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The effects of drugs on Sephadex-induced eosinophilia and lung hyper-responsiveness in the rat

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1 Rats given an intravenous injection of Sephadex particles (0.5 mg of G200 in 1 ml of saline) on days 0, 2 and 5 had a blood eosinophilia which was maximal on day 7.

2 On day 7, broncho-alveolar lavage (BAL) fluids taken from the rats contained an increased number of eosinophils and fewer mononuclear cells but there was no change in the small number of neutrophils. In addition the rats were hyper-sensitive to the increase in resistance to artificial respiration produced by 5-hydroxytryptamine (5-HT), given intravenously, with a shift to the left of the log dose-response curve. Lung parenchymal strips, taken from the rats on days 6, 7 and 8, were hyper-reactive to 5-HT with an increase in slope of the log dose-response curve.

3 Compounds with a wide variety of activities were evaluated for their effects on the blood eosinophilia on day 7 when given before each injection of Sephadex. The eosinophilia was reduced by glucocorticosteroids, β-adrenoceptor agonists, aminophylline, dapsone and phenidone.

4 Dexamethasone, isoprenaline, dapsone and phenidone at doses that reduced the blood eosinophilia also reduced the changes in number of leucocytes in the BAL fluids and the hyper-responsiveness to 5-HT *in vivo* and *in vitro*, except that the effects of dapsone on the hyper-sensitivity to 5-HT *in vivo* did not reach significance. Aminophylline was the least effective of the drugs at reducing the blood eosinophilia and its effects on the other changes did not reach significance. Sodium cromoglycate reduced the BAL eosinophilia but had no effect on the other changes produced by Sephadex.

5 The correlation coefficients between blood eosinophil numbers and reactivity to 5-HT *in vitro* and sensitivity *in vivo* were $r = 0.76$, ($n = 88$; $P < 0.001$) and $r = 0.53$, ($n = 61$; $P < 0.001$) respectively.

6 Doses of dexamethasone, isoprenaline, dapsone and phenidone that reduced the blood eosinophilia when given before each injection of Sephadex were inactive when given up to 8 h after the Sephadex.

7 These data show an association between blood eosinophilia and hyper-responsiveness of the lung. The blood eosinophilia in the rats was triggered within the first few hours of injecting the Sephadex and drugs have been identified which inhibit this trigger.

Introduction

The need for a new treatment for asthma is highlighted by the current increase in prevalence, severity and mortality of the disease in many developed countries (Mitchell, 1985; Friday & Fireman, 1988). It is now recognised that chronic asthma involves an inflammatory response in the lung and that treatments should be directed to reducing this (Barnes, 1989). The inflammatory response is characterized by the presence of the eosinophil which is thought to contribute to the pathology of the disease (Wardlaw & Kay, 1987; Gleich *et al.*, 1988). The peripheral blood eosinophil count in asthma correlates with the severity of the disease and there is a direct relationship between the ability of glucocorticosteroids to reduce blood eosinophil counts in asthma and clinical benefit whether given orally (Horn *et al.*, 1975) or by inhalation (Harris, 1980). Asthmatics have an exaggerated response to a wide variety of stimuli that can produce an increase in resistance to airflow in the lung and the responsiveness of asthmatics to inhaled histamine was found to correlate with the blood eosinophil count (Taylor & Lukza, 1987). The evidence suggests therefore that a compound that reduced eosinophilia in asthmatics would be of clinical benefit.

We have produced a blood eosinophilia in rats by the intravenous injection of Sephadex particles (Laycock *et al.*, 1986). We have used this model as a screen to detect compounds which reduce eosinophilia. The blood eosinophilia was accompanied by an increase in number of eosinophils and a fall in number of mononuclear cells in the broncho-alveolar lavage (BAL) fluids of the rats and, in addition, the lungs of the rats were hyper-responsive to the spasmogenic effects of 5-HT *in vivo* and *in vitro* (Spicer *et al.*, 1989). We have investigated the effects of reducing the blood eosinophilia with drugs on these other changes.

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Methods

Administration of Sephadex and drugs

Sephadex G200, particle size 40 to 120 μm, when fully swollen in water, was suspended in sterile, isotonic saline at 0.5 mg ml^{-1} and stored at 4°C for 48 h; 1 ml of the suspension was injected intravenously into the hind foot vein of Charles Rivers Sprague Dawley rats, 250–350 g, on days 0, 2 and 5. Rats in a control group received saline. Drugs were given before each injection of Sephadex and, in most experiments, with a contact time expected to give maximum activity at the time of the administration of the Sephadex. Six rats, in a control group, were given Sephadex without a drug each time that drugs were evaluated for their effects on leucocyte numbers in the blood or broncho-alveolar lavage fluids.

The drugs were given parenterally as freshly made neutral solutions in isotonic saline or orally as solutions or suspensions in 0.5% methylcellulose at 0.2 ml for every 100 g of body weight.

Broncho-alveolar lavage

Rats were anaesthetized by the intraperitoneal injection of Sagatal at 0.1 ml 100 g^{-1} of body weight. The trachea were cannulated and 1.5 ml of isotonic saline, containing 6 units ml^{-1} of heparin, was injected into the airways from a syringe connected to the cannula. The liquid was gently

sucked back into the syringe and transferred to the centrifuge tube. This was carried out 4 times. The combined washings, (4 to 5 ml), were centrifuged at 150 g for 5 min, the supernatant removed and the pellet resuspended in 500 μl of saline. Samples of 20 μl were used to determine leucocyte counts. The number of cells are quoted as millions per ml of the suspension in the 500 μl of saline.

