

# The effect of treatment with montelukast on levels of serum interleukin-10, eosinophil cationic protein, blood eosinophil counts, and clinical parameters in children with asthma

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**SUMMARY:** Yüksel B, Aydemir C, Üstündağ G, Eldeş N, Kutsal E, Can M, Demirtaş S, Tomaç N. The effect of treatment with montelukast on levels of serum interleukin-10, eosinophil cationic protein, blood eosinophil counts, and clinical parameters in children with asthma. Turk J Pediatr 2009; 51: 460-465.

Interleukin (IL)-10 is an important immunoregulatory and anti-inflammatory cytokine. IL-10 levels are reduced in asthmatic airways. A regulatory mechanism involving IL-4 induced allergen-specific IL-10 production may be defective in allergic subjects, and this defect potentially contributes to more intense inflammation. The aim of this study was to define the effect of treatment with montelukast on serum levels of IL-10, eosinophil cationic protein (ECP), blood eosinophil counts, and clinical parameters (symptom score and lung function tests) in children with mild and moderate persistent asthma. Twenty-five children with mild-to-moderate persistent asthma and 25 nonatopic healthy children as controls were enrolled in the study. Patients were treated with montelukast for four weeks. Lung function tests for forced expiratory volume in 1 second (FEV<sub>1</sub>), peak expiratory flow (PEF), and forced expiratory flow between 25% and 75% (FEF<sub>25-75</sub>) were performed before and after treatment. Serum IL-10, ECP levels, and blood eosinophil counts were determined in both the control group and asthmatic children before and after treatment. The mean serum IL-10 levels were significantly lower before treatment than after treatment (1.75±0.9 pg/ml and 5.49±3.6 pg/ml; p<0.001) and in control subjects (5.6±2.8 pg/ml). After four weeks of treatment with montelukast, the mean blood eosinophil count value (608±73/mm<sup>3</sup> and 469±57/mm<sup>3</sup>; p<0.05) but not the ECP value (33.98±24.3 µg/L and 29.03±19.2 µg/L; p>0.05) was significantly decreased. After treatment with montelukast, all clinical parameters and lung function tests improved. We found no statistical correlations between the serum level of IL-10 and the serum level of ECP, eosinophil count, lung function tests, or clinical scores after treatment with montelukast.

Montelukast caused a statistically significant increase in serum IL-10 levels and decrease in peripheral blood eosinophil counts over the four-week treatment period. Our study indicates that montelukast provides clinical benefits for children with chronic asthma and produces an anti-inflammatory response by increasing serum IL-10 levels.

*Key words:* childhood asthma, eosinophil, interleukin-10, montelukast.

Asthma is one of the most common chronic diseases worldwide, and its prevalence is increasing, especially among children. Asthma is a chronic inflammatory disorder of the airways. Chronically inflamed airways are hyper-responsive; they become obstructed, and airflow

is limited (by bronchoconstriction, mucus plugs, and increased inflammation) when airways are exposed to various risk factors<sup>1</sup>. Asthmatic patients show increased production of cysteinyl leukotrienes (cys-LTs) C<sub>4</sub>, D<sub>4</sub>, and E<sub>4</sub> after allergen challenge<sup>2</sup>.

Cysteinyl leukotrienes are synthesized *de novo* from membrane phospholipids with 5-lipoxygenase and produce their effects via the LTD<sub>4</sub> (cys-LT<sub>1</sub>) receptor<sup>3</sup>. They are potent bronchoconstrictor and proinflammatory mediators that play an important role in the pathophysiology of asthma<sup>4</sup>. The cys-LTs stimulate mucous secretion, slow mucus transport, and decrease the activity of respiratory cilia<sup>5</sup>. In addition, they can induce smooth muscle contraction, cause vasodilatation, and increase vascular permeability<sup>6</sup>.

Montelukast is a potent leukotriene receptor antagonist that is effective in treating asthma symptoms in pediatric patients<sup>7</sup>. Montelukast has been shown to significantly improve forced expiratory volume in 1 second (FEV<sub>1</sub>), reduce nocturnal awakenings, improve the quality of life for children with mild persistent asthma, attenuate the immediate-phase response, and abolish the late-phase response induced by exercise challenge in asthmatic children<sup>7,8</sup>.

