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Pharmacological modulation of eosinophil influx into the lungs of Brown Norway rats [1]

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Abstract

A model of lung inflammation was developed in Brown Norway rats. Intense lung eosinophilia was induced by a single intravenous injection of Sephadex G-200 particles. The eosinophilia observed was preceded by an increase in cysteinyl leukotrienes found in lung lavage fluids. Theophylline and albuterol were tested in the model and found to be inactive, while dexamethasone was effective. Zileuton, a specific leukotriene inhibitor, was found to effectively inhibit leukotriene formation and the influx of eosinophils into the lungs of these Sephadex-treated animals. Studies with specific leukotriene D_4 antagonists of the cysLT$_1$ type receptor indicate that this leukotriene receptor is probably not involved directly in the eosinophilic inflammation. This model appears to be useful in characterizing potential anti-inflammatory effects of inhibitors by evaluating their ability to prevent eosinophil influx into the lung.

Keywords: Pulmonary eosinophilia; Inflammation; Leukotriene B_4; Leukotriene C_4; Leukotriene D_4; Leukotriene E_4; Bronchoalveolar lavage fluid; (Inbred Brown Norway rat)

1. Introduction

Lung inflammation is an important component of human airway diseases and is particularly significant in asthma (Kay, 1991). The eosinophil is an important effector cell in the bronchial inflammation seen in asthmatics (Calhoun et al., 1991). Increased numbers of eosinophils are found in the circulation of asthmatics corresponding to the severity of the disease (Horn et al., 1975). Activation of eosinophils releases several potent inflammatory mediators. Part of the detrimental role of eosinophils in asthma and bronchial hyperresponsiveness has been attributed to the secretion of cationic proteins (Frigas and Gleich, 1986). Leukotrienes have also been proposed as important mediators of bronchial asthma and the lung inflammation associated with that disease (Henderson, 1994). The cysteinyl leukotrienes, leukotriene C_4 and its metabolites leukotriene D_4 and leukotriene E_4, have potent smooth muscle constriction, vasoactive and mucous secretory activities. Recently leukotriene E_4 and leukotriene D_4 have been proposed to have chemotactic properties as well (Laitinen et al., 1993; Spada et al., 1994). Leukotriene B_4 stimulates leukocyte migration, aggregation, adhesion, oxidative burst activity and degranulation (Showell et al., 1982; Spada et al., 1994).

The identification of the primary mediators which induce lung eosinophilia and activate eosinophils once they are located in the lung is an important topic for investigation. We have developed a model of lung eosinophilia in Brown Norway rats to examine the role of various agents on eosinophil recruitment. As expected from clinical and pharmacological studies, theophylline and albuterol were ineffective in this model while steroids were quite effective. Our results with the specific 5-lipoxygenase inhibitor zileuton (Carter et al., 1991) indicate a clear role for leukotrienes in recruiting eosinophils into the lung. The failure of specific cysLT$_1$ receptor antagonists to give inhibition of pulmonary inflammation except at high doses implies that the most important leukotriene is probably leukotriene B_4.

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[1] Some of the data presented here were presented in preliminary fashion elsewhere (Walsh et al., 1994).

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2. Materials and methods

2.1. Materials

Zileuton (*N*-(1-benzo-[b]-thien-2-ylethyl)-*N*-hydroxyurea), CGS-25019c (Cohen and Yagoloff, 1994), MK-0476 (1-((1(*R*)-(3-(2-(7-chloro-2-quinolyl)-(*E*)-ethyl)phenyl-(3-2-(1-hydroxy-1-methyl-ethyl-phenyl)propyl)thio)methyl)-cyclopropane acetic acid sodium salt) (Jones et al., 1995) and ZD 204,219 (4-(5-cyclopentyl-oxycarbonylamino-L-methyldol-3-ylmethyl)-3-methoxy-*N*-*o*-tolylsulphonyl-benzamide) (Krell et al., 1990) were synthesized at Abbott Laboratories (Abbott Park, IL, USA). Theophylline and salbutamol were purchased from Sigma (St. Louis, MO, USA). Urethane (Sigma) was used as a 25% solution in sterile water (Abbott Laboratories). Brown Norway rats (Harlan Sprague Dawley, Prattville, AL, USA) were housed in AALAC accredited facilities and were allowed food and water ad libitum throughout experimental protocols. Rats were lavaged with Dulbecco's Phosphate-Buffered Saline (Gibco BRL, Grand Island, NY, USA) containing 10 u/ml heparin (Sigma). Leukotriene B_4, leukotriene $C_4/D_4/E_4$, prostaglandin E_2 antibodies and leukotriene C_4 tracer were supplied by PerSeptive Diagnostics (Cambridge, MA, USA). Leukotriene B_4 and prostaglandin E_2 tracers were supplied by Cayman Chemical Company, Ann Arbor, MI, USA. Ellman's solution was used for the development of the leukotriene B_4 and prostaglandin E_2 enzyme-linked immunoassay plates. Ellman's solution consists of a mixture of acetylthiocholine iodide and 5,5'-dithiobis(2-nitrobenzoic acid) (both from Sigma) dissolved in a 0.1 M potassium phosphate buffer. *p*-Nitrophenyl phosphate substrate (Sigma) was dissolved in a buffer containing 0.05 M sodium bicarbonate, 1 mM magnesium chloride, and 0.05% sodium azide, pH 9.8. This solution was used for the development of the alkaline phosphate tracer used in the leukotriene $C_4/D_4/E_4$ enzyme-linked immunoassays.

