

A new antihistamine levocetirizine inhibits eosinophil adhesion to vascular cell adhesion molecule-1 under flow conditions

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Summary

Background We previously demonstrated that low concentrations of a new antihistamine levocetirizine inhibited eosinophil transmigration through human microvascular endothelial cells.

Objective Here, the inhibitory effect of levocetirizine on eosinophil adhesion to recombinant human vascular cell adhesion molecule-1 (rhVCAM)-1 was examined under conditions of shear stress using an *in vitro* model of the post-capillary venules.

Methods Eosinophils isolated from normal subjects were pre-incubated with a concentration range of levocetirizine (10^{-6} – 10^{-10} M) or negative dilution control. Resting or granulocyte macrophage-colony stimulating factor (GM-CSF)-stimulated cells were pumped through rhVCAM-1 (10 µg/mL) coated capillary tubes using a microfluidic syringe pump at a precise and constant flow rate (1 dyn/cm²). Images of rolling and firmly adherent eosinophils were captured using real-time video microscopy.

Results Levocetirizine significantly inhibited resting eosinophil adhesion to rhVCAM-1 with maximal effect at 10^{-8} M with an EC₅₀ of 10^{-9} M. Levocetirizine almost abolished resting eosinophil adhesion by the 15 min time-point. GM-CSF significantly enhanced eosinophil adhesion and their ability to flatten on rhVCAM-1. Both phenomena were inhibited by levocetirizine in a dose-dependent manner, at both 5 and 15 min (optimal concentration of 10^{-8} M with an EC₅₀ of 10^{-9} M). Real-time imaging revealed that the effect of levocetirizine on post-adhesion behaviour (detachment, flatness) contributed to its inhibitory action on eosinophil adhesion to rhVCAM-1. In contrast, very late antigen (VLA)-4 mAb inhibited eosinophil adhesion to rhVCAM-1 from the earliest time-points.

Conclusion Physiologically relevant concentrations of levocetirizine inhibit resting and GM-CSF-stimulated firm eosinophil adhesion to rhVCAM-1 under flow conditions.

Keywords adhesion, antihistamines, eosinophils, flow conditions, levocetirizine, VCAM-1, VLA-4

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Introduction

There is much evidence implicating eosinophils as major effector cells in allergic-based disease [1, 2]. Their accumulation is complex involving enhanced eosinophil production and release from the bone marrow followed by a combination of selective adhesion to the post-capillary vascular endothelium and transendothelial migration (TEM) into the surrounding tissues [3, 4]. Adhesion and TEM are mediated by the binding of leukocyte adhesion receptors to their ligands or counter-structures on the post-capillary endothelium with important contributions made by combinations of lipid mediators, chemokines and cytokines. Early adhesive events involve the selectins and these initiate a loose association between eosinophils and the endothelium, a process often termed 'rolling'. Vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) regulate later

and irreversible steps leading to firm attachment and subsequent diapedesis of eosinophils. TEM is followed by chemotaxis that completes eosinophil attraction to inflammatory sites. Selective eosinophil adhesion to the post-capillary venules appears to be dependent to a degree on the very late antigen (VLA)-4 ($\alpha 4\beta 1$)/VCAM-1 pathway [5]. As the major ligand for VCAM-1, VLA-4 is a versatile integrin that can mediate eosinophil tethering, rolling and firm arrest on VCAM-1 [6, 7], therefore supporting all the types of adhesive steps that are thought to be required for eosinophil arrest on vascular endothelia at sites of inflammation.

Mechanistic studies have demonstrated that the second generation H1-antihistamines, in addition to their antihistaminic properties, possess anti-allergic/anti-inflammatory effects. These include inhibition of cell migration, inflammatory cell activation/mediator release, adhesion molecule expression, and leukocyte adhesion to post-capillary endothelium and extravasation [8, 9]. A new antihistamine levocetirizine is the *R* enantiomer of cetirizine dihydrochloride and like its parent compound undergoes minimal hepatic metabolism. Levocetirizine has pharmacodynamically and pharmacokinetically favourable characteristics, including high bioavailability

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ity, rapid onset of action, limited distribution and a low degree of metabolism [10]. Moreover, clinical trials indicate that it is safe and effective for the treatment of allergic rhinitis and chronic urticaria with a minimal amount of untoward effects [11, 12]. We have previously shown that levocetirizine inhibits eotaxin-induced human eosinophil TEM through microvascular endothelial cells [13]. In the present study, we utilized a novel state of the art microflow system and real-time microscopic imaging to examine the inhibitory effects of levocetirizine on resting and granulocyte macrophage-colony stimulating factor (GM-CSF)-stimulated human eosinophil adhesion to recombinant human (rh) VCAM-1 under precise flow rates in conditions mimicking the post-capillary venules.

