Effect of heparin and a low-molecular weight heparinoid on PAF-induced airway responses in neonatally immunized rabbits

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1 We have investigated the effect of an unfractionated heparin preparation, a low-molecular weight heparinoid (Org 10172) and the polyanionic molecule polyglutamic acid against PAF-induced airway hyperresponsiveness and pulmonary cell infiltration in neonatally immunized rabbits *in vivo*.

2 Exposure of neonatally immunized rabbits to aerosolized platelet activating factor (PAF) (80 µg ml⁻¹ for 60 min) elicited an increase in airway responsiveness to inhaled histamine 24 h and 72 h following challenge which was associated with an infiltration of inflammatory cells into the airways, as assessed by bronchoalveolar lavage (BAL).

3 A significant increase in the total numbers of cells recovered from BAL fluid was associated with significantly increased cell numbers of neutrophils, eosinophils and mononuclear cells 24 h following PAF exposure. The numbers of eosinophils and neutrophils in the airways remained elevated 72 h after challenge.

4 The intravenous administration of an unfractionated preparation of heparin (100 units kg⁻¹) or Org 10172 (100 µg kg⁻¹) 30 min prior to PAF exposure significantly inhibited the airway hyperresponsiveness induced by PAF, 24 h and 72 h following challenge. PAF-induced hyperresponsiveness was not significantly affected by prior intravenous administration of polyglutamic acid (100 µg kg⁻¹).

5 The intravenous administration of unfractionated heparin (100 units kg⁻¹), Org 10172 (100 µg kg⁻¹) or polyglutamic acid (100 µg kg⁻¹) 30 min prior to PAF exposure significantly inhibited the expected increase in total cell infiltration.

6 This study shows that unfractionated heparin and a low-molecular weight heparinoid, Org 10172, are capable of inhibiting both the airway hyperresponsiveness and pulmonary cell infiltration induced by PAF in the rabbit.

Keywords: Heparin; low-molecular weight heparin; PAF; airways; inflammation; airway hyperresponsiveness; neonatally immunized rabbit

Introduction

Bronchial asthma is now recognised as an inflammatory disease which is characterized by bronchial hyperresponsiveness, excessive mucus production, mucosal oedema and recruitment of inflammatory cells into the airways. Much attention has been focussed on the role of inflammatory cells such as mast cells, and more recently circulating T-lymphocytes (Corrigan *et al.*, 1988; Kay, 1991; Robinson *et al.*, 1992), in allergic inflammation. Heparin, a major component of the mast cell granule matrix, is widely known as a polyanionic anticoagulant; however, comparatively little work has been done with respect to its anti-inflammatory effects.

It was reported in 1928 that heparin could inhibit features of anaphylaxis (Van der Carr & Williams, 1928), a phenomenon confirmed later by clinical studies which showed that heparin could modulate allergic responses in the skin and respiratory system (Dolowitz & Dougherty, 1960; Dahl, 1962; Boyle *et al.*, 1964; Bowler *et al.*, 1993). In addition, an increased level of an endogenous 'heparin-like material' has been reported in the plasma of allergic subjects (Lasser *et al.*, 1987). Heparin has been shown to inhibit activation of the complement system (Kazatchkine *et al.*, 1981; Ekre *et al.*, 1986), neutrophil chemotaxis (Matzner *et al.*, 1984) and eosinophil infiltration (Hanss *et al.*, 1989; Seeds & Page, 1992). Furthermore, heparin protects against tissue damage induced by cationic leukocyte-derived cationic mediators (Motojima *et al.*, 1989), such as the specific eosinophil-derived mediators major basic protein (MBP), eosinophilic cationic protein and eosinophil peroxidase (Frigas & Gleich,

1986). Heparin has also been shown to inhibit the increased vascular permeability induced by a variety of inflammatory mediators including histamine, bradykinin, prostaglandin E₁ (Carr, 1979) and cationic proteins (Needham *et al.*, 1988; Antunes *et al.*, 1990; Sasaki *et al.*, 1991). Evidence suggests that heparin can inhibit T-lymphocyte activation (Frieri & Metcalfe, 1983), trafficking (Sy *et al.*, 1983; Willenborg & Parish, 1988; Lider *et al.*, 1990) and delayed hypersensitivity responses (Frieri & Metcalfe, 1983; Sy *et al.*, 1983; Lider *et al.*, 1990) via a mechanism unrelated to its anti-coagulant properties.

