Inhibition of Th1/Th17 responses via suppression of STAT1 and STAT3 activation contributes to the amelioration of murine experimental colitis by a natural flavonoid glucoside icariin

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1. Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder in the intestine which involves overproduction of pro-inflammatory cytokines and excessive functions of inflammatory cells. However, current treatments for IBD may have potential adverse effects including steroid dependence, infections and lymphoma. Therefore new therapies or drug candidates for the treatment of IBD are desperately needed. In the present study we found that icariin, a major bioactive compound from plants in Epimedium family, exerted protective effect on intestinal inflammation in mice induced by dextran sulfate sodium. Oral administration of icariin significantly attenuated the disease progression and alleviated the pathological changes of colitis. It also inhibited the production of pro-inflammatory cytokines and expression of p-p65, p-STAT1 and p-STAT3 in colon tissues. Further study showed that icariin dose-dependently inhibited the proliferation and activation of T lymphocytes, and suppressed pro-inflammatory cytokine levels of activated T cells. Moreover, icariin treatment inhibited the phosphorylations of STAT1 and STAT3 in CD4+ T cells, which were the crucial transcription factors for Th1 and Th17 respectively. Taken together, these results indicate that icariin is a potential therapeutic agent for IBD.

Keywords: Icariin, DSS-induced colitis, Th1/Th17, STAT1, STAT3

1. Introduction

Inflammatory bowel disease (IBD), consisting of Crohn’s disease (CD) and ulcerative colitis (UC), is a chronic inflammatory condition of the intestine which not only impairs patients’ quality of life, but also increases the risk of colon cancer. Epidemiology studies show that both CD and UC have a high prevalence of over 200 per 100,000 individuals in the US, which is still increasing over time [1,2]. The pathogenesis of IBD is complex and may involve genetic, environmental and immunological factors. It is suggested that intestinal immune responses activated by bacterial antigens and subsequent overproduction of inflammatory cytokines may play a key role in this disease [3]. CD4+ T cells, also known as T helper (Th) cells, are involved in the process of IBD [4]. Excessive function of Th1 cells which produce interferon-γ (IFN-γ) is generally associated with intestinal inflammation especially in CD. Recently, Th17 cells which are characterized by production of interleukin-17 (IL-17), are also identified as an important pathogenic factor [5]. Therefore, targeting Th1 and Th17 may serve as a potential approach to control intestinal inflammation.

Various animal models have been induced to mimic the process of IBD in human, among which dextran sulfate sodium (DSS)-induced colitis is one of the most commonly used. By oral administration of DSS, mice develop an inflammatory condition in the intestine associated with clinical symptoms (diarrhea, abdominal pain, weight loss), histopathological features and changes in cytokine profile, which resembles IBD in human [6-8]. Here we use this method to induce colonic inflammation in mice and search for potential therapeutic compounds for IBD.

Icariin, a natural flavonoid glucoside isolated from plants in Epimedium family, has been proved to have various pharmacological activities. Studies suggest that icariin has antioxidant [9-11], antidepressant [12-14], anti-inflammatory [15-17] and neuroprotective effects [18-20] in vitro and in vivo. It also shows positive effects on stimulating osteoblast activity in bone tissues [21-23] and promoting cardiovascular functions [24]. In the present study we found that icariin exerted an immunomodulatory activity. It ameliorated DSS-induced colitis in mice by regulating cytokine profiles and signaling pathways of STAT1 and STAT3 in CD4+ T cells, and thus provided a potential therapeutic approach for the treatment of IBD.
2. Materials and methods

2.1. Mice

Female C57BL/6 mice were purchased from Model Animal Genetics Research Center of Nanjing University (Nanjing, China) and housed in specific-pathogen-free (SPF) facility. Mice used in colitis model were 8 weeks of age, 19–20 g. Mice used for in vitro experiments were 6–8 weeks of age. Animal welfare and experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China, 2006) and related ethical regulations of Nanjing University.

