

Original article

The effect of IVX-0142, a heparin-derived hypersulfated disaccharide, on the allergic airway responses in asthma

Background: IVX-0142 is a heparin-derived hypersulfated disaccharide devoid of anticoagulant activity while possessing anti-allergic and anti-inflammatory activity in preclinical studies. In a proof-of-concept study, the allergen inhalation challenge model was used to investigate the effect of IVX-0142 in mild atopic asthma.

Methods: Nineteen subjects, not on controller medications, were randomized to an evaluator-blind, placebo-controlled, cross-over study. The effect of a single nebulized dose of IVX-0142 (80 mg) or placebo administered 30 min prior to allergen inhalation was evaluated on the allergic airway responses, airway responsiveness, and airway inflammation.

Results: When compared with placebo, 14 and 13 subjects experienced a relatively smaller maximum fall in forced expiratory volume in 1 s (maxFEV₁%) for the early airway response (EAR) and late airway response (LAR) with IVX-0142, respectively ($P < 0.01$). The degree of attenuation in the EAR [maxFEV₁% (mean $\pm$ SE) $26.5 \pm 2.8\%$ vs placebo $31.0 \pm 2.8\%$, $P = 0.059$] and LAR ($15.6 \pm 2.9\%$ vs placebo $19.0 \pm 2.9\%$, $P = 0.24$) with IVX-0142, however, was small and did not reach statistical significance compared with placebo. Similarly, a trend in the attenuation of allergen-induced increase in the absolute sputum cell counts was also observed. No difference in the allergen-induced increase in airway hyper-responsiveness and exhaled nitric oxide was noticed.

Conclusions: The majority of mild atopic asthmatics demonstrated a reduction in the EAR and LAR to IVX-0142. However, the treatment effect observed with a single prechallenge dose of IVX-0142 was small and heterogeneous. The potential anti-allergic and anti-inflammatory effects using multiple higher doses need to be evaluated.

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Bronchial asthma is an inflammatory disease of the airways and is characterized by increased airway hyper-responsiveness (AHR) resulting in episodic bronchospasms. The goal of effective asthma therapy is to control

airway inflammation and AHR. Of the current therapeutic agents available, only corticosteroids possess potent anti-inflammatory activity and effectively inhibit AHR. However, long-term use of these drugs is associated with potential side-effects, urging further searches for alternative safer and effective anti-inflammatory agents.

Inhaled heparin and its derivatives have been shown to possess anti-asthmatic properties in clinical models, including the allergen (1, 2), exercise (3), adenosine (4) and distilled water challenge models (5). The protective effect of inhaled heparin resides in the nonanticoagulant fractions as this has been shown to significantly attenuate antigen-induced bronchoconstriction in the allergic sheep model (6, 7). Furthermore a dose-response relationship has been demonstrated between the molecular weight

Abbreviations: AHR, airway hyper-responsiveness; ANCOVA, analysis of covariance; ATS, American Thoracic Society; AUC, area under the curve; 95% CI, 95% confidence interval; DD, doubling dose; EAR, early airway response, maximum % FEV₁ fall $\geq 20\%$ between 0 and 3-h postallergen inhalation; eNO, exhaled nitric oxide level; FEV₁, forced expiratory volume in 1 s; LAR, late airway response, maximum % FEV₁ fall $\geq 15\%$ between 3 and 7-h postallergen inhalation; maxFEV₁% fall, maximum % fall in FEV₁ from baseline; MCC, metachromatic cells; MW, molecular weight; PC₂₀, provocative concentration evoking a 20% fall in FEV₁ from baseline; SE, standard error of the mean; TCC, total cell count.

