THE EFFECT OF BASIC FIBROBLAST GROWTH FACTOR (BFGF) ON REPAIRING INTESTINAL MUCOSA IN ACUTE COLITIS RAT

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ABSTRACT

Objective: Inflammatory bowel disease (IBD) is a chronic and nonspecific inflammatory disease that persist for a long time and decrease the quality of life in patients. In this study, we explored the repair effect of basic fibroblast growth factor (bFGF) on intestinal mucosa in acute colitis rat.

Methods: We established the acute colitis model by TNBS administration in rats. Acute colitis model rats were treated by bFGF with different doses, 60 μg/kg (Low), 80 μg/kg (Medium), 100 μg/kg (High), respectively. Animal weight, stool, food and drink, and activity were observed. Immunohistochemistry (IHC) method was used to detect the expression of Claudin-1, Occludin, Zonula occludens-1(ZO-1), TGF-β1 in intestinal mucosa. The histopathology changes were assessed by HE staining. Ultrastructure of intestinal mucosa were assessed by transmission electron microscope.

Results: bFGF treatment increased the animal weight (p<0.05). The IHC result showed that bFGF obviously increased the expression levels of Claudin-1, Occludin and ZO-1 while attenuated the expression levels of TGF-β1 in intestinal mucosa (p<0.05). In addition, bFGF ameliorated the histological damages, the parameters of injury/necrosis, inflammatory cell infiltration, submucosal edema, and mucosal bleeding, the histological score were all changed by bFGF treatment. Also, microvillus arrangement and cell arrangement in intestinal epithelium were improved after bFGF administration. The results were presented a dose dependent manner.

Conclusion: bFGF have a repairing effect on intestinal mucosa in acute colitis rat, and its mechanisms may be attributed to regulate the expression of transmembrane proteins and anti-inflammatory cytokine.

Keywords: bFGF, intestinal mucosa, acute colitis.

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Introduction

Ulcerative colitis (UC), a chronic inflammatory bowel disease (IBD), is mainly characterized by mainly abdominal pain, diarrhea, nausea, vomiting, fever, even dehydration, electrolyte imbalance and shock1,2. The lesion invades the mucosa of the colon, destroys the mucous membranes and forms ulcers3. It often starts from the left colon and develop from the proximal colon to the entire colon in a continuous manner. The pathogenesis and molecular mechanisms of UC has not been clearly identified and may be involved in bacteria, viral infections, toxins, chemicals, genetic susceptibility and immune disorders4. Due to complex pathogenesis and molecular mechanisms of UC, it was difficult to cure completely and was still considered to be one of the most challenge in clinical practice. In current, the main clinical therapeutic drugs, such as levofloxacin and infliximab, are not widely used because of obviously side effects, unstable efficacy, or high prices5. Therefore, an in-depth and systematic study of the disease is in urgent need.

It has been long recognized that angiogenesis is an important process involved in varieties of pathological entities including chronic inflammation and cancer6. However, the specific relationship between angiogenesis and inflammation process are still unclear. Several studies reported that inflammation may promoted the release of growth factors that can lead to tumor. IBD, as an acute episode, were recognized as
vital inducers of colorectal cancer (CRC)\(^{(7,8)}\). Basic fibroblast growth factor (bFGF) is an 18-kDa poly-peptide\(^{(9)}\). It takes part in various biological processes and has the ability of promoting the epithelial cells growth, angiogenesis, and tissue injury healing.

Therefore, some studies believed that angiogenesis play a key role in biological processes including the ulcer healing, mucosal repair, tissue regeneration, and embryonic development, and the process may be closely related to bFGF\(^{(10)}\). Pharmacologic studies also showed that bFGF could promote the healing of experimental UC and may be a potential use for the treatment of IBD\(^{(11)}\). However, the detailed treatment effect and molecular mechanism are still uncertainly. Some studies suggested that bFGF accelerates healing of injured intestinal mucosa\(^{(12,13)}\). However, the mechanisms were not fully understood. The main purpose of our study was to test the effect of bFGF on acute colitis, and the molecular mechanisms were also explored.

**Methods**

**Animals**

Sprague Dawley rats (SPF grade) with 180 to 220 g body weight, were obtained from Jinan Peng Yue Experimental Animal Breeding Co., Ltd. Rats were housed with the condition of (25±5) °C, (55±5) % relative humidity on a 12-hour light and dark cycle. All experiments in this study were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. And the procedures were all approved by animal Ethics Committee of Laizhou People’s Hospital.

**Model establishment**

Forty rats were divided into the following five groups randomly (n=8): control group, model group, Low bFGF (60 μg/kg weight; ProSpec Bio, Rehovot, Israel) group, Medium bFGF (80 μg/kg) group, High bFGF (100 μg/kg) group. In the control group, the rats did not undergo any operation. The rats in model group and bFGF group were anesthetized by intraperitoneal injection of 3% pentobarbital (50 mg/kg) and sacrificed by cervical dislocation. The colon segment was immediately removed for macroscopic scoring, HE staining and immunohistochemical detection.

