Calcitriol Combined With Budesonide Inhibits the Secretion and Expression of IL-33 in Mouse Airway Smooth Muscle Cells

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Research

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Abstract

Background

Bronchial asthma (asthma) is a chronic respiratory inflammatory disease characterized by reversible airflow limitation and high airway reactivity. Current studies generally show that airway remodeling is the pathologic structural basis for the occurrence of reversible airflow restriction and airway hyper reactivity[1] [2].In the above process, airway smooth muscle cell (ASMC) hyperplasia and hypertrophy are considered to be the main mechanisms of airway remodeling[3]. Calcitriol can be combined with budesonide to more effectively inhibit airway inflammation and airway remodeling and play its role in the treatment of asthma[4, 5].

Methods

The mouse airway smooth muscle cells were divided into blank group, TGF-β1 group, SIS3 group, budesonide group,calcitriol group and drug co-treatment group[6]. The IL-33 concentration in supernatant of each group was detected by ELISA method, and the expression of Smad3, pSmad3 and IL-33 protein in each group was detected by Western blotting method[7].

Results

ELISA showed that the concentration of IL-33 in the supernatant of cell culture in budesonide group was lower than that in the calcitriolgroup, and the concentration of IL-33 in the drugco-treatment group was lower than that in any single drugtreatment group.The expression level of Smad3 protein, pSmad3 protein and IL-33 protein of western blot in the drug co-treatment group were significantly decreased[8, 9].

Conclusions

Calcitriol combined with budesonide can effectively decrease the expression and secretion of IL-33 in mouseairway smooth muscle cells byactivatingTGF-β1/Smad3 signaling pathway [10].

Introduction

Bronchial asthma is a common global chronic inflammatory airway disease with a high incidence in children, which can affect all age groups. There are more than 30 million asthma patients in China, and with the improvement of living standards in recent years. Severe asthma can lead to labor loss, affecting individuals, families, and society in all aspects of life, work, study, and social interaction, with a heavy financial burden. Asthma is a chronic respiratory inflammatory disease characterized by reversible airflow limitation and airway hyper responsiveness[11]. It is composed of various cells such as eosinophils, neutrophils, mast cells, airway epithelial cells, and airway smooth muscle cells[12]. The pathological features of asthma include three aspects, namely airway inflammation, airway remodeling and airway hyper responsiveness. Many studies have shown that airway smooth muscle cells not only play an

important role in airway structure, airway function, and airway remodeling, but also participate in the regulation of airway inflammation by releasing pro-inflammatory or anti-inflammatory mediators and immunomodulatory factors[13]. Transforming growth factor (TGF)-β1/Smad signaling pathway is a classic pathway for airway smooth muscle cells to participate in the pathogenesis of asthma, and plays an important role in the process of airway remodeling. Interleukin (IL)-33 is unanimously recognized as an asthma susceptibility gene in genome-wide association studies and can play a key role in the exacerbation of rhinovirus-induced bronchial asthma. With the deepening of research, vitamin D is considered to be a ring-opening steroid hormone, which is involved in the regulation of multiple system physiological functions such as the immune system, central nervous system, reproductive system and cardiovascular system in the body. 1,25-Dihydroxyvitamin D3 (1,25-(OH)2D3, also known as calcitriol) is the most important active metabolite produced by vitamin D in the body[14]. In recent years, it has been used in anti-inflammatory and as an immunomodulator. The role of stimulating cell differentiation and anti-tumor has received attention. It plays an important role in the treatment of asthma through mechanisms such as immune regulation, anti-infection, regulation of gene expression, and enhancement of glucocorticoid response. This study explored whether calcitriol can cooperate with budesonide by constructing the corresponding bronchial asthma airway smooth muscle cell model[15], and by regulating the TGF-β1/Smad3 signaling pathway to act on airway smooth muscle cells, effectively inhibit airway inflammation and airway remodeling to play its role in the treatment of asthma[16, 17].

Methods

Cell culture

Resuscitated mouse airway smooth muscle cells were inoculated into a sterile culture dish, added with high-sugar DMEM medium containing 10% fetal bovine serum and 1% double antibody, in a cell incubator at 37°C and 5% CO2 Cultivation, when growing to more than 85% of the bottom area of the petri dish, subculture[18].

