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Inhibition of cytokine generation and mediator release by human basophils treated with desloratadine

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Summary

Background Desloratadine is a non-sedating, clinically effective, anti-allergic therapy that has been shown to exhibit anti-inflammatory properties that extend beyond its ability to antagonize histamine at H_1-receptor sites. This latter effect has been shown *in vitro* to be both IgE-dependent and -independent.

Objective In this study, we addressed the ability of desloratadine to inhibit the *in vitro* generation of interleukin (IL)-4 and IL-13 from human basophils while concurrently comparing its efficacy in preventing mediator release by these cells.

Methods Basophil-enriched suspensions were treated with various concentrations of desloratadine for 15 min before stimulating with either anti-IgE antibody, calcium ionophore, IL-3 or phorbol ester. Histamine (fluorimetry), LTC_4 (RIA) and IL-4 (ELISA) were all assayed using the same 4-h culture supernatants. IL-13 (ELISA) was measured in supernatants harvested after 20 h incubation. IL-4 mRNA expression (dilutional RT-PCR) was also examined.

Results Desloratadine was found to be nearly six–seven times more potent in preventing the secretion of IL-4 and IL-13 induced by anti-IgE than it was at inhibiting the release of histamine and LTC_4. These cytokines were equally inhibited by desloratadine following activation with ionomycin despite the lack of an effect on the histamine induced with ionomycin. Desloratadine had a lesser effect regarding inhibition of the IL-13 secreted in response to IL-3 and PMA. There was no evidence that desloratadine mediated its inhibitory effects by causing decreased cell viability. Finally, IL-4 mRNA accumulation was remarkably inhibited, by as much as 80%, following pretreatment with desloratadine.

Conclusion While capable of inhibiting histamine and LTC_4 release by human basophils, desloratadine is more effective at targeting the signals regulating IL-4 and IL-13 generation in these cells. This inhibitory effect on cytokine generation provides additional evidence that this antihistamine exerts anti-inflammatory properties.

Keywords: H_1-receptor, antagonist, cytokine, IgE, basophil, leukotriene, histamine

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Introduction

The clinical efficacy of H_1-receptor antagonists in the treatment of allergic rhinitis and urticaria is thought to result primarily from their competitive antagonism of histamine at H_1-receptor sites located on airway smooth muscle, the vasculature and neural tissues. There has long been evidence, however, that some H_1-receptor antagonists exert anti-inflammatory properties by altering the response of immune cells, which may also account, in part, for their therapeutic effectiveness. Work done some 25 years ago in

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our laboratory showed that antigen-induced histamine release from human basophil leucocyte suspensions was blocked following pre-treatment with first generation antihistamines such as promethazine and chlorpheniramine [1]. In these studies, no correlation was seen between the potency of H_1 receptor-blocking activity and the inhibition of histamine release, suggesting that a mechanism other than receptor antagonism was at work. Many other *in vitro* studies have since followed showing that antihistamines block mediator release from mast cells [2] and, more recently, decrease eosinophil and neutrophil chemotaxis and prevent superoxide production [3]. Additionally, the expression of adhesion molecules has been shown to be down-regulated following exposure to H_1-antagonists, making this a potential mechanism for the decreased cellular inflammation often seen with use of these drugs [4–6].

Desloratadine (DL) is a novel anti-allergic therapy that has been shown to exhibit potent H_1-antagonist activity and may also possess non-histamine-related anti-allergic and anti-inflammatory activity [7]. Initial clinical studies of desloratadine in patients with seasonal allergic rhinitis have shown clinically relevant improvements in both the acute-phase symptoms of rhinorrhea, sneezing, watery eyes and itchy palate, as well as the late-phase symptom of nasal stuffiness/congestion [8]. *In vitro* studies show that desloratadine inhibits histamine release from human basophils induced by both IgE-dependent and -independent mechanisms [9]. These results were later confirmed and extended to the human mast cell in which desloratadine inhibits the release of histamine, tryptase, LTC_4 and PGD_2 [10]. This latter study found no evidence that desloratadine differentially affected the release of these mediators, suggesting that the drug targeted a pathway common for the release of all four products.

While initiating the release of potent inflammatory mediators, cross-linking of the high affinity receptor for IgE (FcεRI) also results in signals that culminate in the generation of pro-inflammatory cytokines [11]. This is particularly true for human basophils, which have been shown to constitute the major source of IL-4 and IL-13 produced in mixed leucocyte cultures. In fact, production of these cytokines by basophils has implied that these cells play a role in modulating a variety of biological activities that are central to the pathogenesis of allergic inflammation [12]. However, there is little information regarding the effects of H_1-antagonists on the generation of cytokines in basophils, or any other cell type. At least one study has reported moderate (50%) inhibition of IL-4 and IL-13 secretion in basophils using relatively high concentrations of the H_1-antagonist, terfenadine [13]. However, this study did not probe into possible mechanisms responsible for this inhibition, including whether the concentrations of drug used were toxic. Desloratadine itself has been shown to inhibit IL-6 and IL-8 secretion by the KU812 (basophilic) and HMC-1 (mast cell) cell lines at nanomolar concentrations [14]. However, since these cell lines are defective in signalling through FcεRI, it is difficult to predict the effect desloratadine has on the IgE-mediated release of these cytokines.