(MW) of these heparin derivatives and their ability to attenuate allergen-induced bronchoconstriction (7). IVX-0142 is a nonanticoagulant, porcine heparin-derived hexa-sulfated disaccharide with a molecular mass of 974.51 g/mol. This novel oligosaccharide has been shown to possess anti-allergic and anti-inflammatory activity in a preclinical allergic sheep model. In this model, a single nebulized dose of IVX-0142, 20 mg (0.65 mg/kg) administered prior to antigen challenge, was shown to significantly attenuate the early airway response (EAR) and late airway response (LAR) and postantigen AHR (8). Moreover, in a phase I study with 32 human healthy volunteers, IVX-0142 was shown to be safe and well-tolerated at nebulized doses up to 80 mg without any evidence of anticoagulation. In the present proof-of-concept study, the effect of inhaled IVX-0142 on allergen inhalation challenge in patients with mild atopic asthma was evaluated. The aim was to examine the effect of a single prechallenge dose of 80 mg on allergen-induced EAR, LAR, AHR, and airway inflammation.

Methods

Mild atopic asthmatics of age 18 years and older with a forced expiratory volume in 1 s (FEV_1) $\geq 70\%$ predicted were screened. Inclusion criteria included nonsmokers with stable asthma (for 6 weeks prior to screening), taking only salbutamol as needed, and who demonstrate a methacholine provocative concentration inducing a 20% fall in FEV_1 (PC_{20}) of < 16 mg/ml. Subjects also had to demonstrate an EAR and LAR to screening allergen inhalation challenge. The same dose of allergen for each subject was used for subsequent challenges. The study was a three-center, randomized, double-dummy, placebo-controlled, two-period crossover study. Each period consisted of three consecutive visits separated by 3–6 weeks washout to allow methacholine- PC_{20} to return within one doubling dose (DD) of screening baseline PC_{20} prior to crossing over. Exhaled nitric oxide (eNO), methacholine test, and sputum induction was performed on days 1 and 3. Allergen was inhaled on day 2 with eNO and sputum induction repeated at 7-h postinhalation. A single dose of nebulized IVX-0142 (80 mg in 4 ml of sterile water) or placebo (4 ml sterile water) was administered 30 min before allergen inhalation. The protocol was approved by the ethics committee of each participating center and informed written consent was obtained from all participants.

Spirometry was performed in accordance with American Thoracic Society (ATS) standards (9) and reference values were taken from Crapo et al. (10). The methacholine test was performed with doubling concentrations of methacholine inhaled at tidal breathing for 2 min (11). The PC_{20} was calculated from the dose–response curve by linear interpolation on a log scale. On-line single-flow eNO was measured at a flow rate of 0.05 l/s as recommended by ATS (12).

Sputum induction and processing was performed as previously described (13). Total cell count (TCC) was determined using a Neubauer haemocytometer chamber (Hausser Scientific, PA, USA). Differential cells were manually counted out of 400 nonsquamous cells and metachromatic cells (MCC) were counted out of 1500 cells.

A modified skin-prick test was used to identify the administered allergen (14) and the concentration that caused a 2 mm wheal. This concentration and the screening methacholine- PC_{20} were used to calculate the PC_{20} -allergen (15, 16). The starting dose of allergen was

three DD below the estimated PC_{20} -allergen and was delivered by a Wright nebulizer (output 0.13 ml/min; Roxon MediTech, Montreal, Canada) for 2 min at tidal breathing. This was followed by FEV_1 measurements at 10-min intervals until the lowest FEV_1 was established. If % FEV_1 fall was $< 20\%$, the next concentration was given, until FEV_1 fell $\geq 20\%$. Then, FEV_1 was measured at 20, 30, 45, 60, 90, 120 min and then hourly until 7-h postallergen inhalation. The EAR was the maximum % FEV_1 (max $FEV_1\%$) fall $\geq 20\%$ from preallergen FEV_1 value between 0 and 3 h, and LAR was the max $FEV_1\%$ fall $\geq 15\%$ between 3 and 7 h postallergen. The area under the curve (AUC) was calculated using the trapezoidal rule and expressed as %change $\times$ h (%*h).

