

Effects of Antihistamines on Leukotriene and Cytokine Release from Dispersed Nasal Polyp Cells

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Summary

In this study the effects of antihistamines on the release of eicosanoids and the pro-inflammatory cytokine tumor necrosis factor alpha (TNF α) were compared. Enzymatically dispersed cells from human nasal polyps served as an in vitro model of chronic respiratory mucosal inflammation. Nasal polyp cells (2×10^6 /ml) were sensitized with human IgE preincubated with azelastine (CAS 58581-89-8), terfenadine (CAS 50679-08-8), levocabastine (CAS 79516-68-0) or cetirizine (CAS 83881-51-0), and stimulated with anti-human immunoglobulin E (IgE). Thromboxane B₂ (TBX₂) and leukotriene C₄ (LTC₄) were measured by radioimmunoassay (RIA), TNF α by enzyme-linked immunosorbent assay (ELISA). Data represent mean values of % inhibition estimated from the untreated positive

control or mean IC₅₀ (n = 5). Azelastine and terfenadine inhibited TNF α release with IC₅₀ values of 6.2 μ mol/l and 4.3 μ mol/l, respectively. Terfenadine reduced TXB₂ release by 37 ± 15 %, and LTC₄ release was decreased by azelastine and terfenadine very potently by 86 % and 100 %, respectively. Azelastine shows anti-inflammatory properties in therapeutically relevant concentrations as assessed by its ability to reduce TNF α release as well as its ability to inhibit LTC₄ production in allergically stimulated human nasal polyp cells.

Zusammenfassung

Wirkung von Antihistaminika auf die Freisetzung von Leukotrien und Cytokin aus dispergierten Zellen nasaler Polypen

In der vorliegenden Studie wurde die Wirkung von Antihistaminika auf die Freisetzung von Eicosanoiden und des

entzündungsfördernden Cytokins „Tumor necrosis factor“ α (TNF α) untersucht. Enzymatisch dispergierte Zellen von menschlichen nasalen Polypen dienten als In-vitro-Modell der chronischen mukosalen Entzündung der Atemwege. Diese Zellen 2×10^6 /ml) wurden mit

Key words

- Azelastine
- Cetirizine
- Leukotriene
- Levocabastine
- Nasal polyps, human
- Terfenadine
- Tumor necrosis factor α

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menschlichem Immunglobulin E (IgE) sensibilisiert. Anschließend wurden sie mit Azelastin (CAS 58581-89-8), Terfenadin (CAS 50679-08-8), Levocabastin (CAS 79516-68-0) oder Cetirizin (CAS 83881-51-0) vorinkubiert. Die allergische Stimulation erfolgte mit Anti-human-IgE. Thromboxan B₂ (TXB₂) und Leukotrien C₄ (LTC₄) wurden mit Hilfe von RIA (radioimmunoassay), TNF α mit ELISA (en-

zyme-linked immunosorbent assay) gemessen. Die Ergebnisse entsprechen einer prozentualen Hemmung im Vergleich zu nicht-behandelten Positivkontrollwerten (n = 5). Azelastin und Terfenadin hemmten die TNF α -Freisetzung mit IC₅₀-Werten von 6,2 μ mol/l bzw. 4,3 μ mol/l. Terfenadin reduzierte die TXB₂-Freisetzung um 37 + 15 %. Die LTC₄-Freisetzung wurde durch Terfenadin und Azelastin

sehr stark, um 86 % bzw. 100 % gehemmt. In therapeutisch relevanten Konzentrationen besitzt Azelastin entzündungshemmende Eigenschaften, die durch die Hemmung der TNF α - bzw. der LTC₄-Freisetzung charakterisiert werden konnten.

1. Introduction

Histamine H₁-receptor antagonists together with topical steroids are the treatment of choice in allergic rhinitis. The phthalazinone derivative azelastine has successfully been introduced in the therapy of allergic rhinitis and conjunctivitis. It has also been demonstrated that azelastine reduces nasal congestion [1–3]. This feature, which distinguishes azelastine from oral antihistamines, is of great interest, because corticosteroids are known to be quite effective in relieving nasal congestion, whereas antihistamines are effective for the sneezing, itching, watery secretion, but not congestion.