In this study, we have investigated the influence of desloradatine on cytokine generation in human basophils by investigating its effects on the production of IL-4 and IL-13. Our focus, however, is directed more at comparing whether this compound differentially affects cytokine production vs. mediator release. To help address this issue, we have tested a variety of stimuli to determine the capacity of desloratadine to inhibit histamine, LTC_4, IL-4 and IL-13 secreted from enriched as well as pure basophil suspensions. Finally, we have also examined the effect desloratadine has on cytokine gene expression to further probe possible mechanisms relating to the anti-inflammatory properties of this antihistamine.

Materials and methods

Special reagents

All reagents were purchased unless otherwise noted: piperazine-N,N′-bis-2-ethanesulphonic acid (PIPES), ionomycin, fMLP, PMA and fetal bovine serum (FBS) from Sigma Chemical Co. (St Louis, MO, USA); RPMI-1640 and Iscove's modified Dulbecco's medium (IMDM) both with L-glutamine and containing 25 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (HEPES), gentamicin, nonessential amino acids (100x) from Life Technologies Inc., (Grand Island, NY, USA); and Percoll from Pharmacia (Piscataway, NJ, USA). The desloratadine used in these experiments was supplied by The Schering-Plough Research Institute. A 0.1-M stock solution was made in DMSO, aliquoted and frozen at -20 °C. All pipes-containing buffers were made from stock 10X PIPES (250 mM PIPES, 1.10 M NaCl, and 50 mM KCL, pH 7.3 and stored at 4 °C). Isotonic Percoll (referred to as 100% Percoll) was made by mixing nine parts Percoll with 1 part 10X PIPES. PIPES/albumin/glucose (PAG) contained one-tenth 10X PIPES, 0.003% HSA and 0.1% D-glucose. PAG-EDTA additionally contained 4 mM EDTA. Percoll solutions used for cell isolation were all made by mixing the appropriate amounts of 100% Percoll with 1X PIPES.

Cell preparation and culture

Subjects were not selected based on allergic status. Mixed leucocyte suspensions containing basophils were prepared either using double-Percoll density centrifugation as

