also significantly decreased hyper-reactivity in mice. In a guinea pig model, IPL576,092 (5 mg/kg/day) significantly protected against allergen-induced increases in lung resistance (11.4 ± 2.3 control versus 4.8 ± 0.5 IPL576,092, area under the curve) and inhibited the increase in lung elastance (280 ± 58 control versus 167 ± 52 IPL576,092, $p < 0.05$). IPL576,092, unlike dexamethasone, did not significantly decrease rat serum corticosterone levels or thymus and spleen weights, supporting a mechanism of action different from classic glucocorticoids. IPL576,092 significantly attenuates characteristics of an asthmatic response, indicating therapeutic potential for this drug class.

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Keywords: Asthma; Therapeutic; Inflammation; Airway hyperresponsiveness; Bronchoconstriction

1. Introduction

Asthma is a chronic, inflammatory disease of the airways affecting approximately 8% of the world's population. The disease is characterized by reversible obstruction and hyperresponsiveness to inhaled stimuli, and is manifested in shortness of breath. There are rising trends in asthma prevalence and severity, with accompanying increases in the morbidity and mortality rates. Current asthma therapies focus on two aspects of the disease, alleviating

the symptomatic bronchoconstriction through relaxation of smooth muscle, and controlling the underlying pathology of inflammation. Bronchodilators are primarily used to reverse an ongoing bronchoconstriction, and while critical for rescue, their effects on inflammation are modest, especially those of the short acting β_2 -selective agonists [1,2]. Although toxicity associated with inhaled β_2 -selective agonists is low, there is some question as to whether they can mask worsening airway inflammation [3], and they usually require co-therapy with anti-inflammatory compounds such as cromoglycates, the anti-leukotrienes, or corticosteroids [4]. Corticosteroids have been the most effective anti-inflammatory in the treatment of asthma for many years. They generally are effective in reducing the inflammation associated with asthma, but have significant side effects which cause concern over their use at high doses or over the long term, especially in children and the elderly [5]. There is considerable demand for the development of

Abbreviations: AHR, airway hyper-responsiveness; BAL, bronchoalveolar lavage; HPA, hypothalamic–pituitary–adrenal; i.p., intraperitoneal; MCh, methacholine; OVA, ovalbumin; p.o., per os; Penh, enhanced pause; q.d., once a day.

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new therapeutics, and the recent introduction of the leukotriene antagonists marked the first new treatment for asthma in 20 years. The leukotriene antagonists have the advantage of oral formulation and they appear safe and well tolerated, although their role in the treatment of asthma is generally limited to add-on therapy in mild-to-moderate asthmatics [6–8]. Some studies suggest that long-acting beta agonists promote superior clinical improvement compared to leukotriene antagonists [9]. Therefore, there is still a compelling need for an anti-asthma drug, which possesses the effectiveness of steroids, but without the steroidal side effects.

Allergic asthma is characterized as a TH₂ driven, IgE mediated disease with clear functional symptoms and underlying lung inflammation. The immediate response to an allergic challenge is bronchoconstriction due to smooth muscle contraction and edema (a result of increased vascular permeability). Among the primary mediators of this response are histamine, thromboxane, prostaglandins, and sulfidopeptide leukotrienes. This is followed by a secondary (or late phase) bronchospasm that is of longer duration and thought to be propagated by numerous mediators released from the inflammatory cells that have infiltrated the airways at this time [10–12]. Many cytokines, chemokines and adhesion molecules are upregulated over this period. These events result in the chronic inflammatory condition of the asthmatic lung, which is characterized by leukocyte infiltration (predominately TH2 lymphocytes and eosinophils), and the release of cytokines and other mediators (including TNF, IL-4, IL-5, IL-13, major basic protein and eosinophil cationic protein). Resultant airway changes include smooth muscle cell hyperplasia/hypertrophy, epithelial damage, goblet cell hyperplasia, and airway hyperresponsiveness. The ability to modify the disease by breaking the cycle of inflammation is crucial to the success of the treatment of asthma.

The IPL576 series of compounds is a novel group of polyhydroxylated sterols based on a marine natural product called contignasterol (IZP-94005) [13]. Contignasterol has been previously shown to inhibit histamine release from rat peritoneal mast cells stimulated by anti-IgE [14], and to inhibit antigen-induced bronchoconstriction in the sensitized guinea pig [15]. IPL576,092 is a synthetic analogue of contignasterol that is easier to synthesize and possesses unique side chain functionalities [16]. We therefore hypothesized that IPL576,092 would suppress acute bronchoconstriction and airway leukocyte infiltration, thereby potentially inhibiting late phase bronchoconstriction and airway hyperresponsiveness. We demonstrate herein that the anti-inflammatory efficacy of IPL576,092 in the Brown Norway rat lung leukocyte infiltration model was strongly supported in the guinea pig, sheep, and mouse by IPL576,092-mediated inhibition of the acute and late phase bronchoconstriction and airway hyperresponsiveness. While the mechanism of action of this compound series is still being investigated, IPL576,092 did not suppress

the HPA axis. Thus, IPL576,092 is significantly efficacious in four different animal models encompassing four different species, suggesting that the activity of IPL576,092 is robust, not unique to a specific protocol or antigenic stimulus, not inductive of a classic steroidal side-effect, and therefore may have potential therapeutic application.