2.2. Induction of eosinophilia

Sephadex G-200 (Pharmacia, Uppsala, Sweden, particle size = 40–120 μm) was prepared as previously described (Lemanske and Kaliner, 1982). Briefly, Sephadex was suspended in sterile saline for 48 h at 4°C at a concentration of 0.5 mg/ml. After being autoclaved for 30 min, 1 ml of the Sephadex solution was injected intravenously, via a tail vein, into male Brown Norway rats (150–200 g). Negative control rats were intravenously injected with 1 ml of sterile saline. Studies were done in most cases over 4 days, designated days 0, 1, 2 and 3 where day 0 was the day of Sephadex injection.

2.3. Pharmacology studies

In studies where drugs were tested the following protocol was used. Drugs were suspended in 0.2% methyl cellulose with a motorized homogenizer equipped with a Teflon coated pestle (TRI-R Instrument, Jamaica, NY, USA). On day 0, 1 h prior to the Sephadex injection, the rats were dosed by oral gavage with either the inhibitor or the vehicle control, 0.2% methyl cellulose. Rats were orally dosed with the drug or vehicle twice a day (b.i.d.) for 4 days.

2.4. Bronchoalveolar lavage

For the Sephadex time-course studies, bronchoalveolar lavage fluid and peripheral blood samples were gathered at 24 h, 2 days, 3 days and 7 days after Sephadex injection. Rats were anesthetized with an intraperitoneal injection of 25% urethane (6 times their body weight (kg) in ml). The trachea was intubated using a polyethylene tube (PE-240). The airways were lavaged twice, via a sterile syringe connected to the tracheal cannula, with 5 ml cold phosphate-buffered saline (PBS) without Ca^{2+} with 10 u/ml heparin. The bronchoalveolar lavage fluid was recovered manually through gentle aspiration. The rats were then euthanized by CO_2 inhalation. Peripheral blood was taken by cardiac puncture and from a lateral vein in the tail. Blood smears were prepared, stained with Wright-Giemsa and differential counts performed. The fluid recovered from the first and second aliquots was pooled and the volume of each sample recorded. A 1 ml aliquot of the bronchoalveolar lavage fluid was added to 2 ml of ice cold methanol and allowed to stand overnight at −20°C. Supernatants of the methanol extract were analyzed for eicosanoids as described below. A 50 μl sample of bronchoalveolar lavage fluid was added to 10 ml Isoton II. A lysing and hemoglobin reagent (Baxter, Deerfield, IL, USA) was added to lyse the erythrocytes. Cells were counted using a Coulter Counter Model Z_F (Coulter Electronic, Hialeah, FL, USA). 150 μl of the remaining bronchoalveolar lavage fluid was cytocentrifuged in a Shandon Cytospin 3 (Shandon Scientific, Cheshire, UK). The cells were stained with Wright-Giemsa and differential cell counts performed.

2.5. Extraction and analysis of eicosaniods

Levels of leukotriene B_4, cysteinyl leukotrienes, and prostaglandin E_2 in the bronchoalveolar lavage fluid were measured by enzyme-linked immunoassay. One ml of bronchoalveolar lavage fluid in 2 ml of methanol was centrifuged at $2000 \times g$ for 15 min at 4°C to remove the precipitated proteins. The supernatants were transferred to clean tubes and dried down under nitrogen. The samples were then reconstituted with 0.5 ml of assay buffer. The enzyme-linked immunoassays were performed using modifications of commercial kits available from Cayman (Ann Arbor, MI, USA) and PerSeptive Diagnostics (Cambridge, MA, USA). Leukotriene B_4 and prostaglandin E_2

enzyme-linked immunoassays were performed using mouse anti-rabbit monoclonal antibody coated 96-well plates. The cysteinyl leukotriene enzyme-linked immunoassay was performed in rabbit anti-mouse monoclonal antibody coated 96-well plates.

