EXPERIMENTAL STUDY

Effect of intra-abdominal boric acid in the experimental adhesion model

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ABSTRACT

BACKGROUND: The continuous advancement in medical and surgical techniques has led to a rise in the frequency of abdominal operations, subsequently increasing the incidence of intra-abdominal adhesions. Over 90% of laparotomies result in postoperative intra-abdominal adhesions. This study investigates the effect of a 5% boric acid solution on the development of intra-abdominal adhesions in rats, using an adhesion model.

METHODS: This study was conducted with two groups: a control group, in which the adhesion model was applied without any treatment, and a boric acid group, which was treated with a 5% boric acid solution. Each group comprised 16 rats. On the 14th postoperative day, the rats were sacrificed, re-explored, and the developed adhesions were evaluated both macroscopically and microscopically. The data from macroscopic and microscopic scoring were analyzed using the Mann-Whitney U test in the IBM Statistical Package for the Social Sciences (SPSS) Statistics 24 program. A p-value of less than 0.05 was considered statistically significant. This research was supported by the Manisa Celal Bayar University Scientific Research Projects Commission.

RESULTS: A statistically significant difference was observed between the boric acid-treated group and the control group, with the boric acid group showing a significant decrease in adhesion development both macroscopically and microscopically ($p<0.05$).

CONCLUSION: In the future, boron could play a significant role in reducing and preventing intra-abdominal adhesions after surgery. This investigation could pave the way for further research into the mechanism by which boric acid prevents the development of intra-abdominal adhesions. Moreover, it is imperative to explore the potential side effects of intra-abdominal boron application at the optimum concentration of the solution.

Keywords: Adhesion; boron; intra-abdominal; postoperative.

INTRODUCTION

As medical and surgical techniques have advanced, the frequency of abdominal operations has increased, leading to a higher incidence of intra-abdominal adhesions. These adhesions can result in common issues such as abdominal pain and constipation. Particularly in women, they may lead to more severe complications like ileus and infertility. It is estimated that more than 90% of intra-abdominal adhesions are the result of abdominal surgeries, and up to 30% of ileus cases in patients with a history of previous abdominal surgery may be attributed to these adhesions.[1-3] Adhesions often remain asymptomatic, their impact largely determined by their location and structure. Yet, for patients who do experience symptoms and develop complications, the mortality rate can reach up to 13%.[4]

Treatment for complications arising from these adhesions

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sometimes necessitates major abdominal surgery. Frequently, operations performed for various indications are prolonged due to the presence of adhesions. Intra-abdominal adhesions, which are bands of connective tissue, form abnormally outside of the normal healing process during the healing of peritoneal injuries. They can develop between organs or peritoneal surfaces. These structures may manifest as thin, non-vascularized, and weak bands; thick, abundant, and hard bands; or direct adhesions of two organ surfaces to each other.[5,6] The formation of adhesion structures is a consequence of the inflammatory response initiated by tissue factors, coagulation factors, and free cells exposed by damage to the mesothelial layer.[3]

Despite advances in surgical techniques and new medical treatments, it is still not possible to completely prevent intra-abdominal adhesions.[7] Minimally invasive surgeries, using laparoscopic or robotic techniques, help reduce the risk of developing intra-abdominal adhesions. These methods focus on preventing the drying of the intestinal surface, avoiding the introduction of foreign bodies such as talcum powder, and minimizing the use of suture materials.[8,9] Various drugs have also been explored to reduce the development of postoperative adhesions. These include corticosteroids, non-steroidal anti-inflammatory drugs, antihistamines, calcium channel blockers, antibiotics, fibrinolytic agents (such as streptokinase and urokinase), colchicine, vitamins, antioxidants, and antibodies targeting proinflammatory cytokines like transforming growth factor-beta (TGF-β). However, most of these drugs are not widely used in clinical practice due to insufficient evidence regarding their effectiveness. Many exhibit the primary feature of reducing the likelihood of adhesion development in only a limited number of clinical cases.[9-11]

Boron was officially recognized as a trace element by the World Health Organization in 1980, after numerous studies.[12,13,14] It has been shown to possess antioxidant, antimicrobial, and anti-inflammatory activities, in addition to activating numerous enzymes.[15]

In this study, based on the established properties of boric acid, we hypothesize that it may have the potential to diminish the formation of postoperative intra-abdominal adhesions, which is a major concern in general surgery practice. This research aims to explore the impact of intraperitoneally administered boric acid.