**Macroscopic score**

Based on Pathological visual observation and histological changes under light microscope, the macroscopic score was evaluated in a blind manner by all the authors: No inflammation (0 score), swelling or redness (1 score), swelling and redness (2 score), 1 or 2 ulcers (3 score), more than 2 ulcers or 1 large ulcer necrosis (5 score), Severe necrosis (6 score).

**Histological evaluation**

Rats colon segment were obtained and fixed, dehydrated and then embedded in paraffin wax. Following, the tissues were cut into 5 μm thick sections, and stained with hematoxylin and eosin (HE) method\(^{(15)}\). Pathological changes were observed under a light microscope (Nikon, Japan). According to the parameters of injury/necrosis, inflammatory cell infiltration, submucosal edema, and mucosal bleeding, the histological score was evaluated in a blind manner by all the authors:
- No change (0);
- Mild (1);
- Moderate (2);
- Severe change (3).

The expression of Claudin-1, Occludin, ZO-1, TGF-β1 in intestinal mucosa by immunohistochemistry

Intestinal mucosal specimens were fixed in 4% paraformaldehyde. After routinely sectioning, the tissues were dewaxing with xylene, dehydration of gradient ethanol, 5 μm-thick sections of intestinal mucosal specimens were placed in citrate buffer solution for antigen extraction, then 3% H2O2 was used to block the antigen for 10 min. The intestinal mucosal specimens were incubated with the following antibodies: Rabbit anti- Claudin-1 antibody (1:1000, ABIN2801934, Abgent, USA), rabbit anti-Occludin antibody (1:200, ABIN687337, Bioss, USA), rabbit anti- ZO-1 antibody (1:100, ABIN671256, Bioss, USA), and rabbit anti- TGF-β1 antibody (1:100, ABIN671256, Bioss, USA) were added and incubat-
ed overnight at 4 °C. Subsequently, secondary antibodies (of Goat anti-rabbit IgG1:1000, #7074, Cell Signaling Technology, USA) with horseradish peroxidase-conjugated were used to incubate. Sections were observed under a 10×40 optical microscope (Olympus, Japan). Aperio Imagescope 11.1 software (Aperio Technologies, Inc. Vista, CA,) was used to count the positive cells, the results were expressed as percentage of positive cells (%).

Transmission electron microscope
Intestinal mucosal specimens were immersed into 2.5% glutaraldehyde at 4°C overnight, then fixed in 1% OsO4 (Sinopharm Chemical Reagent Co., Ltd, China), dehydrated in acetone step by step and embedded in resin (EMbed 812 Embedding Kit, Electron Microscopy Sciences Company, USA). Ultrathin sections (50 nm) were cut on a Leica EMUC7 ultramicrotome (Leica Microsystems GmbH, Germany), stained with lead citrate (Jeol Ltd, Japan), and examined the mucosal changes under the electron microscope of JEM-2010F (JEOL, Japan).

Statistical analysis
Statistical analysis was implemented using SPSS20.0 (SPSS IBM, Armonk, NY USA). Statistical comparisons among groups were analyzed by one-way analysis of variance (ANOVA) and LSD test were used for multiple comparisons. All dates were reported as the mean ± SD, and the level of statistical signification was set at p<0.05.

Results

Therapeutic effect of bFGF on acute colitis Rats.
Animal weight decreased, food and drink declined, activities reduced, loose stool or feces was not formed in model and bFGF groups, and those in control group were normal. Animals in model and bFGF groups showed loose feces, bloody purulent stool, weight decrease, and reduced activity at 4-6 days after model establishment. After administration with bFGF on day 9, the degree of feces and bloody purulent stool is significantly reduced, and body weight rebounds compared with the rats in the control group (p<0.05) (Figure 1). On day 18, animal weight in bFGF groups were (262.5±25.1) g, (281.9±24.9) g, and (308 ± 33.1) g respectively, which were higher than the model group (p<0.05). The change of animal weight in bFGF groups showed a dose dependent manner.

Pathology score of rat colon segment
As shown in Figure 2A, intact membrane glands, normal crypt structure, large number of goblet cells were observed, and no obvious inflammatory cell infiltration was seen. While intestinal mucosal epithelium in the model group showed severe erosion submucosal and muscular thickening, crypt structure basically destroyed, abscesses, goblet cells, neutrophils, lymphocytes, plasma cells and a large number of inflammatory cell infiltration. Administration of bFGF attenuated histopathologic changes of intestinal mucosa. Compared with the control group, the disease activity index of the colon segment in other groups were significantly increased (p<0.05), as well as the macroscopic score and histological score; While compared with the model group, the disease activity index, macroscopic score, and histological score of the colon in other groups in bFGF groups were significantly reduced (p<0.05) (Figure 2B).

Figure 1: The changes of animal weight. Data were presented as mean ± SD; *p<0.05 compared to the control group, #p<0.05 compared to the model group.

