Original Article

Reversible small bowel obstruction in rats

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Abstract: Objective: To establish a novel rat model of reversible mechanical small bowel obstruction and to investigate the phasic alterations of intestinal macroscopic and microscopic characteristics. Method: The rat model of reversible mechanical bowel obstruction was developed by using a flexible polyvinyl chloride ring surgically placed surrounding the terminal ileum and removing the ring after 2 days of obstruction. Rats were sacrificed at different time points (1 and 2 days after obstruction and 1, 3 and 7 days after releasing the obstruction). Macroscopic and microscopic evaluation was performed to assess the injury and recovery from the obstruction. In addition, intestinal claudin-1 mRNA expression was examined by real-time polymerase chain reaction analysis, and serum sIgA levels were determined by enzyme-linked immunosorbent assay. Results: A stable rat model of reversible small bowel obstruction was established. Bowel obstruction induced aggravated macroscopic and pathological changes and increased the damage of the ileum mucosa. After removing the ring, the intestinal mucosa recovered gradually. The epithelium was arranged in order and the structure of the villi was generally normal at 7 days after removal. Further indicators of intestinal mucosa damage and recovery were observed as claudin-1 gene expression and sIgA levels decreased with prolonged obstruction, and gradually increased after relieving the obstruction. Conclusion: This study suggests that the flexible polyvinyl chloride ring can be used to successfully establish a rat model of reversible complete small bowel obstruction, which represents an ideal model for small bowel obstruction research and postoperative recovery from the disease.

Keywords: Small bowel obstruction, rat model, morphology, pathology, intestinal epithelial cells, intestinal immune system

Introduction

Small bowel obstruction (SBO) is one of the most common presentations to emergency general surgery and causes high morbidity and even some mortality [1]. Etiologies include adhesions, hernias, malignancy and others [2]. According to etiology and comorbidities, patients may be selected for early operative intervention or conservative treatment. The previous study suggests that the average hospitalization stay of adhesive SBO patients who required surgery is three times or greater than patients who are managed conservatively and the costs of the former is much higher than the latter [3]. In order to study the morphological and pathological changes in the progress of SBO and postoperative recovery after removing the obstruction, it is relevant to establish a stable reversible mechanical small bowel obstruction model.

Mechanical bowel obstruction can be divided into partial and complete obstruction depending on the severity of the narrowing. Different methods in inducing complete obstruction are currently available. The silk ligation method has been used since 1990 [4] and became the most commonly used for modeling. This was followed by a ligating clip developed in 1998 [5], however, it was not widely used. Recently, some research reported the model could be created by an in vivo pulled-type locking clamp and where clamp was controlled outside the body [6]. A homemade atraumatic clamp made in binding-wire was also described in another study [7].

In previous experiments, most complete bowel obstruction models were usually caused by ligating the intestines with silk threads [8-10]. However, these methods are unstable in practice since tightness and intensity of ligature
cannot be controlled precisely. An overtightened ligation is prone to cause local ischemia and necrosis of the intestine and leads to strangulated obstruction. Besides, the different methods of clip or clamp are difficult to operate and hard to control the external pressure on the bowel wall. Therefore, a ring structure with suitable diameter is more reliable for use in creating a non-strangulating, acute and complete SBO model.

In this study, polyvinyl chloride (PVC) rings were used to develop an acute simple mechanical bowel obstruction model. The flexible PVC ring provided the right balance of hardness and softness and was sufficient to create a complete bowel obstruction while protecting intestine against focal necrosis. The phasic morphological and histological changes of SBO were studied, and postoperative recovery of the bowel wall after removing the ring was investigated. Besides, intestinal claudin-1 mRNA expression and serum sIgA levels were determined to evaluate the effects of SBO on damage and recovery of intestinal epithelial cells, and on the intestinal immune system.

Materials and methods

Animals

A total of 60 healthy adult female and male Wistar rats, weighing between 220 and 260 g, were provided by Beijing HFK Bioscience Co. Ltd. [Licence No. SCXK (JING) 2014-0004; Beijing, China], and were individually housed at 20-22°C with a relative humidity of 50±10% in a light/dark cycle of 12/12 hours, with free access to standard feed and water in ventilated and specific pathogen-free conditions. Before the experiment, the animals were housed adaptively at the Standard Laboratory Animal Center of the Institute of Acute Abdominalgia of Tianjin Nankai Hospital for 1 week. The animal experiment was approved by the Ethics Committee of Tianjin Nankai Hospital.