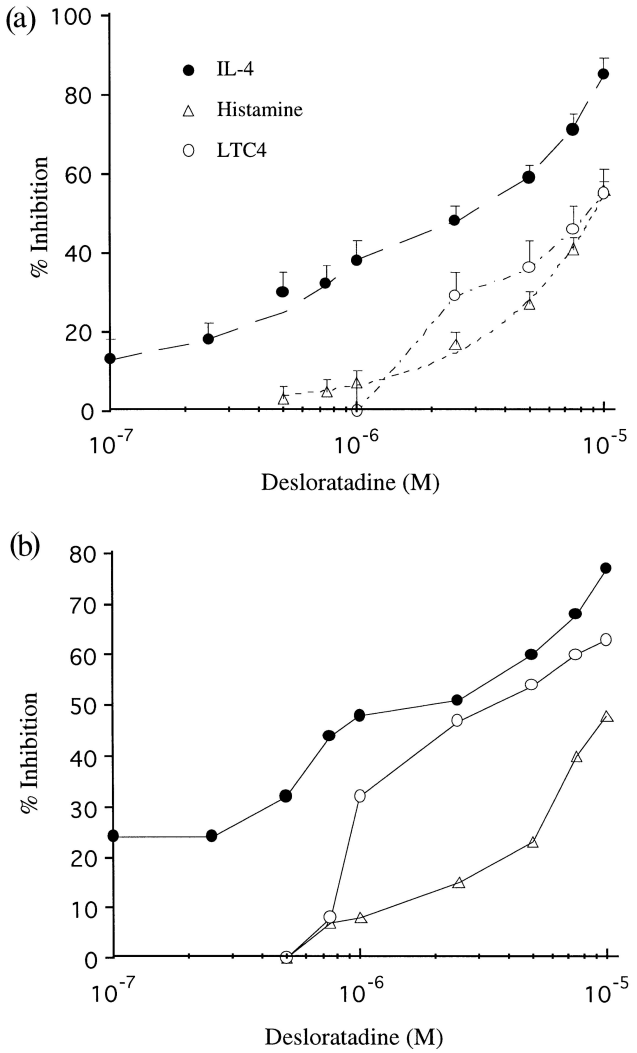

Fig. 1. Effect of desloratadine on histamine, LTC_4 and IL-4 secretion by human basophils. (a) Mixed leucocyte suspensions containing 3–55% basophils were prepared from whole blood using Percoll density centrifugation. Cells were pre-treated for 15 min with the indicated concentrations of desloratadine before activating with anti-IgE antibody (10–20 ng/mL). All three products were measured using the same culture supernatants harvested after 4 h incubation. Values are the mean ± SEM ($n = 5$). Control release for each product: histamine, 40 ± 6% of total; IL-4, 403 ± 207 pg/10^6 basophils; and LTC_4, 860 ± 127 pg/10^6 basophils. (b) Effect of desloratadine on histamine, LTC_4 and IL-4 secretion from human basophils ($n = 1$), 99% purity. Control release for histamine, LTC_4 and IL-4 were 60% of total, 903 pg/10^6 basophils and 854 pg/10^6 basophils, respectively.

described [15], or by a combination of countercurrent elutriation and Percoll density centrifugation protocols [16]. The percentages of basophils obtained using these protocols typically ranged between 5% and 50% and 10–30%, respectively, and were determined by counting Alcian blue positive and negative stained cells on a Spiers-Levy chamber [17]. For some experiments, the basophils were additionally purified to > 99.9% using a negative-selection protocol (Miltenyi Corp., Auburn, CA, USA). For all experiments other than those assessing IL-4 mRNA expression, the cells were cultured in 96-well flat-bottom microtitre plates (in duplicate) using IMDM supplemented with 5% heat-inactivated (56 °C for 30 min) FBS, 1 × non-essential amino acids, and 5 μg/mL gentamicin (C-IMDM). For the analysis of IL-4 mRNA expression, cells in C-IMDM were cultured in autoclaved (RNase-free) 1.5 mL microcentrifuge tubes (see below), since this allowed for a more precise way to quantitatively isolate mRNA without the need for transferring cells from culture wells before extraction. For each condition, leucocyte suspensions containing approximately 100 000–500 000 basophils in 100 μL of C-IMDM were pre-warmed to 37 °C before adding 100 μL of desloratadine concentrations in C-IMDM also pre-warmed to 37 °C. After 15 min pre-incubation, the cells were then activated by adding 50 μL of 5 × (5 times the final concentration) of stimulus. It is important to note here that the concentrations of stimuli used were optimal for IL-4 generation rather than mediator release. This is particularly true for anti-IgE antibody, which has been shown to induce IL-4 at concentrations 10-fold less than those causing optimal histamine release [15].

For harvesting, cultures were centrifuged and cell-free supernatants collected for mediator release and cytokine analysis, as described [15]. Histamine, LTC_4 and IL-4 were all measured in aliquots of culture supernatant taken at 4 h. For histamine, this meant taking 20–50 μL of supernatant and diluting it in 1 mL of PAG buffer containing 1.6% $HClO_4$. After an overnight precipitation at 4 °C, the samples were assayed by automated fluorimetry [18]. LTC_4 was measured by an in-house RIA [19]. IL-4 protein was measured by a commercial ELISA (Biosource International, Camarillo, CA, USA). Culture supernatants were harvested after 20 h incubation for all experiments investigating the effects of desloratadine on IL-13 secretion. IL-13 protein measurements were also made using a commercial ELISA (Immunotech, Westbrook, ME).

RNA isolation and semiquantitative analysis of IL-4 mRNA expression

Cultures for the analysis of IL-4 mRNA were performed in 1.5 mL polypropylene microcentrifuge tubes, as described above. Cells were pre-treated with desloratadine (10 μM) for 15 min prior to activating with anti-IgE antibody (10–20 ng/mL). Total RNA was isolated using the RNAzol protocol (Tel-test Inc., Friendswood, TX, USA) after 2 h

