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Preliminary report

# The mechanism of alopolysaccharide protecting ulceralive colitis

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ABSTRACT

The aim of this study is to explain the mechanism of alopolysaccharide protecting ulceralive colitis in cells and animal models. We divided this study into two parts: cell and animal research parts. In the cell research, HT-29 cells were divided into normal group, model group, aloe polysacchride group and positive drug group, the cell model was used by tumor necrosis factor- α (TNF-α) combine with LPS, detecting IL-6 concentration of difference groups by Elisa testing, Apoptosis of each group was detected by flow cytometry. We detected JAK2 and STAT-3 gene expressions of difference groups by RT-PCR. WB assay was used to detect the expression of JAK2, p-JAK2, STAT-3 and p-STAT3 protein in the cells of each group. In animal experiment, SD rats were divided into normal group, model control group, aloe polysaccharide group and positive drug group. A rat model of colitis was established with 2,4,6- three nitrobenzene sulfonic acid (TNBS). IL-6 concentrations of difference groups were measured by Elisa test, and compared the colon length of difference groups, H&E staining observation of colon tissue changes in each group. We observed JAK2, p-JAK2, STAT-3 and p-STAT3 protein expression in colon tissues by immuno histochemistry and measured JAK2 and STAT-3 gene expression of difference groups by RT-PCR. We found IL-6 concentration and cell apoptosis rate of Model group were significantly up-regulation compared with normal group ($P < 0.05$, *respectively*) in the cell experiment. Compared with Model group, The IL-6 concentration and apoptosis rate of aloe polysaccharide group and positive drug group were significantly down-regulation ($P < 0.05$, *respectively*). In the gene expression, JAK2 and STAT-3 expression of aloe polysaccharide group and positive drug group were higher than model group ($P < 0.05$, *respectively*). JAK2, p-JAK2, STAT-3 and p-STAT3 protein expression of Aloe polysaccharide group and positive drug group were lower than model group. In animal experiment, compared with model group, The serum IL-6 concentration of aloe polysaccharide group and positive drug group were significantly decreased ($P < 0.05$, *respectively*), colon length was improved by drug treated, The HE stain of aloe polysaccharide and positive drug group were significantly improved. In immuno histochemistry, The JAK2, p-JAK2, STAT-3 and p-STAT3 protein expression of aloe polysaccharide and positive drug group was significantly improved, The JAK2 and STAT-3 gene expression of aloe polysaccharide group and positive drug group were signivicantly lower than those of model group ($P < 0.05$, *respectively*). Depending on the results, We supposed Aloe polysaccharide could effectively control the apoptosis of colonic tissues by inhibiting the JAK2/STAT-3 signaling pathway in vovo and vitro.



## 1. Introduction

Ulcerative colitis (UC) is a kind of recurrent chronic nonspecific inflammatory bowel disease, pathogenesis is not yet clear, cytokine IL − 6 and its mediated signaling pathways play a key role in the process of colitis [1,2]. Aloe polysaccharide (AP) extracted from aloe vera and come, for a variety of chemicals, as a result of ulcer has good protection effect, can significantly inhibit the formation of ulcers [3,4]. However, There are relatively limited that aloe polysaccharide on treatment effect and mechanism research of UC. In this study, we research AP improve colitis cell model, and the role of animal models; investigate the protective mechanism of aloe polysaccharide on colitis.

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## 2. Materials and methods

### 2.1. Cell experiments

#### 2.1.1. Experimental materials

HT-29 cell (The Chinese academy of sciences); Sulfasalazine (SASP), McCoy′s5a medium, LPS (Sigma); Fetal bovine serum (Gibco); Recombinant Human TNF-α(Pepro Tech); Aloe polysaccharide (Xi 'an tianrui biological technology co., LTD). Human IL-6 Elisa kit (EB); Primers synthesized by Shanghai sangon; RT-PCR kit (TaKaRa); Rabbit anti rat JAK2, p-JAK2, STAT3 and p-STAT3 (Cell Signaling Technology). Enzyme, electrophoresis system capillary, transblot system, chest, transfer film, the testers, Western blot exposure meter (Bio-Rad, USA); RT-PCR(TaKaRa); UVP Gel imaging system(UVP).

#### 2.1.2. Experimental groups

Dividing the growth phase HT-29 cell into four groups: normal control group (NC); Model control group (MC); aloe polysaccharide treated group (AP) and SASP treated group (SASP). NC were added in McCoy′s 5a without FBS and incubated for 24 h. MC were gave hTNF-α(20 μg $L^{-1}$) to incubate 12 h, after that, adding LPS(1 mg $L^{-1}$) for 12 h; AP and SASP groups were based the above drug intervention and respectively added AP(15 mg $L^{-1}$) or SASP (2 mmol $L^{-1}$) to incubated for 24 h.

#### 2.1.3. Detection IL-6 concentration of HT-29 cell supernatant by Elisa method

Collecting cell supernatant and transfer to Eppnedorf 1.5 mL tube, centrifugal (1600 r $min^{-1}$,5 min), The IL-6 concentration of supermatants were detected by Elisa kit.

