EXPERIMENTAL STUDY

Boric acid is as effective as hyaluronic acid-based agent in preventing intra-abdominal adhesions in a rat model

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ABSTRACT

BACKGROUND: In this experimental rat model, we aimed to investigate boric acid's possible protective effect against the formation of post-operative abdominal adhesions through its anti-inflammatory and antioxidant properties.

METHODS: Forty healthy male albino rats were randomly and evenly allocated to vehicle, hyaluronic acid-based (HA-b) material, boric acid 50 (BA50), boric acid 100 (BA100), and sham groups. Intra-abdominal adhesions were induced by mechanical cecal abrasion. Macroscopic and pathologic assessments of the adhesions were done and tissue tumor necrosis factor-α (TNF-α) and transforming growth factor-β1 (TGF-β1) levels were measured.

RESULTS: Total abdominal adhesion scores were 129.7, 91.07, 53.77, 90.07, and 140.5 for the vehicle, HA-b, BA50, BA100, and sham groups, respectively, with the highest score indicating more severe adhesions. A significant difference in fibrosis scores was noted between both BA50 and BA100, and the sham group (p=0.018). When objective parameters were analyzed, TNF-α levels were significantly lower in the BA50 group than the sham, BA100, and vehicle groups (p=0.01, 0.019, and 0.03, respectively). TGF-β1 levels were also significantly lower in BA50 group than the sham, BA100, and the vehicle groups (p=0.013, 0.016, and 0.05, respectively). No difference was observed for any parameter between BA50 group and HA-b group.

CONCLUSION: Topical boric acid at a dose of 50 mg/kg is found safe and as effective as the hyaluronic acid-based agent in preventing postoperative abdominal adhesions in our rat model.

Keywords: Boric acid; inflammation; intra-abdominal adhesion; peritoneal.

INTRODUCTION

The formation of intra-abdominal adhesions after any abdominal surgical intervention is a well-known entity in the surgical practice. Although other factors such as inflammatory/infectious diseases and abdominal irradiation may also end up with adhesion formation, surgical trauma is the leading cause by far.[1,2] Post-operative abdominal adhesions are known to cause debilitating gastrointestinal symptoms (bloating, constipation, and chronic abdominal pain), female infertility, and life-threatening conditions related to intestinal obstruction.[1–4] The consequences of intra-abdominal adhesions are usually ignored because of the relatively late onset of symptoms after the initial laparotomy.[4] Although trauma reducing minimal invasive surgical techniques are implemented to overcome the adhesion problem, the incidence remains higher than 50% with a hospital readmission rate of 2.2%.[4–6] Thus, the search for adhesion prevention measures is a never-ending challenge for abdominal surgeons.

Several theories have been proposed for the pathogenesis of intra-abdominal adhesion. It has now been understood that fibrous band formation is the cumulative result of consecutive local events: Inflammatory response, coagulatory activation, and fibrinolytic reactions. One or more defective stage through this process may prone a patient to develop postoperative abdominal adhesions. Patient characteristics (age, genetic factors, diabetes, alcohol consumption, etc.) play a great role in the process.[1] Visceral fat is also accused of the catalyzing factor during the inflammatory and oxidative process.

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[7] The initiating event that leads to the formation of fibrous bands is the ischemia of the peritoneal membrane, whether caused by surgical trauma, local inflammation, or irradiation. Reducing oxidative stress may alleviate initial inflammatory response that cascades into problematic adhesions, by attenuating local ischemia.

Boric acid is a naturally occurring boronated compound. Boron (atomic number=5) is a trace element that is widely used in glass, fiberglass, and ceramic making and aerospace and chemical industries. It has anti-inflammatory and antioxidant activity and plays a direct role in calcium metabolism in mammals. Turkey is the leading producer of boronated compounds in the world.[8–10]

In this experimental rat model, we aimed to investigate boric acid's possible protective effect against the formation of post-operative abdominal adhesions through its anti-inflammatory and antioxidant properties.

