International Journal of Surgery 14 (2015) 7–11

Contents lists available at ScienceDirect

International Journal of Surgery

journal homepage: www.journal-surgery.net

Original research

Bromelain: A natural proteolytic for intra-abdominal adhesion prevention☆

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HIGHLIGHTS

- Intraperitoneal adhesions are the major complications of abdomino-pelvic surgery.
- Inflammation, fibrosis and oxidative stress play an important role in adhesion formation process.
- Bromelain has anti-inflammatory, anti-oxidant and proteolytic effects.
- Intraperitoneal administration of bromelain is safe and effective for PPA prevention and reduction.

ARTICLE INFO

Article history:
Received 4 September 2014
Accepted 15 December 2014
Available online 6 January 2015

Keywords:
Adhesion
Abdominal surgery
Bromelain
Proteolytic
Prevention

ABSTRACT

Introduction: Peritoneal adhesions are pathological fibrous connections between peritoneal surfaces resulting from incomplete peritoneal repair. Adhesions cause various health problems ranging from pelvic pain and bowel obstruction to infertility. To date, no effective agent exists for intra-abdominal adhesion prevention. Bromelain is the crude extract of the pineapple and it has fibrinolytic, antithrombotic, and anti-inflammatory properties. Bromelain has been shown to be effective for removing necrotic tissues and has been found to be effective for treating various wounds, inflammatory conditions, and thrombotic pathologies. In the present study, we evaluated bromelain as a novel agent for preventing intra-abdominal adhesions.

Methods: Group 1 (control group): Adhesions were produced by cecal abrasion method, and no treatment was applied. Group 2 (i.p. bromelain-treated group): After adhesion formation, 10 mg/kg/BW of bromelain dissolved in 1 mL saline solution was applied intraperitoneally for 10 days. Group 3 (i.p. saline-treated group): After adhesion formation, 1 mL saline solution was applied intraperitoneally for 10 days. On postoperative day 10, all animals were sacrificed.

Results: All 30 rats survived surgery. Throughout the follow-up period, no complications were observed. Statistically significant differences were found between the groups with regards to macroscopic adhesion scores, inflammation, fibrosis and neo-vascularization ($p < 0.001$, <0.001, $p = 0.001$, $p = 0.002$, respectively). Macroscopic and histopathologic (inflammation, fibrosis, neo-vascularization) adhesion scores were lowest in the bromelain-treated group.

Conclusion: Bromelain, acting through its barrier, anti-inflammatory, antioxidant, and proteolytic effects and without increasing bleeding tendency or having any adverse effects on wound healing, may be a suitable agent for intra-abdominal adhesion prevention.

☆ Bromelain, acting through its barrier, anti-inflammatory, antioxidant, and proteolytic effects and without increasing bleeding tendency or having any adverse effects on wound healing, may be a suitable agent for this pathologic state.

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http://dx.doi.org/10.1016/j.ijsu.2014.12.024
1743-9191/

1. Introduction

Peritoneal adhesions are pathological fibrous connections between peritoneal surfaces resulting from incomplete peritoneal repair [1,2]. Peritoneal healing is a complex process involving hemostasis, inflammation, angiogenesis, formation of granulation tissue, extracellular matrix (ECM) deposition, and tissue remodeling [3,4]. Surgery-related tissue trauma, ischemia, infection, inflammation, and foreign body reaction all intervene in peritoneal healing, and postoperative peritoneal adhesion (PPA) is the inevitable result of any abdomino-pelvic surgery [1,2,5–8]. PPA occurs in 95% of surgeries [1,2].

PPAs cause various health problems ranging from pelvic pain and bowel obstruction to infertility [6,8–10]. Also, during adhesiolysis surgeries or other surgical operations, PPAs cause surgical complications such as intraoperative bowel perforation and ureter and urinary bladder damage [9,10].

To date, no effective agent or technique exists for PPA prevention. Various agents have been tried, but most, such as nonsteroidal anti-inflammatory drugs, fibrinolytics, and antibiotics, have specific activity [11–15]. For an ideal anti-adhesive agent, multiple steps of the pathologic process should be targeted without adversely affecting the peritoneal healing process.

Pineapple (*Ananas comosus*) is a tropical fruit that is consumed as a food and medicinal supplement in several countries [16–18]. Bromelain is the crude extract of the pineapple responsible for its therapeutic effects. Bromelain consists of several endopeptidases, glycoproteins, and carbohydrates. It has fibrinolytic, antithrombotic, and anti-inflammatory properties; these effects have been well documented in animal and human studies [16–20]. Bromelain has been shown to be effective for removing necrotic tissues and has been found to be effective for treating various wounds, inflammatory conditions, and thrombotic pathologies [16–29].

