Inhibition of allergic late airway responses by inhaled heparin-derived oligosaccharides

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Ahmed, Tahir, Jaime Ungo, Min Zhou, and Carlos Campo. Inhibition of allergic late airway responses by inhaled heparin-derived oligosaccharides. J Appl Physiol 88: 1721–1729, 2000.—Inhaled heparin has been shown to inhibit allergic bronchoconstriction in sheep that develop only acute responses to antigen (acute responders) but was ineffective in sheep that develop both acute and late airway responses (LAR) (dual responders). Because the antiallergic activity of heparin is molecular-weight dependent, we hypothesized that heparin-derived oligosaccharides (<2,500) with potential anti-inflammatory activity may attenuate the LAR in the dual-responder sheep. Specific lung resistance was measured in 24 dual-responder sheep before and serially for 8 h after challenge with Ascaris suum antigen for demonstration of early airway response (EAR) and LAR, without and after treatment with inhaled medium-, low-, and ultralow-molecular-weight (ULMW) heparins and “non-anticoagulant” fractions (NAF) of heparin. Airway responsiveness was estimated before and 24 h postantigen as the cumulative provocative dose of carbachol that increased specific lung resistance by 400%. Only ULMW heparins caused a dose-dependent inhibition of antigen-induced EAR and LAR and postantigen airway hyperresponsiveness (AHR), whereas low- and medium-molecular-weight heparins were ineffective. The effects of ULMW heparin and ULMW NAF-heparin were comparable and inhibited the LAR and AHR even when administered “after” the antigen challenge. The ULMW NAF-heparin failed to inhibit the bronchoconstrictor response to histamine, carbachol, and leukotriene D4, excluding a direct effect on airway smooth muscle. In six sheep, segmental antigen challenge caused a marked increase in bronchoalveolar lavage histamine, which was not prevented by inhaled ULMW NAF-heparin. The results of this study in the dual-responder sheep demonstrate that 1) the antiallergic activity of inhaled “fractionated” heparins is molecular-weight dependent, 2) only ULMW heparins inhibit the antigen-induced EAR and LAR and postantigen AHR, and 3) the antiallergic activity is mediated by nonanticoagulant fractions and resides in the ULMW chains of <2,500.

HEPARIN IS A COMPLEX, HIGHLY SULFATED LINEAR POLYSACCHARIDE COMPOSED OF REPEATING 1→3 LINKED L-DURONIC ACID AND GLUCOSAMINE RESIDUES (25, 31, 33). IT IS A MIXTURE OF GLYCOSAMINOGLYCAN POLYMERS WITH AN AVERAGE MOLECULAR WEIGHT OF 12,000–15,000. THE SUGAR SEQUENCE, DEGEE OF SULFATION, AND ITS HIGH-CHARGE DENSITY CONFER ON THE HEPARIN MOLECULE ITS UNIQUE CHEMICAL PROPERTIES AS A PHARMACOLOGICAL MEDIATOR, thus allowing heparin to bind many enzymes and modulate various biological processes (14, 25, 31, 33). THE BIOLOGICAL ACTIONS OF HEPARIN RESULT FROM ITS POLYDISPERSE, HETEROGENEOUS MOLEULAR ORGANIZATION, AND ITS ABILITY TO INTERACT WITH VARIOUS PROTEINS, CAUSING THEIR ACTIVATION, DEACTIVATION, OR STABILIZATION (31, 33). SOME OF THESE INTERACTIONS, SUCH AS THE BINDING OF HEPARIN TO ANTITHROMBIN III, ARE KNOWN TO TAKE PLACE AT SPECIFIC OLIGOSACCHARIDE SEQUENCES WITHIN THE HEPARIN POLYMER (32), WHEREAS OTHER “NON-ANTICOAGULANT” INTERACTIONS ARE ALSO SUSPECTED OF BEING HIGHLY SPECIFIC (24). THE NON-ANTICOAGULANT ACTIONS OF HEPARIN INCLUDE INTERACTION WITH VARIOUS GROWTH FACTORS (10, 24, 39), MODULATION OF CELLULAR PROLIFERATION (13, 52), AND REGULATION OF ANGIogenesis (21). Heparin also modulates various proteases and enzymes (20, 47, 48, 53) and possesses anti-inflammatory, immunoregulatory, and antiallergic activities (18, 30, 38, 49, 51).

