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Original Article

Preventive effects of Tongfu Xiere Enteroclysis Mixture on postoperative intestinal adhesion

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Abstract: Intestinal adhesion is one of the most common complications after abdominal surgeries with high morbidity. Previous studies have shown that it is critical to the maintenance of intestinal epithelial barrier. However, the treatment is still controversial. Traditional Chinese medicine has been used as a adjuvant therapy for postoperative intestinal adhesion (POIA) and its effectiveness is quite satisfactory. Tongfu Xiere Enteroclysis Mixture (TXEM) is a hospital preparation in the First Affiliated Hospital of Guangdong University of Traditional Chinese Medicine and showed a significant curative effect in the treatment of POIA. In order to investigate the effects of TXEM for prevention of POIA and observe the pathogenic mechanism, we ran the experiment. 50 SD rats were randomly divided into five groups, i.e. GI, GII, GIII, GIV, GV. GI and GII were POIA model group administered with high- and low-dose TXEM respectively. GIII was POIA model group administered with normal saline (NS). GIV was sham operation group and Group GV was normal rats as reference, once daily, from the 2nd day after the establishment of POIA for total 14 days. Secretory ImmunoglobulinA (sIgA), Interleukin-4 (IL-4), Interleukin-10 (IL-10), Endothelin-1 (ET-1) and nitric oxide (NO) were measured by immunological and biotechnology methods after the experiment. The pathological morphology in small and large intestine of rats were observed and compared under microscope. According to the adhesion score standard described by Bhatia, GI and GII had less severe than grade1 compared to GIII and reduced the inflammatory responses as indicated by the chronic inflammatory cells in the lamina propria of small intestine and the proliferation and congestion of the blood capillary in the submucosa of large intestine. Tongfu Xiere Enteroclysis Mixture could down-regulate the content of ET-1 and NO and up-regulate the content of IL-4, IL-10 and SIgA in POIA rats ($P<0.05$). Our results demonstrate TXEM can improve the anti-inflammatory action and immune responses in rats and protect intestinal epithelial barrier and prevent the occurrence of inflammation, thus to prevent and treat POIA.

Keywords: Tongfu Xiere Enteroclysis Mixture, postoperative intestinal adhesion, intestinal epithelial barrier, adhesive score, histopathology

Introduction

Intestinal adhesion is a serious complication after abdominal surgeries and lead to a broad spectrum of problems with high morbidity [1]. It occurs in an estimated 93% of patients [2]. Although the aetiology of postoperative intestinal adhesion (POIA) remains unclear, A widely accepted theory suggests that intestinal epithelial barrier have played essential roles in POIA between the blood circulation and the contents of the intestinal lumen by regulating the immune response via paracellular and transcellular pathway [3, 5]. However, no relevant studies to date have focused on the alteration of intestinal epithelial barrier in POIA.

Management of intestinal adhesion is related to correct physiologic derangements [4]. Treatment of patients with intestinal adhesion after operation presents a challenge. The former is addressed by gastrointestinal decompression. In recent years, traditional Chinese medicine is widely used in the treatment of postoperative intestinal adhesion, especially when traditional Chinese and Western pharmacotherapy are combined, unique advantages are shown observably.

Tongfu Xiere Enteroclysis Mixture (TXEM) is an hospital preparation for patients with POIA in the First Affiliated Hospital of Guangdong University of Traditional Chinese Medicine and

Table 1. Adhesion score

Definition	Adhesion score
No adhesions	Grade 0
The ratio of adhesive area/total treated area in the vermiform processes is <50%, and the adhesion is easily to be dissected.	Grade 1
The ratio is ≥50% and the adhesion is easily to be dissected.	Grade 2
Area of the adhesion is out of consideration. Although blunt dissection for the adhesion can be carried out, it is difficult and the intestinal wall will be impaired after the blunt dissection.	Grade 3
Area of the adhesion is out of consideration. The adhesion is fast and cannot be bluntly dissected. Also may have adhesion to other organs (liver).	Grade 4

Table 2. Comparison of adhesive score in various groups

Group	n	Adhesive score				
		0	1	2	3	4
HTXEM	10	0	6	3	1	0
LTXEM	9	0	4	3	2	0
N.S.	8	0	3	3	2	0
Sham	9	6	2	1	0	0
Normal	10	10	0	0	0	0

play a very important role in reducing postoperative complications and improving the quality of life of postoperative patients.