Total and differential leucocyte counts

Samples of blood (20 μl) taken from the tail vein of the rats, or resuspended BAL fluid, were added to 10 ml of Isoton II, and within 30 min, Zaponin (3 drops) was added, to lyse the erythrocytes, 5 min before the determination of total cell counts with a Coulter Counter Model DN. Differential leucocyte counts were carried out by fixing and staining a blood smear on a microscope slide with May-Grunwald and Giesma stains. Smears of BAL fluid were stained with a Wright's and Giesma stain (Speirs & Dreisback, 1956). A minimum of 400 cells was counted on each slide. Blood and BAL fluids were collected between 09 h 00 min and 11 h 00 min.

Preparation of lung strips

Blood samples, for leucocyte counts, were taken from the tail vein of rats which were then stunned and bled and the heart and lungs removed and placed in a modified Tyrode solution at room temperature, containing ($g l^{-1}$): NaCl 8.0, $NaHCO_3$ 1.0, glucose 1.0, NaH_2PO_4 0.032, $MgCl_2$ 0.2, KCl 0.04 and $CaCl_2$ 0.05. The initial 2 to 3 mm of the left lobe was removed and then two consecutive strips were cut at right angles to the bronchus 3 to 4 mm wide. The lung strip was suspended in 4 ml organ baths which contained modified Tyrode solution at 37°C through which was bubbled a mixture of 95% O_2 and 5% CO_2. The response of the tissue was recorded with a UFI isometric transducer and a Kipp and Zonen 2 channel pen recorder. A tension of 1 g was applied to the tissue and the tissue was allowed to stabilize for 1 h. During this time the bathing fluid was changed by upward displacement at 15 min intervals and the tension restored after each wash.

Cumulative dose-response curves of the parenchymal lung strips to 5-hydroxytryptamine

5-Hydroxytryptamine (5-HT), as its creatinine sulphate salt, was dissolved in isotonic saline at a concentration of 800 $\mu g\,ml^{-1}$ of the free base. The solution was then diluted by five-fold serial dilutions seven times to 10.24 $ng\,ml^{-1}$. Volumes of 0.1 ml of each concentration of 5-HT, starting with the most dilute, was added in turn to the organ bath at 3 min intervals, without washing out, to produce a cumulative dose-response curve. The area under the log dose curve was calculated over the concentration range of 5-HT from 1.4×10^{-9} to 1.2×10^{-4} M against the increase in tension over 1 g. Three to four animals were given Sephadex and carrier fluid without drug on each occasion that a drug was evaluated. The results obtained on both lung strips from each animal were used to compare the effects of treatments. Some of the lung strips were weighed after the dose-response to 5-HT was obtained. The strips were placed on blotting paper to remove excess solution before weighing.

Increase in resistance to artificial respiration produced by 5-HT

Resistance to respiratory airflow was measured in anaesthetised artificially respired rats by the overflow method of Konzett & Rossler (1940). Rats were anaesthetized by the intraperitoneal injection of a 25% urethane solution in saline (0.6 ml $100\,g^{-1}$ of body weight). The trachea was cannulated and the animal was artificially respired with a Palmer Ideal respiratory pump set to exceed the normal lung capacity at 90 strokes min^{-1}. The overflow volume was measured with Ugo Basile monitor No. 7020 with a Devices DC 3461 amplifier connected to a Devices MX212 recorder. The carotid artery was cannulated for recording blood pressure with a Bell and Howell type 4-422-0001 physiological pressure transducer connected via a Devices 3552 amplifier to the Devices recorder. The jugular vein was cannulated for intravenous dosing. The level of anaesthesia was maintained to suppress spontaneous respiration, by the intraperitoneal injection of additional urethane when required.

The 100% resistance to respiratory airflow was taken as the overflow volume obtained by momentarily clamping the air supply to the trachea and the zero value was the maximum overflow volume during the normal respiratory cycle. After allowing the animal to stabilize for 30 min, doses of 5-HT were given intravenously in 0.1 ml of saline at 5 min intervals, starting with a dose of 1.5 $\mu g\,kg^{-1}$, as the free base, and increasing by two fold serial amounts until the resistance to respiratory airflow was in excess of 80% of maximum, or a dose of 96 $\mu g\,kg^{-1}$ of 5-HT was reached. The doses of 5-HT in excess of threshold produced a rapid increase in overflow volume which returned to the baseline more slowly and was ended by momentarily clamping the overflow tube if it had not returned to baseline after 3 min. To confirm that the sensitivity of the rat to 5-HT had not changed during the experiment, the dosing with 5-HT was repeated. For each rat, the second dose-response curve was not significantly different from the first. Both sets of results were used to plot two dose-response curves for each rat. Rats were tested in groups and treatments were randomised within the groups, each containing at least one positive and one negative control rat, given either Sephadex or saline, respectively, without drugs. The log dose-response curves for each rat were plotted and the ED_{30} values were estimated.

Hyper-sensitivity and hyper-reactivity

We have used the term hyper-sensitivity to mean a parallel shift to the left of the log dose-response curve, with hyper-reactivity being used to describe an increase in the steepness of the slope, as suggested by Orehek *et al.* (1977), with hyper-responsiveness being used as a general term.

