Therapeutic Effects of Rectal Administration of Basic Fibroblast Growth Factor on Experimental Murine Colitis

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Background & Aims: **Basic fibroblast growth factor (bFGF) is a promising therapeutic agent for various diseases. It remains unclear, however, whether bFGF is effective for the treatment of inflammatory bowel disease. The aim of this study was to examine the efficacy of bFGF on 2 experimental murine colitis models and to investigate its molecular mechanisms.** ***Methods:*** **We evaluated the effects of human recombinant bFGF (hrbFGF) on mice with dextran sulfate sodium (DSS)-induced colitis and mice with trinitrobenzene sulfonic acid (TNBS)-induced colitis as well as normal mice. Body weight, survival rate, and histologic findings of the colonic tissues were examined. Gene expression of tumor necrosis factor (TNF)-α, cyclooxygenase (COX)-2, transforming growth factor (TGF)-β, mucin 2 (MUC2), intestinal trefoil factor (ITF), and vascular endothelial growth factor (VEGF) in the colonic tissues was determined. The proliferation activity of hrbFGF on the colonic epithelium was evaluated by immunohistochemistry.** ***Results:*** **Rectal administration of hrbFGF ameliorated DSS-induced colitis in a dose-dependent manner. Gene expression of TNF-α was significantly reduced in the colonic tissues of mice with DSS-induced colitis treated with hrbFGF, whereas MUC2 and ITF messenger RNA expression was up-regulated. Rectal administration of hrbFGF significantly improved the survival rate of mice with TNBS-induced colitis and partially ameliorated colitis. hrbFGF significantly increased the number of Ki-67–positive cells in the colonic epithelium of normal mice, and up-regulated the gene expression of COX-2, TGF-β, MUC2, ITF, and VEGF in the colonic tissues.** ***Conclusions:*** **Rectal administration of bFGF might be a promising option for the treatment of inflammatory bowel disease.**

Although the cause of inflammatory bowel disease (IBD) remains unclear, it has been suggested that immunologic abnormality has a key role in the pathogenesis of human IBD.[1] Indeed, several immune-regulatory agents, such as corticosteroids, 5-aminosalicylates, immunosuppressants, and anti–tumor necrosis factor (TNF)-α monoclonal antibody, have mainly been used for the treatment of human IBD to control the dysregulated immune response. There are some patients with IBD, however, who are refractory even to the combined use of these agents. In the pathogenesis of IBD, recent clinical and experimental studies have shown that impaired intestinal barrier function permits penetration of toxic and immunogenic factors, which lead to the induction and perpetuation of intestinal inflammation.[2] Therefore, enhancement of intestinal barrier function, which could reduce inflammation caused by decreased uptake of luminal antigens and bacteria, may also provide an effective approach as a novel therapeutic strategy for IBD.

Growth factors have various biologic actions, such as enhancement of cell proliferation, modulation of cell differentiation, and acceleration of cell migration, angiogenesis, and extracellular matrix remodeling.[3,4] A number of growth factors are expressed in the gastrointestinal tract, including members of the epidermal growth factor (EGF) family, the transforming growth factor (TGF)-β family, the fibroblast growth factor (FGF) family, the insulin-like growth factor family, and so on.[5] These growth factors have essential roles in regulating diverse epithelial cell functions, not only to preserve normal homeostasis and the integrity of the intestinal mucosa

Abbreviations used in this paper: bFGF, basic fibroblast growth factor; COX, cyclooxygenase; DSS, dextran sulfate sodium; EGF, epidermal growth factor; FGF, fibroblast growth factor; hrbFGF, human recombinant basic fibroblast growth factor; IL, interleukin; ITF, intestinal trefoil factor; KGF, keratinocyte growth factor; MLN, mesenteric lymph nodes; MUC2, mucin 2; PCR, polymerase chain reaction; PPAR, peroxisome proliferator-activated receptor; TGF, transforming growth factor; TNBS, trinitrobenzene sulfonic acid; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

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but also to repair mucosal injury.[5] Therefore, they might serve as an alternative therapy for patients with IBD. Indeed, 2 clinical trials using growth factors were recently performed in patients with active ulcerative colitis. A phase 2 study of keratinocyte growth factor (KGF)-2 in patients with active ulcerative colitis showed that intravenous administration of KGF-2 at a dose of 1–50 μg/kg was not effective for inducing remission.[6] However, a placebo-controlled trial of EGF enemas in patients with active left-sided ulcerative colitis or proctitis showed that the remission rate in the EGF-treated group was significantly higher than in the group receiving placebo.[7] Thus, it has not yet been determined whether growth factors are useful for the treatment of human IBD.

