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**Effects of baicalin in CD4 + CD29 + T cell subsets of ulcerative colitis patients**

Yu FY *et al.* Effects of baicalin in UC

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**Abstract**

**AIM:** To evaluate the role of baicalin in ulcerative colitis (UC) including CD4+CD29+ T helper cell, its surface markers and serum inflammatory cytokines .

**METHODS:** Flow cytometry was used to detect the percentage of CD4+CD29+ cells in patients with UC. Real time polymerase chain reaction was used to detect expression of GATA-3, forkhead box P3 (FOXP3), T-box expressed in T cells (T-bet), and retinoic acid-related orphan nuclear hormone receptor C (RORC). Western blotting was used to analyze expression of NF-κB p65, p-NF-κB p65, STAT4, p-STAT4, STAT6, and p-STAT6. The concentration of IFN-γ, IL- 4, IL-5, IL-6, IL-10, and TGF-β in serum were determined by ELISA assay.

**RESULTS**: The CD4+CD29+ T cells were lower in the treatment of 40 and 20 μM baicalin than in the treatment of no baicalin. The treatment of 40 or 20 μmol/L baicalin significantly upregulated expression of IL-4, TGF-β1 and IL-10, increased p-STAT6/STAT6 ratio, but downregulated expression of IFN-γ, IL-5, IL-6, RORC, Foxp3, and T-bet, and decreased ratios of T-bet/GATA-3, p-STAT4/STAT4, and p-NF-κB/NF-κB compared to the treatment of no baicalin.

**CONCLUSION**: The results indicate that Baicalin regulates immune balance and relieves ulcerative colitis induced inflammation reaction by promoting proliferation of CD4+CD29+ cells and modulating immunosuppressive pathways.

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**Key words:** Ulcerative colitis; Baicalin; CD4+CD29+; Cytokines; NF-Κb

**Core tip:** Ulcerative colitis is a kind of chronic and non-specific inflammatory bowed disease. researchers believe that several distinguishing factors such as immune systems, genes and environment are likely necessary to result in ulcerative colitis (UC). The study here demonstrated that baicalin might be a potential immune inhibitor. It adjusted immune balance and relieved the inflammatory response caused by UC probably by inhibiting the ratio of CD4+CD29+ cells and immunosuppressive pathways. Our results provided valuable information for further studies on pathogenesis of UC and for the development of new drugs, and also provided a new view of studying T helper cell immune disorder-related diseases including UC.

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**INTRODUCTION**

Ulcerative colitis (UC) is a kind of chronic and non-specific inflammatory bowed disease (IBD) which is recurrent and debilitating. The pathogenesis of UC is not fully understood at present, but researchers believe that several distinguishing factors such as immune systems, genes and environment are likely necessary to result in UC[1]. Among immune systems, T cells (also called T lymphocytes) play a crucial role in developing UC[2]. It is known that the co-expression of CD29 and CD4 can be used as a marker for CD4+ subpopulations. CD4+CD29+ cells primarily assist B cells in producing antibodies and induce CD8+ cell-mediated dissolution of lymphocytes. The increase of CD4+CD29+ cells can result in the high activation of B cells and abnormal immune response. Hence, CD4+CD29+ cells can be used for immunology index for monitoring UC. In 1986, Mosmann *et al*[3] classified murine CD4+ T cells into two groups, T helper type 1 (Th1) and T helper type 2 (Th2), based on the cytokines they produced and their related functional activities. Since then, relationships between Th1/Th2 imbalance and immune diseases have been widely studied. The abnormal Th1/Th2 balance is traditionally considered as one of important immune factors that cause UC[4]. Many researches demonstrated that T-box expressed in T cells (T-bet)/GATA-3 and T helper type 17 (Th17)/ regulatory or suppressor T cells (Treg) balance are critical to Th1/Th2 differentiation. T-bet and GATA3 are specifically expressed on Th1 and Th2 cells, respectively. They respectively regulate Th1 and Th2 cell growth, and both determine the switching of cells from Th0 to Th1/Th2 cells[2]. The fine Th17/Treg balance is crucial for maintenance of a steady state of intestinal immunity[5]. Retinoid-related orphan receptor gamma t (RORγt) has been identified as the lineage-specific transcription factor for Th17 cells. Treg cells can secrete cytokines such as IL-4, IL-10, and TGF-β. It is closely associated with onset of immune diseases. Forkhead box P3 (FOXP3) is a specific nucleic transcription factor for Treg cells and is important in the regulation of differentiation, development, and function of Treg cells[6]. Recently, members of signal transducer and activators of transcription (STAT) family have been reported to be involved in signal transduction of cytokines and cell growth factors, and to regulate human immune response, inflammatory reaction, cell growth and differentiation, playing an important role in pathogenesis of IBD[7]. More information is needed to clarify the role of these immune factors in the UC.