2.6. Determination of drug plasma concentrations

Blood samples were collected at various times following compound administration to Brown Norway rats. Blood samples were centrifuged and the plasma removed and stored frozen until assayed. Plasma samples were thawed, two volumes of methanol added and precipitated plasma proteins removed by centrifugation. Supernatants were injected directly onto a C_{18} reversed-phase column (Little Champ column) and chromatographed using a mobile phase composed of 55% acetonitrile, 10% tetrahydrofuran and 8 mM triethylamine acetate, pH 4.5, at a flow rate of 1 ml/min for MK-0476 and 8% acetonitrile, 8% methanol, 24% tetrahydrofuran and 8 mM triethyl acetate for ZD 204,219. Peaks corresponding to ZD 204,219 and MK-0476 were quantitated by UV absorbance at 265 and 240 nm, respectively, using an external calibration curve.

2.7. Calcium ionophore-induced leukotriene production

Bronchoalveolar lavage was performed as described above. Bronchoalveolar lavage cells were collected from lavage fluid by centrifugation, and the sedimented cells were washed twice in phosphate-buffered saline with heparin and resuspended in Earle's-Hepes, pH 7.4, containing 1 mg/ml bovine serum albumin. Test compounds were added to 120 μl of bronchoalveolar lavage fluid cells (5×10^6 cells/ml) and incubated at 37°C for 15 min. After the addition of A23187 (8.8 μM), the mixture was further incubated for 15 min. The reaction was terminated by the addition of ice-cold methanol to a final concentration of 70% methanol. The precipitated proteins were removed by centrifugation at $1000 \times g$ for 15 min at 0°C. Leukotriene B_4 and cysteinyl leukotrienes were quantified as described above.

2.8. Statistical analysis

To compare the mean responses of the treatment groups, analysis of variance was applied to the variable. Percentage inhibition values were determined by comparing the individual treatment mean values to the mean of the control group. Significance was considered to be established with a P value < 0.05 using Duncan's New Multiple Range comparison. Linear regression was used to estimate ED_{50} values in appropriate assays. Data were analyzed with a Macintosh computer (Apple Computer) with standard statistical packages.

3. Results

3.1. Effects of Sephadex injection in the lung

Following the observations of Walls and Beeson (1972) that a single intravenous injection of Sephadex resulted in blood eosinophilia in Sprague-Dawley rats, we examined the conditions necessary to reproduce this model in Brown Norway rats. In studies comparing the dose of Sephadex required to induce eosinophilia, it was found that 0.5 mg/animal Sephadex G-200 produced significant eosinophilia in rats weighing less than 200 g. In rats weighing over 200 g, a dose of 1 mg/animal Sephadex G-200 was needed to produce significant lung eosinophilia. In larger animals, 0.5 mg/ml Sephadex G-200 did not induce consistent lung eosinophilia. On the basis of these initial results, the dose of 0.5 mg/ml Sephadex G-200 in rats weighing less than 200 g was selected for further experiments.

Normal airway cellular composition in male Brown Norway rats consisted almost entirely of monocytes/macrophages (98 ± 2%) with much smaller percentages of lymphocytes (1%) and eosinophils (1%). Intravenous injection of Sephadex changed the airway cellular composition by dramatically increasing the number of eosinophils from 2.5×10^4 cells/rat (1%) to 1.3×10^6 cells/rat (31 ± 1%). The total number of cells recovered by lavage was also modestly increased from 2.4×10^6 cells/rat to 4.0×10^6 cells/rat at four days after injection. The percentage of neutrophils (2%) and lymphocytes (9 ± 1%) was also increased, whereas the relative number of monocytes/macrophages (58 ± 1%) was decreased as a percentage of cells.