MATERIALS AND METHODS

This study was approved by the Animal Experiments Local Ethics Committee of Manisa Celal Bayar University. It aimed to investigate the impact of a 5% boric acid solution on the development of intra-abdominal adhesions in rats with experimentally induced adhesions.

When designing the experiment, the expected prevalence of intra-abdominal adhesion development was considered to be 90%. It was hypothesized that treatment with a 5% boric acid solution could reduce adhesion formation by 50%. The study was designed with a Type I error rate of 0.05 and a minimum power of 80%. Consequently, it was determined that each group needed to include at least 16 subjects. Accordingly, the subjects were divided into two groups:

• Group 1 (Control group, n=16): An intra-abdominal adhesion model was created.

• Group 2 (Intra-abdominal boric acid solution group, n=16): An intra-abdominal adhesion model was created, followed by the application of a 5% boric acid solution intra-abdominally.

The experiment was financially supported by the Manisa Celal Bayar University Scientific Research Projects Commission. Thirty-two female Wistar-albino rats, averaging a weight of 300-400 g, were obtained from the Manisa Celal Bayar University Experimental Animals Application and Research Center (CBU-DEHAM). The rats were fed a standard pellet diet and given water. After the experiment, they were housed in separate cages in an isolated environment under standard laboratory conditions, which included a 12/12-hour light/dark cycle, a temperature maintained at 21±2°C, and a humidity level of 50%.

The surgical procedures in the experiment were conducted under sterile conditions in the Operating Room of the Manisa Celal Bayar University Experimental Animal Application and Research Center. Prophylactic antibiotics were not administered. For anesthesia, a single dose of intramuscular ketamine (Ketalar®, Pfizer, 20-40 mg/kg) and xylazine (Rompun®, Bayer, 4-8 mg/kg) was used. To maintain normothermia in the rats, both the operating room and postoperative care rooms were heated using air conditioning. Powder-free sterile gloves were the standard in the study. The abdominal skin was shaved, cleaned with 10% povidone, and covered with a sterile green drape. A 3 cm midline incision was then made for the laparotomy to access the abdomen.

Experimental Adhesion Model

For the adhesion model, we utilized the technique of creating abrasions on the serosa of the cecum, a method proven in previous studies for its ease of application. The cecum was located and placed on a sterile, wet sponge. Abrasions were then made using a dry sponge until minimal petechial areas appeared on the cecum's serosa (Fig. 1). After bleeding control was completed, no additional procedures were performed on the control group. In contrast, the experimental group received an intra-abdominal application of 5 cc of a 5% boric acid solution. The peritoneum and abdominal skin were subsequently closed with 3/0 Vicryl and 3/0 silk sutures, respectively.

On the 14th postoperative day, subjects in both groups underwent a second laparotomy with an inverted U-incision. Adhesions were macroscopically scored by an investigator blinded to the group assignments.

Euthanasia of Rats and Collection of Samples in the Laboratory Setting

On the 14th postoperative day, subjects from both groups

Figure 1. Abrasions and petechial areas on the serosa of the cecum.

Figure 2. Grade 4 adhesion that cannot be easily separated between the cecum and anterior abdominal wall in the control group.

were euthanized in the laboratory setting through cervical dislocation under anesthesia to minimize pain, suffering, and distress. After euthanasia, rats that underwent a second laparotomy had their cecum, along with any adhesions that had formed, completely removed for macroscopic examination through an inverted U-shaped incision. The specimens were then placed in containers filled with a 10% formalin solution for transport to the Department of Pathology.