Surgical procedure

The animals were randomized to three groups using a random number table (n = 10 in each group): Sham (sham operation group), S1 (small bowel obstruction for 1 day), S2 (small bowel obstruction for 2 days). The remaining 30 rats underwent small bowel obstruction for 2 days, then the obstruction was relieved and rats were randomized into three recovery groups (each containing 10 rats): R1 (recovery for 1 day), R2 (recovery for 3 days) and R3 (recovery for 7 days).

Before surgery, the animals were fasted for 12 hours, with free access to water. Rats in each group received an intraperitoneal injection of 10% chloral hydrate (0.3 mL/100 g). When surgical anesthesia was achieved, a small midline laparotomy was performed. Proximal colon and ileum with the cecum were taken out with micro-forceps and placed on sterile gauze. A small loop of ileum with mesentery approximately 1 cm distal to the ileocecal valve were selected. The mesentery was carefully incised parallel to the ileum to create a small window. Care was taken not to cut any vasculature. An autoclaved flexible PVC tube (10 mm in length, 4 mm exterior diameter, 3 mm interior diameter) was cut longitudinally to open the tubing and formed a ring structure (Figure 1A). One end of the opened ring was inserted through the mesenteric window and brought into contact with the other. The ring was returned to a completed ring shape surrounding the ileum and closed with a suture (Figure 1B). Then the intestines were carefully placed back in the intraperitoneal cavity. About 1 mL of 5% levofloxacin glucose injection was dripped into the abdominal cavity, followed by closure of the abdomen. The size of the flexible PVC ring was designed to reduce the ability of bolus of material to pass across the site and then induce complete obstruction of the lumen gradually and rapidly. The control group underwent a similar procedure, but no ring used in these animals.

Releasing the obstruction: rats were anesthetized and the abdomen was opened to find the obstruction point, then the flexible PVC ring was removed. This procedure was finished within 3 minutes.

Postoperative care

After surgery, the animals were transferred to the animal room (temperature at 20-22°C and relative humidity of 50±10%). The rats were fasted routinely before the second surgery and were euthanized with 10% chloral hydrate. General status was recorded after operation. The abdomen was cut open to observe morphological changes of the intestine at different points in time. Complications and mortality
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were recorded. In cases of death, explorative laparotomies were performed to investigate causes.

Daily general condition

The rats were housed in individual cages after modeling. Body weight and food intake were recorded around 9 AM each day. Defecation condition and fecal characteristics were observed in each cage. The mental status and death of rats were recorded.

Macroscopic evaluations

The abdominal cavity was opened in U-shaped incision and examined for color, movement and dilatation of the small bowel. A 3 cm long ileal segment 5 cm oral to the obstruction point was cut and assessed morphological changes and tissue damage, using the criteria adapted from Butzner et al. [11] (Table 1). Observed items including adhesions, stenosis, ulceration and bowel wall thickness were graded by two blind-ed pathologists.

Histological evaluations

After Macroscopic evaluations, specimens of ileum were rinsed with pre-cooled 0.9% Normal Saline (Otsuka, Guangdong, China) and immersed in 10% formalin solution (Tianjin Chemical Reagent Company, Tianjin, China) for 24 hours and dehydrated in a graded series of ethanol before embedded in paraffin. Serial sections were stained with Hematoxylin and Eosin (H&E). Each slide was selected for five random visual fields and scored using criteria adapted from Butzner et al., to evaluate the grade of ulceration, inflammation, fibrogenesis and extent of involved bowel wall [11] (Table 2). Finally, histological intestinal mucosal damage was assessed using Chiu’s criteria (Table 3) [12].

