ORIGINAL RESEARCH

Fibrinolytic Responses of the Equine Peritoneum to Abdominal Surgery, Surgical Trauma, and Intraperitoneal Sodium Hyaluronate

Fred J. Caldwell, DVM, MS, Diplomate ACVS,[a] and P.O. Eric Mueller, DVM, PhD, Diplomate ACVS[b]

ABSTRACT

Postoperative abdominal adhesions are known to present clinical challenges to the surgeon. Adhesion formation is a balance modulated by the fibrinolytic system. The key components involved are the tissue plasminogen activators (tPAs) and plasminogen activator inhibitors (PAI-1 and PAI-2). Sodium hyaluronate (HA) has been shown to reduce the incidence and severity of adhesions in horses. The objectives of this study were to measure tPA and PAI-1 activity in equine peritoneum and evaluate the effect of 0.4% HA solution on local tPA and PAI-1 activity. An exploratory laparotomy was performed and local serosal trauma was induced by using an established abrasion model. Our study involved two groups: in the first group (n = 6) 0.4% HA was used in all intestinal manipulations, whereas in the second group (n = 6) sterile saline was used. Parietal peritoneum, jejunal seromuscular biopsies at abraded sites (AJ) and nonabraded sites, and peritoneal fluid samples were taken at time 0- and at 30-minute intervals up to 120 minutes. Peritoneum tPA activity was significantly decreased at 60 and 90 minutes. Interestingly, AJ contained significantly higher tPA activity than nonabraded sites at 30-, 60-, 90-, and 120-minute intervals in control horses. The increase in tPA activity with AJ in treated (HA) horses was significantly attenuated as compared with the control (saline). Detectable levels of PAI-1 activity could not be identified in our samples. The results of our study indicate that exploratory celiotomy in horses is associated with a significant decrease in peritoneal tPA activity, and HA significantly decreases the fibrinolytic response of the jejunum to surgical trauma. Further characterization of these responses will hopefully lead to new pharmacologic strategies for adhesion prevention.

Keywords: Equine; Abdominal; Adhesions; Fibrinolysis; Hyaluronate

INTRODUCTION

Despite considerable efforts to understand the formation and prevention of postoperative adhesions, these complications continue to present clinical challenges to the surgeon. The incidence of clinically significant postoperative abdominal adhesions in horses has been reported to be between 9% and 27%.[1-3] However, the true prevalence of adhesions is unknown because they may be clinically silent or horses demonstrating postoperative pain are often managed medically or euthanized without necropsy evaluation.[3,4]

The fibrinolytic system is the principal modulator of adhesion formation and involves the lysis of fibrin into fibrin degradation products through the action of the enzyme plasmin.[5-10] Plasmin is stored as the inactive substrate plasminogen, which is converted to the active form primarily by tissue plasminogen activator (tPA). Tissue plasminogen activator is present in virtually all tissues and is responsible for 95% of plasmin generation in the human peritoneum.[11]

Fibrinolysis is tightly regulated by plasminogen activator inhibitors type 1 (PAI-1) and type 2 (PAI-2), which are induced by stimuli such as trauma, infection, or endotoxin.[5,12,13] These inhibitors bind to tPA forming inactive complexes. Depressed plasminogen activator activity may result from decreased concentrations of tPA and/or increased expression of plasminogen activator inhibitors.[7,8,10,14-16] The distinction between normal peritoneal healing and adhesion formation lies in the balance of fibrin deposition and degradation. If local fibrinolysis is adequate, fibrinous adhesions are lysed and normal tissue restoration results. However, if local fibrinolysis is insufficient, persistent fibrin becomes infiltrated with fibroblasts and capillaries and develops into permanent fibrous adhesions.[16-19]

From the Department of Clinical Science, College of Veterinary Medicine, Auburn University, Auburn, AL[a]; and Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA[b].
Reprint requests: Fred Caldwell, DVM, MS, Diplomate ACVS, Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL, 36849.
0737-0806/$ - see front matter

doi:10.1016/j.jevs.2010.04.005

Studies in people undergoing abdominal surgery have demonstrated that peritoneal tissue tPA activity decreases and PAI-1 activity increases through the course of the operation.[6,11] Correspondingly, peritoneal fluid concentrations of tPA activity in these patients were significantly increased. These findings suggest that tPA is rapidly released by the visceral peritoneum during intestinal manipulation, and that depressed tissue tPA activity is a primary cause of hypofibrinolysis in these patients.