Heparin is a mixture of anionic sulphated mucopolysaccharide chains with an average molecular weight of 10,000–15,000 Da, composed of sulphated disaccharides. It is heterogeneous with respect to its affinity for anti-thrombin III (AT-III), the most important heparin cofactor present in plasma (Rosenberg *et al.*, 1978). Org 10172, a low-molecular weight heparinoid, is a mixture of sulphated glycosaminoglycans derived from animals intestinal mucosa, with a mean molecular weight of approximately 6,000 Da. Org 10172 consists of approximately 80% low-molecular weight heparin sulphate, 10% dermatan sulphate and approximately 5% each of chondroitin sulphate and a low molecular weight heparin-like component with high affinity for AT-III. In animals studies Org 10172 displayed anti-thrombotic activity with only a small dose-dependent bleeding enhancing capacity compared with equipotent antithrombotic doses of standard heparin (Bradbrook *et al.*, 1987). Org 10172 has been used to investigate the contribution of molecular weight to the activity of heparin on PAF-induced airway changes.

It has been suggested that the ether-linked phospholipid

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platelet activating factor (PAF), plays an important role in the pathogenesis of asthma as it can reproduce many of the features of the disease, both in experimental animals and man (reviewed in Page, 1988). PAF has been employed in this study to induce airway hyperresponsiveness in rabbits as we have previously reported that this is a useful test system for the evaluation of pharmacological agents and is simpler and more robust method than antigen challenge. We have utilized antigen-immunized rabbits in this study as we have previously shown that PAF can induce airway hyperresponsiveness to inhaled histamine in all neonatally immunized rabbits to some degree (Herd *et al.*, 1992), whereas only in some, but not all *normal* rabbits (Coyle *et al.*, 1990; Herd *et al.*, 1992).

Therefore, in the present study we have investigated the effects of heparin and related compounds on PAF-induced airway hyperresponsiveness and pulmonary cell infiltration in neonatally antigen-immunized rabbits *in vivo*.

Methods

Animals

New Zealand White (NZW) rabbits (Froxford Farms, Petersfield, Hants) of either sex were used throughout the study. The immunization procedure of neonatal rabbits was that described previously (Herd *et al.*, 1992). Briefly, rabbits were injected intraperitoneally (0.5 ml) within 24 h of birth with *Alternaria tenuis* extract (1 mg ml⁻¹) in aluminium hydroxide (Al(OH)₃) moist gel. Antigen administration was repeated weekly for the first month and then biweekly for the next two months. The methods described in this study was subject to Home Office approval and performed under the Animals (Scientific Procedures) Act, 1986.

Pulmonary function measurements

Immunized rabbits (2.0–3.75 kg) were pre-medicated with diazepam (5 mg ml⁻¹, 5 mg kg⁻¹, i.p.) and were subsequently treated with Hypnorm (0.4 ml kg⁻¹, i.m.; a mixture of fentanyl citrate 0.315 mg ml⁻¹ and fluanisone 10 mg ml⁻¹), a regime which produces neuroleptanalgesia and is recommended for recovery experiments in laboratory rabbits (Flecknall, 1987). Neuroleptanalgesia was maintained throughout the course of the experiment by administration of 0.2–0.3 ml Hypnorm i.m. approximately every 30 min (Flecknall, 1987). Animals were intubated with an endotracheal tube (3.0 mm i.d., Mallinckrodt Laboratories, Athlone, Ireland), which was connected to a heated Fleisch pneumotachograph (size 00). Measurements of flow, pleural pressure, transpulmonary pressure (the difference between thoracic and pleural pressure) and tidal volume were made according to Herd *et al.* (1992). Measurements of total lung resistance (R_L) and dynamic compliance (C_{dyn}) were calculated by an online respiratory analyser (PMS Version 4.1 Mumed Ltd., London).