Table 1. Canbaz Adhesion Scoring System

Grade	Description of Grade
0	No adhesion
1	1 adhesion band, no vessel, easily separated
2	2 thin adhesion bands, no vessels, easily separated
3	3 thin adhesion bands, no vessels, easily separated
4	>3 thin adhesion bands, easily separated with no vessel or defused adhesion bands with vessels

Macroscopic and Microscopic Examination

Following re-laparotomy, the intra-abdominal adhesions of the subjects were evaluated in a double-blind manner by two researchers using the Canbaz Adhesion Scoring System (Table 1). The specimens underwent microscopic examination by the Department of Pathology to assess the degree of fibrosis and inflammation. Hematoxylin & Eosin and Masson's Trichrome stains were used for this purpose. Adhesions were scored according to fibrosis and inflammation scoring systems (Table 2).

Statistical Analysis

Data were analyzed using the IBM Statistical Package for the Social Sciences (SPSS) Statistics 24 software. Results were evaluated using the Mann-Whitney U test, with a statistical significance level was set at $p<0.05$.

RESULTS

On the 14th postoperative day, the abdomens of the rats were examined via an inverted U incision, and a double-blind macroscopic evaluation was conducted using the Canbaz Adhesion Scoring System. Adhesions developed in all rats in the control group, with Grade 3 adhesions detected in 7 rats and Grade 4 adhesions in 9 rats. The mean macroscopic adhesion score was calculated to be 3.56±0.512 (Fig. 2).

Fewer adhesions developed in the rats in the experimental group compared to the control group. In the experimental

Table 2. Fibrosis and inflammation scoring system (microscopically)

Scoring	Fibrosis Description	Inflamation Description
0	None	None
1	Minimal, weak, mild	Giant cells, lymphocytes, plasma cells
2	Moderate	Giant cells, plasma cells, eosinophils, neutrophils
3	Intense	Large amounts of inflammatory cells and micro-abscesses

Figure 3. Grade 1 adhesion between the cecum and the lateral abdominal wall in the experimental group.

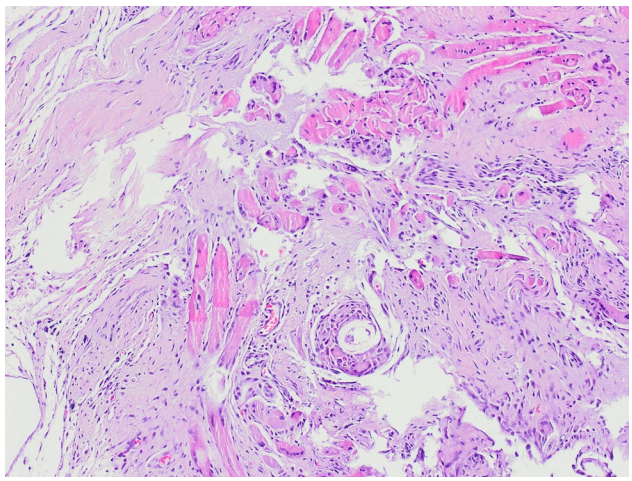

Figure 4. Microscopic view of a sample with intense fibrosis (fibrosis score: 3) from the control group (H&E staining, 10x magnification).

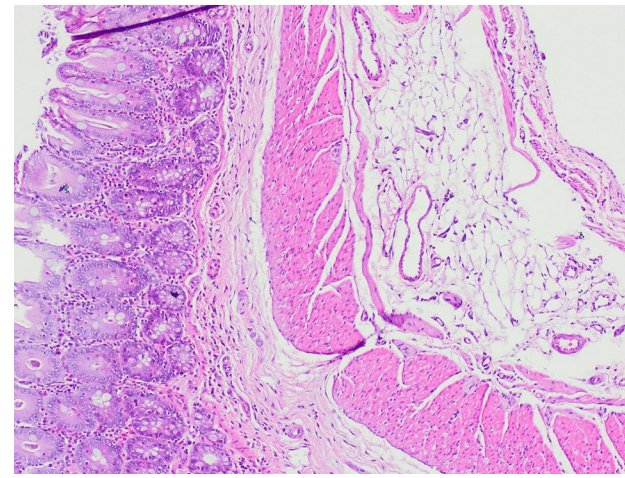

Figure 5. Microscopic view of a sample from the experimental group showing no inflammation (inflammation score: 0) (H&E staining, 10x magnification).

group, no adhesions formed in 4 rats, Grade 1 adhesion occurred in 6 rats, and Grade 2 adhesion in 6 rats. The mean macroscopic adhesion score was calculated to be 1.13±0.806 (Fig. 3).

