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Regulation of stromal cell-derived factor-1 and exhaled nitric oxide in asthmatic children following montelukast and ketotifen treatment

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Abstract

Background: Montelukast and ketotifen are oral anti-allergy medications in asthmatic children. This study investigates the modulation effect of montelukast and ketotifen on children with intermittent to mild persistent asthma as demonstrated by the levels of peak expiratory flow (PEF), asthma scores (AS), exhaled nitric oxide (eNO) and plasma stromal cell-derived factor-1 (SDF-1) concentration in a randomized, prospective study.

Methods: Fifty asthmatic children were enrolled and received 8 weeks of treatment with oral montelukast sodium 5 mg chewable tablet administered once daily, or 1 mg ketotifen, and were followed for a 4-week post-treatment washout period. ENO concentration, AS and PEF were measured before, 2, 4, 6 and 8 weeks after initial treatment, and 4 weeks after cessation of treatment.

Results: Montelukast therapy was showed to improve AS, PEF and eNO within 2 weeks and remained the improvement during the treatment period. Montelukast also significantly decreased plasma SDF-1 levels after 8 weeks of treatment. In contrast, the ketotifen treatment revealed no significant effects in these clinical parameters until 4 and 6 weeks of the therapy, and did not suppress plasma SDF-1 levels after 8 weeks of treatment. To prove whether montelukast directly suppressed SDF-1 induction, we studied effects of montelukast on the LPS-induced SDF-1 expression and SDF-1-induced chemotaxis of monocytic (THP-1) cells. Montelukast, but not ketotifen, could suppress SDF-1 expression and its related chemotaxis on THP-1 monocytic cells.

Conclusions: Leukotriene receptor antagonist, such as montelukast, may be a better non-steroid anti-inflammatory drug for mild childhood asthma in preventing airway inflammation.

Keywords: Exhaled nitric oxide; Ketotifen; Montelukast; Peak expiratory flow; Stromal cell-derived factor-1; THP-1 cell

1. Introduction

Type 2 T helper cell-derived cytokines, such as IL-4, IL-5 and IL-13 are critical in the development and progression of allergic airway diseases [1]. Stromal cell-derived factor-1 (SDF-1), known as CXCL12, is a member of the CXC chemokine family. SDF-1, delivered through the chemokine receptor CXCR4, results in the most efficacious chemoattraction of T lymphocytes [2]. Increased vascularity of bronchial mucosa in asthmatic patients related to the expression of SDF-1 suggested that SDF-1 may play a role in remodeling of the airways via angiogenesis [3]. Neutralization of SDF-1 and CXCR-4 resulted in a greater reduction in both lung allergic inflammation and airway hyperresponsiveness in mice [4]. These results suggested that SDF-1 is critical in allergic airway diseases. Exhaled

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nitric oxide (eNO) is a marker for airway inflammation and correlated with bronchial hyperresponsiveness, sputum eosinophil counts, peak flow variability, and the severity of asthma [5,6]. Measurement of eNO may be a sensitive indicator for accessing the response to anti-inflammatory therapy in children with asthma [7]. Montelukast, a specific leukotriene receptor antagonist, has been proven to improve the symptoms of asthma [8]. Ketotifen is an orally administered mast cell stabilizer and shown to be able to upregulate inducible nitric oxide synthase in an in vitro study [9].

The objective of this study is, therefore, to determine the levels of several inflammatory parameters, eNO, SDF-1, and clinical indicators, such as asthma scores (AS) and peak expiratory flow (PEF) in asthmatic children following montelukast and ketotifen therapy in a double-blind randomized study.

2. Materials and methods

2.1. Study subjects

A total of 50 children with mild persistent asthma between the age of 6 and 14 years were enrolled from the outpatient clinic at our hospital. Informed consent was obtained from the parents in every case. The children recruited for this study had mild persistent asthma (symptoms >1 time a week, PEF $>80\%$, and PEF variability between 20 and 30%), as defined by the National Asthma Education and Prevention Program (NAEPP; Ref. [10]). Subjects who had acute febrile respiratory tract infection, or were receiving corticosteroid, ketotifen or leukotriene receptor antagonist treatment within 4 weeks before the study were excluded [7]. Hospitalized patients were excluded from the study.