Inhaled heparin has been shown to attenuate antigen-induced acute bronchoconstriction in allergic sheep (2), as well as to prevent the bronchoconstrictor response to exercise and antigen in asthmatic subjects (6, 9, 17, 22). The bronchoconstrictor response that results from antigen challenge in vivo may manifest itself not only as an acute early airway response (EAR), but in some patient and allergic animals both as an EAR and a late airway response (LAR) (42). Previous studies have identified pathophysiological differences in the pattern of response between those that develop only EAR (acute responders) and those that develop both EAR and LAR (dual responders) (1, 12, 35, 42). The studies in allergic sheep have demonstrated that, whereas heparin inhibited the antigen-induced bronchoconstriction and airway hyperresponsiveness (AHR) in acute responders, it was ineffective in dual responders (4, 16). Many biological actions of heparin, including the antiallergic activity, are molecular-weight dependent (29, 37). In a previous study in the acute-responder sheep, an inverse relationship between molecular weight and the antiallergic activity of fractionated heparins was observed, and ultralow-molecular-weight (ULMW) heparin (<2,500) was found to be the most potent fraction (37). Although pretreatment with unfractionated and low-molecular-weight (LMW) heparins prevented the antigen-induced AHR in the acute responders, only ULMW heparin attenuated AHR when administered...
“after” the antigen challenge (40). The antiallergic activity of ULMW heparin was also independent of histamine release and indicated possible anti-inflammatory action (40). Based on these observations with ULMW heparin, we have hypothesized that 1) the antiallergic activity of fractionated heparins in the dual-responder sheep may be molecular-weight dependent, and 2) ULMW heparin fractions, with potential anti-inflammatory action, may attenuate the late-phase airway responses. Thus the purpose of this investigation was to study the molecular-weight-dependent effects of “fractionated” and non-anticoagulant heparins on antigen-induced EAR, LAR, and AHR in the dual-responder sheep.

MATERIALS AND METHODS

Animal Preparation

Twenty-four unsedated adult female sheep, with an average weight of 31 kg (27–36 kg), were restrained in the prone position, and their heads were immobilized. All sheep were allergic to Ascaris suum antigen and had previously been shown to develop late-phase bronchoconstriction after inhalation challenge with the antigen. After topical anesthesia of the nasal passages with 2% lidocaine solution, an esophageal balloon catheter was introduced through one nostril and placed in the lower esophagus. Guided by a flexible fiber-optic bronchoscope, a cuffed nasotracheal tube (inner diameter 7.5 mm) was introduced through the other nostril and placed in the lower esophagus. Guided by a flexible fiber-optic bronchoscope, a cuffed nasotracheal tube (inner diameter 7.5 mm) was introduced through the other nostril.

Experimental Techniques

Pulmonary resistance. Pleural pressure was estimated by using an esophageal balloon catheter technique (4, 5). The catheter was so positioned that inspiration produced a negative pressure deflection, and cardiogenic oscillations were clearly detectable on the pressure tracings. In this position, which was between 5 and 10 cm from the gastroesophageal junction, the end-expiratory pleural pressure ranged between −2 and −5 cmH2O in different animals. Lateral pressure in the trachea was measured with a side-hole catheter (inner diameter 2.5 mm), advanced through and positioned distal to the tip of the endotracheal tube. Transpulmonary pressure was defined as the tracheal pressure minus pleural pressure and was measured with a differential pressure transducer (type MP-45; Validyne, Northridge, CA). For measurement of pulmonary resistance (RL), the proximal end of the endotracheal tube was connected to a pneumotachograph (Fleisch no. 1, Dyna Sciences, Bluebell, PA) to obtain flow. The signals of flow and transpulmonary pressure were recorded on a multichannel recorder, which was linked to a model 80–386 DOS personal computer (CCl, Miami, FL) for on-line calculation of RL from the signals of transpulmonary pressures, flow, and volume (obtained by digital integration). Peak flow rates during tidal breathing were usually <1 l/s. The pressure transducer-catheter system was dynamically balanced, and no phase shift was detectable between pressure and flow up to a frequency of 9 Hz. The RL was averaged from at least five consecutive breaths free of swallowing artifacts. Data are expressed as specific RL (sRL), defined as RL × thoracic gas volume. For each animal, the percent change in sRL was also calculated:

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\text{sRL \% change} = \frac{\text{postchallenge sRL} - \text{baseline sRL} \times 100}{\text{baseline sRL}}
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Thoracic gas volume. This was measured by a body plethysmographic technique (4, 5). The endotracheal tube was connected to a solenoid valve that could be activated from outside the plethysmograph. Plethysmographic pressure and lateral mouth pressure measured between the proximal end of the endotracheal tube and the solenoid valve were measured with a differential gauge (model P90D, Pace Engineering) and strain gauge (Statham Instruments, Hato Rey, PR), respectively, and were displayed on an X-Y oscilloscope provided with a template. The plethysmographic pressure was calibrated manually with a 30-ml syringe at a rate similar to sheep’s spontaneous breathing frequency. After the animal was enclosed in the plethysmograph, 1–2 min were allowed for stabilization of plethysmographic pressure. The solenoid was activated at end expiration, and the slope of the first respiratory cycle against the closed airway was taken for the determination of thoracic gas volume, because subsequent efforts usually produced unsatisfactory slopes caused by the animal’s straining against the occluded airway. The mean of three measurements was recorded for each run.

Aerosol delivery system. All aerosols were generated by using a disposable medical nebulizer (Raindrop, Puritan Bennett, Lenexa, KS) that provided an aerosol with a mass median aerodynamic diameter of 3.2 µm (geometric SD 1.9 µm) as determined by an Andersen cascade impactor. The nebulizer was connected to a dosimeter system consisting of a solenoid valve and a source of compressed air (20 psi). The output of the nebulizer was directed into a plastic T piece, which was interconnected between the inspiratory port of the Harvard animal respirator and the endotracheal tube. The solenoid valve was activated for 1 s at the beginning of the inspiratory cycle of the respirator. Aerosols were delivered at a tidal volume of 500 ml and a rate of 20 breaths/min. Various heparin fractions were dissolved in 3 ml of bacteriostatic injection water and administered as an aerosol over 15–20 min.

Bronchial reactivity to carbachol. To assess baseline airway responsiveness, cumulative dose-response curves to inhaled carbachol were performed on experiment day 1 by measuring sRL before and immediately after inhalation of buffered saline and after each administration of 10 breaths of increasing concentrations of carbachol (0.25, 0.5, 2.0, 3.0, and 4.0% wt/vol solution). The bronchoprovocation was discontinued when sRL increased to 400% above the baseline. The cumulative provocative dose of carbachol (in breath units) that increased sRL to 400% above the baseline (PD400) was calculated. One breath unit was defined as one breath of a 1% carbachol solution. Baseline dose-response curves to carbachol were performed in all sheep at least 2 wk after the last exposure to antigen.

Bronchoalveolar lavage. The distal tip of a specially designed 80-cm fiber-optic bronchoscope was wedged into a randomly selected subsegmental bronchus. Lung lavage was performed by an infusion and gentle aspiration of 30-ml aliquots of PBS (pH 7.4) at 39°C by using 30-ml syringes attached to the working channel of the bronchoscope. The effluent was filtered through a single layer of gauze and placed immediately on ice. The volume of the effluent collected from the bronchoalveolar lavage (BAL) was measured and centrifuged at 420 g for 15 min at 4°C. The supernatant was decanted and centrifuged again at 1,000 g at 4°C for 15 min. The supernatant was frozen at −80°C for subsequent histamine analysis.

Histamine RIA. Duplicate aliquots from each BAL sample were used for histamine RIA by using a commercial kit from Immunotech International (AMAC, Westerbrook, ME). The sensitivity of the assay is 0.05–2.0 nM, and coefficient of
variation is <10%. There is <0.1% cross-reactivity with histidine, serotonin, or t-methyl histamine.

Purification of fractionated heparins. Various fractionated and non-anticoagulant fractions of heparins (NAF-heparins) were derived from porcine intestinal mucosa. Medium-molecular-weight (MMW) heparin (KABI-2165, mol wt 5,030) was obtained from Pharmacia (Stockholm, Sweden), whereas LMW heparin (CY216, mol wt 4,270) and ULMW heparin (CY222, mol wt 2,355) were obtained from Sanofi Pharma (Gentilly, France).