Monitoring for adverse events was performed by questioning during each visit; measuring vital signs during and after drug administration (up to 30 min); and measuring serum coagulation factors before and after each treatment dose.

Analysis

Data are presented as mean $\pm$ SE and changes from baseline are provided with 95% confidence intervals (95% CI). Only subjects who completed both study treatments and attended all visits (per protocol) were included in the analysis ($n = 18$). Sputum cell percentages and eNO were log transformed and the methacholine- PC_{20} was expressed as DD change [$\log_2 PC_{20}(\text{preallergen}) - \log_2 PC_{20}(\text{postallergen})$] from baseline. Changes from baseline were compared within and between treatments using analysis of covariance (ANCOVA) with factors: treatment period, treatment, center; baseline as covariate and subject as random factor. A P -value (2-sided) of ≤ 0.05 was accepted as significant.

Results

Nineteen subjects were randomized and their baseline characteristics at screening visit are shown in Table 1. One subject (subject 3) did not complete the two study periods because of personal reasons and was excluded from the per protocol analysis. Baseline values for the two treatment periods were not significantly different (Table 2). Furthermore, IVX-0142 did not cause any significant changes to baseline FEV_1 following dosing. When compared with placebo, 14 and 13 subjects experienced relatively smaller EAR and LAR with IVX-0142, respectively (Fig. 1). Mean serial % FEV_1 fall following the two allergen challenges up to 7 h are shown in Fig. 2. Treatment with IVX-0142 showed a trend toward attenuation of the EAR (max $FEV_1\%$ fall $26.5 \pm 2.8\%$ vs placebo $31.0 \pm 2.8\%$, $P = 0.059$), but not for the EAR-AUC ($-25.3 \pm 3.3\%*h$ vs placebo $-28.4 \pm 3.3\%*h$, $P = 0.21$). The LAR ($15.6 \pm 2.9\%$ vs placebo $19.0 \pm 2.9\%$, $P = 0.24$) and LAR-AUC ($-38.3 \pm 7.1\%*h$ vs placebo $-50.9 \pm 7.1\%*h$, $P = 0.14$) were not significantly different between IVX-0142 and placebo. However, with one extreme outlier removed (subject 6), the treatment effect of IVX-0142 on the EAR ($26.4 \pm 3.0\%$ vs placebo $31.4 \pm 3.0\%$, $P = 0.049$) and the LAR-AUC ($-34.8 \pm 6.8\%*h$ vs placebo $-53.3 \pm 6.8\%*h$, $P < 0.01$) reached statistical significance.

Table 1. Subject characteristics

	Center	Age (year)	Sex	FEV ₁ (% pred.)	Meth-PC ₂₀ (mg/ml)	Inhaled allergen	Allergen dilution
1	1	43	F	111	3.16	Cat	1 : 32
2	1	35	F	82	0.84	Cat	1 : 1024
3	1	21	F	99	0.75	HDM	1 : 128
4	1	28	F	99	0.81	Cat	1 : 8
5	1	29	F	100	15.25	Cat	1 : 16
6	1	29	M	98	5.01	HDM	1 : 64
7	1	29	F	85	1.44	Cat	1 : 256
8	1	26	F	110	2.27	Cat	1 : 32
9	2	52	F	81	5.56	HDM	1 : 16
10	2	24	F	86	14.78	HDM	1 : 8
11	2	24	M	101	7.32	Cat	1 : 64
12	2	23	F	95	0.97	HDM	1 : 128
13	2	39	F	83	0.38	Cat	128
14	2	24	F	116	9.19	HDM	1 : 64
15	2	29	F	97	4.59	HDM	1 : 12 800
16	2	19	F	86	2.11	HDM	1 : 16 000
17	3	44	M	90	3.55	Grass	1 : 64
18	3	23	F	106	9.06	HDM	1 : 128
19	3	23	M	77	3.56	Grass	1 : 128

Abbreviations: F= female; M=male; FEV₁=forced expiratory volume in 1 second; PC₂₀= methacholine concentration causing 20% fall in FEV₁; HDM= house dust mite.