Experimental protocol

On Day 1, airway responsiveness to aerosolized histamine was determined as a measure of lung function, as previously described (Herd *et al.*, 1992). Cumulative dose-response curves were established and the provocation concentration (PC) of histamine which produced 50% increase in R_L (PC₅₀) and 35% decrease in C_{dyn} (PC₃₅) was determined for each rabbit. These calculated values were used as indices of airway responsiveness.

On Day 2, rabbits were injected with 0.5 ml sterile saline (control), unfractionated heparin (100 u kg⁻¹), Org 10172 (100 µg kg⁻¹) or polyglutamic acid (100 µg kg⁻¹) intravenously 30 min prior to exposure of a 2 min aerosol of 0.25% bovine serum albumin (BSA) (the carrier molecule for

PAF). PAF aerosol (80 µg ml⁻¹) was then administered over the following hour, after which time respiratory function was recorded. On Days 3 and 5, airway responsiveness to histamine was determined as on Day 1.

Control studies were performed whereby heparin (100 u kg⁻¹) and Org 10172 (100 µg kg⁻¹) were administered intravenously 30 min prior to a 1 h aerosol challenge of 0.25% BSA. In these experiments, respiratory parameters (R_L and C_{dyn}) were recorded before and 1, 15 and 30 min following the drug administration.

Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) was performed immediately following histamine challenge on Days 1, 3 and 5 as previously described (Herd *et al.*, 1992). Both total and differential cell counts were enumerated from BAL fluid.

Analysis of results

Results of the lung function studies are expressed as mean ± s.e.mean. *In vivo* histamine potency values were derived from measurements of airway resistance (R_L) (PC₅₀) and dynamic compliance (C_{dyn}) (PC₃₅) and are expressed as the geometric means together with upper and lower values for s.e.mean. For statistical purposes PC₅₀ and PC₃₅ values were log₁₀ transformed. One-way analysis of variance was used to analyse the bronchoconstriction data (R_L and C_{dyn}) (expressed as percentage change). Two-way analysis of variance for repeated measures was employed to analyse the histamine potency data prior to and at the 2 time points following PAF administration. The total cell counts and mononuclear cell, eosinophil and neutrophil counts obtained from BAL before, 24 h and 72 h after PAF challenge were analysed by Kruskal-Wallis one-way analysis of variance as the variances were found to be non-homogeneous. Tukey's HSD test was used to determine differences in means when multiple comparisons were made for the parametric data (with analysis of variance) and distribution-free multiple comparisons were used to determine differences in means when multiple comparisons were made for the non-parametric data (Kruskal-Wallis one-way analysis of variance). Results were considered significant if $P < 0.05$.

Drugs

Alternaria tenuis extract was obtained from Greer Laboratories Inc., Lenoir, N.C., U.S.A. (40,000 PNU ml⁻¹, 1 mg ml⁻¹) and aluminium hydroxide (Al(OH)₃) moist gel from FSA Laboratory supplies, Loughborough, Leicestershire. Histamine diphosphate, bovine serum albumin (low endotoxin) and polyglutamic acid (60,000–90,000 mol. wt.) were obtained from Sigma (Poole, Dorset). Platelet activating factor (PAF, C16) was obtained from Novabiochem (Nottingham). Unfractionated heparin was obtained from C.P. Pharmaceuticals. Org 10172 was a gift from Dr T. Moelker, Organon B.V., Oss, The Netherlands. Diazepam was purchased from Roche (UK) and fentanyl citrate (Hypnorm) from Janssen Pharmaceutical Ltd., UK.