2.2. Cells and reagents

Naïve T cells were purified from lymph nodes or spleens of C57BL/6 mice using Mouse Pan T Cell Isolation Kit (Miltenyi Biotec, Auburn, CA) according to the manufacturer’s instructions. Cells were incubated in RPMI 1640 medium supplemented with 100 U/ml of penicillin, 100 µg/ml of streptomycin and 10% NBS under a humidified 5% (v/v) CO2 atmosphere at 37 °C. Icariin (2-(4’-methoxyphenyl)-3-rhamnoso-5-hydroxyl-7-glucosido-8-(3’-methyl-2-butyleny)-4-chromonene, purity > 98%, chemical structure shown in Fig. 1A, purchased from Xi’an Tongjiang Biotechnology Co., Ltd., Xi’an, China) was dissolved at a concentration of 30 mM in 100% DMSO as a stock solution, stored at –20 °C, and diluted with medium before each experiment. The final DMSO concentration did not exceed 0.1% throughout the study (all the control groups are composed of 0.1% DMSO). Dextran sulfate sodium (DSS), cyclosporin A (CsA), sodium carboxymethyl cellulose (CMC), concanavalin A (Con A) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenytlazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO). Antibodies for p-STAT1 and p-STAT3 were purchased from Epitomics (Burlingame, CA). Antibodies for STAT1 and actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibody for STAT3 was purchased from BD Biosciences (San Jose, CA). Antibodies for p-p65 and p65 were purchased from Cell Signaling Technology (Beverly, MA). FITC-anti-CD4, PE-anti-IFN-γ, APC-anti-IL-17A, APC-anti-CD25 were purchased from eBioscience (San Diego, CA). FITC-anti-CD69 was purchased from Biologend (San Diego, CA). Recombinant murine IFN-γ and IL-6 were purchased from Peprotech (Rocky Hill, NJ). Alexa Fluor 594 goat anti-rabbit IgG and 4’,6-diamidino-2-phenylindole (DAPI) were purchased from Invitrogen (Carlsbad, CA). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

2.3. Induction and evaluation of colitis

Mice were fed with 2.5% (wt/vol) DSS dissolved in drinking water for 7 days, and then with normal drinking water for 3 days till sacrificed. Control mice were given normal drinking water without DSS. Icariin (3, 10 mg/kg) and CsA (10 mg/kg) were dissolved in 0.5% CMC solution and orally administered daily from initiation of induction (day 0). Mice were monitored for body weight loss, rectal bleeding and diarrhea. The disease activity index (DAI) which reflects disease severity was calculated as we previously reported [25]. In brief, DAI was calculated as the mean value of the following three parameters: a) body weight loss (0 point = no loss, 1 point = 1–5% loss, 2 points = 5–10% loss, 3 points = 10–15% loss, 4 points = over 15% loss); b) diarrhea (0 point = normal, 2 points = loose stools, 4 points = watery diarrhea); c) hematochezia (0 point = no bleeding, 2 points = slight bleeding, 4 points = gross bleeding).

On day 10 following induction, mice were sacrificed. Entire colon was quickly removed and washed with phosphate-buffered saline (PBS). Colon length was measured. Segments of colon were fixed in formalin, embedded in paraffin, sectioned at 4 µm thickness and stained with haematoxylin and eosin (H&E) for histological analysis.

2.4. Quantitative RT-PCR

Total RNA was extracted from colon tissues and reverse transcribed to cDNA. Quantitative PCR was performed with the ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA) using SYBR Green I dye (Biotium, Inc.). Threshold cycle numbers were obtained using ABI Prism 7000 SDS software version 1.0. PCR cycling conditions were as follows: 1 cycle of 94 °C for 5 min followed by 40 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 45 s. The primer sequences used were as follows: nfα-forward TGAACCTCCGGGTATCGGTCT, reverse AGCCCTTGTCCCTTGAAGAAAC; il-1B forward CAAAGTCCCTTGTGCAAGTA, reverse AAGCCGCAAAGTCATCAGTG; ifn-γ-forward TGAATTTGCAAAATTTTGCGCA, reverse CCGCAACACCTGTGGGAA; il-17A forward TGCAAGAGATGCTGCGGATT, reverse CTCTGTTTAGGCTGTCGC; il-17F forward CCCAGGTCCAGGAAAGACA, reverse CGCAAGACCAGATTCT; β-actin-forward TGCTGTCCCTGATGGC, reverse TTTGATGTCACGCACGATTT. Relative mRNA (mRNA) expression was calculated as a ratio to actin.

2.5. Cytokine assay

Cytokine levels were measured using ELISA kits from Dakewe Biotech Co. Ltd (Shenzhen, China) according to the manufacturer’s instructions.