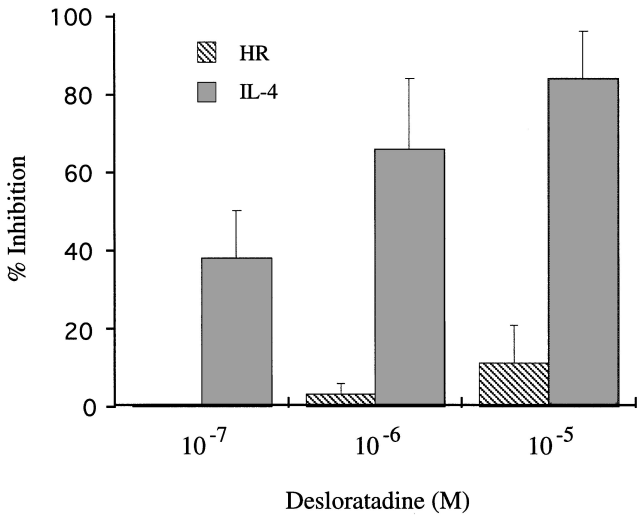

Fig. 2. Effect of desloratadine on histamine release (HR) and IL-4 secretion in response to ionomycin. Basophils suspensions of 42%, 99% and 98% purity were pre-treated for 15 min with 10^{-7}, 10^{-6} and 10^{-5} M desloratadine. Cells were then activated with ionomycin (500 ng/mL) for 4 h. Culture supernatants were harvested and assayed for histamine and IL-4 protein. Values represent the mean ± SEM, $n = 3$. Control levels of IL-4 were 3398, 253 and 348 pg/10^6 basophils. Percentage histamine release in the same cultures was 91%, 25% and 10%, respectively.

incubation, which is the time that IL-4 message expression peaks using IgE-dependent activation [20]. Following isopropanol precipitation, the RNA was washed with 70% ethanol and dried under vacuum. Subsequently, the RNA was resuspended in 25 μL of diethlpyrocarbonate (DEPC)-treated water and stored at − 80 °C. Reverse transcription (RT) and polymerase chain reaction (PCR) were performed with serial dilutions of RNA as previously detailed [16,20] using the GeneAmp RNA PCR kit (Perkin-Elmer Cetus, Norwalk, CT, USA). PCR products were visualized in 3% agrose gels using ethidium bromide staining. As noted elsewhere, two distinct bands for IL-4 were observed. A dominant band was seen with a size of approximately 460 bp. The source of the smaller, fainter band is uncertain, but is thought to be an alternatively spliced form of IL-4 [21]. The two bands are routinely observed using either pure or enriched suspensions of basophils.

Results

In our first series of experiments, we investigated the ability of desloratadine to block IL-4 secretion from basophils triggered by anti-IgE, and compared this inhibition with that seen for histamine and LTC_4 released in the same 4-h culture supernatants. As shown in Fig. 1(a), desloratadine dose-dependently inhibited the release of histamine and LTC_4 at concentrations between 1 and 10 μM. These results, in fact, confirm those previously reported, which showed that desloratadine inhibited mediator release from basophils during a standard 30-min incubation protocol [9]. For comparison, desloratadine was strikingly more potent against IL-4 secretion than for preformed mediator release. Cells pre-treated with 1 μM desloratadine secreted 40% less IL-4 than cells not exposed to drug. Nearly a sevenfold greater concentration of desloratadine was necessary to cause a comparable inhibition in the release of histamine and LTC_4 by these cells. Interestingly, the inhibition of IL-4, unlike that seen for histamine, appeared to plateau in the micromolar range of desloratadine and then reached progressively higher values at concentrations between 1 and 10 μM desloratadine. As shown in Fig. 1(b), this effect was also evident using basophils purified to greater than 99%, indicating that desloratadine was directly affecting basophils for the inhibition of IL-4. Desloratadine also inhibited histamine from pure basophils, much as it did for impure suspensions.

We tested whether desloratadine affected IL-4, histamine and LTC_4 released from basophils activated by other stimuli. Figure 2 shows that the secretion of histamine induced by the calcium ionophore, ionomycin, was unaffected by desloratadine at concentrations of 10^{-7}, 10^{-6} *and* 10^{-5} M. However, the high levels of IL-4 that were secreted after 4 h incubation in response to ionomycin stimulation were remarkably inhibited by the same concentrations of desloratadine. The histamine and LTC_4 made in response to fMLP stimulation was also not inhibited by desloratadine (data not shown), which is consistent with observations reported elsewhere [9]. Unlike ionomycin, f-met peptide does not induce appreciable levels of either IL-4 or IL-13 [15,22], therefore desloratadine was not tested for its ability to inhibit cytokine production induced by this stimulus.

We have previously reported that multiple stimuli induce pharmacologically distinct pathways for the generation of IL-13 in human basophils [23]. In fact, both anti-IgE and ionomycin were found to induce IL-13 secretion that was sensitive to the immunosuppressive drug, FK-506, suggesting that these stimuli utilize a calcineurin-dependent pathway to generate this cytokine. In the same study, the IL-13 made in response to IL-3 or PMA activation was unaffected by FK-506, and only protein generated in response to the phorbol ester was inhibited by PKC inhibitors. Therefore, we addressed whether the IL-13 induced by these stimuli is also differentially affected with desloratadine pre-treatment. Figure 3 shows that IL-13 generated in response to either anti-IgE or ionomycin was inhibited by desloratadine. In fact, there was remarkable similarity in the shape of the concentration–response curve of desloratadine for inhibition of IL-13 induced by either