Currently, the treatment of Western medicine have some shortcomings such as recuring after stopping, long-term drug-taking vice response and undesirable effects of some persistence cases. Thus, searching for a treatment of less side-effect is of importance for UC. Chinese medicine has a significant advantage on treating ulcerative colitis, and has showed an outstanding value of development and broad prospects. Baicalin is a flavonoid isolated from *Scutellaria baicalensis* in Huang Qin Tang (PHY906)[8], and is known to have an effect on multiple biological functions including the ability to modulate pro-inflammatory cytokines and inhibit aldose reductase activity[9]. Recently, researchers have paid more attention on its good therapeutic effect to UC. However, mechanisms involved in its effect are not fully understood.

In our previous study, the CD4+CD29+ T cells are significantly increased in UC patients and rats with heat-dampness[10]. In this study, we further examined the percentage of CD4+CD29+ T cells in an *in-vitro* incubation of cells isolated from peripheral blood of patients with UC. Moreover, we investigated the effects of baicalin on cell proliferation of CD4+CD29+ cells and expression of T-bet/GATA-3 mRNA, T-bet/GATA-3 mRNA, nuclear factors (NF), and cytokines by adding different concentrations of baicalin in the *in-vitro* incubation of cells. The study here would provide valuable information for better understanding the pathogenesis of UC and for developing new drugs.

**MATERIALS AND METHODS**

***Participants selection***

The patients selected for the present study were outpatients and inpatients from the Gastroenterology Department of Nanfang Hospital, Southern Medical University (Guangzhou, China) and Hospital of Guangzhou University of Traditional Chinese Medicine (Guangzhou, China) during June 2010 to January 2011. All the samples used in this study were obtained with approval of the Ethics Committee to the corresponding hospital. The whole procedure of consent was approved and documented by the Ethics Committee. Three groups (UC, D-IBS, and control group) were involved in this study. UC groups consisted thirty-three patients comprised of 18 men and 15 women with a median age of 39 (range: 22-55), and they could be divided into 2 groups (active: *n* =18; inactive=15) according to the modified Williams Disease Activity Index (DAI)[1]. The diagnosis of Irritable bowel syndrome (IBS) patients was based on the Rome III diagnostic criteria[11]. And thirty D-IBS patients were involved including 16 men and 14 women aged 18-60 with an average age of 39. Thirty Healthy examinees including 15 males and 15 females with an average of 42.5 (range: 23-62) were selected as controls.

***Preparation of peripheral blood mononuclear cells***

The serum samples (2 mL) were obtained from fasting participants in the morning and preparation of peripheral blood mononuclear cells (PBMCs) were prepared by use of Ficoll-Hypaque (Miltenyi, Germany) density gradient centrifugation. The serum was diluted by equal volume of RPMI1640 (Gibco, United States). Lymphocyte separation liquid (2 mL) was filled into a 10-mL centrifuge tube. Then, the diluted anticoagulant blood was slowly added into the tube along the wall of tube, and centrifuged at 2500 r/min. After 20 min, the mononuclear cell layer was transferred to a sterile tube by a fresh sterile pipet (capillary tube), gently mixed with five volumes of RPMI1640 and centrifuged at 2000 r/min for 10 min, then washed with RPMI1640 twice. After the supernatant was discarded, the cells were resuspended in RPMI1640 containing 10% fetal bovine serum (Gibco, United States) for lymphocyte count. Then the cells suspension were diluted to 1 × 106 cells/mL for later use.

