Original Article
Effects of intestinal trefoil factor on intestinal mucus barrier in burned mice

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Abstract: Severe burns might cause intense inflammatory response and tissue ischemia and hypoxia, and these effects result in intestinal mucus barrier damage. In this study, we evaluated the effects of recombinant human intestinal trefoil factor (rhITF) on the intestinal mucus barrier after burn injury. The results showed that rhITF could improve the intestinal mucusal damage index, decrease diamine oxidase (DAO) activity, reduce intestinal damage, and thereby alleviate intestinal mucus permeability. Severe burns were associated with subsequent decreases in the mucus thickness and the levels of hexose, and mucin, and rhITF administration might partially reverse these changes. Additional experiments showed that supplementation with rhITF markedly increased the mitochondrial respiratory control rate (RCR) and phosphorus-oxygen ratio (P/O) in intestinal tissue. Moreover, rhITF improved the intestinal mucosal blood flow (IMBF) and the levels of oxygen extraction (Oext), nitric oxide (NO) and ATP. These results suggest that ITF can improve the blood perfusion of the intestinal mucosa after severe burns, promote the transport of glutamine in the intestinal mucosa, improve the energy metabolism of goblet cells, stimulate goblet cell differentiation and maturation, promote the synthesis and secretion of intestinal mucus, and maintain the barrier function of intestinal mucus.

Keywords: ITF, mucus barrier, intestine, burn

Introduction

Severe burns can cause not only extensive damage to the skin and subcutaneous soft tissues but also multiple organ damage due to ischemia, hypoxia and inflammation, and the intestine is one of the most vulnerable organs to burn injury [1]. Burn injury significantly reduces intestinal blood perfusion, resulting in damage to the structure and function of the intestinal mucosa. The intestinal mucosa is the main site responsible for food digestion and absorption, and its barrier function is also the core element responsible for maintaining the environmental stability of the body. Damage to the intestinal mucosal barrier after burn injury can lead to the translocation of bacteria and toxins, and this translocation can result in intestinal infections and high intestinal metabolism, which leads to poor prognosis. The intestinal mucus barrier, an important part of the intestinal barrier system is composed of intestinal mucus. Studies have shown that loss of the mucus barrier can aggravate intestinal damage caused by a variety of pathological factors [2, 3].

The mucus covering the surface of the intestinal epithelium is essentially a hydrated polymer gel with a thickness of 50-800 μm. Mucus is secreted by goblet cells and contains proteins, carbohydrates and lipids, although its core component is mucin [4]. Mucus can play not only the role of lubricant to prevent mechanical damage to the intestinal mucosa but also the role of blocker to prevent bacteria, toxins and various digestive enzymes from coming into contact with the intestinal epithelium [5-7]. In a variety of pathological conditions, an insufficiency of mucus secretion and damage to the intestinal mucus layer caused by changes in the mucus composition are one of the initiating factors that cause intestinal damage [8, 9]. The role of intestinal mucus in maintaining the structure and function of the intestinal mucosa...
has received increasing attention. Previous studies have confirmed that the stability of the intestinal mucus barrier is related to the function of goblet cells [5, 8, 10, 11], particularly intestinal trefoil factor (ITF), which is a growth factor secreted by goblet cells [12-14].

ITF is a specific small-molecule polypeptide composed of 59 amino acids that is secreted by goblet cells. The ITF peptide chain contains a specific sequence of 38-39 amino acids, and the six cysteine residues within this sequence are linked through three disulfides. These bonds are connected to each other to twist and fold the entire peptide chain to form a special “clover-type” spatial structure, and this structure exhibits resistance to protease digestion and acid-base stability, which means that it cannot be destroyed and maintains its biological activity in the complex environment of the gastrointestinal tract [15]. The special “trefoil” spatial structure of ITF can bind to specific sites of oligosaccharides or polysaccharides in mucin, increase the stability of mucin, stabilize the intestinal mucus layer, reduce intestinal damage after burn injury, and promote mucosal repair [16]. However, previous studies have mostly focused on the secretion of ITF by goblet cells and its stabilizing effect on intestinal mucus, and whether ITF can adversely affect the function of goblet cells remains unclear. Previous research has confirmed that ITF reduces the damage to intestinal epithelial cells after burn injury [17, 18], and it has been speculated that ITF might also reduce damage to and improve the function of goblet cells [13, 14, 19]. Therefore, the changes in intestinal goblet cell function after burn injury and the effect of ITF on the energy metabolism of goblet cells were assessed in this study to explore the role of ITF on the maintenance of intestinal mucus from the novel perspective of promoting mucus synthesis and a new barrier mechanism.