MATERIALS AND METHODS

This experimental study was conducted in Animal Research Laboratories of Health Sciences University after the ethical committee of Health Sciences University Gülhane Medical School approval which was granted (2018–18/27). Forty healthy male albino rats (8 weeks old) weighing 275±25 g were randomly and evenly allocated to five groups: Vehicle, hyaluronic acid-based (HA-b) material (sodium hyaluronate/carboxymethylcellulose – Seprafilm®, Genzyme Biosurgery, United States), boric acid 50 (BA50), boric acid 100 (BA100), and control (sham) groups. Animals were kept in standard cages at 21–23°C temperature, ≈60% humidity, and natural 12 h cycles of light and darkness which were provided. Animals were allowed to access tap water and commercial rat chow freely.

All chemicals and phosphate were Sigma-Aldrich® brand. Boric acid (426156) was dissolved in a sterile phosphate solvent (the vehicle, D8537) to obtain a physiological pH (7.30–7.40) which then checked with a pH meter. This solution was titrated with hydrochloric acid or sodium bicarbonate solutions when needed to achieve desired pH levels.

Surgical procedures were performed under general anesthesia induced with intramuscular ketamine (100 mg/kg, Ketalar®, Parke-Davis/Eczacıbası, Turkey) and xylazine hydrochloride (10 mg/kg, Rompun®, Bayer, Mefar, Turkey) administration. After shaving and disinfecting the surgical field with povidone-iodine, a 3 cm midline laparotomy was performed. Cecum was exposed, and a sterile standard toothbrush was used to cause 1.5 cm^2 punctate hemorrhagic areas on the anterior and antimesenteric serosal surfaces of the cecum to induce intraperitoneal adhesions. Nothing further was done in the control group. The abraded cecum wall was sheeted with Seprafilm® in the second group. Boric acid with vehicle solution was administered intraperitoneally, enough to cover the abraded serosal surfaces at the doses of 50 mg/kg and 100 mg/kg in boric acid 50 and boric acid 100 groups, respectively. The vehicle solution alone was applied in the fifth group.

One rat in the control group died on the post-operative 1st day because of the evisceration of the abdominal wound. After 20 days of standard care, the animals were given general anesthesia in the same fashion on the post-operative 21st day. Laparotomy with an inverted U-shape incision was performed. Other than adhesions, internal organs were normal. Macroscopic adhesion scoring (MAS) was done according to Nair et al.'s[11] classification by one of the authors in a blinded fashion (Table 1). Two different tissue samples were obtained from the peritoneal adhesions for histopathological and biochemical analyses. The animals were then sacrificed by exsanguination according to the guidelines of the Canadian Council on Animal Care.

Cecum and surrounding peritoneal tissues were separated after adhesion grading. Tissues then were buffered with a lysis buffer with a protease inhibitor and homogenized in the homogenisator (Heidolph Instruments GmbH&Co., Schwabach, Germany). The homogenates were centrifuged at 10,000 rpm, +4°C. Required protein amounts for ELISA testing were measured with BCA Protein Assay Kit (Thermo Scientific®). Commercially available ELISA kits were used to detect the serum tumor necrosis factor-α (TNF-α – BioLegend®, 438207) and transforming growth factor-β1 (TGF-β1 – Invitrogen®, BMS623/3), following the manufacturers' instructions.

Samples were treated with 10% formalin for 24 h for fixation. Transverse sections containing the whole intestine wall were obtained and immersed in paraffin. Two sections of 5 µm thickness from each group were stained with hematoxylin and eosin (H&E) and Masson's trichrome and evaluated by the same pathologist under light microscopy. Microscopic fibrosis and inflammation were graded according to a semi-quantita-

Table 1. Nair's scoring system for macroscopic abdominal adhesions

Description	Score
No adherence	0
Single adherence between two organs or between an organ and the abdominal wall	1
Two adherences between organs or one organ and the abdominal wall	2
More than 2 adherences between the organs or a massive generalized adherence of the intestine with no adherence to the abdominal wall	3
Generalized adherences between organs and the abdominal wall or massive adherence among all organs	4

Table 2. Microscopic fibrosis grading score

Description	Score
Nil	0
Minimal, loose	1
Moderate	2
Florid, dense	3

Table 3. Microscopic inflammation grading score

Description	Score
Nil	0
Giant cells, occasionally scattered lymphocytes, and plasma cells	1
Giant cells with increased numbers of admixed lymphocytes, plasma cells, eosinophils, neutrophils	2
Many admixed inflammatory cells, microabscesses present	3

tive scoring system introduced by Hooker et al.[12] (Tables 2 and 3).

