Comparison of the hemostatic effects of oxidized cellulose and calcium alginate in an experimental animal model of hepatic parenchymal bleeding

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ABSTRACT

Background: Despite all recent developments, bleeding is still one of the main causes of increasing morbidity and mortality following both trauma and elective hepatic surgery. The main goal of treatment is stop the bleeding immediately. In this study, the hemostatic and histopathological effects of Ankaferd blood stopper (ABS), oxidized cellulose (OC), and calcium alginate (CA) were compared in an experimental liver injury.

Materials and Methods: Forty Wistar albino rats were randomly divided into four groups of ten animals each, receiving 0.9% NaCl, CA, OC, or ABS following liver resection. After 5 days, the samples from the resection site were acquired for histopathological evaluation. The efficacy of the agents was assessed using the hematocrit level and histopathological examination. Statistical analyses were applied.

Results: The amount of bleeding was lowest in ABS-treated rats, followed by those treated with OC, CA, and NaCl, respectively. The difference among the groups was statistically significant ($P < 0.001$). ABS-treated rats also had significantly less necrosis than those receiving OC; other differences in this regard were not significant. Inflammatory status was significantly different between OC- and CA-treated rats ($P < 0.05$) but not among the other groups ($P > 0.05$). No significant difference was determined between the groups regarding granulation ($P > 0.05$).

Conclusion: ABS reduced the volume of bleeding in liver surgery and partial liver resection. The hemostatic effect of CA was limited.

Key Words: Ankaferd blood stopper, calcium alginate, hemostatic, liver, oxidized cellulose

INTRODUCTION

Bleeding of the liver parenchyma, seen following both trauma and elective hepatic surgery, is a serious and difficult-to-manage problem that can be life-threatening. The most important stage in this event, which requires emergency intervention, is the control of bleeding.\(^1\) Halting bleeding by suturing or other classic methods in the liver is almost impossible because of its fragile structure and extreme vascularization.\(^2,3\)

In recent years, many methods to control hepatic hemorrhage have been described and evaluated as successful. These techniques include diathermy, argon beamer coagulation, the Pringle maneuver, total vascular exclusion, and the use of topical agents such as oxidized regenerated cellulose, absorbable gelatin sponge, microfibrillar collagen, and fibrin.
sealant. Topical agents, in particular, are in widespread current use and are highly useful when mechanical and cautery methods cannot be applied. Their hemostatic mechanisms vary; some improve fibrin formation or inhibit fibrinolysis, while other hemostatics are a preparation of a procoagulant agent combined with a transporter molecule such as collagen matrix. In the last 10 years, several studies have reported on the use of Ankaferd blood stopper (ABS) in surgery, which is effective in activating the protein agglutination cascade for hemostasis, but there have been few studies conducted on the control of hepatic bleeding.

In the present study, we compared the efficacy of calcium alginate (CA) and oxidized cellulose (OC), agents that have been previously used in hepatic hemorrhage control, with that of ABS in an experimental model of hepatic parenchyma bleeding and to characterize any subsequent histopathological changes. Our goal was to understand whether ABS and calcium alginate can be used to control morbid or life-threatening hemorrhages that develop during liver surgery or on traumatic liver injury and to observe histopathological changes in hepatic tissue.

**MATERIALS AND METHODS**

This study was conducted at the Experimental Animal Breeding and Research Laboratory of Erciyes University Medical Faculty. Approval for the study was granted by the Animal Experiments Ethics Committee.

**Subjects**

A total of forty outbred Wistar albino male rats aged 6 months and weighing 380 ± 20 g were used in the study.

**Methods**

The rats were randomly separated into four equal groups of ten animals, each of which was subjected to nonanatomic standard liver resection. The topical hemostatic agents used in each group were 0.9% NaCl solution (0.9% Sodyum Klorür®; Eczacıbaşı, Istanbul, Turkey) (as a negative control), CA covering (Sorbalgon®; Hartmann, Heidenheim, Germany), OC (Gelitacel®; Gelita Medical, Amsterdam, the Netherlands), and ABS (ABS®; Trend-Tech Istanbul, Turkey), respectively. In all of the groups, a 2 cm × 2 cm compress soaked in or containing the topical agent was applied to the area of bleeding for a mean time of 3 min at moderate pressure [Figure 1].

**Surgical technique**

Preoperative hematocrit (Htc) values were measured by collecting blood from the tail vein of each rat using a cannula to fill two standard Htc tubes, which were centrifuged at 1500–2000 rpm in an Htc measurement unit.