Materials and methods

Animal model

Preparation and grouping: Fifty six male C57BL6 mice (6 weeks old, 25-28 g) were purchased from the Fujian Medical University Laboratory Animal Centre. Animals were reared in independent cages maintained at 22-25°C with a 12 h light-dark cycle. A standard diet was provided for 1 week prior to the experiment. Mice were randomly assigned to three groups: control group (C), burn group (B), and rhITF treatment group (B+I). All mice received pentobarbital 40 mg/kg for anesthesia and buprenorphine (1 mg/kg body weight) for analgesia. After shaving and fixing an area equivalent to 20% total body surface area, mice in group C were placed in 37°C water for 10 seconds for sham burn; mice in group B and group B+I were placed in 90°C water for 10 seconds to induce full-thickness burn. An intraperitoneal injection of Ringer’s solution (50 mL/kg) was immediately administered for resuscitation. After resuscitation, mice in the B+I group received intragastric rhITF (1 mg/kg.d) twice a day, and mice in other groups received equal volume saline twice a day. Mice in all three groups were anesthetized, and jejunal tissue was harvested after 1, 3, 5 days. All animal experiments were performed in strict compliance with the principles of care and use of experimental animals at the Union Hospital of Fujian Medical University.

Reagents

Recombinant human intestinal trefoil factor (rhITF) was first synthesized by our research group and was recombinantly expressed and fermented by Pichia pastoris. Further analysis verified that the obtained rhITF was completely consistent with its theoretical features. Toxicology experiments also confirmed that the recombinant protein exerts very slight side effects. NaH$_2$PO$_4$ was purchased from Shanghai Biological Engineering Co., Ltd. (Shanghai, China). Citric acid was purchased from Boshide Bioengineering Co., Ltd. (Wuhan, China), and pentobarbital sodium was purchased from Biyuntian Biotechnology Institute (Shanghai, China). Alixinlan (AB) dyeing solution, periodic acid (PAS) dyeing solution, and aldehyde magenta (AF) dyeing solution were purchased from Shenda Biological Products Technology Co., Ltd. (Guangzhou, China), and $^{99m}$Tc-DTPA-HAS was purchased from Isotope Radiation Company (Beijing, China). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Histological assessment

Samples of jejunal tissue (5 cm in length) were harvested from mice under general anesthesia, at different time points after burn injury.
Harvested tissues were fixed in neutral formalin solution (pH 7.4) and embedded in paraffin. Serial sections were prepared for histopathological examination. Histologic changes were observed with hematoxylin-eosin (HE) and mucus staining.

**Mucus sample collection**

The distal 30-cm segment of terminal ileum was identified and transected from the intestinal tract and underlying mesentery. Debris was removed from the external portion of the segment by washing with PBS. Fecal matter was subsequently flushed from the jejunal lumen with PBS. The exterior of the segment was gently compressed in order to collect intestinal mucus, as described by Vesterlund et al [20]. We conducted pilot studies in order to identify a method for collection of mucus that would minimize injury to intestinal villi. First, the intact mucus layer was removed. The intestinal segment was subsequently stained to measure the extent of villous injury. Samples of mucus were homogenized in 500 µl of PBS over ice prior to centrifugation for 30 min at 5000 rpm (Denville 2600; Denville Scientific, Metuchen, NJ). Supernatant was removed and stored at -80°C until further use [9].

**Study indices**

**Intestinal mucosal damage index:** Histopathologic examination of jejunum was performed by HE staining; the slides were observed and photographed with an Olympus microscope. The system for scoring mucosal injury was defined as follows: normal villi, 0 points; presence of a cystic space on the top of the villi, with hyperemic capillaries, 1 point; intraepithelial interstitial enlargement, moderate endothelial edema, central chylous dilation, 2 points; obvious edema in lamina propria, degeneration and necrosis of epithelial cells, abscission on the villous tip, 3 points; epithelial cell layer degeneration, necrosis, shedding, partial villus abscission, nule lamina propria, capillary dilation, hyperemia, 4 points; villus shedding, disintegration of lamina propria, bleeding, ulceration, 5 points.