***Cell culture and treatment***

PBMCs from UC group were plated in a 96-well plate (1 × 105 cells per well) which has incubated with CD3 antibody overnight. And PBMCs were cultured in RPMI1640 containing 10% fetal bovine serum at 37°C in 5%CO2 and stimulated with antibody against CD28. PBMCs were treated with various concentration of baicalin (5 μmol/L, 10 μmol/L, 20 μmol/L and 40 μmol/L), and DMSO as the negative control (Table 1). Three replicates were designed for each treatment.

***Flow cytometry analysis of CD4+CD29+ T cell***

To detect the cell surface expression of a variety of molecules, isolated PBMCs from fresh blood were analyzed by flow cytometry (FACS) using standard staining methods. Briefly, the prepared cell suspension fluid (1 mL) were suspended in PBS (2 mL), centrifuged at 1500 r/min for 10 min, and rinsed twice with suspension fluid. The supernatant was discarded and cells were suspended with PBS to 100 μL, added human CD4 antibody, CD29 antibody, and the other antibodies (about 1.25 μg, suggested by the manual) respectively, and incubated at 4ºC in dark for 30 min. Then, the cell suspension was added with 2 mL PBS, centrifuged at 1500 r/min for 10 min, and washed twice with suspension fluid followed by staining with flow cytometry staining buffer. Then, the percentage of CD4+ and CD29+ T cells was analyzed by the FACS Calibur (Becton Dickinson, Sunnyvale, CA) with appropriate software (CellQuest, BD Biosciences)

***Cell viability assay***

The percentage of viable cells was determined by MTS assay. Cells were collected at different points (0, 24, 48 and 72 h), and then prepared as cell suspensions (1 × 105 cells mL-1), and seeded into 96-well plates at a density of 1 × 104 per well (100 uL). The MTS reagent was added at 1:10 ratio of MTS and culture fluid, and incubated at 37ºC for 4 h. The cell viability was determined by measuring optical density of each sample at 490 nm (OD490) using a microplate reader.

***Quantitative real-time polymerase chain reaction***

Total RNA was extracted in PBMCs by Trizol method, and detected by gel electrophoresis. The RNA concentration and purity were determined by UV-visible spectroscopy (Eppendorf, Germany). The first-strand cDNA was synthesized following the instruction of cDNA synthesis kit. Then it was amplified with specific primers (Table 2) by polymerase chain reaction (PCR). The PCR condition included initial incubation at 50ºC for 2 min, at 95ºC for 2 min, followed by 40 cycles of 95 ºC for 15 s and 60 ºC for 32 s. For melting curve analysis, a melting temperature range of 60ºC-95ºC was used. The fluorescent signal was collected to obtain Ct (threshold cycle) value. Comparative real-time PCR was done in triplicate. Relative expression was calculated using comparative Ct method.

***Western blotting analysis***

The expression of NF-κB p65, p-NF-κB p65, STAT4, p-STAT4, STAT6, and p-STAT6 in UC cells was analyzed by western blotting. Cells were collected and lysed in RIPA (Beytime, China). Equal amounts of proteins from each group were separated by 10% gradient SDS-polyacrylamide gels. The proteins were transferred onto a NC membrane, blocked with 5% fat-free milk powder for 1h and incubated with primary antibodies of NF-κB p65, p-NF-κB p65, STAT4, p-STAT4, STAT6, and p-STAT6 overnight at 4°C. The membrane was rinsed in Tris-buffered saline (TBST) three times, and respectively incubated with secondary antibodies at RT for 1 h. The membrane was washed in TBST again and incubated with substrate. The NC membrane was then rinsed in deionized water. After it was air-dried, the membrane was scanned and the molecular weight and optical density of the aim band was analyzed by gel imaging system.

***ELISA detecting serum levels of IFN-γ, IL- 4, IL-5, IL-6, IL-10 and TGF-β***

The ELISA assay was conducted as the handbook provided by ELISA kit (Jingmei BioTech Co. Ltd., Shenzhen, China). The optical density of each sample at 450 nm (OD450) was detected by an ELISA microplate reader and the concentrations of IFN-γ, IL- 4, IL-5, IL-6, IL-10, and TGF-β levels in serum were determined. A standard curve was calculated using linear dilutions of the BSA supplied by the manufacturer.

