

Short Report

Urinary leukotriene E₄ levels in children with allergic rhinitis treated with specific immunotherapy and anti-IgE (Omalizumab)

Kopp MV, Mayatepek E, Engels E, Brauburger J, Riedinger F, Ihorst G, Wahn U, Kuehr J. Urinary leukotriene E₄ levels in children with allergic rhinitis treated with specific immunotherapy and Anti-IgE (Omalizumab).

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Recently, we were able to demonstrate that Omalizumab, a humanized monoclonal anti-IgE antibody, reduces *in vitro* leukotriene (LT) release of peripheral leukocytes stimulated with allergen in children with allergic rhinitis undergoing allergen immunotherapy. The aim of this study was to investigate the effect of anti-IgE in combination with specific immunotherapy (SIT) on urinary leukotriene E₄ (LTE₄) levels. Children and adolescents with sensitization to birch and grass pollens and suffering from seasonal allergic rhinitis were included in a phase III, placebo-controlled, multicenter clinical study. Within the four-arm study, patients were randomized to receive SIT for either birch or grass pollen and to receive either subcutaneous anti-IgE or placebo for 24 weeks during the pollen season. From a total population of 225 children, we collected three urine samples in a subgroup of 19 children [*n* = 12 boys (63%); mean age 11.8 years; range 7.2–17.5 years; Group A (*n* = 10): SIT (grass or birch) + anti-IgE; Group B (*n* = 9): SIT (grass or birch) + placebo]. Urine samples were collected before, during and at the end of treatment. Endogenous urinary LTE₄ was separated by high-performance liquid chromatography (HPLC) and determined by enzyme immunoassay with a specific antibody. No differences in urinary LTE₄ concentrations were observed between the anti-IgE and the placebo groups before (A: 35.2; B: 36.5 nmol/mol creatinine), during (A: 27.0; B: 29.3) and after treatment (A: 28.9; B: 26.5 nmol/mol creatinine). We conclude that urinary LTE₄ levels are not helpful in monitoring patients treated with anti-IgE and SIT.

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Cysteinyl leukotrienes (LTC₄, LTD₄, LTE₄) are potent lipid mediators derived from arachidonic acid in the 5-lipoxygenase pathway (1). Together with prostaglandins and platelet-activating factor (PAF), the LTs are involved in the pathogenesis of allergic diseases, and individuals with asthma may have enhanced *in vitro* production of LT upon stimulation as well as higher basal excretion of endogenous urinary LTE₄ compared with normal individuals (1–3). LTs mediate a broad spectrum of pro-inflammatory activities, are among the most potent bronchoconstrictors and cause vasodilation, increased microvascular

permeability, exudation of macromolecules and edema as well as neutrophilic chemotaxis and activation (2, 4).

Currently a new strategy in the treatment of allergic disorders is under clinical investigation using a recombinant humanized, monoclonal anti-IgE antibody (Omalizumab). This anti-IgE antibody inhibits the expression of allergic symptoms by preventing IgE binding to IgE-receptor bearing cells. Anti-IgE has been shown to improve symptoms and reduce rescue medication as well as corticosteroid use in patients with allergic asthma (5).

Recently, we conducted a randomized, double-blind trial to assess the efficacy of anti-IgE in combination with specific immunotherapy (SIT) compared with placebo and SIT in children with seasonal allergic rhinitis. We observed that the combined therapy reduced symptom load over the pollen seasons by 48% ($p < 0.001$) compared with SIT alone (6). Supporting the clinical data, we have demonstrated that quantification of *in vitro* LT release in response to allergens is a reliable marker in monitoring the clinical effects of anti-IgE treatment (7). This article reports the endogenous LTE_4 concentration during treatment with anti-IgE.

Methods

Population

During a phase III placebo-controlled clinical multicenter study a total population of 225 children and adolescents were enrolled to investigate the effect of anti-IgE in patients with seasonal allergic rhinitis and a clinical history of birch and grass pollen-induced seasonal allergic rhinoconjunctivitis within the previous 2 years or longer (6). Of these 225 children, we collect three urine samples from 19 children and adolescents in Freiburg.

