

## Basic Study

## Butein effects in colitis and interleukin-6/signal transducer and activator of transcription 3 expression

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### Abstract

**AIM:** To evaluate the effects of butein on inflammatory cytokines, matrix metalloproteinase-9 (MMP-9), and

colitis in interleukin (IL)-10<sup>-/-</sup> mice.

**METHODS:** To synchronize colitis, 8- to 10-wk-old IL-10<sup>-/-</sup> mice were fed pellet-chow containing piroxicam for 2 wk. Subsequently, phosphate-buffered saline or butein (1 mg/kg per day, ip) was injected for 4 wk. Histologic scores, inflammatory cytokines, MMP-9 and phosphorylated signal transducer and activator of transcription 3 (pSTAT3) expressions were analyzed in IL-10<sup>-/-</sup> mice and in Colo 205 cells.

**RESULTS:** Butein reduced the colonic inflammatory score by > 50%. Expression levels of IL-6, IL-1β, interferon (IFN)-γ and MMP-9 were decreased in the colons of mice exposed to butein, whereas other inflammatory cytokines (IL-17A, IL-21 and IL-22) were unchanged. Immunohistochemical staining for pSTAT3 and MMP-9 was significantly decreased in the butein-treated groups compared with the controls. Butein inhibited IL-6-induced activation of STAT3 in Colo 205 cells.

**CONCLUSION:** Butein ameliorated colitis in IL-10<sup>-/-</sup> mice by regulating IL-6/STAT3 and MMP-9 activation.

**Key words:** Butein; Interleukin-6/signal transducer and activator of transcription 3; Colitis; Inflammatory bowel disease; Matrix metalloproteinase-9

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**Core tip:** This study examined if butein, a naturally derived substance, has therapeutic effects in an animal model of inflammatory bowel disease, interleukin (IL)-10<sup>-/-</sup> mice. The results show that butein suppressed bowel inflammation and interfered with the IL-6/signal transducer and activator of transcription 3 and matrix metalloproteinase-9 pathways, suggesting that butein

should be used to treat bowel inflammation-induced colon cancer. Although there have been several *in vitro* studies on tumor cells, there have been no *in vivo* studies examining the effect of butein on colitis in mice.

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## INTRODUCTION

Inflammatory bowel diseases (IBDs), including Crohn's disease (CD) and ulcerative colitis, are chronic diseases that are occasionally complicated by bowel perforations, strictures and fistulas<sup>[1,2]</sup>. The overall risk of colorectal cancer among patients with ulcerative colitis is about ten times higher than that of the general population<sup>[3,4]</sup>. In Asia, including South Korea, the occurrence of colon cancer and IBD has recently increased due to environmental factors such as the influence of Westernized lifestyles. This incidence pattern is expected to continue<sup>[2]</sup>. Immunosuppressive agents and 5-aminosalicylic acid have classically been used as treatments for IBD. Inhibition of tumor necrosis factor (TNF), an inflammatory cytokine, has been considered as a therapeutic target. Many studies have focused on diverse biologic agents to treat IBD, but a completely efficient treatment agent has not yet been discovered. Research continues for the development of new alternative drugs and in clinical trials<sup>[5,6]</sup>.

IBD results from immune modulation abnormalities; helper T cells, in particular, play a critical role in the development of disease. CD is largely associated with abnormal activation of Th1-related cytokines [interleukin (IL)-1 $\beta$ , interferon (IFN)- $\gamma$ , TNF- $\alpha$ , IL-6 and IL-22]; in addition, the importance of Th17-related cytokines in the emergence of CD has recently been highlighted<sup>[7]</sup>. IL-10<sup>-/-</sup> mice are known to be an appropriate animal model for CD due to the similarity of their condition to CD morbidity. In these mice, the manifestation of bowel inflammation is mediated by Th1 cytokines (IL-1 $\beta$ , IFN- $\gamma$ , TNF- $\alpha$  and IL-6), and the ulcerative lesions are found in the proximal portion of the colon. In IL-10<sup>-/-</sup> mice, chronic bowel inflammation can occur without any other stimuli or specific pathogens. Making use of the fact that bowel inflammation occurs after a certain period of time, it is possible to promote inflammation and inflammation-induced tumors with piroxicam, a non-steroidal anti-inflammatory drug. Therefore, the IL-10<sup>-/-</sup> mouse can be a useful animal model for the occurrence of bowel inflammation and inflammation-induced tumors<sup>[8,9]</sup>.

The therapeutic effects of natural substances on inflammation and tumors are being widely studied<sup>[10,11]</sup>. It

has been reported that a number of chemical substances obtained from edible plants with botanical and antioxidant characteristics interfere with tumorigenesis through suppression of the inflammatory reaction<sup>[12]</sup>. However, there has been no agreement in results between actual clinical applications and a large-scale prospective study<sup>[13]</sup>. In addition, numerous mechanisms have been suggested for the anti-inflammatory and anti-tumor effects of such natural substances. Cell signal transmission systems have been actively studied, but it has been difficult to elucidate the mechanism of the therapeutic effects due to the structural diversity<sup>[13]</sup>. *Toxicodendron vernicifluum*, a deciduous tree from the Anacardiaceae family, both grows natively in various places and is cultivated in South Korea. Urushiol, the major constituent, is primarily responsible for the toxicity. Other constituents, such as butein (3,4,2',4'-tetrahydroxychalcone), have been found in *in vitro* studies to have antioxidant and anti-inflammatory effects and to suppress tumor cell proliferation and angiogenesis<sup>[14-18]</sup>. One *in vitro* study demonstrated that butein inhibits the activation of nuclear factor kappa B (NF- $\kappa$ B) through inhibition of TNF- $\alpha$ , IL-6 and IL-8 in human mast cells<sup>[19]</sup>.