After overnight fasting, intramuscular anesthesia was applied, consisting of ketamine hydrochloride (Ketalar®; Pfizer, Istanbul, Turkey) at 100 mg/kg and xylazine (Rompun®; Bayer Istanbul, Turkey) at 10 mg/kg. In addition, analgesia was induced with 0.05 mg/kg intraperitoneal morphine sulfate and maintained with a 75 mg/kg with subcutaneous Ketalar injection. The abdominal midline where the incision was to be made was shaved and the antiseptic 10% povidone iodine. A lamp was used to maintain body heat at 37°C. Before liver resection, a plastic bag was folded into a funnel shape below the liver, and the entire surgical procedure was conducted on a platform at an inclination of 30°. Thus, all of the bleeding throughout the procedure was collected in the bag, and the amount was recorded as perioperative bleeding. The amounts of blood absorbed by the sponges were minimal and were disregarded. This may be a limitation of the study.

The rat liver consists of four lobes: Right and left lateral and medial. After retraction of the right medial and left lateral lobes upward, the left medial lobe was revealed. Without changing the anatomic position of the lobe, it was resected along the longest transverse line [Figures 1 and 2]. All surgical procedures employed sterile knives.

Following 3 min of compression and the expected periods of bleeding, the abdominal midline incision was closed as two layers with 3/0 polypropylene thread, without making any other intervention to the bleeding parenchymal surface. Postoperative Htc was measured using blood collected from the tail vein. Using the same anesthesia technique, 5 days after the surgical procedure,
the left hepatic lobe was removed to obtain samples for histopathological evaluation. No rat died during this period. After the lobectomy, all rats were euthanized with high-dose (300 mg/kg) ketalar.

**Histopathological analysis**
Sections of liver tissue 5 µ in thickness were fixed in formalin, embedded in paraffin, and subjected to hematoxylin and eosin staining. Random tissue samples were evaluated by a single pathologist blinded to the groups. Samples were scored from 1 to 3 according to the inflammation status and formation of granulation tissue as follows: 1, mild; 2, moderate; 3, high. A similar scoring system was used for cell necrosis as follows: <25% of the field at 1/40 magnification was scored as 1; 25%–50% of the field as 2; and >50% of the field as 3.

**Statistical analysis**
The construction of graphics, statistical calculations and analyses were made using MS-Excel, SPSS for Windows v15.00 (SPSS Inc., Chicago, IL, USA) and SigmaStat 3.5 (StatSoft Inc., Tulsa, OK, USA) software. Descriptive statistics were stated as the number (n), percentage (%), and mean ± standard deviation. Statistically significant differences are indicated with an asterisk (*). Conformity of the data to a normal distribution was examined using the Shapiro–Wilk test. To compare more than two groups, variance analysis (ANOVA) or nonparametric Kruskal–Wallis variance analysis was used. To determine the origin of differences, the Student–Newman test was applied to compare differences between amounts of bleeding and preoperative and postoperative Htc values. Dunn’s method was used to assess inflammation intensity, formation of granulation tissue, and cell necrosis. A value of $P < 0.05$ was accepted as statistically significant.

### RESULTS

**Amounts of perioperative bleeding**
The volume of perioperative bleeding was 1.80 ± 0.45 mL in saline-treated, 0.75 ± 0.51 mL in CA-treated, 0.40 ± 0.35 mL in OC-treated, and 0.10 ± 0.04 mL in ABS-treated rats [Table 1]. Bleeding was lowest in rats treated with ABS; the difference from other groups was statistically significant ($P < 0.05$).

**Hematocrit values and differences**
The difference in preoperative and postoperative Htc was 8.00 ± 3.29 in saline-treated, 5.00 ± 5.27 in CA-treated, 4.00 ± 3.06 in OC-treated, and 3.00 ± 2.31 in ABS-treated rats [Table 2]. The decrease in Htc differed significantly between saline- and ABS-treated rats ($P < 0.05$), but differences were not significant between the other groups ($P > 0.05$).