2. Materials and methods

2.1. Lung inflammation in rats

Male Brown Norway rats were sensitized on day zero by intraperitoneal (i.p.) injection of 1 ml sterile saline containing 1.0 mg ovalbumin (OVA) and 100 mg Al(OH)₃ (Sigma, St Louis, MO). One control group of rats was sham sensitized with 1 ml sterile saline alone (i.p.) for determination of baseline leukocyte numbers. Dexamethasone (0.1 mg/kg) or IPL576,092 (0.5, 1, 5, or 10 mg/kg in corn oil) was given orally q.d. for three days prior to challenge (days 19, 20, 21), with the third dose given 2 h before challenge. A final dose was administered 24 h after challenge. On day 21, rats were challenged with nebulized 5% OVA in saline for 5 min via a DeVilbiss ultrasonic nebulizer. The sham sensitized control rats were challenged with saline alone for 5 min. Animals were sacrificed 48 h after challenge with an overdose of sodium pentobarbital (200 mg/kg i.p.), and the lungs were lavaged with cold 2×7 ml phosphate buffered saline. The bronchoalveolar lavage fluid was centrifuged (300×g, 3 min), the supernatant removed, and the pellet resuspended in volumes of 1–5 ml ice cold PBS depending on pellet size (final cell suspension). An appropriate volume of the final cell suspension (containing 240,000 cells) was diluted in ice cold PBS to give a final volume of 240 µl and a final concentration of 1×10⁶ cells/ml (final cytospin suspension). An aliquot of the cell suspension (100 µl; 100,000 cells) was added to a cytospin to prepare cells for staining. Two slides per lavage sample were prepared. The cells were fixed and stained in DifQuik (Damstadt, Germany), and cell types differentiated and enumerated. Total cell counts were corrected for red blood cells using the percentage erythrocytes determined from the cytospins. A minimum of 100 leukocytes per slide was counted. A percentage inhibition greater than 100% indicates that the treatment reduced the number of cells to a level below baseline.

2.2. Airway function in sheep

Airway function in sheep was measured as described previously [17]. Unsedated sheep were restrained. After topical anesthesia of the nasal passages with 2% lidocaine solution, a balloon catheter was advanced through one nostril into the lower esophagus. The animals were then intubated with a cuffed endotracheal tube through the other nostril using a flexible fiber-optic bronchoscope as a guide.

Pleural pressure was estimated with the esophageal balloon catheter (filled with 1 ml of air), which was positioned 5–10 cm from the gastroesophageal junction. Lateral pressure in the trachea was measured with a sidehole catheter advanced through and positioned distal to the tip of the endotracheal tube. Transpulmonary pressure, the difference between tracheal pressure and pleural pressure, was measured with a differential pressure transducer-catheter system. For the measurement of pulmonary resistance, the proximal end of the endotracheal tube was connected to a pneumotachograph. The signals of flow and transpulmonary pressure were recorded on an oscilloscope recorder linked to a computer. Resistance and dynamic lung compliance were calculated from transpulmonary pressure, respiratory volume and flow. Analysis of 10–15 breaths was used for the determination of resistance and compliance. Thoracic gas volume was measured in a body plethysmograph to obtain specific lung resistance (resistance $\times$ thoracic gas volume).

Aerosols were generated using a disposable medical nebulizer. The out-piece from the nebulizer was directed into a plastic T piece, which was interconnected between the animal respirator and the endotracheal tube. Aerosol delivery was controlled with a dosimeter system, which consisted of a solenoid valve and a source of compressed air and was activated for 1 s at the beginning of the inspiratory cycle of the respirator system. All aerosols were delivered at a tidal volume of 500 ml and a rate of 20 breaths/min.

Sheep were treated with aerosolized IPL576,092 for 4 days prior to antigen challenge with the last dose 30 min prior to antigen challenge. Specific lung resistance was measured immediately before and after antigen challenge, and then every hour for 6 h and every 30 min for an additional 2 h, post antigen challenge. To assess airway responsiveness, the cumulative provocative dose of carbachol (in breath units) that increased specific lung resistance four-fold was administered (PC400). One breath unit was defined as one breath of a 1% carbachol solution. Baseline dose-response curves to carbachol were performed in all sheep prior to antigen challenge and again 24 h post antigen challenge.

Ascaris suum extract was diluted with buffered saline to a final concentration of 82,000 protein nitrogen units/ml and was delivered as an aerosol over 20 min (400 breaths). IPL576,092 was dissolved in DMSO and diluted with saline. Carbachol was dissolved in phosphate-buffered saline for aerosolization.

2.3. Airway hyperresponsiveness in mice

Airway hyper-responsiveness to methacholine (MCh; Sigma, St Louis, MO) in OVA sensitized and challenged mice was measured by barometric whole-body plethysmography, as described by [18]. Female BALB/c mice were sensitized on day 1 and 14 by an i.p. injection of 100 μ l sterile saline containing 20 μ g OVA and 2.25 mg Al(OH)₃

(Sigma, St Louis, MO). Sham sensitized mice received 100 μ l sterile saline alone. Mice were challenged in a plexiglass chamber on days 28–30, with aerosolized OVA (5% in saline, 20 min) generated by a DeVilbiss ultrasonic nebulizer (Ultra-Neb 099HC, Somerset, PA). Sham challenged mice were exposed to aerosolized saline for 20 min. On day 31 (24 h after the last OVA challenge), unrestrained, spontaneously breathing mice were placed in plethysmograph chambers (Buxco, Troy, NY: Part # PLY3511) and inspiration/expiration induced changes in box pressure were used to determine the enhanced pause (Penh). Penh correlates closely with traditionally determined pulmonary resistance [18], and is used as a measure of airway responsiveness. A pre-challenge baseline Penh value was recorded for 6 min for each mouse. While in the plethysmograph chambers, mice were challenged (DeVilbiss Pulmosonic nebulizer) for 2 min with aerosolized PBS, followed by doubling concentrations of MCh (0.78, 1.56, 3.125, 6.25, 12.5, 25, 50 mg/ml). Immediately following each challenge, Penh values were again measured for 6 min. Penh values were baseline corrected by subtracting the pre-challenge baseline Penh.