Results

Airway responses

Airway responsiveness to inhaled histamine (both airway resistance R_L and dynamic compliance C_{dyn}) determined on Day 1 were not significantly different in groups of immunized rabbits that received heparin (100 u kg⁻¹), Org 10172 (100 µg kg⁻¹) or polyglutamic acid (100 µg kg⁻¹) compared with the control saline group (Tables 1A and B). The combined mean values were R_L PC₅₀: 11.15 ± 1.14 mg ml⁻¹ and C_{dyn} PC₃₅: 7.96 ± 1.19 mg ml⁻¹ ($n = 27$). There were no

significant differences in baseline absolute values of R_L or C_{dyn} between any of the study days (Table 2).

Single intravenous doses of saline, heparin (100 u kg^{-1}), Org 10172 ($100 \mu\text{g kg}^{-1}$) or polyglutamic acid ($100 \mu\text{g kg}^{-1}$) caused small changes in baseline lung function (R_L or C_{dyn}) measured 1, 15 or 30 min following administration (Table 3).

Table 1 The effect of saline, heparin (100 u kg^{-1}), Org 10172 ($100 \mu\text{g kg}^{-1}$) and polyglutamic acid ($100 \mu\text{g kg}^{-1}$) on airway responsiveness to histamine prior to, and 24 h and 72 h following PAF challenge ($80 \mu\text{g ml}^{-1}$) in immunized rabbits.

		Histamine PC_{50} (mg ml^{-1})		
		Pre	24 h	72 h
Saline	<i>n</i>	9	9	8
	mean	12.11	4.26*	3.78*
	s.e.mean	1.32	1.15	1.24
Heparin	<i>n</i>	6	6	6
	mean	9.29	15.14*†	16.83*†
	s.e.mean	1.35	1.40	1.42
Org 10172	<i>n</i>	6	6	6
	mean	10.21	16.41*†	18.11*†
	s.e.mean	1.33	1.32	1.23
Polyglutamic acid	<i>n</i>	6	6	6
	mean	12.97	4.96*	5.13*
	s.e.mean	1.14	1.14	1.21

* $P < 0.05$ compared with Pre value of same treatment group
† $P < 0.05$ compared with appropriate saline control group

B Histamine PC_{35} is the concentration of histamine (aerosol) (mg ml^{-1}) required to cause a 35% fall in dynamic compliance (C_{dyn}).

		Histamine PC_{35} (mg ml^{-1})		
		Pre	24 h	72 h
Control	<i>n</i>	9	9	8
	mean	9.23	2.99*	3.63*
	s.e.mean	1.37	1.24	1.29
Heparin	<i>n</i>	6	6	6
	mean	5.31	9.55†	9.12
	s.e.mean	1.48	1.35	1.33
Org 10172	<i>n</i>	6	6	6
	mean	5.90	10.14†	9.91†
	s.e.mean	1.53	1.32	1.39
Polyglutamic acid	<i>n</i>	6	6	6
	mean	13.06	4.64*	3.45*
	s.e.mean	1.14	1.18	1.20

* $P < 0.05$ compared with Pre value of same treatment group
† $P < 0.05$ compared with appropriate saline control group

Table 2 Absolute values for baseline lung function (airway resistance R_L) and dynamic compliance (C_{dyn}), Pre, 24 h and 72 h following PAF aerosol ($80 \mu\text{g ml}^{-1}$) in immunized rabbits pretreated with saline, heparin (100 u kg^{-1}), Org 10172 ($100 \mu\text{g kg}^{-1}$) and polyglutamic acid (PGA) ($100 \mu\text{g kg}^{-1}$)

