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Regulating effect of glycyrrhetinic acid on bronchial asthma smooth muscle proliferation and apoptosis as well as inflammatory factor expression through ERK1/2 signaling pathway

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ABSTRACT

Objective: To study the influence of glycyrrhetinic acid (GA) on bronchial asthma (BA) smooth muscle proliferation and apoptosis as well as inflammatory factor expression and its molecular mechanism.

Methods: Male SD guinea pigs were selected and made into asthma models, bronchial asthma smooth muscle cells were cultured and divided into BA group, GA group and GA + LM group that were treated with serum-free RPMI1640 culture medium, serum-free RPMI1640 culture medium containing 50 ng/mL glycyrrhetinic acid, serum-free RPMI1640 culture medium containing 50 ng/mL glycyrrhetinic acid and 100 ng/mL LM22B-10 respectively; normal guinea pigs were collected and bronchial smooth muscle cells were cultured as control group. The cell proliferation activity as well as the expression of proliferation and apoptosis genes, inflammatory factors and p-ERK1/2 was determined.

Results: Proliferation activity value and mRNA expression of *Bcl-2*, *TNF-α*, *IL-4*, *IL-6*, *YKL-40*, protein expression of p-ERK1/2 of airway smooth muscle cell in BA group were significantly higher than those of control group while mRNA expression levels of *Bax*, *caspase-9* as well as *caspase-3* were significantly lower than that of control group ($P < 0.05$); proliferation activity value and mRNA expression of *Bcl-2*, *TNF-α*, *IL-4*, *IL-6*, *YKL-40*, protein expression of p-ERK1/2 of airway smooth muscle cell in GA group were significantly lower than those of BA group ($P < 0.05$) while the mRNA expression levels of *Bax*, *caspase-9* as well as *caspase-3* were significantly higher than those of BA group ($P < 0.05$); proliferation activity value and mRNA expression of *Bcl-2*, *TNF-α*, *IL-4*, *IL-6*, *YKL-40* of airway smooth muscle cell in GA + LM group were significantly higher than those of GA group ($P < 0.05$) while mRNA expression levels of *Bax*, *caspase-9* as well as *caspase-3* were significantly lower that of GA group ($P < 0.05$).

Conclusion: GA can inhibit the proliferation of bronchial smooth muscle cells and reduce the expression of inflammatory factors by inhibiting the phosphorylation of ERK1/2.

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1. Introduction

Bronchial asthma (BA) is the airway chronic disease which is characterized by incompletely reversible airway limitation, the main symptoms are shortness of breath and wheeze, and abnormal bronchial smooth muscle proliferation, massive inflammatory cell infiltration and massive inflammatory factor secretion are the important pathological characteristics in local airway [1–3]. Glycyrrhetinic acid (GA) has the pharmacological activity in regulating inflammatory response, apoptosis and other biological processes [4,5]. In recent years, GA value for treatment of BA has received more and more attention, studies have confirmed that the drug has a delaying and inhibiting effect on the airway remodeling in animal models with asthma, but the specific molecular mechanism is still not clear. The abnormal airway smooth muscle proliferation and massive inflammatory cytokine infiltration mediated by extracellular signal-regulated kinase 1/2 (ERK1/2) signaling pathway are the important molecular mechanisms which causing airway remodeling in the course of BA, and the regulating effect of GA on BA smooth muscle proliferation and apoptosis as well as inflammatory factor expression through ERK1/2 signaling pathway was analyzed in the following research.

2. Materials and methods

2.1. Experimental materials

The experimental animals were male SD guinea pigs that were provided by the Experimental Animal Center of Guangzhou University of Chinese Medicine, and the certificate number was 44005900002663; GA was purchased from Nanjing Jingzhu Bio-technology Co., Ltd., and ERK1/2 agonist LM22B-10 was purchased from MCE Company; the RPMI1640 culture medium and fetal bovine serum for cell culture were bought in Hyclone Company, RNA extraction kits, cDNA synthesis kits and fluorescence quantitative polymerase chain reaction kits were bought in Promega Company, and polyacrylamide, sodium dodecyl sulfate and other reagents for western blot were bought in Sigma Company. Animal experiments passed the ethics of the Guangzhou Yuexiu District Children's Hospital, and the animal experiment operation and animal treatment after execution were carried out according to the regulations.