Cell grouping and processing

Cells were divided into blank group, TGF-β1 group, SIS3 group, budesonide group, calcitriol group, and drug co-treatment group. The blank group was cultured with basic medium for 49 hours; the TGF-β1 group was cultured with TGF-β1 final concentration of 10 ng/mL for 49 hours; the SIS3 group was first cultured with TGF-β1 final concentration of 10 ng/mL for 1 hour, and then changed to SIS3 with a final concentration of 10μmol/L for 48 hours; the budesonide group was first cultured with TGF-β1 with a final concentration of 10ng/mL for 1 hour, and then replaced with budesonide final concentration cultivation of 10μmol/L medium for 48 hours; calcitriol group was first cultured with TGF-β1 final concentration of 10ng/mL medium for 1 hour, and then replaced with calcitriol final concentration of 10μmol/L culture base culture for 48 hours; the drug co-treatment group first cultivated the cells with TGF-β1 final concentration of 10ng/mL medium for 1 hour, and then changed to calcitriol and budesonide final concentration of 10μmol/L medium for 48 hours[19].

Extract the cell supernatant and total protein of each group

Take the cell supernatant collected in a sterile tube, centrifuge for 20min (2000~3000r/min), and carefully collect the supernatant after centrifugation; use a protein extraction kit to extract the total protein of each group.

ELISA method was used to detect IL-33 secretion concentration of mouse airway smooth muscle cells in each group

Samples were added and enzymes were added according to the pre-prepared materials. Incubate at 37°C for 60 min. After the washing solution was prepared, the washing was repeated 5 times and patted dry. The reagent was developed in the dark for 15 min, and then the stop solution was added to stop the reaction (the blue color immediately turned to yellow at this time), the blank wells were zeroed, and the absorbance of each well was measured in sequence at 450 nm wavelength[20].

Western blotting to detect the expression of target protein of each group of cells

According to the volume of target protein molecular weight per hole sample 20 ul, electrophoresis, transfer membrane operation, such as table close after 1 h, 4 ℃ refrigerator overnight incubation resistance, day two resistance at room temperature incubation, washing and ECL luminous drops after the film, under the gel imaging showed stripe grey value analysis was carried on[21].

Statistical analysis

Each experiment is repeated at least three times. Using GraphPad Prism 8.3.0 software, the measurement data conforms to the normal distribution by Shapiro-Wilk (S-W) test, described by . One-way analysis of variance was used to compare the mean difference between multiple groups. $P<0.05$ was considered statistically significant (two-tailed). The Bonferroni method was used for multiple comparisons between groups, $\alpha'=\alpha/6=0.0083$, that is $P<0.0083$ was statistically significant in multiple comparisons.

Results

ELISA results

Blank group [(1.2400±0.1667)pg/mL], TGF-β1 group [(4.1510±0.1564) pg/mL], SIS3 group [(2.3380±0.1887) pg/mL], budesonide group [(2.6829±0.1897) pg/mL], the concentration of IL-33 secreted by mouse airway smooth muscle cells in calcitriol group [(3.0847±0.1894)pg/mL] and drug co-treatment group [(2.4425±0.1946) pg/mL]. The difference was statistical ($F=131.4$, $P<0.05$). Compared with the TGF-β1 group, the secretion of IL-33 in SIS3 group, budesonide group, calcitriol group and drug co-treatment group decreased to varying degrees ($P<0.05$), among which the drug co-treatment group secreted IL-33. The reduction in the budesonide group and the calcitriol group was more significant ($P<0.05$). As shown in Figure 1. The above results indicate that calcitriol combined with budesonide can

effectively reduce the secretion of IL-33 by airway smooth muscle cells, thereby inhibiting airway inflammation.

