Assessment of the Efficacy of Ankaferd Blood Stopper on the Prevention of Postoperative Pericardial Adhesions

Yunus Nazli*, Necmettin Colak1, Mehmet Fatih Alpay1, Hacer Haltas2, Omer Nuri Aksoy1, Ismail Olgun Akkaya1 and Omer Cakir1

1Department of Cardiovascular Surgery, University of Turgut Ozal, Faculty of Medicine, Ankara, Turkey
2Department of Pathology, University of Turgut Ozal, Faculty of Medicine, Ankara, Turkey

Abstract

Objectives: Ankaferd has been used as a blood stopper agent and also it might have anti-inflammatory effects. We investigated the efficacy of the Ankaferd for preventing postoperative pericardial adhesions in an experimental rabbit model.

Materials and methods: Sixteen New Zealand white rabbits were used and categorized into 2 groups as Ankaferd and control groups. Ankaferd group was treated with the sponge which impregnated with Ankaferd solution and applied over the abraded epicardium. The sponge which was impregnated with 0.9% isotonic NaCl solution was applied to the control group by using the same protocol. The scores of adhesion and visibility of coronary vessels were graded by macroscopic examination, and the pericardial tissues were analyzed microscopically in terms of inflammation and fibrosis.

Results: In Ankaferd group; the adhesion scores were significantly higher than the control group (p=0.007). When groups were compared according to the prevalence of fibrosis and degrees of inflammation, the Ankaferd group was found to be statistically significantly different than control group in terms of prevalence of fibrosis (p=0.028).

Conclusion: Topical application of Ankaferd for preventing postoperative pericardial adhesions increased the adhesion and fibrosis scores.

Keywords: Cardiac operation; Ankaferd; Pericardial adhesion

Introduction

Postoperative pericardial adhesion formation occurs very frequently after cardiac surgery and it is an important cause of morbidity and mortality at the time of reoperation. As the number of patients undergoing cardiac operation continues to increase, the number of potential candidates for reoperation has been increasing exponentially. Despite continuous improvements during reoperation in cardiac surgery, the presence of pericardial adhesions not only adds to the operating time but also increases the risk of life threatening injuries to the heart, great vessels, or previously placed coronary bypass conduits by obscuring the true anatomy [1]. Various methods and materials have been investigated to prevent or reduce the severity of the postoperative adhesions in the retrosternal spaces and mediastinal structures. Fibrinolytic agents, histamine antagonists, anti-coagulants, anti-inflammatory drugs (corticosteroids, nonsteroidal), antibiotics and several natural (heterologous pericardium, omentum, peritoneum, amnion, fibrin, gelatin, collagen and hyaluronic acid) and synthetic physical barriers (rubbers, silicon-based materials, cellulose, polytetrafluoroethylene, polyvinyl alcohol and polyester derivatives) have been tried with variable success for such purpose [2-6]. Unfortunately, despite continuous advances and researches, there is still no ideal method to prevent or reduce postoperative pericardial adhesion formation to date.

Ankaferd Blood Stopper® (ABS) (Ankaferd Ilac Kozmetik A.S. Istanbul, Turkey) is a folkloric medicinal plant extract. Ankaferd has been used as a blood stopper agent against various bleedings. It has been approved in the management of bleedings due to external injury and dental surgery by the Ministry of Health in Turkey. It has been used as the topical agent for the prevention of postoperative intra-abdominal fibrosis in the experimental studies, and different results have been obtained [7,8]. To the best of our knowledge, there is no report on the effect of Ankaferd in preventing postoperative pericardial adhesions to date.

The present experimental study was designed to investigate the effect of intrapericardially administered Ankaferd on reducing postoperative pericardial adhesion formation in the rabbit model.