It is well known that azelastine and some other antihistamines may possess properties unrelated to blockade of histamine at its receptor level. In animal studies, it has been demonstrated that in addition to acting as a histamine H₁-receptor antagonist, azelastine also inhibits the production of many chemical mediators of the allergic response [4–9]. Azelastine has been reported to inhibit leukotriene (LT)-mediated acute allergic bronchoconstriction in guinea pigs and passive cutaneous anaphylaxis in rats [10, 11]. In an in vitro study using chopped lungs of actively sensitized guinea pigs, we have shown that azelastine is able to inhibit the allergically induced release of cysteinyl-LTs [12].

There is evidence that azelastine is able to inhibit the release of cysteinyl-LTs from different cell types, including human cells [4, 13–16]. Moreover, polypous tissue samples have been shown to produce more cysteinyl-LT upon stimulation than normal nasal mucosa indicating an altered pattern of arachidonic acid pathways [17]. Therefore, the present study has been performed with enzymatically dispersed cells obtained from human nasal polyps to compare the effect of antihistamines on the release of pro-inflammatory cytokines and eicosanoids. Nasal polyps are a model of chronic respiratory mucosal inflammation [18]. Not only residing cells such as epithelial cells, monocytes, macrophages, and mast cells are contained in this cell culture; also infiltrated cells such as immunocompetent lymphocytes, neutrophil granulocytes and often a high amount of eosinophils can be found. Hence, this culture system ensures an intensive interplay between the different cell populations, thereby providing a suitable model to

study the pharmacologic action of anti-inflammatory and anti-asthmatic drugs [19–22].

2. Materials and methods

2.1. Human nasal polyp cells

Nasal polyps were obtained from patients with nasal polyposis after polypectomy (n = 48). Patients had been free of any medication for at least 2 weeks. The study was approved by the local ethics committee. The polyps were washed in RPMI 1640 (Biochrom, Berlin, Germany), dissected and cell disaggregation was achieved by enzymatic digestion according to Campbell and Bousquet [23]. Briefly, the minced polyps were incubated for 2 h at 37 °C in RPMI 1640 (1 g tissue per 4 ml) containing 2.0 mg/ml protease, 1.5 mg/ml collagenase, 0.75 mg/ml hyaluronidase and 0.05 mg/ml DNase (all enzymes Sigma, Deisenhofen, Germany). Then, the cell suspension was filtered to remove any undigested tissue and the cells were washed 3 times. After an incubation in the presence of human IgE (1 μ g/ml in RPMI 1640 10 % fetal calf serum (FCS), Biochrom) for 1 h at 37 °C, the cells were washed two times, counted and resuspended at a concentration of 2×10^6 cells/ml. Then 1 μ g anti-IgE antibodies (Calbiochem, Bad Soden, Germany) per well were added to the cell suspension (1 ml per well in 6 well plates) to mimic an allergen challenge by cross-linking the receptor bound IgE antibodies. After enzymatic disaggregation, cells were counted in a Neubauer chamber using trypan blue staining. Cell viability always exceeded 95 %.

2.2. Compounds

Azelastine hydrochloride (CAS 79307-93-0) was synthesised by the Chemical Department of ASTA Medica AG (Frankfurt, Germany). Terfenadine (CAS 50679-08) levocabastine hydrochloride (CAS 79547-78-7) and cetirizine hydrochloride (CAS 83881-52-1) were kindly provided by the respective manufacturers.

2.3. Cytokine determination

To determine the effect of antihistamines on cytokine release nasal polyp cells were pre-incubated with 3, 10 and 30 μ mol/l of either azelastine, terfenadine, cetirizine and levocabastine respectively or with vehicle (0.1 % DMSO) for 30 min prior to addition of anti-IgE. After an incubation period of 18 h (37 °C and 5 % CO₂) the cell suspension was centrifuged and the supernatant was stored at –70 °C until determination of TNF α contents by sandwich ELISA using an antibody pair and standard protein from PharMingen (Hamburg, Germany).

2.4. Eicosanoid determination

To determine the effect of antihistamines on eicosanoid release nasal polyp cells were pre-incubated with 10 $\mu\text{mol/l}$ of either azelastine, terfenadine, cetirizine and levocabastine respectively or with vehicle (0.1 % DMSO) for 30 min prior to addition of anti-IgE. After an incubation period of 1 h (37 °C and 5 % CO_2) the suspension was centrifuged; the supernatant was immediately frozen in liquid nitrogen and stored at -70 °C until determination of eicosanoid contents.

