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Involvement of stimulation of α_7 nicotinic acetylcholine receptors in the suppressive effect of tropisetron on dextran sulfate sodium-induced colitis in mice

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ABSTRACT

Ulcerative colitis (UC) involves chronic inflammation of the large intestine. Several agents are used to treat UC, but adverse side effects are remaining problems. We examined the effect of tropisetron as a new type of drug for UC using a dextran sulfate sodium (DSS)-induced model of colitis in mice. We developed a DSS-induced model of colitis and calculated the Disease Activity Index and colon length. We measured myeloperoxidase activity and determined the protein level and mRNA level of cytokines in the colon. DSS-induced colitis was ameliorated by administration of tropisetron and PNU282987. Pre-administration of methyllycaconitine diminished the suppressive effect of tropisetron upon DSS-induced colitis. These findings suggested that α_7 nicotinic acetylcholine receptors (α_7 nAChRs) were related to the suppressive effect of tropisetron on DSS-induced colitis. Additionally, stimulation of α_7 nAChRs decreased the colon level of interleukin-6 and interferon-γ upon DSS administration. Furthermore, stimulation of α_7 nAChRs decreased macrophage infiltration, with expression of α_7 nAChR increased by DSS administration. These results suggest that the underlying mechanism of this suppressive effect on DSS-induced colitis is *via* stimulation of α_7 nAChRs and involves suppression of expression of pro-inflammatory cytokines. Tropisetron could be a new type of therapeutic agent for UC.

1. Introduction

Ulcerative colitis (UC) is a chronic relapsing and remitting inflammatory condition in the large intestine. Diarrhea and bloody stools are typical symptoms of UC. The number of UC patients in Europe, the USA, and Asia has been increasing year by year (1–3). The etiology and pathogenesis of UC have not been defined fully. However, genetic and environmental factors (e.g., flora in the lumen of the intestine) are considered to cause aberrant immune disorders (1–5). In particular, inflammatory cells such as neutrophils and macrophages infiltrate the colonic mucosa and produce a large variety of inflammatory cytokines (e.g., interleukin (IL)-6). Such cytokine release causes activation of inflammatory cells and damage to colon tissue, and results in further infiltration of inflammatory cells (1,2,5). Stopping this negative spiral is a key strategy for UC treatment. 5-aminosalicylic acid, glucocorticoids, immunosuppressants, and infliximab are used for UC treatment. However, several side effects of these agents affect the quality of life of patients, and these drugs cannot produce complete remission in all patients (1–4,6). Hence, new types of drugs are desired for the treatment of UC.

Epidemiologic evidences suggest that cigarette smoking is negatively associated with UC. Nicotine was expected to be a therapeutic agent for UC but the mechanism of this effect is incompletely understood (7,8). In fact, the effect of nicotine administration *via* oral, rectal, and transdermal routes on UC has been shown in clinical studies. However, use of nicotine is limited by its side effects of nausea and headache (9–11).

Stimulation of the vagus nerve attenuates the systematic inflammatory response (12). Stimulation of α_7 nicotinic acetylcholine

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receptors (α_7 nAChRs) on macrophages has anti-inflammatory effects *via* the vagus nerve (13). In a murine model of UC, vagotomy has been shown to aggravate the development of dextran sodium sulfate (DSS)-induced colitis, and nicotine diminishes this effect (14). Electrical stimulation of the vagus nerve has suppressive effect on trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats (15). Furthermore, α_7 nAChR-deficient mice are not affected by stimulation of α_7 nAChRs or vagotomy with regard to the inflammatory response caused by DSS treatment (16). These evidences suggest that the cholinergic anti-inflammatory pathway *via* α_7 nAChRs is a potential target of drug development for UC.

The 5-hydroxytryptamine-3 receptor (5-HT_3R) antagonist tropisetron is used as an antiemetic drug for chemotherapy, and has been reported to have anti-inflammatory effects. Tropisetron inhibits expression of tumor necrosis factor (TNF)-α and IL-1β in lipopolysaccharide (LPS)-stimulated primary human monocytes (17), and inhibits the gene transcription and synthesis of IL-2 in stimulated human T-cells (18). In an animal model of colitis in rats, tropisetron was found to suppress acetic acid- (19) and TNBS-induced colitis (20). These studies suggested that the anti-inflammatory effects of tropisetron are *via* inhibition of 5-HT_3Rs. Tropisetron has been found to be a partial agonist of α_7 nAChRs (21). This evidence is very important for understanding the mechanism of the anti-colitis effect of tropisetron. Also, involvement of α_7 nAChRs in the anti-inflammatory effect of tropisetron has not been studied. Tropisetron could be a safe and beneficial anti-inflammatory drug for the treatment of UC. Here, we examined the effect of tropisetron on the development of DSS-induced colitis in mice.

