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Effects of Ankaferd hemostat on the synovial fluid of patients with osteoarthritis

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Aim: Ankaferd Blood Stopper (ABS) is a novel topical hemostatic agent with antiinfective, wound healing, and antineoplastic properties. In this study, we evaluated the macroscopic and biochemical effects of ABS in the joint fluid of patients with osteoarthritis.

Materials and methods: Synovial fluid samples were obtained from 10 patients with osteoarthritis (5 women, 5 men; mean age 57.6 ± 11.2 years). From each patient, 4 mL of synovial fluid was drawn and put into 2 different serum separator tubes, and 1 mL of ABS was added to the first sample and 1 mL of 0.9% saline was added to the second sample. Macroscopic evaluation and biochemical analyses for glucose, protein, albumin, and lactate dehydrogenase (LDH) were performed.

Results: The addition of ABS into synovial fluid revealed a rapid and apparent reaction resulting in dense coagulation within seconds in all of the samples. No reaction was observed in the saline group. Total protein, albumin, globulin, and LDH levels significantly decreased in the ABS group, while glucose levels remained unchanged.

Conclusion: ABS can work in the synovial fluid, causes rapid frozen gel-like solidification, and decreases the protein component of the synovial fluid. This observation, together with hemostatic and wound healing properties of ABS, might provide an important starting point for testing its actions on distinct hemorrhagic, neoplastic, or inflammatory joint disorders.

Key words: Ankaferd Blood Stopper (ABS), synovial effusion, osteoarthritis

Introduction

Ankaferd Blood Stopper (ABS) is a traditional drug that has been used as a hemostatic agent for centuries in Anatolia. It has recently been approved by the Ministry of Health in Turkey as a topical hemostatic agent for clinical hemorrhages (1-3). ABS is a standardized mixture of the plants *Thymus vulgaris* (dried leaf), *Glycyrrhiza glabra* (dried leaf), *Vitis vinifera* (dried leaf), *Alpinia officinarum* (dried leaf), and *Urtica dioica* (dried root), each of which has some effects on the endothelium, blood cells, angiogenesis, cellular proliferation, vascular dynamics, and/or cell mediators (1). The basic mechanism of the hemostatic action of ABS is the formation of a protein network that provides focal attachment points for very rapid vital erythrocyte aggregation like an "erythrocyte magnet". The ABS-induced protein network enriched with blood cells, particularly erythrocytes, covers the primary and secondary hemostatic system without disturbing individual coagulation factors or platelets (1-5). ABS was as effective as other hemostatic and sealant agents (Glubran 2, FloSeal, and Celox) that have already been licensed for use in controlling kidney surgery bleeding with comparable warm ischemia time and hemostasis time values (6).

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Apart from being an alternative hemostatic medicine for intractable bleedings, ABS has many other effects at the cellular level, including antiinfective (7,8), wound healing (9,10), and antineoplastic properties (11,12), and to restore and maintain tissue homeostasis (1). Recently, ABS also formed aggregates of protein network in the pancreatic fluid and induced frozen gel-like solidification (13). The effect of ABS on synovial fluid has not yet been investigated although its effects on pancreatic fluid (13) and blood plasma (1) disclosed striking in vitro effects transferable to in vivo models. The aim of this study was to assess the macroscopic and biochemical effects of ABS in the joint fluid of patients with osteoarthritis.

Materials and methods

Synovial fluid obtained from 5 women and 5 men (age 57.6 ± 11.2 years) for the effusive complication of knee osteoarthritis was utilized. Following the arthrocentesis, 4 mL of synovial fluid from each patient was put into 2 different serum separator tubes. Then 1 mL of ABS was added to the first sample, and 1 mL of 0.9% saline was added to the second sample of each patient. In the first step, macroscopic evaluation of those saline and ABS groups were performed. Subsequently the tubes were left for 30 min. The tubes were then centrifuged for 15 min at 1000 × *g*. Aliquots were obtained and stored at –80 °C until assaying. Biochemical analyses for glucose, protein, albumin, and lactate dehydrogenase (LDH) were performed by commercially available biochemical tests. The comparisons between the ABS and saline groups were made using the Wilcoxon rank test. $P \leq 0.05$ was considered statistically significant.

Approval for this study was obtained from the Local Ethics Committee.