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Fig. 1. (A) Chemical structure of icariin (ICA, C15H24O15, molecular weight = 676.7); (B) HPLC analysis of icariin. The purity of icariin was confirmed to be 98% by HPLC. Icariin was applied to a C18 column and eluted with CH3OH/H2O (45/55, v/v). The effluents were detected under 270 nm. Column temperature was set at 25 °C and the flow rate was 1 ml/min.
2.6. Intracellular staining

Mesenteric lymph node cells from DSS-colitis mice were collected on day 7 of induction and activated with cell stimulation cocktail (eBioscience, San Diego, CA, containing phorbol 12-myristate 13-acetate (PMA), ionomycin, brefeldin A and monensin) in 37 °C for 4 h. Cultured cells were stained with FITC-conjugated anti-CD4 for 30 min followed by fixation and permeabilization, and then stained with PE-conjugated anti-IFN-γ and APC-conjugated anti-IL-17A for 45 min. Samples were analyzed by flow cytometry.

2.7. Western blot

Proteins were extracted in lysis buffer, separated by SDS-PAGE and electrophoretically transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore Corp., Bedford, MA). The membranes were blocked with 5% nonfat milk for 1 h at room temperature, probed with antibodies overnight at 4 °C, and then incubated with a HRP-coupled secondary antibody. Detection was performed using a LumiGLO chemiluminescent substrate system (KPL, Guildford, UK).

2.8. Immunohistochemistry (IHC)

Paraffin-embedded colon sections were heat-fixed, blocked with 3% H₂O₂, and incubated with specific antibodies overnight at 4 °C. Detection was done using Real Envision Detection kit from GeneTech Company (Shanghai, China) according to the manufacturer’s instructions.

2.9. MTT cell proliferation assay

Purified T cells were cultured in 96-well plates at a density of 3 × 10⁵ cells/well in RPMI 1640 medium (0.2 ml) and stimulated with 5 μg/ml Con A in the presence of various concentrations of icariin for 72 h at 37 °C. 20 μl of MTT (4 mg/ml in RPMI 1640) was added per well 4 h before the end of incubation. MTT formazan production was dissolved by DMSO and the optical density at 570 nm (OD₅₇₀) was measured by a microplate reader.

2.10. Surface CD25 and CD69 analysis

Purified T cells were stimulated with 5 μg/ml Con A for 24 h in the presence of various concentrations of icariin. Cultured cells were stained with specific antibodies for 30 min at 4 °C in the dark and then analyzed by flow cytometry.

2.11. Immunofluorescence

Immunofluorescent assay was performed as we previously reported [26]. Briefly, paraffin-embedded colon sections were incubated with antibodies for p-STAT1 or p-STAT3 overnight at 4 °C, and then with Alexa Fluor 594-conjugated secondary antibody for 2 h. Samples were co-stained with FITC-anti-CD4 and DAPI, and observed under a fluorescence microscope (Olympus, Tokyo, Japan).

2.12. Statistical analysis

All results were expressed as mean ± SEM. Comparisons between groups were evaluated using one-way analysis of variance (ANOVA) followed by Student’s two-tailed t test. P values less than 0.05 were considered statistically significant.

3. Results

3.1. Icariin ameliorated disease progress and pathological changes of DSS-induced colitis in mice

In the present study, we used a mouse model of DSS-induced colitis to evaluate the therapeutic effect of icariin. Mice were challenged with 2.5% DSS, which caused direct colonic mucosal injury and thus led to inflammatory conditions in the intestine, for 7 days. From day 6 mice started to show an increasing severity of symptoms, including dramatic body weight loss, evident rectal bleeding and diarrhea. Compared to the control group, daily administration of icariin at 3 and 10 mg/kg markedly attenuated the body weight loss of DSS-challenged mice (Fig. 2A). Cyclosporin A (CsA) is a potent immunosuppressive agent and has also been used to treat patients of IBD since the mid-1980s. It is highly effective in patients with severe IBD who do not respond to corticosteroid therapy. Here we used CsA as a positive drug. Our results showed that at the same dose of 10 mg/kg, icariin exhibited an even greater therapeutic effect than CsA did (Fig. 2A). Icariin also significantly reduced the disease activity index (DAI), a clinical parameter reflecting the severity of weight loss, rectal bleeding and stool consistency, compared to the control group (Fig. 2B).

