Epidermal Growth Factor for the Repair of Intestinal Injury in Dextran Sulfate Sodium-Induced Colitis Model Mice: A Postprint Study

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Abstract

This study aims to investigate the reparative effect of epidermal growth factor (EGF) on intestinal injury in dextran sulfate sodium (DSS)-induced colitis model mice. Twenty-four 6-week-old BALB/c mice were selected and randomly divided into three groups: normal control group, DSS model control group, and DSS+EGF group. Mice in the normal control group drank tap water; mice in the DSS model control group drank 5% DSS aqueous solution on days 1–7 of the experiment and tap water on days 8–10; mice in the DSS+EGF group were treated the same as the DSS model control group, while receiving subcutaneous injections of EGF twice daily for a total of 10 days. The results showed: 1) Compared with the normal control group, the colon length of mice in the DSS model control group was extremely significantly decreased ($P<0.01$); compared with the DSS model control group, the colon length of mice in the DSS+EGF group was extremely significantly increased ($P<0.01$). 2) Typical ulcers were observed in the colon of mice in the DSS model control group, and the colon damage score (CDS) was extremely significantly higher than that of the normal control group ($P<0.01$); no ulcers were observed in the colon tissue of mice in the DSS+EGF group, and compared with the DSS model control group, CDS was extremely significantly decreased ($P<0.01$). 3) Compared with the normal control group, the concentration of tight junction protein (Occludin) in the colon of mice in the DSS model control group was significantly decreased ($P<0.05$); compared with the DSS model control group, the concentration of Occludin in the colon of mice in the DSS+EGF group was significantly increased ($P>0.05$). 4) Compared with the normal control group, the concentrations of interleukin-2 (IL-2) and interleukin-4 (IL-4) in the colon of mice in the DSS model control group were extremely significantly decreased ($P<0.01$), and the concentration of interleukin-10 (IL-10) was significantly decreased ($P<0.05$); compared with the DSS model control group, the concentrations of IL-2 and IL-4 in the colon of the DSS+EGF group were extremely significantly increased ($P<0.01$), and the

concentration of IL-10 was significantly increased (P<0.05). These results indicate that EGF may repair damaged intestinal tissue and maintain the integrity of the intestinal mucosal barrier by increasing the expression level of intestinal Occludin and regulating intestinal cytokine concentrations toward normal levels.

Full Text

Study on Intestinal Damage Repair by Epidermal Growth Factor in Dextran Sulfate Sodium-Induced Colitis Model Mice

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Abstract

This study investigated the reparative effects of epidermal growth factor (EGF) on intestinal damage in dextran sulfate sodium (DSS)-induced colitis model mice. Twenty-four 6-week-old BALB/c mice were randomly divided into three groups: normal control, DSS model control, and DSS+EGF. The normal control group received regular drinking water throughout the experiment. The DSS model control group was given 5% DSS solution for days 1–7, followed by regular water for days 8–10. The DSS+EGF group received the same DSS treatment plus subcutaneous EGF injections (0.02 mg/kg) twice daily at 08:00 and 16:00 for 10 consecutive days. The results demonstrated: (1) Compared with the normal control group, the DSS model control group exhibited a highly significant reduction in colon length (P<0.01), while the DSS+EGF group showed a highly significant increase in colon length relative to the DSS model control group (P<0.01). (2) The DSS model control group displayed typical colonic ulcers with a highly significant elevation in colonic damage score (CDS) compared to the normal control group (P<0.01). In contrast, the DSS+EGF group showed no ulcers and a highly significant reduction in CDS compared to the DSS model control group (P<0.01). (3) The DSS model control group had significantly lower colonic tight junction protein (Occludin) concentration than the normal control group (P<0.05), whereas the DSS+EGF group showed significantly elevated Occludin concentration compared to the DSS model control group (P<0.05). (4) The DSS model control group exhibited highly significant decreases in colonic interleukin-2 (IL-2) and interleukin-4 (IL-4) concentrations (P<0.01) and a significant decrease in IL-10 concentration (P<0.05) compared to the normal control group. The DSS+EGF group demonstrated highly significant increases in IL-2 and IL-4 concentrations (P<0.01) and a significant increase in IL-10 concentration (P<0.05) compared to the DSS model control group. These findings suggest that EGF may repair damaged intestinal tissue and maintain intestinal mucosal barrier integrity by upregulating Occludin

expression and modulating intestinal cytokine concentrations toward normal levels.