Expression of claudin-1 mRNA

Intestinal claudin-1 mRNA expression was examined by real-time polymerase chain reaction analysis. Total RNA was extracted from intestinal segments (50 mg) (3 cm distal to the obstruction) using TRIzol (Invitrogen, USA) reagent, following the manufacturer’s instructions. First-strand cDNA was synthesized from 1 μg mRNA using a TransScript Fly First-Strand cDNA Synthesis SuperMix kit (TransGen Biotech, China). Real-time PCR was performed with an Applied Biosystems 7500 FAST system using SYBR Green. The primers were synthe-
sized as follows: Claudin1 (forward: 5'-CTGTCGTTGGTGCTGTTCAC-3', reverse: 5'-CCTGATCCCAACCTCAGTAA-3'); GAPDH (forward: 5'-TGTCACCACCAACTGCTTAGC-3', reverse: 5'-GGCATGGACTGTGGTCATGAG-3'). The mRNA expression was quantified by $2^{\Delta\Delta C_{T}}$.

Measurement of sIgA levels

The levels of serum sIgA were measured by enzyme-linked immunosorbent assay, using an ELISA kit (BlueGene, Shanghai, China) following the manufacturer's instructions.

Statistical analysis

Macroscopic and histological scores of SBO and recovery were expressed as mean ± standard deviation and were analyzed by one-way ANOVA with post-hoc LSD tests. A $P$-value <0.05 was considered statistically significant. Analysis was performed using IBM SPSS Statistics version 24 (IBM SPSS, Chicago, Illinois, USA).

Results

Model success

A complete small bowel obstruction was developed in the rat by using the flexible PVC ring surgically placed surrounding the terminal ileum. The rats recovered from anesthesia in 3 hours after the operation. All rats in the Sham group were in good condition. Rats of SBO

<table>
<thead>
<tr>
<th>Table 1. Criteria for scoring macroscopic intestinal damage</th>
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<tr>
<td>Feature</td>
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<td>Adhesions</td>
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<td>Stenosis</td>
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<td>Ulceration</td>
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<td>Bowel wall thickness</td>
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<td>Total score</td>
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<th>Table 2. Criteria for scoring histological intestinal damage</th>
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<tr>
<td>Feature</td>
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<td>Inflammation</td>
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<td>Extent of involved bowel wall</td>
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<td>Total score</td>
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Table 3. Criteria for Chiu’s intestinal mucosal damage

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<tr>
<th>Score</th>
<th>Grading</th>
<th>Criteria</th>
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<tbody>
<tr>
<td>1</td>
<td>Grade 0</td>
<td>Normal villi</td>
</tr>
<tr>
<td>2</td>
<td>Grade 1</td>
<td>Submucosal space on top of villi, capillary congestion</td>
</tr>
<tr>
<td>3</td>
<td>Grade 2</td>
<td>Expanded submucosal space, isolation of intestinal mucosa and submucosa</td>
</tr>
<tr>
<td>4</td>
<td>Grade 3</td>
<td>Isolation of intestinal mucosa and submucosa was extended to bilateral villi</td>
</tr>
<tr>
<td>5</td>
<td>Grade 4</td>
<td>Blunted villi, exposure of lamina propria and vessels, inflammatory infiltration</td>
</tr>
<tr>
<td>6</td>
<td>Grade 5</td>
<td>Digestion and disintegration of lamina propria, with bleeding and ulceration</td>
</tr>
</tbody>
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In the recovery groups, diameter, color and peristalsis of small intestines gradually returned to normal, the gas-liquid accumulation in the bowel lumen gradually disappeared. In the R1 group, intestines were dilated and in poor peristalsis, with congestion and edema and intra-abdominal exudate. In the R3 group, intestinal appearance and abdominal conditions were improved significantly. Congestion and edema in the intestinal wall were alleviated, and the intestines were still mildly dilated. In the R7 group, the appearance of intestines was close to the normal, and the peristalsis was completely restored (Figure 1E-G).

After observing the appearance of the intestines and abdominal cavity, a segment of ileum was cut and assessed for morphological changes and tissue damage. In the obstruction groups, morphological changes included adhesions, stenosis, ulceration and bowel wall thickness in varying degrees. The obstruction ring led to increasing intestinal narrowing of the obstructive site, and scores of stenosis increased significantly after 24 h as proximal intestinal distention and gas-liquid accumulation of intestines. In addition, there was an increase in adhesions and the intestinal wall became gradually thicker. These observed items were getting worse in the first 24 h after removal of obstruction rings but improved gradually in the following time. Figure 2 shows ana-
lytical data of the total scores of macroscopic damage scores (Figure 2).