Shortened colon length is an important feature of colitis. DSS-treated mice showed substantial reduction in colon length compared to the normal group. Icariin at 3 and 10 mg/kg alleviated the situation of colon shortening (Fig. 2C and D). H&E staining revealed severe pathological changes, including mucosal damages and necrosis as well as infiltration of inflammatory cells such as neutrophils and monocytes, in colon samples of DSS-treated mice. Icariin displayed dose-dependent protective effects against these histological damages. Icariin at 3 and 10 mg/kg showed average histological scores of 1.31 and 3.62 respectively, while the DSS group exhibited a high score of 4.5 (Fig. 2E and F).

3.2. Icariin regulated cytokine profiles in colons and T lymphocytes of mice with colitis

Mice with DSS-induced acute colitis display a Th1/Th17-characterized cytokine pattern [8], which shows some similarities to the cytokine profile of human’s IBD. To determine the effect of icariin on cytokine production in mice with DSS-induced colitis, we analyzed the cytokine expression in colons at both mRNA and protein levels. On day 10 following induction with DSS, colons from each group of mice were removed. Total RNA of colons were extracted and analyzed for cytokine mRNA expression using quantitative RT-PCR method. Our results revealed that DSS-treated mice exhibited an aberrant cytokine pattern characterized by overexpression of pro-inflammatory cytokines including tumor necrosis factor-α (TNF-α), IL-1β, IFN-γ, IL-17A and IL-17F, which were significantly suppressed by administration of icariin (Fig. 3A). Moreover, total protein extracted from colon tissues were subjected to ELISA analysis. It was shown that icariin downregulated cytokine levels of TNF-α, IFN-γ and IL-17A in DSS-treated mice (Fig. 3B).

To analyze the effect of icariin on subtypes of T cell population, mesenteric lymph node cells from DSS-treated mice were collected on day 7 of induction for intracellular staining of IFN-γ and IL-17A, which were the respective signature cytokines of Th1 and Th17. DSS treatment resulted in an increased percentage of Th1 and Th17 cells whereas icariin downregulated the proportion of both subtypes (Fig. 3C).
Icariin ameliorated DSS-induced colitis in mice. Colitis was induced by orally giving 2.5% DSS dissolved in drinking water from day 0 to day 7. Changes were examined as described in Materials and methods. (A) Change of body weight during the disease process. (B) DAI of mice. (C) Representative images of a typical colon morphology of each group. (D) Statistics of colon length of each group. (E) Histopathological changes of colons. The original amplification was 200×. (F) Histological scores of colons from each group. All data are presented as mean ± SEM. N = 6 for each group. *P < 0.05, **P < 0.01 vs. DSS group.
mediating showed different experiments about samples chemical p65 colon factors found the DSS treated 4C), 4A), and 4B). We also analyzed the phosphorylation of STAT1 and STAT3, two important transcription factors involved in Th1 and Th17 functions respectively. Results showed that icariin inhibited the activation of STAT1 and STAT3 by suppressing their phosphorylation (Fig. 4A and B). Immunohistochemical assay also revealed a decreased level of p-STAT1, p-STAT3 and p-p65 in colon samples of icariin-treated mice compared to DSS group (Fig. 4C), which is consistent with the data in western blot.

3.3. Icariin downregulated STAT1, STAT3 and p65 phosphorylation in colon tissues of DSS-colitis mice

Nuclear factor kappaB (NF-kB) is one of the major components mediating IBD [27]. So we examined NF-kB p65 levels in colon samples of colitis mice. Western blot of proteins extracted from colon tissues showed an enhanced expression of phosphorylated p65 in DSS-treated mice. Icariin administration markedly inhibited the phosphorylation of p65 (Fig. 4A and B). We also analyzed the phosphorylation of STAT1 and STAT3, two important transcription factors involved in Th1 and Th17 functions respectively. Results showed that icariin inhibited the activation of STAT1 and STAT3 by suppressing their phosphorylation (Fig. 4A and B). Immunohistochemical assay also revealed a decreased level of p-STAT1, p-STAT3 and p-p65 in colon samples of icariin-treated mice compared to DSS group (Fig. 4C), which is consistent with the data in western blot.