Allergic sensitization was evaluated by determination of specific IgE against birch and grass pollen allergen in the children's sera by the Fluoro-Enzyme-Immuno-Assay (FEIA, Pharmacia CAP System, Uppsala, Sweden). Children with a specific IgE equal to or greater than 3.5 kU/l (CAP-RAST class ≥ 2) in response to birch and grass allergen were classified as 'sensitized'.

The participants were randomized to receive SIT (ALK-Scherax, Hamburg, Germany) for either birch or grass pollen, beginning treatment at least 4 months prior to the pollen season. Additionally, these patients were randomized to receive either subcutaneous anti-IgE (Omalizumab 150–300 mg every 4 weeks or 225–375 mg every 2 weeks, based on baseline IgE and bodyweight; Novartis, Nuernberg, Germany) or placebo for 24 weeks during the pollen season. The study protocol was approved by the ethics committee of all participating hospitals and written consent was obtained from patients and parents.

Urine was collected from each patient as a spot sample into a sterile plastic tube. A dipstick was used to rule out urinary tract infection. Two aliquots of 10 ml of urine were immediately frozen and stored at -70°C . Urinary creatinine levels were measured by an Ektachem analyzer (Johnson & Johnson Clinical Diagnosis, Inc.,

Rochester, NY, USA). Urinary LTE_4 was measured as previously described (8). In brief, endogenous urinary LTE_4 was separated by high-performance liquid chromatography (HPLC) and determined by enzyme immunoassay with a specific antibody (Cayman, Ann Arbor, MI, USA) (8).

We compared in our statistical analysis children who received SIT (SIT birch or SIT grass) + anti-IgE (Group A) with children receiving SIT (SIT birch or SIT grass) + placebo.

Results

In this study, we enrolled 19 children from Freiburg [$n = 12$ boys (63%), $n = 7$ girls (37%); mean age 11.8, range 7.2–17.5 years].

There were no differences between group A (SIT + Anti-IgE) and group B (SIT + placebo)

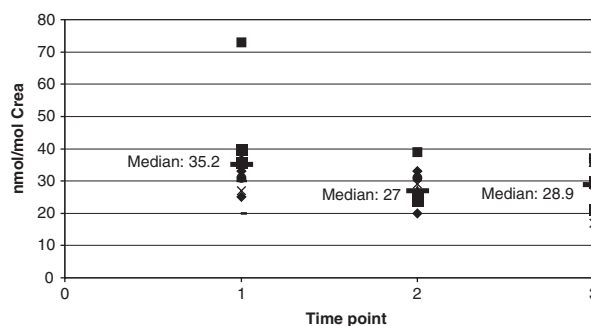


Fig. 1. LTE_4 levels in urine in the group treated with SIT + Anti-IgE. Urinary endogenous LTE_4 concentration (nmol/mol creatinine) before (September 1999; time point 1), during (March 2000; time point 2) and after (July 2000; time point 3) treatment in group A [SIT (grass or birch) + anti IgE; $n = 10$]. The median value is indicated as a bar.

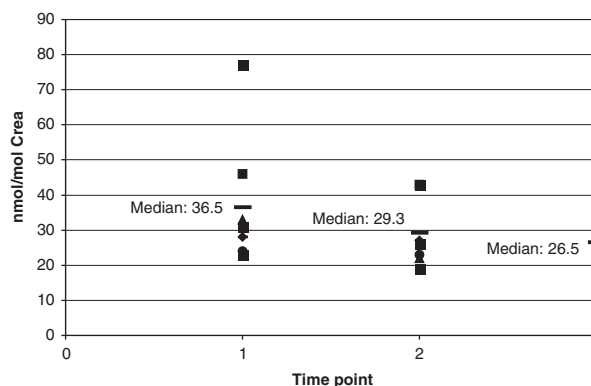


Fig. 2. LTE_4 levels in urine in the group treated with SIT + placebo. Urinary endogenous LTE_4 concentration (nmol/mol creatinine) before (September 1999; time point 1), during (March 2000; time point 2) and after (July 2000; time point 3) treatment in group B [SIT (grass or birch) + placebo; $n = 9$]. The median value is indicated as a bar.

regarding age, sex and the total IgE levels at baseline.