Correlation between number of eosinophils in the blood and the responsiveness to 5-HT

The numbers of eosinophils in the blood of rats was determined from differential leucocyte counts made from blood samples taken from the tail veins of the rats immediately before taking lung strips or anaesthetising the rats for measurement of resistance to artificial respiration. Lung strips were taken on days 6, 7 or 8 and measurements of resistance to artificial respiration were made on day 7. The number of eosinophils in the blood was compared with the area under the log dose-response curve for the lung strips *in vitro* or with the $\log_{10}$ of the ED_{30} value *in vivo*. The mean of the two determinations made for each rat was used. Data from all the rats in the treatment groups shown on Table 3 were used except that data for rats given indomethacin were not used for the correlations between blood eosinophilia and sensitivity *in vivo* since indomethacin appeared to potentiate the sensitivity to 5-HT.

Drugs and chemicals

Sodium cromoglycate was a gift from Fisons. Phenidone (1-phenyl-3-pyrazolidone) was obtained from Sigma. Dexamethasone sodium phosphate solutions were prepared from a Decadron solution, 4 $mg\,ml^{-1}$, purchased from Merck Sharpe and Dohme. Urethane, Giemsa, May Grunwald and Wrights stains were from BDH, heparin sodium (mucous), 1000 u ml^{-1} from Weddel, Isoton 11 and Zaponin from Coulter Electronics, Sagatal (pentobarbitone sodium 60 $mg\,ml^{-1}$) from May and Baker, and Sephadex G200 from Pharmacia. Other compounds were obtained from commercial suppliers.

Statistical analyses

The variability of the results for blood and BAL leucocyte numbers was examined using residual plots following an analysis of variance. Analyses on a logarithmic scale were found to be more appropriate. For BAL neutrophils, analysis was performed on log 10 ($x + 0.005$) because of recorded zero counts. Results for Sephadex-treated control animals varied significantly over experiments and therefore treatments were assessed relative to control means within experiments. For each treatment the difference between its mean value and that for its control was calculated and a weighted mean difference was derived over experiments to allow for unequal replication. The weights used were the reciprocals of the variances of the differences, using a pooled error term from all treatment and control groups. Differences were derived on a logarithmic scale with 95% confidence intervals, which when back transformed represented ratios between geometric means. Additionally P values (2 tailed) were determined for these comparisons. Results were quoted as geometric means with 95% confidence intervals or as arithmetic means with s.e.mean.

Results for areas under the 5-HT dose-response curve on lung strips were analysed in a similar way. Control mean values were found to be consistent over experiments and treatment comparisons were made against a pooled control mean.

The responses to 5-HT *in vivo* were analysed in terms of $\log_{10} ED_{30}$ values comparing each treatment group with the Sephadex-treated controls by Student's t test (unpaired, two-tailed). The mean values and s.e.mean were used to derive geometric means with corresponding 95% confidence intervals.

Correlation coefficients were computed by least square analysis. The significance of the differences between other results was assessed by unpaired Student's t test (two-tailed).

Results

Effects of drugs on Sephadex-induced blood eosinophilia

Rats given Sephadex intravenously on days 0, 2 and 5 had an increase in number of eosinophils in the blood which reached a maximum on days 7 to 8 (Figure 1). There was no change in number of other blood leucocytes at the times measured. Compounds, with a variety of pharmacological activities were tested for their ability to reduce the blood eosinophilia, on day 7, when given before each injection of Sephadex. Most were inactive (Table 1) but the eosinophilia was reduced by the adrenoceptor agonists, isoprenaline, salbutamol, and adrenaline and by aminophylline, glucocorticosteroids, as exemplified by dexamethasone and triamcinolone, and by phenidone and dapsone (Figure 2).

The drugs had no effect on numbers of other leucocytes in the blood except for dapsone and the glucocorticosteroids. Dapsone at $100\,mg\,kg^{-1}$ given orally, 30 min before each injection of Sephadex, increased the number of mononuclear cells (from $9.7 \pm 0.67 \times 10^6\,ml^{-1}$, in control animals given Sephadex alone, to $12.7 \pm 0.9 \times 10^6\,ml^{-1}$, $P < 0.001$, $n = 43$) and neutrophils (from $2.8 \pm 0.27 \times 10^6\,ml^{-1}$ to $3.7 \pm 0.22 \times 10^6\,ml^{-1}$, $P < 0.001$). Dexamethasone at $0.1\,mg\,kg^{-1}$ reduced the number of mononuclear cells (from $11.0 \pm 0.75 \times 10^6\,ml^{-1}$ to $7.5 \pm 0.44 \times 10^6\,ml^{-1}$, $P < 0.001$, $n = 37$), arithmetic mean values ± s.e.mean, but had no significant effect on numbers of neutrophils. The effects produced by triamcinolone at $4\,mg\,kg^{-1}$, p.o., given 4 h before the Sephadex were similar to those produced by dexamethasone.

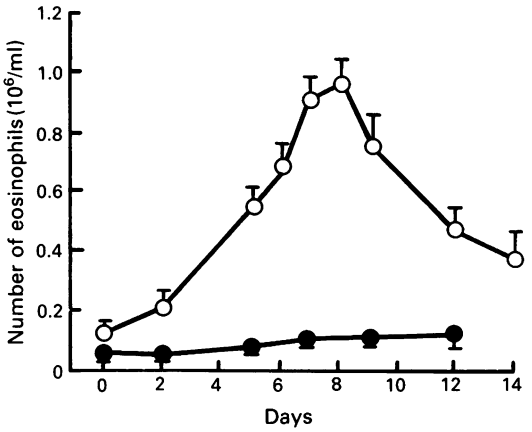

Figure 1 The number of eosinophils in the blood of rats given an intravenous injection of Sephadex, G200, 0.5 mg in 1 ml of saline (○), or saline (●), on days 0, 2 and 5. Blood was collected before the injections on the days when they were given. The values are arithmetic means of $n = 12$ or more for Sephadex-treated rats and 6 or more for rats given saline; s.e.mean shown by vertical bars.