Interleukin (IL)-10-producing type 1 regulatory T cells (Tr1) play an important role in the control of allergic inflammation in several ways. There is a fine balance between the Tr1 and Th2 responses in healthy subjects. Tr1 cells suppress both Th2 cells and effector cells of allergic inflammation, such as eosinophils, mast cells, and basophils, by producing IL-10<sup>9</sup>. Specific Tr1 cells represent the dominant subset against allergens in healthy individuals. In contrast, there is a high frequency of allergen-specific Th2 cells in allergic individuals. Tr1 cells generated *in vitro* by antigen stimulation in the presence of IL-10 prevent Th2 sensitization and IgE production if they are adoptively transferred prior to sensitization<sup>10</sup>. The levels of IL-10 in bronchoalveolar lavage fluid of asthma patients are lower than those in healthy controls, and T cells from asthmatic children produce less IL-10 mRNA than those from healthy children. As observed in a large cohort of allergic and non-allergic children, increased IL-4, IL-5, and IL-13 are associated with allergy. IL-10 is associated with negative allergy skin tests<sup>11</sup>. IL-10 is a potent antiinflammatory and immunosuppressive cytokine that mediates its major immunosuppressive function by inhibiting both the antigen presenting cell function and the production of cytokines by macrophages and dendritic cells, leading to profound inhibition of Th1 cell-mediated

immunity<sup>12</sup>. In mice, IL-10 administration before allergen treatment induced antigen specific T cell unresponsiveness and blocked the generation of allergic inflammation. These findings indicate an association between increased IL-10 production and decreased allergic reactions<sup>13</sup>.

There are limited data regarding the changes in IL-10 levels in the serum of children with asthma. We investigated the effect of four weeks of treatment with montelukast on the serum level of IL-10, blood eosinophil count, eosinophil cationic protein (ECP), severity of asthma symptoms, and lung function tests in children with mild-to-moderate persistent asthma.

## Material and Methods

### Patients

Twenty-five children (14 boys, 11 girls) with mild-to-moderate persistent asthma (18 mild persistent; 7 moderate persistent) and 25 nonatopic healthy children (11 boys, 14 girls) as controls were enrolled in the study. Sixteen (64%) of the asthmatic patients also had allergic rhinitis. The study took place between April and October, when the exposure to dust was at a constant level and all children remained in the same environment. Diagnoses were established based on medical history, physical examination, and atopy according to the Third International Pediatric Consensus<sup>14</sup>, and we did not perform bronchial provocation testing. The atopic status of all patients was defined by positive skin-prick tests for at least one positive response to an allergen (a mean weal diameter  $\geq 3$  mm and  $\geq$  histamine control was defined as positive). Asthma symptoms were evaluated with a screening questionnaire based on the Pediatric Asthma Quality of Life Questionnaire using both daytime and nocturnal asthma symptom diary scales<sup>15,16</sup>. Patients were scored by a single observer before any laboratory measurements were made. For each day and night observation a score of 1-4 was assigned (Table I); this resulted in a minimum possible score of 0 (normal) and a maximum possible score of 4 (symptoms that affect  $>2$  daily activities/disturb sleep all night). The patients were classified according to their asthma symptoms and lung function tests (FEV<sub>1</sub> and peak expiratory flow [PEF]) into

**Table I.** Derivation of Clinical Scores for Asthma Severity

Score	Symptoms
0	No symptoms day/night
1	One or two symptoms in the day/night
2	More than two symptoms in the day/night
3	Symptoms that affect one or two daily activities/disturb sleep most of the night
4	Symptoms that affect more than two daily activities/disturb sleep all night

mild-to-moderate persistent asthma as defined in the Global Initiative for Asthma (GINA)<sup>17</sup>. In the month prior to starting the study, none of the patients had been treated with inhaled corticosteroids, LT antagonists, or long-acting  $\beta_2$ -agonists. No evidence of pulmonary infection was detected, and no patients required antibiotics. The Ethical Review Committees and Institutional Review Boards approved the study, and the parents of all subjects provided written informed consent.

### Study Design

This trial investigated the effects of 5 mg and 10 mg tablets of montelukast of sodium (Singulair; MSD, Whitehouse Station, NJ, USA) on children aged 6-12 or 12-16 years, respectively. The mean age of the children was  $9.4 \pm 2.2$  years. There were three study visits. At the first visit, patients were given a  $\beta_2$ -agonist (Ventolin; GSK, London, UK). They received 100  $\mu$ g four times daily for four weeks as needed for symptomatic relief. In addition, patients were informed of the purpose of the study and were instructed on both the scoring of asthma symptoms at home on a daily diary card and the use of the inhaler. At a second visit four weeks after the first visit, patients were treated with montelukast. Baseline measurements for lung function tests and serum IL-10, ECP, and eosinophil levels were performed during this visit. The third visit occurred after four weeks of montelukast treatment. Blood collection and lung function tests were performed on both the second and third visits. Serum samples were collected into Becton Dickinson serum separator tubes containing no anticoagulant. After clot formation was completed, samples were centrifuged at 3500 rpm for 5 minutes and frozen at  $-80^\circ\text{C}$ . Frozen samples were mixed thoroughly after thawing and recentrifuged before analysis. Repeated freeze-thaw cycles were avoided.