Plasminogen activators and inhibitors have been measured in peritoneal fluid of normal horses and horses presenting for acute abdominal diseases.[13] Tissue plasminogen activator activity was significantly higher in peritoneal fluid of horses with acute abdominal disease as compared with normal horses.[13] The increased tPA activity in the peritoneal fluid of these horses may represent release of tPA from the visceral peritoneum. Local concentrations of peritoneal tissue fibrinolytic activity have not been previously measured in horses. In our preliminary studies, parietal and visceral peritoneal samples collected from three horses undergoing exploratory celiotomy for ischemic intestinal conditions demonstrated a 60% decrease in jejunal tissue tPA activity and a 25% increase in PAI-1 activity, as compared with jejunum from horses euthanized for nongastrointestinal-related conditions (unpublished data). These preliminary studies suggest that hypofibrinolysis may contribute to the pathogenesis of adhesion formation in horses.

Sodium hyaluronate (HA), a naturally occurring hydrophilic polymer, has been proven effective in reducing postoperative adhesions by forming a protective lubricating barrier on the intestinal serosal surface, preventing abrasive manipulative trauma and desiccation, and preserving the integrity of the mesothelium.[20-24] Additionally, HA may modulate adhesion formation by increasing tPA concentrations at the visceral peritoneal surface, thereby promoting mesothelial fibrinolysis.[10] Concentrations of 0.4% HA have been shown to maximally inhibit adhesion formation.[20,21] We have demonstrated that a 0.4% HA solution significantly decreases the incidence and severity of experimentally induced intraabdominal adhesions in horses.[25] The role of fibrinolysis in the pathogenesis of intra-abdominal adhesion formation in horses undergoing exploratory celiotomy and the effect of HA solutions on peritoneal fibrinolytic activity, however, have not yet been evaluated.

In this study, the fibrinolytic response of the equine parietal and visceral peritoneum to routine exploratory celiotomy and surgical trauma was evaluated by measuring tPA and PAI-1 activity in the peritoneum and jejunum. We hypothesized that tPA and PAI-1 activity are present in normal equine parietal and visceral peritoneum, and that abdominal surgery results in peritoneal hypofibrinolysis as evidenced by significant decreases in tPA activity with corresponding increases in PAI-1 activity. Next, we hypothesized that precoating of the small intestine with a 0.4% HA solution will significantly attenuate surgical trauma-induced hypofibrinolysis.

The specific objectives of this study were to (1) measure tPA activity and PAI-1 activity in normal equine parietal and visceral peritoneum during abdominal surgery in horses, and (2) to evaluate the effect of precoating of the small intestine with 0.4% HA solution on the surgically-induced perturbations of tPA and PAI-1 activity. By characterizing the fibrinolytic response of the equine parietal and visceral peritoneum to abdominal surgery and surgical trauma, we hope to provide insight into the development of new pharmacologic strategies for adhesion prevention.

METHODS

Experimental procedures and animal care were approved by the University Institutional Animal Care and Use Committee. Horses were housed in stalls, fed alfalfa hay and a pelleted feed twice daily, and offered water ad libitum. Horses were randomly assigned to one of two experimental groups. In group 1 (control, n = 6) horses, sterile lactated Ringer's solution was used for all intestinal manipulations. In group 2 (HA, n = 6) horses, 0.4% HA solution was applied to the intestine before and during all intestinal manipulations.

Preparation of HA

Sterile, nonpyogenic 0.4% w/w HA solution was prepared in a pH 7 isomolar phosphate buffered saline solution. The HA solution was generously supplied by Dr. Lynn S. Peck, Department of Materials Science and Engineering, University of Florida, Gainesville, FL.

Surgical Procedure

Food was withheld for 12 hours before surgery. Each horse was anesthetized with xylazine hydrochloride (1.1 mg/kg of body weight, IV), followed by ketamine hydrochloride (2.2 mg/kg of body weight, IV) and maintained with sevoflurane in oxygen. The horses were positioned in dorsal recumbency, and the ventral midline prepared for aseptic surgery. Lactated Ringer's solution (10 mL/kg/hr IV) was administered during the surgical procedure.

A 25-cm ventral midline celiotomy was performed. In control (group 1) horses, 2 L of sterile lactated Ringer's solution was used to lubricate the intestine during manipulation. In HA (group 2) horses, 2 L of HA solution was used to lubricate the intestine during manipulation. In all horses, peritoneal and jejunal seromuscular biopsy specimens and a peritoneal fluid sample were taken immediately after opening of the abdominal cavity (time 0), and at 30-minute intervals for a total of 120 minutes. Peritoneal biopsy samples were taken from the parietal peritoneum