The individual values and the median values are presented for group A (Fig. 1) and group B (Fig. 2). No differences in urinary LT concentrations were observed between the anti-IgE and the placebo group before treatment, during the treatment period and at the end of the treatment (Figs 1 and 2). After the treatment period, the median urinary LT levels decreased from 35.2 to 28.9 nmol/mol creatinine in the anti-IgE group and from 36.5 to 26.5 in the placebo group. However, these differences were not statistically significant.

Discussion

Anti-IgE, a humanized monoclonal anti-IgE-antibody, is an effective and safe new therapeutic approach in the treatment of allergic rhinitis as well as bronchial asthma with a potent ability to reduce allergic symptoms as well as the use of rescue medication (5, 6). Recently, we postulated that the quantification of *in vitro* LT release in response to relevant allergens is a valuable marker in monitoring the clinical effects of treatment with anti-IgE (7).

In the present study, we have analyzed the endogenous LTE₄ concentration in urine of these children. Between the anti-IgE and the placebo group there was no significant differences in urinary LTE₄ concentration.

In contrast to these results, we were recently able to show that the combination of SIT and anti-IgE is associated with a significantly reduced *in vitro* LT release after stimulation with allergens parallel to the reduction in symptoms and the use of rescue medication (7). Analysing only data of our current study population (n = 19 out of n = 92), we observed a similar effect on the *in vitro* LT release even in this small subgroup. For these experiments, leukocytes were isolated and after *in vitro* stimulation of the blood cells with grass and birch pollen allergens, LT release (LTC₄, LTD₄, LTE₄) was quantified using an enzyme-linked immunosorbent assay (ELISA) technique (7). In response to grass and birch allergen, the LT release was significant lower after treatment with SIT + anti-IgE (A) compared with SIT + placebo (B) (grass allergen: A: 156; B: 3137 ng/l; p = 0.03; birch allergen A: 108; B: 2900 ng/l; p = 0.04). Comparing the LT levels, we observed no correlation between urinary LTE₄ concentrations and *in vitro* LT production (Spearman's correlation analysis; data not shown).

Together with our recently published data, these findings suggest that *in vitro* LT release but

not urinary LT release mirror the effect of anti-IgE treatment.

The most important difference between these two markers is that LT concentrations of isolated cells primarily reflect the *ex vivo* biosynthesis (9). The CAST-ELISA quantifies newly formed LT of stimulated leukocytes. Obviously the isolated cells showed a decreased capacity to produce LT in response to allergens after treatment with anti-IgE + SIT compared with SIT alone (7). This might be explained by the fact that sufficient anti-IgE treatment results in IgE levels near zero and can also down-regulate the high-affinity IgE receptor (FcεRI) by more than 95% (10). Down-regulation of FcεRI finally led to a diminished LT release of allergen-stimulated effector cells *in vitro*.

In contrast, endogenous urinary LTE₄ may serve as an index of *in vivo* whole body production of LT (9). Therefore other tissues and cells types other than leukocytes contribute to the *in vivo* production of LT synthesized *de novo* from cell membrane phospholipids, LTs are released from the lung tissue, from stimulated mast cells, macrophages, basophils and to a lesser extent from eosinophils. Even if measurement of LTE₄ in urine has the advantage of being a non-invasive sampling technique, which allows frequent collection of samples, our results indicate that endogenous LTE₄ levels in urine do not reflect therapeutic effects of a combined treatment with SIT + anti-IgE. In contrast to the *in vitro* LT concentration of isolated leukocytes (7), the LTE₄ levels in urine are not helpful in monitoring patients treated with anti-IgE and SIT.

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