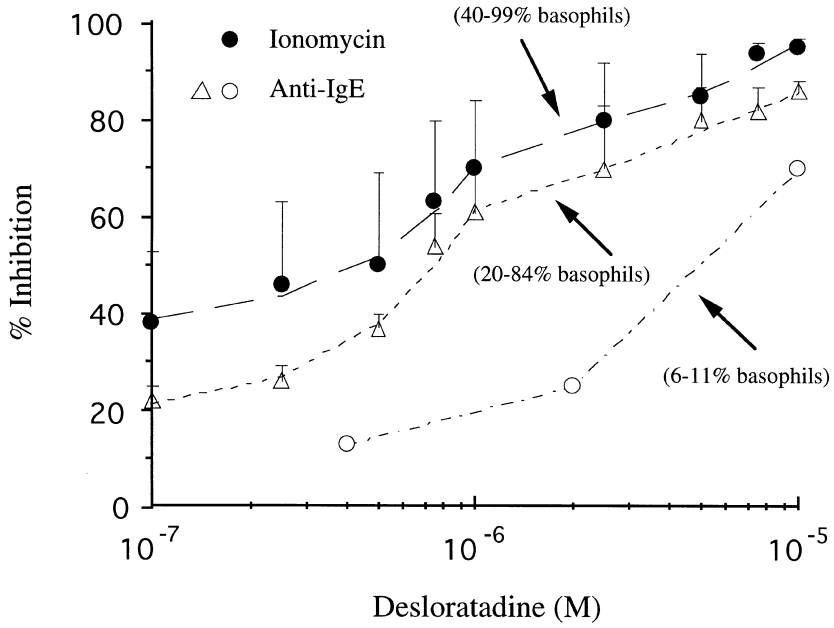

Fig. 3. Effect of desloratadine on IL-13 secretion by human basophils activated by anti-IgE or ionomycin. Basophil suspensions ranging in purity between 6% and 99.9% were pre-treated for 15 min with the indicated concentrations of desloratadine. Cells were activated with either anti-IgE (10–20 ng/mL) or ionomycin (500 ng/mL). Culture supernatants were harvested after 18 h incubation and assayed for IL-13 protein by ELISA. Values with error bars represent the mean ± SEM, $n = 3$. IL-13 protein in control cultures averaged 169 ± 35 and 334 ± 144 pg/10^6 basophils for anti-IgE and ionomycin, respectively. Values indicated by the open circles are the mean for two experiments (control levels of IL-13 were 260 and 230 pg/10^6 basophils).

stimulus. These curves were nearly identical to that seen for the inhibition of IL-4 by desloratadine as shown in Figs 1 and 2. In particular, an initial plateau was seen at 1 μM at the level of 50–70% inhibition. Dose-dependent inhibition of IL-13 continued as desloratadine concentrations were increased up to 10 μM. Interestingly, the response to desloratadine pre-treatment was considerably different in two experiments using impure (6–11%) basophil suspensions. With these cell preparations, desloratadine was considerably less effective in preventing the IL-13 secreted in response to anti-IgE, requiring nearly 10-fold greater concentrations to produce the same IC_{50} seen using more