***Statistical analysis***

All data are expressed as mean ± standard deviation (SD). SPSS13.0 was used to perform one-way analysis of variance (ANOVA) and a *Q* test for all intergroup comparisons. For all analyses, a probability less than an alpha value of 0.05 (*P* < 0.05) was considered to be statistically significant.

**RESULTS**

***Percentage of CD4+CD29+ and CD4+ cell in UC group***

The percentage of CD4**+** and CD4**+**CD29**+** cells was significantly (*P* < 0.01) higher in the UC group than the D-IBS or control group, but showed no significantly (*P* > 0.05) difference between the IBS and control group (Table 3). In UC group, CD4**+** cells was doubled and the percentage of CD4**+**CD29**+** cell showed a fourfold increase compared to D-IBS group and control.

***Effects of baicalin on the proliferation of CD4+CD29+ cells***

To investigate the effect of baicalin on the proliferation of CD4**+**CD29**+** cells, cells were treated with 5 μmol/L, 10 μmol/L, 20 μmol/L, 40 μmol/L baicalin and detected in 0 h, 24 h, 48 h and 72 h by MTS assay. The results presented that the proliferation of cells treated with different concentration of baicalin was obviously repressed (*P* < 0.01) after 24h compared to the control group (Figure 1). Furthermore, flow cytometry was performed 72h after treatment and we found that treatment of 20 μmol/L and 40 μmol/L baicalin significantly (*P* < 0.05) decreased the percentage of CD4**+**CD29**+** cells (Figure 2).

***Effects of baicalin on the expression of GATA-3, FOXP3, T-bet and RORC***

The expression level of GATA-3, FOXP3, T-bet and RORC in UC was detected by quantitative real-time PCR. The results indicated that GATA-3 was significantly (*P* < 0.05) decreased in UC while FOXP3, T-bet and RORC were increased compared to D-IBS group and control (Figure 3A). To test whether these proteins could be regulated by baicalin, we examined their mRNA expression in cells treated with baicalin. We found that GATA-3 mRNA level was obvious (*P* < 0.05) elevated in 5 μmol/L, 10 μmol/L and 20 μmol/L group (Figure 3B). Otherwise, FOXP3, T-bet and RORC mRNA levels were significantly (*P* < 0.05) reduced in 40 μmol/L group.

***Effects of baicalin on the expression of p-STAT4/STAT4 , p-STAT6/STAT6 and p-NK-κB/NK-κB***

The expression level of proteins were detected using western blot assay (Fig. 4A). Compared with the control and IBS group, the expression of the p-STAT6/ STAT6 ratio was significantly decreased (*P* < 0.05) while p-NK-κB/NK-κB and p-STAT4/STAT4 were significantly (*P* < 0.05) increased in UC group (Figure 4B). However, no significant difference (*P* > 0.05) was displayed between control and IBS group. The p-STAT4/STAT4, and p-NK-κB/NK-κB were significantly (*P* < 0.05) increased in the treatment of 40 and 20 μmol/L baicalin when compared with other treatment, but p-STAT6/STAT6 decreased significantly (*P* < 0.05) (Figure 4C).

***Effects of baicalin on the serum level of IL-4, IL-5, IL-6, IL-10, TGF-β1, and IFN-γ***

UC group showed significant (*P* < 0.01) lower level of IL-4, IL-10 and TGF-β1 but significant (*P* < 0.01) higher expression of IFN-γ, IL-6 and IL-5 compared with the control and IBS group (Figure 5A). The concentration of IL-6, IFN-γ and IL-5 were significantly (*P* < 0.05) lower in the treatment of 40 or 20μmol/L baicalin than in the other treatment, but IL-4, IL-10 and TGF-β1 were higher (*P* < 0.05) (Figure 5B).

**DISCUSSION**

CD4+ T cells are important in the regulation of T cells’ immune response to foreign antigens and its own in the occurrence and development of UC[12, 13,14]. CD4+CD29+ T cells are helper T cells which involved in the onset of the autoimmune diseases and can promote the production of B cell growth/differentiation factors in patients with hyperthyroidism[10]. The CD29+ T cells in peripheral blood of patients with gestational diabetes[14,15], allergic rhinitis[14], and acute hepatitis are higher than that in healthy persons[15]. However, little information is found on how baicalin regulates the activity of T cell *via* T cell subsets. Our previous study showed a significant increase in the percentage of CD4+CD29+ T cells in UC patients or rats with heat-dampness, indicating that CD4+CD29+ T cells might be involved in the onset of UC with heat-dampness. In this study, we confirmed that CD4+CD29+ T cells were significantly increased in the UC group than in the IBS and control group in PBMCs isolated from peripheral blood of patients with UC. After cells were treated with baicalin, CD4+ and CD4+CD29+ cells were significantly proliferated. The results indicated that baicalin, as an important component of Huang Qin Tang, might regulate immune function by promoting cell proliferation in the treatment of UC.