Western blotting results

There were statistically significant differences in the expression levels of Smad3 protein, pSmad3 protein and IL-33 protein in the airway smooth muscle cells of blank group, TGF-β1 group, SIS3 group, budesonide group, calcitriol group and drug co-treatment group Significance [(F=2576.0, P<0.05), (F=3538.0, P<0.05), (F=2621.0, P<0.05)]. Compared with TGF-β1 group [(0.8613±0.0066), (0.8274±0.0034), (0.7967±0.0037)], SIS3 group [(0.5377±0.0032), (0.5426±0.0157), (0.5739±0.0085)], budesonide group [(0.6145±0.0135), (0.6263±0.0241), (0.6300±0.0045)], calcitriol group [(0.6244±0.0037), (0.6433±0.0064), (0.6511±0.0026)] and in drug co-treatment group the expression levels of Smad3 protein, pSmad3 protein and IL-33 protein in treatment group [(0.5464±0.0015), (0.5844±0.0078), (0.5914±0.0053)] were all reduced to varying degrees (P□0.05), among which Smad3 in the drug co-treatment group the expressions of protein, pSmad3 protein and IL-33 protein were more significantly decreased than those in budesonide group and calcitriol group (P<0.05). As shown in Figure 2. The above results confirm that calcitriol and budesonide combined can effectively inhibit the expression of IL-33 protein in mouse airway smooth muscle cells through the TGF-β1/Smad3 signaling pathway[22].

Discussion

The incidence of asthma continues to increase globally, and there is currently a lack of specific clinical testing indicators and effective treatments. Studies have found that in the early stage of asthma, due to airway inflammation, airway secretions increase and airway contraction. As the inflammation continues to intensify, the airway is repeatedly damaged and repaired, resulting in substantial structural changes that cause airway remodeling[23]. This process has been considered Airway inflammation is the cause of airway remodeling. However, some studies have shown that early detection of airway remodeling and airway pathological changes can occur in early childhood. It is suggested that airway remodeling may be the same as airway inflammation, which is another independent feature in the natural course of asthma. The period of airway inflammation and airway remodeling are parallel, but there may also be interaction between the two. Plasticity is the continuation and development of airway inflammation, it is necessary to specifically intervene in airway remodeling at an early stage. At present, the relationship between airway remodeling and airway inflammation is still controversial. A large amount of research work has been carried out worldwide to explore the specific logical relationship between asthma airway inflammation and airway remodeling, so as to more accurately study the important effector nuclear specificity of asthma Molecular mechanism, which will play a positive role in formulating new and more effective asthma treatment programs.

At present, the treatment of asthma at home and abroad is emphasizing the control of airway inflammation and the relaxation of bronchial tubes. The commonly used asthma medicines generally

include inhaled glucocorticoids, β2 receptor agonists, aminophylline, and leukotriene receptor antagonists. But the effect of these drugs on airway remodeling is very unclear. In addition, the therapeutic effect of some new drugs, such as anti-TNF-α antibody and anti-IL-5 antibody in airway remodeling needs further study[24].

Calcitriol inhibition is considered to be a very important nutrient in the human body, especially in calcium and phosphorus metabolism and bone metabolism. However, current research believes that calcitriol as a steroid hormone, its role is far more than that. The traditional concept is that calcitriol can only be produced in the kidney, which is the only way to produce it. However, the study found that the catalytic rate-limiting enzyme-1α-hydroxylase during the formation of calcitriol can be widely expressed in many organs in the human body, including: immune system, hematopoietic system, lung, heart, liver, Gonads and pancreas, these organs can locally synthesize calcitriol. At the same time, studies have shown that some organs in the human body contain vitamin D receptor (VDR), so the cells that can express VDR are most likely the target cells of calcitriol. There is evidence that locally synthesized calcitriol can participate in biological behaviors such as immunoregulation, cell growth inhibition, cell differentiation, and cross-linking with other endocrine axes through autocrine and paracrine pathways. Calcitriol plays an important role in the pathogenesis of many chronic diseases, such as diabetes, schizophrenia, multiple sclerosis, cardiovascular diseases, autoimmune diseases, infections, and malignant tumors. However, in terms of the respiratory system, low levels of calcitriol in the body may be associated with respiratory viral infections, chronic obstructive pulmonary disease (COPD), bronchial asthma, interstitial lung disease, and lung cancer. In terms of bronchial asthma, studies have shown that calcitriol may play a role in the occurrence and control of asthma[25], glucocorticoid sensitivity through mechanisms involved in gene regulation, affecting lung development, anti-infection, and enhancing the strength of glucocorticoid response. When calcitriol was used to treat airway smooth muscle cells pretreated with serum from asthma patients, it was observed that calcitriol could effectively inhibit the expression of airway smooth muscle cell nuclear proliferation antigen, and the number of cells from G1 phase to S phase decreased. This suggests that calcitriol may have an inhibitory effect on airway remodeling, but further research is needed.