In order to investigate the effects of Tongfu Xiere Enteroclysis Mixture (TXEM) for prevention of postoperative intestinal adhesion, we developed a series of study.

Materials and methods

Animals and reagents

50 Sprague-Dawlay (SD) rats, weighing 250~300 g, were obtained from the Experimental Animal Center in Guangzhou University of Chinese Medicine (Guangdong, China). Rats were housed individual cages in a restricted-access room with controlled conditions (22 ± 1°C and 65~70% humidity). Food and water were provided ad libitum. The experimental procedures were approved by the Laboratory Animal Care Committee at Guangzhou University of Chinese Medicine. All animals received care according to the Guide for the Care and Use of Laboratory Animals (NIH, Bethesda, MD). The Tongfu Xiere Enteroclysis Mixture (TXEM) was supplied by the First Affiliated Hospital of Guangdong University of Traditional Chinese Medicine (Guangdong, China). The formula was composed of Radix et Rhizoma Rhei, Rhizoma Polygoni Cuspidati, Natrii Sulfas, Fructus Gardeniae, Herba Elephantopi and Caulis Lonicerae in the ratio of 3:3:2:2:1:1, which were submerged in 3.0 L distilled water for 30 min and then decocted twice (2 h per time), then filtered and concentrated to 1 g/mL using a routine method, and stored in a refrigerator at 4°C until use.

Description of experimental groups and the postoperative intestinal adhesion (POIA) rats model

A total of 50 SD rats were randomly divided into five groups (10 rats/group), i.e. GI, GII, GIII, GIV, GV. GI was high-dose (10 mL/kg) Tongfu Xiere Enteroclysis Mixture (HTXEM) group clystered for 14 days after the relief of POIA. GII was low-dose (5 mL/kg) Tongfu Xiere Enteroclysis Mixture (LTXEM) group clystered for 14 days after the relief of POIA. GIII was normal saline (NS) group clystered for 14 days with NS (5 mL/kg) after the relief of POIA. GIV was sham operation (Sham) group clystered for 14 days after the operation with normal saline (5 mL/kg). GV was normal group clystered for 14 days with normal saline (5 mL/kg) only.

Surgical procedures

The rats were fasted overnight before surgery. They were anesthetized with an intraperitoneal injection of 10% chloral hydrate (0.035 ml/kg), a laparotomy was performed on sedated rats using a 3-cm midline incision. The small intestine was gently pulled out of the abdominal cavity and spread. Then used the toothed forceps to clip the duodenum about 0.5 cm every 1 cm, local bleeding for the degree (10 pcs in total). The small intestine was replaced in the abdominal cavity, and the surgical wound was closed using two layer continuous sutures. Sham-operated rats were anesthetized with ether, and a 3-cm midline incision was made. The

Table 3. Comparison of ET, sIgA, NO, IL-4 and IL-10 contents in rats of various groups (mean ± SD)

Group	N	ρ_{ET}/(pg·mL^{-1})	ρ_{sIgA}/(μg·mL^{-1})	ρ_{NO}/(μg·mL^{-1})	ρ_{IL-4}/(pg·mL^{-1})	ρ_{IL-10}/(pg·mL^{-1})
HTXEM	10	0.18 ± 0.06△	0.96 ± 0.16▲	1.30 ± 0.33▲	27.32 ± 2.50△,▲	115.8 ± 49.9▲
LTXEM	9	0.21 ± 0.02△	0.58 ± 0.13△	1.56 ± 0.15▲	18.14 ± 6.05△,▲	116.6 ± 24.6▲
NS	8	0.31 ± 0.01*	0.12 ± 0.05*	2.80 ± 0.46*	7.77 ± 2.46	50.7 ± 31.7△
Sham	9	0.22 ± 0.12	0.46 ± 0.23	1.45 ± 0.16	6.79 ± 1.33	103.2 ± 37.9▲
Normal	10	0.16 ± 0.03	0.45 ± 0.06	1.46 ± 0.11	8.66 ± 4.49▲	96.6 ± 28.1▲

Notes: *P<0.01, compared with the Sham group; △P<0.05, compared with the Sham group; ▲P<0.05, compared with the NS group.

small intestine was gently manipulated, and the surgical wound was closed by two-layer continuous sutures [5]. The rats were allowed to recover postoperatively for 2 days to remove any influence of the anesthesia on our test parameters.

