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Iatrogenic extracellular matrix disruption as a local trigger for postoperative ileus

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ABSTRACT

Background: Active matrix metallopeptidase 9 (MMP-9) disruption of the extracellular matrix (ECM) plays an important role in inflammatory disorders. In this study, we investigated the inflammatory role of MMP-9 and the ECM breakdown product hyaluronan as a trigger for the postoperative intestinal inflammatory response of postoperative ileus.

Methods: We performed a standardized intestinal surgical manipulation on rats to produce ileus assessed by the oral non-digestible fluorescein isothiocyanate–dextran transit assay. We studied isolated intestinal muscularis extracts for mRNA expressions of interleukin 6 (IL-6), MMP-9 and CD44. We quantified peritoneal MMP-9 activity using zymography, and quantified peritoneal fluid and serum for hyaluronan and tissue inhibitor of metalloproteinase 1 levels by enzyme-linked immunosorbent assay (ELISA). We cultured peritoneal macrophages and exposed them to peritoneal fluid or synthetic hyaluronan for ELISA analysis of IL-6 and macrophage inflammatory protein-1α.

Results: Transit was significantly delayed after surgical manipulation, and extracts of the isolated jejunal and colonic muscularis demonstrated a significant induction of IL-6, MMP-9, and CD44 mRNAs compared with controls. Zymography confirmed significant MMP-9 activity in peritoneal fluid compared with controls. Enzyme-linked immunosorbent assay measurements showed a significant up-regulation in hyaluronan and tissue inhibitor of metalloproteinase 1 in the peritoneal fluid and serum. In addition, ELISA and reverse transcriptase–polymerase chain reaction measurements of peritoneal macrophages stimulated with postsurgical peritoneal fluid and synthetic hyaluronan resulted in higher expressions of IL-6 and macrophage inflammatory protein-1α in the macrophage supernatant.

Conclusions: Our results confirm that MMP-9 disruption in the ECM with hyaluronan release and muscularis CD44 receptor induction has the potential to trigger muscularis proinflammatory cascades that cause postoperative ileus. Matrix metallopeptidase 9 inhibition may be a novel therapeutic approach to limit postoperative ileus.

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1. Introduction

The morbidity of postoperative intestinal atony and ileus is widely acknowledged, and its economic burden has been estimated in the United States to be $1 billion annually [1]. Recent studies have shown that prolonged postoperative intestinal ileus is incited by the generation of an enteric molecular inflammatory response that consists of the activation of the dense network of resident muscularis macrophages and their secretion of cytokines (interleukin 6 [IL-6] and tumor necrosis factor α), chemokines, and smooth muscle inhibitory substances via inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). This local inflammatory milieu then causes the increased expression of vascular adhesion molecules and a subsequent recruitment and extravasation of leukocytes into the circular muscle layer, with a further release of potent leukocytic products that perpetuate intestinal atony. Together, these events succeed in delaying gastrointestinal transit, decrease local neuromuscular function, and activate neurogenic inhibitory pathways that suppress motility along the entire gastrointestinal tract for sustained postoperative periods [2–9].

We know from earlier studies that anesthesia, laparotomy, or bowel eventration do not produce a prolonged ileus, but that the physical manipulation of the gut wall itself is necessary to elicit this detrimental response [3,4]. This suggests that physical manipulation of the gastrointestinal tract leads to a disruption in the interstitial extracellular matrix (ECM), which maintains the cohesiveness of the numerous cellular constituents of the muscularis externa. The aims of this study were to analyze intestinal ECM breakdown via matrix metallopeptidase 9 (MMP-9) activity, and to explore the potential of its breakdown product hyaluronan as an inflammatory trigger that participates in the postoperative inflammatory response generated by the dense network of normally quiescent muscularis macrophages, which leads to the deleterious state of postoperative ileus.

The key role of MMP-9 and tissue inhibitor of metalloproteinase 1 (TIMP-1) in the progression, prognosis, and treatment of gastrointestinal cancers is being intensively investigated [10,11]. A significant body of evidence has also developed in the inflammatory bowel disease literature over the past decade, indicating that increased mucosal MMP-9 activity during episodes of inflammation participates in the destruction of the epithelial barrier, which subsequently exposes the immunologically active lamina propria to bacterial antigens, resulting in the production of proinflammatory mediators that further aggravate the pathology [12,13]. Interestingly, ECM fragments of hyaluronan have been shown to be directly proinflammatory in cell culture conditions on peritoneal macrophages via nuclear factor-κB activation [14] through CD44 receptor binding and on endothelial cells through *TLR4* ligand activity [15].

To our knowledge, however, no previous study has focused on the pathophysiological role of MMP-9 activity on the ECM within the intestinal muscularis in any disease. In this study, we show that manipulation-induced postoperative ileus is associated with a rapid and sustained induction of MMP-9 activity within the postsurgical muscularis externa, which results in a dramatic increase in peritoneal hyaluronan levels bathing the gut wall. Furthermore, the receptor for hyaluronan (CD44) is postoperatively induced and postoperative peritoneal fluid and exogenous hyaluronan possess significant proinflammatory potential on cultured peritoneal macrophages. These data indicate that limiting the proinflammatory activity of MMP-9–generated hyaluronan may be a novel approach to limiting the morbidity of postoperative ileus.

2. Methods

2.1. Animals

We purchased Sprague-Dawley male rats (280–320 g) from Charles River Laboratories (Sulzfeld, Germany) and maintained them in a pathogen-free animal facility at the University of Bonn with standard rat chow and tap water supplied ad libitum. They were allowed to acclimatize at least 5 d before experimental manipulation. The District Government of Köln, Germany, approved the animal protocol.

2.2. Intestinal manipulation

We subjected the entire intestine of the animals to a standardized, moderate surgical manipulation (SM) as described previously [16]. In brief, we anesthetized animals with continuous isoflurane inhalation (DeltaSelect, Pfullingen, Germany) and performed a midline abdominal incision. We eventrated the cecum and small bowel, placed them onto moist gauze outside the abdominal cavity, and kept them moist with saline. Next, we manipulated the entire small bowel and colon using moist sterile cotton applicators in a standardized fashion. After manipulation, we replaced the intestine and closed the laparotomy with two layers of continuous sutures. Age-matched, non-manipulated, naive animals without surgery served as controls ($n = 6$/group).

2.3. In vivo gastrointestinal transit

We measured gastrointestinal transit in controls and manipulated animals 24 h postoperatively by evaluating the gastrointestinal distribution of fluorescein-labeled dextran (molecular weight = 70,000; Sigma-Aldrich, Munich, Germany) as previously described [17]. For statistical analysis, we calculated a geometric center (GC) for the median distribution of fluorescein-labeled dextran along the gastrointestinal tract as previously described [17].

2.4. Peritoneal fluid and serum

Postoperatively, after 0, 3, 6, and 24 h, we obtained peritoneal fluid (2 mL/animal) and preserved it—at −20°C ($n = 6$). In brief, we injected sterile saline intraperitoneally at the defined time points after abdominal surgery in manipulated or control animals and recollected and analyzed peritoneal fluid for ECM components and cytokines. We withdrew venous blood samples (8 mL/animal) from the different animal groups at the defined time points from the inferior vena cava, centrifuged