3.4. Icariin suppressed T lymphocyte proliferation and activation in vitro

Since previous results showed that icariin affected activation of major transcription factors in Th1 and Th17, we further examined icariin’s effect on T cells in vitro. Using a HPLC assay, we found that the concentration of icariin in the serum of mice treated with 10 mg/kg of icariin daily for 3 days reached a peak at about 3 μM. Therefore we performed most of the in vitro experiments within the concentration range of 0.3–10 μM. We first examined the effect of icariin on T lymphocyte proliferation. Naïve T cells collected from C57BL/6 mice were incubated with different concentrations of icariin for 72 h together with mitogen concanavalin A (Con A) which stimulated T cells for activation. MTT assay showed that icariin dose-dependently inhibited the proliferation of Con A-activated T cells (Fig. 5A). It is notable that icariin did not exert any significant suppressive effect on naïve T cells until reaching a high dose of 30 μM (Fig. 5B), indicating a relatively low cytotoxicity.

Expression of surface CD25 and CD69 induced by Con A is one of the hallmarks of activated T cells. Upon stimulation with Con A, T cells expressed enhanced levels of CD25 and CD69 compared to the control group. When treated with icariin, the percentage of CD25+ and CD69+ T cells was markedly reduced in a dose-dependent manner (Fig. 5C-5E), suggesting that activation of T cells was suppressed by icariin.

Furthermore, we examined icariin’s effect on apoptosis of activated T cells using Annexin V-FITC and propidium iodide (PI) staining. Flow cytometric analysis revealed an increase of apoptotic T cells occurred in icariin-treated group (Supplementary Fig. S1). However, the cell cycle progression of activated T cells was not affected by icariin treatment (Supplementary Fig. S2). Therefore, the mechanism of icariin’s immunosuppressive activity needs to be further explored.

3.5. Icariin suppressed pro-inflammatory cytokine production of activated T cells

Next we examined icariin’s effect on cytokine production of activated T cells. Naïve T cells were cocultured with Con A and various concentrations of icariin for 48 h. Cell culture supernatants were collected for cytokine analysis. Con A-activated T cells produced an augmented level of pro-inflammatory cytokines including IFN-γ, IL-6, IL-17A, TNF-α and IL-2, while icariin...
treatment dose-dependently suppressed these cytokines (Fig. 6). In addition, icariin did not show significant effect on the level of anti-inflammatory cytokine IL-10, whereas CsA suppressed its production (Fig. 6).

3.6. Icariin inhibited STAT1 and STAT3 activation in T cells in vivo and in vitro

Results from Fig. 4C showed that icariin inhibited STAT1 and STAT3 phosphorylation in colon tissues of DSS-colitis mice. In order to further determine its effect on STAT1 and STAT3 activation, in vitro tests were performed using T cells derived from naïve lymph node cells of C57BL/6 mice. When stimulated with Th1 cytokine IFN-γ, the phosphorylation of STAT1 in T cells was markedly upregulated. Icariin treatment inhibited the level of phosphorylated STAT1 both dose- and time-dependently (Fig. 7A and C). In addition, the phosphorylation of STAT3 upon IL-6 stimulation, which is typically associated with Th17 differentiation, was also inhibited by icariin in a dose- and time-dependent manner (Fig. 7B and D).

To further confirm icariin’s effect on STAT1 and STAT3 phosphorylation, immunofluorescent assay was performed on paraffin-embedded colon sections of mice with DSS-induced colitis. Co-staining of CD4-FITC and p-STAT1 or p-STAT3 showed that p-STAT1 and p-STAT3 positive cells were mostly colocalized with CD4+ cells in colon tissues. Fewer infiltrating
CD4⁺ T cells, as well as cells expressing p-STAT1 or p-STAT3, were found in icariin-treated group, suggesting that icariin downregulated the phosphorylations of STAT1 and STAT3 of infiltrating CD4⁺ T cells in colon tissues of colitis mice (Fig. 7E and F).