Results

The addition of ABS into synovial fluid revealed a rapid and apparent reaction resulting in dense coagulation within seconds in all of the samples (Figure 1A and 1B). No reaction was observed in the saline group.

Biochemical analysis revealed a significant decrease in the protein components of plasma in the ABS group (Table). The decreases were observed in all of the protein components, namely albumin and

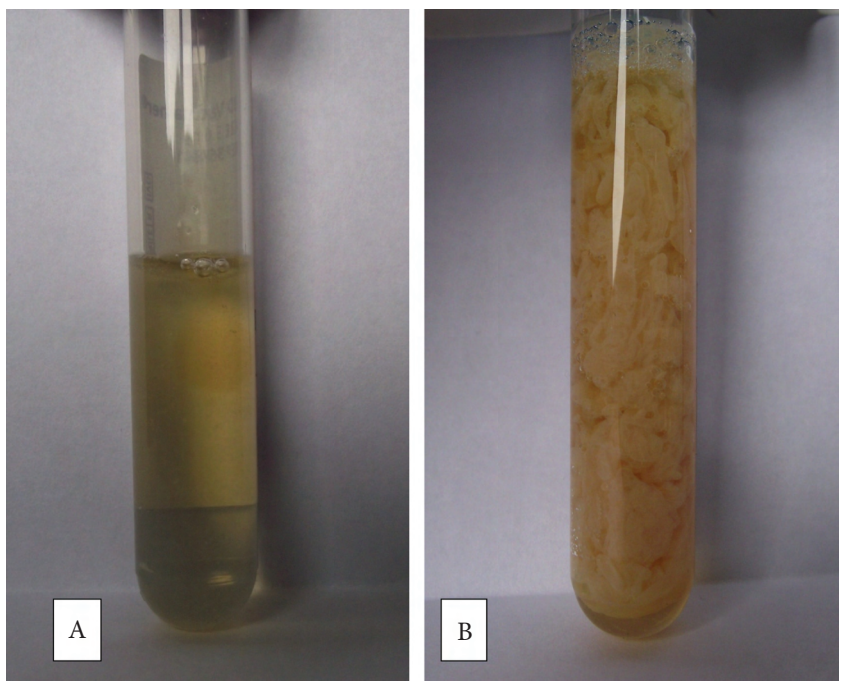

Figure 1A. The macroscopic figure of the synovial fluid before the addition of ABS.
Figure 1B. The macroscopic figure of the synovial fluid after the addition of ABS.

Table. Comparison of biochemical parameters in the ABS group and the control saline group (n = 10). All of the values are presented as median (min-max).

	After the addition of SF	After the addition of ABS	P
Glucose, mg/dL	61 (27-129)	63 (33-121)	0.47
Total protein (g/dL)	2.5 (1.5-5.2)	0.2 (0.1-1.8)	0.008
Albumin (g/dL)	1.5 (0.9-2.2)	0.1 (0-1.1)	0.011
Globulin (g/dL)	1.3 (0.6-3.1)	0.2 (0-0.9)	0.012
LDH (U/L)	78 (39-210)	2.0 (0-6)	0.012

ABS: Ankaferd blood stopper, LDH: lactate dehydrogenase.

globulin, as well as enzymatic component represented by LDH ($P < 0.05$ for each parameter). However, glucose levels remained unchanged ($P > 0.05$).

Discussion

We investigated macroscopic and biochemical effects of ABS in the synovial fluid of patients with osteoarthritis. We macroscopically observed rapid frozen gel-like solidification, and the biochemical analyses showed decreases in protein, albumin, globulin, and LDH levels, while glucose levels remained unchanged.

Previous studies demonstrated that the addition of ABS to plasma and serum caused rapid formation of an encapsulated protein network, which provided focal points for erythrocyte aggregation. Furthermore, they observed decreases in plasma fibrinogen, protein, albumin, and globulin levels without any changes in the clotting factors (factors II, V, VII, VIII, IX, X, XI, and XIII). Therefore, rather than affecting an individual clotting factor, ABS acts by forming a protein mesh and affects the entire physiological hemostatic process that controls bleeding (2-5). ABS can also work in other tissue fluids such as pancreatic fluid. Recently, ABS-induced frozen gel-like solidification in the pancreatic fluid, and cytopathological examination demonstrated a cluster of aggregated proteinous material of the pancreatic fluid. Biochemical analyses revealed decreases in amylase, protein, and albumin levels (13).