Computer software was used for statistical analyzes (SPSS Statistics for Windows, version 22.0. Armonk, NY: IBM Corp). A non-parametric test (Kruskal–Wallis H) was used to seek any possible significant difference between the groups. The level of significance was set to $p<0.05$. After detecting a difference for at least one variable among the groups, a post hoc test (Tamhane-T2) was used to complete the analysis.

RESULTS

A total of five parameters were evaluated to assess post-operative intra-abdominal adhesion formation in this animal experiment model. Three of them (MAS, inflammation, and fibrosis) were partially subjective and the other two (biochemical levels of TNF-α and TGF-β1) were objective. BA50 group had the lowest intra-abdominal adhesion scores for each parameter. Total abdominal adhesion scores were 129.7, 91.07, 53.77, 90.07, and 140.5 for the vehicle, HA-b, BA50, BA100, and sham groups, respectively, with the highest score indicating more severe adhesions (Table 4). Macroscopic adhesion between the cecum and adjacent intra-abdominal organs is shown in Figure 1.

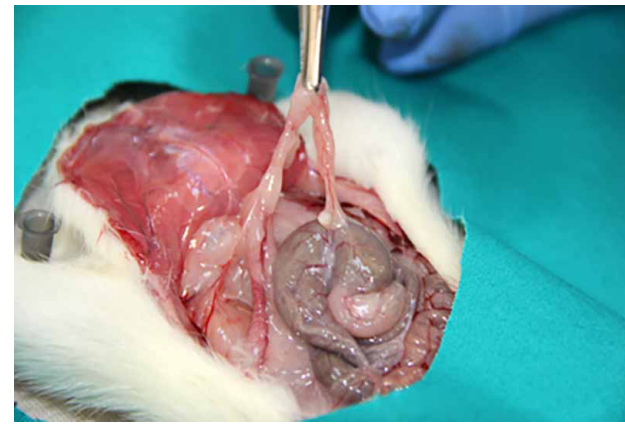

Figure 1. Post-operative intra-abdominal adhesion.

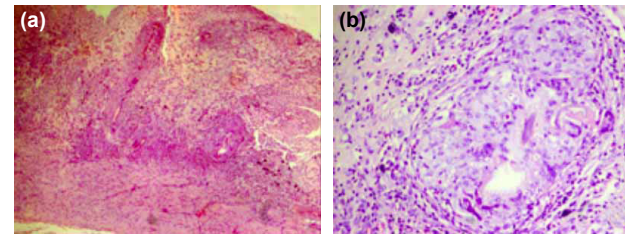

Figure 2. Inflammation on pathological examination. **(a)** Transmural severe inflammation (H&E, ×40). **(b)** Giant cells, eosinophils, neutrophils, and plasmocytes are seen in the inflammation (H&E, ×400).

MAS and inflammation scores did not mark a significant difference among groups. A significant difference in fibrosis scores was noted between both BA50 and BA100 and the sham group (p=0.018). Figure 2a and b shows inflammation, and Figure 3 shows fibrosis on pathological examination.