**Histological evaluations**

The histological findings in ileal tissues are presented in Figure 3. In the Sham group, the small intestinal epithelium was arranged in order, the structure of the villi was normal. In the S1 group, mild derangement of intestinal mucosa was observed, with slightly uneven villus heights and partial widening, mucosa edema and inflammatory cell infiltration. In the S2 group, there was significant damage of the small intestinal mucosa, with partial villous atrophy, separation of submucosa and muscular layer and capillary congestion in the villi. Partial villous fusion and epithelium shedding appeared in the mucosal epithelium, with inflammatory cells infiltration and focal hemorrhage. There were fibrous tissue proliferation, vasodilation and hyperemia in sub-mucosa, as well as serositis and mesenteritis (Figure 3A-C).

In the recovery groups, all of the above pathological changes were returned to normal gradually. Compared to S3 group, the R1 group showed improved mucosal appearance with a smaller gap between villi. Intestinal villi were still in different heights and partial widening. Interstitial edema and vascular congestion were observed in the intestinal mucosa and submucosa, with separation of submucosa and muscular layer. In the R3 group, the mucosal appearance was significantly improved. A small number of intestinal villi were uneven in height and inflammatory cell infiltration declined. In the R7 group, the epithelium is arranged in order and the structure of the villi was generally normal. There were a few scattered inflammatory cells within the layers of muscles and submucosa (Figure 3D-F).

**Figure 3.** Histological micrographs of intestinal tissue sections and intestinal mucosal damage scores. A-F. Sham, S1, S2, R1, R3 and R7 groups, respectively. H&E staining, 40×. G. The histopathological score of intestines in ulceration, inflammation, fibrogenesis and extent of involved bowel wall. H. Chiu’s intestinal mucosal injury scores in rats. The numbers 0, 1, 2, 3, 5, 9 on the abscissa respectively indicate Sham, S1, S2, R1, R3, R7 group. Data are shown as means ± SD, n = 8-10, *: P<0.05 vs. Sham group, #: P<0.05 vs. the preceding group.
Histological evaluation and Chiu’s intestinal mucosal damage scores gradually increased with prolonged obstruction duration and continued to rise 24 h after relieving the obstruction. However, the scores decreased significantly in S3, S7 groups, and had no differences in Chiu’s score between Sham and R7 groups (Figure 3G, 3H).

**Intestinal claudin-1 mRNA expression**

There were no differences in the expression of claudin-1 mRNA between Sham and S1 groups (P>0.05), but it was significantly reduced at 48 h of obstruction (P<0.05). Expression continued to decline within 24 h after relieving obstruction and began to increase from 3 to 7 days (P<0.05). R7 group returned to normal with no significant differences compared to the Sham group (Figure 4A).

**Change in serum sIgA levels**

Serum sIgA levels were significantly decreased with prolonged obstruction time and continued to decrease in the first 24 h after relieving obstruction (P<0.05). The levels began to elevate sharply from 1 to 3 days but remained unchanged by 7 days. There were still significant differences between Sham and R7 groups (P<0.05) (Figure 4B).

**Discussion**

Mechanical small bowel obstruction is a common situation for emergency laparotomy, and enhanced recovery after SBO surgery remains an ongoing challenge [13, 14]. A reliable animal model that simulates SBO patients undergoing surgical treatment is an important component in elucidating the strategies of promoting postoperative rehabilitation. This study established a novel animal model of reversible SBO to observe histological and morphological changes at different time points as the disease progressed and recovered.

There are many studies in animal models for incomplete intestinal obstruction and this has been formed as a more mature model, but less complete for small bowel obstruction. Ring structure materials such as polyethylene strips, silicone rings and polyurethane bands were reported to create partial bowel obstruction animal models [15-17]. The diameter of the ring was usually 1-2 mm larger than the intestinal luminal diameter. When the ring was relatively too small, it could create an acute complete bowel obstruction and lead to early death [17]. Therefore, ring structure materials with suitable diameter can be used in creating a reliable, complete SBO that is valuable and feasible.

In this study, flexible PVC rings with the optimal size proved to be appropriate and accessible to establish a complete small bowel obstruction animal model. Research has shown that the diameter of the ileum of rats weighted 200-300 g is about 4.3±0.3 mm [18]. We used flexible PVC tube with a specific size to form a ring (10 mm in length, 4 mm exterior diameter, 3 mm interior diameter) and the ring was 1-2 mm smaller than the intestinal luminal diameter. The model would fail if the ring were too large or too small. Too large a ring may create less blockage in the ileum where the obstruction is only partial, whereas too small a ring may induce severe intestinal ischemia and mucosal damage, leading to early death.

