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Anti-fibrotic effects of a novel small compound on the regulation of cytokine production in a mouse model of colorectal fibrosis

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ABSTRACT

Intestinal fibrotic stricture is a major complication of inflammatory bowel disease. Despite its clinical importance, anti-fibrotic therapy has not been implemented. Transforming growth factor-β (TGF-β) is considered to be a major factor contributing to tissue fibrosis. We have previously shown that the administration of a small compound, HSc025, which promotes the nuclear translocation of YB-1 as a downstream effector of IFN-γ and antagonizes TGF-β/Smad signaling, improves fibrosis in several murine tissues. In this study, we evaluated the anti-fibrotic effect of HSc025 on colorectal fibrosis in TNBS-induced murine chronic colitis. Daily oral administration of HSc025 (3, 15 and 75 mg/kg) suppressed collagen production and decreased the severity of colorectal fibrosis in a dose-dependent manner. In addition, the local production of TGF-β was decreased after HSc025 treatment, whereas that of IL-13 and TNF-α was not affected. HSc025 administration maintained the level of IFN-γ production, even at a late stage when IFN-γ production was lost without the drug treatment. These results demonstrate that HSc025 could be a therapeutic candidate for intestinal fibrosis in inflammatory bowel disease that acts by altering the local production of cytokines, as well as by directly suppressing collagen production.

1. Introduction

Inflammatory bowel disease (IBD), which comprises Crohn's disease (CD) and ulcerative colitis (UC), is a chronic inflammatory disorder of the gastrointestinal tract of unknown etiology. Chronic inflammation often results in tissue fibrosis, especially in CD, frequently leading to extensive local intestinal fibrosis and mechanical obstruction. Approximately 40% of CD patients with ileal disease develop clinically apparent strictures [1]. According to different therapeutic approaches used in clinical practice, the strictures may be subdivided into fibrotic and inflammatory forms, as well as mixed forms. For fibrotic strictures, medical treatment is ineffective; therefore, surgical resection and endoscopic dilation are the only reasonable treatment options [2]. Conversely, inflammatory strictures may be improved by anti-inflammatory therapy through the reduction of inflammation-mediated edema [3]. In particular, the success of anti-tumor necrotic factor-alpha (TNF-α) antibodies fuels the hope of altering the natural course of CD. However, some recent epidemiological data have shown that after adopting of anti-TNF-α antibodies as a novel treatment for CD, the overall rates of bowel resections have either remained relatively stable or decreased moderately [4]. Because the existing therapeutic approaches are insufficient to completely solve this problem, a specific anti-fibrotic therapy for stricture complications in CD patients is needed [5].

From studies of fibrotic disease in several tissues (liver, lung, kidney and skin), transforming growth factor-β (TGF-β) and its intracellular mediators, Smad proteins, are well known to be the principal factors that lead to tissue fibrosis. Additionally, the overexpression of TGF-β in the intestine has been reported to result in the development of intestinal fibrosis [6]. We have also shown that interferon-γ (IFN-γ) and its downstream effector, Y-box binding protein-1 (YB-1), inhibit transcription of the *COL1A2* gene by its

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direct binding to the IFN-γ responsive element (IgRE) and the interruption of a key binding interaction between Smad3 and the p300 co-activator on the TGF-β-responsive element (TbRE) [7,8]. Moreover, adenovirus-mediated YB-1 overexpression under the control of a collagen enhancer significantly suppresses the progression of hepatic fibrosis and enhances the anti-fibrotic effects of IFN-γ [8]. Furthermore, a novel small compound, HSc025, promotes the nuclear translocation of YB-1 (Fig. S1) and improves skin, lung and liver fibrosis [9].

Based on these findings, in this study, we demonstrated anti-fibrotic effects of the oral administration of HSc025 in an intestinal fibrosis model, occurring through a reduction in TGF-β and enhancement of IFN-γ production, as well as direct effects on TGF-β/Smad signaling. These observations provide novel insights into possible treatment strategies for intestinal fibrosis in IBD.

2. Materials and methods

2.1. Mice

Female 6-week-old BALB/c mice obtained from CLEA Japan Inc. (Tokyo, Japan) were maintained under specific pathogen-free conditions. All experiments were approved by the Animal Experimentation Committee (Tokai University, Kanagawa, Japan).