The effect of intravenous Sephadex G-200 particles on the appearance of eosinophils in the bronchoalveolar lavage

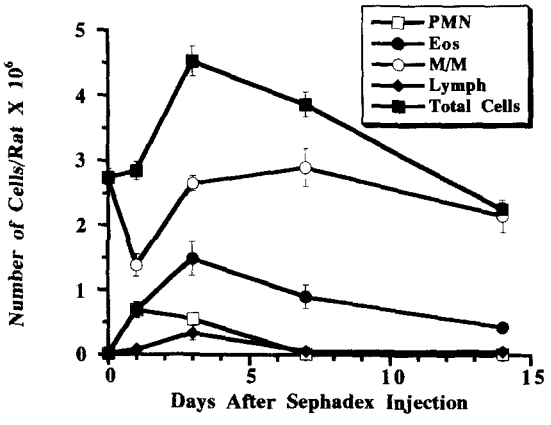

Fig. 1. Time-course of cellular composition after Sephadex injection in Brown Norway rats. Sephadex was injected intravenously on day 0 and lung lavages were performed at days indicated. Cell composition was determined as described in methods. PMN = polymorphonuclear cells; Eos = eosinophils; M/M = monocytes or macrophage; Lymph = lymphocytes. Each data point is the mean ± S.E.M. of 8 animals.

fluid was monitored over 14 days with measurements made at days 0, 1, 3, 7 and 14 (Fig. 1). Infiltration of eosinophils into the lung was maximal by day 3 (an approximate 52-fold increase over baseline counts) with lower but significant eosinophil levels seen at day 7. The number of eosinophils returned to near control levels by day 14. Increased numbers of neutrophils were seen somewhat earlier than for eosinophils (day 1) and were at baseline by day 7. Lymphocytes were increased at day 3 but not at the other time points.

In addition to these cellular changes, there was also an increase in the concentration of immunoreactive leukotrienes present in the bronchoalveolar lavage fluid. The levels of cysteinyl leukotrienes (leukotriene $C_4/D_4/E_4$) were found to be significantly increased over control animals on day 1 and 3 after Sephadex injection (day 3: controls, 1.2 ± 0.12 ng/rat vs. Sephadex, 3.6 ± 0.39 ng/rat; Fig. 2). The levels of cysteinyl leukotrienes decreased more rapidly than did the eosinophilia, reaching baseline levels at day 7. In some experiments, leukotriene B_4 was increased in bronchoalveolar lavage fluid from Sephadex-treated animals over controls but this was not seen consistently. Sephadex increased the levels of the cyclooxygenase product prostaglandin E_2 in bronchoalveolar lavage fluid, compared to control animals at day 3 (controls, 5.12 ± 0.64 ng/rat vs. Sephadex, 7.0 ± 0.5 ng/rat). At earlier days the difference between control and sephadex animals was even smaller. Thus day 3 had both elevated eosinophil and eicosanoid levels and therefore was chosen for pharmacological studies.

3.2. Calcium ionophore stimulation of rat bronchoalveolar lavage fluid cells

In order to examine the capacity of bronchoalveolar lavage fluid cells to make leukotrienes, rat bronchoalveolar lavage fluid cells were collected from Sephadex-injected rats and from saline-injected controls. These cells were then stimulated in vitro with calcium ionophore A23187 (8.8 μM). The two populations of cells were found to be very different in both spontaneous production of leukotrienes and the capacity to form leukotrienes after stimulation. Cells taken from saline controls had very low baseline leukotriene B_4 production (2–20 pg/10^6 cells) and yielded no more than 2-fold increases in leukotriene formation following calcium ionophore challenge. The cells had leukotriene formation capacity however, since the addition of 10 μM arachidonic acid with ionophore challenge gave 1–3 ng/ml leukotriene B_4/10^6 cells. The cells from Sephadex-treated animals produced large amounts of leukotriene B_4 (7.6 ng/10^6 cells) as well as cysteinyl leukotrienes (2.1 ng/10^6 cells) after ionophore challenge. These cells had a basal production of leukotriene B_4 of 0.4 ng/10^6 cells, which was stimulated 17-fold by the addition of A23187. Addition of arachidonic acid (10 μM) gave only modest increases in leukotriene B_4 formation (8 ng/10^6 cells). Zileuton was able to reduce leukotriene formation induced by calcium ionophore A23187 in a dose-dependent fashion (Fig. 3), giving an IC_{50} of 1.8 (1.6–2.0) μM against leukotriene B_4 and 2.1 (1.6–2.6) μM for cysteinyl leukotrienes.

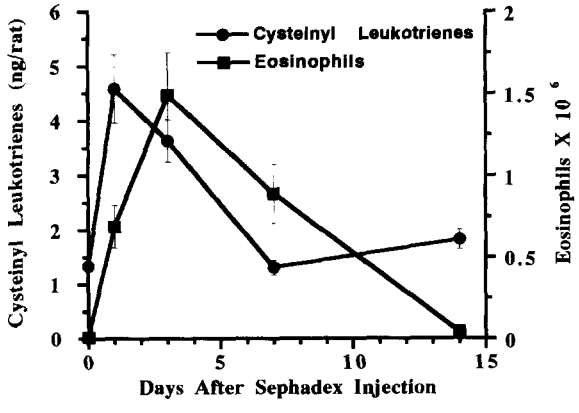

Fig. 2. Time-course of lung eosinophilia and leukotriene formation after Sephadex injection in Brown Norway rats. Sephadex was injected intravenously and lung lavages were performed at days indicated. Each data point is the mean ± S.E.M. of 8 animals. Leukotriene measurements were performed on an aliquot of the same lavage fluid as the cell counts.

