Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Intestinal alkaline phosphatase decreases intraperitoneal adhesion formation

*Sanjiv K. Hyoju, MD, Sara Morrison, MD, Sarah Shireen Gul, MD, Mohammad Hadi Gharedaghi, MD, PhD, Mehran Najibi, MD, Konstantinos P. Economopoulos, MD, PhD, Sulaiman R. Hamarneh, MD, and Richard A. Hodin, MD**

Department of Surgery, Massachusetts General Hospital, Harvard School of Medicine, Boston, Massachusetts

ARTICLE INFO

Article history:
Received 20 July 2016
Received in revised form
24 August 2016
Accepted 1 September 2016
Available online xxx

Keywords:
Intestinal alkaline phosphatase
Postoperative adhesions
Cytokines

ABSTRACT

Background: Damage to the peritoneum initiates an inflammatory response leading to the formation of adhesions, which subsequently cause significant morbidity in some patients. Intestinal alkaline phosphatase (IAP) is a gut enzyme capable of detoxifying various inflammatory mediators such as lipopolysaccharide, lipoteichoic acid, CpG DNA, and ATP. In this study, we aimed to examine the anti-inflammatory effects of IAP on postoperative adhesions in mice.

Methods: C57BL/6 mice were subjected to a midline laparotomy and then six musculoperitoneal buttons (MPBs) were created by pinching and ligating the peritoneum and underlying muscle. The buttons were half-excised and E-cauterized, and then cecal abrasion was performed. Five hundred microliters of vehicle with IAP 5000 U or vehicle alone were applied over the peritoneal cavity. In some experiments, the mice were euthanized on the first and second postoperative day (POD), and cytokines analysis was done on the MPB, peritoneal tissue, and peritoneal fluid. In separate experiments, the mice were sacrificed on the 21st POD, and adhesion to each button was scored based on type and tenacity.

Results: IAP group mice had significantly lower adhesion scores compared with controls (21.5 ± 1.7 *versus* 13.2 ± 1.3; $P = 0.0014$, $n = 15$). MPB from IAP group mice had significantly lower interleukin-1β and tumor necrosis factor-α protein level compared to control mice (105.66 ± 4.5 *versus* 69.8 ± 4.8 *versus* pg/mg, $P = 0.0001$; 45.25 ± 2.8 pg/mg *versus* 24.88 ± 4.1 pg/mg; $P = 0.0007$, $n = 10$). IAP treatment significantly decreased interleukin-1β and tumor necrosis factor-α mRNA expression in MPB in the first POD (1.14 ± 0.25 *versus* 0.33 ± 0.07; $P = 0.0068$; 1.33 ± 0.31 *versus* 0.33 ± 0.08; $P = 0.0064$, $n = 10$).

Conclusions: Application of IAP during laparotomy could represent a novel approach to prevent postoperative adhesions.

Introduction

Postoperative peritoneal adhesions, a form of peritoneal wound healing, are a common occurrence after abdominal surgery. Adhesions are associated with significant morbidity in some patients, accounting for a majority of all cases of intestinal obstruction.[1] Furthermore, repeat abdominal surgery becomes technically difficult secondary to adhesions, which

* *Corresponding author*. Department of Surgery, Massachusetts General Hospital, Harvard Medical School, 15 Parkman Street, Boston, MA 02114. Tel.: +1 617 724 2570; fax: +1 617 724 2574.
E-mail address: RHODIN@mgh.harvard.edu (R.A. Hodin).

0022-4804/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2016.09.006

can prolong operating times and increase the risk of complications such as inadvertent enterotomies.[2,3] During an abdominal operation, the peritoneum can be injured by various modes such as direct manual handling, electrocauterization, suturing, or ischemia. In all modes of injury, an inflammatory response is first initiated locally in the peritoneal cavity and then becomes further propagated through a process orchestrated by cytokine signaling.[4]