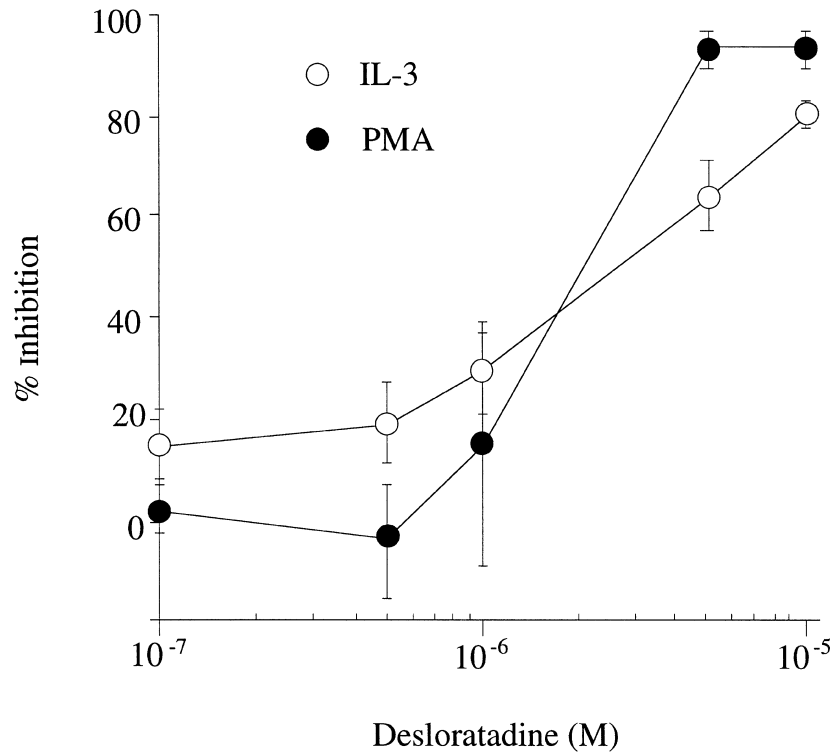

Fig. 4. Desloratadine inhibits IL-13 secretion from basophils activated with IL-3 and PMA. Basophils (up to 94% purity) were prepared from whole blood using double Percoll density centrifugation and negative selection protocols. Cells were pre-treated with the indicated concentrations of desloratadine before adding IL-3 (100 ng/mL) or PMA (2 ng/mL). Culture supernatants were harvested after 18 h incubation and assayed for IL-13 protein by ELISA. Values represent the mean ± SEM, $n = 3$–4. The amount of IL-13 secreted in the absence of desloratadine averaged 570 ± 142 pg/10^6 basophils for IL-3 activation and 216 ± 120 pg/10^6 basophils for PMA induction.