Keywords: epidermal growth factor; intestine; damage repair; colitis

Introduction

The intestine serves not only as the primary site for nutrient digestion and absorption but also as a crucial immune organ, playing vital roles in mucosal immunity, intestinal barrier function, and growth and development [1]. However, in practical production settings, various stressors, bacteria, viruses, drugs, and nutritional factors can cause intestinal damage in animals, leading to structural changes, altered secretion of cytokines and antibodies in the gut mucosal immune system, and compromised intestinal barrier function. These disruptions severely affect normal physiological functions and production potential, causing substantial economic losses in animal husbandry [2].

Epidermal growth factor (EGF) is an important member of the growth factor family, consisting of a single-chain polypeptide of 53 amino acids [3]. EGF is primarily secreted by the submandibular glands, pancreas, and duodenal Brunner' s glands, and is released into saliva, duodenal fluid, milk, blood, and amniotic fluid [4]. EGF possesses multiple important biological functions. As a potent mitogen, it stimulates cell division and proliferation in various tissues, increases intracellular DNA and protein synthesis, promotes maturation and regeneration of epithelial tissues, inhibits gastric acid secretion, and facilitates ion exchange [5-6]. Research has shown that EGF in breast milk is a critical growth factor promoting gastrointestinal development in newborn animals [7]. Knockout of the EGF receptor during fetal or neonatal periods in mice leads to death, and surviving postnatal mice eventually succumb to hemorrhagic enteritis, demonstrating the essential role of EGF in intestinal growth and development [8]. Exogenous EGF supplementation may represent an important approach for improving intestinal structure and function and repairing damaged intestinal tissue. Therefore, this study employed DSS-induced colitis mice as a model to investigate the effects of EGF on intestinal morphology, permeability, and cytokine concentrations, evaluating its reparative effects on damaged intestine to provide a theoretical basis for EGF application in animal production research.

1.1 Experimental Materials

DSS (molecular weight 36,000–50,000) was purchased from MP Biomedicals (Shanghai). Ten percent neutral formalin (100 mL of 40% formaldehyde, 4.0 g sodium dihydrogen phosphate, 6.5 g anhydrous disodium hydrogen phosphate, 900 mL distilled water) was obtained from Hangzhou Changqing Chemical Co., Ltd. ELISA kits for interleukin-2 (IL-2), tumor necrosis factor- (TNF-), interleukin-4 (IL-4), interleukin-10 (IL-10), and tight junction protein (Occludin) (catalog numbers H003, H052, H005, H009, and H264) were purchased

from Nanjing Jiancheng Bioengineering Institute.

1.2 Experimental Animals and Grouping

Twenty-four 6-week-old specific-pathogen-free BALB/c mice weighing 18–20 g were purchased from Shanghai Slack Laboratory Animal Co., Ltd. The animals were housed under standard conditions with a temperature of 24±1°C, relative humidity of 40–70%, and a 12 h light/dark cycle, with free access to food and water. Mice were randomly divided into three groups (n=8): normal control, DSS model control, and DSS+EGF. The normal control group received regular drinking water throughout the 10-day experiment. The DSS model control group received 5% DSS solution for days 1–7, followed by regular water for days 8–10. The DSS+EGF group received identical DSS treatment plus subcutaneous EGF injections (0.02 mg/kg) at 08:00 and 16:00 daily for 10 days. Mice were carefully monitored daily for mental status, activity, coat appearance, and fecal characteristics.

1.3 Sample Collection and Processing

On day 11, blood was collected from the infraorbital vein to obtain serum. Mice were euthanized by cervical dislocation, and the abdominal cavity was opened along the midline. The intestinal tract was removed, and colon length was measured from the cecal end to the anus. A 1 cm segment of colon tissue was fixed in 10% neutral formalin at 4°C, while the remaining colon was rinsed with sterile saline and immediately stored at -70°C.

1.4 Colonic Morphological Observation and Damage Scoring

Fixed colonic samples were routinely dehydrated, paraffin-embedded, sectioned, and stained with hematoxylin-eosin (HE) for microscopic examination of colonic morphology. Colonic damage score (CDS) was assessed using the method described by Dieleman et al. [9] as follows: inflammatory severity was graded 0–3 for acute and chronic inflammation; infiltration extent was graded 0–4; crypt damage and repair were graded 0–4. The extent of inflammation was estimated as: 1) 1–25%, 2) 26–50%, 3) 51–75%, and 4) 76–100%. Each parameter score was multiplied by the extent of inflammation, and the CDS was calculated as the sum of all parameter scores.