The MMW NAF-heparin (SR-80258) was prepared by periodate degradation of standard heparin (Sanofi Pharma). It has an average molecular weight of 6,500, an antithrombin and antifactor Xa activity of 36 IU/mg. Purification of NAF-heparins has been described previously (11).

Purification of NAF-heparins (KABI-2226) was prepared by Dr. L. O. Andersson (Pharmacia) by nitrous acid depolymerization of porcine heparin. It has an average molecular weight of 2,400, activated partial thromboplastin time activity of 2 I/U, and antifactor Xa activity of 36 I/U/mg. Purification of NAF-heparins has been described previously (11).

Carbachol, histamine, and leukotriene D4 (LTD4; CY222, mol wt 2,355) were obtained from Sanofi Pharma (Gentilly, France). LMW heparin (CY216, mol wt 4,270) and ULMW heparin (CY222; 0.25, 0.5, and 1 mg/kg), whereas group V (n = 7) was pretreated with ULMW NAF-heparin (KABI-2226; 0.25, 0.5, and 1 mg/kg).

Postantigen administration of ULMW heparin fractions. To evaluate the effect of "postantigen" administration of ULMW heparin fractions on LAR and AHR, the above-mentioned 3-day protocol was repeated in each sheep in groups IV and V. However, various doses of ULMW heparin (CY222; 0.25, 0.5, 1 mg/kg) or ULMW NAF-heparin (KABI-2226; 0.25, 0.5, and 1 mg/kg) were administered immediately after the postantigen measurements of sRL.

Experimental Protocol

For every protocol, each animal was studied on 3 different experiment days. On experiment day 1, baseline bronchial reactivity to carbachol was determined. On experiment day 2, the antigen challenge was performed without or after treatment with different fractionated heparins; whereas on experiment day 3 (24 h postantigen), the bronchial reactivity to carbachol was redetermined.

Antigen-induced bronchoconstriction. For the control experiments, baseline bronchial reactivity to carbachol was determined on experiment day 1. On experiment day 2, after the baseline measurements of sRL, each animal was challenged with aerosolized Ascaris suum antigen. Repeat measurements of sRL were obtained within 5 min after the antigen challenge and serially for up to 8 h for demonstration of EAR and LAR. Twenty-four hours after the antigen challenge, bronchial reactivity to carbachol was redetermined as an index of postantigen AHR.

Effect of LMW and MMW heparin fractions. Each sheep served as its own control, and the above-mentioned 3-day protocol was repeated at least 2 wk apart. In each sheep, a carbachol dose-response curve was performed on experiment day 1 to determine the baseline PD400 of carbachol. On experiment day 2, the measurements of sRL were obtained before and 30 min after pretreatment with fractionated MMW or LMW heparins, and the sheep were then challenged with Ascaris suum antigen. Repeat measurements of sRL were obtained within 5 min after the antigen challenge and serially for up to 8 h for demonstration of EAR and LAR. Twenty-four hours after the antigen challenge, bronchial reactivity to carbachol was redetermined as an index of postantigen AHR.

Effect of LMW and MMW Heparins

LMW heparin (CY-216, n = 3) failed to inhibit the antigen-induced EAR and LAR. Peak early and peak late sRL increased by 255 ± 106 (SE) and 160 ± 20%, respectively, with antigen alone, which was not significantly different from the peak increases of 210 ± 80 and 140 ± 13%, respectively, observed after pretreatment with CY-216 (Fig. 1).

MMW fractions of heparin also failed to inhibit the antigen-induced EAR and LAR. Peak early and peak late sRL increased by 224 ± 51 and 156 ± 21%, respectively, after pretreatment with MMW heparin (KABI-2165, n = 3), which was not different from 225 ± 64 and 148 ± 26%, respectively, increases in sRL observed with antigen alone (Fig. 1). Similarly, MMW NAF-heparin (SR-80258, n = 3) had no effect on antigen-induced peak early [change (Δ)] in sRL = 262 ±
20 vs. 231 ± 29%) or peak late responses (ΔsRl = 204 ± 24 vs. 184 ± 21%) (Fig. 1). LMW heparin, MMW heparin, and MMW NAF-heparin failed to modify the antigen-induced AHR. PD400 decreased from 22 ± 4 to 12 ± 3 breath units 24 h postantigen, indicating antigen-induced AHR. This was not prevented by treatment with LMW heparin (13 ± 3 breath units), MMW heparin (10 ± 2 breath units), or MMW NAF-heparin (9 ± 2 breath units).