Materials and Methods

The study protocol was approved by Ankara Education and Research Hospital, Ethics Committee for Animal Researches, Ankara, Turkey. Sixteen New Zealand white rabbits weighing 2.5 to 3.0 kg were anesthetized with 35 mg/kg ketamine hydrochloride and 5 mg/kg xylazine administered intramuscularly [9,10]. After disappearance of the pedal reflex in the hindlimbs, the rabbits were placed in the supine position on a heated operating table and their temperature maintained at 39°C by monitoring their rectal temperature [11,12]. The pedal reflex was checked every 5 minutes throughout the surgical procedure. A venous line was established in the ear and a saline solution was infused at a rate of 3 mg/kg/h. Propylactic antibiotic (cefazolin sodium 40 mg/kg) was given intravenously just before the operation. All had continuous two-lead electrocardiograph monitoring during the operative procedure.

*Corresponding author: Yunus Nazli, Alparslan Turkes caddesi No: 57, 06510, Emek/Ankara, Turkey, Tel: +903122035186; Fax: +903122213670; E-mail: yununazli@gmail.com

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pediatric facial mask in which oxygen gas flowed at a rate of 200 ml/min was placed on each rabbit [13].

The surgical procedure was performed in a sterilized fashion. After a midline muscle and skin incision was made over the sternum, the xiphoid process was carefully detached from the sternal part of the diaphragm. A median sternotomy was then performed; the median incision went straight down from the xiphoid process towards the jugular notch of the sternum exactly along the midline of the sternum so that injury to the parietal pleura was avoided. Sternal retractors were used to spread the sternal edges and maintain surgical exposure. The epicardium and pericardial pericardium related to right ventricle atrium, right and left ventricle were abraded with ten vertically reciprocal movement of dry gauze in order to create local inflammation [13].

The rabbits were divided into 2 groups: The Ankaferd group was treated with the sponge which had soaked with 2 ml concentrations of ABS solution (Ankaferd Blood Stopper® ampul, 2 ml, Istanbul, Turkey) and applied over the abraded epicardium for 5 minutes (n=8). Subsequently, this sponge was removed over the epicardium. The abrasive areas of the epicardium were irrigated immediately with enough saline to get rid of the remaining ABS after the application of sponges. In the control group; the sponge which had soaked with 0.9% isotonic NaCl solution (serum fizyolojik, 0.9% NaCl, 5 ml/ampul Adeka, Turkey) was applied onto the surface of the abraded epicardium for 5 minutes (n=8). After the 5 minutes, sponge was removed over the epicardium. The investigators were blinded during to application of the Ankaferd or saline. Suture was closed with three interrupted sutures using 3-0 nylon with a cutting needle. The rabbits were allowed to recover. During the surgical procedure, all rabbits exhibited spontaneous respiration and loss of the pedal reflex.

The rabbits were sacrificed 2 weeks after surgery with a lethal dose of pentobarbital (150 mg/kg) (Nembutol, IE Ulagay, Istanbul, Turkey). The heart and pericardium were removed en bloc. Specimens were fixed in 10% formaldehyde, embedded in paraffin and sectioned into 4 µm slices which were stained with Hematoxylin and Eosin to assess the inflammatory reaction, fibrosis degree and to check for remnants of the pericardial substitute in the 2 groups.

Macrosopic examination

The heart and pericardium were removed with the anterior chest wall from the body with heart en bloc. The severity of the pericardial adhesions and visibility of coronary vessels were evaluated by same two blinded observers and scored. The following qualitative grading system was used to evaluate the tenacity of the adhesions: (0) no adhesions; (1) mild adhesions (transparent filmy adhesions separable by lifting the pericardium from the myocardium without dissection); (2) moderate adhesions (fibrous and easily separated by blunt dissection); (3) severe adhesions (thick, requiring aggressive blunt dissection); (4) very severe adhesions (multiple thick adhesions requiring aggressive dissection that damaged adherent tissue) [14]. In addition, another grading system was also used to evaluate the visibility of the coronary arteries: 0=clearly visible, 1=blurred, 2=completely obscured [6].