In recent years, studies have shown that Th17 is closely associated with Treg in cell differentiation and both can be transformed to one another. They are independent in the immune response but sometime show a close relationship. Disruption of the balance between Th17 and Treg resulted in diseases. It is reported that IL-23, its receptor IL-23R, and RORC play a key role in the differentiation and maintenance of Th17 and are closely related with the pathogenesis of UC[16-20]. It is also reported that the T-bet and GATA-3 balance is crucial to the regulation of Th1/Th2 differentiation. T-bet and GATA-3 can form self-activated feed-back regulation circle. They formulate a dynamic regulation network of its own and the other subpopulation specific transcription factors[21,22]. T-bet can directly bind to GATA-3 and interfere GATA-3 mediated Th2 response[23-25].

Our previous study demonstrated that Huang Qin Tang can regulate Th17/Treg balance and relieve inflammatory reaction of rats with heat-dampness UC by regulating expression of FOXP3, RORC, IL-17, IL-6, IL-10, and TGF-β, which might be one of the immune regulative mechanisms in the treatment of UC with heat-dampness by Hang Qin Tang. In this study, we further detected cytokines in blood by ELISA assay and found that the UC group had higher concentrations of IFN-γ, IL-6 and IL-5 but lower concentrations of IL-4, IL-10 and TGF-β1 than IBS and control group. Baicalin treatment, especially in 20 and 40 μmol/L, resulted in a decrease of IFN-γ, IL-5, and IL-6 and an increase of IL-4, IL-10 and TGF-β1. The expression of RORC and FOXP3 mRNA in PMBCs detected by PCR were higher (*P* < 0.05) in the UC group than the control and IBS group. When cells were treated by high concentrations of baicalin, the expression of RORC showed a significant decrease compared with FOXP3, which led to significant lower ratio of RORC/FOXP3 and indirectly regulated the balance of Th17/Treg differentiation. qRT-PCR results indicated higher T-bet and lower GATA-3 expression in the UC group, so the T-bet/GATA-3 ratio was significantly higher compared with the control group. The high T-bet expression could promote the differentiation of Th1 cells and the production of cytokines. However, the low GATA-3 expression could not promote the shifting of cells from Th0 to Th2 cell, and hence could result in high level of Th1 expression in the body, which was consistent with Hwang[26]. The treatment of 40 μmol/L baicalin displayed a significantly decrease in T-bet/GATA-3 ratio by highly decreasing T-bet. As a result, high concentrations of baicalin could reduce the ration of T-bet/GATA-3, inhibit the secretion of cytokines (IFN-γ, IL-5, IL-6), but increase concentrations of IL-4, IL-10 and TGF-β1. Thus, we proposed a novel view that baicalin might be a novel therapy target by regulating the differentiation of Th0 into Th1/Th2 in the treatment of UC. It also suggested that baicalin could correct Th1/Th2 imbalance by regulating expression of T-bet and GATA-3.