When objective parameters were analyzed, TNF-α levels were significantly lower in the BA50 group than the sham, BA100, and the vehicle groups (p values are 0.01, 0.019, and

Table 4. Mean ranks of the evaluated variables (Kruskal-Wallis-H)

Groups	n	Mean MAS rank	Mean inflammation rank	Mean fibrosis rank	Mean TNF-α rank	Mean TGF-β1 rank
Vehicle	8	22.94	24.5	24.38	30	27.88
HA-b	8	20.44	19.88	20.5	15.25	15
BA50	8	10.88	13.63	12.5	7.63	9.13
BA100	8	18.06	13.63	12.5	22.63	23.25
Sham	7	28.79	29.57	31.57	25.14	25.43

MAS: Macroscopic adhesion score; TGF: Transforming growth factor-β1.

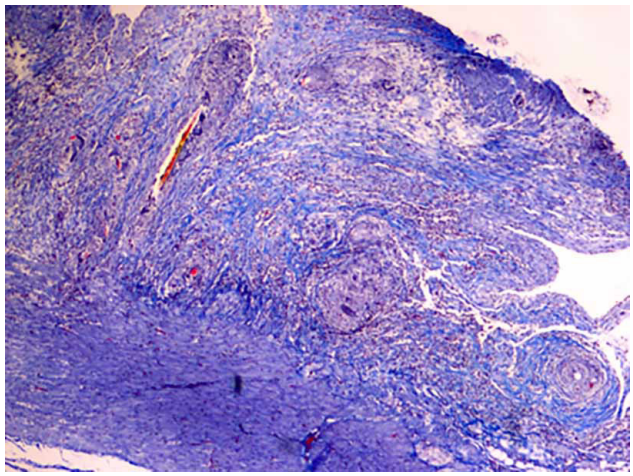

Figure 3. Mild-severe fibrosis through the intestinal wall (Masson's trichrome, ×40).

Table 5. Tamhane-T2 test results of the variables with a significant difference

Variables	Mean difference	Compared groups	p value
Fibrosis	1,57143	BA50 versus sham	0.018
	1,57143	BA100 versus sham	0.018
TNF-α	6,43	BA50 versus vehicle	0.03
	5,2575	BA50 versus sham	0.01
	6,73696	BA50 versus BA100	0.019
TGF-β1	391,23750	BA50 versus vehicle	0.05
	367,82393	BA50 versus sham	0.013
	275,62375	BA50 versus BA100	0.016

TGF: Transforming growth factor-β1.

0.03, respectively). TGF-β1 levels were also significantly lower in BA50 group than the sham, BA100, and the vehicle groups (p values are 0.013, 0.016, and 0.05, respectively) (Fig. 4). No significant difference between BA50 group and HA-b group was observed for any parameter (Table 5).

DISCUSSION

Post-operative intra-abdominal adhesions occur in almost all of the patients that undergo abdominal or pelvic surgery.[4,5] Although most of the patients remain clinically "silent," some of them develop vague symptoms (abdominal cramping, early satiety, and bowel habit changes) that are usually not attributed to abdominal adhesions.[1,2,4] Pelvic adhesions are held responsible for more than 20% of secondary female infertility and known to increase the risk of ectopic pregnancy.[1,13,14] The most fearsome and life-threatening complication of abdominal adhesions is the intestinal obstruction. More than half of all small bowel obstructions are caused by intra-abdominal adhesions.[15] A retrospective cohort study with a follow-up period of 10 years has shown that almost 6% of more than 20,000 patients reviewed were readmitted because of adhesion-related complications, whom more than half required a laparotomy. This rate exceeded 30% when "possibly adhesion-related" complications were considered.[5]

The pathway to post-operative abdominal adhesions is paved with increased inflammatory reactions and the imbalance between pro- and antifibrinolytic activities. TNF-α is a well-understood pro-inflammatory cytokine mostly derived from activated macrophages. Its elevated levels indicate a strong inflammatory response.[1,14,16] TGF-β1 expression is also increased after inflammation and tissue injury.[17,18] TGF-β1 inhibits degradation of fibrin by the mesenchymal cells and contributes antifibrinolytic activity, resulting in fibrosis.[19] The cumulative effect of TGF-β1 and TNF-α promotes fibrosis induced by inflammation and trauma and stimulates fibrous adhesions.[7] We analyzed tissue levels of TNF-α and TGF-β1 to determine inflammation and fibrosis more objectively.

