

RESEARCH ARTICLE

The effects of montelukast on eosinophilic gastroenteritis in a mouse model

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Abstract

Gastrointestinal eosinophilic (EG) is a rare and heterogeneous condition characterized by patchy or diffuse eosinophilic infiltration of gastrointestinal tissue. Pharmacological study so far has demonstrated that montelukast, an oral leukotriene receptor antagonist, might be considered in patients with this disease. The aim of this study was to evaluate the effect of montelukast on oral ovalbumin (OVA) allergen induced EG inflammation in mice and to suggest some mechanisms underlying this effect. Twenty-four mice were divided into three experimental groups: PBS control, OVA group, and montelukast treated group. The mice were sensitized intraperitoneally and challenged intragastrically with OVA, and were treated with montelukast. Gastrointestinal symptoms were observed after challenged intragastrically with OVA. Eosinophils count in blood, serum OVA specific IgE and gastrointestinal histology were evaluated. Montelukast could significantly reduce the severity of oral allergen-induced eosinophilic inflammation, villous atrophy, and associated symptoms of weight loss associated with diarrhea. Montelukast also could ameliorate OVA-induced gastrointestinal pathological lesions, which was associated with the decrease of IgE and LTD4 levels, and this might be one of the important mechanisms of montelukast that protected gastrointestinal injury from EG. These findings indicated that montelukast therapy may be a novel therapeutic approach for EG and other eosinophil-mediated diseases.

Keywords

Eosinophilic gastroenteritis, montelukast, mouse

History

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Introduction

Eosinophilic gastroenteritis (EG) is an uncommon chronic disease of the gastrointestinal tract that is characterized by abdominal pain, nausea, and diarrhea¹. EG can be classified into three types (mucosal, muscular, and subserosal) based on which level of the gut wall is predominantly involved. Eosinophilic infiltration of the gut wall is pathognomonic of the disease². The collaborative efforts of gastroenterologists, allergists, and immunologists have made significant advances in understanding of the immunopathogenesis of EG in recent years. Recently, Urek et al. have reported that leukotriene receptor antagonists may be useful as steroid sparing agents in patients with the serosal form of EG³.

Montelukast is a selective and competitive leukotriene receptor antagonist that is used primarily to treat asthma⁴. Moreover, different authors describe inhibition of the eosinophils infiltration after the use of montelukast in different animal and human models, mainly in the respiratory system. It is currently used in the treatment of eosinophilic gastroenteritis by blocking the inflammatory effects of eosinophils. However, studies report mixed results in induction of clinical and histologic remission, and the mechanism is not clear.

Meanwhile, there are not reports on the effects of montelukast in experimental EG models. Here, we have used a mouse model of oral mice ovalbumin allergen induced gastrointestinal eosinophilic inflammation to determine whether montelukast could inhibit intestinal eosinophilic inflammation and to suggest some mechanisms underlying this effect.

Materials and methods

Drugs and reagents

Montelukast was provided by Hangzhou Merck pharmaceutical Co., Ltd. (Hangzhou, China); OVA (grade V) was obtained from Sigma-Aldrich (St. Louis, MO); inject alum was from Sigma-Aldrich.

Experimental design

Female BALB/c mice obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Animals were divided into three groups ($n=8$ in each); phosphate buffered saline (PBS) control, Ovalbumin (OVA) control and Montelukast treated groups. During the entire study period, the animals were housed in cages under hygienic conditions and placed in a controlled environment with a 12-h light/dark cycle at $23 \pm 3^\circ\text{C}$ and 40–70% humidity.

Gastrointestinal eosinophilic inflammation was carried out according to previous procedure⁵. Briefly, animals were

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sensitized intraperitoneally on day 0 and day 14 (50 µg of OVA adsorbed to 1 mg of aluminum hydroxide adjuvant) (OVA, grade V, Sigma; aluminum hydroxide, Sigma) and challenged intragastrically (50 mg per animal) on six occasions with OVA on days 28, 30, 32, 35, 37 and 39 to induce gastro-intestinal eosinophilic inflammation. PBS control was sensitized and challenged with PBS instead of OVA. MK-treated mice were administered with montelukast (gavage, 3 mg/kg) once daily from d 26 to d 39.

On day 40, blood samples were collected from different groups of mice for blood eosinophil quantitation and elisa assay. The mice, then, were exsanguinated by cervical dislocation and their gastrointestinal tract processed for histopathology using a light microscope and an image analysis system.

