ORIGINAL RESEARCH

Dietary fiber alleviates intestinal barrier dysfunction in post-trauma rats

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Abstract

Background: Damage to the intestinal barrier often occurs following severe trauma. It has been reported that enteral nutrition with dietary fiber (DF) could mitigate impairment of the intestinal barrier and might therefore be effective in clinical application; however, the conclusions from existing trials are controversial and the nature of the protective mechanism is far from clear. This study investigated the protective mechanism of dietary fiber on intestinal barrier in rats under bilateral closed femur fracture.

Methods: Twenty-four Sprague-Dawley rats were divided into four groups: normal control without any manipulation, trauma control with normal feeding, DF and dietary fiber-free (NF) groups fed with Nutrison Fibre and Nutrison, respectively. The later two groups were further divided into 1, 4, 7 and 10 days post-trauma groups.

Results: The trauma caused body weight decline, promoted bacterial translocation, and decreased immune function. The levels of portal vein endotoxin in DF group was significantly lower than in NF group ($p=0.013$). Levels of both serum TNF- α and IL-6 on post-trauma day 10 showed no statistical differences between DF and NF groups. The incidence of bacterial translocation recovered to normal in DF group. Only secreted immunoglobulin a (sIgA) levels in DF group was higher than in NF group ($p=0.005$).

Conclusion: Early enteral nutrition with dietary fiber could alleviate damage to intestinal barrier function and decreased the incidence of bacterial translocation caused by trauma and endotoxemia in rats under extra-abdominal trauma.

List of Abbreviations

DF	dietary fiber
sIgA	secreted immunoglobulin A
SCF	short-chain fatty acids
EEN	early enteral nutrition
TC	trauma control
NC	normal control
NF	dietary fiber-free
PTD	post-trauma day
TNF- α	tumor necrosis factor α
IL-6	interleukin 6
MLNs	mesenteric lymph nodes
BT	bacterial translocation

Severe trauma is accompanied by prolonged negative nitrogen balance, disrupted endocrine balance, delayed wound healing and decreased immune function. Systemic inflammatory response may be partly re-

sponsible for numerous complications; including sepsis, multiple organ failure and unregulated hypermetabolism leading to protein-caloric malnutrition.^{1,2} It has been reported that the integrity of the gastrointestinal tract is an important factor in the pathogenesis of the systemic inflammatory response and sepsis. Resuscitation and nutritional support strategies for preserving gut mucosal integrity have been strongly promoted³ and it has been shown that early enteral feeding protects intestinal barrier function in traumatized patients.⁴ The exact mechanism for this protection is not yet known; however, increased visceral circulation and mucosal perfusion, and enhancement of mucous gel production may contribute.^{5,6}

Fiber supplementation during enteral nutrition with liquid formula diets has been recommended to normalize bowel function and improve feeding tolerance. Dietary fiber (DF) is a category of carbohydrates that cannot be ingested by endogenic digestive enzymes. Short-chain fatty acids (SCFAs), products of carbohydrate fermentation and primary important energy substances for colonic mucosal epithelium, are essential to maintain the metabolism and regeneration of epithelia, protect epithelial structure and function, promote water and sodium absorption and regulate bowel function. Thus, DF has been widely recommended as a critical part of enteral nutrition in the last 20 years; this despite some controversial conclusions from existing trials.^{7,8} In our study, we have used the bilateral closed femur fracture model in rats, observed the effects of DF in early post-trauma enteral nutrition (EEN), and proposed a potential therapeutic mechanism for DF.

Methods

Animals

All studies were conducted in compliance with the Guide for the Care and Use of Laboratory Animals, the Animal Research Committee of Nanjing University. Male, adult Sprague-Dawley rats, weigh-

ing 220-260 g, were housed in metabolic cages and acclimatized to their environment (12-h light/dark cycle) for at least 2 days before experiments. All animals were fed with rat chow (Animal Feed Co., Shanghai, China) and water *ad libitum*.

Experimental design

All animals were randomly assigned to four experimental groups as follows: normal control (NC) without any manipulation, trauma control (TC) with normal feeding, dietary fiber and dietary fiber-free (NF) groups fed with Nutrison Fibre and Nutrison respectively. The later two groups were sacrificed on post-traumatic days 1, 4, 7 and 10, and blood and tissues were collected.