		R_L (cmH ₂ O/(l s ⁻¹))			C_{dyn} (ml cmH ₂ O ⁻¹)		
		Pre	24 h	72 h	Pre	24 h	72 h
Total	mean	33.38	32.73	32.22	7.46	7.75	7.58
(<i>n</i> = 27)	s.e.mean	1.97	2.06	2.18	0.49	0.46	0.42
Saline	mean	26.05	28.05	31.67	7.47	7.60	7.77
(<i>n</i> = 9)	s.e.mean	2.57	2.63	5.41	0.90	0.96	0.71
Heparin	mean	33.49	36.18	33.06	7.02	7.27	6.23
(<i>n</i> = 6)	s.e.mean	3.90	2.57	3.93	0.94	0.94	0.90
Org	mean	40.04	40.34	36.33	6.59	7.54	7.40
(<i>n</i> = 6)	s.e.mean	4.27	5.79	3.61	0.50	0.74	0.41
PGA	mean	34.63	28.70	30.91	9.20	8.62	8.86
(<i>n</i> = 6)	s.e.mean	1.41	3.06	1.49	0.94	0.57	0.95

These changes were significantly less than those achieved following PAF administration (Table 4). The acute bronchoconstriction induced by PAF was not significantly different from groups of rabbits pretreated with saline, heparin (100 u kg^{-1}), Org 10172 ($100 \mu\text{g kg}^{-1}$) or polyglutamic acid ($100 \mu\text{g kg}^{-1}$) (Table 4).

Airway responses to inhaled histamine (PC_{50} or PC_{35}) were not significantly different in animals treated with heparin (100 u kg^{-1}), Org 10172 ($100 \mu\text{g kg}^{-1}$) or polyglutamic acid ($100 \mu\text{g kg}^{-1}$) 30 min prior to BSA aerosol at 24 h or 72 h following challenge compared with Pre-BSA values (Table 5).

There was a significant increase in airway responsiveness to inhaled histamine (R_L and C_{dyn}) in immunized rabbits 24 h and 72 h after PAF exposure compared to pre-PAF level in the control saline-treated group ($P < 0.05$) (Tables A and B). In rabbits pretreated with either heparin (100 u kg^{-1}) or Org 10172 ($100 \mu\text{g kg}^{-1}$), PC_{50} and PC_{35} values were significantly different from saline control values 24 h following PAF challenge (Tables 1A and B). Furthermore, in rabbits that received heparin or Org 10172, PC_{50} values 24 h and 72 h post PAF were significantly increased. Pretreatment of rabbits with polyglutamic acid ($100 \mu\text{g kg}^{-1}$) had no significant effect on PAF-induced hyperresponsiveness. R_L and C_{dyn} were significantly different at 24 h and 72 h compared with Pre-PAF values (Tables 1A and B). PC_{50} and PC_{35} values were not significantly different from the saline control group at 24 h and 72 h following PAF challenge (Tables 1A and B).

Bronchoalveolar lavage

There were no significant differences in total or differential cell counts between any of the pretreatment groups prior to PAF exposure (Table 6). Total cell counts were significantly elevated in BAL fluid 24 h and 72 h after PAF exposure in control saline-treated rabbits ($P < 0.05$). This was reflected as significant increases in the numbers of neutrophils, eosinophils and mononuclear cells at 24 h and 72 h following challenge ($P < 0.05$, Table 6).

In rabbits pretreated with heparin and Org 10172, total cell counts in BAL fluid at 24 h and 72 h after PAF exposure were significantly reduced compared with the saline-treated controls ($P < 0.05$, Table 6). Numbers of neutrophils and eosinophils were significantly less than those observed in the control group 24 h and 72 h following PAF challenge ($P < 0.05$, Table 6).

In rabbits pretreated with polyglutamic acid, numbers of total cells and eosinophils were significantly different from those in the control animals 24 h and 72 h following PAF exposure ($P < 0.05$, Table 6). The number of neutrophils recovered from polyglutamic acid-treated rabbits was significantly less than that recovered from control animals at 24 h following PAF challenge only ($P < 0.05$, Table 6).

