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Intraarticular Ankaferd blood stopper application increases cartilage degeneration: an experimental study

İbrahim KAYA[1], Deniz GÜLABİ[2,*], Murat YILMAZ[1], Ali BAŞ[3], Gültekin Sıtkı ÇEÇEN[2], Nurullah ŞENER[1]

[1]Department of Orthopedics and Traumatology, Haseki Training and Research Hospital, İstanbul, Turkey
[2]Department of Orthopedics and Traumatology, Dr. Lütfi Kırdar Kartal Training and Research Hospital, İstanbul, Turkey
[3]Department of Orthopedics and Traumatology, Ağrı State Hospital, Ağrı, Turkey

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Background/aim: Ankaferd blood stopper (ABS) is a mixture of certain ratios of 5 different plant roots (*Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum*, and *Urtica dioica*). The aim of this study is to evaluate the histopathological effects of ABS on articular cartilage in vitro.

Materials and methods: Twenty-one albino Sprague Dawley rats were randomly allocated to 3 groups: 0.1 mL of saline was injected in the first group, 0.1 mL of ABS was injected in the second group, and 0.1 mL of blood and 0.1 mL of ABS were injected in the third group. One month later all rats were sacrificed. Specimens were obtained for histopathological evaluation.

Results: Significant results were detected in the groups with respect to International Cartilage Repair Society and synovial proliferation scores ($P < 0.05$ and $P < 0.01$). According to inflammatory cell infiltration and fibrin formation scores, there was no significant difference between group 1 and group 2 ($P < 0.01$), although there was significant difference between group 3 and the other groups ($P > 0.05$).

Conclusion: ABS and hemarthrosis had toxic effects on knee cartilage. The side effects were increased with the combination of hemarthrosis and ABS. As a result, ABS had unexpected effects on experimental hemarthrosis.

Key words: Hemarthrosis, Ankaferd, cartilage

1. Introduction

In intraarticular bleeding in patients with trauma, specific damage is caused that is associated with inflammatory cell infiltration and the fibrin formation effects of the accumulation of blood products (1–3).

Ankaferd blood stopper (ABS) is a compound obtained from the mixture of certain ratios of 5 different plant roots (*Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum*, and *Urtica dioica*) (1). The properties of ABS as a powerful hemostatic agent, which prevents peritoneal adhesions and has positive effects in the early stage of bone healing, have been reported in several studies (2,3). Various clinical studies have shown the use of ABS as an effective hemostatic agent in ear, nose, throat, and dentistry practice, and in the gastrointestinal and cardiovascular systems (4,5). Its antihemorrhagic properties have been shown by several authors in in vivo and in vitro studies (4,5). The clotting effect of proteins in the blood as a result of the interaction established with fibrinogen is demonstrated with the aggregation of red cells (6–8). In addition, various studies have shown ABS to have antiinflammatory and antioxidant properties (3,6).

Traumatic hemarthrosis is a frequently encountered orthopedic problem. Recurrent hemarthrosis has an adverse effect on the chondral side of the joint. This adverse effect is due to the blood and blood products in the joint (9,10). The application of hyaluronic acid, impaction ultrasound, low-dose laser therapy, and IL-4 and IL-10 administered together has been shown to reduce joint degeneration in joints with hemarthrosis in in vitro studies (9–11). The effect on joint cartilage of ABS intraarticular injection used for a range of hemorrhages is not known as of yet (12).

The purpose of the present study was to examine the effect of ABS on the cartilage of the joint. We aimed to research the suitability of ABS for use in patients with hemarthrosis by performing a histopathological examination of the changes in the intraarticular structures following intraarticular injection of ABS.

* Correspondence: dgulabi@yahoo.com

2. Materials and methods

2.1. Study design

Approval for the study was granted by the Animal Research Committee of İstanbul University. The study was conducted at the Experimental Research Center (DETAM, confirmation no.: 2012/43). The histological examination was performed at the İstanbul University Oncology Institute Pathology Laboratory. A total of 21 healthy male albino Sprague Dawley rats, weighing 250–300 g, were included in the study. The rats were randomly allocated to 3 groups; isotonic serum was administered to group 1 (n = 7), ABS only to group 2 (n = 7), and blood and ABS to group 3 (n = 7).

