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INTERVENTIONAL RADIOLOGY

ORIGINAL ARTICLE

Splenic artery embolization with Ankaferd blood stopper in a sheep model

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PURPOSE
Splenic artery embolization is a minimally invasive therapeutic procedure utilized in a number of disorders. Ankaferd blood stopper (ABS) is a novel hemostatic agent with a new mechanism of action independent of clotting factors. We aimed to investigate the safety and efficiency of ABS for splenic artery embolization in a sheep model.

METHODS
Seven adult female sheep were included in the study. Selective celiac angiography was performed using a 5F diagnostic catheter and then a 2.7F hydrophilic coating microcatheter was advanced coaxially to the distal part of the main splenic artery. Under fluoroscopic guidance, 6 mL mixture composed of half-and-half ABS and contrast agent was slowly injected. Fluoroscopy was used to observe the deceleration and stagnation of the flow. Control celiac angiograms were obtained immediately after the embolization. After the procedure, the animals were observed for one day and then sacrificed with intravenous sodium thiopental.

RESULTS
Technical success rate was 100%. None of the animals died or experienced a major systemic adverse event during the procedure. All of the spleens appeared dark on macroscopic examination due to excessive thrombosis. Microscopically, the majority of the splenic sinusoids (90%–95%) were necrotic.

CONCLUSION
In our study, splenic artery embolization by ABS was found to be safe and effective in the short-term. Further studies are needed to better understand the embolizing potential of this novel hemostatic agent.

Splenic embolization (SE) is an interventional procedure that is considered to be a viable alternative to splenectomy (1). Splenic arterial interventions are usually performed to treat portal hypertension, hypersplenism, splenic injury due to abdominal trauma, splenic arterial aneurysm, and splenic neoplasms (2). However, most of the current embolizing agents have various limitations such as unequal permeation, local diffusion, injury on the surrounding tissues, as well as high costs. For this reason, alternative approaches with different biological materials that could eliminate the potential limitations of the current embolization techniques are strongly needed in clinical practice.

Ankaferd blood stopper (ABS; Ankaferd Drug Inc.) is an herbal extract that has been used as a folk hemostatic agent for centuries in Anatolia. ABS has been approved by the Ministry of Health of Turkey as a topical hemostatic agent for use in mucocutaneous bleeding. ABS is a standardized mixture of dried leaves of the plants *Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum*, and dried root of the plant *Urtica dioica*, each of which has some effects on the endothelium, blood cells, angiogenesis, cellular proliferation, vascular dynamics, and/or cell mediators (3). ABS has a novel hemostatic mechanism, which has never been described before. ABS forms a protein network that provides focal attachment spots for very rapid vital erythrocyte aggregation (4). The ABS-induced protein network enriched with blood cells, particularly erythrocytes, encompasses the primary and secondary hemostatic systems without disturbing individual coagulation factors or platelets. ABS has been found to be as effective as other hemostatic and sealant agents, such as Glubran 2, FloSeal, and Celox, that have already been licensed for use in controlling kidney surgery bleeding (5). ABS has been used for the management of clinical hemorrhages, when the convention-

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al control of bleeding by ligature and/or hemostatic measures is insufficient. Successful use of ABS has been reported for a number of clinical bleeding disorders including epistaxis (6), gastrointestinal bleeding (7, 8), bladder hemorrhage (9), and dental surgery. We previously reported successful and safe arterial embolization of sheep kidney with ABS (10).

Here, we aimed to investigate the safety and efficiency of splenic embolization by ABS in sheep.

Methods

Seven adult healthy female sheep (≥1-year-old) that were free of any parasitic infestation were included. The animals weighed 52–64 kg. University ethical committee approved the study protocol. All animals were treated according to the Principles of the Laboratory Animal Care of the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals [NIH Publication No. 80–23 (revised 1985)]. All animals underwent complete veterinary examination before being included in the study. Sheep were observed for 24 hours prior to the embolization procedure, and no food was given for at least 12 hours before the procedure. Transcatheter embolization of the spleen was performed in all animals. Animals were observed for 24 hours after completion of the procedure. Vital signs were recorded. Animals were sacrificed thereafter with intravenous sodium thiopental, and spleen was examined for any macroscopic changes related to embolization and sent for pathologic examination.

Main points

- Splenic embolization is an interventional procedure that is considered to be a viable alternative to splenectomy.
- Ankaferd blood stopper (ABS) is an herbal extract, and is a standardized mixture of dried leaves of the plants *Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum, and dried root of the plant Urtica dioica*.
- Endovascular splenic artery embolization is effectively used in diseases of the spleen. This experimental study showed that ABS is a safe and effective agent for splenic artery embolization in a sheep model.
- ABS could be an alternative embolic agent in splenic artery embolization pending further large experimental and clinical studies.