General surgical principles such as gentle tissue handling, introducing less foreign body in the peritoneal cavity, keep-

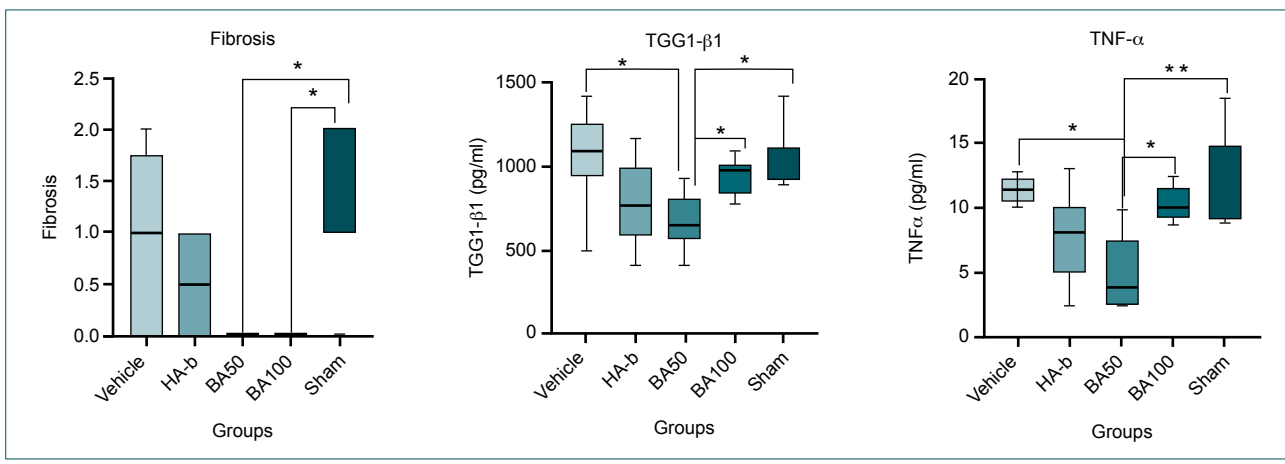

Figure 4. Significant differences in fibrosis, transforming growth factor-1 (TGF1)-β1, and TNF-α levels among study groups (*indicates p<0.05 and **indicates p<0.01).

ing the surgical field moist, and careful hemostasis appear theoretically reasonable but they do not seem to alter the formation of post-operative adhesions.[15,20,21] Laparoscopy is thought to minimize surgical injury to the tissue and causes a less severe inflammatory process.[15] There are conflicting results in the literature and it has been proposed that CO2 pneumoperitoneum itself might be inducing adhesion formation as well.[22]

HA-b barriers (especially HA-cellulose combination) are the most studied and well-known bioabsorbable antiadhesion barriers and frequently used for comparison with the novel agents and materials.[15,21,22] Most prospective randomized studies documented a significant reduction in the severity of post-operative intra-abdominal adhesions with these products but their negative effect on anastomoses was also acknowledged.[22,23]

Mechanical barriers and topical chemical agents have widely investigated in prevention post-operative intra-abdominal adhesions. Fibrinolytic agents, anti-inflammatory agents, antibiotics, crystalloids, hydrogen-rich saline, icodextrin, liquid paraffin, mitomycin-C, phospholipids, and even honey have been evaluated, in most experimental studies for their anti-adhesion properties.[15,16,21–27] A more recent experimental study found thermosensitive hydrogel (xyloglucan) effective in preventing early-onset intra-abdominal adhesions.[28] *Bletilla striata*, Chinese medicinal herb, is found promising in alleviating inflammatory process and reducing the formation of abdominal adhesions.[29] However, their clinical use remained limited. This might be attributed to the designs of the experimental studies investigating the aforementioned chemical and organic agents which mostly compared the chemical agents' effect with control groups. We compared boric acid's efficacy with both sham and HA-b material, which is widely used in clinical practice to reduce post-operative abdominal adhesions. As a cheaper substitute, BA could be considered for clinical use. If backed up with further experimental studies comparing BA with other antiadhesive agents, clinical trials could be initiated on human subjects.