Children and young adults (age range, 6–14 years) who visited our health examination clinic or underwent routine check-ups of visual acuity and revealed no history of allergies or respiratory disease within 4 weeks prior to beginning the study were recruited for comparison.

2.2. Study design

The protocol was approved by the Clinical Trial Review Board of Tri-Service General Hospital. Asthmatic children were followed for a 2-week run-in period during which information regarding symptoms and beta-agonist usage was collected by diary where entry criteria were met. This study was conducted in a randomized, double-blinded fashion. Patients who were eligible for the study were randomly assigned to receive either ketotifen or montelukast for 8 weeks at an 1:1 ratio using a computer-generated randomization code. To reach a double-blind design, ketotifen and montelukast were ground to powder and prepared in capsules indistinguishable containing 1 mg of ketotifen (Zaditen, Sandoz, Canada) or 5 mg of montelukast (Singulair; Merck & Co), and were followed for a 4-week post-treatment washout period. eNO concentration, AS and PEF were measured before, 2, 4, 6 and 8 weeks after initial treatment, and 4 weeks after cessation of treatment. The AS, modified from a previous study [11], were based on clinical assessment for symptoms of coughing, wheezing, chest tightness and difficulty sleeping. In this scoring system, a score of 0–3 was assigned to each variable. The final scores ranged from 0 to 12, with 12 being the most severe condition. PEF was measured by using a peak flow meter (Astech Co, Port Washington, NY). The eNO level was measured by using a fast-response chemiluminescence analyzer (NOA 280; Seivers Instrument Inc., Boulder, CO) with the validated, single-breath technique as previously described [7]. Blood samples were collected before and after 8 weeks of treatment.

2.2.1. Preparation of monocytic THP-1 cells

The human monocytic cell line THP-1 (American Type Culture Collection, Rockville, MD) was cultured in RPMI 1640 medium (Sigma Chemical Co., St. Louis, MO) supplemented with 10% fetal bovine serum, 100 U/ml of penicillin, 100 μg/ml of streptomycin at 37 °C and 5% CO_2 in a humidified incubator. Cells were centrifuged and resuspended in fresh media in 24-well plates at a concentration of 10^6/ml for 24 h before experimental use.

2.2.2. ELISA assay

THP-1 cells were counted and stimulated with 0.2 μg/ml of lypopolysaccharide (LPS) with or without the pre-treatment of the cells with varying doses of montelukast, zafirlukast (AstraZeneca) and ketotifen (0.1–100 μM) for 2 h. Cell-free supernatants were collected 48 h after stimulation and stored at −80 °C. The SDF-1 concentrations of cell supernatants and plasma were determined using commercially available ELISA-based assay systems (R&D System). Assays were performed using the protocols recommended by the manufacturer.

2.2.3. Chemotactic assay

Monocyte chemotaxis was measured by using a 24-well Micro Chemotaxis Transwell (Corning Costar, Cambridge, MA). The cells were resuspended at 3×10^6/ml in the presence or absence of montelukast, zafirlukast, budesonide and ketotifen at various concentrations (0.1–100 μM) for 2 h and then loaded into the upper chamber of the Micro Chemotaxis chamber. The chemoattractant SDF-1 (R&D Systems) (20 ng/ml in RPMI medium with 1 mg/ml of bovine serum albumin) was added to the lower chamber. The lower and upper chambers were separated by a polycarbonate membrane (5-μm pore size). The THP-1 cells were left to transmigrate for 2 h at 37 °C in a humidified atmosphere with 5% CO_2. After the 2-h incubation, the number of monocytes that migrated to the lower compartment was determined by counting the cells under light microscopy.

To analyse the relationship between mitogen-activating protein kinases (MAPK) and SDF-1-induced chemotaxis of THP-1 cells, three MAPK inhibitors, including the