Immunoreactive TXB_2 and cysteinyl-LT (calculated using a LTC_4 standard curve and expressed as LTC_4 equivalents [24]) were determined as described previously [25, 26], using [5, 6, 8, 9, 11, 12, 14, 15(n) ^3H] TXB_2 and [14, 15, 19, 20- ^3H (n)] LTC_4 (both from New England Nuclear, Vienna, Austria) as radioligands and synthetic TXB_2 and LTC_4 (both from Cayman, Ann Arbor, USA) as standards, respectively.

2.5. Analysis of results

For determination of the percent inhibition of mediator release by antihistamines the results were compared to the positive control and the average inhibition for each concentration was calculated. Data are given in mean \pm SD. The IC_{50} values were determined using an in-house programmed PC software package (EDX 2.1).

3. Results

3.1. Effect of antihistamines on $\text{TNF}\alpha$ release from nasal polyp cells

The finding that azelastine may act via mechanisms different from blocking the histamine receptor prompted us to investigate its anti-inflammatory potency. Therefore the ability of azelastine to reduce the allergically triggered release of the pro-inflammatory cytokine $\text{TNF}\alpha$ was tested and compared to that of terfenadine, levocabastine and cetirizine. Nasal polyp cells, which were only sensitized with human IgE and stimulated with anti-human IgE, served as positive control and released 400 to 500 pg/ml $\text{TNF}\alpha$ into the supernatant 18 h after addition of the stimulus. Azelastine and terfenadine inhibited the release of $\text{TNF}\alpha$ concentration-dependently and were very potent in this respect, since they almost completely prevented $\text{TNF}\alpha$ release at 30 $\mu\text{mol/l}$ (Fig. 1). Both antihistamines were equally effective with an IC_{50} value of 6.2 $\mu\text{mol/l}$ for terfenadine and 4.3 $\mu\text{mol/l}$ for azelastine. At a concentration of 3 $\mu\text{mol/l}$ levocabastine and cetirizine were as effective as terfenadine in inhibiting $\text{TNF}\alpha$ release by 40 % and 35 %, respectively. However, an increase in the concentration of either antihistamine hardly enhanced their inhibitory action (Fig. 1).

3.2. Effect of antihistamines on TXB_2 and LTC_4 release from nasal polyp cells

To further investigate possible actions of antihistamines which are not mediated through blockade of histamine receptors we determined the influence on the production of arachidonic acid metabolites. To investigate whether the cyclic or the linear pathway of arachidonic acid metabolism was influenced, we determined TXB_2

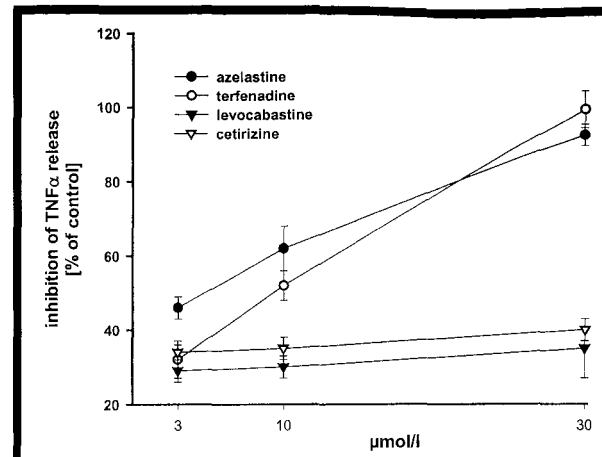


Fig. 1: Inhibition of allergically induced release of $\text{TNF}\alpha$ from enzymatically dispersed nasal polyp cells. Nasal polyp cells (2×10^6 ml) were sensitized with human IgE for 1 h. 30 min prior to stimulation with anti-human IgE 3, 10, or 30 $\mu\text{mol/l}$ azelastine, terfenadine, levocabastine and cetirizine were added respectively. 18 h after anti-IgE addition supernatants were removed and frozen at -70 °C until cytokine determination with ELISA. Data represent mean values of % inhibition estimated from the untreated positive control \pm SD (n = 5).

and LTC_4 in parallel. Positive control nasal polyp cells, which were only sensitized with human IgE and stimulated with anti-human IgE, released 320 pg/ml TXB_2 and 350 pg/ml LTC_4 . Azelastine, terfenadine, levocabastine or cetirizine were given to the nasal polyp cells at a concentration of 10 $\mu\text{mol/l}$ 30 min before addition of the allergic stimulus anti-IgE. Of all the antihistamines investigated only terfenadine reduced TXB_2 release by 37 \pm 15 % as compared to the positive control assessed one hour after addition of anti-IgE. On average neither