The material of the rings is an essential factor of this model. Polyethylene rings, flexible PVC ring and latex rings were tried in our previous research, and the material of flexible PVC was the most suitable. The material of polyethylene is relatively hard, and it was difficult to operate on when suturing the ring with silk thread, which could cause damage to the intestine. Conversely, the latex ring was relatively soft,
and its good compliance led to being able to pass gas and a small amount of intestinal contents after 48 hours of obstruction, delaying the formation of complete obstruction. Flexible PVC has a good balance of hardness and softness. This material is used for a wide variety of medical devices in hospitals and is chemically nonreactive [19]. Therefore, flexible PVC ring is more reliable for use in creating SBO because of its properties, accessibility and relatively low cost.

The ring induced partial obstruction at the beginning when fecal pellets were blocked, but gas or liquid stool could still pass. As time progressed, the intestinal wall became more and more edematous, and no substance could pass through the point of narrowing [20]. This complete SBO model was successfully developed within 48 hours and showed good stability, without causing intestinal ischemia and damage, and promoted postoperative recovery after relieving the obstruction. In addition, the ring is easy to remove and causes little or no damage to the rat, which makes the reversible mechanical small bowel obstruction model feasible and stable. Our previous work showed that the mortality rates clearly increased if the obstruction was relieved at 72 hours, this was likely caused by severe ischemia and necrosis of bowel wall, followed by intestinal ischemia-reperfusion and intestinal barrier function loss [21, 22]. Therefore, the ring was removed at 48 hours of obstruction.

Macroscopic evaluation and histological evaluation were used to assess intestinal damage in the SBO groups. In accordance with the findings in other reports, the Sham group showed slight adhesions and inflammation of intestines and no clear evidence of intestinal damage [6]. In contrast, obstructed rats showed dilation, congestion, edema and abnormal motility of the bowel wall in macroscopic evaluation and showed villus alteration, inflammation of intestinal mucosa in histological evaluation. Statistically significant differences in the scores of tissue damage and mucosal damage were observed in the obstructed rats compared with the Sham group (P<0.01). Damage of the intestines still existed on the first day after relieving the obstruction, but significantly improved by 3 days and was generally normal by 7 days.

SBO can cause a time dependent damage to the small-intestine mucosal barrier with subsequent systemic pathophysiological changes [23]. Molecular changes in the gut mucosal barrier occur with prolonged obstruction duration and return to normal gradually after relieving the obstruction. This study determined intestinal claudin-1 mRNA expression to evaluate the effects of SBO on damage and recovery of intestinal epithelial cells and serum sIgA levels to the function of intestinal immune system.

Claudin-1 is a composition of tight junctions between intestinal epithelial cells, an integral membrane protein and an important regulator of epithelial barrier function [24]. Tight junctions, the most apical cell-cell adhesions, constitute continuous seals around cells that act as a physical barrier [25]. In the present study, the intestinal mechanical barrier was severely damaged with prolonged obstruction indicated by the decrease in expression of claudin-1, then entering a repair process after relieving the obstruction as the gradual increase of claudin-1 expression. These findings suggest that there is a balance between intestinal epithelial cell damage and recovery regain after relieving the obstruction.

Secretory IgA (sIgA) is an abundant immunoglobulin that is produced and secreted by mucous membranes [26]. It promotes humoral immunity at the intestinal mucosa and prevents enteropathogenic pathogens from inhibiting adherence and invasion of mucosal tissues [27]. In this study, the levels of sIgA decreased with prolonged obstruction and increased slowly after relieving the obstruction. These changes reflect serious damage to the intestinal mucosal immune barrier due to bowel obstruction and cannot be restored immediately even the obstruction is removed.

In summary, this method can be used to establish a novel model of reversible small bowel obstruction successfully. Animals showed disruption and restoration of intestinal homeostasis during the procedure. This in vivo reversible obstruction model provides a viable therapeutic target for the treatment of SBO and postoperative recovery.

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Disclosure of conflict of interest

None.

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