Methods

Reagents

Percoll was supplied by Pharmacia (Milton Keynes, UK) and the CD-16 immunomagnetic beads purchased from Miltenyi Biotec (Surrey, UK). VLA-4 mAb (HP2/1) was from Serotec Ltd (Kidlington, Oxon, UK) and isotype-matched control was from Dako Ltd (Cambridgeshire, UK). Both were mouse IgG1 isotype. Human VCAM-1 and GM-CSF were purchased from R&D Systems (Abingdon, UK). Bovine serum albumin (BSA) and Hank's Balanced Salt (HBSS) solution containing Ca^{2+} and Mg^{2+} were purchased from Sigma (Poole, Dorset, UK). Levocetirizine was provided by UCB Pharma (Brussels, Belgium).

Eosinophil isolation

Blood was obtained from normal donors or individuals with a history of mild allergic disease with an eosinophilia not greater than 0.5×10^6 eosinophils/mL who were not taking any medication for their condition for at least 1 month before venesection and who gave informed consent. Eosinophils were purified using dextran sedimentation and centrifugation on Percoll gradients followed by CD16-dependent negative immunomagnetic selection as described [14]. Isolated eosinophils were >99% pure with >98% viability as judged by trypan blue exclusion. Before use in the adhesion assay eosinophils were suspended in flow assay medium (filtered HBSS containing Ca^{2+} and Mg^{2+}) at a final concentration of 0.5×10^6 /mL.

Adhesion assays under flow conditions

This study used a novel microflow system consisting of a novel syringe pump with microfluidic biochip and flow sensor controlled by a PC using dedicated software (Cellix, Department of Physics, Trinity College, Dublin, Ireland). The system is described in full elsewhere [15]. Briefly, the

study (1 dyn/cm^2) mimic those found in the post-capillary venules during inflammatory processes. The biochip comprises of a flow splitter that splits the flow into eight different channels. Each channel mimics the dimensions of the post-capillary venules and these are coated with the rh adhesion protein of interest (see below). The microfluidic syringe pump is vital to the use of small diameter channels as standard syringe pumps are incapable of delivering the required low flow rates. Microchannels were coated overnight at 4°C with rhVCAM-1 ($10 \mu\text{g/mL}$ in HBSS containing Ca^{2+} and Mg^{2+}), rinsed twice and incubated with heat denatured 1% BSA in HBSS for 1–2 h at room temperature to reduce non-specific eosinophil adherence to plastic. Following rinsing of the chambers, the cell adhesion assay was performed at 37°C . Flow chambers were connected to the syringe pump and mounted on a controlled heater stage of an inverted microscope (Nikon TE2000-U; Nikon, Richmond, UK) equipped with $\times 20$ phase objective, and a Sony digital video camera (DCR-TRV250E; Sony, Thatchan, UK) connected to a JVC TV monitor (JVC, London, UK). Eosinophils were pre-incubated at 37°C in a water bath for 10 min before incubation with/without levocetirizine treatment (10^{-6} – 10^{-10} M) for a further 20 min, then cells were infused under a shear stress 1 dyn/cm^2 for 15 min. For experiments with GM-CSF-stimulated (10 ng/mL) eosinophils the cytokine was added to the warmed cells at the same time as levocetirizine or diluent control and incubated at 37°C for 20 min prior to commencing the flow assay. For all experiments, images of eosinophil interaction with rhVCAM-1 in real time were recorded by the digital video camera.