Surgical procedure

Following overnight fasting, rats were anesthetized with ketamine (100mg/kg), administered intraperitoneally. Bilateral closed femur fractures were caused by dropping a weight (750 g) from a height of 80 cm. Abdominal fur was clipped, and the skin was disinfected with alcohol. Under aseptic conditions, the abdomen was opened using a midline incision. A sterile fenestrated silastic gastrointestinal catheter (1.6 mm inner diameter/2.4 mm outer diameter; Xinya Co., Shanghai, China) was used as a gastrostomy tube, with one end placed through the anterior gastric wall into the duodenum. The other end of tube was placed through a subcutaneous tunnel, externalized at the cervical region of the back, and attached to a free swivel device. Immediately after surgery, all catheterized rats were placed in metabolism cages and connected to an infusion pump (WZ50-D; Medical Apparatus Co., Zhejiang, China).

Nutrition support

EEN began 3-5 hours following the placement of the gastrostomy tube. The DF group received 0.75 g DF daily, while the NF group received no DF. The other

TABLE 1. Comparison of the nutrition elements of Nutrison and Nutrison Fibre

	Nutrison (21.5 g)	Nutrison fibre (100 ml)
protein	4.0	4.0
nitrogen	0.6	0.63
fat	3.9	3.89
seed fat	3.9	
linoleic acid	1.7	1.025
linolenic acid		0.205
carbohydrate	12.3	12.3
mineral matter	0.5	0.48
vitamins	0.03	0.029
dietary fiber	0	1.5
energy	100 Kcal	100 Kcal
protein	16%	16%
fat	35%	35%
carbohydrate	49%	49%
osmotic pressure	320 mOsm/L	250 mOsm/L
pH	6.8	6.6

elements of EEN were identical. All rats received isocaloric total enteral nutrition solutions (200 kcal/kg, 50 ml per day) from the free swivel device using an infusion pump. The composition of total enteral nutrition solutions (prepared by Nutricia Co., Wuxi, Jiangsu, China) is shown in Table 1.

Body weight changes

Body weight was measured on post-trauma day (PTD) 1, 3, 7, and 10.

Serum cytokine and endotoxin measurements

Blood was obtained from the inferior vena cava and portal vein at the time of sacrifice. Serum samples were analyzed for TNF- α and interleukin 6 (IL-6) by enzyme-linked immunosorbent assay according to manufacture's instructions (BioSource International, Camarillo, CA).

Blood was obtained from the inferior vena cava and portal vein at the time of sacrifice. Serum endotoxin levels were quantified using tachypleus amebocyte lysate (Bio Center, Shanghai, China).

Estimate of bacteria translocation

On PTD1, 3, 7 and 10, respectively, six rats per group were anesthetized, the mesenteric lymph nodes (MLNs) and liver were aseptically harvested: 0.5 gram of tissues of MLNs and 2 g liver tissue were homogenized in 1.0 and 2.0 ml of sterile isotonic saline using tissue grinders respectively, and 0.01 ml of homogenate of each tissue sample was plated on blood and chocolate agar plates, then incubated 24-48 hours at 37°C. Bacteria levels of more than 100 per gram was considered positive.⁹

Measurements of secreted immunoglobulin a (sIgA) and mucin of gut

Samples were obtained by scraping mucosa of cecum with microscope slide. Samples were then diluted with saline (10ml per gram), kept overnight at -4 °C and centrifuged for 5 minutes at 3000 rpm. The supernatant (100 ul) was aspirated and the concentration of sIgA was measured by enzyme-linked immunosorbent assay as previously described.¹⁰

Equal amounts of cecum and saline were mixed sufficiently, centrifuged for 10 minutes at 2000 rpm, then the supernatant was aspirated. Chemical colourimetry was used and the concentration of mucin was observed using a 751 spectrophotometer (Shanghai Optical Instrument Factory, Shanghai, China) as previously described.¹¹

Statistical Analysis

Results are expressed as mean values $\pm$ SD. Student's t test was used to determine the significance of group differences and Chi square test was used to analyse bacterial translocation data. $P < 0.05$ was considered statistically significant.

Results

Post-trauma recovery

Bilateral closed femur fracture and damage of local soft tissue were observed immediately following

TABLE 2. Bacteriology tests in lymph nodes and liver following trauma

Groups	MLNs				Liver			
	PTD1	PTD4	PTD7	PTD10	PTD1	PTD4	PTD7	PTD10
NC	0	0	0	0	0	0	0	0
TC	5/6	0	0	0	6/6	0	0	0
DF	5/6	2/6	0*	0*	6/6	3/6	0*	0*
NF	5/6	3/6	2/6	0*	6/6	5/6	3/6	1/6*

* P<0.001 vs. the same group PTD1

trauma. On post-trauma day five, animals displayed local hyperemia, edema, patchy congestion and punctiform necrotic foci without obvious infection. No statistical difference was observed between DF and NF groups for body weight decline.