IPL576,092 (5 mg/kg), dexamethasone (1 mg/kg, Sigma, St Louis, MO), or vehicle only (distilled water), were administered by oral gavage (50 μ l) on five consecutive days: 2 days before challenge (days 26 and 27) and on the three days of OVA challenge (days 28–30, 2 h before challenge). Since mice are not as sensitive to dexamethasone as rats, a 10-fold higher dose of dexamethasone was used in the mouse airway hyperresponsiveness model than was used in the Brown Norway rat lung inflammation model.

2.4. Bronchoconstriction in guinea pigs

Female Cam–Hartley guinea pigs were sensitized to OVA using a modification of the method described by [19]. Guinea pigs were exposed to an aerosolized solution of 1% OVA in saline for 15 min on two consecutive days via a DeVilbiss nebulizer (Ultra-Neb 099HC), and given a single intradermal injection of 3 μ g OVA in saline on day 1. Using this protocol, animals were found to be at peak sensitivity approximately 14 days after the initial exposure, and therefore were challenged with OVA at this time. The animals were weighed, anaesthetized with ketamine (50 mg/kg i.p.) and xylazine (10 mg/kg i.p.) and maintained on 1% halothane delivered via a nose cone. The left carotid artery was cannulated with PE90 tubing containing 200 U/ml heparin in saline. A tracheostomy was performed and a fluid-filled cannula (PE 160) was inserted approximately 7 cm into the esophagus. The animal was positioned in a plethysmograph and the trachea attached to a fixed stainless steel tracheal tube in the body box. The carotid cannula was attached to a pressure transducer for monitoring of blood pressure and heart rate. The guinea pig was paralyzed with pancuronium bromide (0.8 mg/kg) and ventilated with air at a frequency of

60 Hz and tidal volume of 3 ml using a Harvard small animal ventilator (Harvard Inst., St Laurent, QC).

Data were collected for 20 s periods at a sampling rate of 100 Hz on a computer-linked physiological recording system using DIREC physiological software (Raytech Inst., Vancouver, BC) and analyzed using ANADAT software (RHT, Infodat, Inc. Montreal, Que.). Pulmonary resistance and elastance values were obtained from the volume, flow and pressure signals according to the method of [20], using an isovolumetric multi-point regression model for analysis [21], and calculated as absolute changes in lung resistance (R_L ; cm H_2O /ml/s) or lung elastance (E_L ; 1/ml/cm H_2O). Elastance is the inverse of compliance, which is a measure of the distensibility of the lung, i.e. how lung volume changes as a result of pressure. Volume and pressure signals were calibrated before each set of experiments following standard procedures.

Several lung function measurements were obtained over a 5–10 min period to ensure a steady baseline. Animals were required to have a baseline resistance below 0.35 cm H_2O /ml/min before challenge in order to be included in the study. The animal was then challenged with OVA (2% in saline) administered in six tidal breaths as a nebulized aerosol at a flow rate of 5 l/min. Pulmonary and cardiovascular function was continually monitored throughout the experiment, although data were collected at specific time-points after antigen challenge (10 s, 1, 2, 3, 4, 5, 10, 20, 30 min).

IPL576,092, rolipram or vehicle was administered under light halothane anesthesia by oral gavage (5 mg/kg/day q.d.) in 300 μ l polyethylene glycol-200 for 4 days prior to challenge with the final dose administered 2 h prior to antigen challenge. Rolipram was used instead of dexamethasone in this model, because guinea pigs are relatively steroid insensitive and inhibition of antigen-mediated lung function changes in guinea pigs requires toxic doses.

2.5. HPA axis study

Male Brown Norway rats were treated orally for 4 days with either vehicle, dexamethasone (0.1 mg/kg/day), or IPL576,092 (5 or 50 mg/kg/day). Compounds were administered as a suspension in 300 μ l of corn oil by oral gavage. Animals received drug in the morning, and were sacrificed 6–8 h after the final administration in the afternoon, when corticosterone levels are normally elevated. Animals were sacrificed by anesthesia with sodium pentobarbital followed by exsanguination via cardiac puncture after a 30 min period in which to allow any hormone spike due to the stress of handling to normalize. Blood was collected in tubes and allowed to clot on ice. Blood was then centrifuged for 10 min at 1500 rpm, and the serum collected and frozen at -80°C for subsequent analysis of corticosterone levels. The adrenals, thymus, and spleen from each animal were excised, trimmed, blotted and weighed.

Corticosterone concentrations in rat serum were determined using a commercially available radioimmunoassay kit according to the manufacturer's instructions (Coat-A-Count Rat Corticosterone Assay, Diagnostic Products Corporation, Los Angeles, CA). Briefly, 50 μ l of standard or unknown and 1.0 ml of ^{125}I rat corticosterone were added to rat corticosterone antibody-coated tubes in duplicate. The tubes were incubated at room temperature for 2 h, decanted thoroughly to remove all visible traces of moisture, and then counted for 1 min each in a gamma counter. All duplicate counts were averaged and represented as a percent of the zero standard counts. Percent bound for the standards was plotted against concentration, and corticosterone values for the unknowns were determined by interpolation from the resulting standard curve.