Effect of ULMW Heparin

ULMW heparin (CY222, n = 8) inhibited the antigen-induced EAR, LAR, and AHR in a dose-dependent manner. Peak early and peak late sRl increased by 266 ± 40 and 160 ± 11%, respectively, with antigen alone. Pretreatment with CY222 inhibited the EAR by 2.3, 25, and 32% at doses of 0.25, 0.5, and 1 mg/kg, whereas LAR was inhibited by 7.5, 56, and 57%, respectively (Fig. 2). CY222 also inhibited the antigen-induced AHR in a dose-dependent fashion. PD400 decreased from 22.5 ± 2.5 to 10.4 ± 1.5 breath units 24 h postantigen; this was inhibited by 25, 98, and 77% at 0.25, 0.5, and 1 mg/kg doses of CY222, respectively (Fig. 3).

Effect of ULMW NAF-Heparin

ULMW NAF-heparin (KABI-2226, n = 7) also inhibited the antigen-induced EAR, LAR, and AHR. Peak early and peak late sRl increased by 326 ± 35 and 183 ± 27%, respectively, with antigen alone. Pretreatment with KABI-2226 inhibited the EAR by 0.4, 5.4, and 50% at doses of 0.25, 0.5, and 1 mg/kg, whereas LAR was inhibited by 42, 82, and 57%, respectively (Fig. 4). KABI-2226 also inhibited the postantigen AHR in a dose-dependent manner. The PD400 decreased from 23 ± 2 to 11 ± 2 breath units 24 h postantigen; this was inhibited by 0.3, 125, and 70% at 0.25, 0.5, and 1 mg/kg doses of KABI-2226, respectively (Fig. 5).

Postantigen Administration of ULMW Heparin

Administration of ULMW heparin (CY222) and ULMW NAF-heparin (KABI-2226) after the antigen challenge also inhibited the LAR and antigen-induced AHR. Postantigen administration of CY222 (n = 6) at a dose of 1 mg/kg inhibited the LAR by 82% (Fig. 6), whereas AHR was inhibited by 71% (Fig. 7). Postantigen administration of KABI-2226 (n = 7) at a dose of 1 mg/kg inhibited the LAR by 78% (Fig. 6), whereas AHR was inhibited by 106% (Fig. 7).

Administration of LMW heparin (CY216) and MMW NAF-heparin (SR-80258) after the antigen challenge had no effect on LAR or AHR (data not shown).

Agonist-induced Bronchoconstriction

Inhaled ULMW NAF-heparin (KABI-2226) failed to modify the bronchoconstrictor responses to histamine (n = 3), carbachol (n = 3), and LTD4 (n = 3). The ΔsRl values with vs. without pretreatment with KABI-2226 were 353 ± 6 vs. 378 ± 4 (P = not significant (NS)), 338 ± 46 vs. 333 ± 21 (P = NS), and 210 ± 35% vs. 186 ±
30% (P = NS) for histamine, carbachol, and LTD₄, respectively.

Histamine Release in BAL (n = 6)

Baseline concentrations of histamine ranged between 0.41 ± 0.2 and 1.18 ± 0.2 nM/ml on 2 experiment days. Segmental antigen challenge caused a marked increase in BAL histamine (8.54 ± 7.1 nM/ml) that was not inhibited by pretreatment with KABI-2226 (9.34 ± 6.3 nM/ml).

DISCUSSION

We have previously shown that inhaled heparin selectively inhibited the airway effects of antigen only in the acute-responder sheep, whereas in dual responders heparin was ineffective (4, 16). The results of this study in the dual-responder sheep demonstrate that 1) the antiallergic activity of inhaled fractionated heparins is molecular-weight dependent, 2) only ULMW heparins attenuate EAR and LAR without inhibiting antigen-induced histamine release in BAL, and 3) the antiallergic activity is mediated by NAF and resides in the ULMW chains of ~2,500.

It has recently been shown that the antiallergic activity of fractionated heparins in the acute-responder sheep is molecular-weight dependent (37). The ULMW heparin was the most potent fraction and inhibited the antigen-induced bronchoconstrictor response and AHR in a dose-dependent fashion (37). Although pretreatment with unfractionated heparin and LMW heparins prevented the antigen-induced AHR in the acute responders, only ULMW heparin inhibited EAR and LAR in a dose-dependent manner. *Significantly different from antigen control (P < 0.05).