4. Discussion

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder in the gastrointestinal tract. It has a high prevalence worldwide and is a well-established risk factor of colorectal cancer.
Current treatments for IBD, including corticosteroids and immunomodulators, have been proved effective in reducing symptoms. However, they also have potential side effects including steroid dependence [28], serious infections and increasing risk of lymphoma [29,30]. Therefore, novel strategies with fewer adverse effects are needed. Crohn’s disease (CD) and ulcerative colitis (UC) are the two major forms of IBD. While sharing many similarities in clinical manifestation and histopathological features, CD and UC...
also have some differences: CD may affect any part of the gastrointestinal tract while UC is usually restricted to the colon and the rectum; CD exhibits transmural inflammation in the intestine while inflammation in UC is limited mostly to mucosa; CD is often classified as a Th1-mediated disease while UC is thought to be Th2-mediated. Yet some studies also suggest Th2 profiles in CD and Th1 profiles in UC [31,32], indicating that both Th1 and Th2 may play roles in CD and UC. DSS-induced acute colitis is a widely used animal model of IBD which shows features of both CD (transmural inflammation with disseminated lymphoid follicles, focal lesions) and UC (regular rectal localization) [7]. Mice with DSS-induced acute colitis display a predominant cytokine pattern of Th1/Th17 type [8], which shows resemblance to the cytokine profile of human’s IBD. In the present study, we used DSS-induced acute colitis model to mimic the process of IBD in human. We found that icariin, a natural flavonoid glucoside isolated from plants in Epimedium family, ameliorated intestinal inflammation in mice induced by DSS due to its immunoregulatory property. At doses of 3 and 10 mg/kg/day, oral administration of icariin markedly recovered the body weight loss and attenuated the disease progress in mice with DSS-induced colitis. It also improved pathological changes including colon shortening and infiltration of inflammatory cells in colon mucosa. Moreover, it exhibited a regulatory effect on cytokine profiles in colon and mesenteric lymphocytes as it downregulated the levels of pro-inflammatory cytokines. All these data suggest that icariin can be used as a beneficial agent for the treatment of IBD.

It is well known that the pathology of IBD involves imbalance of pro-inflammatory and anti-inflammatory cells. Excessive effector T cell function is an important part in the disease process. In this study we investigated the immunosuppressive effect of icariin on T cell proliferation and activation, and found that icariin suppressed cell proliferation, as well as expression of cell activation marker CD25 and CD69, in T cells in response to mitogen stimulation. This immunosuppressive activity can be partly attributed to its ability to induce apoptosis of activated T cells as icariin-treated T cells exhibited an increased proportion of apoptotic cells compared to Con A-treated control cells.

Classical Th1/Th2 pathways play a critical role in IBD pathogenesis. Th1 cells are characterized by their production of IFN-γ, and are important in immune responses to intracellular pathogens and tumors. However, dysregulation of Th1 functions may lead to autoimmunity and inflammatory diseases including IBD. Our study demonstrated that icariin targeted Th1 functions by inhibiting STAT1 activation, the major transcription factor involved in Th1 responses. Colon samples from icariin-treated mice showed decreased level of phosphorylated STAT1 as assessed by western blot, immunohistochemistry and immuno-fluorescence. In vitro studies also showed that icariin suppressed STAT1 phosphorylation of T cells stimulated with Th1 cytokine IFN-γ. Therefore, icariin is effective in controlling aberrant Th1 functions.

Other than Th1 cells, accumulating data have suggested that Th17 cells which produce IL-17, are also a crucial mediator of IBD. Infiltration of Th17 cells, along with elevated Th17 cytokine production, is found in intestinal mucosa of IBD patients. Therefore, targeting Th1 and Th17 simultaneously, rather than only one of the two subsets, might be a feasible strategy to control IBD. From this perspective, icariin is a more effective drug candidate for IBD because of its suppressive activity on Th17 functions. Colitis mice treated with icariin showed decreased levels of Th17 cytokines such as IL-17A and IL-17F in colon tissues. Moreover, icariin inhibited the activation of STAT3, which is a crucial transcription factor in Th17 responses, both in vivo and in vitro. These data confirm the efficacy of icariin on intestinal inflammation.

In conclusion, our study explored the therapeutic effect of icariin on DSS-induced colitis model. Administration of icariin significantly attenuated the inflammatory conditions in mice with colitis. The mechanism of icariin’s effect involved inhibition of effector T cell functions as it suppressed the proliferation and activation of T cells and downregulated pro-inflammatory cytokine levels of activated T cells. Moreover, inhibition of activation of STAT1 and STAT3, major transcription factors of Th1 and Th17 respectively, contributed to the amelioration action of icariin. These results collectively suggest that icariin can be an effective candidate compound for the treatment of IBD.

Conflict of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bcp.2012.12.002.

References