**Diamine oxidase (DAO) activity in plasma:** The DAO activity in plasma was determined as described previously [21]. In the final volume of 3.8 mL, containing 3 mL of phosphate buffer (0.2 M, pH 7.2), 100 µL (4 µg) of horse radish peroxidase, 100 µL (500 µg) of odiainisidine, 500 µL of plasma, and finally 100 µL (175 µg) of cadaverine. After samples were mixed thoroughly, they were incubated for 30 min at 37°C. DAO activity was measured at absorbance of 436 nm.

**Measurement of mucin levels:** To quantitatively absorb the mucus sample (1 ml), 4 ml of Coomassie brilliant blue G-250 solution was added to the sample tube, and the mixture was mixed well and incubated for 2 min. A standard solution of bovine serum albumin with a concentration of 10 mg/ml was prepared, and the multiple dilution method was used to obtain solutions with concentrations of 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml, and 0 mg/ml. These solutions were mixed with 1 ml of 0.9% NaCl solution, and 4 ml of Coomassie brilliant blue solution was then added. The O.D. values of the various standard and sample tubes were then recorded using a spectrophotometer (λ = 595 nm). Using absorption value of the standard tube as the ordinate and the concentration as the abscissa, the standard curve was then drawn on coordinate paper. Based on the O.D. value of each sample tube, its corresponding concentration was found from the curve, and the final protein concentration (mg/ml) was calculated as sample concentration × 25.

**Respiratory control rate (RCR) and phosphorus-oxygen ratio (P/O):** The mitochondria of intestinal epithelial cells were isolated using the Watford Masola method [22], and the mitochondrial respiratory activity was determined using the Estabrood method. The mitochondria to be tested were placed in a 1.5-ml reaction tube at a constant temperature of 30°C and measured with a Clark oxygen electrode. After 1 min of incubation, 100 µl of mitochondria, 0.75 mol/L sodium glutamate and 10 µl of sodium malate were added. After the resulting mixture was incubated for 1 min, 0.17 mol/L ADP was added, and the change in the oxygen content in the solution was measured. The difference in the breathing state was ST3 (state III breathing rate refers to the rate of oxygen consumption in the presence of ADP) and ST4 (state VI breathing rate is the rate of oxygen consumption after ADP is exhausted). The entire measurement was strictly performed at low temperature and completed within 2 h, and
the breathing control was calculated using the following formula: Rate (RCR) = ST3/ST4 and phosphorus-oxygen ratio (ADP/O, P/O).

**Oxygen extraction and measurement of the oxygen uprate rate (Oext):** For blood gas analysis and routine blood assessment, 1 ml of blood was directly drawn from the abdominal aorta of each mouse, and another 1 ml of blood was obtained from the superior mesenteric vein. The Oext was calculated according to the following formula: (SaO₂ * Hba-SvO₂ * Hbv)/SaO₂ * Hba (SaO₂: arterial oxygen saturation. Hba: arterial hemoglobin. SvO₂: venous oxygen saturation. Hbv: venous hemoglobin).

**Nitric oxide (NO) determination:** The metabolites downstream of nitrite were detected using the Griess reaction, which allows the indirect measurement of the amount of NO. Briefly, 50 μl of cell culture fluid/protein lysate and 50 μl of Griess reagent A (1% sulfanilamide and 5% phosphoric acid; Sigma, USA) were added to each well of a 96-well plate, and the plate was incubated for 5 min. Subsequently, 50 μl of Griess reagent B (0.1% N-(1-naphthyl)ethylene-diamine; Sigma, USA) was added, and the plate was incubated for 5 min. The supernatant was collected by centrifugation, and the absorbance value was measured at 540 nm using a Thermo Scientific Varioskan Flash instrument. Additionally, different concentrations of NaNO₂ were tested and used as standards for comparison.

**ATP levels in intestinal mucosa:** A mortar and pestle were used to convert intestinal tissue to powder form. This powder was then transferred to a test tube containing 0.6 N perchloric acid. After extracting the metabolites, the extraction was neutralized with a mixture of KOH and K₂CO₃. Then the extraction was centrifuged at 8000 rpm for 15 min at 4°C. 10 mL of supernatant was subjected to high performance liquid chromatography with UV/VIS-152 (Gilson, France). The results were expressed as micromole ATP per gram protein.