### Biochemical Analyses

Serum IL-10 levels were measured with a chemiluminescent immunometric assay using a DPC Immulite 1000 analyzer with commercial kits (Bio DPC, Los Angeles, USA; sensitivity limit 1.0 pg/ml, expected 95<sup>th</sup> percentile value 9.1 pg/ml). Serum ECP levels were measured by UniCAP 100 (Pharmacia & Upjohn Diagnost icsAB, Uppsala, Sweden; sensitivity limit 0.5  $\mu$ g/L, expected 95<sup>th</sup> percentile value 13.3  $\mu$ g/L) based on a fluoroimmunoassay. The number of eosinophils was measured by an automated hematology analyzer Coulter LH 750 (Beckman Coulter, USA) and expressed as the number of cells per cubic mm.

### Statistical Methods

The Wilcoxon matched pairs signed rank test was used to evaluate two related study samples before and after treatment. The comparisons between asthma patients and normal subjects were completed with the Mann-Whitney U rank sum test. The correlation study was performed using the Pearson correlation test.

### Results

All 25 patients completed the study. The characteristics of the patients and healthy control group who completed the study are given in Table II. The values of serum IL-10, ECP levels, and blood eosinophil counts in the patients and control group are also shown in Table II. The clinical scores were significantly improved for both day ( $2.92 \pm 0.75$  and  $0.64 \pm 0.35$ ;  $p < 0.05$ ) and night ( $1.44 \pm 0.49$  and  $0.32 \pm 0.32$ ;  $p < 0.05$ ) after treatment. Montelukast significantly improved asthma control (based on asthma symptom score), FEV<sub>1</sub>, PEF, and forced expiratory flow between 25% and 75% (FEF<sub>25-75</sub>), and it significantly decreased blood eosinophil count after four weeks of treatment. Four weeks of treatment with montelukast significantly increased the level of serum IL-10 in children with asthma. Montelukast had no effect on ECP levels in the serum. The value of serum IL-10, ECP, and blood eosinophil counts as well as the results of lung function tests before and after montelukast treatment for asthma are shown in Table III. We found no statistical correlations between serum IL-10 and eosinophil counts, ECP, lung function tests, or clinical scores.

**Table II.** Baseline Characteristics and Levels of Serum IL-10, ECP, and Blood Eosinophil Counts for Patients and Controls\*

	Patients (n=25) #	Control (n=25)	p
Age (years)	9.4±2.2	10.3±1.8	>0.05
Gender (F/M)	11/14	14/11	
Weight (kg)	31.18±10.9	32.48±10.8	>0.05
Height (cm)	130.5±13.0	137.9±9.5	>0.05
Duration of asthma (years)	5.7±1.8		
Allergic rhinitis (%)	16 (64%)		
Eosinophil (mm <sup>3</sup> )	608±73	278±30	p<0.001
ECP (µg/L)	33.98±24.3	13.4±11.1	p<0.001
IL-10 (pg/ml)	1.75±0.9	5.6±2.8	p<0.001

ECP: Eosinophil cationic protein. IL: Interleukin.

\*Values are presented as the mean with the standard deviation (±SD)

#Second visit measurements.

Statistically significant results are indicated in bold.

**Table III.** Effect of Treatment with Montelukast on Levels of Serum IL-10, ECP, IgE, and Blood Eosinophil Counts and Lung Function Tests\*

	Before Treatment#	After Treatment &	p
IL-10 (pg/ml)	1.75±0.9	5.49±3.6	<0.001
ECP (µg/L)	33.98±24.3	29.03±19.2	0.27
Eosinophil (mm <sup>3</sup> )	608±73	469±57	<0.05
FEV <sub>1</sub> (%)	85.9±17.9	98.8±13.1	<0.001
PEF (%)	72.6±16.1	87.6±12.3	<0.001
FEF <sub>25-75</sub> (%)	88.6±26.3	106.7±18.3	<0.001

IL: Interleukin. ECP: Eosinophil cationic protein. FEV<sub>1</sub>: Forced expiratory volume in 1 second. PEF: Peak expiratory flow. FEF<sub>25-75</sub>: Forced expiratory flow between 25-75%.