1.5 Determination of Colonic Occludin and Cytokine Concentrations

Colonic tissue was accurately weighed, homogenized with saline in an ice-water bath, and centrifuged. The supernatant was collected, and enzyme-linked immunosorbent assay (ELISA) was used to determine colonic Occludin concentration and cytokine concentrations (IL-2, TNF- , IL-4, and IL-10).

1.6 Data Processing and Statistical Analysis

Data were analyzed using SPSS 17.0 software with one-way ANOVA followed by Duncan' s multiple comparison test. Results are expressed as mean ± standard deviation. $P<0.05$ was considered statistically significant, and $P<0.01$ was considered highly significant.

2.1 Clinical Manifestations in Mice

The normal control group exhibited normal mental status, appetite, and activity with glossy coats and no bloody stools. Following 5% DSS administration, the DSS model control group became lethargic and less active from day 2 onward. As the experiment progressed, mice developed dull coats, reduced food intake, and diarrhea with bloody stools, with all mice showing severe hematochezia by day 6. The DSS+EGF group maintained relatively normal mental status and activity, though a few individuals showed reduced feed intake.

2.2 Colon Length in Mice

[Figure 1: see original paper] shows that colon length in the DSS model control group was highly significantly reduced compared to the normal control group ($P<0.01$), decreasing by 39.27%. The DSS+EGF group also showed reduced colon length compared to the normal control group ($P<0.01$), decreasing by 21.01%. However, compared to the DSS model control group, the DSS+EGF group exhibited a highly significant increase in colon length ($P<0.01$), increasing by 23.04%.

Data bars with the same letter superscripts indicate no significant difference ($P>0.05$), different lowercase letters indicate significant difference ($P<0.05$), and different uppercase letters indicate highly significant difference ($P<0.01$). This applies to all subsequent figures.

2.3 Colonic Morphology and CDS in Mice

[Figure 2: see original paper] presents HE staining results. The normal control group showed clear colonic architecture with distinct layers, intact mucosal layer and epithelial cells without shedding, and no inflammatory cell infiltration or ulcers in the lamina propria. The DSS model control group exhibited disrupted mucosal and epithelial integrity with typical ulcers, epithelial hyperplasia at ulcer margins, extensive inflammatory cell infiltration in the lamina propria and submucosa, and lymphoid follicle formation. Mice receiving EGF subcutaneous injections showed clear colonic architecture with complete mucosal and epithelial repair, no ulcers, and only minimal inflammatory cell infiltration in the lamina propria.

CDS results revealed that the DSS model control group had highly significantly elevated scores compared to the normal control group ($P<0.01$). The DSS+EGF group showed significantly higher CDS than the normal control group ($P<0.05$)

but highly significantly lower CDS compared to the DSS model control group (P<0.01), decreasing by 71.88%, indicating substantial improvement in colonic damage.

2.4 Colonic Occludin Concentration

[Figure 3: see original paper] demonstrates that colonic Occludin concentration in the DSS model control group was significantly lower than in the normal control group (P<0.05), decreasing by 30.53%. In contrast, the DSS+EGF group showed significantly elevated Occludin concentration compared to the DSS model control group (P<0.05), increasing by 39.49%, with no significant difference from the normal control group (P>0.05).

2.5 Colonic Cytokine Concentrations

shows that colonic TNF- concentration did not differ significantly among groups (P>0.05). Compared to the normal control group, the DSS model control group exhibited highly significant reductions in IL-2 and IL-4 concentrations (P<0.01), decreasing by 29.78% and 36.59%, respectively, and a significant reduction in IL-10 concentration (P<0.05), decreasing by 24.07%. The DSS+EGF group showed highly significant increases in IL-2 and IL-4 concentrations (P<0.01), increasing by 41.10% and 31.56%, respectively, and a significant increase in IL-10 concentration (P<0.05), increasing by 31.17%, compared to the DSS model control group. No significant differences were observed between the DSS+EGF and normal control groups for IL-2, IL-4, or IL-10 concentrations (P>0.05).

Discussion

The intestine is a vital organ for nutrient digestion and absorption and the body' s largest immune organ. Its tightly structured morphology maximizes barrier function (biological, physical, and chemical barriers) to prevent entry of toxins, bacteria, antinutritional factors, and other harmful substances, representing the first line of defense against foreign invaders. However, numerous detrimental factors in production settings compromise animal intestinal health, causing morphological alterations, dysregulated secretion of cytokines and antibodies in the gut mucosal immune system, impaired intestinal barrier function, and subsequent damage to other tissues and organs, severely affecting animal growth performance and health. Maintaining intestinal health represents a critical research priority in animal husbandry.