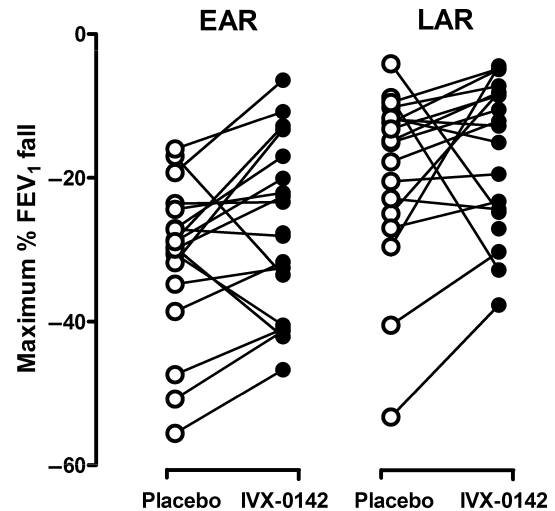
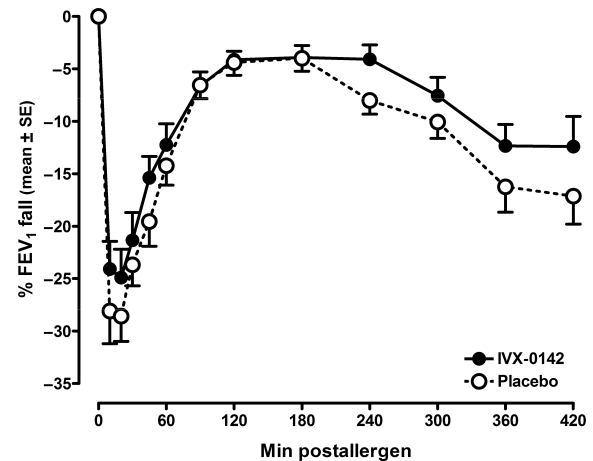
Table 2. Baseline variables for the two treatment periods

	Placebo	IVX-0142
FEV ₁ (l)	3.29 ± 0.16	3.30 ± 0.16
FEV ₁ postdosing (l)	3.33 ± 0.16	3.37 ± 0.15
Meth-PC ₂₀ (mg/ml)	4.35 ± 0.98	3.86 ± 0.83
eNO (ppb)	43.0 ± 6.0	48.8 ± 7.7
TCC (10 ⁶ cells/ml)	2.04 ± 0.39	2.05 ± 0.33
Eosinophils (10 ⁴ cells/ml)	4.06 ± 1.31	5.12 ± 1.41
Neutrophils (10 ⁴ cells/ml)	73.59 ± 17.14	87.64 ± 26.44
MCC (10 ⁴ cells/ml)	0.03 ± 0.01	0.08 ± 0.03
Eosinophils (%)	3.72 ± 1.47	3.14 ± 1.07
Neutrophils (%)	36.04 ± 5.62	41.88 ± 6.30
MCC (%)	0.02 ± 0.01	0.04 ± 0.02

Abbreviations: eNO = exhaled nitric oxide, TCC = total cell count; MCC = meta-chromatic cells.

Allergen-induced changes in methacholine-PC₂₀, although significant when compared with baseline, were not significantly different between IVX-0142 and placebo (0.7 ± 0.3 DD vs placebo 1.0 ± 0.3 DD, $P = 0.2$) (Fig. 3).

Allergen inhalation induced a significant rise in eNO, sputum eosinophils%, neutrophils% and MCC% at 7 and 24 h which were not significantly different and were similar in magnitude between the two treatment arms (Table 3). In contrast, allergen inhalation resulted only in a significant rise in the absolute TCC, eosinophil, and neutrophil counts with placebo but not with IVX-0142 at 7 h (Fig. 4). However, the difference in the observed changes from baseline was not significant between the two treatments (Table 3). At 24-h postallergen inhalation, the changes from baseline were similar for both


Figure 1. Maximum % FEV₁ fall during EAR and LAR following allergen inhalation for individual subjects ($n = 18$).