2. Materials and methods

2.1. Chemicals

DSS (molecular weight, 5000; sulfur content, 15–20%), hydrogen peroxide, and Mildform were obtained from Wako Pure Chemicals (Osaka). Tropisetron, hexadecyltrimethylammonium (HTAB), O-dianisidine dihydrochloride, and myeloperoxidase (MPO) from human leukocytes were purchased from Sigma–Aldrich (Saint Louis, MO, USA). PNU282987 and methyllycaconitine were obtained from Tocris Bioscience (Bristol, UK). FSC22 was purchased from Leica Biosystems (Saint Louis, MO, USA). α-bungarotoxin, Alexa Fluor 488 Conjugate, Alexa Fluor 594 Anti-goat Anti-rat Immunoglobulin (Ig)G, and ProLong® Gold Antifade Reagent with Diamidino-2-phenylindole (DAPI) were obtained from Life Technologies Japan (Tokyo). Anti-CD11b antibody was purchased from Abcam (Cambridge, UK). Mouse IL-1 beta ELISA Ready-SET-Go!® and each enzyme-linked immunosorbent (ELISA)

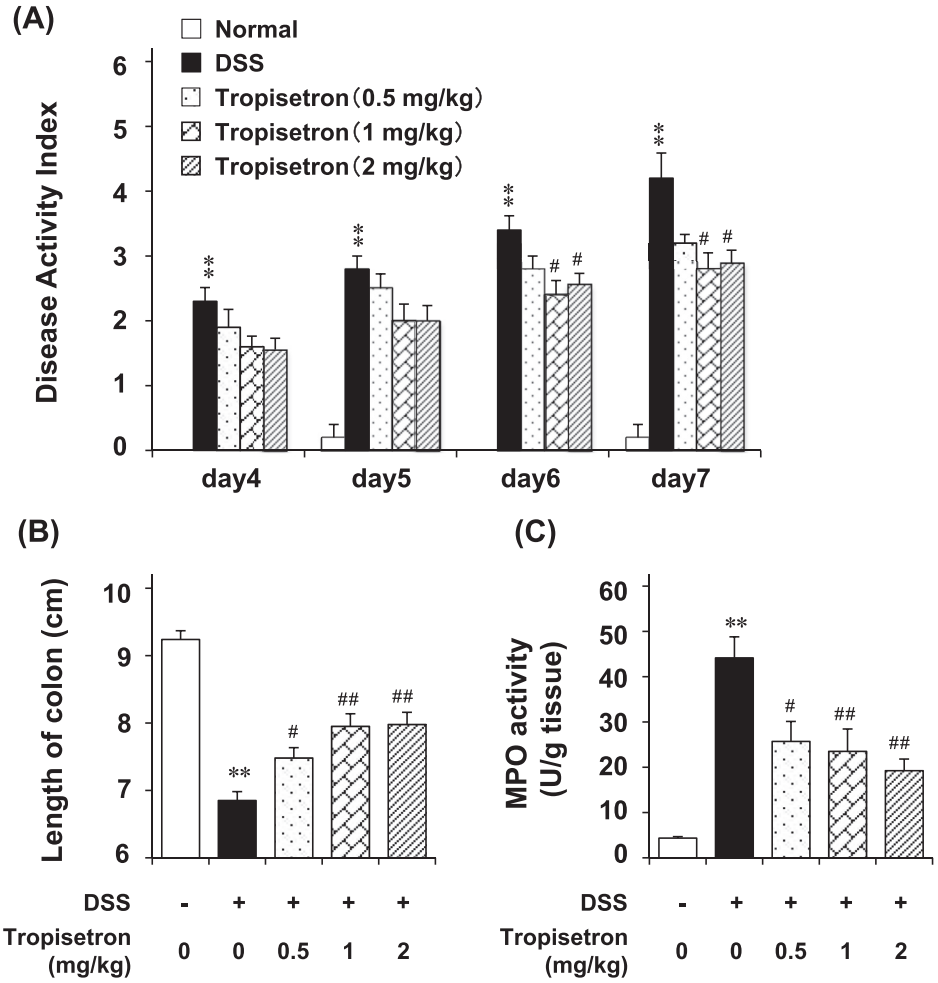

Fig. 1. Effect of tropisetron on development of DSS-induced colitis. 6 week old mice were treated with 5% DSS for 7 days, and intraperitoneally administered tropisetron (0.5 mg/kg; n = 10, or 1 mg/kg; n = 10, or 2 mg/kg; n = 9) once daily. Disease Activity Index was measured daily (A). The length of the colon (B) and colonic MPO activity (C) were determined at the end of the experimental period. Statistical significances versus normal (n = 5) or DSS (n = 10) were calculated by Steel's test (Disease Activity Index) or Dunnett's test (Length of colon and MPO activity). Values are mean ± S.E.M. **, $P < 0.01$ (versus normal), #, $P < 0.05$ or ##, $P < 0.01$ (versus DSS) were considered significant.