Fig. 3. Effect of pretreatment with aerosolized ULMW heparin on antigen-induced airway hyperresponsiveness (AHR). Data are means ± SE of cumulative provocating dose of carbachol (in breath units) that increased sRL by 400% (PD₄₀₀₀). Antigen challenge caused a marked increase in AHR 24 h postantigen, which was inhibited by ULMW heparin in a dose-dependent manner. Significantly different (P < 0.05) from *baseline and + postantigen.

Fig. 4. Effect of pretreatment with aerosolized ULMW NAF-heparin on antigen-induced early- and late-phase bronchoconstrictor responses (EAR and LAR). Data are means ± SE of %increase in sRL from BSL without (○) and after pretreatment with ULMW NAF-heparin (©). ULMW NAF-heparin inhibited EAR and LAR in a dose-dependent manner. *Significantly different from antigen control (P < 0.05).

Fig. 4. Effect of pretreatment with aerosolized ULMW NAF-heparin on antigen-induced early- and late-phase bronchoconstrictor responses (EAR and LAR). Data are means ± SE of %increase in sRL from BSL without (○) and after pretreatment with ULMW NAF-heparin (©). ULMW NAF-heparin inhibited EAR and LAR in a dose-dependent manner. *Significantly different from antigen control (P < 0.05).
The results of the present study further extend our previous observations and demonstrate that the antiallergic activity of fractionated heparins in the dual-responder sheep is critically dependent on molecular weight. Only ULMW heparins (<2,500) attenuated the antigen-induced EAR, LAR, and AHR, whereas unfractionated heparin (4, 16), MMW heparin, and LMW heparin were ineffective. The antiallergic activity of ULMW heparins was dose dependent; whereas 0.25 mg/kg inhaled dose was ineffective, 0.5 and 1 mg/kg doses caused marked inhibition of EAR, LAR, and AHR. The ULMW heparins were equally effective in inhibiting LAR and postantigen AHR, whether administered before or after the antigen challenge. In this regard, the activity of inhaled ULMW heparins is comparable to glucocorticosteroids, which can inhibit LAR and AHR whether administered before or after the antigen challenge (15, 44), and considerably different from cromolyn sodium, which is only effective when administered before the antigen challenge (15, 44).

Inhaled unfractionated heparin has been shown to attenuate exercise- and antigen-induced acute bronchoconstrictor response in human subjects (6, 9, 17, 22); however, its effects on LAR are not well characterized. O'Donnel et al. (43) failed to show any inhibitory effect of heparin on LAR. In contrast, Diamant et al. (17) observed that inhaled heparin had no effect on EAR, whereas LAR was inhibited by 27% as assessed by area under the curve. However, Diamant et al. used very high and multiple doses of heparin (total dose = 400,000 units), and a nonspecific effect of such a high dose could not be excluded. Furthermore, no protective effect on postantigen AHR to histamine was observed.

Previous studies in human subjects and sheep have suggested that the antiallergic activity of inhaled heparin is independent of its anticoagulant properties, as activated partial thromboplastin time activity was not prolonged (2, 6, 9, 17, 22). Recent studies have confirmed this hypothesis and showed inhibition of allergic airway responses in the acute-responder sheep by NAF-heparins (3, 11). In the acute-responder sheep, all NAF-heparins inhibited allergic airway responses in a dose-dependent fashion, and ULMW NAF-heparin was the most potent fraction (11). In contrast, the present study in the dual-responder sheep shows that only ULMW NAF-heparin inhibited the EAR, LAR, and AHR, whereas HMW and MMW NAF-heparins were ineffective. The dose-response data showed that the antiallergic activity of ULMW NAF-heparin (KABI-2226) was comparable to that of ULMW heparin (CY222) and showed comparable potency on LAR and
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Fig. 7. Effects of ULMW heparin (A) and ULMW NAF-heparin (B) administered after antigen challenge on antigen-induced AHR. Data are means ± SE of PD400. Antigen challenge caused a marked increase in AHR 24 h postantigen, which was prevented by ULMW heparin and ULMW NAF-heparin administered after antigen challenge. Significantly different (P < 0.05) from *baseline and + postantigen.