Several proinflammatory interleukins have been studied for their potential role in adhesion formation. During acute inflammation in the immediate postoperative period, proinflammatory cytokines interleukin-1 (IL-1), IL-6, IL-8, and tumor necrosis factor-alpha (TNF-α) are found to be increased in peritoneal fluid.[5,6] In both animal and human studies, the cytokines are found to interact extensively with the fibrinolytic pathway and can contribute directly or indirectly to the remodeling of the extracellular matrix.[7,8] Cytokines not only promote inflammation and coagulation but also act to decrease fibrinolytic capacity by stimulating the release of plasminogen activator inhibitor[9] and suppress the production of tissue plasminogen activator in the peritoneal cavity.[10,11] The disruption in the balance of the fibrinolytic system favors adhesion formation and remodeling. It has been speculated that interruption or manipulation of key cellular processes early in the inflammatory cascade might help to diminish downstream events, including the ultimate fibrotic end point thought to be primarily responsible for adhesion formation.[4]

Our team has demonstrated the anti-inflammatory properties of intestinal alkaline phosphatase (IAP). Specifically, we have found that this naturally occurring brush border enzyme detoxifies a variety of bacterial toxins, including LPS, CpG DNA, and flagellin.[12] We have demonstrated that IAP prevents high-fat diet induced metabolic syndrome by decreasing serum levels of TNF-α, IL-1β, thereby lowering systemic levels of inflammation.[13] Endogenous IAP expression is decreased in patients with inflammatory bowel disease,[14] and exogenous IAP has been found to exert a protective effect in murine[13] and rat[14] models of colitis. We have previously safely administered this enzyme intraperitoneally in a mouse model of peritonitis and found that it enhanced survival by decreasing local inflammation and preventing remote organ damage.[15]

Based on its potent anti-inflammatory properties, we hypothesized that intraperitoneal application of IAP could be used to prevent postoperative adhesion formation.

Materials and methods

Animal experiment

Ten to 12-week-old, male, C57BL/6 WT mice were purchased from Charles River Laboratories and maintained in accordance with the guidelines prepared by the institutional animal care and use committee (IACUC) at Massachusetts General Hospital (MGH). The animal protocol was reviewed and approved by the IACUC at MGH. All mice were housed in filter-top cages under standardized laboratory conditions and acclimatized for 72 h before all experiments. Mice were maintained in a temperature-controlled room (22°C-24°C) with a 12-h light/12-h dark diurnal cycle with food and water *ad libitum*.

Surgical procedure

Mice were injected with buprenorphine 0.05-0.1 mg/kg subcutaneously at 30 min preoperatively followed by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). The abdomen was shaved and painted with 10% povidone-iodine solution, followed by 70% ethanol solution. The abdomen was subsequently draped, and the peritoneal cavity was opened with 1.5-cm midline incision. Musculoperitoneal buttons (MPBs) were created without and with different modification as described in Section 2.3. The midline incision was closed with 5-0 Prolene suture, interrupted, in two layers. During the postoperative period, mice were recovered on a heating pad and 1-mL 0.9% NS injection was given subcutaneously to assist in fluid resuscitation. Injection of buprenorphine 0.05 mg/kg was given subcutaneously during the immediate postoperative period and was continued every 8 h until the second postoperative day (POD). All mice were returned to their cages after full recovery from anesthesia and were given normal chow diet and autoclaved water. On the 21st POD, all the mice were euthanized, and adhesions from the abdominal viscera to each button were scored and charted as described in Section 2.4 by a separate researcher blinded to the experimental groups.

Peritoneal adhesion model development

Initial experiments were performed to develop mouse adhesion model that produces robust and reproducible peritoneal adhesion. In first model, six MPBs were created and subsequently manipulated as described in the following paragraphs in different adhesion models (Fig. 1A and B). All the mice were sacrificed on POD 21. Number of adhesion-positive MBPs and adhesion-negative MBPs were counted in each mouse of each model and recorded for analysis.

(A) MPB creation model (C; $n = 3$)

Six peritoneal buttons were created on the inner abdominal wall; three on each side, 5 mm apart and 5 mm lateral to midline. To create the buttons at the designated points, parietal peritoneum, along with underlying muscle, was pinched with a hemostat and a controlled upward traction was maintained. A needle was then passed through the pinched tissue below the hemostat and tied with 5-0 silk suture by using pressure sufficient to create constriction and hold the tissue in position.

(B) MPB creation and cut model (CC; $n = 5$)

MPB were created as described in group C, but the lower half of the buttons were excised with a number 11 surgical blade immediately below the hemostat.

(C) MPB creation, cut, and cauterization model (CCC; $n = 5$)

MPB were created and cut as described in group CC. The buttons were E-cauterized over the cut surface for 2 s with bipolar cautery bipolar electrocautery (Radionics, Bipolar