Other methods (1 Measurement of the permeability of intestinal mucous membrane. 2 Mucosal thickness. 3 Measurement of mucosal hexose levels. 4 Intestinal mucosal blood flow (IMBF). 5 Hematoxylin and eosin (HE) staining. 6 High iron diamine-Alcian blue (HID-AB) staining. 7 Periodic acid Schiff (PAS) staining. 8 Examination of intestinal tissue sections using aldehyde fuchsine (AF) staining) are shown in the annex (Supplementary Materials).

**Statistical analysis**

All the data are expressed as the mean ± standard deviation (SD). Correlations between variables were assessed by two-way analysis of variance (ANOVA). Because the experimental design involved repeated measures, the significance of the two variables (variable 1: treatment; variable 2: time) and their interaction were simultaneously tested by repeated measures analysis of variance. All statistical analyses were performed with SPSS 22.0. P < 0.05 was considered statistically significant.

**Results**

**Effects of rhITF on the intestinal mucosal damage index, diamine oxidase (DAO), and permeability**

A mouse severe burn injury model was established to assess whether ITF can effectively reduce intestinal mucus damage. In this study, the intestinal mucosal damage index, which was used for the histopathological examination of the jejunum, was determined by HE staining. After burn injury, the intestinal mucus appeared hyperemic and edematous, with mucosal exfoliation and ulceration. Compared with group B, group B+I showed a significantly decreased intestinal mucosal damage index on postburn days 3 and 5 (Figure 1A). DAO is a sensitive indicator of intestinal damage, and plasma DAO activity was significantly increased after damage to the intestine and then decreased over time, but the levels were still significantly higher than those found in group C until 5 days after burn injury. DAO activity was significantly lower in group B+I than in group B on day 5 after burn injury (Figure 1B). After burn injury, the permeability of the murine intestinal mucous membrane increased significantly and remained higher than that of group C until 5 days after burn injury. Significantly lower permeability was found in group B+I than in group B during days 3 through 5 after burn injury (Figure 1C).

**Changes in the mucosal thickness and levels of hexose and mucin**

The thickness of the intestinal mucus layer decreased after burn injury, and on days 3 and
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5 after burn injury, the mucus layer of group B+I was significantly thicker than that of group B (Figure 2A). The levels of hexose reflect the degree of mucus maturity. After burn injury, the levels of hexose in intestinal mucus tended to decline, and significantly higher levels were found in group B+I than in group B on days 3 through 5 after burn injury (Figure 2B). Compared with the levels in group C, the mucin levels in the intestinal mucus decreased gradually after injury. At days 3 and 5 after injury, the mucin levels in group B+I were significantly higher than those in group B (Figure 2C).

Effects of rhITF on RCR and P/O

The mitochondrial RCR was decreased by different degrees from days 1 to 5 after burn injury but was significantly higher in group B+I than in group B from days 3 to 5 after burn injury (P < 0.01, Figure 3A). The mitochondrial P/O levels in the intestinal mucosa decreased from days 1 to 5 after burn injury. Significant recovery was observed with rhITF supplementation; in particular, the mitochondrial P/O content in the intestine was restored to normal levels within 3 days after injury. In addition, the mitochondrial P/O in group B+I was significantly higher than that in group B on days 3 and 5 after burn injury (P < 0.05, Figure 3B).

Effects of rhITF on the intestinal mucosal blood flow (IMBF), Oext, NO and ATP

In this study, the IMBF was decreased to 75% of baseline on day 1 after burn injury and then increased slowly but remained lower than baseline on day 5 after injury. Group B+I presented significantly higher IMBF than group B from days 3 to 5 after burn injury (Figure 4A). The Oext level decreased significantly from day 1 to 5 after burn injury, and comparisons with group
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B showed that rhITF supplementation might enhance the Oext level on days 3 and 5 after burn injury (P < 0.05, Figure 4B). The NO level in the intestinal mucosa was increased on day 5 after burn injury but was significantly higher in group B+I than in group B on days 3 and 5 postburn injury (P < 0.01, Figure 4C). The ATP content in the intestine was decreased by different degrees from days 1 to 5 after burn injury. Significant recovery was observed with rhITF supplementation; in particular, the ATP content in the intestine was restored to normal levels 3 days postburn injury. The ATP content in group B+I was significantly higher than that in group B on days 3 and 5 after burn injury (P < 0.01, Figure 4D).

