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Abstract: Montelukast, widely used in the treatment of asthma, is a selective and potent oral cysteinyl leukotriene-1 receptor antagonist. In this study, we investigated the effects of montelukast on oxidative stress and antioxidant defense in childhood asthma by measuring malondialdehyde and the paraoxonase activity. Twenty-five children with mild to moderate atopic asthma and 25 nonatopic children as controls were enrolled in the study. Asthmatic children were treated with montelukast, 5 mg tablets, for one month. Serum paraoxonase, malondialdehyde and high-density lipoprotein (HDL) cholesterol levels were measured before and after treatment. Serum paraoxonase and paraoxonase/HDL ratios were significantly increased after montelukast treatment. These parameters were significantly higher when compared with the normal subjects. Although montelukast treatment caused an enhancement in serum malondialdehyde, this increase did not reach statistical significance between the groups. The present study clearly demonstrates that montelukast increases paraoxonase activity in children with bronchial asthma. However, for better understanding of this enhancement, additional in vivo and in vitro studies are required.

Key Words: Montelukast, asthma, paraoxonase

Astımlı Çocuklarda Montelukast Tedavisinin Serum Paraoksonaz Aktivitesi Üzerine Etkisi

Amaç: Montelukast, astım tedavisinde yaygın olarak kullanılan selektif ve etkili oral sistein lökotrien 1 reseptör antagonistidir. Bu çalışmamızda, malondialdehit düzeylerini ve paraoksonaz aktivitesini ölçerek, çocukluk çağı astım hastalarında oksidatif hasar ve antioksidan defans üzerine montelukast'ın etkilerini araştırdık. Hafif ve orta derecede atopik astımlı 25 çocuk hasta grubu olarak ve 25 non-atopik sağlıklı çocuk kontrol grubu olarak çalışmaya dahil edildi. Astımlı çocuklar bir ay boyunca 5 mg montelukast ile tedavi edildi. Serum paraoksonaz, malondialdehit ve HDL kolesterol seviyeleri tedavi öncesi ve sonrası ölçüldü. Montelukast tedavisi sonrası serum paraoksonaz ve paraoksonaz/HDL oranı anlamlı bir artış gösterdi. Kontrol grubu ile kıyaslandığında bu parametreler belirgin olarak yüksek bulundu. Montelukast tedavisi serum malondialdehit düzeylerinde bir artışa neden olmakla beraber, bu değişim istatistiksel olarak anlamlı değildi. Bu çalışmada montelukast'ın bronşial astımlı çocuklarda serum paraoksonaz aktivitesini etkin biçimde arttırdığı görüldü. Bu konuda yapılacak in vivo ve in vitro çalışmaların bu mekanizmayı aydınlatma konusunda faydalı olabileceğini düşünüyoruz.

Anahtar Sözcükler: Montelukast, astım, paraoksonaz

Introduction

Asthma is a chronic airway disease. Although the exact mechanism of the pathogenesis of asthma is unknown, it is characterized with chronic inflammation by an activation of inflammatory cells, generation of inflammatory mediators, and epithelial cell shedding (1).

The inflammatory cells infiltrating the airways, such as macrophages, neutrophils, and eosinophils, release increased amounts of reactive oxygen species (ROS) in asthmatic patients (2). Increased production of ROS leading to an imbalance between the oxidative stress and antioxidant defense systems causes an oxidative injury in asthma (3). ROS can produce many of the pathological properties of asthma, including increased airway reactivity and secretions, production of chemoattractant molecules, and increased vascular permeability (4-6). These oxygen intermediates also affect lipid and lipoproteins and result in their oxidation yielding oxidation products. Paraoxonase, a constituent of

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high-density lipoprotein (HDL) recognized as an antioxidant enzyme, metabolizes and detoxifies biologically active lipid peroxides.

Montelukast is a potent, selective reversible oral cysteinyl leukotriene-1 (CysLT1) receptor antagonist and is widely used in the treatment of asthma (7). Many studies have established the determinants of oxidative stress and antioxidant defense in children with asthma. However, to our knowledge, this is the first study to compare antioxidant effects of montelukast through the paraoxonase activity.

Based on these findings, we investigated the effects of montelukast on oxidative stress and antioxidant defense in childhood asthma by measuring malondialdehyde and the paraoxonase activity.

Materials and Methods

Twenty-five children with mild to moderate atopic asthma and 25 nonatopic children as controls were enrolled in the study. The diagnosis was established on the basis of medical history, physical examination and atopy according to the Third International Pediatric Consensus (8); bronchial provocation test was not performed. 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 > 3 mm was defined as positive control and diluent was considered as negative control). Asthma symptoms were evaluated by a single observer before any laboratory measurements were made.

Asthma symptoms were evaluated with a screening questionnaire based on the Pediatric Asthma Quality of Life Questionnaire and both daytime and nocturnal asthma symptom diary scales (9,10). Patients were scored by a single observer before any laboratory measurements were made. For each of the day and night observations, a score of 1–4 was assigned (0: No symptoms day/night; 1: One or two symptoms day/night; 2: More than two symptoms day/night; 3: Symptoms that affect one or two daily activities/disturb sleep most of the night; and 4: Symptoms that affect more than two daily activities/disturb sleep all night), resulting in a possible minimum score of 0 and a maximum score of 4.

The patients were classified into mild to moderate asthma according to the symptoms. In the month prior to the study, none of the patients was treated with inhaled corticosteroids, leukotriene antagonist or long-acting β agonist. No evidence of pulmonary infection was detected and no patients required antibiotics. The Ethical Review Committee and Institutional Review Boards approved the study and all of the subjects gave written informed consent from their parents.