Levels of serum endotoxin

The levels of portal vein endotoxin decreased gradually after EEN. The endotoxin level in DF group was lower than NF group on PTD7 (0.128 ± 0.056 vs. 0.256 ± 0.098 , $P=0.013$). On PTD10, the level recovered to normal in DF group, while it in NF group was still higher than normal NC group (0.222 ± 0.079 vs. 0.114 ± 0.003 , $P=0.021$). The change of endotoxin level in inferior vena cava exhibited a similar appearance.

BT of MLNs and liver

No BT occurred in NC group but appeared in most post-trauma rats' livers. After EEN, the incidence of BT decreased gradually. DF and NF groups recovered to normal on PTD7 and PTD10 respectively. The incidence of BT in MLNs was 100% (6/6), and decreased quickly after EEN. The incidences of DF and NF

groups were 50% (3/6) and 83.5% (5/6). The DF group recovered to normal (0/6) on PTD7, while the NF group did not recover (Table 2). The translocated bacteria, in order of frequency, were *E. coli*, *Enterococcus* and other gram-negative bacteria.

Levels of serum TNF- α and IL-6

The levels of serum IL-6 and TNF- α increased significantly in traumatized groups and then decreased gradually with EEN (Table 3). Levels of serum TNF- α and IL-6 on PTD 10 showed no statistical differences between DF and NF groups.

Levels of sIgA and mucin of gut

Fasting and trauma led to a significant decrease of the concentration of sIgA and mucin in gut, then recovered gradually after EEN (Table 4). In comparison with the NF group, only sIgA levels were significantly higher in DF group ($P=0.005$) on PTD10.

Discussion

The gut is a complex cellular and secreted barrier which separates the external environment from the host tissues. The intestinal barrier includes physical diffusion barriers, regulated physiological and enzymatic barriers, and immunological barriers; all of which are under neurohormonal control and therefore possible targets for influence by stress. The epithelial barrier is one of the most important non-immunological components; it exerts an important physiological defense by secreting fluid and mucus, together with sIgA, into the lumen to dilute, wash away, and bind noxious substances. This defense

TABLE 3. Levels of serum TNF- α and IL-6 in post-trauma rats

Groups	IL-6 (pg/ml)				TNF- α (ng/ml)			
	PTD1	PTD4	PTD7	PTD10	PTD1	PTD4	PTD7	PTD10
NC	29.6 $\pm$ 10.1				98.7 $\pm$ 23.5			
TC	58.5 $\pm$ 13.7				355.8 $\pm$ 58.4			
DF	65.3 $\pm$ 18.2	55.4 $\pm$ 12.0	36.5 $\pm$ 8.9†	28.8 $\pm$ 9.5	334.1 $\pm$ 45.2	268.5 $\pm$ 76.3	246.2 $\pm$ 48.9†	145.3 $\pm$ 67.8
NF	60.2 $\pm$ 18.9	58.4 $\pm$ 11.2	45.9 $\pm$ 7.5‡	30.5 $\pm$ 8.9	359.0 $\pm$ 29.1	333.8 $\pm$ 67.9	299.7 $\pm$ 39.8	210.4 $\pm$ 60.1§

†: $p=0.026$ vs. NF group for IL-6, $p=0.035$ vs. NF group for TNF- α , ‡: $p=0.014$ vs. NC group, §: $p<0.001$ vs. NC group

TABLE 4. sIgA and mucin concentrations in intestinal mucosa

Groups	sIgA (ng/g)				Mucin (mg/g)			
	PTD1	PTD4	PTD7	PTD10	PTD1	PTD4	PTD7	PTD10
NC	0.352±0.02				42.3±11.5			
TC	0.189±0.07				16.2±7.1			
DF	0.190±0.06	0.250±0.05	0.343±0.07¶	0.359±0.05¶	17.4±3.3	30.4±6.8**	34.9±8.4	43.0±6.5
NF	0.188±0.05	0.188±0.05	0.230±0.08	0.271±0.05Δ	16.8±6.4	22.4±5.5	31.8±6.9	35.4±6.8

¶: vs. NF group p=0.002 on PTD7, p=0.005 on PTD10; Δ: p=0.003 vs. NC group; **: p=0.023 vs. NF group

mechanism is not fully understood, and factors such as molecular size and physiochemical properties of the permeating substances might play a major role in selectivity.^{12,13}

