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*Original Research*

# Preventive effect of verapamil and sodium citrate on the formation of postoperative peritoneal adhesion

Verapamil and sodium citrate in postoperative peritoneal adhesion

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**Abstract**

Aim: Calcium is an important component in the inflammatory response to injury, and it has been reported to have an adhesion-reducing effect. In this study, we aimed to examine the effects of verapamil, a calcium channel blocker, and sodium citrate, when used alone or in combination, on adhesion formation.

Material and Methods: Thirty female Wistar-albino rats with an average body weight of 225 g were used in the study. The rats were divided into five groups as follows: six rats in each group: Sham group (n=6): Laparotomy alone; Group A: Control group (abrasion) (n=6): Laparotomy + cecal abrasion; Group B: Verapamil alone (n=6): Laparotomy + cecal abrasion + verapamil; Group C: Sodium citrate alone group (n=6): Laparotomy + cecal abrasion + sodium citrate; Group D: Dual therapy group (n=6): Laparotomy + cecal abrasion +verapamil + sodium citrate.

Results: The adhesion score was lower in Groups B and D compared to Group A (p=0.015 and p=0.015, respectively). Groups B, C and D had lower inflammation intensity scores compared with Group A (p=0.006, p=0.025 and p=0.011, respectively). Groups B and D groups had significantly lower inflammation intensity scores compared to Group A (p=0.010 and p=0.019, respectively).

Discussion: Based on the results of our study, it was concluded that the combined use of verapamil and sodium citrate did not increase the effectiveness of the prevention of intra-abdominal adhesions compared to their use separately.

**Keywords**

Postoperative Adhesions, Verapamil, Sodium Citrate, Fibrosis, Inflammation

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## Introduction

Postoperative peritoneal adhesions (PPA) is a condition that occurs in more than 90% of patients undergoing abdominal surgery. PPAs can cause chronic abdominal pain, repeated bowel obstruction and female infertility, which require repetitive surgical interventions that leads to morbidity and mortality, as well as high costs [1, 2]. Complications occurring as a result of bowel obstructions due to adhesions requiring surgery include iatrogenic intestinal injuries, transition from laparoscopy to laparotomy, intraoperative bleeding, enteric fistula formation, surgical site infection, and prolonged hospitalization [3].

Postoperative peritoneal adhesion formation is very common and may occur following any intraperitoneal procedure. Peritoneal adhesions are irritation of the peritoneum secondary to peritoneal infection or abdomino-pelvic surgery. Their prevalence after major abdominal surgery has been reported to be between 63-97% [4].

Peritoneal healing is different from that of the skin. In skin healing, epithelial cells proliferate from the periphery toward the center of the skin wound to induce re-epithelization. Whereas, the peritoneum is mesothelialized simultaneously regardless of the size of the injury with new mesothelium being developed from islands of mesothelial cells. Reepithelization of large skin injuries takes longer than small skin injuries. The parietal peritoneum completes its re-mesothelialization within five to six days, and the visceral peritoneum within five to eight days [5].

The formation of PPA is a complicated process that includes some biochemical events such as fibrinolysis, inflammation, angiogenesis and healing [6]. Damage or trauma in the peritoneal cavity results in ischemic regions within minutes and coagulation pathways and immune system become activated. This, in turn, causes the migration of repair cells including inflammatory cells such as neutrophils and macrophages and fibroblasts to the injury areas and a fibrin mesh is created through coagulated blood [7]. Studies have shown that fibroblasts separated from adhesions are considerably different from the normal peritoneum in terms of characteristics of proteins that are involved in the cell functioning, including migration, proliferation, transportation and adhesion [8]. Thus, inflammation, fibroblast and fibrin formation activities play critical roles in the mechanisms underlying the formation of adhesion [9].