2.2. Induction of colitis

Colonic inflammation was induced by intrarectal injection by 3.5-F catheter instillation of a 2% solution of 2,4,6-trinitrobenzene sulfonic acid (TNBS) (Research Organics, Cleveland, OH) in 50% ethanol under light anesthesia with isoflurane. The dose of TNBS solution was sequentially increased in the range of 0.4–1.6 mg by 8 (0.4, 0.4, 0.8, 0.8, 1.2, 1.2, 1.6 and 1.6) weekly injections, as described in Fig. S1A. The mice were deprived of food for 24 h before TNBS instillation. The mice were held in a vertical position for 60 s after the intrarectal injection. The animals in the ethanol (ETOH) group were given the same volume of 50% ethanol by intrarectal injection. The animals were weighed at the beginning of the study and weekly thereafter before each TNBS administration (Fig. S2A). Tissues and cells were harvested at three days after the completion of the 8 injections (day 52). The colon was opened and snap frozen for the preparation of frozen sections. In some experiments, formalin-fixed paraffin-embedded sections were prepared for hematoxylin and eosin (H & E) or collagen (Sirius red) staining.

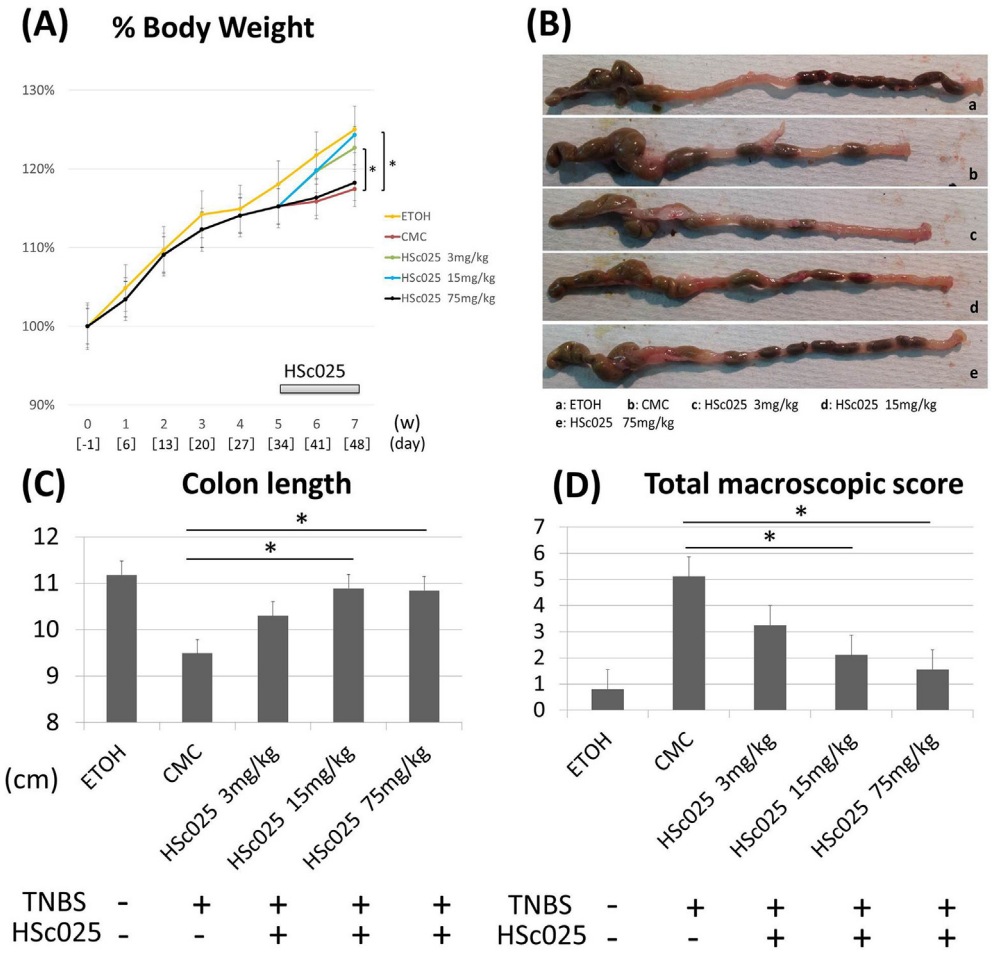

Fig. 1. HSc025 ameliorates the severity of TNBS-induced murine colitis. Murine colitis was induced by the intrarectal administration of TNBS in 50% ethanol, as described in the Materials and Methods section. Mice were treated with daily oral administration of different doses (3, 15, or 75 mg/kg) of HSc025 or vehicle (CMC) during the last 2 weeks (d35-d52) of the 8-week experimental period. (A) The percent change in body weight of each group was determined weekly by comparing the current weight with that before the experiment. (B) Representative examples of colon tissue from all of the groups are shown. (C) The length of the colon tissue in each group was measured. (D) Analysis of the total macroscopic scores was performed based on adhesion, thickness, strictures, dilation, mucosal edema, and mucosal ulcerations. The data are expressed as the mean ± SD (n = 9–10 in each group, *P < 0.05).