2.2. Experimental study

At 30 min preoperatively, cefazolin sodium 20 mg/kg was administered intramuscularly as antibiotic prophylaxis, and an additional dose was given 8 h postoperatively to terminate the antibiotic prophylaxis procedure. General anesthesia was applied to all the subjects with a peritoneal injection of a mixture of 5 mg/kg xylazine hydrochloride (Rompun; Bayer Healthcare, Leverkusen, Germany) and 6 mg/kg ketamine HCL (Ketalar, Pfizer, İstanbul, Turkey). Under aseptic conditions and in order to create experimental hemarthrosis, 0.1 mL of saline was injected into the knees of group 1 and 0.1 mL of ABS was injected into the knees of group 2. In group 3, 0.1 mL of blood was obtained from the tail of the rats, and this was injected into their knees together with 0.1 mL of ABS. After awakening, the animals were allowed free movement in cages with a 12-h light-dark cycle in an air-conditioned environment at 22 ± 1 °C.

After 1 month, the animals were sacrificed with a high-dose phenobarbital injection and the distal femur and proximal tibia were cut, protecting the knee joint. Samples were taken from the knees of the sacrificed animals for histopathological examination of the cartilage status. After 48 h of fixation in 10% neutral buffered formalin, the samples were left for 12 h in 10% formic acid in 0.1 M citrate for decalcification. After the decalcification process, sections were taken from the length of the femoral condyle. For histopathological examination of the cartilage, slices of 5 μm in thickness were prepared in paraffin from the sections and stained with hematoxylin and eosin (H&E) and toluidine blue.

A light microscope was used for the evaluations. To avoid any person-related differences, all variables were evaluated by a single histopathologist blinded to the study. In the histopathological evaluation, the synovial proliferation, inflammation, and amount of fibrin were scored by the histopathologist on a scale from 0 to 3 (Figures 1–3). These findings were scored according to the percentage of the area covered: 0%–1% of area covered = 0, 1%–30% = 1, 30%–60% = 2, >60% = 3. Changes in the cartilage were scored from 0 to 6 according to the International Cartilage Repair Society (ICRS) osteoarthritis and cartilage histopathology classification system, as it is a straightforward system that has been used in both experimental and clinical studies (13).

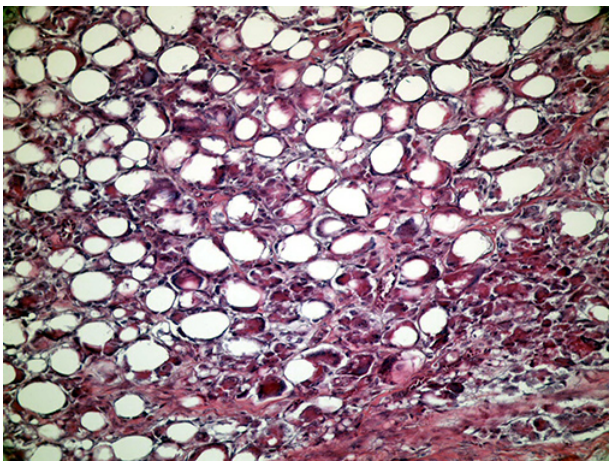

Figure 1. Pathology slice after ABS injection in a knee with hemarthrosis. The joint surface shows advanced irregularity and areas of joint cartilage that have lost full layer continuity, formation of fibrous tissue formed from young mesenchymal cells in these areas, and synovial cell proliferation in the joint space and many foreign giant cells (H&E, 200×).