2.6. Animal care

Female Cam-Hartley guinea pigs (375–425 g) were obtained from Charles River (St Constant, QC). Male Brown Norway rats (325–375 g) and female Balb/c mice were obtained from Harlan (Indianapolis, IN). All animals were housed in climate controlled quarters and given food and water ad libitum. Some of the studies were done with different vehicles as vehicle was optimized for each model. Multiple studies with different vehicles have been done in the same model and no difference has been seen in either PK or efficacy. All protocols were approved by the University of British Columbia Animal Care Committee, and were conducted in accordance with the Canadian Council for Animal Care.

2.7. Data analysis

Cell infiltration data in the Brown Norway rat model were analyzed by one-way ANOVA, followed by Dunnett's post hoc multiple comparison procedure.

Allergic response data in the sheep were separately assessed as Early Response (0–4 h) and Late Response (4–8 h). Area under the curve was calculated for each individual animal using WinNonLin, and control versus treated animals compared for statistical differences using two-tailed student's *t*-test. Airway hyperresponsiveness in the sheep was tested by comparing PC400 baseline versus PC400 post allergen challenge using a paired, two-tailed student's *t*-test.

Data from the mouse airway hyperresponsiveness study were corrected by baseline Penh subtraction and plotted as mean $\pm$ standard error of mean. Data were analyzed by one-way ANOVA followed by a student's *t*-test at each concentration of MCh.

In the guinea pig model, resistance and elastance were calculated from the following variables: flow, volume and pressure (elastance = pressure/volume, resistance = pressure/flow) using ANADAT. For each time point, data were collected over a 20 s interval. Area under the curve for 1–10 min post challenge was calculated with WinNonlin

software (Pharsight, Cary, NC) and statistical significance determined with a one-way ANOVA followed by Dunnett's post hoc (all-pairwise) comparison. All statistical analysis was done with SigmaStat (SPSS Science, Chicago, IL) using a $p \leq 0.05$ to determine statistical significance.

For the HPA axis study, data were analyzed by one-way ANOVA, followed by Dunnett's multiple comparison procedure.

3. Results

3.1. Lung inflammation in rats

The OVA-sensitized Brown Norway lung model is an established model of airway leukocyte infiltration that was utilized to demonstrate the anti-inflammatory effects of IPL576,092. Ovalbumin challenge of vehicle treated control rats induced more than a five-fold increase in total leukocytes in the bronchoalveolar lavage fluid compared to saline challenged animals, which was comprised of significant increases in the number of macrophages, eosinophils, neutrophils, and lymphocytes.

IPL576,092 at 1, 5 and 10 mg/kg p.o. significantly suppressed the OVA-induced increase in total leukocytes infiltrating the airways in response to allergen challenge (Fig. 1, Panel A). IPL576,092 at 10 mg/kg produced the greatest inhibition of total leukocyte ($73 \pm 7\%$ inhibition, $p < 0.05$), macrophage ($94 \pm 6\%$ inhibition, $p < 0.05$) and lymphocyte ($72 \pm 7\%$ inhibition, $p < 0.05$) infiltration (Fig. 1). At 5 and 1 mg/kg there was also significant inhibition of these cell types in a dose dependent manner. There was a trend towards inhibition of eosinophil and neutrophil accumulation in the BAL, which was greatest following administration of 10 mg/kg IPL576,092 (52 ± 9 and $55 \pm 15\%$ inhibition, respectively) and which was lost at 0.5 mg/kg. However, this inhibition of eosinophils or neutrophils did not reach statistical significance due to a higher standard deviation in these cell populations.

Dexamethasone at 0.1 mg/kg p.o. significantly inhibited the infiltration of total leukocytes ($90 \pm 5\%$ inhibition), eosinophils ($96 \pm 2\%$ inhibition), macrophages ($84 \pm 10\%$ inhibition), neutrophils ($92 \pm 3\%$ inhibition) and lymphocytes ($99 \pm 4\%$ inhibition) (Fig. 1).

3.2. Airway function in sheep

IPL576,092 caused a significant reduction in the early phase of allergen induced increase in specific lung resistance ($63 \pm 4.6\%$ inhibition) and significantly attenuated the late phase response ($84 \pm 4.6\%$ inhibition) as assessed by AUC 0–4 h and AUC 4–8 h, respectively (Fig. 2, Panel A). Mean area under the curve 0–8 h significantly decreased from 1335.1 ± 130.1 to 335.9 ± 55.7 (arbitrary units, $p < 0.05$, Table 1). Hyperresponsiveness to carbachol was measured by the PC400 (the number of breath units of

carbachol required to increase the airway resistance four-fold). IPL576,092 completely blocked the AHR to carbachol (pre-challenge PC400 not significantly different from post-challenge PC400, $2.2 \pm 5.7\%$ change), whereas control animals showed a significant hyperresponsiveness with a $62.2 \pm 2.6\%$ decrease in the PC400 ($p < 0.05$) (Fig. 2, Panel B).

3.3. Airway hyperresponsiveness in mice

Ovalbumin challenge of sensitized, vehicle treated (water) control mice induced more than a two-fold increase in Penh (4.07 units) compared to saline challenge of sham sensitized mice (1.75 units, $p < 0.05$) following challenge with 50 mg/ml MCh (Fig. 3). Treatment with either IPL576,092 at 5 mg/kg or dexamethasone at 1 mg/kg resulted in a similar decrease in responsiveness to MCh, to a level which approximated the sham sensitized mice. Inhibition of airway hyperresponsiveness was observed at the four highest doses of MCh, with statistical significance achieved at the two highest doses of MCh ($p < 0.05$).