Real-time microscopic imaging and analysis

At the end of each experiment, adherent cells were counted manually from the video images throughout the whole channel (50 fields of view) at the 5 and 15 min time-points. A 15 min time-point was chosen on the basis of broad reference to published *in vivo/vitro* flow assay experiments [16–21]. In these, most arrested leucocytes transmigrated through endothelial cells within 10–20 min. Therefore, in the present study, any changes (cell detachment, flatness level, etc.) taking place in adhered cells were likely to be between the 5 and 15 min time-points and thus would be most representative of the steps preceding transmigration. Flat-tened cells were distinguished by phase contract microscopy and also counted. Figs 1 and 3 are representative experiments with each panel showing one field of view.

Percentage adhesion was calculated as follows:

$$\frac{\text{Number of adhered eosinophils}}{\text{Number of eosinophils initially added}} \times 100$$

Percentage detachment was calculated as follows:

$$\frac{\text{Number of adhered eosinophils at 5 min} - \text{number of adhered eosinophils at 15 min}}{\text{Number of adhered eosinophils at 5 min}} \times 100$$

microfluidic syringe pump allows very accurate flow rates to be achieved that are more reproducible and consistent than anything currently available. Importantly, flow rates are very low (5 pL/min to $10 \mu\text{L/min}$) and those used in the present

Percentage flatness was calculated as follows:

$$\frac{\text{Number of phase} - \text{dark eosinophils}}{\text{Number of total adhered eosinophils}} \times 100$$

Effects of levocetirizine on eosinophil $\alpha 4$ receptor expression

Resting or GM-CSF (10 ng/mL) stimulated eosinophils were pre-treated with increasing concentrations of levocetirizine. Immunostaining and flow cytometry were performed as previously described [14]. Briefly, treated and control eosinophils (2×10^5) were immunostained with the relevant antibodies or isotype-matched control at saturating concentrations for 40 min at 4 °C. Following a wash and staining with an appropriate fluorescein isothiocyanate (FITC)-conjugated secondary mAb, the cells were analysed on a flow cytometer (FACScan; Becton Dickinson, Franklin Lakes, NJ, USA) with eosinophils cells gated on the basis of their forward and side scatter profile with any cell debris excluded from analysis.

Fluorescence and confocal microscopy

Resting or GM-CSF (10 ng/mL) stimulated eosinophils, pre-treated with different concentrations of levocetirizine for 30 min at 37 °C, were fixed in 0.5% formaldehyde for 5 min, washed and immobilized on polylysine-coated slides. After blocking non-specific binding sites with 10% BSA for 15 min, the slides were stained with the relevant antibodies or isotype-matched control at saturating concentrations for 1 h at 4 °C, followed by being stained with an appropriate fluorescein isothiocyanate (FITC)-conjugated secondary mAb for 30 min. The slides were washed, mounted and sealed in mounting medium, and stored in the dark at 4 °C until viewed using a fluorescent or confocal microscope. Eosinophils were pre-treated with eotaxin (100 ng/mL) for 5 min under the same conditions served as a positive control to demonstrate redistribution of VLA-4 receptors.

Statistical analysis

All data are presented as mean $\pm$ SEM (standard error of mean) and where n is given, this represents the number of experiments each performed in duplicate. Statistical analysis was by the unpaired two-tailed Student's t -test where a P -value of <0.05 was considered significant.

Results

Effect of levocetirizine on resting and GM-CSF-stimulated eosinophil adhesion to VCAM-1 under flow

Preliminary experiments established that optimal adhesion by unstimulated eosinophils to rhVCAM-1 at a shear stress of 1 dyn/cm² was seen at a coating concentration of 10 μ g/mL per channel (around 10% of total cells added) and that eosinophil adhesion peaked at 5 min and plateaued thereafter (data not shown). This was due to the fact that the majority of cells were pumped through the microchannel over the first 5 min of each study period. Thereafter, the number of eosinophils entering the microchannel was negligible. Thus, differences in the number of adherent eosinophils between the 5 and 15 min time-points mainly reflected the level of detachment under flow conditions. Unstimulated eosinophils were observed to 'roll' on the rhVCAM-1 with negligible interactions with the BSA negative control coating observed. Some cells rolled throughout the observation period, some