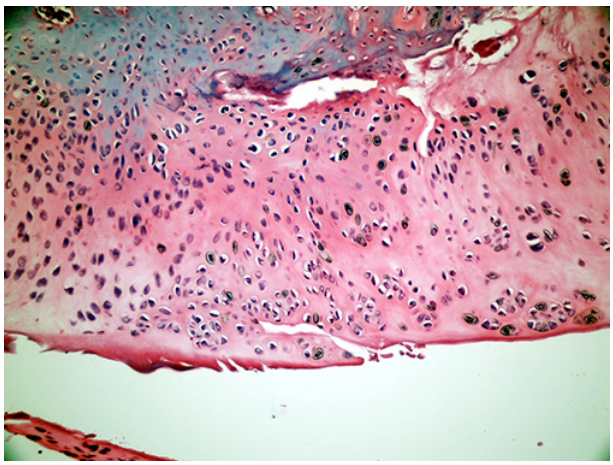

Figure 2. Histopathological examination after ABS application to the right knee. On 3 joint surfaces, minimal irregularity and small superficial fissures in the joint cartilage, synovial proliferation, and scattered macrophages (H&E, 200×).

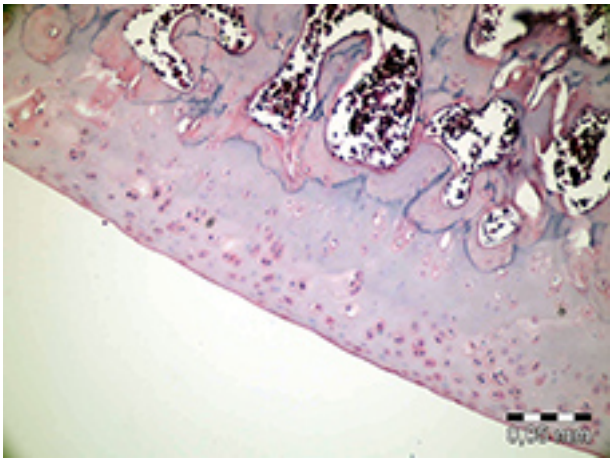

Figure 3. Control group: joint surface of normal appearance and joint cartilage of natural structure (H&E, 200×).

2.3. Statistical analysis

SPSS 15.0 (SPSS Inc., Chicago, IL, USA) was used for the evaluation of the study data. In addition to the use of descriptive statistical methods (mean, standard deviation, median) to evaluate the data, in the comparison of parameters not showing normal distribution between the groups, the Kruskal–Wallis test was used, and the Mann–Whitney U test was used to determine from which group the difference originated. $P < 0.05$ was accepted as statistically significant.

3. Results

The signs of cartilage degeneration, inflammation, and fibrosis were higher in the hemarthrosis + Ankaferd group. A statistically significant difference was seen between the groups in the ICRS stages ($P < 0.05$). As a result of the paired comparison applied to determine from which group the difference originated, the mean cartilage damage of the hemarthrosis + Ankaferd group was statistically significantly higher than that of the Ankaferd group and the control group. The cartilage damage of the Ankaferd group was determined as statistically significantly higher than that of the control group (Table).

A statistically significant difference was determined between the groups in the levels of synovial proliferation ($P < 0.01$). As a result of the paired comparison applied to determine from which group the difference originated, the level of synovial proliferation of the hemarthrosis + Ankaferd group was statistically significantly higher than that of the Ankaferd group and the control group. The level of synovial proliferation of the Ankaferd group was determined to be statistically significantly higher than that of the control group.

A statistically significant difference was determined between the groups in the levels of fibrin ($P < 0.01$). As a result of the paired comparison applied to determine from which group the difference originated, the level of fibrin of the hemarthrosis + Ankaferd group was statistically significantly higher than that of the Ankaferd group and the control group. No significant difference was determined between the Ankaferd group and the control group ($P > 0.05$).

A statistically significant difference was determined between the groups in the levels of inflammation ($P < 0.01$). As a result of the paired comparison applied to determine from which group the difference originated, the level of inflammation of the hemarthrosis + Ankaferd group was statistically significantly higher than that of the Ankaferd group and the control group. No significant difference was determined between the Ankaferd group and the control group ($P > 0.05$).

4. Discussion

The most significant finding of this study is that the use of ABS for hemarthrosis in the knee joint has a negative effect on the cartilage.

ABS is a traditional herbal essence medication that has been used for many years in Anatolia. Due to its

Table. Evaluation of cartilage degeneration, synovia, fibrin, and inflammation in the groups.