Macroscopic assessment

The abdominal cavity was inspected through a straight incision. Adhesions were identified, and graded using the classification described by Bhatia et al. [6] (**Table 1**).

Measurement index

After intraperitoneal anesthesia using 1% sodium pentobarbital, serum was prepared by taking rats abdominal aorta blood, and serum Interleukin-4 (IL-4), Interleukin-10 (IL-10), Endothelin-1 (ET-1) levels were tested by ELISA; Then all rats were sacrificed by decapitation and the small and large intestine were removed for analysis. Terminal ileum were washed repeatedly using 2 mL of saline, the fluid was removed, and then SIgA level was measured by ELISA. Total NO production in bowel tissues was determined by measuring the concentration of nitrate and nitrite, a stable metabolite of NO, by modified Griess reaction method. The activity was examined following the manual of the Total Nitric Oxide Assay Kit (Beyotime Institute of Biotechnology).

Histology

The small and large intestine were fixed in 15% buffered-formalin and examined under a light microscope after embedding in paraffin, sectioning, and staining with hematoxylineosin (HE).

Statistical analysis

The software package SPSS version 17.0 (SPSS, Chicago, IL, United States) was used for statistical analyses. One-way analysis of variance was used for comparing values obtained in three or more groups. Frequency variables were compared using the χ^2 test. Data are expressed as mean ± SD and P<0.05 was regarded as statistically significant.

Animal care and use statement

The animal protocol was designed to minimize pain or discomfort to the animals. The animals were acclimatized to laboratory conditions (22 ± 1°C, 12 h/12 h light/dark, 65%-70% humidity, ad libitum access to food and water) for two weeks prior to experimentation. Enema administration was carried out with animals, using 12# straight gavage needles. All animals were euthanized with an intraperitoneal injection of 10% chloral hydrate (0.035 ml/kg) for tissue collection.

Results

Macroscopic assessment

Most rats survived in the experiment and reached the end-point of observation in an apparently healthy condition except for four rats in which one rats from LTXEM group (died on the 5th postoperative day), two rats from N.S group (died on the 4th and 7th postoperative day) and one rats from Sham group(died on the 6th postoperative day). All macroscopic assessments are shown in **Table 2**. Adhesions that are more severe than grade1 in HTXEM group were obviously decreased than LTXEM, N.S. and Sham groups.

Effects of TXEM on the inflammation of POIA in SD rats

TXEM down-regulated the content of ET-1 and NO in POIA rats: Compared with the Sham group, the ET-1 and NO levels in the N.S. group