Intestinal ischemia reperfusion is an important initiating event under severe pathophysiological conditions, and it results in not only intestinal injury but also secondary organ dysfunction, as the release of destructive proinflammatory cytokines such as TNF- α and endotoxin into circulation causes systemic inflammatory response syndrome and endotoxemia, a leading cause of morbidity and mortality. The gastrointestinal response to severe trauma is characterized by mucosal atrophy, changes in digestive absorption, and increased intestinal permeability. Mucosal permeability also parallels increases in gut epithelial apoptosis. Decreased intestinal blood flow and changes associated with increased gut permeability have also been shown to occur following trauma.¹⁴⁻¹⁶

Luminal nutrition is important for maintenance of gastrointestinal mucosal structure and function.¹⁷ In particular, SCFAs, metabolic products of anaerobic bacterial fermentation of DF and resistant starch, are very important as the preferred respiratory fuel of the colonocytes.¹⁸ A generous intake of dietary fiber reduces risk for developing the following diseases: coronary heart disease, stroke, hypertension, diabetes, obesity, and certain gastrointestinal disorders. Furthermore, increased consumption of DF improves serum lipid concentrations, lowers blood pressure, improves blood glucose control in diabetes, promotes regularity, aids in weight loss, and appears to improve immune function.¹⁹⁻²¹ Gomez *et al*²² showed that SCFAs could protect the intestinal barrier and prevent

BT; however, there have been few reports concerning whether DF can affect the permeability of the intestinal mucosa and prevent or decrease BT after an extra-abdominal trauma. Hence, the bilateral closed femur fracture model of rats was used for further research on the protective effects of DF.

Trauma-induced damage to intestinal barrier, resulting in weight loss, BT and endotoxemia were well demonstrated in our study, and DF appears to ameliorate the intestinal barrier dysfunction and decreased the incidence of BT.²³⁻²⁵ Nakamura *et al*.²⁶ reported that DF could effectively reduce the intestinal bacterial translocation in burned rats. Besides, EEN with DF also had anti-inflammatory effects for decreasing the TNF- α and IL-6 levels in rats. These protective effects may be due to a number of factors. (1) DF could stimulate the distal intestine and colon directly, and improve enterocyte proliferation and cytokinetics.²⁷ (2) DF has been shown to induce the production and release of intestinal trophic factors. Fiber stimulates trophic gut hormones, and trophic gut hormones improve intestinal morphology and function, so intestinal permeability could be maintained. (3) DF could prevent bacterial overgrowth, especially for enteric bacilli, prevent the disruption of gut microflora and inhibit the adherence of bacteria to epithelial cells after trauma.^{8,28,29} (4) SCFAs, the major anion of the intestinal lumen and gastrointestinal-specific fuels, could promote intestinal adaptation and intestinal (colonic) Na⁺ absorption. It has been reported that SCFAs can maintain mucosal function, decrease apoptosis and promote cytoprotection, which might be associated with the induction of cytoprotection heat shock proteins. Moreover, it exhibits anti-inflam-

matory effects via inhibition the expression of TNF- α and interferon γ .^{31,31} Inan *et al.*³² demonstrated that SCFAs inhibited colonic inflammatory responses via nuclear factor- κ B. Thus, EEN, in combination with DF, could ameliorate the gut blood flow, maintain the structure of intestinal barrier and reduce intestinal permeability.

One of the major protective immune mechanisms for the intestinal tract is the synthesis and secretion of sIgA and mucin. sIgA and mucin inhibit the adherence of bacteria to epithelial cells and prevents bacterial colonization and multiplication. In addition, sIgA neutralizes bacterial toxins and viral activity and blocks the absorption of antigens from the gut while the intestinal mucus layer covers the gastrointestinal epithelium to form an effective barrier against harmful luminal microflora. Our study revealed that DF could promote sIgA and mucin secretion and stimulate the immunological defense of the gut.^{9,28}

A meta-analysis of randomized controlled trials by Yang⁸ showed no evidence that DF prevented infection in clinical EEN, though they did show that length of hospital stay was reduced.

As is well known, rats are better able to digest these carbohydrates than humans, so the species-specific variability should not be ignored. Extending this work to pigs or to a larger mammal (smaller species-specific variability) would provide more data more applicable to the clinical setting.

Conclusion

In post extra-abdominal traumatized rats, EEN with DF appears to effectively regulate immunological reactions and promote secretion of sIgA and mucin, leading to significant recovery of intestinal barrier function and a reduced incidence of BT. To date, there have been very few clinical trials with DF, and most reports have been anecdotal or uncontrolled. Large samples and multicenter studies are needed to explore cellular and molecular mechanisms of DF, and evaluate the potential effects in a clinical setting.

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