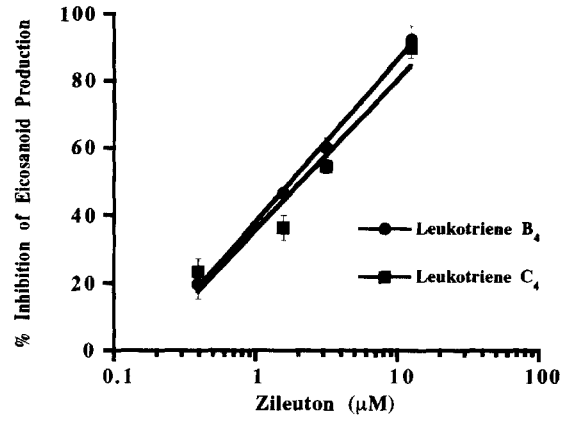

Fig. 3. Inhibition of bronchoalveolar lavage fluid cell leukotriene formation by zileuton. Lavage cells were stimulated to make leukotrienes by the addition of calcium ionophore A23187 for 30 min. Data are the average of duplicate incubations.

3.3. Effects of agents on Sephadex-induced eosinophilia

Zileuton, a selective inhibitor of 5-lipoxygenase, was used to investigate the importance of leukotrienes in the eosinophilia elicited by Sephadex injection. The compound was previously shown to be an effective inhibitor of leukotriene formation in vivo (Carter et al., 1991). Zileuton was given by oral gavage at various doses 1 h before injection of Sephadex and twice a day for the duration of the study (4 days). Rats treated with zileuton had significantly lower leukotriene B_4 levels in their bronchoalveolar lavage fluid compared to vehicle-treated animals (data not

shown). The cysteinyl leukotrienes were found to be inhibited significantly ($P < 0.05$) in the presence of zileuton at oral doses ranging from 3 to 100 mg/kg compared to those animals who received no zileuton (Fig. 4a). Zileuton inhibited the leukotriene levels in a dose-dependent fashion. Data from two dose response studies gave a mean oral ED_{50} of 5.5 mg/kg. Treatment with zileuton had no effect on prostaglandin E_2 levels even at the highest dose tested (100 mg/kg). In conjunction with its effects on the 5-lipoxygenase products, treatment with zileuton dose dependently suppressed the number of eosinophils in bronchoalveolar lavage fluid in Sephadex-treated animals (Fig. 4b). The mean ED_{50} for inhibiting the influx of eosinophils in two experiments was 11.5 mg/kg. Zileuton at the high (100 mg/kg) dose also inhibited the influx of neutrophils and in one of two experiments modestly decreased lymphocyte number. Thus zileuton inhibited both leukotriene formation and lung eosinophilia induced by Sephadex in a parallel manner (Fig. 4).

An attempt was also made to probe the role of leukotriene B_4 in the eosinophilia model with a recently described specific BLT receptor antagonist, CGS-25019c (Cohen and Yagoloff, 1994). This compound (10 mg/kg) was inactive against the formation of leukotrienes and eosinophil influx (data not shown). In order to confirm that