AHR, whether administered before or after the antigen challenge.

Our previous findings in the acute-responder sheep have demonstrated that the inhibitory effects of unfractionated heparin and fractionated heparins on antigen-induced histamine release in BAL are also molecular-weight dependent (11, 40). In the acute-responder sheep, unfractionated heparin and LMW heparin not only prevented the allergic bronchoconstriction and AHR, but also inhibited the antigen-induced histamine release in BAL (40). In contrast, ULMW heparin and ULMW NAF-heparin prevented the allergic bronchoconstriction and AHR without inhibiting the histamine release in BAL (11, 40). The results of the present study are consistent with the previous observations and demonstrate that ULMW NAF-heparin also failed to inhibit the antigen-induced histamine release in BAL of dual responders.

The mechanism of the antiallergic activity of ULMW heparins is not known at present. It failed to modify the bronchoconstrictor response induced by the smooth-muscle agonists, including histamine, carbachol, and LTD_4, thus excluding a direct effect on airway smooth muscle. It has been proposed that the antiallergic activity of unfractionated heparin in the acute-responder sheep may be mediated by inhibition of inositol 1,4,5-trisphosphate (IP_3)-dependent mast cell mediator release (7, 19). The inhibition of IP_3 binding to its receptors by heparin is molecular-weight dependent, and the inhibitory activity decreases as the size of the heparin chain is reduced below 18 monosaccharide units (50). Whereas 10- to 14-monosaccharide fractions had substantially lower activity, the 8-monosaccharide fractions (mol wt < 2,500) had none. Failure of ULMW NAF-heparin to inhibit antigen-induced histamine release in BAL fluid is consistent with these observations. Thus it is unlikely that the antiallergic activity of ULMW heparins is related to inhibition of IP_3-dependent mast-cell mediator release. The attenuation of allergic airway responses and AHR, in both the acute- and the dual-responder sheep, without inhibition of histamine release, suggests that the in vivo activity of ULMW heparin may be mediated by an unknown anti-inflammatory mechanism.

The glycosaminoglycan heparins have been shown to possess anti-inflammatory properties including anti-complement action (51), modulation of T lymphocytes (30), inhibition of neutrophil chemotaxis and free radical generation (27, 38), as well as eosinophil influx (49). It has been reported in the guinea pig and rabbit that unfractionated heparin and a LMW heparinoid (ORG-10172) can prevent antigen- and platelet-activating-factor-induced eosinophil influx (49). Recent evidence shows that heparin strongly binds various cytokines, including tumor necrosis factor, interleukin (IL)-2, IL-4, IL-5, and IL-8 (26, 28, 34, 45), and decreases the blood clearance of interferon-γ (36). It has also been suggested that unfractionated heparin and heparin oligosaccharides are effective L- and P-selectin inhibitors and demonstrate anti-inflammatory activity in vivo (41). Although molecular-weight dependence of the anti-inflammatory properties of heparin has not been well studied, our data suggest that selective inhibition by ULMW heparins of EAR, LAR, and AHR in the dual-responder sheep may be related to its potent anti-inflammatory activities.

Molecular-weight dependency of the biological actions of heparin has been observed previously (14, 29, 37, 50). It is well established that both the degree of sulfation and molecular chain length influence the anticoagulant activity of heparin (14, 29). Compared with unfractionated heparin, LMW heparins have a reduced binding and uptake by Kupffer cells and endothelial cells, which may partly account for their prolonged anticoagulant activity (8, 23). Redini et al. (46) also showed that the ability of heparin-derived oligosaccharides to inhibit leukocyte elastase was inversely related to the molecular chain length. The various biological actions of heparin show either reduced or increased activity with lower molecular weight but were observed in the parent unfractionated heparin. The results of the present study are novel and demonstrate unmasking of a biological action by ULMW heparins (i.e., inhibition of LAR), which was not observed in the unfractionated heparin or heparin fractions with a molecular weight of >2,500. The ULMW heparins have the highest glycosaminoglycan content, the lowest molecular weight, and the highest percentage of chain length < 2,500. It is possible that the anti-allergic and anti-inflammatory activities of heparin-derived oligosaccharides reside in the chain length of < 2,500.
REFERENCES