There is no established treatment method to prevent the formation of PPAs. To date, streptokinase, recombinant tissue plasminogen activators, antioxidants, mechanical barrier agents (sodium hyaluronate + carboxymethylcellulose, sodium hyaluronate + phosphate- buffered saline, collagen foil + polypropylene mesh, agar films) have been studied [10]. Sodium citrate has been shown to reduce adhesion formation by inhibiting fibrin deposition [11]. Calcium channel blockers have been used in animal models based on the idea that calcium is an important component in the inflammatory response to injury, and it has been reported to have an adhesion-reducing effect [12].

The objective of this study was to examine the effects of verapamil, a calcium channel blocker, and sodium citrate, when used alone or in combination, on adhesion formation.

## Material and Methods

Before the beginning, the study protocol was approved by the Sakarya University Animal Experiments Local Ethics Committee (decision dated 04/07/2018, number 21). The study was conducted in accordance with the relevant ethical principles of the Declaration of Helsinki.

### *Selection of Experimental Animals*

Thirty female Wistar-albino rats weighing 225 g on average were used in the study. The rats were obtained from Sakarya University Faculty of Medicine Experimental Medicine Applications and Research Center. The rats were followed up in metabolic cages under standardized laboratory conditions (day/night: 12/12 hours, temperature 21±2 oC, humidity 50%), and fed with pellet food and water. The surgeries were performed in the intervention room of the research center under aseptic conditions. Antibiotic prophylaxis was not administered.

### *Anesthesia and Surgical Procedure*

The anterior abdomen of the rats was shaved before the surgical incision. Xylazine (Rompun®, Bayer 5 mg/kg) and ketamine (Ketalar®, Parke Davis and Co. Inc., 50mg/kg) were intraperitoneally used as anesthetic agents. The abdominal skin was shaved and cleaned with 10% povidone-iodine. The rats were placed on their backs and fixed to the table using the anterior and posterior extremities with a plaster tape without creating tension. Perforated sterile drapes were covered in a way to target the cleaned, shaved midline skin area, and a 5 cm laparotomy incision was made. Anesthesia was maintained by intermittent intraperitoneal injection of Ketamine.

### *Subject Animal Groups*

The rats were divided into five groups as follows, with six rats in each group:

Sham group (n=6): Laparotomy alone.

Group A: Control group (abrasion) (n=6): Laparotomy + cecal abrasion

Group B: Verapamil alone group (n=6): Laparotomy + cecal abrasion + verapamil

Group C: Sodium citrate alone group (n=6): Laparotomy + cecal abrasion + sodium citrate

Group D: Dual therapy group (n=6): Laparotomy + cecal abrasion +verapamil + sodium citrate

After laparotomy and cecal abrasion, 10 ml of verapamil (diltiazem hydrochloride) was applied intraperitoneally in 10 ml/kg saline and sodium citrate at a dose of 1 ml/kg to cover the intra-abdominal cavity, while in dual therapy verapamil + sodium citrate was administered once in the same doses and waited for 10 minutes, then aspirated and the abdomen was closed. The rats, which were kept under observation until the effect of the anesthesia wore off, were then placed back in their metabolic cages.

### *Sample Collection*

Intra-abdominal tissue samples were taken from the rats by re-laparotomy on the 7th postoperative day. These tissues were selected from the area where adhesion formation was observed, and if adhesion did not occur, they were selected from the anterior surface of the cecum and adjacent peritoneal tissue.

### *Histopathological Analysis*

Each of the resection materials was fixed in formalin. Sections

were then taken from the adhesion areas associated with the peritoneum and embedded in paraffin blocks. Sections of 5 microns were stained with Hematoxylin and Eosin. The preparations were evaluated by a pathologist under a light microscope. The presence of adhesion was evaluated primarily in the macroscopic examination of the resected materials. In the histomorphological examination, the degree of fibrosis and the intensity of inflammation were evaluated. While the fibrosis evaluation was based on the density of collagen fibers and fibroblasts, the inflammation evaluation was based on the density of inflammatory cells (macrophage, neutrophil, lymphocyte, plasma cell, eosinophil). Classification of fibrosis and inflammation is given in Table 1.