Matrix metalloproteinase (MMP), an enzyme that degrades zinc-dependent gelatin matrices, serves an important role in inflammatory cell infiltration, cytokine activation and tissue injury, reformation and recovery. MMP-9 is specifically known to be closely associated with rheumatoid arthritis, atherosclerosis, colon cancer and IBD. The suppressive effects that butein has on MMP-9 activation have been reported in an *in vitro* study using prostate cancer cells<sup>[20,21]</sup>. However, to our knowledge, no study has yet been conducted that examines the therapeutic effects of butein on bowel inflammation and colon cancer. This study, therefore, aims to evaluate the interfering effects of butein on inflammatory cytokines and MMP-9 in IL-10<sup>-/-</sup> mice and ultimately to examine the therapeutic effects of butein.

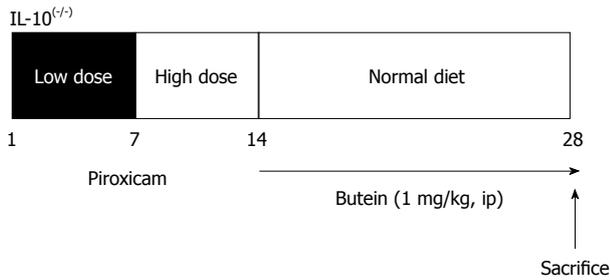
## MATERIALS AND METHODS

### Experimental animals

IL-10<sup>-/-</sup> mice were purchased from Jackson Laboratories (Bar Harbor, ME, United States). All mice were housed and bred in the animal care facility of Korea University Guro Hospital.

### Reagents

Butein and piroxicam were purchased from Sigma-Aldrich Inc. (St. Louis, MO, United States). Butein was dissolved in dimethylsulfoxide and was diluted in phosphate-buffered saline (PBS) before use. Antibodies against phosphorylated signal transducer and activator of transcription 3 (pSTAT3)/STAT3 and  $\beta$ -actin were purchased from Cell Signaling Co. (Beverly, MA, United States), anti-MMP-9 was purchased from Abcam Inc. (Cambridge, United Kingdom), anti-BrdU was purchased from Accurate Chemical (Westbury, NY, United States),



**Figure 1** Experimental protocol for the interleukin-10<sup>-/-</sup>-piroxicam colitis model. IL: Interleukin; ip: Intraperitoneal injection.

GAPDH and donkey anti-rabbit antibodies were purchased from Santa Cruz Biotechnology Inc. (Dallas, TX, United States), and anti-rabbit and mouse-HRP-labeled polymers were purchased from Dako of Agilent Technologies (Glostrup, Denmark).

### Colitis induction

To synchronize and accelerate colitis, 8-wk-old IL-10<sup>-/-</sup> mice were treated with piroxicam as previously described<sup>[22,23]</sup>. In brief, a lower dose of piroxicam (60 mg/250 g chow) was administered for 7 d followed by a higher dose of piroxicam (80 mg/250 g chow) for 7 d. Mice were then placed on normal chow for the remainder of the experimental period. On days 15–28, 1 mg/kg of butein or PBS was administered daily to mice, and mice were sacrificed on the 28<sup>th</sup> day (Figure 1). This experiment was performed in accordance with the guidelines of the Korea University Animal Ethics Committee.

### Colitis assessment

Entire colons were dissected longitudinally and made into Swiss rolls. The tissues were fixed in 10% formalin for 14–16 h, embedded in paraffin and 4- $\mu$ m sections were cut and stained with hematoxylin and eosin. The degree of colitis was assessed using the inflammation scoring system as described previously with slight modifications<sup>[24]</sup> (Table 1).

### Cell lines and cultures

The human colon cancer cell line, Colo 205, was purchased from the American Type Culture Collection (Manassas, VA, United States). Cells were cultured in RPMI media containing 1% penicillin-streptomycin (Sigma) and 10% heat-inactivated fetal bovine serum in 5% CO<sub>2</sub> at 37 °C. Cells were seeded at  $9 \times 10^5$  cells/mL in 60-mm dishes and then treated with IL-6 (25 ng/mL) and the indicated concentrations of butein.

### Colic epithelial cell isolation

Colonic epithelial cells were isolated as previously described<sup>[25]</sup>. Mouse colonic tissues were washed using cold PBS and opened longitudinally. Colons were irrigated with cold Ca<sup>2+</sup>- and Mg<sup>+</sup>-free Hank's balanced salt solution (CMF-HBSS). The tissue was then transferred to 5 mL CMF-HBSS containing 10 mmol/L