\*Values are presented as the mean with the standard deviation (±SD)

# Second visit measurements.

& Third visit measurements.

Statistically significant results are indicated in bold.

## Discussion

There are many endogenous mediators involved in the inflammation of asthma. Cys-LTs seem to play an important role in this process. The leukotriene modifiers are safe new agents for the treatment of asthma. Montelukast, a new specific LTD<sub>4</sub> receptor antagonist, binds competitively and selectively to cys-LT<sub>1</sub> receptors to block the proinflammatory effects of cys-LTs. It thus protects against the allergen-induced early and late airway responses in asthma<sup>18</sup>. Some studies have shown that montelukast significantly improves both FEV<sub>1</sub> and the quality of life, reduces nocturnal awakenings, and decreases airway inflammation in children with mild persistent asthma<sup>7,19-22</sup>. Our study showed that treatment with montelukast (following 4 weeks of β<sub>2</sub>-agonist administration per day) improved FEV<sub>1</sub>, PEF, and FEF<sub>25-75</sub> values and clinical symptom

scores in children with mild and moderate persistent asthma. The improvement in FEV<sub>1</sub>, PEF, and FEF<sub>25-75</sub> values seen in response to montelukast in our study is better than that seen in response to daily doses of 400 µg salbutamol. In the literature, there are two studies similar in design to our study. They demonstrated that similar changes in lung function tests occurred after treatment with montelukast<sup>20,21</sup>.

The normal respiratory tract is characterized by elevated concentrations of IL-10<sup>23</sup>. Thus, IL-10 production in the lungs of non-asthmatic patients may play a role in limiting pathology-inducing inflammatory Th2 responses<sup>12</sup>. In the literature, there are two studies describing changes in IL-10 levels in the serum of children with asthma. Stelmach et al.<sup>20,21</sup> showed that treatment with montelukast significantly increased serum levels of IL-10 and decreased

ECP, blood eosinophil count, and bronchial hyper-reactivity. In our study, before-treatment serum IL-10 levels were significantly decreased in comparison to those of the control group. We observed significantly increased serum levels of IL-10, decreased blood eosinophil counts, and improved FEV1 and clinical symptom scores after treatment with montelukast; however, we observed no decrease in serum ECP levels. These data suggest possible reasons for the anti-inflammatory effects of montelukast.

Some studies suggest that IL-10 may be a marker for monitoring asthma<sup>24,25</sup>. Stelmach et al.<sup>21</sup> demonstrated that one possible method by which montelukast contributes to the inhibition of inflammation is by increasing IL-10 levels. Our study showed that patients treated with montelukast had increased serum levels of IL-10 and improved asthma symptoms.

The specific mechanisms by which cys-LTs promote eosinophilia are unknown, but they may involve direct chemotaxis of eosinophils in inflamed tissues, up-regulation of adhesion molecules on eosinophils and the vascular endothelium, effects on eosinophilopoiesis, and the release of eosinophil precursors from the bone marrow or enhanced eosinophil survival<sup>20</sup>. In 201 children with moderate asthma (40% of whom were using inhaled corticosteroids), treatment with montelukast for eight weeks produced significantly greater reductions in blood eosinophil counts than placebo; concentrations of serum ECP remained unchanged<sup>26</sup>. Diamant et al.<sup>18</sup> showed that montelukast protects against the allergen-induced early and late airway responses in asthma, but this effect was not accompanied by significant changes in sputum eosinophil or ECP concentrations. Stelmach et al.<sup>22</sup> showed a significant reduction in blood eosinophils and serum ECP levels after treatment with montelukast. In our study, we observed significant decreases in blood eosinophil counts, but serum ECP levels were not significantly decreased after four weeks of treatment with montelukast.

In conclusion, our study indicates that montelukast administered once daily over a four-week period provides clinical benefits for children with mild-to-moderate persistent asthma. Although treatment with montelukast significantly increased serum IL-10 levels,

decreased blood eosinophil counts, and showed improvement in clinical findings, there was no significant correlation between the serum level of IL-10 and ECP level, eosinophil count, lung function tests, or clinical symptom scores. Our results suggest the possibility that montelukast may have effects on the parameters of inflammation in asthmatic children. Therefore, further clinical trials in larger groups of patients with asthma are required to understand this relationship more fully.

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