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BASIC RESEARCH

Evaluation of the effect of pyrrolidine dithiocarbamate in suppressing inflammation in mice with dextran sodium sulfate-induced colitis

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Abstract

AIM: To evaluate the effect of pyrrolidine dithiocarbamate (PDTC; an NF-κB inhibitor) administered at low (50 mg/kg) and high (100 mg/kg) doses in suppressing colitis in mice with dextran sodium sulfate (DSS)-induced colitis.

METHODS: Mice were divided into a DSS-untreated group (normal group), DSS-treated control group, DSS+PDTC-treated group Ⅰ (low-dose group), and DSS+PDTC-treated group Ⅱ (high-dose group). In each group, the disease activity index score (DAI score), intestinal length, histological score, and the levels of activated NF-κB and inflammatory cytokines (IL-1β and TNF-α) in tissue were measured.

RESULTS: The DSS+PDTC-treated group Ⅱ exhibited suppression of shortening of intestinal length and reduction of DAI score. Activated NF-κB level and IL-1β and TNF-α levels were significantly lower in DSS+PDTC-treated group Ⅱ.

CONCLUSION: These findings suggest that PDTC is useful for the treatment of ulcerative colitis.

Key words: Ulcerative colitis; DSS-induced colitis; Pyrrolidine dithiocarbamate; NF-κB; Mice

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INTRODUCTION

Nuclear factor-κB (NF-κB) is a transcription activation factor that moves from the cytoplasm into the nucleus following appropriate extracellular signaling. It is thought to play roles in inflammation, immune reactions, carcinogenesis and apoptosis. NF-κB is composed of a family of genes with a high degree of homology. Five subtypes of NF-κB have been identified, including NF-κB 1 (p50), NF-κB 2 (p52), Rel A (p65), Rel B, and c-rel, which form hetero- or homo-dimers. Hetero-dimers of p65 and p50 are most abundant in cells. NF-κB is usually found in the cytoplasm conjugated to an inhibitory protein termed IκB. Phosphorylation of IκB by IκB kinase (IKK) following inflammatory signal transduction leads to degradation of IκB *via* proteosome, resulting in the transfer of NK-κB into the nucleus and its activation there[1].

Pyrrolidine dithiocarbamate (PDTC) is a NF-κB inhibitor. Various studies have been performed in attempts to suppress inflammation mediated by the NF-κB pathway[2,3]. Németh *et al*[4] reported that treatment of intestinal epithelial cells with PDTC *in vitro* suppressed the activity of NF-κB. All studies thus far reported, involving evaluation of the efficacy of PDTC in suppressing intestinal inflammation, were performed *in vitro*, and no such *in vivo* study has been reported. The present study was undertaken to evaluate the efficacy of intraperitoneally administered PDTC in suppressing inflammation in mice with dextran sodium sulfate (DSS)-induced colitis *in vivo*.

MATERIALS AND METHODS

Preparation of a mouse model of DSS-induced enteritis

Six-week-old female BALB/c mice (SLC, Shizuoka) were used for this study. DSS with a molecular weight of 5000 (Nacalai Tesque, Kyoto) was dissolved in tap water to obtain 5% DSS solution. Mice were allowed free access to

Table 1 Disease activity index score

Score	Weight loss (%)	Stool consistency	Occult/grossbleeding
0	(-)	Normal	Normal
1	1-5		
2	6-10	Loose	Guiac (+)
3	11-15		
4	> 15	Diarrhea	Gross bleeding

The disease activity index = (combined score of weight loss, stool consistency and bleeding)/3. Normal stools = well formed pellets; Loose = pasty stool which do not stick to the anus; Diarrhea = liquid stools that stick to the anus.

Table 2 Histological disease score

Grade 0	Normal colonic mucosa
Grade 1	Loss of one-third of the crypts
Grade 2	Loss of two-third of the crypts
Grade 3	The lamina propria is covered with a single layer of epithelium and mild inflammatory cell infiltration is present
Grade 4	Erosions and marked inflammatory cell infiltration are present

Randomly selected 8 fields (magnified 100 times) in each section were inspected and graded as above by a pathologist who was blinded to the treatment protocol. The mean in each section was calculated by scoring the grades in 8 fields.