*Statistical analysis*

Data obtained in this study were statistically analyzed using the SPSS version 25.0 (SPSS, Statistical Package for Social Sciences, IBM Inc., Armonk, USA). Descriptive statistics were expressed as medians and interquartile range (25-75) for continuous variables, while categorical variables were presented as number and percentage (%). The presence of a statistically significant difference in adhesion development was evaluated using the Likelihood Ratio test, and then the situations causing the difference were determined by using Fisher's Exact Probability test. The significance of the difference between the groups in terms of inflammation intensity, fibrosis, and total histopathology scores was evaluated with the Kruskal-Wallis test. The groups causing the difference were determined by using the Dunn-Bonferroni test if the Kruskal-Wallis test results were found to be significant. P- values <0.05 were considered statistically significant.

*Ethical Approval*

Ethics Committee approval for the study was obtained.

## Results

No macroscopic adhesion was observed in the rats in the sham group who underwent laparotomy. Macroscopic adhesion formation was observed from the antimesenteric side of the cecum to the peritoneal surface in all rats in the control group (Group A), which underwent cecal abrasion with laparotomy. Macroscopic adhesion was observed in only one rat in verapamil given group (Group B), where verapamil alone was administered intraperitoneally after laparotomy and cecal abrasion. Macroscopic adhesion was observed in two rats in sodium citrate given group (Group C), where sodium citrate alone was administered intraperitoneally after laparotomy and cecal abrasion. Macroscopic adhesion was observed in only one rat in verapamil+ sodium citrate given group (Group D), where both verapamil and sodium citrate were administered intraperitoneally after laparotomy and cecal abrasion. Histopathological evaluation of the rats with adhesion is given in Table 2 according to the groups.

There was a statistically significant difference in terms of adhesion between the groups (p=0.003), and the rate of adhesion was lower in Groups B and D (p=0.015 and p=0.015, respectively). Although the rate of adhesion was lower in Group C compared with Group A, the difference did not reach statistical significance (p=0.061). There was no statistically significant difference between Groups B and D and between Groups C and D (p>0.999) (Figure 1).

There was a statistically significant change in the inflammation intensity scores according to the study groups (p=0.002). Groups B, C and D had lower inflammation intensity scores (p=0.006, p=0.025 and p=0.011, respectively). There was no statistically significant difference between Groups B and C, Groups B and D, and Groups C and D in terms of inflammation intensity scores (p>0.999) (Figure 2).

There is a statistically significant change in the fibrosis scores according to the study groups (p=0.006). Groups B and D had lower inflammation intensity scores (p=0.010 and p=0.019, respectively). Although fibrosis score was lower in the Group C compared to Group A, the difference did not reach statistical significance (p=0.100). There was no statistically significant difference between Groups B and C, Group B and D, and Groups C and D in terms of inflammation intensity scores (p>0.999) (Figure 3).