Clinical observations and body weight

All animals were observed twice daily for clinical signs. Body weight was measured pre-test, then weekly and at sacrifice after fasting.

Blood eosinophil quantitation (EOS)

Peripheral blood was collected from different groups of mice, and blood smears were prepared and air-dried. Slides were stained with hematoxylin-eosin (H&E) and eosinophil count was performed under a light microscope.

Elisa assays

At the end of the experiment, blood was collected and serum was separated by centrifugation, and the levels of IgE and LTD4 in serum were measured using ELISA kits according to manufacture instructions (R&D Systems China Co. Ltd., Shanghai, China).

Histological analysis of stomach and jejunum

The stomach and jejunum were removed, and fixed with 4% paraformaldehyde solution for 24 h, embedded in paraffin, and 5-µm tissue sections were prepared and stained with H&E for morphologic analysis. The analysis of slides was performed by an investigator blinded to the study group.

Statistical analysis

All values were presented as mean ± SD. One-way ANOVA was used for statistical analysis of the differences between groups. $p < 0.05$ was considered statistically significant.

Results

Clinical observations and body weight

In OVA-challenged mice, villous atrophy and diarrhea were obviously found. Asthenia, hypoactivity and piloerection were also noticed in these animals. However, treatment with montelukast almost ameliorate all of them to near normal. Throughout the study, the body weights of OVA-challenged animals were shown to be consistently low ($p < 0.01$), but this adverse effect was blocked by treatment with montelukast ($p < 0.01$, Figure 1).

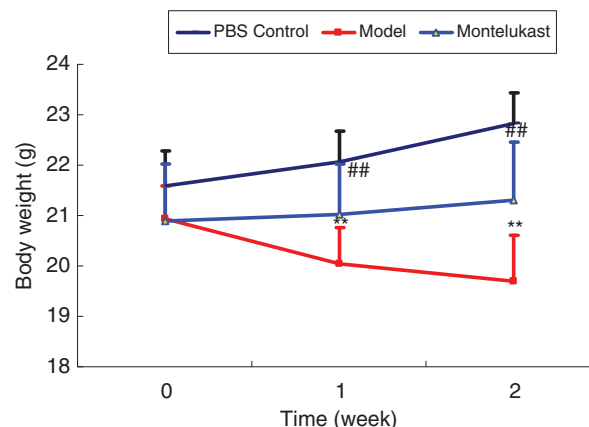


Figure 1. The effects of montelukast on the body weight of OVA-challenged animals. All values were presented as mean ± SD. One-way ANOVA was used for statistical analysis of the differences between groups. ## $p < 0.01$ versus PBS Control; ** $p < 0.01$ versus Model group.

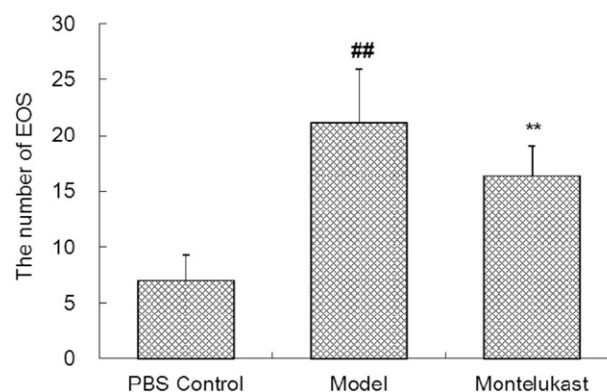


Figure 2. The effects of montelukast on the level of blood eosinophil in OVA-challenged-animals. All values were presented as mean ± SD. One-way ANOVA was used for statistical analysis of the differences between groups. ## $p < 0.01$ versus PBS Control; ** $p < 0.01$ versus Model group.

Blood eosinophil quantitation (EOS)

In model group, the level of blood eosinophil was significantly increased, indicating that the model of eosinophilic gastroenteritis has been established. However, continuous treatment with montelukast could significantly improved this parameter ($p < 0.01$, Figure 2). These results strongly suggested that montelukast significantly reduces levels of eosinophilic inflammation in a mouse model.

Elisa assays

After 2 weeks as shown in Figure 3, the levels of IgE and LTD4 were increased significantly in OVA-challenged mice ($p < 0.01$). However, administration of montelukast to oral OVA-challenged mice significantly reduced these levels ($p < 0.01$).