STAT6 and STAT4 belong to STAT family and they respectively participate in the differentiation of Th cells into Thl and Th2[7,27,28]. STAT4 is a transcription factor that regulates Th1 differentiation and hence its activation can cause inflammation. A study showed that the expression of p-STAT4 is high in colitis mucosa tissue of patients with UC, CD, and irritable bowel syndrome, but is highest in patients with UC. Other studies also showed that p-STAT4 expressed in UC tissues is significantly higher than normal tissues. The p-STAT4 means that STAT4 is in an active state, indicating that STAT4 is playing a role in pathogenesis of UC and the high expression of STAT4 could result in the inflammation of UC. STAT6 is one of key transcription factors that mediate Th2 and the activated STAT6 is of anti-inflammation effect. It is reported that STAT6 in colonic mucosa tissues of patients with UC is not significantly different from that of the healthy person, but it can be greatly decreased after the Chinese medicine is used, and the clinical symptoms of UC can be improved by inhibiting STAT6[29]. We detected expression of STAT4, STAT6, p-STAT4, p-STAT6, and the related cytokines of PBMC in UC cells by western blotting and found that showed p-STAT4/ STAT4 ratio was significantly higher but p-STAT6/ STAT6 ratio was lower in the UC group than the healthy control group and IBS group. In *in-vitro* culture of cells treated with baicalin, the treatment of 40 and 20 μmol/L baicalin significantly decreased p-STAT4/STAT4 ratio but increased p-STAT6/STAT6 ratio compared with groups of medium only, DMSO, 5 μM baicalin and 10 μmol/L baicalin.

Many researchers believed that immune dysfunction was a key factor that resulted in IBD. Neurath *et al*[29] firstly reported the existence of NF-κB activation in inflammatory intestine tissues in a TNBS-induced IBD animal model[30]. Schreiber *et al*[31] confirmed that NF-κB is inactive in normal human intestine tissues but is highly active in intestine tissues of patients with UC or CD and it is important for the occurrence and development of UC[32,33]. Zhang *et al*[34] reported that baicalin, used for the treatment of rats with severe pancreatitis, can reduce concentrations of the toxin and TNF- in blood, relieve intestine mucosa damage, decrease production of NF-κB, inhibit inflammation reaction, and hence protect intestine mucosa. We here detected expression of NF-κB and p-NF-κB in PBMCs of patients with UC and found that the UC group significantly increased p-NK-κB expression and p-NK-κB/NK-κB ratio but decreased NK-κB expression. In the i*n-vitro* culture of PBMCs treated with baicalin, 40 μmol/L baicalin significantly lowered p-NK-κB/NK-κB ratio, indicating that baicalin could and regulate immune balance and relieve inflammation response caused by UC by decreasing p-NK-κB/NK-κB ratio.

The study here demonstrated that baicalin might be a potential immune inhibitor. It adjusted immune balance and relieved the inflammatory response caused by UC probably by inhibiting the ratio of CD4+CD29+ cells and immunosuppressive pathways[35]. Our results provided valuable information for further studies on pathogenesis of UC and for the development of new drugs, and also provided a new view of studying T helper cell immune disorder-related diseases including UC.

**COMMENTS**

***Background***

Ulcerative colitis (UC) is a kind of chronic and non-specific inflammatory bowed disease (IBD) which is recurrent and debilitating. The pathogenesis of UC is not fully understood at present, but researchers believe that several distinguishing factors such as immune systems, genes and environment are likely necessary to result in UC. Among immune systems, T cells (also called T lymphocytes) play a crucial role in developing UC.

***Research frontiers***

Baicalin is a flavonoid isolated from *Scutellaria baicalensis* in Huang Qin Tang (PHY906), and is known to have an effect on multiple biological functions including the ability to modulate pro-inflammatory cytokines and inhibit aldose reductase activity. Recently, researchers have paid more attention on its good therapeutic effect to UC.

***Innovations and breakthroughs***

In authors previous study, the CD4+CD29+ T cells are significantly increased in UC patients and rats with heat-dampness. In this study, authors further examined the percentage of CD4+CD29+ T cells in an *in-vitro* incubation of cells isolated from peripheral blood of patients with UC. Moreover, we investigated the effects of baicalin on cell proliferation of CD4+CD29+ cells and expression of T-bet/GATA-3 mRNA, T-bet/GATA-3 mRNA, nuclear factors (NF), and cytokines by adding different concentrations of baicalin in the *in-vitro* incubation of cells. The study here would provide valuable information for better understanding the pathogenesis of UC and for developing new drugs.

***Applications***

The study results suggest that Baicalin regulates immune balance and relieves ulcerative colitis induced inflammation reaction by promoting proliferation of CD4+CD29+ cells and modulating immunosuppressive pathways. The study here provided valuable information for further studies on pathogenesis of UC and for the development of new drugs.