**Table 1** Histologic scores

Grade 0	Normal tissue
Grade 1	One or a few multifocal mononuclear cell infiltrates in the lamina propria Minimal epithelial hyperplasia Slight to no depletion of mucus from goblet cells
Grade 2	Lesions tended to involve more of the intestine than grade 1 lesions Several multifocal, mild inflammatory cell infiltrates in the lamina propria Mild epithelial hyperplasia and mucin depletion Small epithelial erosions
Grade 3	Lesions involved a large area of the mucosa or were more frequent than grade 2 lesions Moderate inflammation with the involvement of submucosa Mixture of mononuclear cells as well as neutrophils, and crypt abscesses Moderate epithelial hyperplasia and mucin depletion Ulcers were occasionally observed
Grade 4	Lesions usually involved most of the intestinal section and were more severe than grade 3 lesions Severe inflammation including mononuclear cells and neutrophils with transmural involvement Marked epithelial hyperplasia Crypt abscesses and ulcers

dithiothreitol (1:100; Sigma-Aldrich Inc.) and 50 nmol/L calyculin A (1:200; Wako, Richmond, VA, United States) and incubated in rotator for 30 min at 4 °C. The tissue was then transferred to another 5 mL CMF-HBSS solution containing 1 mmol/L ethylenediaminetetraacetic acid and 50 nmol/L calyculin A, and incubated at 4 °C for 1 h. Colonic tissues were removed from the tube, and epithelial cells were isolated by centrifugation at 300 rpm for 5 min. The supernatant was removed, and the remaining cells were snap-frozen in liquid nitrogen and maintained at -70 °C for future analysis.

### Immunohistochemistry

For the proliferation assay, 1 mg 5-Bromo-2'-Deoxyuridine (BrdU; Sigma-Aldrich Inc.) was injected into mice 2 h before sacrifice. Formalin-fixed, paraffin-embedded sections were processed for immunohistochemistry. Paraffin-embedded slides were deparaffinized and hydrated. Antigen retrieval was performed using Target retrieval solution in a decloaking chamber followed by staining with antibodies against pSTAT3 (1:100), BrdU (1:200) or MMP-9 (1:100) followed by anti-rabbit or anti-mouse-HRP-labeled polymers. Sections were developed using 3,3'-diaminobenzidine tetrahydrochloride and counterstained with hematoxylin.

### Western blot

Protein was extracted from isolated colonic epithelial cells and Colo 205 cells using protein extraction buffer (Fisher Thermo Scientific Inc., Waltham, MA, United States) as described by the manufacturer. Extracted protein concentrations were measured using the BCA method, and 30  $\mu$ g protein samples were separated on a 10% sodium dodecyl sulfate-polyacrylamide gel, transferred to

**Table 2** Primer list

Name		Sequence
IL-6	F	AGT TGC CTT CIT GGG ACT GA
	R	TCC ACG ATT TCC CAG AGA AC
IL-1b	F	GCC CAT CCT CTG TGA CTC AT
	R	AGG CCA CAG GTA TTT TGT CG
IL-22	F	CAA CTT CCA GCA GCC ATA CA
	R	GTT GAG CAC CTG CTT CAT CA
IL-17A	F	TCC AGA AGG CCC TCA GAC TA
	R	AGC ATC TTC TCG ACC CTG AA
IFN- $\gamma$	F	ACT GGC AAA AGG ATG GTG AC
	R	TGA GCT CAT TGA ATG CTT GG
MMP-2	F	CAG ACT TGT CCC GTT TCC AT
	R	GGT GCT GAC TGC ATC AAA GA
MMP-9	F	CGT CGT GAT CCC CAC TTA CT
	R	AAC ACA CAG GGT TTG CCT TC
IL-21	F	GGC AAC ATG GAG AGG ATT GT
	R	AAG CAG GAA AAA GCT GAC CA
IL-23R	F	CAT GAC TTG CAC CTG GAA TG
	R	GCT TGG ACC CAA ACC AAG TA
IL-12 $\beta$ (P.40)	F	AGG TCA CAC TGG ACC AAA GG
	R	TGG TTT GAT GAT GTC CCT GA

IFN: Interferon; MMP: Matrix metalloproteinase; F: Forward; R: Reverse; IL: Interleukin.

nitrocellulose membranes and blocked with Tris-buffered saline containing 0.05% Tween-20, 5% nonfat milk and 5% fetal bovine serum. The primary antibodies were applied overnight at 4 °C. Antibodies against STAT3 (1:1000) and GAPDH served as the sample loading controls. After extensive washing, the membranes were incubated for 1 h in corresponding horseradish peroxidase-coupled secondary antibodies (donkey anti-rabbit 1:1000). The chemiluminescence reaction was developed using a West Pico reagent (Pierce, Rockford, IL, United States).

### RNA isolation and quantitative real-time polymerase chain reaction

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to quantify the expression of mRNA for cytokines and MMP-9 with the expression of GAPDH for endogenous control. Total RNA from the proximal colon of each mouse was extracted using Trizol (Invitrogen of Thermo Fisher Scientific Inc.), and cDNA was synthesized using the iScript™ cDNA synthesis Kit (Roche, Basel, Germany). qRT-PCR was performed using the LightCycler 480 SYBR Green I Master kit (Roche) according to the manufacturer's instructions with the primers summarized in Table 2.

### Statistical analysis

Statistical analysis was performed using Student's *t*-test or the Mann-Whitney *U* test. Results are expressed as mean  $\pm$  SE.

## RESULTS

### Butein treatment ameliorates colitis in IL-10<sup>-/-</sup> mice

It has been established that induction with piroxicam

for 2 wk accelerates and synchronizes the onset and severity of colitis in IL-10<sup>-/-</sup> mice with marked transmural inflammation and ulceration in the proximal colon<sup>[8,23]</sup>. During the colitis experiment, mice treated with butein exhibited a greater body weight gain and longer colonic length (Figure 2A and B). Histologic analysis demonstrated that treatment with butein (1 mg/kg) for 2 wk after cessation of the 2-wk piroxicam administration significantly reduced the inflammatory score with no visible deep ulceration and lessened inflammatory cell infiltrates (Figure 2C and D).