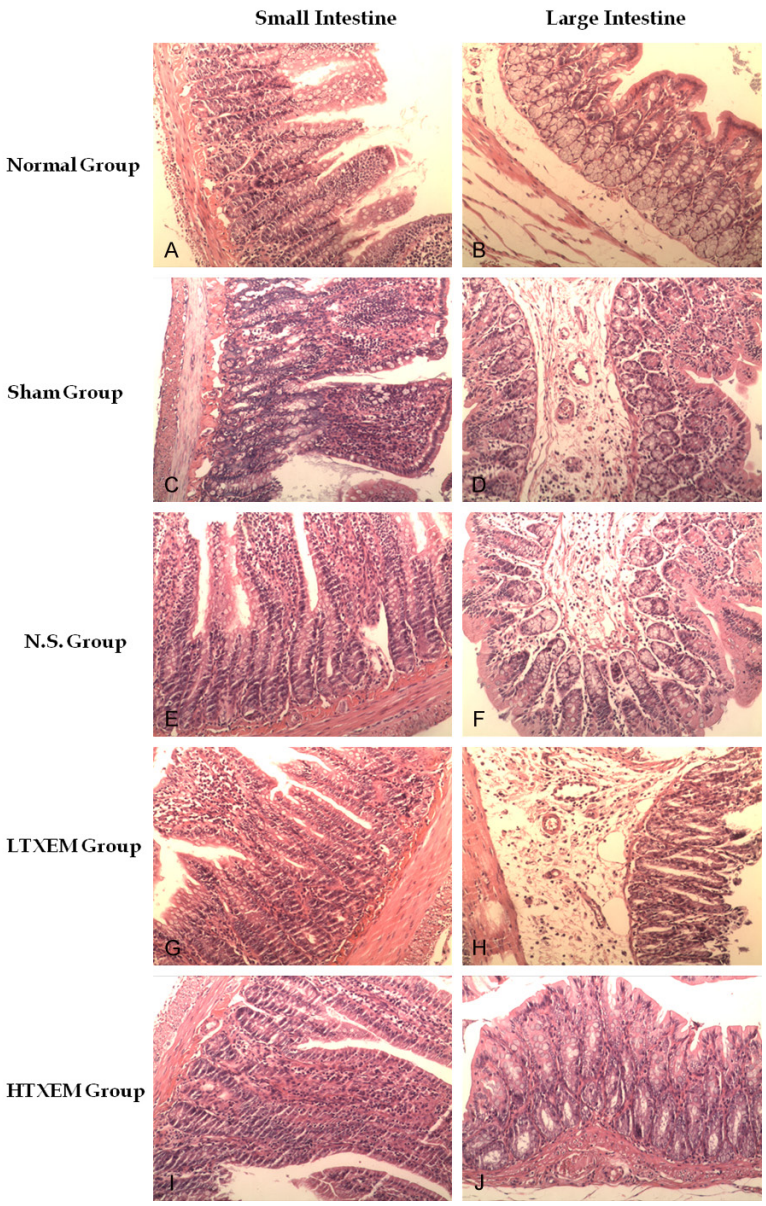

Figure 1. Histopathology assessment results of the five groups (hematoxylin and eosin stain, × 200). A, B. The normal group. C, D. The sham group. E, F. The N.S group. G, H. The LTXEM group. I, J. The HTXEM group. A. The submucosa and the lamina propria in small intestine of the normal group was intact. B. The submucosa could be seen with few infiltrations of inflammatory cells in large intestine of the normal group. C. The lamina propria in small intestine of the sham group could be clearly seen with infiltrations of inflammatory cells. D. Both the submucosa and the lamina propria were found with inflammatory edema and the blood capillary congested in large intestine of the sham group. E. The lamina propria in small intestine of the N.S group could be obviously seen with a lot infiltrations of inflammatory cells. F. Both the submucosa and the lamina propria were obviously seen with a lot infiltrations of inflammatory cells in the small and large intestine of the N.S group. G. There were few inflammatory cells in the lamina propria of small intestine in LTXEM group compared with N.S group. H. There were few inflammatory cells in the lamina propria and congestion of the blood capillary in the submucosa of large intestine in LTXEM group compared with N.S. group. I. There were few inflammatory cells in the lamina propria of small intestine in HTXEM group compared with LTXEM group. J. There were few inflammatory cells in the lamina propria of large intestine in HTXEM group compared with LTXEM group.

significantly increased ($P < 0.01$). Howerer, the ET-1 levels in the LTXEM and HTXEM groups significantly decreased compared with the Sham group ($P<0.05$). Moreover, the NO levels were significant decreased compared with the N.S. group ($P<0.05$), no matter in LTXEM or HTXEM groups (**Table 3**).

TXEM up-regulated the content of IL-4, IL-10 and SIgA in POIA rats: The results revealed that SIgA, IL-4 and IL-10 are consistently in lower levels in N.S. group. Compared with the N.S. group, the SIgA levels are increased in Sham and Normal groups, moreover, the SIgA levels in the LTXEM and HTXEM are significantly increased ($P < 0.05$). Similarly, compared with the N.S. group, the IL-4 and IL-10 in LTXEM and HTXEM groups are consistently increased ($P<0.05$). Furthermore, the IL-4 levels in LTXEM and HTXEM groups are also significantly increased when compared with the Sham group ($P<0.05$). In addition, compared with the N.S. group, the IL-10 are significantly increased in other four groups ($P<0.05$), and increased more in LTXEM and HTXEM groups than Sham and Normal groups (**Table 3**).