***Terminology***

Baicalin have an effect on multiple biological functions including the ability to modulate pro-inflammatory cytokines and inhibit aldose reductase activity. Baicalin regulates immune balance and relieves ulcerative colitis induced inflammation reaction by promoting proliferation of CD4+CD29+ cells and modulating immunosuppressive pathways.

***Peer review***

This is a good descriptive study in which the authors analyzed the preventive effect of Baicalin regulates immune balance and relieves ulcerative colitis induced inflammation reaction by promoting proliferation of CD4+CD29+ cells and modulating immunosuppressive pathways.

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**Table 1** **Treatments designed for the *in-vitro* culture of peripheral blood mononuclear cells**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **No.** | **1** | **2** | **3** | **4** | **5** | **6** |
| Treatment | Cell (PBMCs of UC group) | DMSO | 5 μmol/Lbaicalin | 10 μmol/L baicalin | 20 μmol/L baicalin | 40 μmol/L baicalin |

**Table 2** **Polymerase chain reaction primers used in this study**

|  |  |
| --- | --- |
| **Gene name** | **Primer** |
| GATA3 | Forward | AGTTGGCCTAAGGTGGTTG |
| Reverse | CACGCTGGTAGCTCATACAC |
| FOXP3 | Forward | CAGGATCTGAGGTCCCAACA |
| Reverse | TTTGGCAAGGCAGTGTGTGT |
| T-bet | Forward | CTGCATATCGTTGAGGTGAA |
| Reverse | GTAGGCAGTCACGGCAATGA |
| RORC | Forward | CAAGACTCATCGCCAAAGCA |
| Reverse | CAGTGCTGAAGAGCTCCTTG |
| 18S | Forward | CCTGGATACCGCAGCTAGGA |
| Reverse | GCGGCGCAATACGAATGCCCC |

**Table 3 Percentage (%, mean ± SD) of CD4+CD29+ cells in peripheral blood of participants of each group**

|  |  |  |
| --- | --- | --- |
| **Groups** | **CD4+ cells** | **CD4+CD29+ cells** |
| UC group | 41.56 ± 3.6 a | 8.33 ± 1.25a |
| D-IBS group | 20.12 ± 3.4 | 2.42 ± 1.12 |
| Control group | 19.37 ± 4.1 | 1.91 ± 2.35 |

a*P* < 0.05 *vs* control group.

**Figure 1 Cell proliferation rate (%) of CD4+CD29+ cells affected by baicalin *in-vitro*.**b*P<* 0.01 *vs* control group (cell).

****

**Figure 2 Percentage of CD4+CD29+ cells detected by flow cytometry at 72 h after cells were incubated in different concentration of baicalin *in vitro*.** a*P <* 0.05 *vs* control group (cell).



**Figure 3 Relative expression of GATA-3, T-bet, FOXP3 and RORC by qRT-PCR.** A: GATA-3, T-bet, FOXP3 and RORC expression level in tissues from normal, D-IBS and UC patients. b*P <* 0.01 *vs* control group (normal). B: GATA-3, T-bet, FOXP3 and RORC expression level in cells treated with 5 μmol/L, 10μmol/L, 20 μmol/L and 40 μmol/L baicalin. d*P <* 0.01 *vs* control group (cell).



**Figure 4** **Expression of proteins in tissues and cells treated with different concentration of baicalin by western blot assay.** A: western blot assay was performed to detect the expression level of STAT-, STAT4, NF-κB and their phosphorylated proteins in tissues and cells treated with baicalin. GAPDH was used as the control; B: The ratio of p-STAT6/STAT6, p-STAT4/STAT4 and p-NF-κB/NF-κB in normal, D-IBS and UC tissues; C: The ratio of p-STAT6/STAT6, p-STAT4/STAT4 and p-NF-κB/NF-κB in cells treated with 5 μmol/L, 10 μmol/L, 20 μmol/L and 40 μmol/L baicalin. f*P <* 0.01 *vs* control group.



**Figure 5 Relative expression of several cytokines in tissues and cells treated with different concentration of baicalin by ELISA assay.** A: Cytokines were detected in normal, D-IBS and UC serum samples; B: Relative expression of cytokines in the supernatant of cells treated with 5 μmol/L, 10 μmol/L, 20 μmol/L and 40 μmol/L baicalin; f*P <* 0.01 *vs* control group.