rolled for various distances and then spontaneously arrested. Others arrested immediately after tethering. A portion of the adhered cells spontaneously detached, returning to the flow. Only a minority of total unstimulated eosinophils pumped through the microchannel (around 10%) remained adherent to the rhVCAM-1-coated channel at the end of the assay. The majority of unstimulated eosinophils bound to rhVCAM-1 remained in phase-bright, i.e. remained rounded, whereas some bound eosinophils changed shape and spread on the coated surface. Fig. 1 is a representative experiment demonstrating resting eosinophil adhesion to rhVCAM-1 at 5 (Fig. 1a) and 15 min (Fig. 1b) to be very similar. Levocetirizine (10^{-8} M) had a modest effect at 5 min (Fig. 1c) but almost abolished eosinophil adhesion at 15 min (Fig. 1d). Adhesion was almost totally inhibited by eosinophil pre-treatment with anti-VLA-4 mAb at 5 and 15 min, which confirmed that eosinophil adhesion to rhVCAM-1 was mainly mediated through $\alpha 4$ integrins (Figs 1e and f). Levocetirizine inhibited eosinophil adhesion in a dose-dependent manner with maximal inhibition seen at a concentration of 10^{-8} M with an EC₅₀ of 10^{-9} M (Fig. 2a). Time-course studies in real time revealed that levocetirizine treatment resulted in modest but significant ($P < 0.05$) detachment of adherent eosinophils from the rhVCAM-1 coated plastic at 5 min and their return to the flow in a dose-dependent fashion (Fig. 2b). We observed greater inhibition of eosinophil adhesion to rhVCAM-1 under flow at the later time-point of 15 min. In contrast, untreated cells did not become detached over the same time period. VLA-4 mAb inhibited

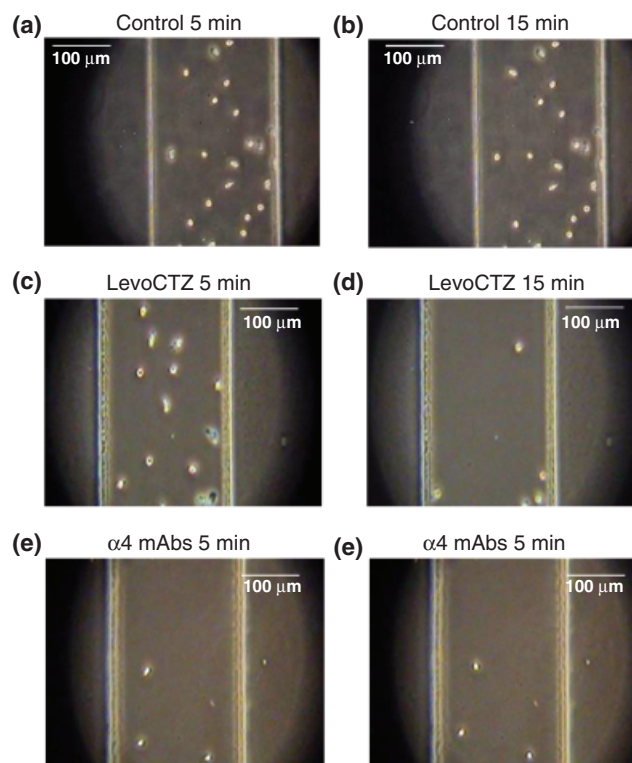


Fig. 1. Representative experiment showing the effects of levocetirizine and an anti-VLA-4 mAb on eosinophil adhesion to rhVCAM-1 (10 μ g/mL) under flow (1 dyn/cm²). Each panel depicts the same phase-contrast field of view ($\times 200$) at different time-points with their respective treatments.