	Ankaferd		Hemarthrosis + Ankaferd		Control		P
	Mean ± SD	Min–max (median)	Mean ± SD	Min–max (median)	Mean ± SD	Min–max (median)	
Stage of cartilage damage	2 ± 1.41	1–5 (2)	4.14 ± 1.57	2–6 (5)	0 ± 0	0–0 (0)	0.024*
Synovial proliferation	1 ± 0.58	0–2 (1)	2.86 ± 0.38	2–3 (3)	0 ± 0	0–0 (0)	0.001**
Fibrin	0.57 ± 0.53	0–1 (1)	2.43 ± 0.53	2–3 (2)	0 ± 0	0–0 (0)	0.001**
Inflammation	0.14 ± 0.38	0–1 (0)	1.71 ± 0.95	0–3 (2)	0 ± 0	0–0 (0)	0.006**

Kruskal–Wallis test; *: $P < 0.05$; **: $P < 0.01$.

hemostatic effect, ABS is widely used (and approved by the Turkish Ministry of Health) particularly in dentistry and in the cardiovascular system, the digestive system, and stopping bleeding in internal organs (14–16). In an experimental model, İşler et al. (3) showed the positive contribution of ABS to the early stages of bone healing. It was reported that this effect was due to the antioxidant and antiinflammatory properties of the plant essence.

In a clinical study by Fernandez-Palazzi et al. (9), successful results were reported for the administration of rifampicin to 39 hemophilic joints with hemarthrosis. In the experimental rat model of a study by van Meegeren et al. (10), cartilage degeneration was seen to decrease with the intraarticular injection of IL-4 and IL-10. In a rabbit model used by Ravaanbond et al. (11), impaction ultrasound and low-dose laser were shown to have successful results in hemarthrosis treatment.

Iron and cytokines produced by hemarthrosis caused damage to the synovium and cartilage by stimulating neoangiogenesis of the hematoma in the joint (17,18). In an experimental study by Parsons et al., it was determined that autologous blood injected within the joint resulted in a reduction of proteoglycan in the cartilage and in cartilage damage (19). Jansen et al. (18) reported from an experimental dog model that damage was determined in the articular cartilage analysis 24–48 h after puncture. This resulted from the inflammatory reaction, which started in the synovial tissue of the intraarticular hematoma (18). In the present study, a statistically significant increase was observed in the histopathological examination in cartilage damage, synovial proliferation, fibrin, and inflammation. This can be considered to result from the inflammatory process starting from the synovial response triggered by the hematoma products, as reported by Jansen et al. (18). Nevertheless, there are opposing views to this, as some authors have emphasized the antiinflammatory and antioxidant properties of *G. glabra*, which is contained in ABS (8,20–22). Immunochemical analyses are required to better understand this effect.

A statistically significant difference was observed in the parameters of the stage of cartilage damage and synovial proliferation in the histopathological examination of the knees without hemarthrosis, which received an ABS injection, compared to the control group. This may have resulted from the reaction against ABS, which was treated as a foreign substance by the body. A study by Okumuş et al. (20), which examined the histopathological changes in local tissue of rats, reported that there was a significant inflammatory response in surrounding tissues with 0.1 mL of ABS spray. Similar results were reported by Odabaş et al. (21). In another experimental model by Cömert et al. (2) on the effect of ABS on intraperitoneal adhesions, greater acute and chronic inflammatory changes were seen in the ABS-applied group compared to the control group. In an experimental study by Beyazit et al. (22), it was reported that ABS increased inflammation in Wistar albino rats. In the present study, increase in inflammation, stage of cartilage damage, and synovial proliferation were determined with a dose of 0.1 mL of ABS solution applied intraarticularly.

The limitations of the present study include the lack of immunochemical analysis of inflammation precursors such as IL-1, IL-6, NO, PG-E, and TNF-alpha. In addition, the follow-up period was short and there was no observation of results from different doses.

Small sample size and lack of power analysis, radiological outcome analysis, and toughness analysis of the cartilage also constitute limitations of the present study.

In conclusion, the use of ABS in intraarticular hemarthrosis showed a negative effect on joint cartilage damage created by blood and blood products. However, more detailed in vivo and in vitro studies related to the intraarticular application of ABS would be useful.

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