Effects of drugs on changes in the BAL fluids produced by Sephadex

Most of the cells in the BAL fluids of negative control rats, given saline instead of Sephadex, were mononuclear cells, less than 5% of the cells were neutrophils and no eosinophils were detected. Eosinophils were found, on day 7, in the BAL fluids of rats given Sephadex, at the time of the peak in blood eosinophil numbers. At this time there was also a fall in number of mononuclear cells but there was no change in the number of neutrophils. Drugs which reduced the blood eosinophilia were tested for their effects on the changes in the BAL fluids. Doses of dexamethasone, isoprenaline, aminophylline, dapsone and phenidone which reduced the blood eosinophilia when given before each injection of Sephadex also reduced the increase in number of eosinophils in the BAL fluids, although the reduction produced with aminophylline did not reach significance. These drugs also reduced the fall in number of mononuclear cells in the BAL fluid but again this did not reach significance with aminophylline. Sodium cromoglycate, which had no effect on the blood eosinophilia, produced some reduction of the eosinophilia in the BAL fluids but did not prevent the fall in number of mononuclear cells.

Table 1 Compounds that had no effect on the increase in number of eosinophils in the blood of rats given Sephadex

Compound	*Dose* ($mg\,kg^{-1}$)	*Route*	*Time* (h)	*Compound*	*Dose* ($mg\,kg^{-1}$)	*Route*	*Time* (h)
Aspirin	300	p.o.	1	Noradrenaline	0.5	s.c.	0.17
Atropine	10	s.c.	0.5	Papaverine	10	s.c.	0.5
Chloroquine	100	p.o.	1	Phentolamine	2.5	s.c.	0.5
Cyproheptadine	4	s.c.	1	Phenoxybenzamine	2	s.c.	0.5
Sodium cromoglycate	100	s.c.	0.17	Phenylephrine	4	s.c.	0.5
Indomethacin	5	p.o.	1	Propranolol	5	s.c.	0.25
Ketotifen	20	p.o.	0.25	Quinidine	50	p.o.	0.5
Mepacrine	100	p.o.	1	Quinine	100	p.o.	0.5
Mepyramine	20	s.c.	0.5	Verapamil	50	p.o.	0.25

Rats were given Sephadex, G200 0.5 mg in 1 ml of saline, intravenously on days 0, 2 and 5. The numbers of eosinophils in the blood were counted on day 7. Compounds were given at the stated dose, route and time before each injection of Sephadex.

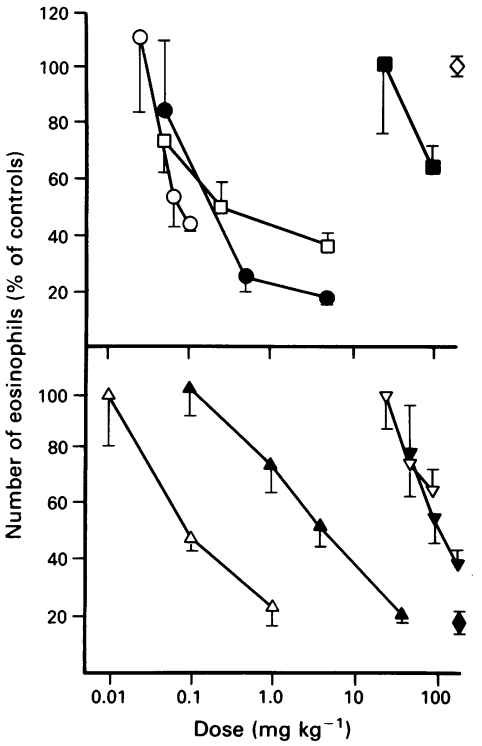

Figure 2 The effect of drugs on the number of eosinophils in the blood of rats on day 7 after the i.v. injection of Sephadex, G200, 0.5 mg in 1 ml of saline on days 0, 2 and 5. The drugs were given by the stated route and time before each injection of Sephadex: isoprenaline (○), salbutamol (●) and adrenaline (□) s.c. 10 min; aminophylline (■) p.o. 30 min; dexamethasone (△) and triamcinolone (▲) p.o. 4 h; phenidone (▽) and dapsone (▼) p.o. 30 min. The values are given as a percentage of the mean values for similar numbers of rats given Sephadex but no drug in the same experiments. For typical groups of control rats given Sephadex but no drug (◇) or saline only (◆) the number of eosinophils in the blood, as 10^6 ml^{-1} were 0.86 ± 0.05, $n = 53$, and 0.14 ± 0.01, $n = 56$ as arithmetic means ± s.e.mean. The drugs had no effect on the number of other leucocytes in the blood except for dapsone and the glucocorticosteroids (see text). Points represent arithmetic means and the vertical lines s.e.mean, $n = 6$ to 43.

Indomethacin had no effect on any of the changes in leucocyte numbers. The number of neutrophils remained low for all the treatments. Aminophylline and isoprenaline significantly reduced the number of neutrophils but the numbers in the Sephadex-treated controls were low and not different from the saline controls, so that this reduction is unlikely to be of importance and is probably due to the inaccuracy that results from counting cells in such low numbers (Table 2).