Figure 2: Effects of bFGF on pathology score of rat colon segment. A. Rat colon pathology pictures (×400); B. Disease, macroscopic, histological scores of rats. Data were presented as mean ± SD; *p<0.05 compared to the control group, #p<0.05 compared to the model group.
The expression of Claudin-1, Occludin, ZO-1 and TGF-β1

As shown in Figure 3A, ZO-1 and Occludin proteins in the control group were evenly distributed in the apical part of intestinal epithelial cells and accumulated in a honeycomb or spot shape; while ZO-1 and Occludin proteins in model group were scattered in the colonic mucosa at the top of intestinal epithelial cells, but its staining is unevenly distributed even fades. Claudin-1 and TGF-β1 proteins in the control group were localized in the intestinal mucosa, and mainly distributed on the margins of the intestinal epithelial cells. The expression of Claudin-1 and TGF-β1 proteins in the rats intestinal mucosa of the model group were positive. Cell intensity and range were significantly lower than that of the control group, and the distribution was scattered. The expression of Claudin-1, Occludin, ZO-1 in model group were significantly decreased than the control group (p<0.05), and TGF-β1 was significantly increased(p<0.05); Administration with bFGF increased the expression levels of Claudin-1, Occludin, ZO-1, while downregulated the levels of TGF-β1 in a dose dependent manner (Figure 3B-E).

Ultrastructure of intestinal mucosa

As shown in Figure 4, in the control group, on the cell apical surface of the lumen, there were multiple long microvilli of adjacent columnar absorptive cells, which had an elliptical base electron emission nucleus and prominent nucleoli. The basal layer cytoplasm contains abundant mitochondria and rER, and adjacent cells strongly stick together through composite connections. Intestinal villi in intestinal epithelial cells were showed well-arranged, clear endoplasmic reticulum and mitochondria. In the model group, the number of microvilli decreased, the length and arrangement were irregular, and the gap between epithelial cells expanded. Administration of bFGF attenuated ultrastructural changes of intestinal mucosa in a dose dependent manner.

Discussion

Over the past decade, the incidence of IBD has been on the rise over. Large scale studies showed patients with IBD suffered from a poor quality of life and economic impact, so the therapy is gaining increasing attention in the world. UC is the most prevalent of the IBD TNBS-induced acute colitis was found to be coincided with UC behaviors in human, so it was used to investigate the effect of bFGF on IBD in this study. The current study revealed that bFGF have a repair effect on acute colitis in rats, and this beneficial effect might be associated with regulating the related protein expression.

Acute colitis lead to a poor quality of life. The indicator about life quality including animal weight, stool, food and drink, and activity of rats was observed. animal weight decreased, food and drink declined, activities reduced, loose stool or feces was not formed in model and bFGF groups.
The results indicate that acute colitis model in rats were established successfully. On day 18, animal weight in bFGF groups obviously increased than the model group. The results demonstrated that administration with bFGF relieved symptoms of acute colitis in rats. The histological evaluation and transmission electron microscope also showed that bFGF attenuated histopathologic and ultrastructural changes of intestinal mucosa induced by acute colitis in a dose dependent manner.

It was well known that angiogenesis is one of the most important component of UC pathogenesis. Inflammation induced by UC increase growth factors which can promote carcinogenesis. Inflammatory cells produce oxygen radicals that induce DNA damage, which could lead to an increase in the chance of tumor growth\(^{(19)}\). Therefore, the drugs which can promote angiogenesis may be a potential means for UC treatment. A previous study demonstrated that bFGF could repair the destruction of the blood-brain barrier caused by occlusion of the middle cerebral artery through increasing the expression of adhesion molecules and transmembrane proteins in the blood-brain barrier\(^{(19)}\). Tight junctions (TJs) which are the vital part of intestinal epithelial barrier. It was consisted of transmembrane proteins (such as Occludin, claudin) and zonula occludens proteins (ZO)s\(^{(20)}\). Claudin-1, as a tightly connected transmembrane protein has a similar molecular topology with Occludin. There are 4 transmembrane domains with their N and C-terminal are in the cell, and the C-terminal could attach with ZO-1 proteins\(^{(21)}\). The tight junction cytoplasmic attachment protein ZO-1 is located in the cytoplasm, whose N-terminus is connected to the C-terminus of occludin and claudin inner region, while the C-terminus is connected to cytoskeletal actin in the cytoplasm\(^{(22)}\). The decrease of Claudin-1, Occludin and ZO-1 can affect the stability of cell-to-cell tight junctions and the integrity of cell functions. TGF-β1 is an anti-inflammatory cytokine and play an important role in immunological homeostasis and inflammatory responses\(^{(23)}\). The results in this study indicated that bFGF had a repair effect on intestinal mucosa in acute colitis rat by regulating the expression of transmembrane proteins and decreasing inflammatory.

In summary, this study indicated that bFGF had a repairing effect on intestinal mucosa in acute colitis rat. The mechanisms may be attributed to regulate the expression of transmembrane proteins and anti-inflammatory cytokine. This may suggest therapeutic potential of bFGF for IBD.

References


Conflict of interest statement: The authors report no conflicts of interest. The authors alone are responsible for the content of this manuscript.

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