Figure 2. Mean fall in FEV₁ following allergen challenge with placebo and IVX-0142 pretreatment ($n = 18$).

treatments with significant elevations of TCC and eosinophil absolute count but not for neutrophil count.

Overall nebulized IVX-0142 was well-tolerated with only one subject complaining that he was suffering from a mild headache associated with drug administration which was self-limited. No major adverse events were reported and no significant change in the measured coagulation factors was detected with IVX-0142 treatment.

Discussion

In the majority of atopic asthmatic dual responders, we found pretreatment with a single dose of nebulized IVX-0142 (80 mg), a heparin-derived hypersulfated disaccharide, reduced the EAR and LAR compared with placebo. However, there was heterogeneity in the treat-

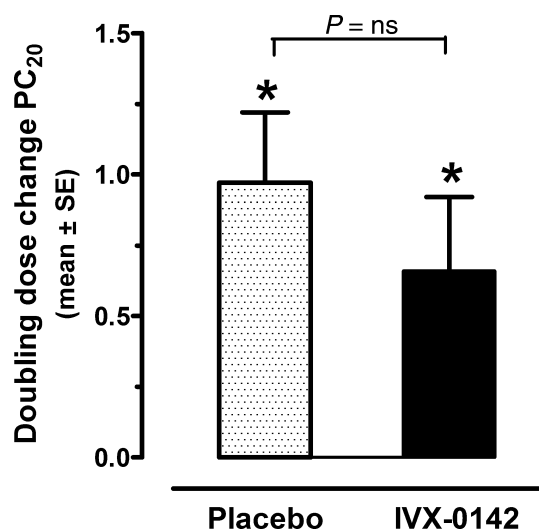


Figure 3. Doubling dose change in methacholine- PC₂₀ 24-h postallergen. The change from preallergen value was significant at $P < 0.05$ (*) but not significant (ns) between treatments ($n = 18$).

ment response and the overall magnitude of the effect was modest and did not reach statistical significance. There was also a trend to reduction in the allergen-induced increase in the number of granulocytic cell influx into the airways with IVX-0142. Together, these findings suggest that this novel agent has potential anti-inflammatory activity that may be useful in the treatment of allergic asthma.

Heparin is a complex highly sulfated polysaccharide mixture of large glycosaminoglycan polymers with an average MW of 12 000–15 000 Da. As a result of the sugar sequence, degree of sulfation, and high charge density, heparin has the ability to interact with a number

of proteins and modulate various biological processes. In the physiological state, heparin is synthesized exclusively in mast cells of the lung, intestine, and liver, and is thought to have a role in host defense (17). Clinically, heparin is important for its potent anticoagulant property, which resides in a specific pentasaccharide sequence allowing it to bind to antithrombin III. Heparin is also known to possess nonanticoagulant activities because of various distinctive oligosaccharide sequences, allowing it to bind to growth factors, cellular mediators, and adhesion molecules (18). In asthmatic patients, pretreatment with inhaled heparin has been shown to reduce the acute bronchoconstriction response to exercise (3), inhaled allergen (1, 2), adenosine (4), and water challenges (5). The prevailing hypothesis for this effect is the ability of heparin to inhibit inositol 1,4,5-triphosphate-mediated intracellular calcium release and mast cell degranulation (19). This anti-allergic effect on the acute bronchoconstrictor response has been shown to be inversely related to the MW of heparin and its derivatives (6, 7, 20, 21). Moreover, in the allergic sheep model, treatment with only ultra-low MW nonanticoagulant fractions of heparin (<2500 Da) demonstrated significant attenuation of the EAR, LAR, and AHR in both acute and dual responders when given before or after antigen challenge, suggesting that these oligosaccharides also possess anti-inflammatory activity (7, 22, 23). In contrast, the higher MW heparins are able to only attenuate the EAR in acute responders, an effect that was associated with a reduction in allergen-induced increase in histamine levels in bronchoalveolar fluid (7, 23), whereas ultra-low MW heparins failed to inhibit the rise in histamine levels, suggesting their mode of action was independent of mast cell degranulation (22). The anti-allergic and anti-inflammatory activity of the ultra-low MW heparins has since been shown to reside in a