3.4. Antigen-induced bronchoconstriction in guinea pigs

Control animals showed a consistent baseline resistance and elastance over time, which increased significantly within 1 min of challenge with antigen. Calculated lung resistance peaked at 3 min post antigen challenge and recovered close to baseline values by 30 min. IPL576,092 administered orally on four consecutive days (q.d.), with the last dose 2 h prior to the antigen challenge, did not affect baseline resistance (Fig. 4). After challenge by OVA, there was significant inhibition of bronchoconstriction in the IPL576,092 treated animals based on area under the curve (Table 2). The protective effect was measured as a significant inhibition of OVA-mediated increase in lung resistance (Fig. 4, Panel A, $p < 0.05$) and a marked tendency towards inhibition of the increase in lung elastance which did not reach statistical significance (Table 2; Fig. 4, Panel B). Effects of IPL576,092 on airway resistance and elastance were compared with the PDE4 inhibitor rolipram, which also significantly improved lung function.

3.5. HPA axis study

Serum corticosterone levels in animals receiving dexamethasone (0.1 mg/kg/day) were significantly reduced (41.6 ± 24.7 ng/ml) compared to control animals (340.7 ± 60.9 ng/ml, $p < 0.05$, Fig. 5). There were no significant effects on corticosterone levels due to either 5 or 50 mg/kg/day IPL576,092 administered orally for 4 days. This effect of dexamethasone was accompanied by a significant decrease in the weights of the thymus and spleen ($p < 0.05$), but no statistically significant decrease in the weights of the adrenal glands (Table 3). Tissue weights were

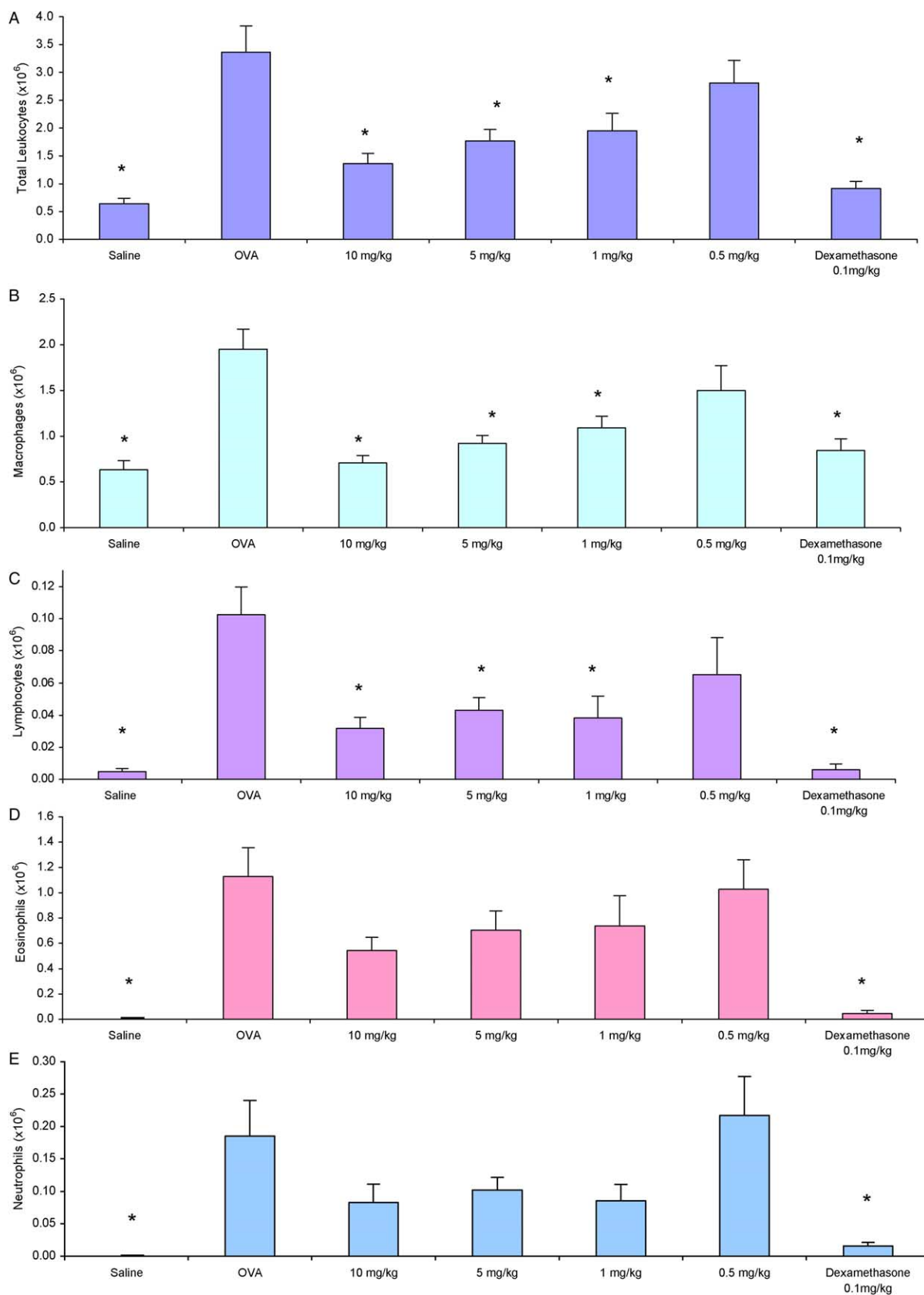


Fig. 1. Effects of IPL576,092 administered once daily for 4 days on the numbers of total leukocytes (A), macrophages (B), lymphocytes (C), eosinophils (D) and neutrophils (E) in the bronchoalveolar lavage following OVA sensitization and challenge of Brown Norway rats. Values represent the mean $\pm$ SEM $\times 10^6$, $n=10-16$. * indicates significant difference ($p < 0.05$) from OVA challenged, no drug.