### Butein treatment inhibits inflammatory cytokines and MMP-9 in the colons of IL-10<sup>-/-</sup> mice

Previous studies have shown that colitis in IL-10<sup>-/-</sup> mice is induced by dysregulation of Th1-mediated cytokines, including IL-1 $\beta$ , IFN- $\gamma$  and IL-6. The Th1 cytokines, IFN- $\gamma$ , IL-1 $\beta$ , IL-6, IL-21, IL-23 and IL-12 $\beta$  were heavily upregulated in IL-10<sup>-/-</sup> mice, together with significantly increased MMP-9, two weeks after the last administration of piroxicam. IL-10<sup>-/-</sup> mice that received 2-wk-treatment with butein demonstrated significantly reduced expression of *IFN- $\gamma$* , *IL-1 $\beta$* , *IL-6* and *MMP-9* mRNA in the proximal colon, while there was no effect on the expression of *IL-17a*, *IL-21*, *IL-22* and *MMP-2* (Figure 3).

### Butein treatment results in reduced STAT3 and MMP-9 expression in the colons of IL-10<sup>-/-</sup> mice

Knowing that STAT3 is part of a major intrinsic pathway for inflammation and inflammation-associated cancers that are mediated and activated by cytokines, chemokines and other mediators including IL-6, IL-1 $\beta$  and macrophage colony-stimulating factor, we investigated STAT3 activity in the colons of IL-10<sup>-/-</sup> mice. Increased STAT3 activity was noted in inflamed colonic epithelial cells, which was inhibited by butein treatment, as noted both in immunohistochemistry and Western blots (Figure 4).

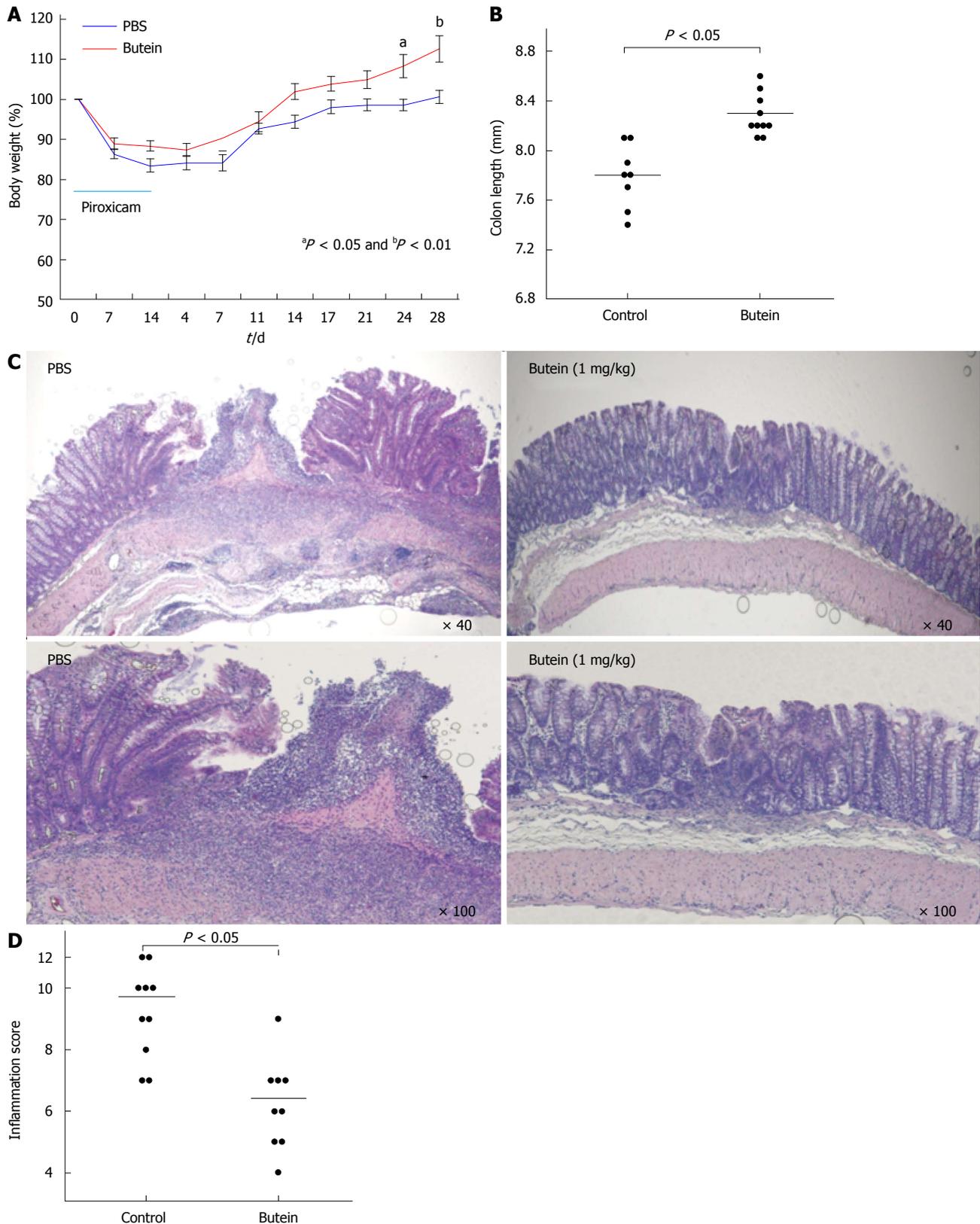
Butein treatment suppressed MMP-9 expression, which was noted in adjacent inflammatory cells in mice with colitis and ulcerations in control mice. To evaluate proliferation, a BrdU incorporation assay was performed by immunohistochemical analysis. There was no significant difference between the control and butein treatment groups (Figure 5).