Morphological effects on the intestinal mucosa

In this study, HE histochemical staining was used to observe the morphological changes in the intestinal tissue structure after burn injury under an optical microscope. Compared with group C, group B showed obvious pathological changes, including intestinal mucosal edema, capillary congestion, and villi shortening 1 day after injury and villi rupture, necrosis, shedding, lymphocyte infiltration, submucosal congestion and edema 5 days after injury. Compared with group B, the damage in group B+I was alleviated to varying degrees, and the damage observed in group B+I mainly manifested as villous swelling, mucosal congestion and edema, and capillary congestion (Figure 5). These findings indicate that rhITF can reduce mucosal damage after burn injury and maintain the structure of the intestinal mucosa. To observe the changes in the composition of intestinal mucus after burn injury, the changes in acidic mucus, sulfated mucus and neutral mucus were assessed using three staining methods, HID-AB, AF and PAS. The histochemical staining of mucus showed that burn injury damaged goblet cells, reduced their ability to synthesize mucus and significantly altered the chemical composition of mucus. Both intracellular (goblet cells) and extracellular (intestinal lumen), the degree of staining of the three mucus forms was significantly reduced by burn injury; in particular, the number of goblet cells with strong positive staining of HID-AB, PAS and AF was significantly
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Discussion

Large-area deep burns can cause intense stress and continuous inflammation, which can lead to multiple organ damage. The intestine is very sensitive to ischemia, hypoxia and inflammation and is one of the most vulnerable organs after burn injury [1, 23]. As demonstrated in this study, the intestinal mucosa showed obvious pathological changes after burn injury, and these were characterized by intestinal mucosal edema, capillary congestion, and villi shortening. During the course of the disease, the intestinal mucosa exhibited varying degrees of villi rupture, necrosis, shedding, lymphocyte infiltration, submucosal congestion and edema (Figure 5). Correspondingly, the activity of DAO, a serological indicator of intestinal mucosal injury, also increased significantly after burn injury, and the permeability of the intestinal mucosa was significantly higher after injury than before injury (Figure 1). These findings showed that the structure of the intestinal tissue was obviously affected after severe burn injury and that the injury also damaged the barrier function of the intestinal mucosa. In addition, we found that the intestinal mucus barrier also significantly altered after burn injury. Compared with those in group C, the intestinal mucosal thickness and hexose levels in group B were significantly reduced (Figure 2A, 2C), and the content of mucin decreased to 50% of baseline on day 1 after injury and further decreased over time (Figure 2B). In addition, histochemical staining of intestinal mucus showed that burn injury caused damage to and reduced the number of goblet cells, reduced the ability of these cells to synthesize mucus, and significantly altered the chemical composition of mucus (Figures 6-8).

ITF is secreted by intestinal goblet cells maintaining at a stable level, which does not require additional exogenous ITF. This study confirmed...
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that rhITF can reduce the degree of intestinal injury after burn injury. rhITF treatment was administered after burn injury, and the intestinal mucosal injury index of burned mice was significantly reduced by this treatment on the day 3 after injury (Figure 1). The same result was found in the permeability of the mucosa (Figure 1). As an important indicator of intestinal mucosal injury, significantly lower DAO activity was obtained 5 days after rhITF treatment, and the difference was statistically significant (Figure 2, P < 0.01). The level of mucin, the main component of mucus, was significantly higher on days 3 to 5 after rhITF treatment compared with that in group B, and this difference was statistically significant (Figure 2B, P < 0.01 or 0.05). Goblet cells that secrete mucus are differentiated from crypt pluripotent stem cells and mature from crypts upward. The immature goblet cells in the crypt mainly secrete neutral mucus, and the content of acidic mucus is an important indicator of goblet cell maturation [24]. This study found that burn injury increased the secretion of neutral mucus (Figure 7) but decreased the secretion of acidic mucus, particularly sulfated mucus (Figures 6, 8); in contrast, rhITF treatment increased the secretion of acidic mucus and decreased that of neutral mucus. These findings show that rhITF can reduce the damage to goblet cells and promote the differentiation and maturation of goblet cells. Our experimental results indicate that the mechanism through which rhITF maintains intestinal mucus secretion involves reducing intestinal damage and maintaining the structure and function of goblet cells [13, 14, 25, 26], but more importantly, this mechanism might be related to improving the energy metabolism of intestinal epithelial cells after burn injury.

The process of mucus synthesis in goblet cells is complicated but mainly consists of glycosyl-
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Figure 7. Intestinal tissue in groups B and B+I on days 1 and 5 after burn injury, compared with control. PAS staining.