Table 3. Mean changes (95% CI) from baseline at 7- and 24-h postallergen inhalation with placebo and IVX-0142 for eNO and sputum variables

	Time (h)	Placebo	n	IVX-0142	n	Treatment difference*
eNO† (ppb)	7	42.0 (35.8, 49.3)	18	44.5 (38, 52.2)	18	$P = 0.50$
	24	69.9 (59.6, 81.9)	18	64.6 (55.1, 75.8)	18	$P = 0.36$
TCC (10^6 cells/ml)	7	2.0 (0.9, 3.1)	17	0.6 (−0.5, 1.7)	16	$P = 0.059$
	24	1.2 (0.09, 2.3)	16	1.5 (0.4, 2.6)	16	$P = 0.63$
Eosinophils (10^4 cells/ml)	7	45.8 (16.2, 75.5)	16	20.8 (−9.5, 51.1)	15	$P = 0.08$
	24	50.4 (20.3, 80.5)	15	37.0 (6.2, 67.8)	14	$P = 0.36$
Neutrophils (10^4 cells/ml)	7	138 (56.6, 219.4)	16	44 (−40, 128.1)	15	$P = 0.10$
	24	39.1 (−44.8, 123)	15	53 (−33.9, 139.9)	14	$P = 0.81$
MCC (10^4 cells/ml)	7	0.14 (−0.4, 0.7)	14	0.45 (−0.1, 1.0)	14	$P = 0.40$
	24	0.20 (−0.3, 0.7)	14	0.65 (0.1, 1.2)	14	$P = 0.23$
Eosinophils† (%)	7	7.8 (4.3, 14.2)	16	6.2 (3.3, 11.7)	15	$P = 0.44$
	24	8.1 (4.4, 14.9)	15	6.2 (3.2, 11.6)	14	$P = 0.37$
Neutrophils† (%)	7	37.3 (29.1, 47.8)	16	33.6 (26, 43.4)	15	$P = 0.54$
	24	29 (22.5, 37.5)	15	24.8 (19, 32.3)	14	$P = 0.38$
MCC† (%)	7	0.07 (0.02, 0.2)	13	0.08 (0.03, 0.2)	14	$P = 0.80$
	24	0.05 (0.02, 0.2)	14	0.09 (0.03, 0.3)	14	$P = 0.46$

*Difference in the change from baseline at 7 and 24 h between IVX-0142 vs placebo.

†Data presented in geometric mean (95%CI).