### Butein inhibits STAT3 phosphorylation induced by IL-6 in human Colo 205 cells

We investigated the effects of butein on the modulation of IL-6/STAT3 activation *in vitro* using Colo 205 cells. Exposure to IL-6 for different times and at different concentrations increased phosphorylation of STAT3. Butein treatment suppressed the phosphorylation of STAT3 induced by IL-6 (25 ng/mL) at a concentration of 10  $\mu$ mol/L (Figure 6).

## DISCUSSION

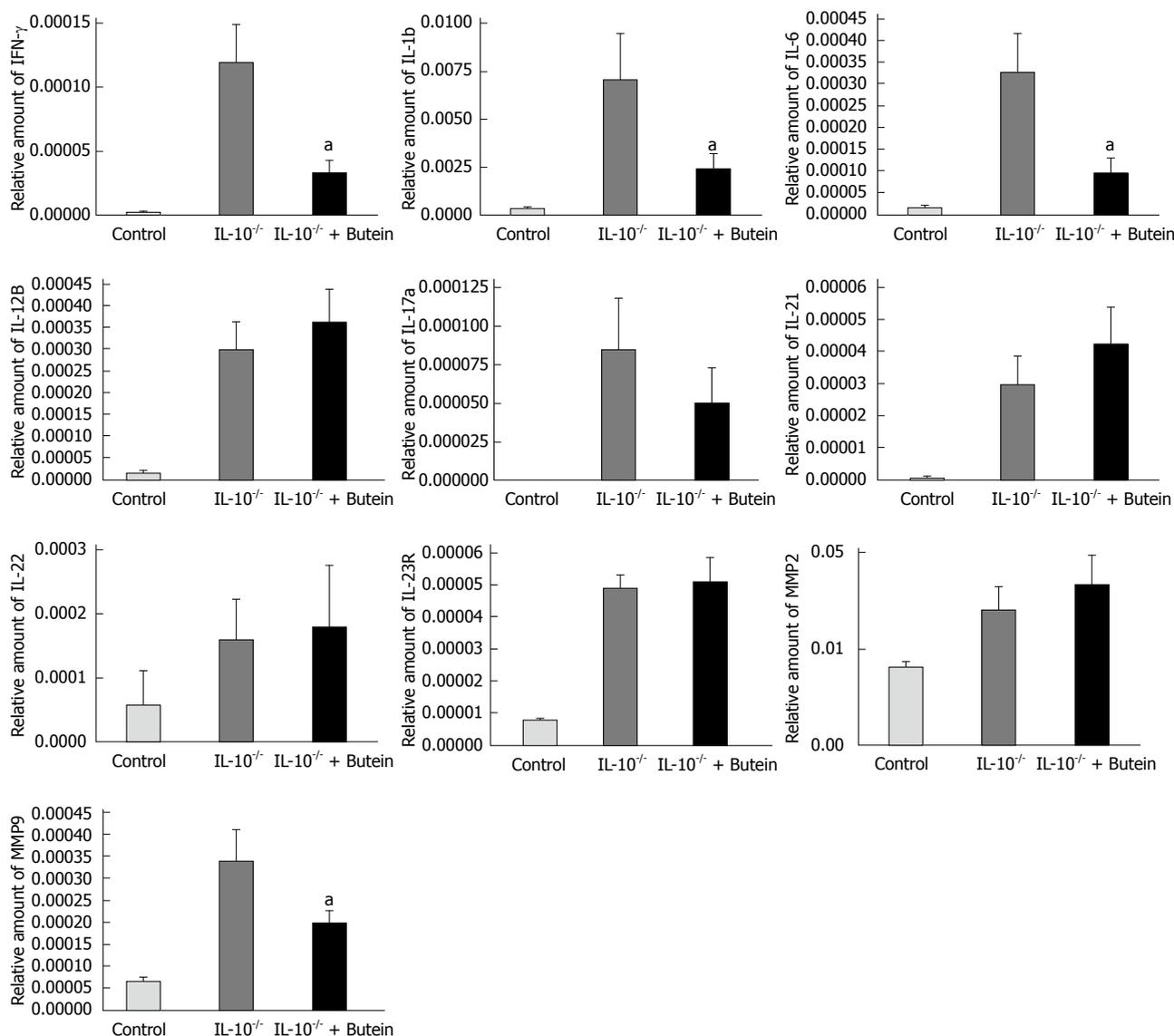
The objective of this study was to examine whether



**Figure 2** Butein treatment ameliorates colitis. A and B: Body weight and colon length between the two groups revealed a significant difference; C: Hematoxylin and eosin stain of Swiss rolls showing ulcers and inflammation; D: Histologic score of mice treated with butein or phosphate-buffered saline (*n* = 9 per group). PBS: Phosphate-buffered saline.

butein, a naturally derived substance, had therapeutic effects in IL-10<sup>-/-</sup> mice, an IBD model. The occurrence

of IBD significantly decreased in mice injected with butein; butein blocked the IL-6/STAT3 signal trans-

