The involvement of extracellular matrix remodeling and up-regulated TNF-α in asthma rat and the interventions of montelukast sodium

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Objective: The present research aimed to explore the involvement of extracellular matrix remodeling and up-regulated TNF-α in asthma rat and the interventions of montelukast sodium. Methods: Clean SD rats were divided into 3 groups: control, model and drug intervention. The expression of TNF-α, MMP2, MMP9 and its inhibitor TIMP1 was detected by Western Blotting. Results: The expression of TNF-α, MMP2, MMP9 and its inhibitor TIMP1 was increased in asthma lung when compared with control. These abnormalities were normalized by the medication of montelukast sodium with a statistical difference when compared with model group. Conclusions: Extracellular matrix remodeling and up-regulated TNF-α were participated in the pathogenesis of asthma lung injury and montelukast sodium alleviates the injury by normalizing those abnormal proteins expression.

1. Introduction

Bronchial asthma is the clinical common disease of which the onset has the familial aggregation and is easy to be affected by genetic and environmental factors[1-3]. Asthma may be affected by various precipitating factors such as infections, smoking, climate change and drugs. However, currently, the exact pathogenesis of asthma is still unknown. Matrix metalloproteinase is the main enzyme involved in degradation and remodeling of extracellular matrix. The up-regulated MMPs protein involves in the onset progress of various diseases, especially the pathologic change in lung tissue[4-6]. The present study aimed to analyze the involvement of extracellular matrix remodeling and up-regulated TNF-α in asthma rat and the amelioration of montelukast sodium.

2. Materials and methods

2.1. Animal and group

SD rats of clean grade weighting 220-240 g were purchased from Shanghai SLRC Laboratory Animal Co. Ltd. All the rats were fed adaptively for 1 week with free access to food. Rats were randomly divided into three groups: control, model and drug intervention groups (n=8). Sensitization injection and atomization were used to establish the rat model of asthma. Drug intervention group was given montelukast sodium for treatment. The rat model of asthma was established with the following process. The inner thighs of rats were subcutaneously injected with 1 mL sensitization agent on Day 1 and Day 8. From Day 15, atomization was performed for 56 d. After the experiment, the liquid nitrogen of lung tissue was isolated and kept for further use.

2.2. Instruments and reagents

Apparatus for vertical polyacrylamide gel electrophoresis was purchased from Bio Rad (CA, USA). Transfer membrane apparatus was purchased from Beijing Liuyi Biotechnology Co., Ltd. Refrigerated centrifuge was purchased from Eppendorf, Germany. Pipette was the production of Eppendorf, Germany. The primary antibodies were purchased from Santa Cruz Biotechnology, Inc., USA. The secondary antibodies were purchased from Abcam, USA. DAB kit was purchased from Tiangen Biotech (Beijing) Co., Ltd.
2.3. Detection of protein expression by Western Blotting

Lung tissue protein was extracted strictly followed by the instruction. BCA method was used for the total protein quantitative. Equivalent total protein was used for electrophoresis under 80 V voltage for 3 h. After electrophoresis, the protein was transferred to cellulose acetate membrane by half-dry transfer membrane method, and was sealed with skim milk for 2 h. After washing the membrane with PBS buffer, primary and secondary antibodies were used respectively to incubate the cellulose membrane, and the optical density was detected after developing and fixing. GAPDH protein was the internal reference.

2.4. Detection of the expression of related gene mRNA by fluorogenic quantitative PCR

High-purity total RNA Rapid Extraction Kit, TRIzol and chloroform were all purchased from Tiangen Biotech (Beijing) Co., Ltd. M-MLV reverse transcriptase first-strand synthesis system for RT-PCR was purchased from Invitrogen. Primers of housekeeping genes GAPDH, objective gene primers (TIMP1and TIMP2), and SYBR Premix Ex TaqTM were purchased from TaKaRa. Primer sequences are as follows: TIMP1: Forward: 5’-CGACATAGACGGCATCCAG-3’, Reverse: 5’-CTGTCGGCTGTGGTTCAGT-3’; TIMP2: Forward: 5’-CCCAGAGGTCTTTTTCCGAG-3’, Reverse: 5’-CCAGCCCATGATGGTTCTGAT-3’; GAPDH: Forward: 5’-AACGACCCCTTCATTGAC-3’, Reverse: 5’-TCCACGACATACTCAGCAC-3’.

2.5. Statistical analysis

Dada was analyzed with SPSS18.0 statistical software. Measurement data was expressed as mean±SD. One-way analysis of variance was used for the comparison among groups. P<0.05 was considered as significantly different.