Cellular mechanisms for the antiadhesion properties of TXEM

Histopathology revealed significant morphologic differences in inflammatory response. Similar changes in the small intestine were observed in the Normal (**Figure 1A**) and sham groups (**Figure 1C**). It could be clearly seen with infiltrations of chronic inflammatory cells in

the lamina propria. As shown in **Figure 1D**, the large intestine in the sham group, both the submucosa and the lamina propria were found with inflammatory edema and the blood capillary congested compared with the normal group (**Figure 1B**) which indicated that the changes were a response to the surgical procedure. Tongfu Xiere Enteroclysis Mixture treatment reduced the inflammatory responses as indicated by the chronic inflammatory cells in the lamina propria of small intestine and the proliferation and congestion of the blood capillary in the submucosa of large intestine (**Figure 1G**, **1J**) compared with N.S group (**Figure 1E**, **1F**). High-dose Tongfu Xiere Enteroclysis Mixture treatment showed a significant curative effects in the treatment of POIA compared with LHTXEM group (**Figure 1G**, **1J**).

Discussion

Intestinal adhesion is a common problem after abdominal surgery and cause symptomatic complications, such as intestinal obstruction, pain and infertility which was characterized as an fibrin deposition because of the imbalance between fibrin deposition and fibrin degradation [7, 8]. Although minimally invasive surgery have been improved to reduce postoperative adhesions formation markedly compared with open surgery, it still remains an inevitable event of most abdominal procedures [9-11]. Enormous measures have been used to manage these problems, but the treatment is still controversial.

Traditional Chinese medicine has evolved over past 5,000 years to prevent and treat human disease [12]. Chinese herbal medicine has been frequently used to treat postoperative adhesions formation [13]. Tongfu Xiere Enteroclysis Mixture is an anti-adhesion agent which effectively protects against postoperative adhesions formation and inflammation, with several lines of evidence including reduced adhesion scores from direct assessment of adhesions, histopathology measurement of intestinal tract, lymphocytes and vascular proliferation, decreased circulating concentration of blood vessel regulating factors, up-regulated expression levels and secretion of various anti-inflammatory cytokines and immunoglobulin in serum and cecum.

Endothelin (ET)-1, a vasoconstrictor peptide, which was originally identified at endothelial cells and played a physiological role in the modulation of gastrointestinal function [14, 15]. It has been implicated in the integrity of the intestinal epithelial barrier that was closely associated with the severity of intestinal damage and may be a modulator that lead to the intestinal mucosal barier dysfunction as a potent vasoconstrictor and an inflammatory agent [16]. Our study showed that Tongfu Xiere Enteroclysis Mixture could decrease the expression of ET-1 at the POIA rats model and pretect the intestinal mucosal barier function.

The mucosal film of intestinal tract is formed primarily by mucins and contains globular proteins like secretory IgA (SIgA). SIgA plays an important role in immune response to the absorbed proteinaceous films [17]. It is the first line of denfense against microorganisms through selective adhesion to M cells in intestinal Peyer's patches [18]. Therefore it become the key index to observe the intestinal mucosal barier function. Tongfu Xiere Enteroclysis Mixture could prevent the invasion of toxin by up-regulated SIgA expression levels and reduce the intestinal mucosal injury.

Nitric Oxide(NO) is an mediator of immune and inflammatory responses and is highly implicated in intestinal inflammation [19]. It can regulate mucosal integrity in response to noxious stimuli and modulate intestinal movements [20]. Clystered with Tongfu Xiere Enteroclysis Mixture could reduce the NO content of intestinal mucosa and prevent any further damage to intestinal epithelial barrier.

Cytokines such as interleukin-4 (IL-4) and interleukin-10 (IL-10) apear to influence the vascular endothelium of the intestinal tract. Within the lamina propria of the intestine, IL-4 and IL-10 can stimulate IgA production on the mucosal surface and reduce the expression of intracellular adhesion molecule on the vascular endothelium [21]. Tongfu Xiere Enteroclysis Mixture could modulate intestinal epithelial barrier function in POIA models by up-regulating the expression levels and secretion of various anti-inflammatory cytokines like IL-4 and IL-10.

Although we have found Tongfu Xiere Enteroclysis Mixture could promote the recovery of postoperative intestinal adhesion significantly and had the ability to promote the restoration of intestinal epithelial barier function,

the precise mechanisms involved are still to be elucidated Tongfu Xiere Enteroclysis Mixture as a adjuvant therapy for postoperative intestinal adhesion needs to be investigated in the future.

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Disclosure of conflict of interest

None.

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