2.2. Experimental methods

2.2.1. Asthma model establishment

A total of 15 experimental animals were collected and made into asthma models as follows: 1 mL saline containing 1 mg ovalbumin and 100 mg aluminum hydroxide was intraperitoneally injected on the 1st and 8th day of the experiment. The experimental animals were put in atomization box for challenge from the 15th day, and ultrasonic nebulizer was used to spray into 8 mL saline solution containing 1% ovalbumin, which lasted for 30 min. It was done once every other day and continued for 8 wk. Another 15 experimental animals were collected, intraperitoneal injection of 1 mL saline was done on the 1st and 8th day of the experiment, and atomization of 8 mL saline solution was provided from the 15th day.

2.2.2. Cell culture

After animal models were made, the guinea pigs were executed to collect the airway smooth muscle tissue. It was cut into tissue blocks about 1 mm × 1 mm × 1 mm and inoculated in petri dishes. Tissue block adherence method was used to culture bronchial smooth muscle cells, and the cells were digested and sub-cultured with trypsin after the cell density reached about 80%. The 3–6 generation of cells were collected and inoculated within the culture plate and divided into control group, BA group, GA group and GA + LM group, and the treatment methods were as follows: Control group were bronchial smooth muscle cells of normal guinea pigs, inoculated in culture plate and then treated with serum-free RPMI1640 medium; BA group, GA group and GA + ERK1/2 agonist LM22B-10 group (LM group) were bronchial smooth muscle cells of guinea pigs with asthma, and the treatment methods were as follows: BA group were treated with serum-free RPMI1640 culture medium, GA group were treated with serum-free RPMI1640 culture medium containing 50 ng/mL GA and GA + LM group were treated with serum-free RPMI1640 culture medium containing 50 ng/mL GA and 100 ng/mL LM22B-10.

2.2.3. Cell viability detection

The density of digested cells was adjusted to 4×10^3/mL, the cells were added in 96-well culture plate with 200 μL/well and treated with different conditions for 24 h, then 10 μL CCK-8 detection liquid was added in each culture well and evenly mixed. Cells were placed in the incubator and continuously incubated for 2 h. At last, the absorbance at 450 nm wavelength was measured at microplate reader and used as the cell proliferation activity value.

2.2.4. RNA expression detection

The density of digested cells was adjusted to 6×10^5/mL, the cells were added in 96-well culture plate with 1.5 mL/well and treated with different conditions for 24 h, RNA extraction kit was used to separate the RNA in the cells, cDNA synthesis kit was used to synthesize the RNA into cDNA by reverse transcription, fluorescence quantitative polymerase chain reaction kit was used as last for the amplification of *Bcl-2*, *Bax*, *caspase-9*, *caspase-3*, *TNF-α*, *IL-4*, *IL-6* and *YKL-40*, and the mRNA expression of above genes were calculated according to amplification curve.

2.2.5. Protein expression detection

The density of digested cells was adjusted to 6×10^5/mL, the cells were added in 96-well culture plate with 1.5 mL/well and treated with different conditions for 24 h. Then RIPA lysate was added to extract protein. The protein sample was mixed with loading buffer and added in polyacrylamide-sodium dodecyl sulfate gel sampling well, 5% skim milk was used to close NC membrane after the completion of vertical electrophoresis and electrophoretic transfer. The p-ERK1/2 and ERK1/2 antibodies were incubated for the nigh. The next day, the HRP-labeled second antibodies were incubated for 1 h and then developed on the visualizer to obtain the protein bands of p-ERK1/2 and ERK1/2 respectively. Image J software was used to calculate the gray value and the ratio of p-ERK1/2 and ERK1/2, the ratio of p-ERK1/2 and ERK1/2 of the control group was set as 1 to calculate the protein expression of p-ERK1/2 in other groups of cells.

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