In the present study, we evaluated bromelain as a novel agent for preventing PPA because of its multiple beneficial effects (antioxidant, fibrinolytic, and anti-inflammatory effects).

2. Methods

Thirty Wistar albino female rats of reproductive age (weight, 200–250 g) were used as a model. The study was approved by the Zonguldak Bulent Ecevit University Experimental Animal Research Ethics Committee. The animals were treated in a humane manner in accordance with the Declaration of Helsinki. Water and food were provided ad libitum. Animals were obtained from the Zonguldak Bulent Ecevit University Experimental Animal Research Laboratory. The rats were randomly divided into three equal groups (10 rats in each group).

All animals were anesthetized with 50 mg/kg ketamine and 12.5 mg/kg xylazine injections.A 3 cm vertical midline incision was made for laparotomy. The cecum was identified, and the serosa of the cecum was rubbed with a gauze sponge until punctuate hemorrhage occurred. Then the cecum was placed in its abdominal location. Abdominal incisions were closed continuously with 3/0 prolene suture.

2.1. Experimental groups

Group 1 (control group): Adhesions were produced as described above, and no treatment was applied. ***Group 2*** (i.p. bromelain-treated group): After adhesion formation, 10 mg/kg/BW of Bromelain 10 mL/kg/BW (Nutraxin, Emek Mahallesi Ordu Cadddesi Sivat Yolu N0:9 Sancaktepe-Istanbul) dissolved in 1 mL saline solution was applied intraperitoneally (by using a 26-gauge syringe) for 10 days. ***Group 3*** (i.p. saline-treated group): After adhesion formation, 1 mL saline solution was applied intraperitoneally for 10 days.

Table 1
Macroscopic adhesion scoring.

	Macroscopic adhesion scoring
0	No adhesion
1	Fine adhesions, split with spontaneously or weak traction
2	Firm adhesions, split with moderate traction
3	Dense adhesion, split with blade dissection

2.2. Macroscopic evaluation of adhesion scores

On postoperative day 10, all animals were sacrificed. A surgeon who was blinded to the groups scored the adhesions using the macroscopic scoring system published by Evans et al. [13] (Table 1). Sample adhesion scores are given in Fig. 1a–d.

2.3. Histopathologic evaluation

Histopathologic evaluation of specimens was performed by a pathologist who was blinded to the experimental groups. Samples (1 cm^2) of cecum tissue and adjacent tissues were collected and fixed in formalin solution. Following dehydration and paraffinization, the tissues were cut into sections (5 μm thick) and stained with hematoxylin and eosin. Samples were examined under a light microscope. Inflammation, neo-vascularization, and fibrosis were scored.

Statistical analysis was performed with SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Results are expressed as median (min–max). Differences among the groups were analyzed using the Kruskal–Wallis test. Dual comparisons among groups with significant values were evaluated with the Bonferroni-adjusted Mann–Whitney U-test. A P value of less than 0.05 was considered statistically significant for all tests.

3. Results

All 30 rats survived surgery. Throughout the follow-up period, no complications were observed. The macroscopic and histologic adhesion scores of the three groups are summarized in Table 2.

30% of animals had grade 2 adhesion and 70% of animals had grade 3 adhesion in Group 1. In Group 2, 10% of animals had grade 2 adhesion and 40% had grade 1 adhesion, the remainder had no adhesion. In Group 3, 80% of animals had grade 2 adhesion and 20% of animals had grade 3 adhesion. Statistically significant differences were found between the groups ($p < 0.001$) and are shown in Table 3. Macroscopic adhesion scores were lowest in the bromelain-treated group. Although the scores were lower in the saline-treated animals than in controls, the difference was not statistically significant (Table 3).

The degree of inflammation was scored on a scale of 0–3: grade 0 (normal cell count), grade 1 (slight increase in polymorphonuclear cells), grade 2 (moderate increase in polymorphonuclear cells), and grade 3 (severe increase in polymorphonuclear cells) (Fig. 2a). Neo-vascularization was evaluated as grade 0 (no neo-vascularization), grade 1 (formation of 1–2 vessels), grade 2 (formation of 3–9 vessels), and grade 3 (formation of ≥10 vessels) (Fig. 2b). The degree of fibrosis was evaluated as grade 0 (no fibrosis), grade 1 (slight increase in young fibroblasts), grade 2 (moderate increase in young fibroblasts), and grade 3 (severe fibrosis) (Fig. 2c). Histologic evaluation revealed a statistically significant difference among the three groups. Inflammation, fibrosis, and neo-vascularization scores were lowest in the bromelain-treated animals (<0.001, $p = 0.001$, $p = 0.002$,