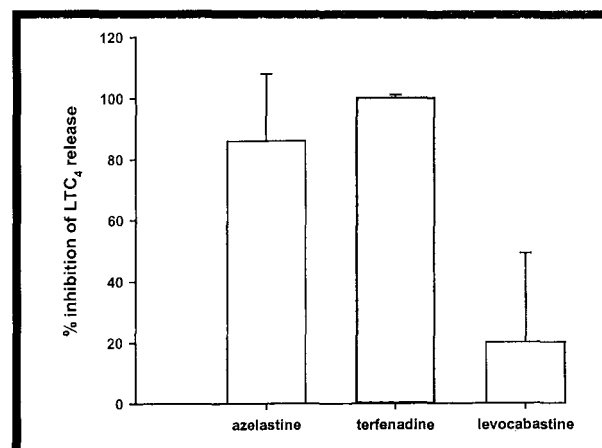


Fig. 2: Inhibition of allergically induced release of LTC_4 from enzymatically dispersed nasal polyp cells. Nasal polyp cells (2×10^6 ml) were sensitized with human IgE for 1 h. Thirty minutes prior to stimulation with anti-human IgE 10 $\mu\text{mol/l}$ azelastine, terfenadine and levocabastine were added respectively. One hour after anti-IgE addition supernatants were removed, shock frozen in liquid nitrogen and stored at -70 °C until LTC_4 determination. Data represent mean values of % inhibition estimated from the untreated positive control \pm SD (n = 5).

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azelastine, levocabastine or cetirizine showed an effect on TXB_2 production. Cetirizine had also no influence on the production of LTC_4 , and levocabastine only slightly inhibited this mediator by 20 % (Fig. 2). Azelastine and terfenadine, on the other hand, very potently decreased LTC_4 release by 86 % and even 100 % respectively.

4. Discussion

The knowledge of allergic rhinitis has been enriched in recent years by the investigation of different mediators, cells and mechanisms involved in the pathophysiology. Certain cytokines, such as $\text{TNF}\alpha$, as well as leukotrienes have become matters of further scientific interest.

$\text{TNF}\alpha$ is a candidate cytokine relevant to the pathogenesis of allergic rhinitis through its capacity for up-regulating the expression of endothelial cell adhesion molecules, mediating granulocyte chemoattraction, and activating eosinophils, mast cells and T cells [27, 28]. Moreover, $\text{TNF}\alpha$ is localised in nasal mast cells and is released in rhinitis patients upon allergen challenge [29]. $\text{TNF}\alpha$ is increased in the nasal epithelial lining fluid and in nasal secretions of patients suffering from rhinitis [30, 31]. Recently, it has been demonstrated that human mast cells and eosinophils are a source of $\text{TNF}\alpha$ [32, 33].

In the present study we were able to show the anti-inflammatory action of azelastine in a human in vitro model most suitable to investigate the interaction of various cells populations involved in allergic and inflammatory processes. Cells gained from nasal polyps include epithelial cells, monocytes, macrophages and mast cells as well as infiltrating cells such as lymphocytes, neutrophils and many eosinophils. Upon sensitisation with human IgE and subsequent stimulation with anti-human IgE to mimic allergen contact the cells secrete $\text{TNF}\alpha$. Azelastine, in contrast to levocabastine and cetirizine, very potently inhibited the release of $\text{TNF}\alpha$. In its effect on $\text{TNF}\alpha$ terfenadine was equally effective as azelastine.

LTs play an important role in nasal congestion, secretion and cell infiltration, in other words, in the development of the chronic allergic inflammation [34, 35]. Intense production of arachidonic acid metabolites in nasal polyp tissue samples has been shown by Jung et al. [36]. In agreement with previous data [23], cells available in nasal polyps produce LTs and cytokines upon allergen stimulation. Recent studies have suggested that antihistamines, widely used in the treatment of symptoms of patients with allergic rhinitis, may also possess anti-inflammatory properties.

Loratadine and its active metabolite, descarboxyethoxyloratadine, help to stabilise mast cells, as evidenced by their ability to inhibit the release of histamine and LTs [37]. There is evidence that loratadine inhibits LT biosynthesis [38]. LTC_4 release by peripheral blood basophils and tissue mast cells was inhibited by

loratadine in the micromolar range [39, 40]. It has also been shown that loratadine (0.25–25 $\mu\text{mol/l}$) inhibits $\text{TNF}\alpha$ -induced LTB_4 production in peripheral neutrophil granulocytes [41]. There is no evidence in the literature that loratadine might inhibit $\text{TNF}\alpha$ release. However, it has been shown that loratadine can inhibit the release of IL-6 and IL-8 from human mast cells and endothelial cells to a certain degree [42, 43]. It is believed that mizolastine is effective in the treatment of nasal obstruction due to its inhibitory action on LT synthesis [38].