No significant differences in mononuclear cell numbers were evident following PAF in any of the 3 drug treatment

Table 3 Percentage change in airway resistance (R_L) and dynamic compliance (C_{dyn}), 1, 15 and 30 min following i.v. administration of heparin ($100 \mu\text{g kg}^{-1}$) and Org 10172 ($100 \mu\text{g kg}^{-1}$) in immunized rabbits

		R_L ($\text{cmH}_2\text{O}/(1 \text{ s}^{-1})$)			C_{dyn} ($\text{ml cmH}_2\text{O}^{-1}$)		
		1 min	15 min	30 min	1 min	15 min	30 min
Heparin	mean	5.13	11.70	15.90	-3.73	-8.68	-7.23
($n=3$)	s.e.mean	1.27	4.65	1.35	8.07	10.28	9.16
Org	mean	2.32	0.30	4.03	-8.80	-11.00	-3.60
($n=3$)	s.e.mean	3.19	7.67	10.70	3.83	3.62	6.74

Table 4 Percentage change in airway resistance (R_L) and dynamic compliance (C_{dyn}) following PAF aerosol ($80 \mu\text{g ml}^{-1}$) in immunized rabbits pretreated with saline, heparin (100 u kg^{-1}), Org 10172 ($100 \mu\text{g kg}^{-1}$) and polyglutamic acid ($100 \mu\text{g kg}^{-1}$)

		R_L	C_{dyn}
Saline	mean	65.88	-44.84
($n=9$)	s.e.mean	6.85	5.50
Heparin	mean	38.18	-27.22
($n=6$)	s.e.mean	7.03	7.87
Org 10172	mean	40.60	-32.47
($n=6$)	s.e.mean	13.34	7.28
Polyglutamic acid	mean	56.37	-38.77
($n=6$)	s.e.mean	9.57	7.90

groups. Numbers of mononuclear cells in these groups of animals were all significantly less than numbers recovered from control rabbits at 24 h and 72 h following PAF ($P<0.05$, Table 6).

Therefore, the total cell infiltration induced by PAF was significantly inhibited by heparin, Org 10172 and polyglutamic acid treatment at 24 h and 72 h post challenge, as was the recruitment of eosinophils and mononuclear cells. The infiltration of neutrophils was similarly inhibited by all drug pretreatments at 24 h and 72 h following PAF with the exception of that observed 72 h post PAF in animals that received polyglutamic acid ($P<0.05$, Table 6). In addition, no significant differences were found to exist between the effects of heparin, Org 10172 or polyglutamic acid on PAF-induced cell infiltration.

Table 5 The effect of saline, heparin (100 u kg^{-1}) and Org 10172 ($100 \mu\text{g kg}^{-1}$) on airway responsiveness to histamine prior to, and 24 h and 72 h following BSA challenge (0.25%) in immunized rabbits.

		PC_{50} R_L (mg ml^{-1})			PC_{35} C_{dyn} (mg ml^{-1})		
		Pre	24 h	72 h	Pre	24 h	72 h
Saline	mean	10.66	11.24	12.69	5.56	5.92	4.77
	s.e.mean	1.52	1.22	1.04	1.33	1.14	1.11
	n	6	6	6	6	6	6
Heparin	mean	9.08	8.53	6.28	5.83	6.20	5.61
	s.e.mean	1.24	1.25	1.24	1.14	1.12	1.17
	n	7	7	5	7	7	5
Org	mean	7.36	7.77	8.03	7.50	7.38	7.59
	s.e.mean	1.06	1.06	1.08	1.10	1.09	1.10
	n	5	5	5	5	5	5

PC_{50} is the concentration of histamine (aerosol) (mg ml^{-1}) required to cause a 50% increase in airway resistance (R_L). PC_{35} is the concentration of histamine (aerosol) (mg ml^{-1}) required to cause a 35% fall in dynamic compliance (C_{dyn}).