Light microscopic examination

After the macroscopic scoring, the paraffin-embedded heart tissues (the segments of the pericardium and heart especially from the site of abrasion, which had been marked with prolene stitch) were cut (4 µm thick) and then stained with hematoxylin and eosin. Histopathological evaluation was performed by one pathologist who was blinded to the study groups. The severity of the inflammatory reaction was based on the quantification of inflammatory cells (i.e. neutrophils, plasma cells, lymphocytes) and inflammatory foci. The scoring schemes of Lu et al. [15] were used to grade inflammation: (0) no cell infiltration; (1) sparse, focal infiltration of lymphocytes and plasma cells; (2) focal infiltration of neutrophils, plasma cells and lymphocytes; and 3: diffuse infiltration of neutrophils, plasma cells and lymphocytes) and fibrosis: (0: no fibrous reaction; 1: sparse, focal fibrous connective tissue, hyalinization and fibrin deposition; 2: a thin layer of focal fibrous connective tissue, hyalinization and fibrin deposition; and 3: a thick layer of focal fibrous connective tissue, hyalinization and fibrin deposition).

Power and sample size

The sample size of our study was calculated with G’Power (G*Power Ver. 3.00.10, Franz Faul, Universität Kiel, Germany, http://wwwpsycho.uniduesseldorf.de/aap/projects/gpower/) statistical packages. The required sample size for 80% power, α=0.05 Type I error, β=0.20 Type II error and f=0.70 effect size was calculated as 16 including 8 New Zealand white rabbits in each group. To protect the study from potential lost to follow-ups, one more rabbit was included in each group and the study was completed with a sample size of 18.

Statistical analysis

Data coding and statistical analyses were conducted with SPSS (version 15; SPSS Inc., Chicago, IL, USA). Following the entering of rabbits’ data into the computer, all the necessary diagnostic checks and corrections were performed. Conformity of the measured values to normal distribution was examined graphically and using a Shapiro Wilks test. In presenting descriptive statistics, numbers and percentages were used for categorical variables, medians and ranges were used for non-normally distributed variables. The Mann Whitney U test was used to compare the median values of the groups. Chi-square test was performed to evaluate the difference in the proportion of rabbits in the groups. Groups that are close to each other were combined and the 2 × 2 table chi-square test was created. The Likelihood ratio test was used for the comparison of these groups. Two-tailed test of p ≤ 0.05 was considered statistically significant.

Results

All animals tolerated the procedure with no apparent postoperative complications. Results were analyzed in terms of macroscopic and microscopic findings.

Macroscopic findings

A total of sixteen rabbits were evaluated for grading of pericardial adhesions by macroscopic findings. In six cases of control group and one case of Ankaferd group, pericardial adhesions were split by blunt dissection (Figure 1A). In contrary; the seven of the Ankaferd group and the two of the control group were associated with tight adherences to the sternum and the rest of the pericardium requiring sharp dissection (Figure 1B). There was statistically significant difference between the Ankaferd group and control group in terms of the adhesion score (Ankaferd group vs. control group: 3 (2-4) vs. 2 (1-3), p=0.007) (Figure 2). There was no statistically significant difference between the Ankaferd group and control group in terms of the visibility of coronary vessels score (Ankaferd group vs. control group: 1 (1-2) vs. 1 (1-2), p=0.105) (Table 1). When the prevalence of pericardial adhesion was compared, there was a positive trend in the odds ratio for severe to very severe adhesion in the Ankaferd group (Ankaferd group vs. control
Figure 1: (A) Two weeks after the initial operation, the adhesion formation in the control group was (more easily dissected) significantly low between the epicardial and pericardial surfaces. (B) The severe pericardial adhesions, which were difficult to dissect out, were observed between the epicardium and the mediastinal tissues in the Ankaferd group.

Figure 2: Difference of the pericardial adhesion score in the two groups.

Table 1: Results of the macroscopic and microscopic scores between control group and Ankaferd group.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Ankaferd group (n: 8) Median (Range)</th>
<th>Control group (n: 8) Median (Range)</th>
<th>Z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macroscopic scores</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pericardial adhesions</td>
<td>3 (2-4)</td>
<td>2 (1-3)</td>
<td>2.680</td>
<td>0.007</td>
</tr>
<tr>
<td>Visibility of coronary vessels</td>
<td>2 (1-2)</td>
<td>1 (1-2)</td>
<td>2.000</td>
<td>0.105</td>
</tr>
<tr>
<td><strong>Microscopic scores</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammation</td>
<td>2 (2-3)</td>
<td>3 (1-3)</td>
<td>1.061</td>
<td>0.382</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>3 (3-3)</td>
<td>3 (1-3)</td>
<td>1.852</td>
<td>0.234</td>
</tr>
</tbody>
</table>

NS: not statistically significant (P>0.05).