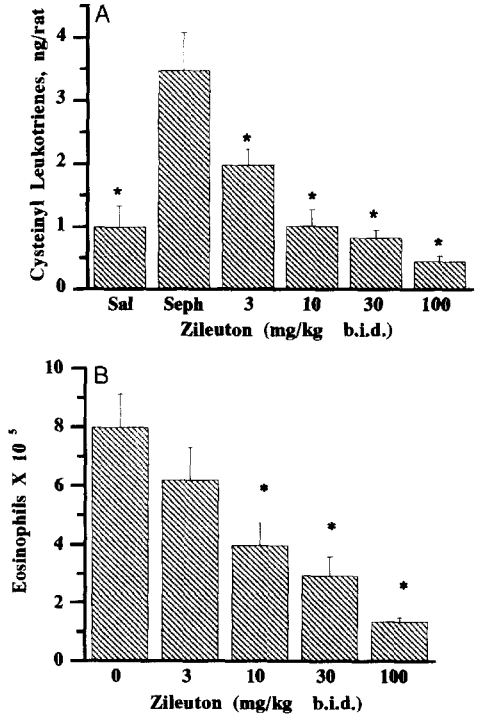

Fig. 4. (a) Effect of oral doses of zileuton on lung cysteinyl leukotriene concentrations in Sephadex treated animals. * Indicates result of treatment statistically different from Sephadex treated animals at the 0.05 level. Each dose level and control is the mean ± S.E.M. of 8 animals. (b) Effect of oral doses of zileuton on lung eosinophilia in Sephadex treated animals. * Indicates result of treatment statistically different from Sephadex treated animals at the 0.05 level. Each dose level and control is the mean ± S.E.M. of 8 animals.

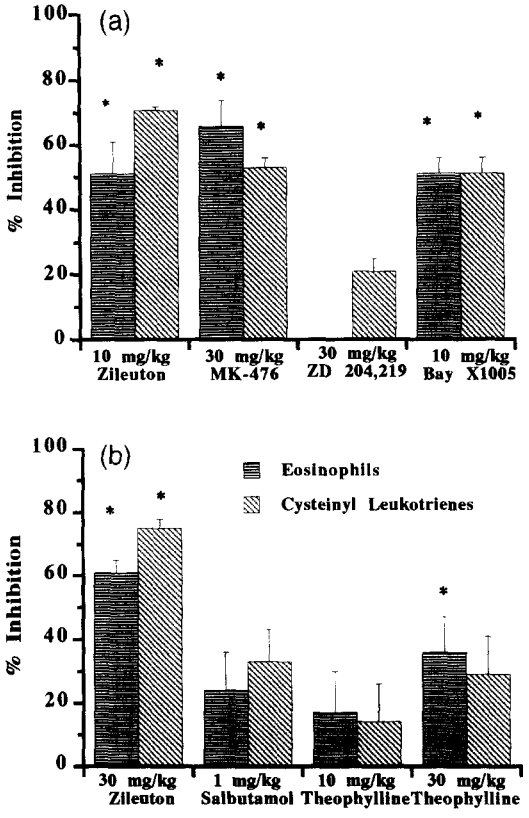

Fig. 5. (a) Comparison of the inhibition of leukotrienes and eosinophil influx in Sephadex treated animals dosed with zileuton, ZD 204,219, MK-476 and Bay X 1005. Drugs were given by oral gavage, at the doses noted, 2 h prior to Sephadex injection and every 12 h thereafter through the 4 day time-course. * Indicates result of treatment statistically different from Sephadex treated animals at the 0.05 level. Each dose level and control is the mean ± S.E.M. of 8 animals. (b) Comparison of the inhibition of leukotrienes and eosinophil influx in Sephadex treated animals dosed with zileuton, theophylline and albuterol. Drugs were given by oral gavage 2 h prior to Sephadex injection and every 12 h thereafter through the 4 day time-course. Doses used were 30 mg/kg for zileuton, 1 mg/kg for albuterol and 10 (a) and 30 (b) mg/kg for theophylline. * Indicates result of treatment statistically different from Sephadex treated animals at the 0.05 level. Each dose level and control is the mean ± S.E.M. of 8 animals.

inhibition of leukotrienes is important in this model, Bay X1005 (Muller-Peddinghaus et al., 1993) was tested. As shown in Fig. 5a, this compound showed similar activity and potency to zileuton.

Two recently described potent $cysLT_1$ receptor antagonists, ZD 204,219 and MK-0476, were also examined in this model. Both compounds were given by oral gavage twice a day throughout the testing period. ZD 204, 219 gave inconsistent results at 10 mg/kg. Accordingly it was dosed at 30 mg/kg where it was again inactive against both eosinophil influx and leukotriene formation (Fig. 5a). MK-0476, given at 10 mg/kg, was also inactive. At 30 mg/kg, the compound inhibited both eosinophil influx and cysteinyl leukotriene in bronchoalveolar lavage fluids (Fig. 5a).

Compounds which are currently used in the treatment of

asthma were also examined in the model. In contrast to the data with zileuton, theophylline, a non-specific phosphodiesterase inhibitor, and albuterol, a selective β-agonist, were both much less active in this model (Fig. 5b). Theophylline, in one study, gave modest inhibition of eosinophilia at 30 mg/kg but this was not repeated in a second study. Albuterol had no effect on eosinophilia and neither compound inhibited lung leukotriene levels significantly (Fig. 5b). Dexamethasone, used as an example of steroid therapy, had remarkable potency inhibiting both eosinophilia and leukotriene formation in bronchoalveolar lavage fluid. Greater than 50% inhibition of both parameters was seen at a dose of 0.03 mg/kg (data not shown). Less than 50% inhibition was seen on both parameters at 0.01 mg/kg.