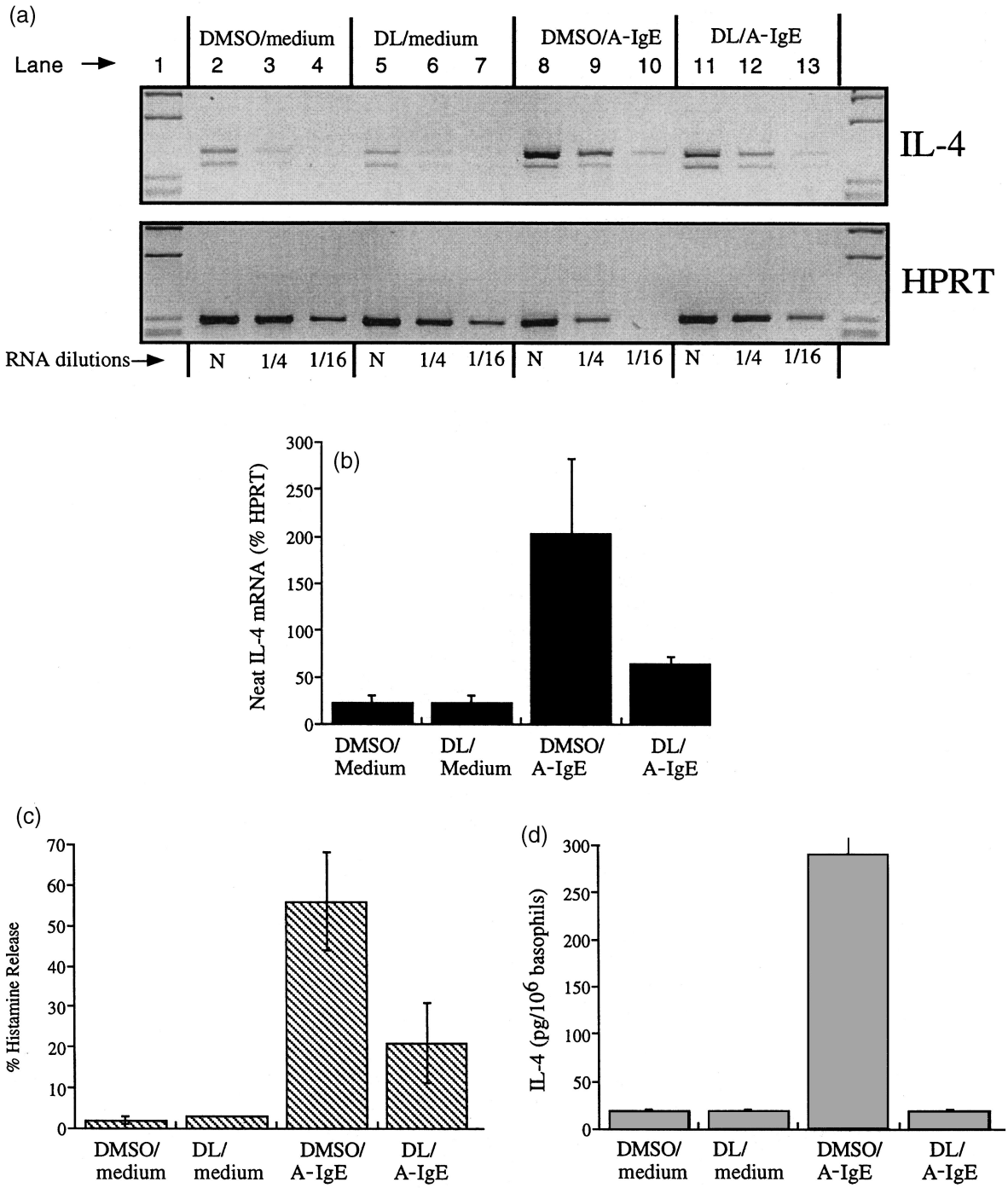

Fig. 5. Inhibition of IL-4 mRNA expression in basophils pre-treated with desloratadine (DL). Cells were incubated for 15 min with desloratadine (10 μM) or DMSO (1 : 10 000) before receiving anti-IgE (10 ng/mL) or medium alone. Total RNA was isolated after 2 h and used for dilutional RT-PCR analysis to compare message expression for IL-4 and the housekeeping gene, hypoxanthine phosphoribosyl transferase (HPRT) (a). Densitometric analysis is shown for undiluted RNA expression (b). The inhibition of histamine and IL-4 secreted in the supernatants after 2 h are shown for comparison, (c) and (d), respectively. Values represent the mean ± SEM, $n = 3$.

pure basophil suspensions. Figure 4 shows that desloratadine also inhibited the IL-13 made in response to IL-3 or PMA, albeit to a lesser extent and with inhibition curves that appeared more log-linear. In fact, the drug inhibited IL-3-mediated IL-13 production with an IC_{50} of approximately 2 μM, which was nearly five-fold that necessary to inhibit the IL-13 secreted with anti-IgE or ionomycin activation. Desloratadine inhibited the IL-13 induced by PMA in similar fashion, with inhibition seen between 1 and 10 μM concentrations of drug.

Unlike histamine and LTC_4, both of which are released from basophils within minutes after activation, IL-4 protein is first detected after 1–2 h and IL-13 some 4–8 h after activation. Therefore, it seemed possible that the kinetics of mediator release vs. cytokine production might account for the differences in their sensitivity to desloratadine, particularly if cell lysis was a factor. However, we saw no evidence that 10 μM desloratadine, even when combined with stimulus for 18 h, caused cell death as assessed by trypan blue exclusion in pure basophil cultures. In fact, viability ranged from 90% to 98% ($n = 2$), with the lowest percentages observed after 18 h incubation, and these did not differ in cultures containing desloratadine vs. controls. More than 95% of the cells did stain with trypan blue when cultured with 100 μM desloratadine alone. Furthermore, histamine was detected in these cultures, indicating that this concentration of desloratadine caused cell lysis (data not shown). Similar results showing histamine release have been observed with a host of H_1 antagonists [1].

Finally, to investigate the possibility that desloratadine inhibits cytokine secretion from basophils by affecting the accumulation of mRNA, we examined the effect of desloratadine (10 μM) pre-treatment on the expression of IL-4 message. For these experiments, we used a dilutional RT-PCR protocol to provide a semiquantitative analysis. Panel (a) of Fig. 5 clearly shows that IL-4 mRNA expression was induced in cells activated with anti-IgE (lanes 8–10) relative to cells not receiving stimulus (lanes 2–4). Pre-treatment with desloratadine resulted in a substantial decrease of the induced cytokine message (lanes 11–13), while having no effect on the expression of the housekeeping gene, HPRT. Panel (b) shows the average densitometric analysis of three experiments, indicating that desloratadine pretreatment caused approximately an 80% reduction in the IL-4 message accumulated with anti-IgE activation. For comparison, desloratadine (10 μM) also inhibited the histamine and IL-4 protein secreted into the supernatants of these cultures, as shown in panels (c) and (d), respectively.

Discussion

There is increasing evidence that allergic rhinitis is a local manifestation of a systemic inflammatory process that is mediated by a variety of cytokine, chemokine and cellular responses. Desloradatine is a non-sedating, anti-allergic therapy that has demonstrated potent H_1-antagonist activity as well as having histamine-independent effects on cells that mediate allergic reactions. Our findings support the concept that desloratadine possesses inhibitory activity against the generation of IL-4 and IL-13 from human basophils. Others have shown that desloratadine prevents IgE-mediated histamine release and LTC_4 generation by human basophils and our results confirm these findings [9,10]. However, we show that desloratadine is remarkably (six–seven-fold) more effective at inhibiting IL-4 and IL-13 induced by anti-IgE than it is at blocking histamine and LTC_4 released in these cultures. Interestingly, desloratadine inhibited IL-4 and IL-13 with a dose–response curve that plateaued at 1 and 10 μM concentrations. This effect was not seen for mediator release and occurred only for cytokine produced in response to anti-IgE or ionomycin. While the nature of this inhibition is presently unknown, we predict that desloratadine targets at least two signals along a common pathway triggered by both stimuli. Thus, at concentrations below 1 μM desloratadine the inhibition may result from disruption of a signal involved only in cytokine generation, while at concentrations above 1 μM, a second signal may also be affected, resulting in inhibition of pre-formed mediator release for most stimuli.