Cetirizine substantially reduced the release of LTs from blood leukocytes of children with allergic rhinitis [44], and in a model of nasal antigen challenge in humans it significantly decreased LTC_4 levels [45]. However, the data obtained in humans are contradictory. In a model of allergen-induced early phase for instance cetirizine did not influence the elevations of nasal LTC_4 levels [46].

In previous experiments, azelastine has been shown to inhibit the Ca^{2+} -ionophore A23187-induced LT release with IC_{50} values between 1.1 and 36.5 $\mu\text{mol/l}$ depending on animal species and cells used [15, 16, 47–49]. Various studies on actively or passively sensitized lung preparations of human or guinea-pig origin showed that azelastine potently inhibits release of cysteinyl-LTs after allergen or anti-IgE challenge with IC_{50} values in the range of 14 $\mu\text{mol/l}$ to 272 $\mu\text{mol/l}$ and was superior to other antiallergic/antiasthmatic drugs (i.e. ketotifen, terfenadine, DSCG) [4, 12, 47, 50]. The potent inhibition by azelastine may be partly a result of the inhibition of 5-LOX (5-lipoxygenase), since in rat basophilic leukaemia cell homogenate 5-HETE (12(S)-hydroxy-5-cis-8-cis-10-trans-14-cis-eicosatetraenoic acid) formation was inhibited by azelastine [49]. Studies on isolated murine peritoneal cells showed that azelastine significantly inhibited 5-LOX activity ($\text{IC}_{50} = 11 \mu\text{mol/l}$), but not LTC_4 -synthase or LTA_4 -hydrolase activity [14]. Furthermore, azelastine exerted little effect on 12-LOX activity and did not influence COX activity and TXA_2 -synthesis in human platelets and guinea pig lung homogenates [12, 48]. Chand and Sofia suggested that azelastine may inhibit the Ca^{2+} -dependent translocation of 5-LOX from cytosol to the nuclear envelope or it might be a FLAP (5-lipoxygenase activating protein) inhibitor rather than directly effecting 5-LOX [51]. Others indicate that azelastine reduces LT production by inhibiting PLA_2 and LTC_4 synthase [52].

After a single treatment with azelastine, there was a significant reduction in the median level of cysteinyl-LT concentration in patients suffering from rhinitis or patients who underwent nasal challenge [53]. In this study we were able to confirm this anti-inflammatory action of azelastine on human nasal polyp cells. Azelastine (10 $\mu\text{mol/l}$) almost completely abolished the release of LTC_4 from polyp cells after allergic stimulation. TXB_2 production, on the other hand, was not affected. Hence azelastine, as has been shown for other cell

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 had no effect on its
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 The effect on 12-LOX
 activity and TXA₂-
 release in guinea pig lung
 tissue suggested that
 azelastine might affect
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 oxygenase) [51].
 The effect on 12-LOX
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LT production
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 In conclusion, there was a
 significant effect of cysteinyl-
 LT release from rhinitis or
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 study, the anti-inflammatory
 effect of azelastine on
 mast cells was estab-
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 sults showed that
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 by other cell

types, specifically inhibits the linear pathway of arachi-
 donic acid metabolism in our cells culture system prob-
 ably by influence on the 5-LOX or possibly on PLA₂
 (phospholipase A₂) or LTC₄ synthase, while leaving the
 cyclic pathway unaffected. Terfenadine in comparison
 to azelastine proved to be more potent in inhibiting
 both LTC₄ and TXB₂. Although TXB₂ release was not as
 strongly decreased as LTC₄, terfenadine still is not as
 specific for the linear arachidonic acid pathway. This
 specificity seems to be a unique feature of azelastine.
 However, the exact mechanism of the anti-inflamma-
 tory property of azelastine remains to be elucidated.

In summary, the present study shows the anti-in-
 flammatory action of azelastine in therapeutically rele-
 vant concentrations as assessed by its ability to reduce
 TNFα release as well as inhibit LTC₄ production in aller-
 gically stimulated human nasal polyp cells.

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