3. Results

3.1. Up-regulated TNF- α expression of the lung tissue in rat model of asthma and the intervention of montelukast sodium

The present study showed that the up-regulated TNF- α expression of the lung tissue in asthma group was significantly higher than that of in control group, which had significant difference (P<0.01). Meanwhile, compared with model group, the abnormal TNF- α protein of the lung tissue in the intervention group obtained remarkable recovery, which had significant difference (P<0.01) (Figure 1).

3.2. Detection of up-regulated MMP2 protein expression of the lung tissue in rat model of asthma and the intervention of montelukast sodium by gelatin zymography

Gelatin Zymography detection found that MMP2/9 protein expression of matrix metalloproteinase of the lung tissue in rat model of asthma were significantly higher than that of in control group, which had significant difference (P<0.01). Meanwhile, compared with model group, the abnormal MMP2/9 protein of the lung tissue in drug intervention group obtained remarkable recovery, which had significant difference (P<0.01) (Figure 2).

3.3. Up-regulated mRNA expression of TIMP1/2 gene of the lung tissue in rat model of asthma and the intervention of montelukast sodium

The present study showed that the expression of mRNA TIMP1/2 protein of matrix metalloproteinase of the lung tissue in rat model of asthma was significantly higher than that of in control group, which had significant difference (P<0.01). Meanwhile, compared with model group, the abnormal mRNA TIMP1/2 gene of the lung tissue in the model rat of asthma got remarkable recovery, which had significant difference (P<0.01) (Table 1).
Compared with control, **$P<0.01$; Compared with model, ##$P<0.05$.

4. Discussion

Bronchial asthma is a chronic nonspecific inflammatory disease involved by a variety of cells and cell groups, which will cause severe damage in patients’ respiratory system and health. But currently, the exact mechanism of onset is still unclear. Montelukast sodium is a selective leukotriene receptor antagonist, which has specificity to inhibit cysteine leukotriene receptor and is suitable for the treatment and prevention of asthma[7-9]. During the analysis of extracellular matrix remodeling, up-regulated TNF-\(\alpha\) expression involved asthmatic lung tissue mechanism in rats and intervention of montelukast sodium, we found that compared with control group, TNF-\(\alpha\), matrix metalloproteinase MMP2, MMP9 and the expression of inhibitor TIMP1 of the lung tissue in model group were significantly increased, while the abnormal expression of the above proteins in the lung tissue of drug intervention group got remarkable recovery. Hence, in the onset progress of asthmatic model rats involved by extracellular matrix remodeling and up-regulated TNF-\(\alpha\) expression, montelukast sodium mainly improved the abnormal lung tissue of asthma rats by ameliorating the degradation and remodeling of extracellular matrix and up-regulated TNF-\(\alpha\) expression.

Extracellular degradation mechanism and remodeling is the common pathological process of a variety of diseases, especially pulmonary disease[10,11]. In the research of imiquimod on the lung tissue damage of chronic asthma model in mice, it was found that airway inflammation and remodeling of mice in chronic asthma group was obviously heavier than that of in control group, and the MMP9 expression of the lung tissue was significantly increased, while after the treatment of imiquimod, airway anomalies in mice got some recoveries, and the abnormal expression of MMP9 was also significantly improved, indicating that imiquimod reduced the airway anomalies of mice with chronic asthma by ameliorating the abnormal expression of matrix metalloproteinase[12]. In the study of lung tissue of bronchiectasis model rats, it was found that the expression of MMP9 and TIMP1 in the lung tissue of model group was significantly increased, and the drug Qingjinkangyuoxin can significantly recover the above abnormal proteins to play a role of the improvement of bronchiectasis[13]. In elderly patients with chronic obstructive pulmonary disease, it was also found that the ratio of serum of MMP9 and MMP9/TIMP1 was higher than that of in normal group, which indicates that the degradation and remodeling of extracellular matrix are correlated with the onset of elderly patients with COPD[14]. In the present study, the expression of related enzymes MMP2/9 and TIMP of extracellular matrix remodeling in the lung tissue of asthma model rats was significantly increased, which indicated that extracellular matrix remodeling involved in the lung damage process of asthma model rats. TNF-\(\alpha\) involved in the onset progress of a variety of models such as pulmonary fibrosis and chronic obstructive pulmonary disorder[15,16].

In the present study, we also found that TNF-\(\alpha\) expression of lung tissues in model group was significantly higher than that of in control group, and montelukast sodium can significantly recover the abnormal expression of the protein.

Therefore, extracellular matrix remodeling and up-regulated TNF-\(\alpha\) were participated in the pathogenesis of asthma lung injury and montelukast sodium alleviates the injury by normalizing those abnormal proteins expression.

References