In this study, we observed the effect of calcitriol combined with budesonide on the expression and secretion of IL-33 in airway smooth muscle cells. 10ng/mL TGF-β1 was used to stimulate airway smooth muscle cells into a proliferating and secreting state. The negative control used TGF-β1/Smad3 signaling pathway blocker SIS3 to specifically block the modified signaling pathway. The expression and secretion of smooth muscle cells were significantly reduced, and the difference was statistically significant ($P<0.05$). The drug treatment components are budesonide group, calcitriol group and drug co-treatment group. The first two are single drug stimulation. The results show that the three groups can reduce the expression and secretion of IL-33 in airway smooth muscle cells. The difference has statistics ($P<0.05$), but the reduction effect of the budesonide group was more obvious than that of the calcitriol group, and the experimental results of the drug co-treatment group showed that compared with the single-use drug group, budesonide and calcitriol. The combination can more effectively inhibit the expression and

secretion of IL-33 in mouse airway smooth muscle cells, and this process plays a role through the TGF-β1/Smad3 signaling pathway.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Competing interests

Not applicable.

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Authors' contributions

ZN and ZWW designed experiments.ZQ and CQJ did experiments and provided experimental data.ZQ and LQS analyzed data.CQJ and HXJ wrote the manuscript.ZQ ,CQJ and ZYM edited the manuscript.All authors read and approved the final manuscript.