In the microscopic examination of the control group, no fibrosis developed in 1 rat, mild fibrosis was observed in 7 rats, moderate fibrosis in 5 rats, and severe fibrosis in 3 rats (Fig. 4). The mean fibrosis score was calculated to be 1.63±0.885. Inflammation was detected in all rats in this group, with Grade 1 inflammation observed in 6 rats, Grade 2 in 4 rats, and Grade 3 inflammation in 6 rats. The mean inflammation score was calculated to be 2±0.894. In the microscopic examination of the experimental group, fibrosis did not develop in 7 rats, mild fibrosis was observed in 7 rats, moderate fibrosis in 1 rat, and severe fibrosis in 1 rat. The mean fibrosis score was calculated to be 0.75±0.856. Inflammation was not detected in 10 rats in this group, Grade 1 inflammation was found in 5 rats, and Grade 3 inflammation in 1 rat (Fig. 5). The mean inflammation score was calculated to be 0.50±0.816.

Upon analyzing the statistical data, a significant decrease was observed in the macroscopic adhesion scores ($p<0.05$), as well as in the microscopic inflammation ($p<0.05$), and fibrosis scores ($p<0.05$) in rats treated with a 5% boric acid solution compared to the control group.

Table 3. Adhesion, fibrosis and inflammation scores of the control and experimental groups. (C: control group, E: experimental group)

Rat		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
C	Adhesion	3	4	4	3	3	3	4	3	4	3	4	4	3	4	4	4
	Fibrosis	2	3	3	1	1	1	2	1	2	2	1	1	0	3	1	2
	Inflammation	1	2	3	1	1	1	3	3	3	2	3	3	2	1	2	1
E	Adhesion	1	1	0	2	1	1	2	0	2	1	2	2	2	0	1	0
	Fibrosis	1	1	0	0	2	0	3	0	1	1	0	1	1	0	0	1
	Inflammation	0	0	0	0	1	1	3	0	0	1	1	0	0	0	0	1

DISCUSSION

Numerous studies in the literature focus on the mechanisms of formation and prevention of intra-abdominal adhesions, which can lead to complications such as ileus and infertility in women. These studies include both intra-abdominal and systemic approaches. Many techniques have been identified through these studies to address this concern in a large population. The most critical aspects of these techniques involve reducing contact between mechanical barriers and serosal surfaces, as well as preventing fibrin deposition. An ideal mechanical barrier should be safe and effective, remain in place without the need for fixation throughout the remesothelization process, and be completely absorbed by the abdomen at the end of the healing process. Anti-adhesion treatments should avoid causing intra-abdominal infections, anastomotic leaks, and fistula formations, and should not impair wound healing.

In the literature, studies investigating the effect of boric acid on intra-abdominal adhesions are limited. This study was planned based on the antioxidant, anti-inflammatory, and antifibrotic effects of boric acid found in previous studies. In the study conducted by H. Bozkurt et al., it was demonstrated that the antifibrotic effect of 5% boric acid could reduce epidural fibrosis.[16] This study aimed to reduce intra-abdominal adhesions, whose basic formation mechanisms include inflammation and fibrosis, through a similar mechanism.