this solution as drinking water for 7 d to prepare a mouse model of DSS-induced colitis. PDTC (Sigma), dissolved in distilled water, was administered intraperitoneally to mice at dose levels of 100 and 50 mg/kg. Mice were divided into four groups. In the DSS-untreated group (normal group, n = 5), each mouse was allowed free access to tap water for 7 d. In the DSS-treated control group (control group, n = 5), each animal was allowed free access to 5% DSS solution, supplied as drinking water, for 7 d, and underwent intraperitoneal administration of physiological saline immediately before and 3 d after the start of DSS treatment. In the DSS+PDTC-treated group Ⅰ (low-dose group, n = 5), each animal was allowed free access to 5% DSS solution for 7 d and underwent intraperitoneal administration of PDTC (50 mg/kg) immediately before and 3 d after the start of DSS treatment. In the DSS+PDTC-treated group Ⅱ (high-dose group, n = 5), each animal was allowed free access to 5% DSS solution for 7 d and underwent intraperitoneal administration of PDTC (100 mg/kg) immediately before and 3 d after the start of DSS treatment (Figure 1).

Analysis of DAI score of colitis, evaluation of intestinal shortening, and histological evaluation

On the d 7 of the experiment, each animal was weighed to check for weight loss, and the appearance of feces and severity of bloody stool were also checked, followed by calculation of the DAI score according to the method reported by Murthy *et al*[5] (Table 1). Each mouse was then sacrificed and the large intestine was immediately removed for measurement of intestinal length and evaluation of intestinal shortening. Furthermore, the histological score of HE-stained specimens of the distal segment of the colon was determined in accordance with the method for scoring reported by Cooper *et al*[6] (Table 2).

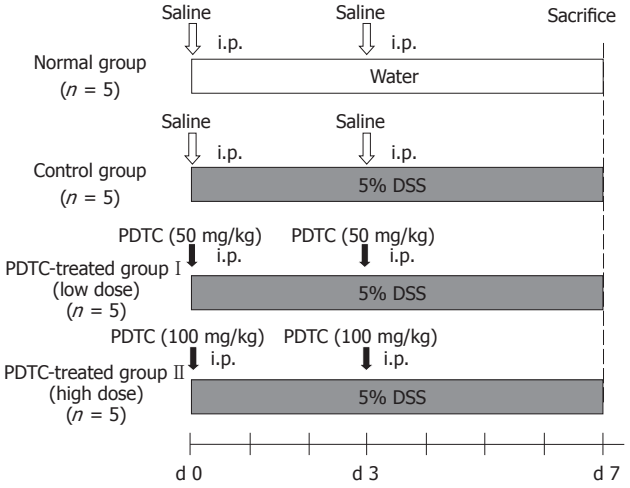

Figure 1 Method of DSS colitis model and administration of PDTC.

Measurement of cytokine and NF-κB activity in colorectal tissue

On the d 7 of the experiment, a protein extract from the distal segment of the intestine of each sacrificed mouse was obtained, using a PARIS Kit (Ambion). The levels of the inflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) in the extract were measured by ELISA, using the Quantkine Mouse IL-1β/IL-1F Immunoassay Kit and Mouse TNF-α/TNFSF1A Immunoassay Kit (R&D Systems, Minneapolis, MN, USA). The experiment was repeated on other mice in the same fashion as described above, except for the timing of sacrifice (the d 6 instead of the d 7 of the experiment). A nuclear extract from the distal segment of the intestine of each sacrificed mouse was obtained using a Nuclear/Cytosol Fractionation Kit (Biovision). The level of NF-κB in the extract was measured by ELISA, using a Mercury™ TransFactor Kit (NF-κB p65, BD Biosciences), to evaluate activation of NF-κB.

Statistical analysis

The data was analyzedsis using the t-test. mean ± SE values are presented for each parameter.

RESULTS

Evaluation of enteritis (DAI score and intestinal shortening)

Compared to the control group, both DSS+PDTC-treated group Ⅰ and DSS+PDTC-treated group Ⅱ exhibited marked reduction of weight loss, improvement in appearance of feces, and alleviation of bloody stool. DAI score, an indicator of the severity of intestinal inflammation, was 0 in the normal group, 2.73 ± 0.16 in the control group, 1.73 ± 0.27 in DSS+PDTC-treated group Ⅰ, and 0.67 ± 0.30 in DSS+PDTC-treated group Ⅱ. Thus, significant suppression of inflammation was noted in DSS+PDTC-treated group Ⅰ and DSS+PDTC-treated group Ⅱ compared to the control group ($P < 0.05$). Suppression was strongest in DSS+PDTC-treated group Ⅱ ($P < 0.01$) (Figure 2).