Effects of drugs on lung hyper-responsiveness

Rats given Sephadex had increased sensitivity to the increase in resistance to artificial respiration produced by the intravenous injection of 5-HT as shown by a shift to the left of the 5-HT log dose-response curve as compared to that for control rats given saline (Figure 3a). *In vitro* parenchymal lung strips taken from Sephadex treated rats were hyper-reactive to 5-HT in that they responded over the same dose range of 5-HT as did strips from control rats but the slope of the log dose-response curve was steeper (Figure 3b). The results can be expressed as mg tension per mg of tissue weight rather than as mg tension. When expressed in either way the differences in the response of lung strips obtained from untreated to Sephadex-treated rats were similar. The area under the log dose-response curve for tissue from untreated rats, as a percentage of that for tissue from Sephadex-treated rats, was $27 \pm 2\%$ as mg tension and $26 \pm 4\%$ as mg tension per mg of tissue ($n = 22$ and 15 respectively, as arithmetic means ± s.e.mean).

The effects on the hyper-responsiveness of the lungs to 5-HT of drugs that reduced blood eosinophilia were studied. Dexamethasone, isoprenaline, aminophylline, dapsone and phenidone were given to the rats before each injection of Sephadex at doses at which they reduced the blood eosinophilia. The drugs reduced the hyper-reactivity to 5-HT *in vitro* and hyper-sensitivity *in vivo* (Figure 3, Table 3), with the exception that the apparent reduction in hyper-sensitivity *in vivo* produced by aminophylline and dapsone did not reach significance and aminophylline had little effect on hyper-reactivity *in vitro*. Sodium cromoglycate and indomethacin had no effect on any of the parameters.

Blood eosinophil numbers for each rat were compared with either the reactivity of lung parenchymal strips to 5-HT or with the sensitivity to 5-HT *in vivo*. There was good corre-

Table 2 The effects of drugs on the changes in number of leucocytes in the broncho-alveolar lavage (BAL) fluids of rats produced by Sephadex

		Number of leucocytes					
		Mononuclear cells		*Neutrophils*		*Eosinophils*	
Treatment	n	× 10^6/ml	% of controls	× 10^6/ml	% of controls	× 10^6/ml	% of controls
Controls							
Sephadex, no drug	43	1.22	100	0.05	100	0.54	100
Saline, no drug	12	2.48	198 (148 to 264)***	0.03	45 (20 to 103)	0.00	0
Dexamethasone (0.1 mg kg^{-1}, p.o., 4 h)	12	1.96	133 (100 to 178)*	0.12	64 (28 to 145)	0.07	12 (7 to 21)***
Isoprenaline (0.1 mg kg^{-1}, s.c., 10 min)	6	2.03	152 (101 to 229)*	0.01	22 (6 to 70)*	0.09	17 (8 to 37)***
Aminophylline (100 mg kg^{-1}, p.o., 30 min)	6	1.82	137 (91 to 206)	0.01	25 (7 to 80)*	0.28	57 (27 to 120)
Dapsone (100 mg kg^{-1}, p.o., 30 min)	30	1.67	137 (114 to 166)***	0.08	80 (47 to 136)	0.06	11 (8 to 15)***
Phenidone (100 mg kg^{-1}, p.o., 30 min)	17	1.58	131 (103 to 166)*	0.02	84 (42 to 167)	0.18	44 (28 to 68)***
Sodium cromoglycate (100 mg kg^{-1}m s.c., 10 min)	10	0.92	83 (60 to 114)	0.05	95 (38 to 237)	0.31	48 (27 to 87)*
Indomethacin (5 mg kg^{-1}, p.o., 1 h)	6	1.55	117 (77 to 176)	0.01	32 (10 to 101)	0.61	123 (58 to 259)

Drugs were given at the dose route and time stated before each injection of Sephadex, 0.5 mg in saline, i.v., on days 0, 2 and 5. BAL washings were collected on day 7, cells were separated by centrifugation and suspended in 0.5 ml of saline. The values as 10^6/ml represent the number in this suspension and are geometric means. For each drug treatment, leucocyte numbers were compared with the values for a similar number of control rats given Sephadex, on the same occasion, to give the % of control values together with 95% confidence intervals in parentheses. Comparisons were by analysis of variance on a logarithmic scale, as described in the test, P values are quoted for these comparisons. The control values given are for typical controls given Sephadex or saline. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