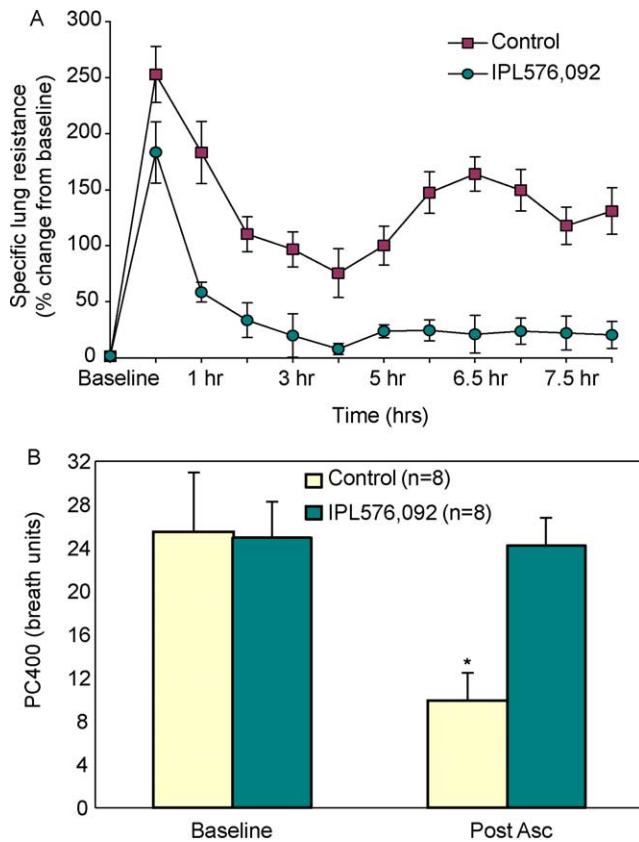


Fig. 2. The effect of IPL576,092 (400 μ g/kg/day by inhalation for 4 days) on allergen-induced changes in specific lung resistance (Panel A) and airway hyperresponsiveness to carbachol (Panel B) in sheep sensitized to *Ascaris suum*. Values represent the mean $\pm$ SE mean, $n=8$. * indicates significant difference ($p<0.05$) from baseline.

not significantly decreased by treatment with IPL576,092 at either dose level.

4. Discussion

The results presented here show that IPL576,092 suppressed many of the key features that occur in the airways of asthmatic individuals in response to an antigen challenge. IPL576,092 significantly inhibited the pulmonary infiltration by leukocytes and improved lung function by inhibiting both the acute and late phase bronchoconstriction, and the associated airway

Table 1

Specific lung resistance as measured by areas under the curve for the early airway response (0–4 h) and the late airway response (4–8 h) to allergen challenge in sensitized sheep

	Area under the curve (EAR and LAR), mean $\pm$ SE		
	0–4 h	4–8 h	0–8 h
Control	553.1 $\pm$ 173.2	782.1 $\pm$ 247.6	1335.2 $\pm$ 367.9
IPL576,092	207.1 $\pm$ 71.9*	128.9 $\pm$ 101.3*	335.9 $\pm$ 157.6*

Animals received 400 μ g/kg/day by inhalation for 4 days. * Indicates significant difference ($p<0.05$) from control, $n=8$.

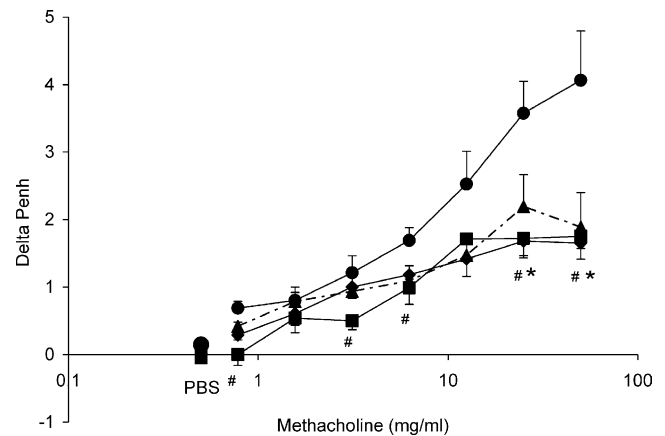


Fig. 3. The response of OVA sensitized and challenged (●, ▲, ◆) or sham sensitized and challenged (■) BALB/c mice exposed to increasing doses of MCh. Mice were treated with vehicle (●, ■) ($n=8$), 5 mg/kg IPL576,092 (▲) ($n=8$), or 1 mg/kg dexamethasone (◆) ($n=4$). * IPL576,092 and dexamethasone significantly different from OVA sensitized and challenged group. # sham sensitized and challenged group significantly different from OVA sensitized and challenged group. Values are expressed as the mean $\pm$ SEM for delta Penh (baseline subtracted Penh).