Experimental studies suggest that boric acid has anti-inflammatory, antioxidant, antimicrobial, and anticarcinogenic properties.[8–10,30] Boric acid took its place also in clinical practice. Two review studies concluded that topical boric acid was effective in the treatment of chronic otitis media and vulvovaginal candidiasis.[31,32] Boric acid has not been investigated as a potential anti-adhesion agent ever before.

We used two different doses of boric acid and compared the results with sham, HA-b material, and vehicle groups. In a study evaluating oral boric acid's preventive effect on nephrolithiasis, moderate doses of boric acid (50 mg/kg) were found superior to higher doses (100 mg/kg).[33] TNF-α and TGF-β1 levels were significantly lower in the BA50 group than the sham, BA100, and vehicle groups. Although subjective parameters (MAS and pathological inflammation and fibrosis) were also in favor of BA50 group, the differences were not statistically significant. We observed no difference between BA50 and HA-b groups.

Conclusion

Topical boric acid at a dose of 50 mg/kg is found as effective as sodium hyaluronate/carboxymethylcellulose in preventing post-operative abdominal adhesions in our rat model. The therapeutic potential, optimal dosage, and the safety of boric acid should further be investigated in other experimental studies before designing any clinical trials.

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Ethics Committee Approval: This experimental study was approved by the ethics committee of Health Sciences University Gülhane Medical Faculty (2018-18/27).

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Conflict of Interest: None declared.

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DENEYSEL ÇALIŞMA - *ÖZET*

Borik asit sıçan modelinde oluşturulan karın içi adezyonların önlenmesinde hiyaluronik asit bazlı ürün kadar etkili

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AMAÇ: Bu deneysel sıçan modelinde, borik asidin anti-enflamatuvar ve antioksidan özellikleri ile ameliyat sonrası abdominal adezyon oluşumuna karşı olası koruyucu etkisini araştırmayı amaçladık.
GEREÇ VE YÖNTEM: Kırk adet sağlıklı erkek albino sıçan rastgele ve eşit olarak taşıyıcı, hiyaluronik asit bazlı (HA-b) materyal, borik asit 50 (BA50), borik asit 100 (BA100) ve kontrol grupları olarak ayrıldı. Karın içi adezyonlar mekanik çekal abrazyon ile indüklendi. Adezyonların makroskopik ve patolojik değerlendirmesi yapıldı ve doku tümör nekroz faktörü-α (TNF-α) ve transforme edici büyüme faktörü-1 (TGF-β1) seviyeleri ölçüldü.
BULGULAR: Toplam abdominal adezyon skorları araç, HA-b, BA50, BA100 ve kontrol grupları için sırasıyla 129.7, 91.07, 53.77, 90.07 ve 140.5 idi ve en yüksek skor daha ciddi adezyonlara işaret ediyordu. Hem BA50 hem de BA100 ile kontrol grubu arasında fibrozis skorlarında anlamlı bir fark olduğu görüldü (p=0.018). Objektif parametreler incelendiğinde, TNF-α seviyeleri BA50 grubunda kontrol, BA100 ve araç gruplarından anlamlı olarak düşüktü (sırasıyla, p=0.01, 0.019 ve 0.03). TGF-β1 düzeyleri BA50 grubunda kontrol, BA100 ve araç gruplarından anlamlı olarak düşüktü (sırasıyla p=0.013, 0.016 ve 0.05). BA50 grubu ve HA-b grubu arasında herhangi bir parametre için fark gözlenmedi.
TARTIŞMA: Sonuç olarak, sıçan modelimizde ameliyat sonrası abdominal adezyonlarını önlemede 50 mg/kg dozunda topikal borik asit güvenli ve hiyaluronik asit bazlı ajan kadar etkili bulunmuştur.
Anahtar sözcükler: Borik asit; enflamasyon; karın içi adezyon; peritoneal.

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