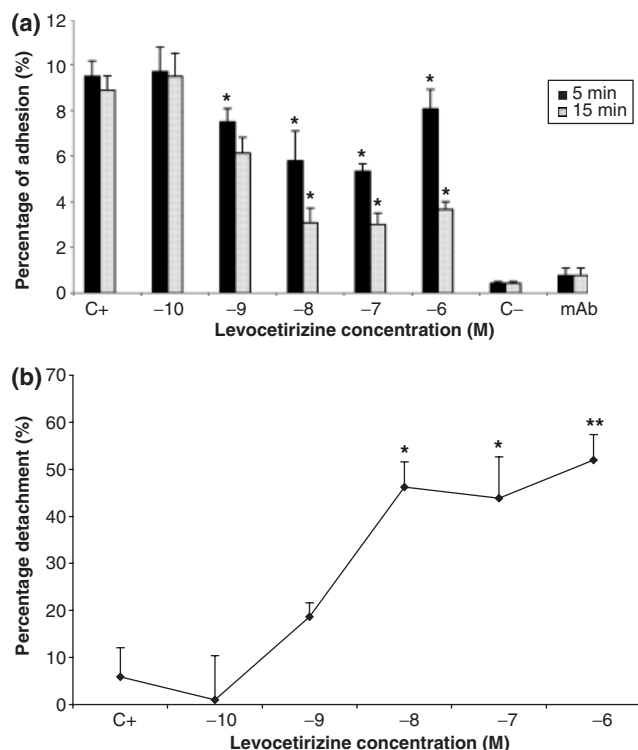


Fig. 2. (a) Dose response of the effect of levocetirizine on unstimulated eosinophil adhesion to rhVCAM-1 under flow at 5 and 15 min after the start of the flow assay. Data are from cell counts of 50 fields of view for each microchannel; (b) effect of increasing concentrations of levocetirizine on resting eosinophil detachment level between the 5 and 15 min time-points of the flow assay. In a and b, each bar/point represents the mean $\pm$ SEM of at least four independent experiments. * $P < 0.05$, ** $P < 0.005$ vs. group without treatment (C+). C- denotes bovine serum albumin (BSA)-coated microchannel; mAb denotes anti-VLA-4.

all eosinophil adhesion to rhVCAM-1 from the earliest time-points. (Figs 1e and f, 2a). Pre-treatment of eosinophils with anti-CD11b (data not shown) had no effect on adhesion to VCAM-1 further confirming that adhesion was mainly mediated through α -4 integrins.

We next examined the effect of a concentration range of levocetirizine on GM-CSF-stimulated (10 ng/mL – optimal concentration established in preliminary experiments) eosinophil adhesion under flow to rhVCAM-1. Figure 3 is a representative experiment illustrating that pre-incubation of eosinophils with GM-CSF increased the adhesion level by more than 50%, and also rapidly generated significantly more flattened cells at both the 5 and 15 min time-points. Levocetirizine significantly inhibited GM-CSF stimulated eosinophil adhesion to VCAM-1 and the ability of eosinophils to flatten on rhVCAM-1, in a dose-dependent manner, at both 5 min and 15 min. An example of a phase-dark flattened cell at 5 min (thick arrow) returning to a phase-bright phenotype at 15 min (thin arrow) is shown in Fig. 3. The data are summarized in Fig. 4a with optimal inhibition of adhesion observed at a levocetirizine concentration of 10^{-8} M with an EC_{50} of 10^{-9} M. However, a greater effect on GM-CSF-dependent eosinophil flattening was observed at 15 min (Fig. 4b). Pre-treatment of GM-CSF-stimulated eosinophils with a CD11b mAb had no measurable effect (data not shown).

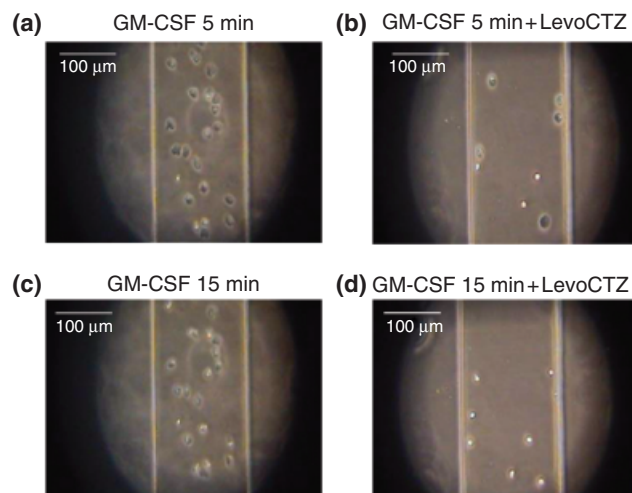


Fig. 3. Representative experiment showing the effect of levocetirizine (10^{-8} M) on granulocyte macrophage-colony stimulating factor (GM-CSF)-enhanced eosinophil adhesion to VCAM-1 under flow (1 dyn/cm²). GM-CSF treated eosinophils rapidly flattened on the rhVCAM-1 (a and b). Both adhesion and flattening were inhibited by levocetirizine (c and d). Each panel depicts the same phase-contrast field of view ($\times 200$) at different time-points with their respective treatments. The thick arrow indicates a phase-dark flattened cell at 5 min while the thin arrow showed that the same cell had returned to phase-bright at 15 min.