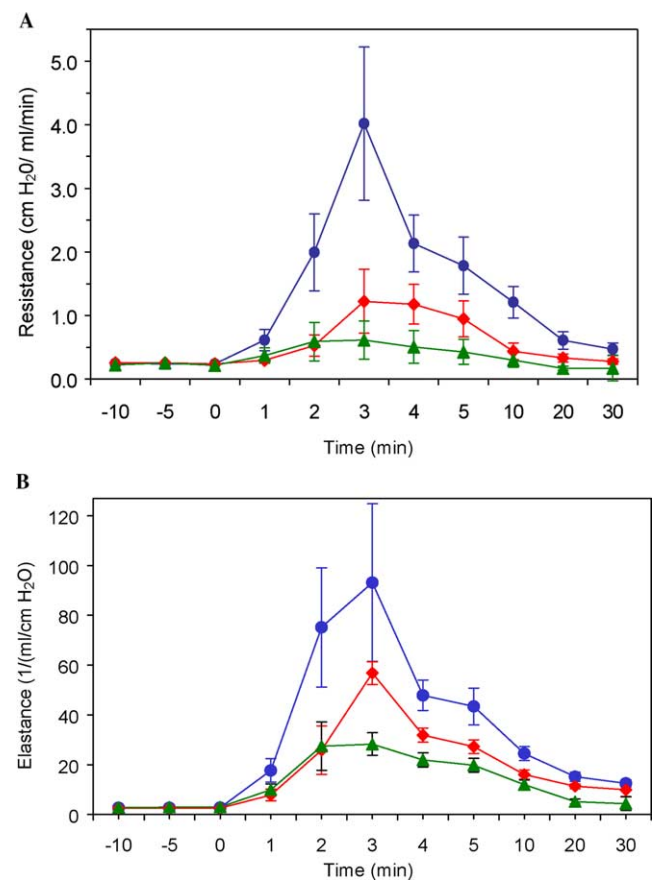


Fig. 4. Effect of IPL576,092 and rolapram, administered orally (5 mg/kg once per day for 4 days) prior to challenge, on allergen-induced changes in lung resistance (Panel A) and elastance (Panel B) in sensitized guinea pigs. Data is presented as mean $\pm$ SEM, $n=5-7$. Rolapram (▲5 mg/kg) and IPL576,092 (◆5 mg/kg) significantly different from vehicle control (●), $p<0.05$, based on area under curve.

Table 2

Areas under the curve (1–10 min) for the bronchoconstriction response to allergen challenge in sensitized guinea pigs

Group	Resistance, mean $\pm$ SEM (1–10 min)	Elastance, mean $\pm$ SEM (1–10 min)
Control	11.4 $\pm$ 2.3	280 $\pm$ 58
IPL576,092	4.8 $\pm$ 1.5*	167 $\pm$ 52
Rolipram	2.4 $\pm$ 1.2*	76 $\pm$ 25*

Animals received 5 mg/kg/day by oral gavage for 4 days. Values represent the mean $\pm$ SEM, n = 5–7. * Indicates significant difference (p < 0.05) from control.

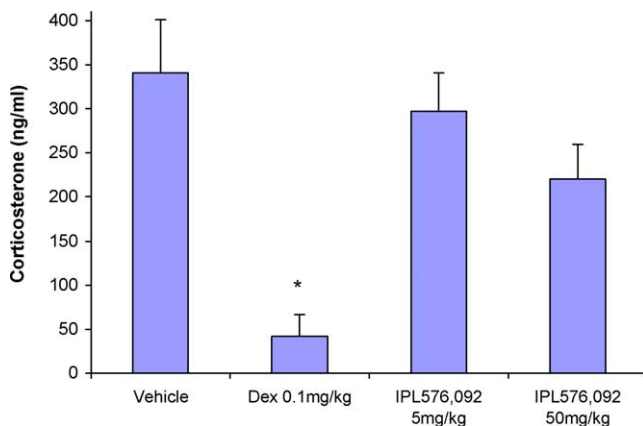


Fig. 5. Effect of IPL576,092 and dexamethasone (dex) on serum corticosterone levels of Brown Norway rats. Ovalbumin sensitized rats were treated orally for 4 days with either vehicle (corn oil), dexamethasone, or IPL576,092. Data represent means $\pm$ SEM of six determinations assayed in duplicate. * Significantly different from vehicle (p < 0.01).

hyperresponsiveness that follows allergen challenge *in vivo*. These findings suggest that IPL576,092 may be beneficial in the treatment of allergic airway diseases.

Most leukocyte types are implicated at some stage in the pathogenesis of asthma. While the mechanisms responsible for the late phase bronchoconstriction are not fully understood, evidence points to a complex cascade of cell infiltration and mediator release from multiple cell types, especially lymphocytes, neutrophils and eosinophils, as fundamental to the response [22–26]. Mounting evidence suggests that T lymphocytes, possibly in conjunction with eosinophils, and dominant cytokines such as IL-13, may have a prominent role as effectors of AHR [27–31], with eosinophils and epithelial cells implicated in airway remodeling [32,33]. The inhibition of leukocyte infiltration

to the lungs is thought to be critical in an inflammatory disease such as asthma.