Table 6 Number of total cells and different cell counts recovered from bronchoalveolar lavage (BAL) fluid before (Pre) and 24 h and 72 h following exposure of PAF ($80 \mu\text{g ml}^{-1}$) in immunized rabbits pretreated with saline, heparin (100 u kg^{-1}), Org 10172 ($100 \mu\text{g kg}^{-1}$) and polyglutamic acid (PGA) ($100 \mu\text{g kg}^{-1}$)

		$\times 10^5 \text{ cells ml}^{-1}$							
		Total	Neutrophils		Eosinophils		Mononuclear cells		
Control (saline)	Pre ($n=9$)	1.01 (0.3-2.0)	0.024 (0.002-0.09)		0.007 (0.0-0.054)		0.99 (0.30-1.98)		
	24 h ($n=9$)	5.94 (0.9-12.6)*	3.451 (0.38-8.42)*		0.141 (0.032-0.253)*		2.40 (0.49-5.88)*		
	72 h ($n=8$)	3.25 (0.9-5.8)*	1.146 (0.19-3.63)*		0.101 (0.023-0.464)*		2.01 (0.68-4.21)*		
Heparin	Pre ($n=6$)	0.94 (0.45-1.8)	0.049 (0.005-0.143)		0.003 (0.0-0.009)		0.89 (0.44-1.73)		
	24 h ($n=6$)	1.37 (0.6-2.4)*†	0.608 (0.15-0.96)*†		0.021 (0.003-0.048)*†		0.78 (0.45-1.63)†		
	72 h ($n=6$)	1.23 (0.7-2.1)†	0.557 (0.34-1.01)*†		0.015 (0.0-0.024)*†		0.66 (0.32-1.07)†		
Org 10172	Pre ($n=6$)	0.85 (0.4-1.4)	0.008 (0.004-0.018)		0.001 (0.0-0.06)		0.84 (0.39-1.39)		
	24 h ($n=6$)	1.47 (0.6-2.4)*†	0.943 (0.32-1.62)*†		0.020 (0.007-0.032)*†		0.56 (0.28-0.78)†		
	72 h ($n=6$)	1.33 (0.8-1.8)*†	0.400 (0.04-0.81)*†		0.007 (0.0-0.018)*†		0.94 (0.75-1.35)†		
PGA	Pre ($n=6$)	0.92 (0.4-2.1)	0.035 (0.006-0.06)		0.001 (0.0-0.006)		0.90 (0.34-2.07)		
	24 h ($n=6$)	1.35 (0.7-1.8)*†	0.754 (0.51-1.12)*†		0.011 (0.0-0.042)*†		0.57 (0.18-1.12)†		
	72 h ($n=6$)	1.58 (1.1-2.4)*†	0.658 (0.41-0.92)*		0.007 (0.0-0.012)*†		0.92 (0.52-1.46)†		

Results are expressed as means with range in parentheses

* $P<0.05$ compared with Pre value of same treatment group.

† $P<0.05$ compared with respective control value (saline treated group).

Discussion

Our results show that unfractionated heparin and the low-molecular weight heparinoid Org 10172 can inhibit airway hyperresponsiveness induced by aerosolized PAF in neonatally immunized adult rabbits. It has been reported that heparin can bind histamine, which possibly may explain the effect we have seen *in vivo* since we have used histamine as a marker of airway responsiveness. Nonetheless, neither heparin nor Org 10172 had any effect on the airway responsiveness to histamine following BSA challenge, suggesting that the observed inhibitory action of heparin and Org 10172 is not attributable merely to the ability of these drugs to bind histamine. Furthermore, from our results we suggest that the ability of heparin to inhibit airway hyperresponsiveness is unlikely to be related solely to its highly anionic nature since similar inhibitory effects were not observed with the linear anionic molecule polyglutamic acid. However, we cannot rule out the contribution of the negatively charged nature of heparin as it is plausible that any charge effect is dependent on the charge being presented in a conformationally constrained manner.