Embolization technique

Following placement and securing of the animal on the angiography table, xylazine hydrochloride (0.2 mg/kg) was applied via intramuscular route for sedation and analgesic purposes. After shaving the groin region, povidone iodine and appropriate draping were used to attain a sterile condition. Ultrasound-guided micropuncture technique was used to place a vascular sheath (5F) to the right femoral artery. Abdominal aortography was performed using a 5F pigtail catheter (Johnson & Johnson, Cordis Europe) to visualize the celiac trunk (Fig. 1a). Digital arteriography of the spleen was performed by initially obtaining an anteroposterior celiac arteriogram (Sos Omni catheter, Angiodynamics). Additional anteroposterior and oblique projections of the spleen were obtained after selective catheterization of the main splenic artery (Fig. 1b). Coaxial 2.7F microcatheter was advanced to the distal main splenic artery (Progreat microcatheter, Terumo) (Fig. 1c). A stopcock was attached to the microcatheter. Isotonic saline was used to irrigate the lumen of the catheter. Commercially available preparation of liquid ABS was used for embolization. Under fluoroscopic guidance, 3 mL of ABS mixed with 3 mL of nonionic contrast agent was slowly injected in approximately one minute through the microcatheter until a resistance to injection secondary to embolization was observed. Slow flow and stagnation were visualized with fluoroscopy. Mean volume of 5±0.5 mL of ABS-contrast dye mixture was used to embolize the spleens. Microcatheter was withdrawn as soon as embolization was confirmed in control splenic angiograms (Fig. 1d). Manual compression was applied to groin region after sheath extraction to prevent bleeding.

After the embolization procedure, spleens of the sacrificed animals were harvested with the splenic artery preserved,

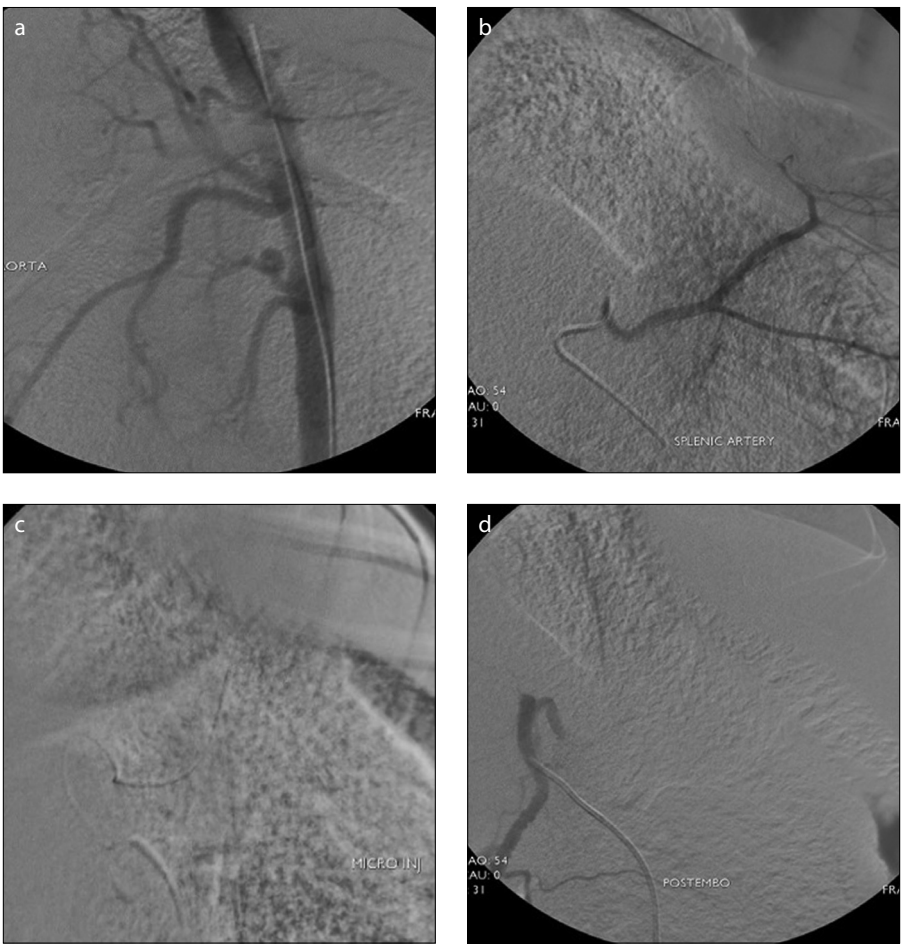

Figure 1. a–d. Angiography images showing embolization of the splenic artery. Panel **(a)** shows abdominal aortic angiography. Panel **(b)** shows selective diagnostic angiogram of the splenic artery before embolization. Panel **(c)** shows the position of the microcatheter. Panel **(d)** shows the splenic artery stump in a control angiogram, which was performed immediately after the embolization.

and the organs were sent for pathologic examination to be evaluated by a seasoned pathologist who had experience with reticuloendothelial system pathology.