Basic fibroblast growth factor (bFGF, also known as FGF-2) was initially regarded as a potent angiogenic factor because it induces endothelial cell proliferation, migration, and smooth muscle cell proliferation.[8,9] Previous studies have shown that administration of bFGF improved myocardial infarction with developing collateral vessels in experimental models of ischemic heart disease.[10,11] Moreover, based on its pleiotropic function, which has important roles in the differentiation and/or function of the skin, the eye, and the nervous system,[12] bFGF has already been administered as a potential therapeutic agent in various experimental models.[13–20] Those experimental studies showed that bFGF stimulated wound repair in various organs. Additionally, FGF-2 knockout mice show reduced reepithelialization and collagen deposition after skin injury.[21] Those data strongly suggested that bFGF plays a pivotal role in the repair process of wound injury. In the gastrointestinal tract, bFGF enhances epithelial cell proliferation and restitution as well as stem cell survival after radiation injury to the intestine.[22,23] These data suggest that bFGF might be a promising agent in the treatment of mucosal injury in human IBD.

Therefore, in the present study, we examined the efficacy of rectal administration of human recombinant bFGF (hrbFGF) on 2 experimental murine colitis models (ie, dextran sulfate sodium [DSS]-induced colitis and trinitrobenzene sulfonic acid [TNBS]-induced colitis) and investigated the molecular mechanisms of actions of hrbFGF on healing intestinal mucosal injury.

Materials and Methods

Animals

Female C57BL/6 mice (8–10 weeks old; Japan SLC, Inc, Shizuoka, Japan) and female SJL/J mice (10–11 weeks old; Charles River Japan, Inc, Kanagawa, Japan) were used for the experiments. They were fed with standard laboratory chow and tap water ad libitum. All mice were housed in specific pathogen–free conditions in the animal facility of Kyoto University. The studies were approved by the animal protection committee of our institution.

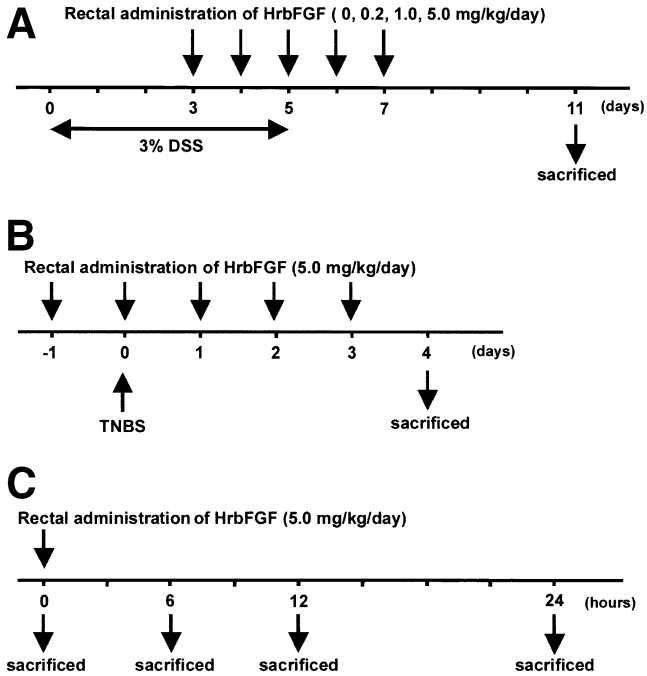

Figure 1. Experimental protocols of the study. (*A*) Study with DSS-induced colitis mice; (*B*) study with TNBS-induced colitis mice; and (*C*) study with normal mice.

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DSS-induced colitis model. *Induction of colitis.* To induce colitis, C57BL/6 mice were given 3% DSS (mol wt, 36–50 kilodaltons; ICN Biomedicals, Inc, Aurora, OH) in their drinking water for 5 days (from day 0 to 4). On day 5, they were switched to regular drinking water. Normal control mice received regular drinking water throughout the experiment.

Treatments. Forty mice with DSS-induced colitis were divided into 4 groups (n = 10 in each group) and treated with rectal administration of hrbFGF as follows: 0 (no medication), 0.2, 1.0, or 5.0 mg · kg^{-1} · day^{-1}. Another 10 mice were used as normal controls without DSS treatment. hrbFGF was a generous gift from Kaken Pharmaceutical Co Ltd (Tokyo, Japan). hrbFGF was diluted in 150 μL phosphate-buffered saline (PBS) and rectally administered once a day for 5 consecutive days starting from 3 days after the initiation of DSS treatment (Figure 1*A*). After each rectal administration of hrbFGF, mice were kept in an inverted position for 30 seconds. Both normal and nontreated colitis-induced mice received PBS as a vehicle solution via the rectum. Body weight was measured daily throughout the experiment. All mice were monitored for 6 additional days after DSS treatment and killed on