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Figures

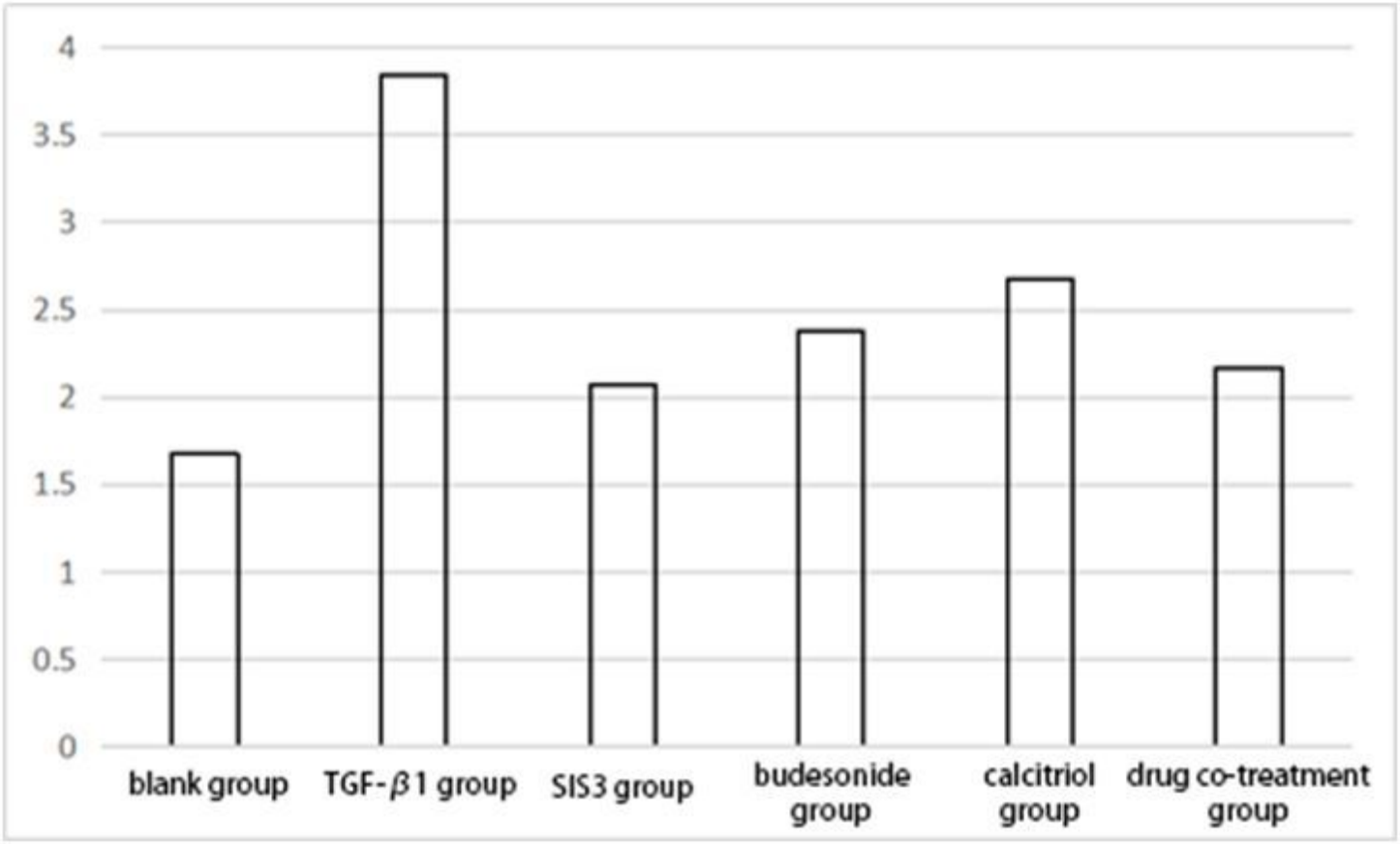

Figure 1

IL-33 concentration in supernatant after treating mouse airway smooth muscle cells with different drugs

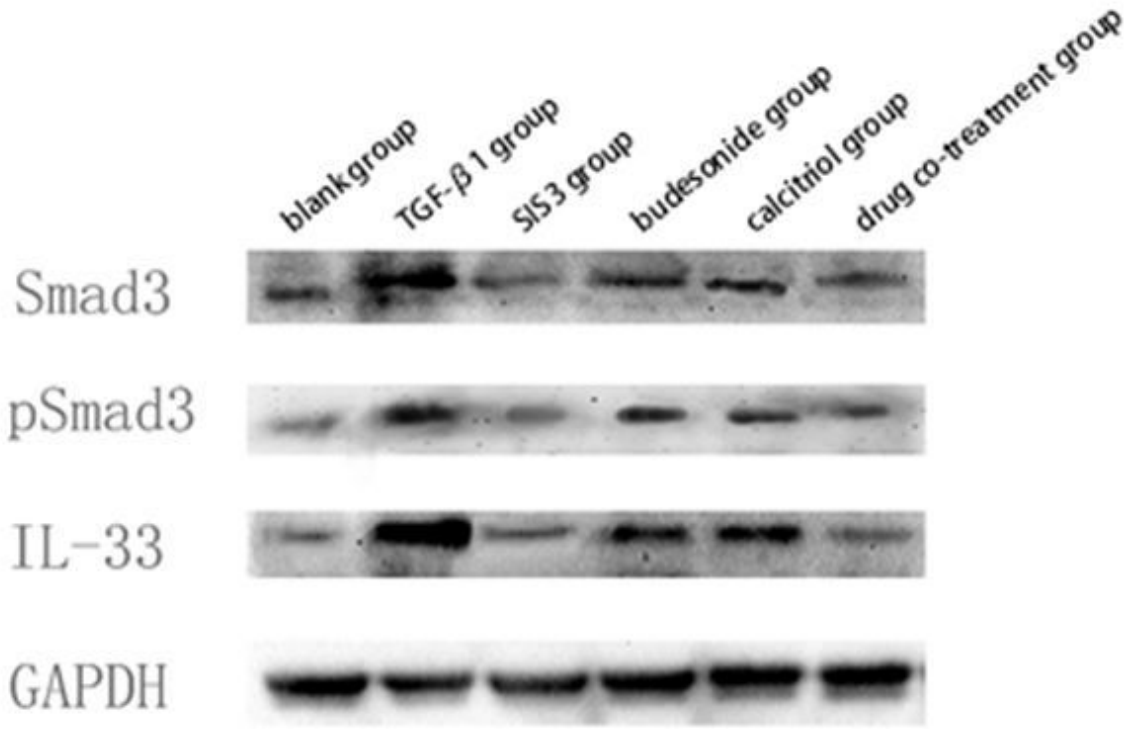

Figure 2

Effect of different drug treatments on IL-33, pSmad3 and Smad3 expressions in mouse airway smooth muscle cells