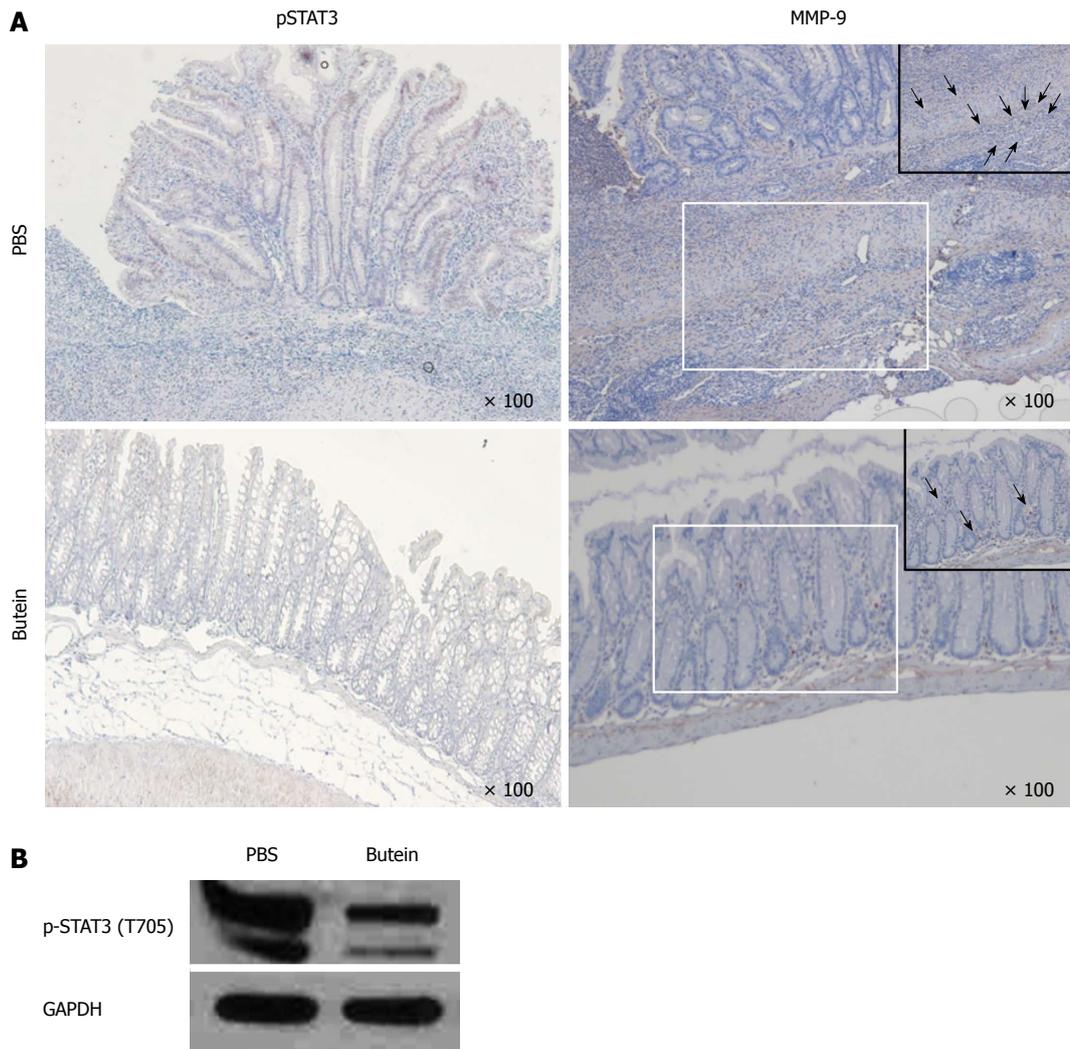


**Figure 3** Expression of cytokines and matrix metalloproteinases in the colons of interleukin-10<sup>-/-</sup> mice. Relative expression levels of mRNA were determined by real-time polymerase chain reaction. Data are expressed as mean ± SE; n = 5 per group; <sup>a</sup>P < 0.05 vs control. IL: Interleukin.

mission pathway and suppressed MMP-9 activation. Butein, a major constituent of *Toxicodendron vernicifluum*, can also be found in the stems of *Semecarpus anacardium* and in the heartwood of *Dalbergia odorifera*, as well as other plants. Previous studies have reported that butein has anti-oxidant, anti-inflammatory and antitumor effects, and that it suppresses angiogenesis<sup>[26-29]</sup>. Butein is known to suppress cell proliferation and promote apoptosis in both solid and hematologic tumors; it is also less toxic than urushiol, another constituent of *Toxicodendron vernicifluum*<sup>[30,31]</sup>. Butein's effects on tumor cells arise as a result of suppressing c-Src and JAK1/JAK2 activation, thus inhibiting the IL-6/STAT3 pathway. Butein also directly inhibits the expression of Bcl-xL, Bcl-2 and cyclin D1, the target genes of STAT3<sup>[31]</sup>. STAT3 plays an important role in cytokine receptor transmission, which is a system that connects a membrane receptor to nuclear transcription and is principally activated by gp130-related cytokines, the most representative of which is IL-6.