Histological analysis of stomach and jejunum

On histopathological examination, stomach tissues from OVA-challenged mice showed widespread structure disorder, thinning, fracture and diffused cloudy swelling as compared with PBS control group. Infiltration of the submucosa,

muscularis propria, and subserosal connective tissue by eosinophils were all abnormal in these animals. In addition, mucosal edema, focal necrosis and infiltration by eosinophils into lamina propria as well as muscularis propria were also obvious in OVA-challenged mice jejunum. After treated with montelukast, gastrointestinal tissues became typical of normal structure or mild architectural damage (Figure 4).

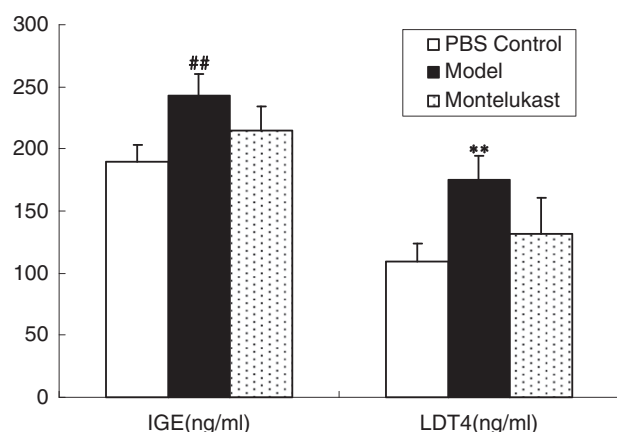
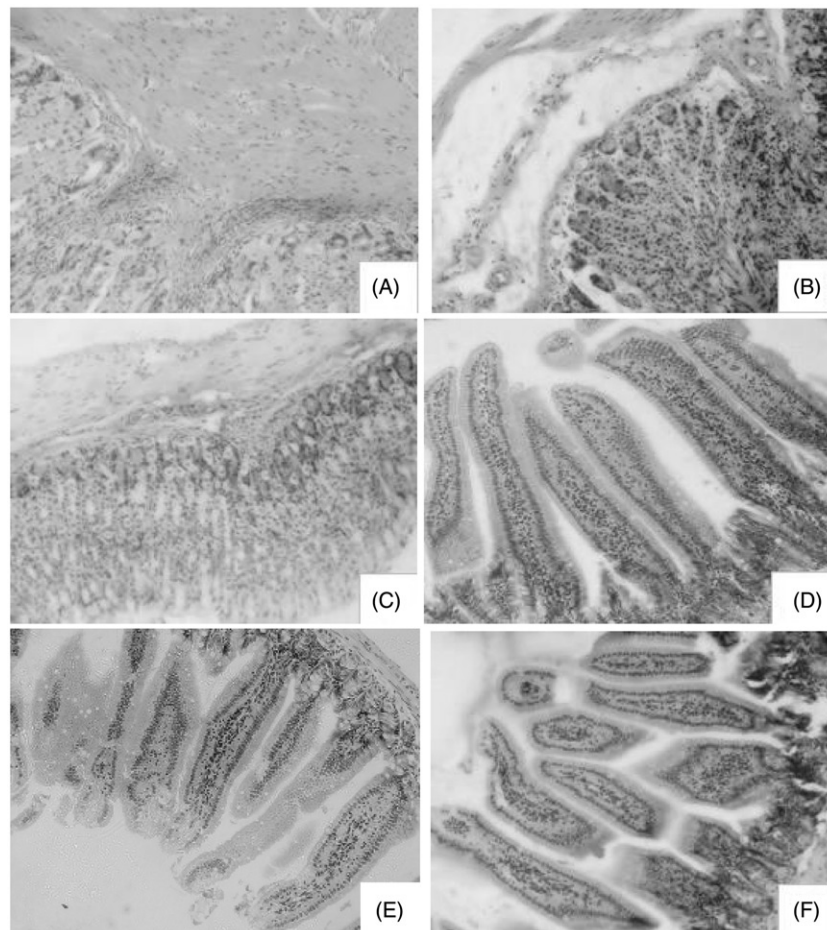


Figure 3. The effects of montelukast on the levels of IgE and LDT4 in OVA challenged-animals. All values were presented as mean $\pm$ SD. One-way ANOVA was used for statistical analysis of the differences between groups. ^{##} $p < 0.01$ versus PBS Control; ^{**} $p < 0.01$ versus Model group.

