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Recombinant human lubricin for prevention of postoperative intra-abdominal adhesions in a rat model

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ABSTRACT

Background: Postoperative intra-abdominal adhesions are a major cause of morbidity and mortality and contribute to a heavy burden on health care resources. At present, numerous introduced adhesion prevention products have demonstrated some benefit but none are consistently effective. The aim of this study was to examine the effectiveness of recombinant human lubricin in preventing intra-abdominal adhesion formation.

Materials and methods: A total of 62 male Wistar Albino rats were randomly assigned to the study. Six rats were used to the initial pilot study and 56 rats were randomized into four groups: (1) control cecal abrasion; (2) treatment cecal abrasion with 0.5 mg/mL lubricin solution; (3) control cecal enterotomy and primary closure; and (4) treatment cecal enterotomy and primary closure with 0.5 mg/mL lubricin solution. Rats were sacrificed at 3 d and 21 d postoperatively for the pilot and main studies, respectively. Macroscopic and microscopic adhesion severity was graded by blinded investigators.

Results: For the pilot study, all six rats successfully reached the end point indicating safety of the lubricin gel. In the main randomized study, adhesions in the treated cecal abrasion group were significantly reduced both macroscopically ($P = 0.001$) and microscopically (fibrosis $P = 0.009$, inflammation $P < 0.0001$), when compared with the control group. In the cecal enterotomy group, adhesions were reduced for the treatment group in macroscopic ($P = 0.011$) and microscopic grading (fibrosis $P = 0.500$, inflammation $P = 0.206$) compared with the control group.

Conclusions: Recombinant human lubricin significantly reduced both macroscopic and microscopic intra-abdominal adhesions in the cecal abrasion group. The cecal enterotomy group showed modest macroscopic adhesion reduction. Future study using higher

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concentration of lubricin solution are needed to investigate its toxicity and more profound antiadhesion properties in significant operations.

Introduction

An adhesion is defined as a band of scar tissue which binds two parts of tissue together when they should remain separate. Development of intraperitoneal adhesions after abdominal surgery remains an almost inevitable consequence. Approximately, 90% of patients who undergo laparotomies develop adhesions, and 5%-20% of them require treatment related to the complications.[1,2] Adhesion-related complications are often associated with significant morbidity and mortality. It is a heavy burden on health care resources with more than 300,000 annual hospitalizations in USA, accounting for nearly 850,000 d of inpatient care and 1.3 billion US dollars in expenditure.[3] Despite the introduction of minimally invasive surgical techniques, such as laparoscopic surgery, having helped reduce adhesion formation consequences of postoperative adhesions remain high.[2]

Currently, there are number of adhesion prevention products which have been shown to reduce some degree of adhesion formation, but several of them are associated with increased incidence of anastomotic leaks, delayed wound healing, and intra-abdominal sepsis. Seprafilm (hyaluronic acid/carboxymethylcellulose) has been shown to reduce the incidence, extent, and severity of adhesions but also caused increased anastomosis leak and intra-abdominal abscess rate. Another product, 4% icodextrin solution, reduced incidence of adhesive small bowel obstruction but did not reduce relaparotomy rate.[4,5]

At present, no product has been proven to completely block the mechanism of adhesion formation and demonstrate effectiveness in all types of surgery. Therefore, developing a versatile, robust, and easy-to-use product that is more effective and safe in preventing adhesions is essential.

Lubricin is a mucinous glycoprotein acting as a lubricating agent found on articular cartilages.[6,7] It also has profound antiadhesive physical properties. Chang *et al.* demonstrated strong repulsive interactions between two surfaces due to lubricin's adsorbing and amphiphilic properties to either hydrophobic or hydrophilic surfaces using atomic force microscopy.[8,9] Furthermore, the biological antiadhesive property of lubricin was demonstrated by Enlert *et al.*[8] where lubricin prevented fusion of opposing surfaces of articulating cartilages. Lubricin also has been proven to reduce adhesions for flexor tendon reconstruction surgery in animal models.[10-12] However, other studies also showed the undesirable effect of inhibiting physiological repair mechanisms of the cartilage,[8] which can potentially delay intra-abdominal wound healing postoperatively. At present, lubricin has a variety of medical applications, including eye drops, although its use as an antiadhesive product within the abdomen has yet to be fully established. Based on the antiadhesive properties of lubricin, we hypothesize that recombinant human lubricin is effective in preventing or reducing abdominal adhesion formation.

Material and methods

Materials

Purified recombinant human lubricin solution was supplied by the Lubris company, LLC (San Diego, CA). About 0.5 mg/mL of lubricin solution was prepared in 1X phosphate-buffered saline with 0.05% polysorbate-20, pH of approximately 7.0 and 0.22 micron filtering. Previous studies used 0.2 mL of 0.26 mg/mL lubricin administration on tendon repair and 0.04 mL of 0.3 mg/mL lubricin injection into intra-articular space, but administration of lubricin solution into intra-abdominal cavity has not previously been performed.[7-9] 1-mL lubricin per 100 g body mass was used per animal as recommended by our animal ethics committee's standard protocol for new experiments without previous established data. The solution was kept at 3°C before the planned use.

Animals and preoperative preparation

The study was approved by the University of Adelaide and South Australia Pathology animal ethics committees with the care of animals performed under the established guidelines set by these institutions. Adult male Albino Wister rats used in the study were provided by the University of Adelaide Laboratory Animal Services from around 10 wk of age, weighing between 300 and 400 g. Standard rodent food and water were provided ad libitum. Animals were housed at a constant room temperature with a 12 h light and dark cycle. Their welfare was monitored daily by experienced animal technicians using a standard observation chart. The animals were under observation for at least 48 h before inclusion in the study.

The initial pilot study on six rats was conducted to test the toxicity of 0.5-mg/mL lubricin solution, with half (three rats) receiving cecal abrasion and the remaining half receiving cecal enterotomy. All had lubricin solution administered before closing the abdominal wall, and were kept for 72 h before euthanasia. After successful outcomes of the pilot study, the main study divided 56 animals randomly into four groups; the first group received cecal abrasion without treatment, the second group received cecal abrasion with lubricin solution, the third group received cecal enterotomy and primary closure without treatment, and the fourth group received cecal enterotomy and primary closure with lubricin solution.

Surgical procedures

All rats underwent general anesthesia using a sealed chamber to deliver 3%-4% continuous inhaled isoflurane and 2 L/min of oxygen. Animals were consequently positioned for surgery and anesthesia was maintained with isoflurane and oxygen. Prophylactic subcutaneous amoxicillin (25 mg/kg) antibiotic injection and subcutaneous buprenorphine (0.05 mg/kg) were