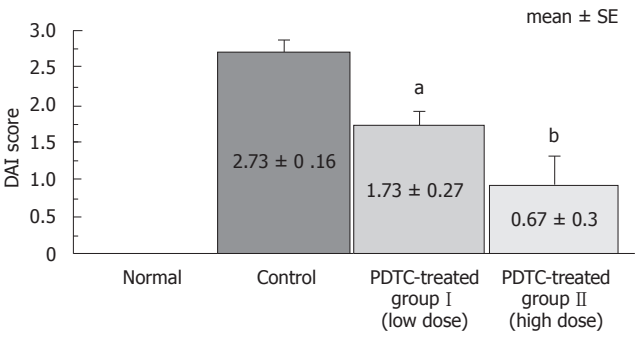

Figure 2 The effect of PDTC on clinical indices (DAI). $^{a}P < 0.05$, $^{b}P < 0.01$, *vs* control group.

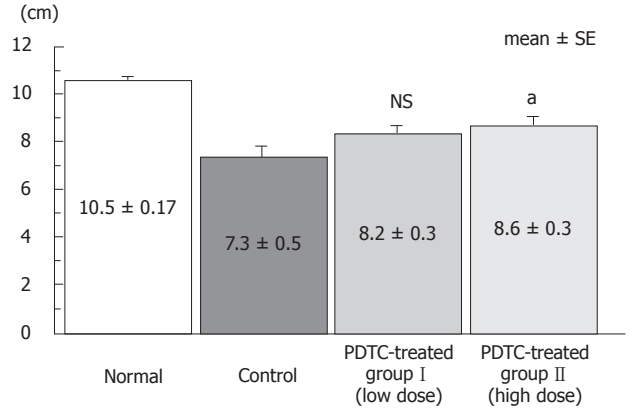

Figure 4 The effect of PDTC on length of colon. $^{a}P < 0.05$, P = NS, *vs* control group. NS: not significant.

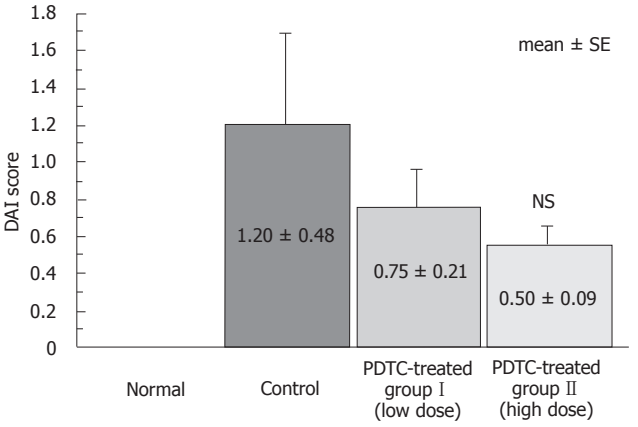

Figure 6 The effect of PDTC on histological disease score. P = NS *vs* control group. NS: not significant.

The effect of DSS on the severity of intestinal shortening caused by intestinal inflammation was evaluated. Pictures of intestine from the normal group, the control group, and DSS+PDTC-treated groups Ⅰ and Ⅱ are shown in Figure 3. Intestinal length was 10.5 ± 0.17 cm in the normal group, 7.3 ± 0.5 cm in the control group, 8.2 ± 0.3 cm in DSS+PDTC-treated group Ⅰ, and 8.6 ± 0.3 cm in DSS+PDTC-treated group Ⅱ. Thus, shortening of the intestine was not significantly suppressed in the

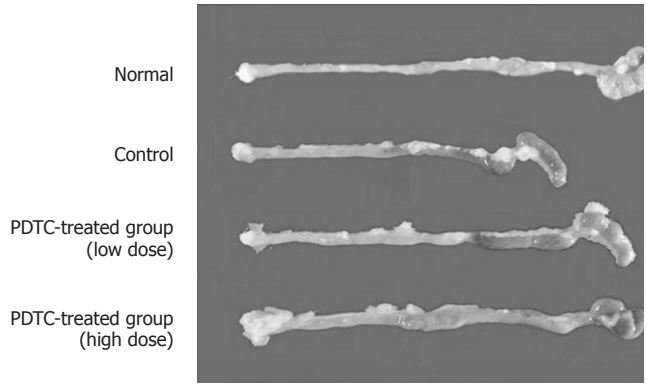

Figure 3 Comparison of length of colon in different groups.