Table 2: The distribution of the macroscopic and microscopic scores between control group and Ankaferd group.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Ankaferd group n (%)</th>
<th>Control group n (%)</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macroscopic scores</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pericardial adhesions</td>
<td>Mild - Moderate (1+2)</td>
<td>1 (12.5)</td>
<td>6 (75)</td>
<td>6.904</td>
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<td></td>
<td>Severe - Very severe (3+4)</td>
<td>7 (87.5)</td>
<td>2 (25)</td>
<td></td>
</tr>
<tr>
<td>Visibility of coronary vessels</td>
<td>Blurred (1)</td>
<td>1 (12.5)</td>
<td>5 (62.5)</td>
<td>4.557</td>
</tr>
<tr>
<td></td>
<td>Completely obscured (2)</td>
<td>7 (87.5)</td>
<td>3 (37.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Microscopic scores</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammation</td>
<td>None - Mild (0+1)</td>
<td>0 (0.0)</td>
<td>1 (12.5)</td>
<td>1.453</td>
</tr>
<tr>
<td></td>
<td>Moderate - Severe (2+3)</td>
<td>8 (100)</td>
<td>7 (87.5)</td>
<td></td>
</tr>
<tr>
<td>Fibrosis</td>
<td>Mild - Moderate (1+2)</td>
<td>0 (0.0)</td>
<td>3 (37.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Severe (3)</td>
<td>8 (100)</td>
<td>5 (62.5)</td>
<td>4.857</td>
</tr>
</tbody>
</table>

NS: not statistically significant (P>0.05).

Table 3: The distribution of the macroscopic and microscopic scores between control group and Ankaferd group.

Discussion

One of the primary long-term postoperative concerns after a sternotomy is the formation of pericardial adhesions during the healing process. These adhesions are still an important cause of

Ankaferd group and the control group in terms of the severity of fibrosis (p=0.234) (Table 1). However, the severe fibrosis was present in 100% of the Ankaferd group and 62.5% of the control group. When the groups were compared according to the prevalence of fibrosis and the degree of inflammation, the statistically significant difference between groups only in terms of the prevalence of fibrosis was found in the Ankaferd group (p=0.028) (Figures 3A and 3B). There were no statistically significant difference between Ankaferd and control group with regard to degree of inflammation (p=0.220) (Figures 4A, 4B and Table 2).

Microscopic findings

There were no statistically significant difference between the group; 87.5% vs. 25%, respectively) (Table 2). When the groups were compared according to the degree of pericardial adhesions and the visibility of coronary vessels score, there were statistically significant difference between the Ankaferd group and control group (p=0.009, p=0.033, respectively) (Table 2). In our study, there was no infection or delayed healing at the wound site. Results of the macroscopic and microscopic scores and distribution of the scores between control group and Ankaferd group are shown in Tables 1 and 2.
morbidity and mortality in cardiac surgery [16]. Pericardial adhesions can attach heart to the undersurface of the sternum and neighboring structures, compromise right ventricular contraction, and restrict left ventricular diastolic filling. They can also obscure the anatomy of the heart, coronary arteries, and great vessels, coronary grafts if present, immensely complicating the procedure at the reoperation by prolonging the operation time, and potentially escalating the serious mediastinal injury during the reentry [17,18].