#### 2.1.4. Apoptosis analysis assay

Cells of difference groupus were washed for at least 2 times using PBS solution. Then the cells were fixed with 70% ethanol for 30 min and rinsed 2 times with PBS solution. Subsquently, cells were labeled with annexin V-FITC and propidium iodide (PI) and incubated at room temperature for 30 min. At last, the flow cytometry (Becton Dickinson, New Jersey, USA) was performed for cell apoptotic rate detection. Tests were repeated three times.

#### 2.1.5. RT-PCR

Taking HT-29 cell, using TRIzol to cleavage, Chloroform extraction, precipitation of ISO, washing by 75% ethanol, dissolved in DEPC, and keeped in − 80 °C. Taking 2 μg total RNA reverse transcription for cDNA. Primer sequence:

JAK2:F:5′-GGAATGGCCTGCCTTACAATG-3′

R: 5′-TGGCTCTATCTGCTTCACAGAAT-3′;

STAT-3: F: 5′-CACCTTGGATTGAGAGTCAAGAC-3′

R: 5′-AGGAATCGGCTATATTGCTGGT-3′;

GAPDH: F: 5′-AGGTCGGTGTGAACGGATTTG-3′

R: 5′-GGGGTCGTTGATGGCAACA-3′;

RT-PCR reaction system: SYBR Premix Ex Taq(2×): 12.5 μL; F and R primer: 0.5 μL; cDNA: 1 μL; DEPC:9.5 μL. Reaction conditions: 95 °C for 2 min,95 °C for 5 s,57 °C for 30 s, 45 cycles. Results expressed by CT value. Gene expression in all CT gene GAPDH gene value minus CT value, relative expression with $2^{-\Delta\Delta Ct}$.

#### 2.1.6. Western blot

Collecting HT-29 cells from difference groups, Total protein was extracted at low temperature, and the concentration of protein was determined by BCA method, Samples were separated by 30 μg mass volume fraction, separated by SDS-PAGE, and transferred to the membrane. According to 1: 1000 diluted − 4 DEG C, shaking incubated overnight, washed thoroughly, according to 1:3000 diluted two anti shaking, at room temperature were incubated for 2 h, fully washing, plus ECL developer.

#### 2.1.7. Animal experiment

*2.1.7.1. Experimental materials.* Male SD rats (40), SPF, 7–9 week ages, Body weight (200 ± 50) g. 2, 4, 6-trinitrobenzenesulfonic acid (TNBS, Sigma), and the concentration is 5%(w/v).

*2.1.7.2. Experimental grouping.* Dividing the rats into 4 groups by random number table method, NC group: Giving normal saline (0.1 mL/10 g) to gavage; MC group: Making model rats by TNBS and giving normal saline (0.1 mL/10 g) to gavage; AP group: Based on MC group and adding AP (15 mg/kg); SASP group: Based on MC group and adding SASP (20 mg/kg). There were 10 rats in every group.

*2.1.7.3. Model making.* All rats in the model before the 24 h, in addition to the normal group of rats, and the rest were given ether anesthesia, before making the mold TNBS 25 mg dissolved in 0.25 mL 50% ethanol, As for choosing the diameter of sterile polyethylene 2 mm rats after complete anesthesia into the Escherichia, about 8 cm deep and TNBS- ethanol enema, after the end of the anus to maintain high for about 30s. At the end of the second days after the start of the drug treatment, 1 times a day, continuous 10d.

*2.1.7.4. H&E staining.* Samples were taken and fixed, and the samples were stained by HE, and the samples were observed by optical microscope.

*2.1.7.5. Elisa testing.* The venous blood was taken from each group of rats, and transferred to the Eppnedorf 1.5 mL tube, centrifugal (1600 r $min^{-1}$,5 min), The IL-6 concentration of supermatants were detected by Elisa kit.

*2.1.7.6. Immuno histochemical assay.* Baking dewaxing and hydration; PBS(phosphorylation antibody TBS) washed by 3 times; Microwave repair; The sodium citrate buffer were immersed in boiling pH6.0 microwave has the fire continue to heat 20 min in the microwave oven, cooled to room temperature; PBS(phosphorylation antibody TBS) washed by 3 times; Blocking endogenous peroxidase: adding 3% peroxide solution, incubation at room temperature 30 min; PBS(phosphorylation antibody TBS) washed by 3 times; Serum enclosed: drop plus normal sheep serum room temperature incubation 30 min, light thrown to the serum, do not need to rinse; incubation: adding the antibody and incubate; Color: add freshly prepared DAB solution, under the microscope observation 1min–5 min, control the color; washing, counterstained with hematoxylin 1 min; Dehydrated, transparent and mounting.

*2.1.7.7. RT-PCR.* We measured the mRNA expressions of JAK2 and STAT3 in colonic tissue, the method as like above.

*2.1.7.8. Statistical analysis.* Data are displayed as the mean ± SD. All statistical analyses were performed using SPSS 17.0 statistical software (SPSS, Chicago, IL, United States). *T*-test was used to evaluate the difference between groups. A value of $P < 0.05$ was considered statistically significant.