Synovial fluid is an ultrafiltrate of plasma, with only small amounts of higher molecular weight proteins such as fibrinogen, complement, globulin, and other immunoglobulins, and its glucose level is nearly equal to that of blood. Synovia fluid is supplemented with high-molecular weight, saccharide-rich molecules, especially hyaluronans, produced by type B synoviocytes. Normally, synovial fluid contains few cells (<200/mm^3), mainly chondrocytes and synoviocytes, and the polymorphonuclear leukocyte (PNL) percentage is low (<25%) (14,15). A small quantity of joint fluid is present in the normal knee. Normal fluid is difficult to aspirate, and it is an ethical issue to aspirate joint fluid from asymptomatic individuals. On the other hand, joint effusion is often observed in osteoarthritis, which is regarded as a joint disease with a low inflammatory component. Synovial effusion in patients with osteoarthritis is noninflammatory in nature, with transparent clarity, normal glucose levels, 50-1000 cells <200/mm^3, and low PNL percentage (15). Hence, we preferred to use the synovial fluid of osteoarthritis patients in order to minimize the confounding effect of inflammatory cells and mediators on the effects of ABS. All of the synovial fluid samples in our study were transparent, and protein, LDH, and glucose levels were compatible with noninflammatory synovial effusion. After the addition of ABS, the synovial fluid demonstrated rapid frozen gel-like solidification, supporting the hypothesis that ABS can work in other tissue fluids, together with decrements in the levels of protein components of the synovial fluid. Our observation suggests that ABS could promote the aggregation of both the protein components as well as high-molecular weight saccharide-rich hyaluronic acid in synovial fluid.

ABS could help to control bleeding in defective hemostasis. Two animal models supported this hypothesis. In the first model, rats were treated either

with warfarin (2 mg/kg) or a vehicle (0.9% NaCl), orally before bilateral hind leg amputation. ABS was administered topically to 1 of the amputated legs. Topical ABS administration to the amputated leg shortened the duration of bleeding markedly in both the untreated and warfarin-treated rats. Furthermore, the amount of bleeding in the ABS-administered amputated leg showed an approximate 50% decrease compared to the warfarin-treated group (16). In the second model, the rats were pretreated with acetylsalicylic acid or enoxaparin sodium or did not receive any anticoagulant. ABS was administered to the cut tails of the studied animals. Topical ABS administration reduced both the duration and the amount of bleeding in the acetylsalicylic acid-treated and enoxaparin-treated animals (17). Those 2 in vivo studies provide important data that ABS has a therapeutic potential for the management of patients with deficient hemostasis in clinical medicine. This hypothesis was further supported by a number of case reports, which demonstrated that topical ABS application helped to control bleeding in patients with disseminated intravascular coagulation, hemophilia A, von Willebrand disease, hereditary thrombocytopenia, and Glanzmann thrombasthenia (18-23).

In addition to its hemostatic effect, ABS also has wound healing properties. Topical ABS reduced excisional bleeding in the experimental burn injury model (10). In an animal model of bone defects experimentally created in the right and left tibiae, over 60% of the defects treated with ABS were free of inflammation, the ABS-treated group showed lower fibrosis rate than the untreated control group, and the defects treated with ABS showed more intense new bone formation and less occurrence of necrosis, suggesting that ABS decreased the inflammation, necrosis process, and increased the new bone formation in early bone healing period (9).

Hemarthrosis is a frequent problem in patients with hereditary hemorrhagic diatheses, especially in hemophilia A, and can subsequently cause progressive arthropathy due to fibrosis or ankylosis. The management of this condition requires expensive factor replacement, which is not always satisfactory (24). Our results and previous experience with ABS, demonstrating its hemostatic effects in patients with defective hemostasis and wound healing properties, suggest that ABS might offer an alternative therapeutic approach for those patients. In vivo experiments with animal models of hemarthrosis should be performed to test this hypothesis.

Conclusion

In summary, we demonstrated for the first time that ABS can work in the synovial fluid, causes rapid frozen gel-like solidification, and decreases the protein component of synovial fluid. This observation, together with hemostatic and wound healing properties of ABS, might provide an initial step for developing an intraarticular agent for the management of hemarthrosis, especially in patients with defective hemostasis.

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