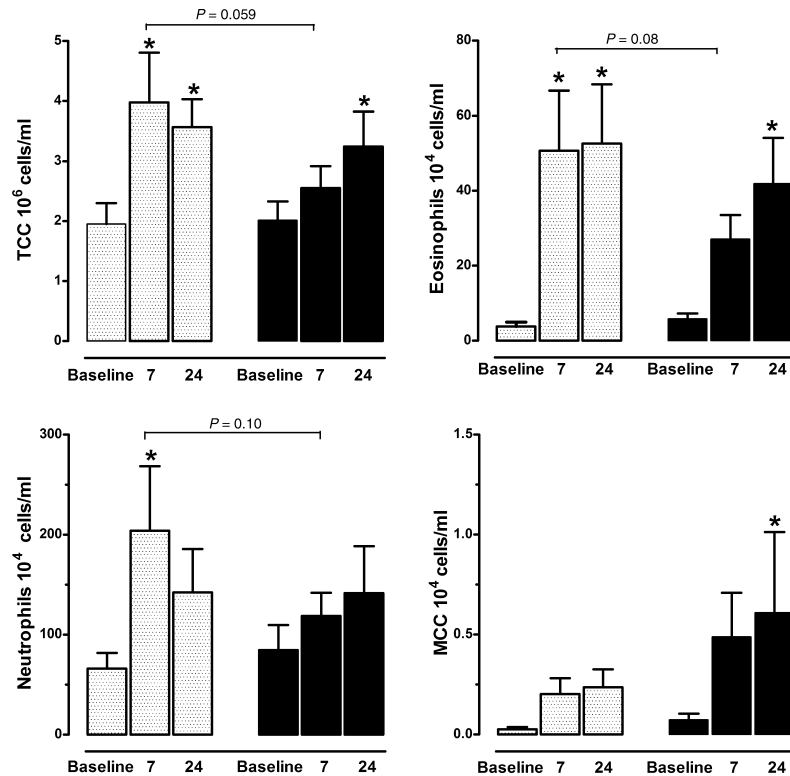


Figure 4. Sputum absolute cell counts at baseline, 7- and 24-h postallergen inhalation. Data are presented as mean $\pm$ SE. Placebo is represented by open and IVX-0142 by closed bars. * $P < 0.05$ compared with preallergen value.

tetrasaccharide sequence (24). In addition to inhibiting the LAR and AHR in the allergic sheep model, heparin-derived oligosaccharides, including a tetrasaccharide, have also been shown to inhibit eosinophil influx in bronchoalveolar lavage in a murine model of asthma (25).

In addition, equally important to the immunomodulatory properties of heparin and its derivatives is the charge density and the degree and selective sites of sulfation. Selective removal of some O-sulfates from unfractionated heparin has been shown to eliminate the anticoagulant action but preserve the inhibitory activity against neutrophil proteases, complement activation, and proliferation of airway smooth muscles (26). At the same time, maintenance of charge density (through N-sulfation) allow these modified heparins to conserve its ability to reduce eosinophil trafficking into the airways following allergen challenge in animal studies (27). On the contrary, hyper-sulfation of heparin derivatives increases the affinity for adhesion molecule binding and inhibition of leukocyte migration into tissues (28).

IVX-0142 is a porcine heparin-derived ultra-low MW (980 Da), hypersulfated disaccharide devoid of anti-coagulant activity. Its structure consists of 1 $\rightarrow$ 4-linked fully sulfated glucosamine and uronic acid residues each with O-sulfates at C2, C3, and C5. In preclinical allergic sheep model, IVX-0142 exhibits anti-allergic and anti-inflammatory activity. Single doses of IVX-0142 (5, 10,

20 mg) administered before antigen challenge in dual-responder sheep resulted in significant attenuation of the EAR, LAR, and AHR in a dose-dependent manner (8). The present study is the first to examine the immunomodulatory effects of IVX-0142 on the allergic-induced airway responses in subjects with mild atopic asthma. Our findings suggest that IVX-0142 has a beneficial effect on the EAR and LAR in the majority of subjects even if the magnitude of the mean difference relative to placebo did not reach statistical significance. This may in part be explained by the heterogeneity in the treatment response and the small sample size studied. As this was a pilot multicenter allergen challenge study, there were inadequate data available at the time, on the variability of the allergen-induced airway responses between centers and subjects. Therefore, the sample size was calculated based on previous single center experiences which may have led to a conservative estimate of the between-subject and between-center variability (29). Consequently, the statistical analysis was case-sensitive as, with one extreme outlier removed, the difference between active and placebo treatments in the EAR and LAR reached statistical significance. Nonetheless, there was an observed trend for a treatment effect of IVX-0142 on the airway responses with and without the outlier included. Furthermore, although the dose of IVX-0142 used in the present study was nearly double the highest