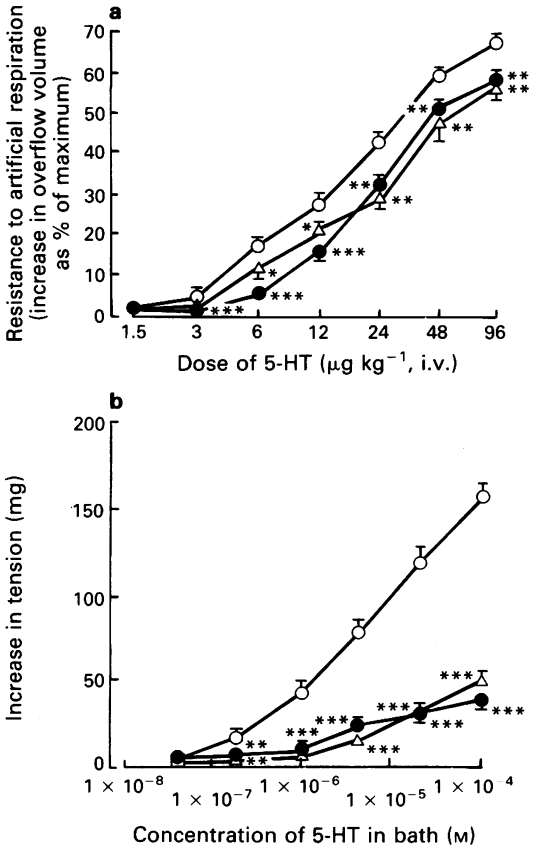

Figure 3 Effect of dexamethasone on the increase in lung responsiveness to 5-hydroxytryptamine (5-HT) produced in rats given Sephadex (a) *in vivo* and (b) on lung parenchymal strips *in vitro*. Rats were given Sephadex G200, 0.5 mg in 1 ml of saline (○) or saline alone (●), i.v., on days 0, 2 and 5. Other rats were given dexamethasone orally at 0.1 mg kg^{-1}, 4 h before each dose of Sephadex (△). Measurements *in vivo* were made on day 7 and lung strips were taken on days 6, 7 and 8. Numbers of rats for *in vivo* and *in vitro* determinations respectively were, for rats given: Sephadex, 15, and 22; saline, 20 and 21; dexamethasone and Sephadex, 6 and 7. Points represent arithmetic means and vertical lines s.e.mean. Student's *t* test (2-tailed) was used to assess the significance of the difference from rats given Sephadex: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

lation between blood eosinophil numbers and reactivity *in vitro*, as measured by the area under the log dose-response curve $r = 0.76$ ($n = 80$; $P < 0.001$). The correlation was less between blood eosinophil numbers and sensitivity *in vivo*, as measured by the $\log_{10}$ of the ED_{30} value, $r = -0.53$ ($n = 61$; $P < 0.001$). This was also less than the value reported previously when only Sephadex- and saline-treated animals were compared (Spicer *et al.*, 1989). In this study when the values in these control rats were compared the correlation was increased, $r = -0.66$ ($n = 25$; $P < 0.001$).

The drugs could have a direct effect on the responsiveness of the lungs of rats to 5-HT. Therefore dexamethasone, isoprenaline, aminophylline, dapsone and phenidone were given to groups of 6 rats at the same dose and dosage regimen as outlined in Table 3 except that saline was given to the rats instead of Sephadex. A control group of 6 rats was given saline only. When the rats given the drugs were compared with this control group the log dose-response curves for the effects of 5-HT given intravenously and for its effects on lung parenchymal strips were not different (data not shown). Thus the drugs by this dosage regimen, did not antagonize the effects of 5-HT.

Effects of drugs on the blood eosinophilia when given after each dose of Sephadex

Drugs which reduced the eosinophilia when given before the Sephadex showed a reduced activity when given after Sephadex. Of the drugs tested, dexamethasone could be given for the longest period of time after the Sephadex and still be active but it produced no significant inhibition when given at 8 h (Table 4).

Discussion

Effect of drugs on eosinophilia

The blood eosinophilia in rats given Sephadex was reduced by glucocorticosteroids, β-adrenoceptor agonists, aminophylline, dapsone and phenidone. It is remarkable that the first three of these are used for the treatment of asthma and dapsone has been shown to have a steroid sparing effect in asthmatics (Berlow *et al.*, 1990).

At the time of the peak in the blood eosinophilia, BAL fluids taken from the rats contained an increased number of eosinophils, a reduced number of mononuclear cells but there was no change in the small number of neutrophils. The glucocorticosteroid, dexamethasone, the β-adrenoceptor agonists, isoprenaline, dapsone, and phenidone reduced these changes at the same doses at which they reduced the blood eosinophilia. Aminophylline appeared to have similar effects but these did not reach significance. Aminophylline was the least effective of these drugs at reducing the blood eosinophilia. Sodium cromoglycate, whilst it had no effect on the blood eosinophilia or the fall in number of BAL mononuclear cells, did reduce the BAL eosinophilia.

Others have studied the effects of drugs on a BAL eosinophilia in the guinea-pig produced by the inhalation of PAF or antigen. Similar findings, to ours were that the eosinophilia was reduced by dexamethasone, aminophylline and sodium cromoglycate (Sanjar *et al.*, 1990a,b). However in these studies, in contrast to our findings in the rat, ketotifen was effective and no activity was demonstrated for salbutamol. Others found that while antigen induced BAL eosinophilia in the guinea-pig was reduced by methylprednisolone, no activity was demonstrated for ketotifen (Havill *et al.*, 1990). The finding of activity for a drug in one study but not in another could be due to differences in dosage regimens. For example; in the two studies in which salbutamol failed to reduce the BAL eosinophilia in the guinea-pig it was given over a period of days from an implanted minipump so that tolerance may have developed.

Sodium cromoglycate has been reported to reduce the number of eosinophils in the BAL fluids in asthmatics (Diaz *et al.*, 1984) and the ability of β-adrenoceptor agonists, aminophylline and glucocorticosteroids to produce an eosinopenic effect in man has been known for many years (Ohman *et al.*, 1972). In addition, asthmatics can show tolerance to the eosinopenic effects of β-adrenoceptor agonists (Reed *et al.*, 1970).