Adhesion formation occurs as a consequence of the inflammatory response to surgical trauma, which can start within a few hours of surgery as routine surgical procedures include tissue handling including abrasion, desiccation, ischemia, hemorrhage, exposure to foreign material and overheating by lamps [18]. Histological examination of pericardial tissues from animals undergoing cardiac surgical procedures indicates that damage to the mesothelium (especially by abrasion) is adhesiogenic [19,20]. It is known that mesothelial cells are responsible for fibrinolytic properties of the coelomic cavities and the damage to these cells is the trigger for adhesion formation [5]. Although the mechanism of adhesion formation is not fully understood, fibroblast activity and inflammatory responses are believed to be important in the pathogenesis of adhesion formation according to the previous several studies. The inflammatory response is a complex pathophysiological process including many chemical and cytokine mediators that will cause extravascular plasma leakage (as a consequence of the increased vascular permeability) and formation of fibrin. This leads to the formation of serosanguineous exuda, which in turn initiates adhesion formation [16]. Fibrin provides a framework for fibroblast proliferation, synthesis of collagen and adhesion formation [18]. Increasing of reactive oxygen species (ROS) after endothelial tissue damage which occurs during open surgery can play a role in postoperative adhesion formation. Evidence showed that ROS scavengers could reduce adhesion formation in animal models [16,21]. Subsequently, if these initial adhesions are not lysed, they are organized into fibrous adhesions by activated fibroblasts. However, in a state of an imbalance between fibrin deposition and dissolution, deposited fibrin may persist and fibrinous adhesions may develop [16,21].

Unfortunately, despite continuous advances and researches, an ideal method and material to decrease postoperative pericardial adhesion formation have not yet been found. A number of antiadhesive interventions have been developed and many have been tested clinically and experimentally in cardiac applications. Various methods and agents have been used with controversial results [22]. Some studies have focused primarily on substitutes (autogenous, heterogenous, and synthetic) providing a barrier between epicardium and pericardium and agents have been used with controversial results [22]. Some studies [23] have also been shown to decrease pericardial adhesion formation [16,26,27].

ABS is an herbal extract attained from 5 different plants: Thymus vulgaris (thyme), Glycyrrhiza glabra (licorice), Vitis vinifera (unripe grape), Alpinia officinarum (galangal), and Urtica dioica (stinging nettle). It has been folklorically used in traditional Turkish medicinal practice. ABS represents an alternative treatment modality for many kinds of bleeding that are resistant to conventional methods. Today, topical ABS has been used and has provided positive results in spontaneous or secondary bleedings (gastrointestinal, orthopedic, nasal, dermal) due to body injuries, traumas, and minor or major surgical interventions and wound healing [28]. Besides its homeostatic activity, Kocak et al. [29] reported that Ankaferd might also have anti-inflammatory effects [7]. Tests have demonstrated its safety, efficacy, sterility, and non-toxicity for external usage [8,30].

Al et al. showed that Ankaferd have not efficient in reducing postoperative intraabdominal adhesions [7]. Conversely, Cömert et al. reported that there was less intraperitoneal adhesion formation in the Ankaferd group than in the control group [8]. However, the safety and non-toxicity of Ankaferd for intrapericardial usage and the effects of Ankaferd on postoperative pericardial adhesion have yet to be discussed. In the present study, fibrosis score measurements showed no statistically significant difference between the Ankaferd group and the control group (p=0.234). But when the groups were compared according to the prevalence of fibrosis, there were statistically significant difference between groups (p=0.028) and fibrosis scores were significantly higher in the Ankaferd group. The results of our study showed that topical application of Ankaferd could increase the pericardial adhesion after abusive injury onto the epicardial surface in rabbit model. Addition to the pericardial adhesion and visibility of coronary vessels score, the histological evaluation was used to evaluate the effect of Ankaferd on inflammation and fibrosis in rabbit model. However, there were no statistically significant difference between groups in terms of inflammatory scores and degree of the inflammation (p=0.382, p=0.220, respectively). Hence, the efficacy on inflammation was not observed in the histological evaluation.

In conclusion, we applied Ankaferd to reduce postoperative pericardial adhesion in the experimental rabbit model. The use of Ankaferd increased the adhesion and fibrosis scores. However, the efficacy on inflammation was not demonstrated. Further studies with Ankaferd are necessary to evaluate its efficacy in adhesion formation prevention in cardiac surgery.

Conflicts of Interest

The authors had no conflicts of interest to declare in relation to this article.

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