STAT3, a principal mechanism of the inflammatory reaction and inflammation-related malignant tumors, is involved in the inflammatory reaction and inflammation-related malignant changes from the initial stage to tumor progression. STAT3 modulates the activity of NF-κB and is activated by the IL-6/JAK signal pathway<sup>[32-34]</sup>. The IL-6/JAK/STAT3 signal system promotes tumorigenesis by inducing cellular or epigenetic changes that follow intracellular inflammation. IL-6/JAK/STAT3, activated by gp130-related IL-6, IL-22, cytokines, and other growth factors, is found to be active in multiple malignant tumors, such as multiple myeloma, lymphoma, hematologic malignancies, breast cancer and prostate cancer.

In this study, *IL-6* mRNA expression was increased in IL-10<sup>-/-</sup> mice with bowel inflammation, and STAT3 activation was also observed in colonic epithelial cells with inflammation and ulcers following the inflammation. Butein inhibited the expression of STAT3 in epithelial



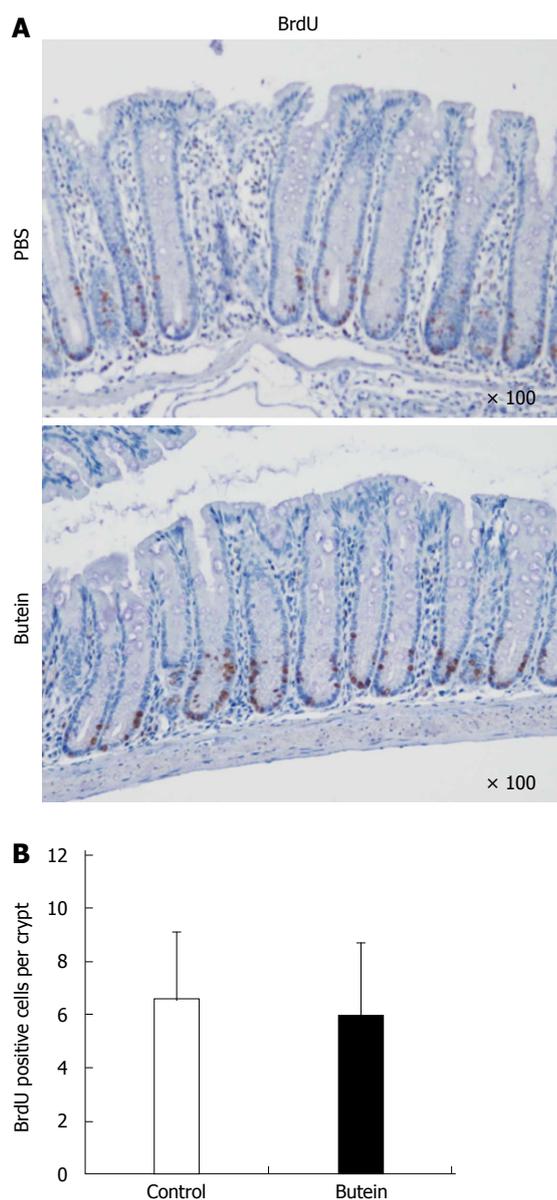
**Figure 4** Butein treatment decreased mucosal expression of phosphorylated signal transducer and activator of transcription 3 and matrix metalloproteinase-9 expression in interleukin-10<sup>-/-</sup> mice. **A:** Immunohistochemical staining for phosphorylated signal transducer and activator of transcription 3 (pSTAT3) and matrix metalloproteinase (MMP)-9 detected nuclei of epithelial cells or stromal cells of interleukin (IL)-10<sup>-/-</sup> mice (black arrows); **B:** Western blot analysis revealed reduced pSTAT3 protein expression in intestinal epithelial cells of butein-treated IL-10<sup>-/-</sup> mice ( $n = 3$ ). PBS: Phosphate-buffered saline.

cells, which was demonstrated by immunohistochemical staining and Western blot analysis. We also found that MMP-9 expression in the colonic tissues was blocked by butein. *In vitro* experiments showed that IL-6-activated MMP-9 was highly concentrated, and this activity was blocked by butein. MMP-9 is closely connected with tissue remodeling and tumor metastasis, and is secreted with MMP-2 from tumor cells, inflammatory cells and cell matrix cells<sup>[35]</sup>. In a previous *in vitro* study, butein was reported to inhibit the activation of MMP-9<sup>[36]</sup>. Similarly, we found that butein blocked the increased expression of MMP-9 in inflammatory cells, and infiltration of the muscle layer in bowel inflammation or inflammation-induced ulcers.

Here, we present the first *in vivo* study examining the therapeutic effects of butein in an animal model of bowel inflammation. There were a few limitations to our study. First, there was no confirmatory analysis of MMP-9 with zymography for analyzing MMP-9 activation after butein treatment. Second, we did not determine whether

the protein expression of IL-6 matched mRNA levels. Third, we had other limitations related to our experiment methods. These shortcomings will be further modified in future studies. Finally, no quantitative analysis of inflammatory cytokine activation was undertaken, and the analysis of the signal transmission system was limited to epithelial cells. It is reasonable to state that IL-10<sup>-/-</sup> mice are not an appropriate model to study the proliferation and recovery of epithelial cells, as they are a model principally defined by the degree of inflammatory cytokine expression. This limitation could be overcome by conducting additional experiments with other study models. In doing so, the therapeutic effects of butein could be evaluated more accurately.