Eosinophilia and lung hyper-responsiveness

Hyper-responsiveness of the lungs, to spasmogens, has been produced in animals by a variety of techniques, most of which involve producing inflammation in the lung. In many of the studies the hyper-responsiveness was associated with a cellular infiltration, often of neutrophils (Smith, 1989). However, it is only in the dog that there is strong evidence that the neutrophil is the cause of the hyper-responsiveness (O'Byrne *et al.*, 1984). In other species such as the rabbit (Coyle *et al.*, 1988), guinea-pig (Silbaugh *et al.*, 1987) and rat (Pauwels *et al.*, 1986), the airway responsiveness could remit whilst a neutrophilia was still present in the lung. An eosinophilia in the lung has been associated with a lung hyper-responsiveness in sheep (Abraham, 1987), monkey (Grundel *et al.*, 1990) and rabbit (Coyle *et al.*, 1988). In the guinea-pig, inhaled antigen produced an eosinophilia in the BAL fluids and an *in vivo* hyper-reactivity to inhaled spasmogens. However, there was no direct relationship between the two since the hyper-reactivity could be reduced by drugs which had no effect on the BAL

Table 3 The effects of drugs on the blood eosinophilia and lung hyper-responsiveness to 5-hydroxytryptamine (5-HT) *in vitro* and *in vivo* produced in rats given Sephadex

Treatment	Number of eosinophils % of value in Sephadex-treated controls	Response to 5-HT *in vitro* AUC	Response to 5-HT *in vivo* EC_{30} ($\mu g\,kg^{-1}$ i.v.)
Controls			
Sephadex, no drug	100	100	12.3 (9.8 to 15.5)
Saline, no drug	14 (11 to 18)***	27 (21 to 34)***	26.1 (22.0 to 30.9)***
Dexamethasone ($0.1\,mg\,kg^{-1}$, p.o., 4 h)	47 (38 to 59)***	35 (25 to 49)***	22.8 (15.8 to 32.8)**
Isoprenaline ($0.1\,mg\,kg^{-1}$, s.c., 10 min)	36 (25 to 52)***	53 (39 to 72)***	22.5 (16.7 to 30.4)**
Aminophylline ($100\,mg\,kg^{-1}$, p.o., 30 min)	62 (39 to 99)*	80 (56 to 114)	15.5 (11.6 to 20.8)
Dapsone ($100\,mg\,kg^{-1}$, p.o., 30 min)	50 (40 to 62)***	45 (32 to 62)***	14.6 (10.6 to 20.2)
Phenidone ($100\,mg\,kg^{-1}$, p.o., 30 min)	66 (52 to 83)***	65 (48 to 89)**	19.8 (16.0 to 24.4)**
Sodium cromoglycate ($100\,mg\,kg^{-1}$, s.c., 10 min)	87 (63 to 118)	108 (76 to 154)	12.6 (10.3 to 15.3)
Indomethacin ($5.0\,mg\,kg^{-1}$, p.o., 1 h)	98 (62 to 156)	108 (77 to 154)	8.4 (6.5 to 10.7)

Drugs were given at the stated dose route and time before each injection of Sephadex, 0.5 mg in 1 ml of saline, i.v., on days 0, 2 and 5. Blood was taken and sensitivity to 5-HT *in vivo* was determined on day 7. Lung strips were taken on days 6, 7 or 8. For each drug-treatment the eosinophil numbers were compared with the values for a similar number of control rats given Sephadex on the same occasion. The means for the area under the log dose-response curves (AUC) were compared with the pooled control values for rats given Sephadex. Comparisons were made by an analysis of variance on a logarithmic scale as described in the text. *P* values are quoted for these comparisons. The EC_{30} values are the i.v. doses increasing the overflow volume to 30% of the maximum and are geometric means. The $\log_{10}$ of the EC_{30} values were compared by unpaired Student's *t* test, two tailed, with the values in Sephadex-treated rats. Number of rats, for each drug treatment, were 12 to 43 for blood eosinophils 6 to 7 for AUC and for EC_{30} values, with two determinations for each rat, numbers for Sephadex-treated controls were 24 for AUC and 15 for EC_{30} values. 95% confidence intervals are given in parentheses. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

eosinophilia and conversely the eosinophilia could be reduced by drugs which had no effect on the hyper-reactivity. In one study ketotifen, PAF receptor antagonists and prednisolone reduced the hyper-reactivity but only prednisolone reduced the eosinophilia (Havill *et al.*, 1990). These results were made somewhat confusing by the finding in another study that ketotifen had the converse effect in that it reduced the eosinophilia but not the hyper-reactivity and whilst dexamethasone reduced the eosinophilia it had no effect on the hyper-reactivity (Sanjar *et al.*, 1990b).

In our study in the rat we found a close association between a blood eosinophila and the reactivity of lung strips to 5-HT. Lung strips, taken from the rats given Sephadex at the time of the peak in blood eosinophilia, were hyper-reactive to 5-HT with an increase in the slope of the log dose-response curve. Drugs that reduced the eosinophilia also reduced the hyper-reactivity to 5-HT except that the effects of aminophylline on the hyper-reactivity did not reach significance. Rats given Sephadex also had an hyper-sensitivity to the increase in resistance to artificial respiration produced by the intravenous injection of 5-HT with a shift to the left of the log dose-response curve. The effects of reducing the eosinophilia with drugs on the hyper-sensitivity were less clear cut. Whilst dexamethasone, isoprenaline and phenidone reduced the hyper-sensitivity, the effects of dapsone and aminophylline did not reach significance, at doses at which they reduced the blood eosinophilia. The correlation between blood eosinophil numbers and lung hyper-responsiveness was greater *in vitro* than *in vivo*. This could be due to the variability of the measurements *in vivo* and the correlation was better when results in non-drug treated control rats were used. The nature of the smooth muscle in the lung showing the hyper-responsiveness to 5-HT is not known and the extent of the involvement of respiratory or vascular tissue is being investigated.