Effect of levocetirizine and/or GM-CSF on eosinophil expression of VLA-4

Treatment of eosinophils with levocetirizine (10^{-6} – 10^{-10} M) or GM-CSF (10 ng/mL) or both, for 30 min at room temperature had no effect on the eosinophil expression of VLA-4 as assessed by flow cytometry (data not shown). Immunostaining and fluorescent microscopy of eosinophils incubated with levocetirizine (10^{-6} – 10^{-10} M) and/or GM-CSF (10 ng/mL) revealed no evidence of VLA-4 receptor redistribution compared with control eosinophils (Fig. 5). Eosinophils pre-treated with eotaxin (100 ng/mL) for 5 min under the same conditions, served as the control to show the redistribution of the VLA-4 receptors (Fig. 5e). Eosinophils were also treated with GM-CSF or eotaxin and fixed at sequential time-points (5, 10, 20 and 30 min). Eotaxin induced a dynamic change of the VLA-4 receptor distribution on the cell surface, while cells treated with GM-CSF did not display any redistribution of VLA-4 receptors during the whole period. (data not shown) Near identical results (not shown) were obtained using confocal microscopy.

Discussion

Our previous work has demonstrated that levocetirizine inhibits eotaxin-induced eosinophil TEM through microvascular endothelial cells [13]. That study utilized static adhesion with no element of flow present. There is general agreement that the introduction of flow conditions to *in vitro* models of leukocyte adhesion is crucial to the generation of data relevant to the *in vivo* situation. Here we utilized a novel microflow system, syringe pump and dedicated software and real-time analysis [15] to examine the effect of levocetirizine on resting and GM-CSF-stimulated eosinophil adhesion to rhVCAM-1 under flow conditions. We used a flow rate of

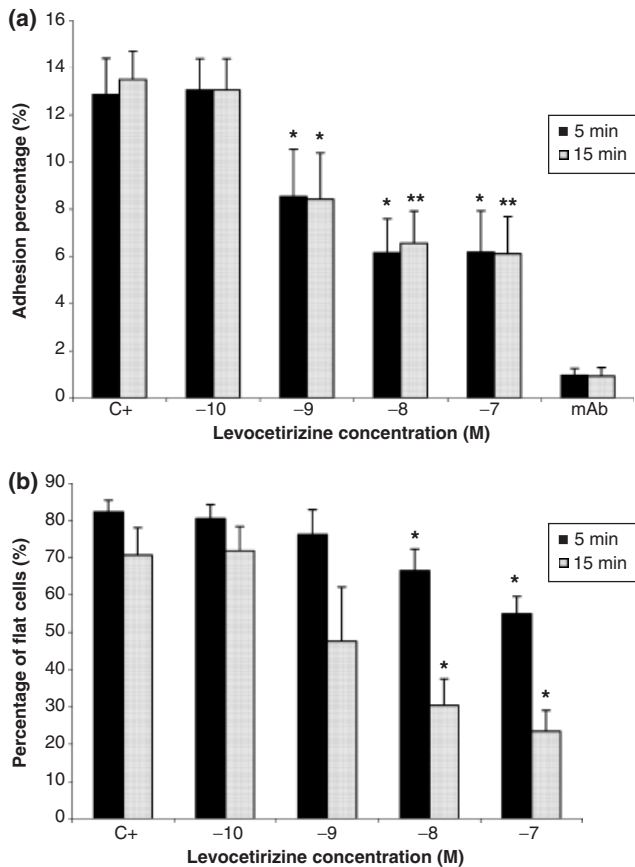


Fig. 4. (a) Dose response of the effect of levocetirizine on granulocyte macrophage-colony stimulating factor (GM-CSF)-stimulated eosinophil adhesion to rhVCAM-1 under flow at 5 and 15 min after the start of the flow assay. Data are from cell counts of 50 fields of view for each microchannel; (b) effect of increasing concentrations of levocetirizine on GM-CSF-dependent eosinophil flattening between the 5 and 15 min time-points of the flow assay. In a and b, each bar represents the mean $\pm$ SEM (standard error of mean) of at least four independent experiments. mAb denotes anti-VLA-4. * $P < 0.05$, ** $P < 0.005$ vs. GM-CSF-stimulated cells (C+).