...of all epithelial cells in the intestine can provide an overall understanding of the energy metabolism of goblet cells because goblet cells are essentially a specialized intestinal epithelial cell. The population of intestinal epithelial cells isolated in this study contain a large number of goblet cells, and the changes in their energy metabolism are consistent. This study found that severe burn injury significantly reduced Oext and ATP synthesis in intestinal epithelial cells (Figure 4) and significantly decreased the mitochondrial RCR (Figure 3) and P/O (Figure 3) in intestinal epithelial cells. The significant reduction of the RCR and P/O indicated that the electron transport function and oxidative phosphorylation function of mitochondria were damaged, which results in reduced production of ATP. The normal physiological function of mitochondria depends on an adequate blood supply, good gas diffusion and exchange, and barrier-free mitochondrial O\textsubscript{2} utilization [27]. After burn injury, the blood flow of the intestinal mucosa is significantly reduced, and obvious intestinal edema can be observed, which prevents oxygen and nutrients from entering cells and oxygen diffusion in the tissues. These findings provide an important explanation for the inhibition of mitochondrial respiratory function observed with mitochondrial dysfunction. Mitochondrial dysfunction interfering Cell oxygen utilization and energy synthesis are mutually causal and form a vicious circle. Our experimental results showed that rhITF treatment significantly increased the synthesis of ATP in intestinal epithelial cells compared with that found in group B (Figure 4), and this finding is consistent with the significant improvements in the mitochondrial respiratory function.
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The experimental results showed that ITF can significantly improve goblet cell energy metabolism and maintain the secretion of intestinal mucus. This study also found that severe burn injury significantly decreased the intestinal mucosal NO content and IMBF and that the administration of rhITF significantly increased these variables (Figure 4). The same results have also been confirmed in other studies [28, 29]. We thus infer that ITF can improve intestinal mucosal blood perfusion by promoting NO synthesis and by providing oxygen and energy needed for maintaining the energy metabolism of goblet cells. The energy demand of the intestinal mucosa is particularly important. Glutamine is the most important energy substance and plays an important role in maintaining the energy metabolism of the intestine [10, 11]. Glutamine has two nitrogen side chains, amino and amide, and this structure provides carbon and nitrogen sources for the rapid growth and differentiation of intestinal epithelial cells, including goblet cells [30, 31]. Nitrogen sources can be used as substrates for intestinal mucus synthesis, and carbon sources can provide energy for mucus synthesis [32-36]. Our previous studies have found that the transport of glutamine in intestinal epithelial cells is impaired after burn injury, which leads to a decrease in the glutamine utilization rate and affects the synthesis of energy in intestinal epithelial cells. The administration of ITF can significantly increase the ability of intestinal epithelial cells to transport glutamine, increase their utilization of glutamine, and improve the energy metabolism of these cells, and these effects promote the synthesis and secretion of mucus by goblet cells and maintain the intestinal mucus barrier [18]. Therefore, we infer that ITF maintains the intestinal mucus barrier function by promoting the transport of glutamine in intestinal epithelial cells and thereby improving the energy metabolism of intestinal epithelial cells.

Figure 8. Intestinal tissue in groups B and B+I on days 1 and 5 after burn injury, compared with control. AF staining.
In summary, ITF can improve the blood perfusion of the intestinal mucosa after severe burn injury, promote the transport of glutamine in the intestinal mucosa, improve the energy metabolism of goblet cells, stimulate the differentiation and maturation of goblet cells, and promote the synthesis and secretion of intestinal mucus, which ultimately results in maintenance of the barrier function of intestinal mucus.

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Disclosure of conflict of interest

None.

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References

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[22] Masola B and Ngubane NP. The activity of phosphate-dependent glutaminase from the rat small intestine is modulated by ADP and is dependent on integrity of mitochondria. Arch Biochem Biophys 2010; 504: 197-203.


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Supplementary materials

Measurement of the permeability of intestinal mucous membrane

1480 KBq/kg of $^{99m}$Tc DTPA-HAS was injected into the mice via the caudal vein, and the mice were decapitated 30 min later. Scintillation counting of intestinal lavage solution was carried out. Permeability of the intestinal mucous membrane at various phrases after burn injury was normalized to the level of flux across the intestinal mucous membrane in control mice. In the study a decay control was set to adjust the counts.