Figure 4. The effects of montelukast on histological analysis of stomach and jejunum (H&E, $\times 200$). Intragastric OVA challenge induced gastrointestinal tissue damage and infiltration of inflammatory cells when compared with control group. Widespread structure disorder, thinning, fracture and diffused cloudy swelling were observed in the tissue of stomach and jejunum. Treatment with montelukast significantly ameliorated the OVA-induced morphologic changes. A: PBS Control, stomach; B: Model group, stomach; C: Montelukast group, stomach; D: PBS Control, jejunum; E: Model group, jejunum; F: Montelukast group, jejunum.



Discussion

EG is a rare and heterogeneous condition characterized by patchy or diffuse eosinophilic infiltration of gastrointestinal tissue, first described by Kaijser in 1937^{6,7}. Pharmacological study so far has demonstrated that montelukast, an oral leukotriene receptor antagonist, might be considered in patients with this disease². However, up to now, few system studies on its gastrointestinal protective effects have been carried out.

Mouse models of allergen induced gastrointestinal eosinophilic inflammation have shed important light on the mechanisms of eosinophil trafficking to the gastroenteritis^{8–10}. In the present study, we investigated the effects of montelukast on eosinophilic gastroenteritis in OVA-challenged mice model. Our findings suggest that montelukast markedly reduces levels of eosinophilic inflammation, decreased the levels of IgE and LDT4 levels, and ameliorated gastrointestinal tissue damage.

EG is characterized by the presence of eosinophilic infiltration that can involve distinct digestive tract layers¹¹. The localized eosinophilic infiltrates may cause crypt hyperplasia, epithelial cell necrosis and villous trophy. The eosinophils are important in pathophysiology and impairment of the intestinal mucos due to eosinophil derived cytotoxic proteins. In the present study, montelukast could significantly improved the level of blood eosinophil in OVA-challenged mice. These results strongly suggested that montelukast

markedly attenuated EG as demonstrated by a significant reduction eosinophilic infiltration.

Shabnam Foroughi et al. have suggested that IgE-mediated processes play a major role in the generation of eosinophilic inflammation in EG¹². They also indicated that Anti-IgE might be a potential therapy for Eosinophil-associated gastrointestinal disorders¹². Moreover, montelukast could decrease the serum IgE level^{13,14}. In light of the previous findings, we hypothesize that montelukast can reduce IgE level associated with EG. In the present study, we found that OVA specific IgE in the blood were increased significantly in the mice challenged with OVA compared with the control groups. However, treatment with montelukast significantly attenuated it to near normal level. The overall results might be rational to understand the beneficial effects of montelukast on EG. This protective effect might be due, in large part, to the suppression of IgE and eosinophilic inflammation.

Eosinophils are the main source of the cysteinyl leukotrienes, such as LTD₄, which is lipid mediators that play major roles in the pathogenesis of allergic inflammation¹⁵. LTD₄ has been shown to be a potent and specific chemotactic factor for human eosinophils *in vitro*, which has been completely inhibited by a selective leukotriene antagonist¹⁶. Evidence for the role of cysteinyl leukotrienes in airway eosinophilic inflammation and montelukast greatly suppresses eosinophil infiltration in both pulmonary interstitium and bronchoalveolar lavage fluid of asthmatic mice have been reported¹⁷. But there were few reports about the role of cysteinyl leukotrienes in gastrointestinal eosinophilic inflammation. Our finding suggested that treatment with montelukast produced modifications in eosinophilic inflammation with a decrease in the levels of LTD₄ in OVA-challenged mice. With previous observations and our findings, a possible explanation could be that montelukast prevented EG partially due to inhibition of LTD₄ *via* suppression of eosinophilic inflammation.

On histopathological examination, gastrointestinal tissue damage and infiltration of inflammatory cells, when compared with control group, were observed in OVA-challenged mice. Widespread structure disorder, thinning, fracture and diffused cloudy swelling were also observed in these animals. Our results indicated that montelukast restored most of these pathological lesions, indicating the gastrointestinal protective action of test substance.

In summary, we have demonstrated in a mouse model of food allergen induced gastrointestinal eosinophilic inflammation that montelukast could significantly reduce the severity of oral allergen-induced eosinophilic inflammation, villous atrophy, and associated symptoms of weight loss associated with diarrhea. Montelukast also could ameliorate gastrointestinal pathological lesions in OVA-challenged mice model, which was associated with the decrease of IgE and LTD₄

levels, and this might be one of the important mechanisms of montelukast that protected gastrointestinal injury from EG. These findings indicated that montelukast therapy may be a novel therapeutic approach for EG and other eosinophil-mediated diseases.

Conflict of interest

The authors declare that there are no conflicts of interest.

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