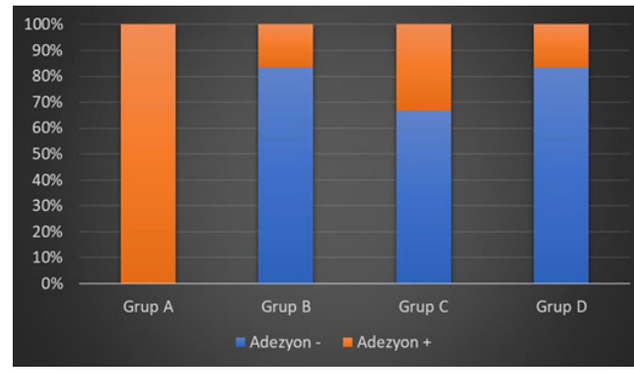


**Figure 1.** Frequency distribution of adhesions observed in the groups.

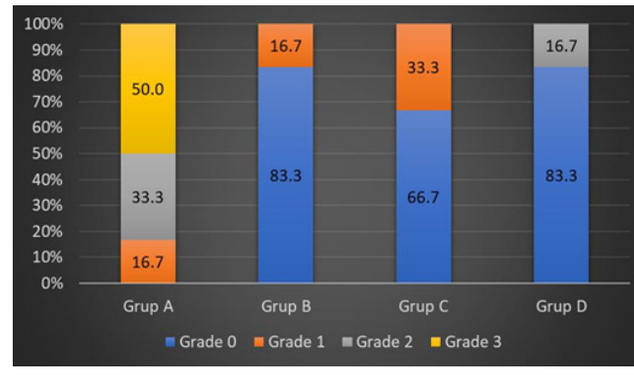


**Figure 2.** Distribution of inflammation intensity scores according to the groups.

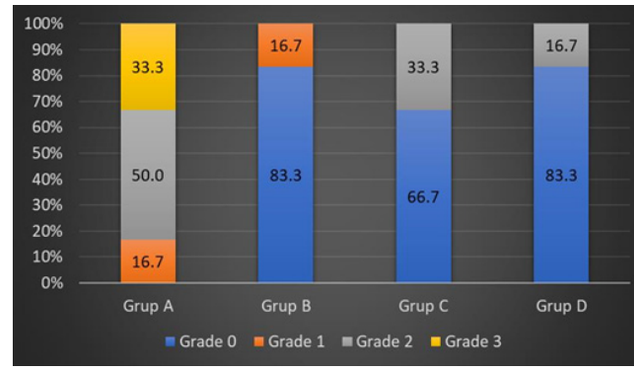


**Figure 3.** Distribution of fibrosis scores according to the groups.

**Table 1.** Histopathological classification of fibrosis and inflammation according to classification of Zografos et al. [13]

| Fibrosis Classification | | Inflammation Classification | |
|---|---|---|---|
| Grade 0 | No fibrosis | Grade 0 | No inflammation |
| Grade 1 | Mild fibrosis | Grade 1 | Mild inflammation |
| Grade 2 | Moderate fibrosis | Grade 2 | Moderate inflammation |
| Grade 3 | Severe fibrosis | Grade 3 | Severe inflammation |

**Table 2.** Histopathological evaluation of rats with adhesion.

| | Inflammation intensity | Fibrosis | Total Scor |
|---|---|---|---|
| Group A (Rat 1) | 1 | 1 | 2 |
| Group A (Rat 2) | 3 | 3 | 6 |
| Group A (Rat 3) | 2 | 2 | 4 |
| Group A (Rat 4) | 2 | 2 | 4 |
| Group A (Rat 5) | 3 | 2 | 5 |
| Group A (Rat 6) | 3 | 3 | 6 |
| Group B (Rat 2) | 1 | 1 | 2 |
| Group C (Rat 1) | 1 | 2 | 3 |
| Group C (Rat 6) | 1 | 2 | 3 |
| Group D (Rat 4) | 2 | 2 | 4 |

## Discussion

Although intra-abdominal adhesions are usually asymptomatic, they can cause intestinal obstruction, fistula development, chronic abdominal pain, dyspareunia, infertility, ureteral obstruction, postoperative bleeding, and serious complications during reoperation [14]. Postoperative adhesions are thought to cause infertility in 15-20% of women [15]. The most common cause of intra-abdominal adhesions is previous abdominal surgery. Adhesions and related complications after surgery constitute a significant increase in workload and economic burden. Surgery due to intra-abdominal adhesions is usually difficult and complication rates are high. Intestinal obstruction is an important clinical outcome of adhesions, resulting in significant morbidity and mortality and high costs [16]. Colorectal operations are the most important procedures related to intraabdominal adhesion, accounting for 35.3% to 46.8% of the total clinical workload or costs attributable to postoperative adhesion-related obstruction [17]. In a study by Van der Krabben et al., intestinal injury occurred in 51 patients during the separation of adhesions in 270 patients who underwent reoperation [16].

Various measures can be taken to minimize adhesion formation. These measures include using a careful and appropriate surgical technique, adequate hemostasis, use of the omentum as a protective cover, avoiding excessive manipulation of tissues, not leaving ischemic tissue in the abdomen, short cutting of suture materials such as linen and silk that may form granulomas, keeping substances such as talc and starch away from the abdomen, repairing peritoneal defects without tension if possible, leaving the defect open if tension occurs, not using excessive suture materials, taking into account that bacteria are also among the etiological factors, fighting the infection, and preventing the intestines from drying out and losing water during surgery [4, 18].