**Histopathological results**
Effects of the various hemostatic agents on the formation of tissue necrosis, inflammation, and formation of granulation tissue were examined by scoring histopathological sections. In an area of 1/40 magnification, <25% necrosis was seen in 10% of saline-treated rats, 20% of CA-treated, 50% of OC-treated, and 0% of ABS-treated rats. In an area of 1/40 magnification, 25%–50% necrosis was seen in 50% of saline-treated rats, 50% of CA-treated, 50% of OC-treated, and 80% of ABS-treated rats. In an area of 1/40 magnification, more than 50% necrosis was seen in 40% of saline-treated rats, 30% of CA-treated, 0% of OC-treated, and 20% of ABS-treated rats. A statistically significant difference was determined between saline- and OC-treated rats ($P < 0.05$) but not between the other groups ($P > 0.05$) [Table 3].

Mild inflammation was determined in 50% of saline-treated, 20% of CA-treated, 80% of OC-treated, and 60% of ABS-treated rats. Moderate inflammation was determined in 20% of saline-treated, 60% of CA-treated, 20% of OC-treated, and 40% of ABS-treated rats. Severe inflammation was not observed in OC- or ABS-treated rats and was observed in 30% of saline-treated and

![](image)

**Table 1: Perioperative bleeding during liver resection**

| Hemostatic treatment | Number of rats (n) | Mean perioperative bleeding volume mean ± SD (mL) |
|----------------------|-------------------|-----------------------------------------------|
| Saline *             | 10                | 1.80 ± 0.45                                   |
| Calcium alginate *   | 10                | 0.75 ± 0.51                                   |
| Oxidized cellulose * | 10                | 0.40 ± 0.35                                   |
| Ankaferd blood stopper * | 10               | 0.10 ± 0.04                                   |

*P < 0.001. SD: Standard deviation

**Table 2: Change in hematocrit following liver resection**

| Hemostatic treatment | Number of rats (n) | Htc Preoperative ± SD | Htc Postoperative ± SD | Htc difference ± SD |
|----------------------|--------------------|-----------------------|------------------------|---------------------|
| Saline *             | 10                 | 55.00 ± 2.06          | 48.00 ± 3.17           | 8.00 ± 3.29         |
| Calcium alginate     | 10                 | 55.00 ± 2.89          | 50.00 ± 2.01           | 5.00 ± 5.27         |
| Oxidized cellulose * | 10                 | 55.50 ± 2.67          | 49.00 ± 2.49           | 4.00 ± 3.06         |
| Ankaferd blood stopper * | 10               | 54.00 ± 3.37          | 52.00 ± 2.87           | 3.00 ± 2.31         |

All values mean ± SD. *P = 0.024. SD: Standard deviation, Htc: Hematocrit
20% of AC-treated. The difference in inflammation between OC- and CA-treated rats was statistically significant \((P < 0.05)\), but differences between the other groups were not \((P > 0.05)\) [Table 3].

Mild granulation was observed in 30% of saline-treated, in 20% of CA-treated, in 30% of OC-treated, and in 10% of ABS-treated rats. Moderate granulation was determined in 60% of saline-treated, in 60% of CA-treated, in 70% of OC-treated, and in 90% of ABS-treated rats. Severe granulation was determined in 0% of saline-treated, in 20% of CA-treated, in 0% of OC-treated, and in 0% of ABS-treated rats. No statistically significant differences were found between the groups \((P > 0.05)\) [Table 3].

### DISCUSSION

In 1897, Elliot stated that the liver was a dispersed structure full of blood vessels that could not hold sutures; therefore, it was not possible to successfully repair a large injury.\(^{[2,3]}\) This view encompasses the anatomic structure of the liver because there are no smooth muscle cells in the hepatic parenchyma and very little collagen tissue. These features are important in bleeding for two reasons. First, smooth muscle does not contract, preventing vasoconstriction; second, the resistance formed by parenchymal sutures with collagen fibers is not of sufficient benefit.\(^{[6]}\)

There are two basic approaches for the treatment of bleeding in all parenchymal organs, including the liver: Reducing the amount of blood reaching the parenchyma and preventing bleeding from damaged vessels.\(^{[7]}\) The latter category includes topical hemostatic agents. Of these agents, several studies have been conducted related to CA and OC. The studies related to ABS are much fewer in number, and to the best of our knowledge, the current study is the first to compare it to CA and OC.