4. Discussion

Several methods have been used to induce lung eosinophilia in the rat using a variety of foreign particles (Walls and Beeson, 1972; Schriber and Zucker-Franklin, 1974). Walls and Beeson studied the induction of systemic and lung eosinophilia in Sprague-Dawley rats following intravenous injection of Sephadex particles (Walls and Beeson, 1972). These studies showed that the response to foreign particles evoked an increase in both circulating and infiltrating eosinophils. Since those observations, a number of researchers have examined a variety of Sephadex protocols to induce both blood and lung eosinophilia in several strains of rats (Laycock et al., 1986; Schriber and Zucker-Franklin, 1975; Spicer et al., 1990; Cook et al., 1989). We chose to work with the Brown Norway strain because it has been shown to respond to antigen challenge with eosinophilia (Rossi et al., 1993). No significant blood eosinophilia was observed using our protocol in these rats although in some studies 2–3-fold increases were observed. These were not statistically significant changes however, indicating a difference in the response of this strain compared to Sprague-Dawley or Wistar strains. However, a vigorous lung eosinophilia was found after a single intravenous injection of Sephadex G-200 particles. The lung eosinophilia was accompanied by an elevation of leukotrienes. This elevation of bronchoalveolar lavage fluid eosinophils reached a maximum 3 days after the Sephadex injection. Leukotriene formation peaked at day 1, before maximum increases in eosinophils were detected. This time-course is consistent with a possible role for these autocoids in attracting eosinophils into the lung. Interestingly, the level of cysteinyl leukotrienes also decreased prior to the drop in the number of eosinophils. In addition to the marked eosinophilia observed, a modest increase of neutrophils and lymphocytes was also seen after Sephadex injection.

The time-course studies suggest that an initial step of the Sephadex-induced eosinophilia is the triggering of elevated levels of leukotriene production from resident lung cells, such as macrophages or mast cells. Increased levels of leukotrienes appear to contribute to the chemotaxis of eosinophils into the lung. Both leukotriene B_4 and the cysteinyl leukotrienes have been shown to be chemotactic for human eosinophils (Spada et al., 1994). Parallel information on rat eosinophils is not available. The proposal that leukotrienes are critical factors in the lung eosinophilia seen after Sephadex injection is supported by the nearly complete inhibition of lung eosinophilia in animals given zileuton and by the inhibition seen with Bay X1005, a leukotriene inhibitor with a distinctly different mechanism. In the zileuton-treated animals, the inhibition of lung eosinophilia was accompanied by a parallel inhibition of leukotriene formation in lung lavage fluid. Interestingly, the potency observed with zileuton in this model was somewhat greater than that seen in more neutrophil-dependent inflammation models. For example, Carter et al. observed that zileuton inhibited neutrophil influx in response to an Arthus reaction in the pleural cavity of Sprague-Dawley rats at oral doses 3–5-fold higher than those seen here (Carter et al., 1991). In addition, the inhibition observed with the Sephadex model was more extensive than that seen in the Arthus reaction where the maximal inhibition of neutrophil influx seen was 50%. In the Sephadex model, eosinophil influx into the lung could be inhibited 80–90% by zileuton. These data indicate that the Sephadex response is more leukotriene dependent than the Arthus response. It is unclear whether these differences arise from the different rat strains used, the challenge (Sephadex vs. antigen), or the cells responding to the challenge (eosinophils vs. neutrophils).

ZD 204,219 and MK-0476 were used to define the role of leukotriene D_4 in Sephadex-induced eosinophilia. Both compounds have nanomolar potency in antagonizing the binding of leukotriene D_4 to the $cysLT_1$ receptor antagonist (Krell et al., 1990; Jones et al., 1995). The compounds have also been reported to have significant activity in various leukotriene D_4-dependent animal models at low doses and are currently being tested in man at doses of less than 1 mg/kg. We were unable to get significant effects with ZD 204,219 even at 30 mg/kg. The data with MK-0476 were less clear because significant inhibition was seen at 30 mg/kg although not at 10. In order to understand the data with MK-0476 better, plasma concentrations of both compounds were measured after a 30 mg/kg dose was given twice daily. Plasma levels of MK-0476 peaked at 5 μM 1 h after dosing, dropping to 10-fold lower values 8 h after the first dose. ZD 204,219 gave more constant and higher plasma levels peaking at 14 μM. It is possible that at 30 mg/kg MK-0476 has other less specific effects in this model unrelated to $cysLT_1$ receptor antagonism. Indeed preliminary data indicates that MK-0476 (3–10 μM) does inhibit leukotriene B_4 formation in ionophore challenged human neutrophils (E. Otis and R. Bell, unpublished observations). Thus it appears

that leukotriene D_4 acting on $cysLT_1$ receptors probably plays only a minor part if any in the eosinophilic inflammation seen in this model. Unfortunately $cysLT_2$ receptor antagonists are not yet available to examine the possible role of this receptor type.