Mucosal thickness

The Alcian blue combination method was used to infer mucosal layer thickness Buffer 0.2 M NaH$_2$PO$_4$, 0.1 M citric acid, pH 5.8 was combined with 1 g/L Alcian blue solution buffer, Ten centimeters of ileum was measured, then washed in isotonic saline solution and then immersed in 10 mL of staining solution. After incubation at room temperature (RT) for 2 h, the intestinal segment was removed, and approximately 5 mL of staining solution was centrifuged at 3000 rpm for 15 min. The OD value of the supernatant was measured at $\lambda = 615$ nm, using buffer as blank and Alcian blue solution as standard.

Alcian blue combination (mg) = $10 - (\text{OD}_{\text{sample}}/\text{OD}_{\text{standard}}) \times 10$.

Measurement of mucosal hexose levels

Mouse intestinal mucus was diluted and centrifuged to remove impurities. Mucosal hexose levels were determined with phenol sulfone. 1 mL of sample was added to 1 mL of 6% phenol. Ten minutes later, 5 mL of 98% sulfuric acid was added. Colorimetry was performed 30 min later, at a wavelength of 495 nm. The standard curve was plotted using D-galactose as standard.

Intestinal mucosal blood flow (IMBF)

IMBF was detected by microcirculatory Doppler blood flow meter. Laparotomy was performed under superficial anesthesia. After 0.5 cm of intestinal wall was obtained from the opposite side of the hollow mesentery, the probe was gently placed in contact with the mesenteric side wall. Results after stabilization were recorded, and average values were calculated after measurements had been performed in triplicate.

Hematoxylin and eosin (HE) staining

Intestinal tissue specimens were removed from 10% formaldehyde solution after 24 hr and rinsed with saline. Filter paper (Yiyuan Machine Tool Accessory Co., Ltd, Yantai, China) was used to gently and carefully wipe surface liquid. Specimens were dehydrated overnight in 95-100% alcohol in an automatic hydro-extractor (TP1020, Leica, Wetzlar, Germany). Intestinal tissue was sectioned into 3-µm slices with a pathologic tissue embedding machine (HistoCore Arcadia, Leica, Wetzlar, Germany). Slices were gently placed onto glass slides using forceps, and any folds were stretched. Slices were then dewaxed with xylene (twice, each time for 10 min). Sections were rinsed with saline, stained for 5 min with hematoxylin, and rinsed, then stained for 30 s with 0.5% eosin. Sections were then dehydrated with alcohol and dealcoholized with xylene. Neutral gum and coverslips were applied. Sections were examined using light microscopy with $\times$ 400 magnification (Leica DM1000, Leica, Wetzlar, Germany) and photographed.

High iron diamine-Alcian blue (HID-AB) staining

Diamine solution was prepared by dissolving N, N-dimethyl-p-phenylenediamine monohydrochloride (20 mg) and N, N-dimethyl-m-phenylenediamine dihydrochloride (120 mg) in distilled water (50 mL). High iron diamine solution was also prepared as described by using 40% (w/v) ferric chloride prepared from anhydrous resublimed salt and the diamine solution. After staining for 24 hr at RT, sections were rinsed 3 times with tap water. Sections were then dehydrated, cleared, and mounted after exposure to 1%
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(w/v) Alcian blue in 3% acetic acid for 30 min at RT. HID-AB stain was graded on a three-point scale from - to ++ (- = no glands positive; + = only luminal mucin positive; ++ = intracytoplasmic mucin positive).

*Periodic acid Schiff (PAS) staining*

PAS and Alcian blue staining were performed with a Periodic Acid-Schiff kit (395B-1KT, Sigma-Aldrich, St Louis, MO, USA). After deparaffinization and rehydration, periodic acid solution was applied for 5 min at RT. Schiff reagent was then applied for 15 min at RT, followed by hematoxylin counter staining. PAS-Diastase staining was performed using the same kit, with the addition of α-amylase (0.2 g to 40 mL water). Slides were then placed in a microwave at 600 W for 25 s and rinsed in water for 5 min.

*Examination of intestinal tissue sections using aldehyde fuchsin (AF) staining*

Paraffin sections were washed with AF dye solution for 5 min, then gently washed in distilled water for 20 min or until the dye was removed. Normal dehydration yielded transparent sections, and the dehydrating water used for paraffin sections was washed with AF dye for 5 min. AF has strong affinity for the sulfate group on mucopolysaccharide. Therefore, strongly sulfated mucus is dark purple, while weakly sulfated mucus is purple.