These agents act by distinct mechanisms. CA becomes a moist gel, transferring calcium, and sodium ions from

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**Table 3: Necrosis, severity of inflammation, and formation of granulation tissue**

|                         | Saline (%) | Calcium alginate (%) | Oxidized cellulose (%) | Ankaferd blood stopper (%) |
|-------------------------|------------|----------------------|------------------------|--------------------------|
| **Necrosis, n (%)**     |            |                      |                        |                          |
| Score 1                 | 5 (50)     | 1 (10)               | 2 (20)                 | 0                        |
| Score 2                 | 5 (50)     | 5 (50)               | 5 (50)                 | 8 (80)                   |
| Score 3                 | 0          | 4 (40)               | 3 (30)                 | 2 (20)                   |
| **Severity of inflammation, n (%)** |            |                      |                        |                          |
| Mild                    | 5 (50)     | 2 (20)               | 8 (80)                 | 6 (60)                   |
| Moderate                | 2 (20)     | 6 (60)               | 2 (20)                 | 4 (40)                   |
| Severe                  | 3 (30)     | 2 (20)               | 0                      | 0                        |
| **Formation of granulation tissue (P>0.05), n (%)** |            |                      |                        |                          |
| Mild                    | 3 (30)     | 2 (20)               | 3 (30)                 | 1 (10)                   |
| Moderate                | 6 (60)     | 6 (60)               | 7 (70)                 | 9 (90)                   |
| Severe                  | 1 (10)     | 2 (20)               | 0                      | 0                        |

\(^{[P=0.031, ~P=0.048]}\)

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**Figure 3:** (a) saline \((×40)\), (b) calcium alginate \((×40)\), (c) oxidized cellulose \((×100)\), (d) Ankaferd blood stopper \((×100)\). H and E stained histopathological sections showing areas of necrosis (yellow arrows), inflammation (red arrows), and granulation (green arrows)
the alginate to the tissue. The hydrophobic effects and the five plant extracts in ABS trigger a cascade caused by protein agglutination; blood components are also recruited to this network.

In a study by Henderson et al., calcium alginate (CA) was not found to have a benefit over standard sterile gauze for controlling bleeding following tooth extraction in children. Both Ingram et al. and Henderson et al. concluded that CA had no effect on bleeding in clinical studies. In the current study, the amount of bleeding in the CA group was lower than that of the control group, a result that could be explained by the early termination of bleeding because of high Htc levels in the rats. The effects of triggering clotting factors by administering calcium ion in the CA environment are considered to be limited in individuals with no blood disorders.

In the current study, CA was found to be the least effective agent with respect to both perioperative bleeding and change in Htc. However, it was more effective than saline.

In a study by Davidson et al., a hepatic resection model was created in pigs using OC with fibrin filling (Vivosat® Istanbul, Turkey). In comparison with the control group, OC significantly reduced bleeding. Karakaya et al. used a hepatic laceration model to compare the hemostatic effects of ABS and OC and concluded that ABS was as effective as OC in providing hemostasis. In another rat hepatic resection study by Aysan et al., ABS significantly reduced bleeding compared to 9% NaCl solution. Kurt et al. used endoscopy and biopsy to evaluate a 52-year-old male with an initial diagnosis of distal cholangiocarcinoma. Bleeding ceased after the application of 15 mL ABS to the biopsy site. Tuncer et al. used endoscopic ABS to stop variceal bleeding (in 18 s) in the esophageal fundus of a patient with cirrhosis.

In the current study, ABS was determined to be more effective than CA and OC in halting bleeding. CA and OC were effective compared to saline but were not as effective as ABS either in terms of the amount of perioperative bleeding or change in Htc. Evaluation of the histopathological changes associated with use of these agents in the hepatic tissue formed the second leg of this study. Although the necrosis, granulation formation and inflammation scores of the ABS group were higher than those of the CA and OC groups, there was no statistical significance. These high scores in the ABS group could be associated with the hemostatic effect.

However, it is possible that the five different plant extracts found in the composition of ABS could lead to a greater inflammatory response in the hepatic parenchyma. In the histopathological examination, the relatively lower fibrosis, severity of inflammation, and adhesions in ABS-treated than in OC-treated rats were seen to be caused by the foreign body reaction. Similarly, the difficulty of dissection revealed less macroscopic adhesion in ABS-treated than in OC-treated rats. A normal histological appearance was determined in the remaining parenchyma of the surface in contact with ABS [Figure 3a-d]. Even if it is not possible to make a definitive judgment because liver function tests were not evaluated, the imaging findings suggest that no structural damage occurred in adjacent tissues.

CONCLUSION

This study demonstrates that ABS is more effective than OC, CA, and saline in achieving hemostasis and reducing blood loss in the rat hepatic resection model of liver injury. It also caused fewer intraperitoneal adhesions and was associated with the features of better regeneration in this model.

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Conflicts of interest
There are no conflicts of interest.

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