Eosinophils release various inflammatory mediators such as leukotrienes (Jorg et al., 1982) along with several basic proteins. Human cells have been found to release large amounts of leukotriene C_4 in vitro (Borgeat et al., 1984; Henderson et al., 1984; Tamura et al., 1988). Our results showed a temporal elevation of leukotriene B_4, cysteinyl leukotrienes, prostaglandin E_2 and eosinophils in bronchoalveolar lavage fluid in response to the Sephadex insult. The cysteinyl leukotrienes found in bronchoalveolar lavage fluid at day 3 appear to be predominantly derived from the elicited eosinophils since the dominant change in cell composition in bronchoalveolar lavage fluid after Sephadex is the increase in these cells. Alternatively, resident mast cells and macrophages could also contribute if activated. Our examination of the capacity of the bronchoalveolar lavage fluid cells to make leukotrienes indicated that the Sephadex challenge not only changed the cellular composition in the lung but apparently the activation state and capacity to form leukotrienes of the cells as well, since unstimulated cells from Sephadex-treated animals produced much higher basal levels of leukotrienes than cells from saline-treated animals. Ionophore-stimulated cells from Sephadex-treated animals produced significant amounts of both leukotriene C_4 and leukotriene B_4. Bronchoalveolar lavage fluid cells from saline-treated control animals, in contrast, gave low amounts of leukotrienes, even after ionophore challenge, unless supplemented with exogenous arachidonic acid.

The model described here has advantages over other similar models for inducing airway eosinophilia accompanied by an increase in inflammatory mediators. For example, in contrast to the model developed by Laycock et al., a single Sephadex injection is sufficient to induce a marked eosinophilia and the time required is considerably shorter, 3 versus 14 days (Laycock et al., 1986). In our hands it is much more reproducible than the antigen models used in Brown Norway rats (Ellwood et al., 1992). In addition, recent results indicate the possibility of combining Sephadex injection with antigen challenge (Rossi et al., 1993; M. Namovic and R. Harris, unpublished observations). Of particular significance is the observation that the model is remarkably leukotriene dependent. We interpret these data to indicate that at least with Sephadex challenge, leukotrienes provide a necessary function in attracting, activating or retaining eosinophils in the lung. Clearly other substances such as IL-5 (Takatsu et al., 1994) and eotaxin (Jose et al., 1994) also play important roles in causing lung eosinophilia. The interaction of these interesting mediators with leukotrienes is currently being examined in these laboratories.

Recently several anti-leukotriene agents have shown promise in the treatment of asthma (Harris et al., 1995). These data show a clear role for these agents in blocking bronchoconstriction. Only preliminary data are available in man about the possible anti-inflammatory effects of such agents (Kane et al., 1994, Wenzel et al., 1995). The data shown here for zileuton would indicate that leukotriene biosynthesis inhibitors could have anti-inflammatory effects in the human lung. In contrast, albuterol and theophylline, agents currently used in asthma therapy, were not active against either leukotriene formation or lung eosinophilia in this animal model of lung inflammation. These data are consistent with the findings of Spicer et al. (1990) who showed these two agents to be ineffective in blocking blood eosinophilia induced by Sephadex injections. ZD 204,219, a specific $cysLT_1$ receptor antagonist, and CGS 25019c, a BLT receptor antagonist, were both inactive in the model. Although some activity was observed for MK-0476 at higher doses, it was not as potent as zileuton. Whether our observations in the rat model will translate to human asthma awaits clinical data with specific BLT and $cysLT_1$ receptor antagonists.

In conclusion, these studies describe an efficient method of eliciting lung eosinophilia which can be modulated with leukotriene biosynthesis inhibitors. These results suggest that 5-lipoxygenase products play a significant role in the Sephadex-induced airway eosinophilia in rats and imply a possible role for leukotrienes in lung eosinophilia in man. Furthermore, this model should be an interesting tool to study the mechanisms of airway eosinophilia and the potential role of these cells in the development of bronchial hyperresponsiveness.

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