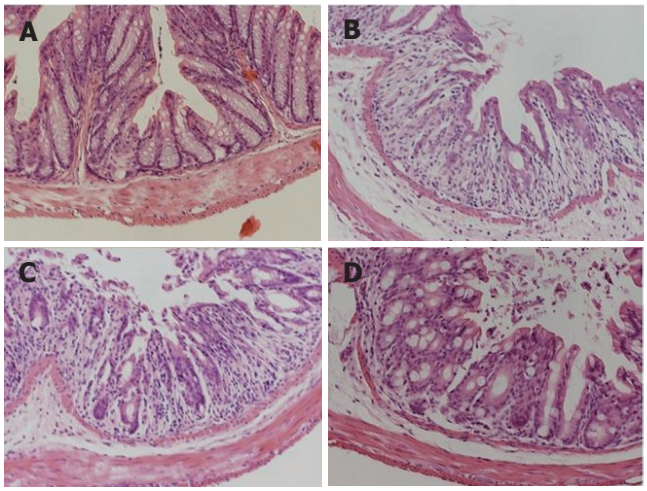

Figure 5 Histological findings of distal colon in mouse. **A**: normal; **B**: control; **C**: PDTC-treated group Ⅰ (low dose); **D**: PDTC-treated group Ⅱ (high dose).

DSS+PDTC-treated group Ⅰ, but was in the DSS+PDTC-treated group Ⅱ ($P < 0.05$) (Figure 4).

Histological evaluation

The histological score for the distal segment of the colon was 0 in the normal group, 1.20 ± 0.48 in the control group, 0.75 ± 0.21 in the DSS+PDTC-treated group Ⅰ, and 0.50 ± 0.09 in the DSS+PDTC-treated group Ⅱ. Compared to the normal group (Figure 5A), the control group exhibited marked erosion of the lamina propria mucosae, disappearance of glandular epithelium, inflammatory cell infiltration, and other related findings (Figure 5B). In the DSS+PDTC-treated group Ⅰ (Figure 5C), the findings of evaluation of inflammation did not differ markedly from those in the control group. In the DSS+PDTC-treated group Ⅱ (Figure 5D), erosion, disappearance of glandular epithelium, inflammatory cell infiltration, and other abnormalities tended to be less severe than those in the control group, although none of these differences was statistically significant (Figure 6).

Activated NK-κB level in colonic tissue

NF-κB levels in nuclear extract from distal colonic tissue were compared among groups. The level was 0.790 ± 0.191 ng/μg in the normal group, 0.938 ± 0.229

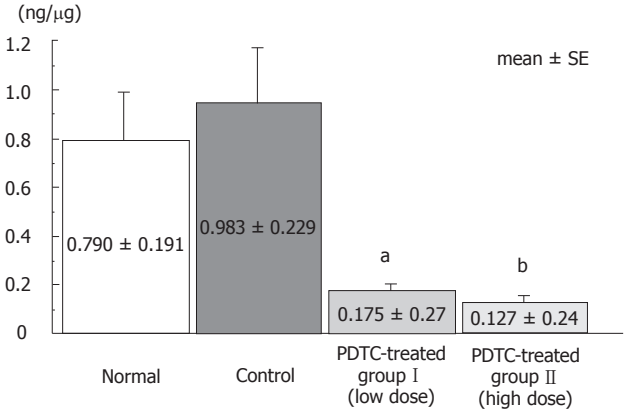

Figure 7 The effect of PDTC on NF-κ concentration. $^{a}P < 0.05$, $^{b}P < 0.01$, *vs* control group.