dose (20 mg, 0.65 mg/kg) administered in the preclinical study (8), higher doses or multiple dosing may have reduced the heterogeneity and or increase the observed treatment response. In this regard, kinetic studies have shown that the protective effect afforded by a single prechallenge dose of inhaled unfractionated heparin against allergen (1000 U/kg) (30), exercise (80 000 U) (31) and AMP (60 000 U) (4) challenges may last up to 3 h. In contrast, multiple dosing given before and after allergen challenge may prolong the protective effect up to 8 h (2–3 h after the last dose) (2). The pharmacokinetic properties of ultra-low MW heparins have not been extensively studied. However, compared with unfractionated heparin, low MW heparins have shown lower binding affinity to endothelial cells and reduced nonrenal cellular clearance resulting in increased bioavailability and prolonged biological activity (32). For this reason, we anticipated the duration of action and bioavailability of IVX-0142, an ultra-low MW heparin, to be prolonged. However, our finding of a lack of effect on the allergen-induced AHR and airway inflammatory cell counts at 24 h suggests perhaps the duration of action of IVX-0142 may not extend up to 24-h postdrug dosing. Alternatively, the total dose (80 mg) used in the present study may have been too low. In allergic asthma, inhaled unfractionated heparin at a dose of approximately 300 U/kg was not shown to be effective against allergen-induced airway responses (33) and a dose of 1000 U/kg only slightly protected against the early response to intra-dermal and inhaled allergen (1). However, at a higher cumulative dose following repeated inhalation of unfractionated heparin given before and after challenge (1000 U/kg, every 2-h up to five doses), there was an average reduction of 40% (not statistically significant) of the EAR and a significant inhibition of 36% of the LAR to inhaled allergen (2). Whether a larger treatment effect could be achieved with higher doses or frequent dosing (or both) may be further clarify in future studies comparing single (preallergen) vs multiple dosing (before and after allergen) with low and high doses IVX-0142.

The mechanism underlying the effect on the EAR and LAR observed with IVX-0142 is presently unclear. In keeping with previous findings (1–3, 31), we found that the effect on the allergen-induced airway responses was independent of the anticoagulant effect, as the coagula-

tion parameters were unaffected by IVX-0142. Similarly, IVX-0142 did not cause any significant bronchodilation indicating that it had no direct antagonism on airway smooth muscle (4, 22). However, our finding, that pretreatment with IVX-0142 reduced the significant postallergen increase in TCC, eosinophil, and neutrophil absolute counts (which increased with placebo) at 7 h, suggests that IVX-0142 has an anti-inflammatory effect by means of reducing the influx of inflammatory cells into the airways. In contrast, there were similar changes in the percentages of eosinophils, neutrophils, and MCC with IVX-0142 and placebo indicating that the usual preferential response by these cells to allergen was unaffected. Together, we speculate that IVX-0142 mediates its effect predominantly through the interruption of processes involved in cellular transmigration of inflammatory cells into tissues rather than by direct inhibition of specific chemo-attractants. This is in keeping with the *in vitro* evidence supporting the ability of heparin and its derivatives to inhibit leukocyte–endothelial adhesion and to prevent the movement of leukocytes into inflamed tissues by binding and blocking of P- and L-selectins (34, 35). It is also in accordance with the structural–functional relationship that has been demonstrated for highly sulfated heparin derivatives, possessing greater P-selectin inhibitory capacity and greater reduction in leukocyte extravasation (28). Alternatively, other anti-inflammatory activities (36) that have been proposed for heparin and its derivatives, including anti-complement action, modulation of T lymphocytes and direct binding, and inactivation of mediators from activated cells, may also involve and warrant further exploration.

In conclusion, we have shown that a novel and well tolerated heparin-derived hypersulfated disaccharide administered via nebulization exhibits potential anti-inflammatory activity. Further studies are needed to explore the magnitude of the treatment response using higher doses and or multiple dosing and its potential efficacy in the treatment of allergic asthma.

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