Numerous researchers have reported that boron possesses anti-inflammatory and antioxidant properties. Specifically, it has been shown that boric acid inhibits lipopolysaccharide (LPS)-induced tumor necrosis factor-alpha (TNF-α) release in human THP-1 monocytic leukemia cells,[17] and fructoborate decreased interleukin-1 beta (IL-1β) release from LPS-stimulated murine macrophage RAW 264.7 cells.[18] Animal studies have demonstrated that boric acid and borates reduce oxidative stress induced by injections of alcohol,[19] arsenic trioxide,[20] and sheep red blood cells.[21] Furthermore, supplementation with calcium fructoborate, the natural and soluble form of boron (sugar-borate ester) found in fresh fruits and vegetables, was found to reduce C-reactive protein (CRP) in subjects with a serum concentration of more than 3.0 mg/L. [22,23]

The results of this study indicate that boric acid is effective in reducing adhesions macroscopically ($p<0.05$). In addition to findings from various studies in the literature, this study also microscopically detected a significant reduction in both fibrosis and inflammation ($p<0.05$ for both).

Given the anti-inflammatory, antifibrotic, and antioxidant effects of boric acid documented in the literature, this study has demonstrated a reduction in postoperative intra-abdominal adhesions.

Limitations

The results found in animal studies may not be the same as effects on humans. Additional studies are required to understand the potential side effects of boric acid when in direct contact with the peritoneum and visceral organs.

CONCLUSION

Boron could play a crucial role in the reduction and prevention of intra-abdominal adhesions post-surgery in the future. This study serves as a pioneering investigation into the mechanisms by which boric acid prevents the development of intra-abdominal adhesions. Furthermore, exploring the potential side effects of intra-abdominal boron application and determining the optimum concentration of the solution are imperative.

Ethics Committee Approval: This study was approved by the Manisa Celal Bayar University Ethics Committee (Date: 14.01.2020, Decision No: 77.637.435).

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DENEYSEL ÇALIŞMA - *ÖZ*

Deneysel adeyon modelinde, entraabdominal borik asidin etkisi

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AMAÇ: Bu çalışmada; batın cerrahilerinin giderek daha fazla sayıda yapılmasıyla sıklıkla karşılaşılan ve ciddi mortalite ile morbidite sebebi olan intraabdominal adezyonların azaltılabilmesi için; adezyon modeli oluşturulmuş ratlarda, %5 borik asit solüsyonu uygulamasının batın içi adezyon gelişimine etkisini araştırmak amaçlanmıştır. Borik asidin daha önce kanıtlanmış olan antiinflamatuvar, antioksidan ve antifibrotik etkileri ile intraabdominal adezyon gelişimini azaltacağı düşünülmüştür.

GEREÇ VE YÖNTEM: Çalışma her biri 16 adet rat içeren 2 grupta (adezyon modeli oluşturulmuş kontrol grubu ve %5'lik borik asit solüsyonu uygulanmış deney grubu) yapıldı. Operasyon sonrası 14. günde ratlar sakrifiye edilip tekrar eksplore edildi ve gelişen adezyonlar makroskopik ve mikroskopik olarak değerlendirildi. Makroskopik ve mikroskobik skorlamalar sonucunda elde edilen veriler IBM SPSS Statistics 24 programında Mann-Whitney U testi ile değerlendirildi. İstatistiksel anlamlılık düzeyi "$p<0.05$" olarak belirlendi.

BULGULAR: İstatistiksel veriler incelendiğinde borik asit uygulanan grupta adezyon gelişiminde makroskopik ve mikroskopik olarak anlamlı bir azalma olduğu görüldü ($p<0.05$).

SONUÇ: Bu çalışmanın sonucunda; deneysel adezyon modelinde intraabdominal %5' lik borik asit solüsyonu uygulamasının, postoperatif intraabdominal adezyonları azaltmada anlamlı etkiye sahip olabileceği gösterilmiştir. Bu etkinin borik asidin antiinflamatuvar, antioksidan ve antifibrotik etkilerinden kaynaklandığı öngörülmektedir. Bu çalışma, borik asidin intraabdominal adezyonları azaltma mekanizmasının ve ideal dozun araştırılması için öncü olacaktır.

Anahtar sözcükler: Adezyon; boron; intraabdominal; postoperatif.

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