1 dyn/cm² to mimic that found in the inflamed post-capillary venules [6, 7]. Inhibition of both resting and GM-CSF stimulated adhesion was observed at low physiologically relevant concentrations of levocetirizine. These findings support earlier studies that demonstrated inhibitory effects by levocetirizine's parent compound, cetirizine, on eosinophil adhesion or related events at similarly low concentrations [22, 23]. These inhibitory effects at low drug concentrations are important findings as many of the reported *in vitro* anti-inflammatory effects for many second generation antihistamines were only achieved at concentrations several order of magnitude higher than could be achieved *in vivo* casting doubt on their relevance to the clinical setting [8, 9]. Indeed, it could be argued that in some *in vitro* studies, which utilized very high antihistamine concentrations, the reported inhibitory effects were a consequence of drug toxicity. In this respect we have previously demonstrated that high concentrations of cetirizine or levocetirizine had no effect on eosinophil viability or apoptosis [13].

The inhibitory effect of levocetirizine on eosinophil adhesion to rhVCAM-1 appeared to be on post-adhesion behaviour, i.e. detachment and degree of cell flattening.

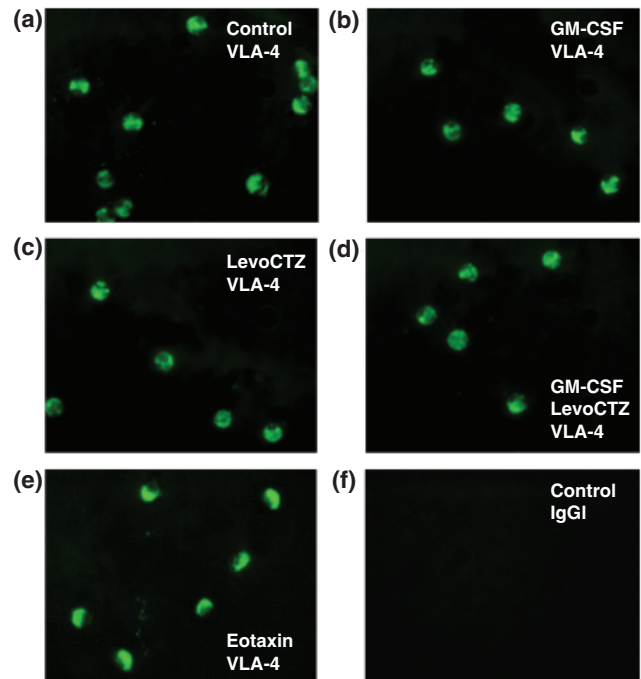


Fig. 5. Effect of granulocyte macrophage-colony stimulating factor (GM-CSF) and/or levocetirizine (10^{-8} M) on eosinophil VLA-4 receptor expression assessed by immunofluorescence microscopy. Eosinophils were cultured with 10 ng/mL GM-CSF and/or levocetirizine (10^{-6} – 10^{-10} M, here only 10^{-8} M shown for simplification) for 30 min, and VLA-4 receptors were labelled with fluorescein isothiocyanate (FITC), followed by being topped with mounting medium. There was no difference in the level of $\alpha 4$ expression by eosinophils cultured with either (a) or GM-CSF (b) or levocetirizine (c) or both (d). Eotaxin (100 ng/mL) for 5 min under the same conditions served as a positive control to show the redistribution of the VLA-4 receptors (e). Control isotype-matched IgG1 Ab (in place of the primary Ab) produced no staining with FITC (f).

Levocetirizine treatment resulted in unstimulated adherent eosinophils detaching from the rhVCAM-1 and returning to the flow. We observed a similar effect with levocetirizine-treated eosinophils stimulated with GM-CSF as the flattened cells either returned to phase-bright i.e. were more rounded or became totally detached from the rhVCAM-1 coated microchannel. In contrast, a mAb specific for VLA-4 abolished resting and GM-CSF-stimulated eosinophil adhesion to rhVCAM-1 from the earliest time-points. It can be appreciated, therefore, that if levocetirizine were to exert a comparable effect on eosinophil adhesion *in vivo* this would be on adherent cells; promoting either their return to the microcirculation or inhibiting their migration through the endothelium.