IPL576,092 generally did not show selectivity with respect to cell type in its inhibition of leukocyte accumulation in the bronchoalveolar fluid from the rats. Macrophage and lymphocyte infiltration were significantly reduced by IPL576,092, while there was a strong but non-significant trend towards inhibition of eosinophil and neutrophil infiltration. In other similar studies with IPL576,092 in the rat (not shown), significant inhibition of eosinophils has typically been observed. The time of lavage post-challenge was chosen from preliminary experiments to coincide with the eosinophil and lymphocyte peaks (48 h), and was approximately 24 h past the neutrophil peak in this model, making it difficult to assess the effect on neutrophils.

The anti-inflammatory effect of IPL576,092 was strongly supported in the guinea pig, sheep and mouse by the inhibition of the acute and late phase bronchoconstriction, and the AHR. Thus, it is encouraging that the effects of IPL576,092 are significant in four different animal models encompassing four different species, suggesting that the efficacy of IPL576,092 is robust and not unique to a specific protocol or antigenic stimulus. Functional enhancement is critical in the treatment of asthma, and it should be noted that even small improvements in lung function in the clinic can lead to significant improvements in quality of life [34].

The inhibitory effect of IPL576,092 on early bronchoconstriction could be the primary mode of action leading to inhibition of leukocyte infiltration, late phase bronchoconstriction and AHR, although the relationship of the acute events in an allergic response to the chronic inflammatory state is unclear. While the acute events begin a cascade of release and upregulation of inflammatory mediators, prevention of acute responses with agents such as mast cell stabilizers leads only to a modest effect on the chronic aspects of asthma [35,36]. The models employed in this study use prophylactic dosing, i.e. exposure to IPL576,092 prior to antigen challenge, since the intended dosing regimen of IPL576,092 is designed for prophylactic therapy, not as a rescue therapy. The inhibitory effect of IPL576,092 on early bronchoconstriction in the guinea pig could suggest a reduction in the need for β -agonist rescue medication. Moreover, the sheep model of airway disease involves chronic sensitization and multiple challenges over the life of the sheep, indicating that IPL576,092 is efficacious in chronic models of airway disease.

Table 3

Effect of IPL576,092 and dexamethasone on tissue weights from Brown Norway rats

Tissue (mg)	Vehicle	Dexamethasone 0.1 mg/kg	IPL576,092 5 mg/kg	IPL576,092 50 mg/kg
Adrenals	0.059 $\pm$ 0.008	0.046 $\pm$ 0.004	0.053 $\pm$ 0.005	0.061 $\pm$ 0.003
Thymus	1.30 $\pm$ 0.05	0.46 $\pm$ 0.03*	1.68 $\pm$ 0.09*	1.47 $\pm$ 0.06
Spleen	2.43 $\pm$ 0.11	1.41 $\pm$ 0.11*	2.32 $\pm$ 0.10	2.39 $\pm$ 0.06

Ovalbumin sensitized rats were treated orally for 4 days with either vehicle (corn oil), dexamethasone, or IPL576,092. Adrenal weight is the average of both glands. Data represent mean $\pm$ SEM of tissue weights normalized to body weight from six animals. * Significantly different from vehicle, p < 0.05.

Despite its steroidal backbone, treatment with IPL576,092 for 5 days did not affect serum corticosterone levels or gland weights even at 10 times the efficacious anti-inflammatory dose in vivo. In contrast, dexamethasone induced decreases in serum corticosterone and gland weights were observed at the dose required for efficacy. The data suggests that these effects of dexamethasone occur at low doses of the compound, and argue against IPL576,092 acting as a weak glucocorticoid. Other studies have shown that IPL576,092 does not competitively bind the glucocorticoid receptor (data not shown), supporting this conclusion. Therefore, it is also possible that IPL576,092 will not cause the undesirable clinical side effects associated with long term glucocorticoid therapy. As the mechanism of action of IPL576,092 is not yet known, it is unclear whether IPL576,092 has one or multiple mechanisms that are responsible for the inhibition of the early phase bronchoconstriction versus the reduction in leukocyte activation and infiltration, thereby ameliorating the late phase response and AHR.

While IPL576,092 was not as efficacious as dexamethasone in the preclinical models that were studied, this very potent comparative compound has limited clinical usefulness. While steroids are clinically effective in the treatment of asthma, oral use of steroids, particularly that of dexamethasone, is restricted to acute situations as toxicity compromises long-term use. Even in the inhaled form, steroids are associated with numerous side effects, such as retardation of growth in children and HPA axis suppression. Dexamethasone (and rolipram, which is not used clinically) nonetheless serve as useful comparators preclinically as examples of different modes of action and for validation of the animal models. There is still a need in asthma for a therapeutic that is orally bioavailable and efficacious, but that has a high safety profile. While the preclinical activity of IPL576,092 did not inhibit allergen-induced responses by 100%, the activity was comparable on a dose basis to rolipram head-to-head in the guinea pig model, and to the anti-leukotriene agents in similar rat and sheep models [37,38]. Most treatments, such as the leukotriene receptor antagonists, also do not completely inhibit antigen-induced responses in preclinical models at therapeutic doses, and yet are effective and safe in the appropriate patient sub-group. IPL576,092 has undergone phase one clinical trials and was found to be generally safe and well tolerated.

These data demonstrate that IPL576,092 exerts a protective effect against allergen-induced bronchoconstriction and lung inflammation in animal models of asthma, and may not have the side effects associated with synthetic glucocorticoid therapy, suggesting therapeutic potential for this class of drug in the treatment of asthma.

Acknowledgements

Financial support: Inflazyme Pharmaceuticals, Ltd. *Meeting abstracts:* Kasserra C, Harris P, Dumdei E. Prevention of

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