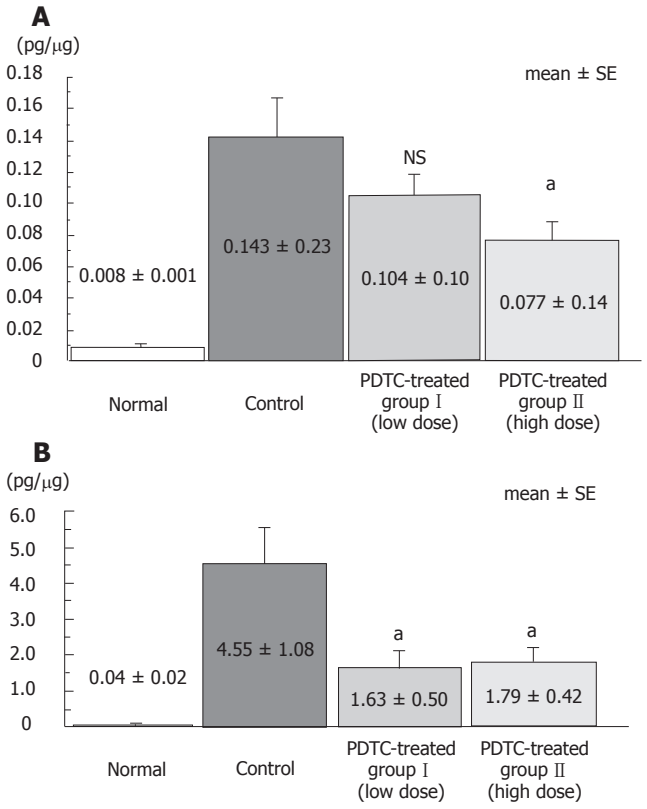

Figure 8 **A:** The effect of PDTC on IL-1β cytokine concentration. $^{a}P < 0.05$, P = NS, *vs* control group. NS: not significant; **B**: The effect of PDTC on TNF-α cytokine concentration. $^{a}P < 0.05$ *vs* control group.

ng/μg in the control group, 0.175 ± 0.27 ng/μg in the DSS+PDTC-treated group Ⅰ, and 0.127 ± 0.24 ng/μg in the DSS+PDTC-treated group Ⅱ. This parameter was thus slightly higher in the control group than in the normal group, and significantly lower in the DSS+PDTC-treated group Ⅰ ($P < 0.05$) and the DSS+PDTC-treated group Ⅱ ($P < 0.01$). These findings suggest that treatment with PDTC suppressed activation of NF-κB (Figure 7).

IL-1β and TNF-α levels in colonic tissue

Levels of inflammatory cytokines IL-1β and TNF-α in distal intestinal tissue were compared among groups. IL-1β level was 0.008 ± 0.003 pg/μg in the normal group, 0.143 ± 0.52 pg/μg in the control group, 0.104 ± 0.32 pg/μg in the DSS+PDTC-treated group Ⅰ, and 0.077 ± 0.23 pg/μg in the DSS+PDTC-treated group Ⅱ. Thus, this parameter tended to be lower in the DSS+PDTC-treated group Ⅰ than in the control group, although this difference was not statistically significant. However, this parameter was significantly lower in the DSS + PDTC treated group Ⅱ ($P < 0.05$) than in the control group (Figure 8A). TNF-α level was 0.04 ± 0.06 pg/μg in the normal group, 4.55 ± 2.41 pg/μg in the control group, 1.63 ± 1.12 pg/μg in the DSS + PDTC treated group Ⅰ, and 1.79 ± 0.94 pg/μg in the DSS+PDTC-treated group Ⅱ. Thus, this parameter was significantly lower in the DSS + PDTC treated group Ⅰ and the DSS+PDTC-treated group Ⅱ than in the control group ($P < 0.05$) (Figure 8B).

DISCUSSION

Ulcerative colitis is an inflammatory bowel disease whose for which the etiology has not yet been fully clarified. Many studies of it have been carried out using animal models of enterocolitis. Numerous reports have been published concerning experiments using animal models of ulcerative colitis, i.e. rodent with DSS-induced colitis[7-9].

It is thought that NF-κB is associated with the expression of various cytokines, chemokines, and adhesion factors in inflammation. NF-κB p65 protein has been reported to exhibit increased expression in nuclear extracts from local mucosal tissue specimens of patients with inflammatory bowel disease[10,11]. Recently reports have been published to evaluate the inflammation suppressing effect of antisense oligonucleotide for NF-κB (p65) administered to mice with TNBS-induced enteritis (a model of Crohn's disease)[12] and mice with DSS-induced colitis (a model of ulcerative colitis)[13] or ischemia-reperfusion injury of the small intestine[14].

It has been reported that NF-κB binds to the DNA sequence termed "κB sequence" and induces expression of the genes regulated by this sequence. NF-κB is usually present in an inactive form, bound to IκB, within the cytoplasm. If IκB is detached, NF-κB can move into the nucleus to become activated. Of the IκB family members, IκB-α has recently been shown to play roles in the signaling triggering the entry of κB into the nucleus[15]. PDTC is a kind of antioxidants known to inhibit NF-κB. PDTC has been reported to suppress NF-κB activity more powerfully than any other drug of the dithiocarbamate family. Cuzzocrea *et al*[17] reported that PDTC suppresses the transfer of NF-κB from the cytoplasm into the nucleus by inhibition of the IκB-α degradation[16].