GM-CSF likely contributes to eosinophil accumulation in inflamed tissues by stimulating the proliferation and differentiation of eosinophils in the bone marrow, enhancing eosinophil adhesion and TEM, and prolonging their persistence in the tissue via inhibition of apoptosis [1]. As a cytokine expressed by inflamed endothelium [24], GM-CSF may contribute to the preferential accumulation of eosinophils *in vivo* in part via enhancement of eosinophil TEM [25]. GM-CSF and other cytokines appear to increase eosinophil adhesion to immobilized VCAM-1 by altering the functional state of eosinophil-expressed VLA-4 from a low-affinity state to a high-affinity state rather than upregulating VLA-4 expression or receptor redistribution [26, 27]. Levocetirizine

did not appear to exert its inhibitory effect via a direct effect on eosinophil adhesion molecule expression as no concentration of the drug had any significant effect on VLA-4 expression or receptor clustering by resting or GM-CSF-stimulated eosinophils. Rather levocetirizine appears to exert its inhibitory effects on unstimulated and GM-CSF stimulated eosinophil adhesion to rhVCAM-1 through modulation of VLA-4 affinity. However, the exact mechanism by which levocetirizine exerts its effect on eosinophil adhesion to VCAM-1 under flow remains to be determined.

VLA-4 mediates both early events of eosinophil adhesion (rolling), and later processes (arrest, firm adhesion). It has been suggested that receptor clustering controls the tethering and rolling interactions of the low-affinity resting receptor, whereas firm attachment and arrest is associated with receptor activation and affinity change [28, 29]. Since affinity regulation is a major factor that governs the avidity of cell adhesion mediated by the $\alpha 4$ integrin, the inhibitory action of levocetirizine on eosinophil adhesion to rhVCAM-1 is not likely due to its effect on cell rolling. Activation of the transcription factor NF- κ B is a requirement for adhesion molecule expression [30] and low concentrations of cetirizine downregulated NF- κ B expression in a bronchial epithelial cell line [31]. Whether NF- κ B contributes to the effect of levocetirizine on eosinophil adhesion to VCAM-1 under flow remains to be elucidated, as do those signalling pathways responsible for the inhibitory effect of levocetirizine on firm eosinophil adhesion to rhVCAM-1 under flow. Strategies aimed at blocking the $\alpha 4\beta 1$ integrin may provide a suitable target for preventing eosinophil accumulation in the lung. For example, small peptide antagonists of the integrins $\alpha 4\beta 1$ and $\alpha 4\beta 7$ (TR14035 and BIO1211) have had positive effects in animal models of asthma [32] and are currently in Phase II trials for efficacy in asthma [33]. However VLA-4 blockade may be potentially harmful as it may disrupt physiologically beneficial functions of the receptor. Our data suggest that levocetirizine exerts its inhibitory effect on eosinophil adhesion to rhVCAM-1 via modulating VLA-4 affinity state and not by receptor blockade. Whether levocetirizine inhibits resting or cytokine stimulated eosinophil primary tethering to selectins or firm adhesion to other endothelial ligands such as ICAM-1 is currently under examination.

Other studies have demonstrated *in vitro* anti-inflammatory effects by levocetirizine at relevant drug concentrations including inhibition of eotaxin production by endothelial cells [34] or inhibition of ICAM-1 and major histocompatibility complex (MHC) class I expression by IFN- γ -stimulated keratinocytes together with modulation of histamine-dependent release of GM-CSF and chemokines by these cells [35]. Neither of these studies nor the findings presented here examined whether these effects were special to levocetirizine. The question as to whether the phenomenon described here is a class effect exhibited by other second-generation antihistamines can only be answered by performing comprehensive comparative experiments and these will be the subject of a future report. However, as most antihistamines have documented anti-inflammatory effects [8, 9], it seems likely that the phenomenon reported here for levocetirizine is a class effect.

In conclusion, we demonstrate that physiologically relevant concentrations of levocetirizine inhibit both resting and GM-CSF-stimulated firm eosinophil adhesion to VCAM-1 under

flow conditions in an *in vitro* model of the post-capillary venules. These demonstrate further anti-inflammatory effects by levocetirizine that may provide important clues for developing novel therapy aimed at blunting eosinophil-induced inflammation in allergic-based disease.

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