This trial investigated the effects of montelukast sodium (Singulair, MSD, NJ, USA) 5 mg tablets in children. There were three study visits. At the first visit, we enrolled 25 patients and gave them β_2-agonist (Ventolin, Glaxo Wellcome, London, UK) 100 µg four times daily for one month. They were informed of the purpose of the study and were told how to score asthma symptoms and use the inhaler. At the second visit, subjects were treated with montelukast. The third visit was after one month of the treatment. Blood collection and lung function test were performed on the same day of 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 five minutes and frozen at –80°C. Frozen samples were mixed thoroughly after thawing and recentrifuged before analysis. Repeat freeze-thaw cycles were avoided.

Malondialdehyde measurement was assayed on UV-1601 Shimadzu spectrophotometer by Hunter's method with standard 1,1,3,3 tetra-etoxypropane and results are expressed as nmol/ml (11). Paraoxonase activities were measured according to Gan et al. (12), using paraoxon as substrate, in the presence of 1 mM Ca^{+2} in 100 mM Tris-HCl buffer (pH 8.0) and results were expressed as U/L. Serum HDL-cholesterol levels were measured by enzymatic colorimetric methods with commercially available kits on Roche Integra 800.

The study group comparisons were assessed by Wilcoxon matched pairs signed rank test to evaluate two related samples before and after treatment. The comparisons between asthma patients and normal subjects were done by Mann-Whitney U rank sum test.

Results

The demographic characteristics of the subjects are outlined in Table 1 and there was no significant difference

between groups. After the treatment period, all patients experienced significant improvements in symptom scores and forced expiratory volume in 1 second (FEV1) levels (Table 2).

Serum paraoxonase and paraoxonase/HDL ratios were significantly increased after montelukast treatment. These parameters were significantly higher when compared with the normal subjects. Although montelukast treatment caused an enhancement in serum malondialdehyde, this increase did not reach statistical significance between the groups (Table 3).

Discussion

Montelukast is regarded as an effective and reliable drug of choice in the treatment of bronchial asthma (13). It has been demonstrated that montelukast reduced the number of eosinophils in bronchoalveolar lavage fluid in experimental allergic asthma and in peripheral blood, bronchial mucosa, and sputum of asthmatic subjects (14,15). Treatment period with montelukast did not significantly affect neutrophil chemotaxis or phagocytosis nor the elevated superoxide production (16). Gurer et al. (17) found that montelukast treatment increased the phagocytic and intracellular killing activity of polymorphonuclear leukocytes in patients with bronchial asthma.

Malondialdehyde level is an indicator of lipid peroxidation of the membranes that results from oxidative damage. In our study, malondialdehyde concentrations were higher after montelukast treatment and these levels were found to be higher than those of the control group, but this increase did not reach statistical significance between the groups. This observation suggests that elevated lipid peroxidation may partly be due to the free radicals generated by leukocytes or neutrophils and that montelukast is enough to prevent further increases in lipid peroxidation.

In a recent study, Ekmekci et al. (18) studied the role of paraoxonase in adult asthmatic patients and no significant difference was found between the control and patient groups. Similarly, an insignificant decrease was detected in children during bronchial asthma exacerbation (19). In accordance with these findings, in the present study, there was no difference in the plasma levels of paraoxonase before montelukast treatment compared with those of the control group. Collectively, these observations suggest that bronchial asthma causes no significant change in serum paraoxonase levels.

Paraoxonase is predominantly produced in the liver (20). Feingold et al. (21) showed that tumor necrosis factor (TNF)-α administration decreased both paraoxonase activity and mRNA levels in the hamster liver. As reported by Kumon (22), paraoxonase was

Table 1. Demographic characteristics of the subjects.

	Patients (n=25)	Controls (n=25)
Male/Female	11/14	14/11
Age (years)	9.4±0.4	10.3±0.4
Weight (kg)	31.2±.4	32.5±2.2
Disease duration (years)	5.7±0.2	-

Table 2. Clinical and functional measurements.

	Before Montelukast (n=25)	After Montelukast (n=25)
FEV1 (%)	85.9±3.6*	98.9±2.6
Symptom scores (day)	2.9±0.8*	0.7±0.4
Symptom scores (night)	1.4±0.5*	0.3±0.3

* P<0.05, compared to Montelukast-treated group.

Table 3. Comparison of parameters before and after treatment and in control subjects.

	Before Montelukast (n=25)	After Montelukast (n=25)	Control (n=25)
Paraoxonase (U/L)	211.0±123.2*	323.2±189.7**	230.5±108.7
HDL-cholesterol (mg/L)	486±116	496±128	503±108
Paraoxonase/HDL (U/mg)	4.7±3.4*	6.9±4.2**	4.9±2.8
Malondialdehyde (nmol/ml)	4.2±0.2	4.7±0.6	4.6±0.4

* P<0.05, compared to Montelukast-treated group.
** P<0.05, compared to Control group.

downregulated by TNF-α, and this was concordant with the result of paraoxonase mRNA expression by HepG2 cells in response to the cytokines. The findings of our previous trial in asthmatic children revealed that montelukast significantly reduced the serum concentration of TNF-α (23). In this study, after one month of treatment with montelukast, we observed a significant increase in paraoxonase activity. These findings and the results of the present study suggest that the increase in serum paraoxonase activity may be due to a reduction in TNF-α synthesis or secretion which directly causes liver cells to increase paraoxonase mRNA.

In conclusion, the present study clearly demonstrates that montelukast, a CysLT1 receptor antagonist, increases paraoxonase activity in children with bronchial asthma. However, for better understanding of this enhancement, additional in vivo and in vitro studies are required.

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