In the present study, BALB/c mice were treated with 5% DSS solution to induce acute colitis. DAI score was markedly higher in the control group than in the normal group. Histologically, the rectum of the control group exhibited marked inflammatory cell infiltration and erosion. As in a previous study using rats with DSS-induced colitis[18], the present study revealed marked increases in IL-1β and TNF-α, known to produce primarily by activated macrophages in the control group. In the DSS+PDTC-treated group Ⅱ (mice intraperitoneally

administered PDTC at a high dose, 100 mg/kg), suppression of intestinal shortening and improvement of DAI score were noted, accompanied by reduction of inflammatory cytokines IL-1β and TNF-α. Suppression of inflammation was most marked in DSS+PDTC-treated group Ⅱ, in which NF-κB level in rectal nucleus extract was lower than in any other group. These findings demonstrate that treatment with PDTC suppresses the activity of NF-κB in the intestine, and suggest that treatment with PDTC reduces intestinal NF-κB activity and suppresses the production of IL-1β and TNF-α, resulting in alleviation of DSS-induced colitis.

However, on histopathological examination, no significant suppression of inflammation was noted in the DSS+PDTC-treated group Ⅰ (low dose) or the DSS+PDTC-treated group Ⅱ (high dose). It has been reported that NF-κB is associated with cyclin D during the cell cycle and that p65/p50 hetero-dimer (a NF-κB molecule) binds to the NF-κB binding site of the cyclin D promoter, possibly resulting in appropriate regulation of cyclin D and triggering of the start of the cell cycle[19,20]. This indicates that suppression of NF-κB with PDTC will suppress progression of the cell cycle, resulting in delay of cell proliferation and tissue repair. Thus, in the present study, in which inflammation was not satisfactorily controlled, the mucosal damage arising from inflammation was found not to have been alleviated when examined histologically, because suppression of NF-κB had suppressed regene-ration of the damaged mucosa.

These findings suggest that suppression of NF-κB activity by PDTC can delay the healing of mucosal tissue defects (erosions or ulcers) arising from inflammation, but that it can strongly suppress the expression of inflammatory cytokines (IL-1β and TNF-α), resulting in significant alleviation of colitis.

We conclude that PDTC is promising as a drug clinically useful for the treatment of ulcerative colitis.

COMMENTS

Background

In inflammatory bowel disease, the inflammatory cytokines IL-1β and TNF-α play important roles in inflammatory reactions in the intestinal mucosa. NF-κB is one of the factors that induces expression of these inflammatory cytokines. The present study was aimed to evaluate the effect of NF-κB inhibitor PDTC administered at low (50 mg/kg) and high (100 mg/kg) doses in suppressing DSS-induced colitis in mice.

Research frontiers

Various studies have been performed in attempts to sup-press inflammation mediated by the NF-κB pathway. Németh *et al* reported that treatment of intestinal epithelial cells with PDTC *in vitro* suppressed the activity of NF-κB. All studies thus far reported, involving evaluation of the efficacy of PDTC in suppressing intestinal inflammation, were performed *in vitro*, and no such *in vivo* study has been reported. The present study was undertaken to evaluate the efficacy of intraperitoneally administered PDTC in suppressing inflammation in mice with DSS-induced colitis *in vivo*.

Innovations and breakthroughs

These findings suggest that suppression of NF-κB activity by PDTC can delay the healing of mucosal tissue defects (erosions or ulcers) arising from inflammation, but that it can strongly suppress the expression of inflammatory cytokines (IL-1β and TNF-α), resulting in significant alleviation of colitis.

Applications

Therefore, PDTC may be promising as a drug clinically useful for the treatment of ulcerative colitis.

Terminology

Pyrrolidine dithiocarbamate (PDTC) is a NF-κB inhibitor. Various studies have been performed in attempts to suppress inflammation mediated by the NF-κB pathway. Nuclear factor-κB (NF-κB) is a transcription activation factor which moves from the cytoplasm into the nucleus following appropriate extracellular signaling. It is thou-ght to play roles in inflammation, immune reactions, carcinogenesis and apoptosis.

Peer review

This study is well constructed and well documented. They clearly presented the effects of PDTC on DSS-induced colitis in mice.

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