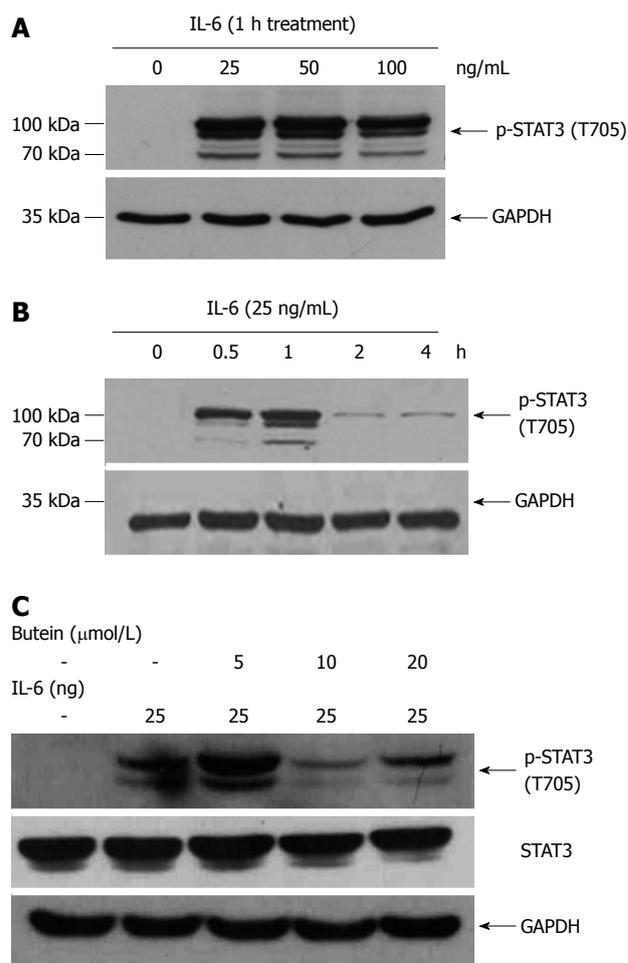
The results of this study regarding the interfering effects of butein on the IL-6/STAT3-MMP-9 pathway are similar to those of an *in vitro* study that used established malignant tumor cells. Considering that the suppression of bowel inflammation and the recovery capability of the injured mucous membrane are critical



**Figure 5 Immunohistochemistry for BrdU incorporation.** A and B: Immunohistochemistry for BrdU incorporation revealed no significant difference in proliferation of the intestinal epithelial cells between the two groups. PBS: Phosphate-buffered saline.

for IBD treatment, the findings obtained from the BrdU analysis indicating that the cell proliferation necessary for the recovery of the mucous is not affected by butein treatment further supports the clinical applicability of butein in treating IBD.

Chronic inflammation is a major mechanism of malignant tumors. The incidence of malignant tumors in the colon and the small intestine is significantly increased with IBD such as ulcerative colitis and CD. The IL-6/STAT3 pathway is an important pathway for the initiation of inflammation-mediated malignant tumors, and MMP-9 is an essential signal transmission system related to the metastasis of malignant tumors. In IBD patients, the expression of IL-6 is increased, and the expression of MMP-9 is known to serve a critical role in pathogenesis.



**Figure 6 Butein inhibited the interleukin-6-induced activation of signal transducer and activator of transcription 3 in Colo 205 cells.** A: Colo 205 cells were treated with various concentrations of interleukin (IL)-6; B: Colo 205 cells were treated with IL-6 for various times; C: Expression levels of phosphorylated signal transducer and activator of transcription 3 (pSTAT3), were measured by Western blot analysis after pretreating Colo 205 cells with IL-6 (25 ng/mL) for 1 h prior to butein treatment for 24 h at various concentrations (*n* = 3).

Our results point to the possibility of applying butein to bowel inflammation-induced colon cancer, as butein suppressed bowel inflammation and interfered with the IL-6/STAT3 and MMP-9 pathways in IL-10<sup>-/-</sup> mice. It is therefore important to further investigate the effects of butein on the occurrence of bowel inflammation-related colon cancer.

## COMMENTS

### Background

The therapeutic effects of natural substances on inflammation and tumors are being widely studied. Butein has been found in *in vitro* studies to have antioxidant and anti-inflammatory effects and to suppress tumor cell proliferation and angiogenesis.

### Research frontiers

The suppressive effects that butein has on matrix metalloproteinase-9 (MMP-9) activation have been reported in an *in vitro* study using several cancer cells. However, no study has yet to be conducted that examines the therapeutic effects of butein on bowel inflammation and colon cancer. This study, therefore,

aims to evaluate the interfering effects of butein on inflammatory cytokines and MMP-9 in interleukin (IL)-10<sup>-/-</sup> mice, an inflammatory bowel disease model, and ultimately to examine the therapeutic effects of butein.

### Innovations and breakthroughs

The results suggest that butein could be used to treat bowel inflammation-induced colon cancer, as it suppressed bowel inflammation and interfered with the IL-6/signal transducer and activator of transcription 3 (STAT3) and MMP-9 pathways in IL-10<sup>-/-</sup> mice. To our knowledge, while there have been several *in vitro* studies on tumor cells, this is the first *in vivo* study to show the effect of butein in mice with colitis.

### Applications

The study results suggest that it is important to represent the inhibitory effects of butein on the occurrence of bowel inflammation-related colon cancer by regulating IL-6/STAT3 and MMP-9 activation.

### Peer review

The authors examined whether butein, a naturally derived substance, had therapeutic effects in IL-10<sup>-/-</sup> mice, a model of inflammatory bowel disease. Butein blocked the IL-6/STAT3 signal transmission pathway and suppressed MMP-9 activation. The results are interesting and may represent the effects of butein on the occurrence of bowel inflammation-related colon cancer.

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