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Time-dependent alterations of gut wall integrity in small bowel obstruction in mice

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ABSTRACT

Background: Small bowel obstruction (SBO) is one of the most common disorders in surgical emergency departments. Without resolution of the obstructed bowel segments, patients may develop multiorgan failure. The aim of this study was to investigate whether morphological damage of the intestinal wall during SBO may lead to molecular translocation and how this may impair intestinal motility.

Methods: C57Bl6 mice were laparotomized, and the small intestine was ligated 5 cm oral to the coecum for SBO. Controls received minilaparotomy only. Animals were sacrificed 3 h, 9 h, and 24 h after SBO. Morphological changes were evaluated on hematoxylin and eosin histology by a standardized score. Intestinal motility was determined by recording intraluminal pressure of the small intestine *in vitro*. Permeability was measured by fluorospectroscopy and ELISA of blood samples after oral gavage with fluorescein isothiocyanate (FITC)-dextrane and horse radish peroxidase. Data are mean ± SD.

Results: Three hours after SBO, FITC-dextrane uptake was increased to 187.6 ± 15.2 ng/mL compared to controls ($P = 0.011$). At 9 h, uptake of horse radish peroxidase (23.0 ± 8.6 ng/mL, 9.0 ± 6.3 ng/mL, $P = 0.039$) and FITC-dextrane (86.8 ± 17.8 ng/mL, 62.0 ± 1.6 ng/mL, $P = 0.029$) was higher compared to controls. Motility was increased to 162.2 ± 20.2 area under the curve (AUC) compared to 121.3 ± 20.3 AUC in controls, $P = 0.009$ and an increased histology score was observed at 9 h (3.2 ± 1.8 *versus* 0.6 ± 0.7, $P = 0.003$). Twenty-four hours after SBO, histology score was 3.8 ± 1.7, which was higher than 0.9 ± 0.7 in controls ($P = 0.001$). Intestinal motility was decreased 24 h after SBO compared to sham controls (146.0 ± 21.4 AUC *versus* 198.9 ± 21.2 AUC, $P = 0.003$).

Conclusions: SBO entails a time dependent epithelial damage to the mucosa. In parallel, molecular changes in the gut mucosal barrier occur as early as 3 h after the onset of SBO with a subsequent increase in permeability. Initial intestinal hypermotility is followed by a decrease in motility.

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Introduction

Mechanical ileus or small bowel obstruction (SBO) is a frequent problem in surgical emergency departments. Potential underlying conditions are adhesions, stenosis secondary to intestinal inflammation, hernia, or tumors.[1] Without resolution of SBO, patients may develop bacterial translocation or perforation with subsequent risk of multiorgan failure.[2-4]

Although clinically SBO is well described, there is not a lot of experimental data of SBO animal models available. To develop possible targets of treatment, it is crucial to examine simple experimental models that reduce possible confounders.

Bowel obstruction entails multiplication of the bacterial population by 100 to 1000 fold depending on the site of obstruction. Although in normal gut segments the highest number of bacteria was found in the colon, bacterial overgrowth was more pronounced in the ileum compared to large bowel segments in bowel obstruction.[5]

In a previously published study, we showed that SBO leads to an inflammatory response followed by a secondary sensitization of afferent nerve fibers after the administration of different chemical stimuli. This inflammatory response is characterized by a leukocyte infiltration into the tunica muscularis of the small bowel and elevated interleukin-6 expression 9 h after the induction of SBO.[6]

Given these findings, one can hypothesize that gut barrier dysfunction may occur during SBO. In consequence, macromolecular antigens may be able to translocate through the mucosal barrier in a similar fashion as described in different colitis models.[7-9] Hence, altered integrity of the bowel wall and translocation may be a key factor in pathophysiology of SBO. The aim of the present study was, therefore, to investigate whether morphological damage of the small bowel wall in SBO animals may be followed by molecular translocation through mucosal barrier and how this may impair gastrointestinal (GI) motor function.

Methods

Animal model

The experimental model was described previously.[6] In brief, male (C57BL6, body weight 25-30 g, mean age 7 wk) mice were deeply anesthetized. Operation was performed through a midline minilaparotomy. After identifying the ileocecal area, a tie was placed 5 cm orally to the ileocecal valve. Then the abdomen was closed by a running suture.

In contrast, the sham group was treated by midline minilaparotomy and subsequent closing with a running suture without inducing a mechanical ileus.

Recovery after surgery happened quickly, and the animals began to drink and to eat.

Subgroups of mice were sacrificed by neck fracture 3, 9, and 24 h after inducing SBO. Each group consisted of six mice. Approval for the experiments was obtained by Regierung of Oberbayern project number 129/09. All animal experiments complied with the ARRIVE guidelines and were performed in accordance with the EU Directive 2010/63/EU for animal experiments.

Microscopic morphological changes

A separate piece of ileum, 3.5 cm orally of the placed tie, was stained for hematoxylin and eosin (H&E) to visualize structural changes of the bowel wall. In this study, a standard H&E staining protocol was used.[10] Microscopic morphological changes of the small bowel wall were analyzed according to the previously published standardized score by Hausmann *et al.*[11,12] by an observer blinded to the type of pretreatment. In brief, the Hausmann score represents the sum of inflammatory infiltration and epithelial damage to the mucosa with a maximum of eight points.

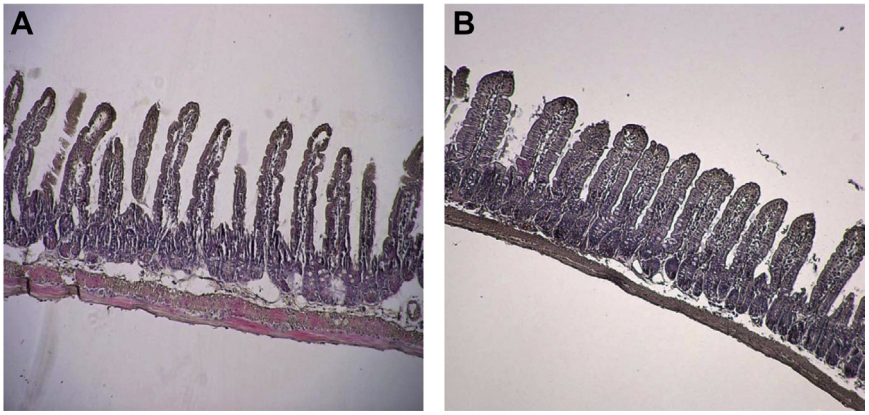

Fig. 1 – H&E stains of ileal bowel segments 9 h postobstruction (A) compared to 9 h sham control (B) (10×). Note the loss of goblet cells and destroyed crypts in the postobstruction bowel segments compared to (B) Even a detachment of mucosal and tunica muscularis can be seen. (Color version of figure is available online.)