Table 4 Effects of drugs on the blood eosinophilia when given after each dose of Sephadex

Drugs	Time (h)	Eosinophils (% of controls)
Dexamethasone	1	34.3 ± 6.3
	4	60.8 ± 7.8
	8	81.2 ± 11.6
Isoprenaline	8	105.0 ± 19.0
Dapsone	1	54.6 ± 7.3
	4	82.0 ± 14.2
Phenidone	0.5	63.7 ± 10.7
	1	83.1 ± 15.3

The drugs were given at the same dose and route as shown on Table 3, but at the time stated after each injection of Sephadex on days 0, 2 and 5. Blood was taken on day 7. The numbers of eosinophils are given as a percentage of the mean value in control rats tested at the same time and given Sephadex but no drug; 6 rats were used for each test and control except for isoprenaline when 4 rats were used. The values are expressed as arithmetic mean ± s.e.mean.

In the rats given Sephadex there was a marked correlation between the number of blood eosinophils and the reactivity of the lungs, to 5-HT, *in vitro* which suggests, but does not prove, a causal relationship. The eosinophil can secrete a variety of cytotoxic and spasmogenic materials which are potential mediators of the hyper-reactivity, such as basic proteins, peroxidase, leukotriene C_4, PAF and 15-lipoxygenase products (Wardlaw & Kay, 1987). Tracheal smooth muscle of guinea-pigs and dogs treated with major basic protein from eosinophils was hyper-reactive to spasmogens (Flavåhan *et al.*, 1988; Brofman *et al.*, 1989).

If the eosinophil is causal of the lung hyper-responsiveness it may be the number of activated eosinophils rather than the total numbers that are important. Eosinophils taken from the peritoneal cavities of rats given three injections of Sephadex were activated when compared with eosinophils from rats given saline, or a single injection of Sephadex, as shown by enhanced cytotoxicity in a variety of assays (Cook *et al.*, 1987). It was assumed that the eosinophils would be activated in the BAL fluids of guinea-pigs given antigen by inhalation, since they had crossed several body compartments (Sanjar *et al.*,

1990b). However, this applies also to the eosinophils in the peritoneal cavity of the rat and since it is possible to detect difference in levels of activation in peritoneal eosinophils the same could apply to eosinophils in the lung.

The lung hyper-responsiveness of the rats given Sephadex has similarities with that shown by asthmatics. Asthmatics have a mixed hypersensitivity and hyper-reactivity to the acute bronchoconstrictor effects of inhaled histamine with a shift to the left and an increase in the slope of the log dose-response curve (Snashall, 1987) and, in two recent studies, lung tissues from asthmatics was found to be hyper-reactive to spasmogens (De Jongste *et al.*, 1987; Bai, 1990).

Mechanism of drug action

The mechanism by which the drugs reduce the eosinophilia is not known. It is possible that some compounds are active because they produce endogenous glucocorticosteroid release. However dexamethasone reduced the eosinophilia at doses at which it reduced the number of mononuclear cells in the blood and this was not found with the other active compounds. Phenidone is an inhibitor of the cyclo-oxygenase and lipoxygenase pathways of arachidonic acid metabolism (Blackwell & Flower, 1978). Inhibitors of the cyclo-oxygenase pathway such as indomethacin and aspirin had no effect on the eosinophilia suggesting that inhibition of lipoxygenase might be a relevant activity. The other active compounds have been also shown to be capable of inhibiting the release of lipoxygenase products: β-adrenoceptor agonists and theophylline (Orange & Austen, 1971), glucocorticosteroids (Blackwell *et al.*, 1980), and dapsone (Bonney *et al.*, 1983). The possible involvement of lipoxygenase products in the triggering of the eosinophilia warrants further study.

Mechanism of eosinophilia

Sephadex particles when injected intravenously into rats, embolised the lung vasculature to form a localised inflammatory reaction or granuloma (Walls & Beeson, 1972). The number of granuloma in the lung was not reduced by isoprenaline, dexamethasone or dapsone when given before each injection of Sephadex at doses at which they inhibited the blood eosinophilia and therefore they were not active merely because they prevented the embolisation of the vasculature. The mature granuloma contained mainly mononuclear cells but for the first few hours the predominant cell was the neutrophil (Cook *et al.*, 1989). Drugs which reduced the eosinophilia, when given before the Sephadex, were inactive when given 8 h afterwards. The changes produced by the Sephadex during the first few hours after being injected must therefore trigger the eosinophilia. These changes may be those taking place in the early stages of the formation of the granuloma involving interactions between leucocytes, particularly neutrophils, and vascular endothelial cells. In the lungs a large number of vascular endothelial cells are close to the external environment and 60 to 75% of the neutrophils in the blood are marginated in the lung vasculature (Worthen *et al.*, 1987), and are therefore suitably placed for this type of interaction. It was reported in 1953, that lung tissue from guinea-pigs, which had been previously given an intraperitoneal injection of antigen-antibody complexes, when placed into the peritoneal cavity of normal guinea-pigs produced an eosinophilia, suggesting that the lungs could be a site of formation of eosinopoietic factors (Samter *et al.*, 1953).

In conclusion these studies provide data showing an association between eosinophilia and hyper-responsiveness of the lung. The eosinophilia in the rats was triggered within the first few hours of injecting the Sephadex and drugs have been identified which inhibit this trigger. The mechanism by which the eosinophilia is produced warrants further study.

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