EGF belongs to the growth factor family and plays important roles in promoting gastrointestinal epithelial cell proliferation and differentiation, as well as intestinal tissue growth and development, making it a key regulator of intestinal homeostasis [10]. Postpartum EGF in milk is the primary nutritional factor stimulating intestinal cell proliferation and maturation in newborn animals [11]. EGF significantly increases small intestinal weight, length, and DNA content in neonatal rats and accelerates maturation of goblet cells in early embryonic

intestines [12-13]. In this study, subcutaneous EGF administration highly significantly increased colon length and maintained relatively intact colonic architecture with complete mucosal and epithelial repair, minimal inflammatory infiltration, and highly significantly reduced CDS. These findings indicate that EGF alleviated colon shortening and preserved relative integrity of colonic epithelial structure.

The mechanical barrier constitutes the most important component of intestinal mucosal barrier function. Intercellular tight junctions form the foundation of the mechanical barrier, preventing entry of bacterial endotoxins and toxic macromolecules into the internal environment and playing a crucial role in maintaining intestinal epithelial barrier integrity [14]. Occludin is the primary functional protein in tight junctions between intestinal endothelial cells and is closely associated with intestinal tight junctions and barrier function. Reduced Occludin concentration leads to intestinal barrier dysfunction [15]. Following ischemic intestinal injury in rats, increased permeability and mucosal barrier damage occur early, and high-dose subcutaneous EGF significantly increases Occludin concentrations in the jejunum and ileum [16]. When tissues are damaged, endogenous EGF synthesis increases, but tissue EGF content is generally low. As disease progresses, EGF may become depleted, exacerbating injury, and exogenous EGF supplementation can reduce tissue damage [17]. In this study, 5% DSS administration significantly reduced colonic Occludin concentration, while EGF injection significantly increased it. These results demonstrate that DSS increased intestinal permeability and compromised barrier function, whereas exogenous EGF delivery significantly reduced mucosal permeability and protected epithelial barrier function, consistent with HE staining observations.

Imbalance between pro-inflammatory and anti-inflammatory cytokines is considered a key pathogenic mechanism in inflammatory bowel disease, and abnormal cytokine expression is closely associated with colitis development and progression [18]. Administration of 2.5% DSS for 6 days significantly increased IL-6 and TNF- mRNA expression while highly significantly decreasing IL-4 mRNA expression in colonic inflammatory tissues [19]. Rats receiving 5% DSS showed significantly elevated colonic TNF- and IL-6 concentrations and significantly reduced IL-2, IL-10, and transforming growth factor- (TGF-) concentrations [20]. The exact mechanism of DSS-induced colitis remains unclear but may involve macrophage dysfunction, gut microbiota imbalance, altered cytokine concentrations, DSS effects on colonic epithelial DNA synthesis, inhibition of cell proliferation, and disruption of the intestinal mucosal barrier [21]. IL-2 is an important cytokine in immune responses with bidirectional regulatory properties. Reduced IL-2 concentration can impair cellular immune function and decrease local defensive capacity of colonic mucosa, exacerbating tissue damage [22]. IL-4 inhibits production of IL-1 , IL-6, IL-8, and TNF- by mononuclear macrophages and promotes production of IL-1 receptor antibodies, playing an important role in maintaining intestinal immunity and suppressing inflammatory responses [23]. IL-10 mediates reciprocal regulation between Th1 and Th2 cells and inhibits release of TNF- , IL-1, and IL-6 while suppressing inflammation [24]. In this

study, the DSS model control group showed highly significant reductions in IL-2 and IL-4 concentrations and a significant reduction in IL-10 concentration. Subcutaneous EGF administration highly significantly increased IL-2 and IL-4 concentrations and significantly increased IL-10 concentration, restoring these cytokines to normal ranges. These findings indicate that EGF can regulate IL-2, IL-4, and IL-10 concentrations in colitis mice, suppress intestinal inflammation, improve colitis symptoms, and exert protective and reparative effects on damaged intestine.

In conclusion, subcutaneous EGF administration improved colonic morphology, reduced CDS, prevented DSS-induced reduction in Occludin concentration, and modulated IL-2, IL-4, and IL-10 concentrations in DSS colitis mice, demonstrating protective and reparative effects on damaged intestine.

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Note: Figure translations are in progress. See original paper for figures.