In agreement with the findings reported by others [9], we found desloratadine to be less effective at preventing mediator release induced by IgE-independent stimuli, such as ionomycin, fmlp and phorbol ester. In fact, we found no evidence that the histamine and/or LTC_4 released with these stimuli were inhibited by any of the desloratadine concentrations tested (data not shown). In contrast, desloratadine was quite effective in blocking the production of IL-4 induced by ionomycin and the IL-13 generated in response to either ionomycin, PMA or IL-3. As noted above, desloratadine equally inhibited the IL-4 and IL-13 induced by ionomycin or anti-IgE with inhibition curves that plateaued at 1 and 10 μM concentrations of drug. However, the same pattern and potency of inhibition was not observed for the inhibition of IL-13 induced by IL-3 or PMA. Two lines of evidence make this an interesting observation with regard to potential mechanisms of action. First, desloratadine has been shown to inhibit the mobilization of calcium initiated by IgE cross-linking in rat basophilic leukaemia (RBL) cells [24]. In human basophils, both anti-IgE and calcium ionophore actively mobilize cytosolic calcium, whereas IL-3 has little to no direct effect and phorbol ester inhibits calcium changes induced by anti-IgE [25]. With regard to cytokine production, both anti-IgE and ionophore induce IL-4 and IL-13 that are inhibited by the immunosuppressive drug,

FK506, suggesting that these stimuli utilize a calcium-dependent calcineurin pathway for the generation of these cytokines. However, this drug does not block the IL-13 made in response to IL-3 or PMA, both of which are thought to activate separate pathways [23]. Thus, desloratadine may inhibit IgE-dependent cytokine secretion and mediator release by simply preventing changes in cytosolic calcium. This effect, however, does not fully explain why desloratadine inhibited cytokine induced by ionomycin while having no effect on mediator release. Since IgE-crosslinking and Ca^{2+} ionophores appear to activate calcineurin in basophils, it is possible that desloratadine targets a factor within pathways initiated by this phosphatase.

By using pure basophil suspensions we were able to investigate for the first time whether desloratadine mediates its inhibitory effects on basophil mediator release and cytokine production directly and/or by affecting cell viability. However, we could detect no evidence, as assessed by trypan blue exclusion, that the decreased IL-4 and IL-13 secreted was accompanied by increased cell death during the 4–20-h incubations. This was true even for 10^{-5} M desloratadine, which caused up to complete inhibition of cytokine secretion. However, cell lysis was observed at 10^{-4} M and this is likely to explain why others have reported the release of histamine using desloratadine at this concentration [9].

Our study also demonstrates that desloratadine inhibits the accumulation of cytokine mRNA, raising the concept that this compound can negatively regulate factors important for cytokine gene transcription. Although our experiments focused on IL-4 and not IL-13, there is a body of evidence indicating that the two are similarly regulated when conditions involving FcεRI cross-linking are used [24]. Since the protein for each cytokine was similarly inhibited by desloratadine, we thought it appropriate to only investigate IL-4 mRNA expression. Interestingly, desloratadine pre-treatment resulted in nearly 80% inhibition of the IgE-mediated increase of IL-4 message. However, the secretion of protein for this cytokine in the same cultures was below detection, thus inhibited by $>95\%$. Once again this may imply that desloratadine targets multiple pathways in the generation of cytokines, affecting more than just signals important for gene transcription. In fact, it remains possible that transcription is not at all affected but that mRNA is made unstable in some way and that this accounts for the inhibitory action of desloratadine. If so, the instability must be specific for cytokine mRNA, as our results demonstrated that housekeeping gene (HPRT) expression is unaffected by desloratadine.

It is uncertain whether the inhibitory effects of desloratadine on cytokine generation and mediator release seen in these *in vitro* studies have any relevance to the clinical efficacy of this drug. However, it has recently been reported that desloradatine therapy significantly improves nasal stuffiness/congestion in subjects with seasonal allergic rhinitis [26]. Nasal stuffiness has traditionally been associated with the allergic late-phase inflammatory reaction and is resistant to treatment by the second-generation antihistamines. Thus, given the fact that these two cytokines help regulate the synthesis of IgE and activate endothelium for VCAM-1-mediated eosinophil transmigration, and that IL-4 promotes the development of the T-helper 2 phenotype, these findings raise the possibility that desloratadine can mediate anti-allergic activity, not only by inhibiting mediator release, but also by blocking the generation of pro-inflammatory cytokines.

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