In order to prevent PAAs, liquid and solid barriers including hyaluronate carboxymethyl cellulose, oxidized regenerated cellulose, icodextrin 4% solution, and polyethylene glycol have been studied [19]. Mechanical barriers can prevent the formation of postoperative adhesions by keeping the peritoneal surfaces isolated for 5-7 days necessary for the formation of re-mesothelialisation. They can prevent contact between damaged areas for the first several days [4]. Chemical agents are usually used to prevent the organization of the persisting fibrin, by inhibition of fibroblastic proliferation. Many chemical agents are used for this purpose such as non-steroidal anti-inflammatory drugs (NSAIDs), calcium channel blockers, antioxidants, corticosteroids, histamine antagonists, antibiotics, fibrinolytic agents, anticoagulants, hormones, vitamins, selective immunosuppressors and colchicines [4]. Jafari Sabet et al. reported that intraperitoneal injection of streptokinase for 10 days reduced the rate of PPA formation [20]. Hosseini et al. showed that adding streptokinase (100,000 IU/kg equivalent to 20,000 IU) to the abdominal cavity after flushing with normal saline could significantly prevent PPA formation on the 7th day and postoperative first 1 month [21]. It has been shown that verapamil injected intraperitoneally at a dose of 0.1 mg/kg has a significant pharmacological effect against excessive connective tissue production in the hemoperitoneum [22]. In another rat model, Deng et al. showed that verapamil reduced the formation of PPAs [23].

In the present study, we investigated the effects of using sodium citrate, which has calcium-binding properties, and verapamil, which is a calcium channel blocker alone or in combination on the formation of PPAs. It was observed that macroscopic adhesion did not occur with re-laparotomy on the postoperative 7th day of the rats who underwent only laparotomy. Peritoneal tissue samples taken from this group were examined under a light microscope, and signs of inflammation and fibrotic reaction were not observed. Upon this, we determined our control group as the group in which we performed cecal abrasion following laparotomy.

We preferred the method of creating abrasion with a scalpel on the cecum wall and the peritoneal face adjacent to it. We tried to provide standardization with the principle of creating abrasion with the same surface area in rats by a single surgeon [24]. We observed that macroscopic adhesion developed in all groups of rats with re-laparotomy on the 7th postoperative day. In the examination of this group with H&E staining under a light microscope, the subjects with the lowest 2 and the highest 6 total inflammation intensity and fibrosis scores were detected. We administered verapamil alone to one group, sodium citrate alone to one group, and one group received both intraperitoneally, in which we performed laparotomy and cecal abrasion. While macroscopic adhesion was observed in only one subject in the groups in which we used verapamil and both agents together, macroscopic adhesion was observed in two subjects in the group in which we used sodium citrate.

In histopathological examinations consisting of the inflammation intensity, fibrosis intensity and scores obtained from these evaluation criteria, it was concluded that the

separate use of verapamil and sodium citrate did not provide a statistically significant superiority to each other. When the data of the group using verapamil and sodium citrate together were compared with the data of the group using verapamil and sodium citrate separately, no statistically significant difference was observed. In paired comparisons, inflammation intensity and histopathological results were superior to the control group in all groups.

*Conclusion*

In conclusion, the combined use of verapamil and sodium citrate did not increase the effectiveness of the prevention of intra-abdominal adhesions compared to using them separately. Although it was not statistically significant, the fibrotic and inflammatory changes observed in one rat in the verapamil alone group and in two rats in the sodium citrate alone group suggest that verapamil may be superior to sodium citrate in preventing adhesions. The result obtained in the group in which the two agents were used together suggests that the effect of verapamil was dominant. However, further studies are needed to reach firm conclusions.

***Scientific Responsibility Statement***

*The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.*

***Animal and human rights statement***

*All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.*

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***Conflict of interest***

*None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.*

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