Heparin has been previously shown to inhibit PAF-induced airway hyperresponsiveness and eosinophil infiltration in guinea-pigs (Hanss *et al.*, 1989). While in the present study both heparin and Org 10172 inhibited the infiltration of inflammatory cells into the airways following PAF challenge, it appears that the inhibitory effect of heparin on PAF-induced airway hyperresponsiveness is not dependent on this anti-inflammatory effect as polyglutamic acid substantially inhibited cell infiltration without inhibiting the associated airway hyperresponsiveness. These results, separating the induction of bronchial hyperresponsiveness from inflammatory cell infiltration, support previous work in this model with other drug classes (Spina *et al.*, 1991; Herd *et al.*, 1992) and in other experimental (Ladenius & Biggs, 1989; Kings *et al.*, 1990; Sanjar *et al.*, 1990; Matsuse *et al.*, 1991) and clinical (Lundgren *et al.*, 1988; Gibson *et al.*, 1989) situations, showing that eosinophils may not be a prerequisite for the induction of airway hyperresponsiveness.

Others have reported that heparin can modulate allergen-induced airway hyperresponsiveness in the guinea-pig via a mechanism related to reversing the effects of the eosinophil-derived cationic protein MBP on M_2 receptor function on

airway ganglia (Fryer & Jacoby, 1991). Again it is unlikely that such a mechanism explains the inhibitory effect of heparin on PAF-induced airway hyperresponsiveness in the present study as, unlike the experiments in guinea-pigs, the inhibitory effect of heparin against airway hyperresponsiveness could not be modulated by polyglutamic acid. Furthermore, the experiments in the guinea-pig used very high doses of heparin in comparison with those reported here. Recently heparin has been reported to inhibit Type 1 hypersensitivity reactions in allergic sheep, which has been suggested to be via an inhibitory effect on mast cell degranulation (Ahmed *et al.*, 1992). However, such a mechanism is unlikely to account for the inhibitory effects of heparin on PAF-induced airway hyperresponsiveness as rabbits have very few mast cells and their Type I hypersensitivity reactions are mediated principally by basophils or 'basophiloid' cells.

Heparin has also been reported to inhibit both exercise and antigen-induced bronchoconstriction, possibly by altering inositol trisphosphate (IP_3) levels (Ahmed & Danta, 1992). In the present experiments however, these observations were not supported as heparin did not inhibit PAF-induced bronchoconstriction. These findings furthermore suggest that the inhibition of PAF-induced airway hyperresponsiveness and cell infiltration by heparin cannot be explained by heparin acting merely as a PAF antagonist. We have previously reported that PAF-induced airway hyperresponsiveness and eosinophil infiltration in the rabbit is platelet dependent, being substantially reduced in animals rendered thrombocytopenic (Coyle *et al.*, 1990). It is therefore of interest that two platelet-derived products, platelet factor 4 (PF_4) (Chihara *et al.*, 1988) and the related member of the IL-8 supergene family, RANTES (Kameyoshi *et al.*, 1992) have been recently reported to be chemoattractant for eosinophils. Heparin has long been known to inhibit the actions of the PF_4 (Block *et al.*, 1980), a cationic protein shown to be released following antigen challenge in sensitized rabbits (McManus *et al.*, 1979) and human asthmatics (Knauer *et al.*, 1981; Averill *et al.*, 1992), raising the possibility that part of the inhibitory actions of heparin on PAF-induced eosinophil infiltration is via the neutralization of PF_4 .

In summary, our results provide clear evidence that heparin and Org 10172 possess anti-inflammatory activity in the lung and the ability to inhibit PAF-induced airway hyperresponsiveness in the rabbit.

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