Results

Ultrasound-guided femoral arterial catheterization and splenic arterial embolization procedures were technically successful in all animals studied. Postembolization control angiograms showed complete occlusion of the splenic artery and no blood flow in the spleen. The technical success rate was 100%. On macroscopic examination, embolized spleens looked enlarged and hemorrhagic. Thrombus formed by ABS in the splenic artery was compact and hard compared with thrombi observed elsewhere. On microscopic examination, diffuse sinusoidal necrosis was evident in the embolized spleen specimen (Fig. 2). More than 90% of the spleen was necrotic.

Discussion

Our results showed that ABS is an effective and safe agent in splenic artery embolization in an animal model. Technical success rate was 100%. To the best of our knowledge, ABS has never been used to embolize the splenic artery, and this paper is the first report of such use in the sheep.

Percutaneous vascular embolization is a main area of interest in the interventional radiology departments. Embolization is used in several different situations in daily practice such as target organ embolization, embolization of acute hemorrhages due to various causes, embolization of vascular malformations and aneurysms.

Splenic artery embolization is used increasingly in the setting of nonoperative management of splenic diseases. The novel methods and improvements in radiologic imaging and interventional radiology techniques might be responsible for this increased use. Two different procedures, namely proximal and distal, are applied in splenic artery embolization (11). The rate of success of splenic artery embolization procedures is reported between 73% and 100% in the relevant literature (12). The technical success rate was 100% in our study.

The selection of the proper embolizing agent depends upon the type of the disease that will be treated, the diameter of the vessel undergoing occlusion, and the aim of the therapy. Today, numerous different embolizing materials are available for clinical use. Many of these agents can also be used for splenic embolization purposes.

Embolization agents can be grouped into three main categories: agents providing mechanical occlusion, particular agents, and liquid agents. Balloons, coils, and Amplatzer vascular plug belong to the mechanical occlusive agents, while polyvinyl alcohol, microspheres, autologous blood clot, and gelfoam are examples of particular agents. Sclerosing agents such as N-butyl cyanoacrylate (NBCA) glue, onyx, and alcohol are among the examples of liquid embolization agents (13).

Each embolizing agent has its advantages and disadvantages. A coil is a piece of wire that is looped in various shapes and sizes. Coils provide a basic frame upon which clot formation occurs, and they usually have the addition of some fibers (made of wool, nylon fibers, polyester, silk) to increase thrombogenicity (14). Inability to attain the desired form due to oversized coil and coil migration due to small size are among the disadvantages of coil embolization. In addition, infection and vessel wall injury can be seen with these agents (15).

Amplatzer vascular plug is costly. Moreover, it may lead to vessel injury and migration of the plug. Amplatzer vascular plug has inherent limitations when used in small caliber vessels such as those in the spleen (16–18).

Polyvinyl alcohol particles (PVAs) are made from a PVA foam sheet that is vacuum dried and shaped into particles. Their size varies between 100 and 1100 μm, and the most commonly used sizes for embolization are 355–500 μm and 500–710 μm. PVAs provide permanent occlusion by adherence to the vessel wall, causing stagnation of flow, in addition to lodging in the smallest vessel into which they will fit. The major disadvantage of PVAs is their tendency to aggregate, occluding vessels more proximally than might be expected based on the stated size. Particle clumping can also cause catheter occlusion, which is preventable by dilution of particles, proper suspension, and slow infusion. In addition, PVAs can accumulate in the catheter hub and theoretically cause subsequent nontarget embolization when the catheter is flushed (15, 19).

Gelfoam has the ability to absorb and retain blood and fluid several folds greater than its own weight in its lacunae. Gelfoam usually provides a temporary occlusion and recanalization may develop three weeks to three months after the embolization procedure. If contaminated with air during the preparation, Gelfoam may lead to development of infections. As with other liquid agents, operator should be wary of reflux during the procedure (13, 20, 21).

NBCA glue is a liquid agent that rapidly solidifies when gets in contact with ionic liquids such as blood and saline. It has to be used with caution due to technical problems with handling the material, and com-

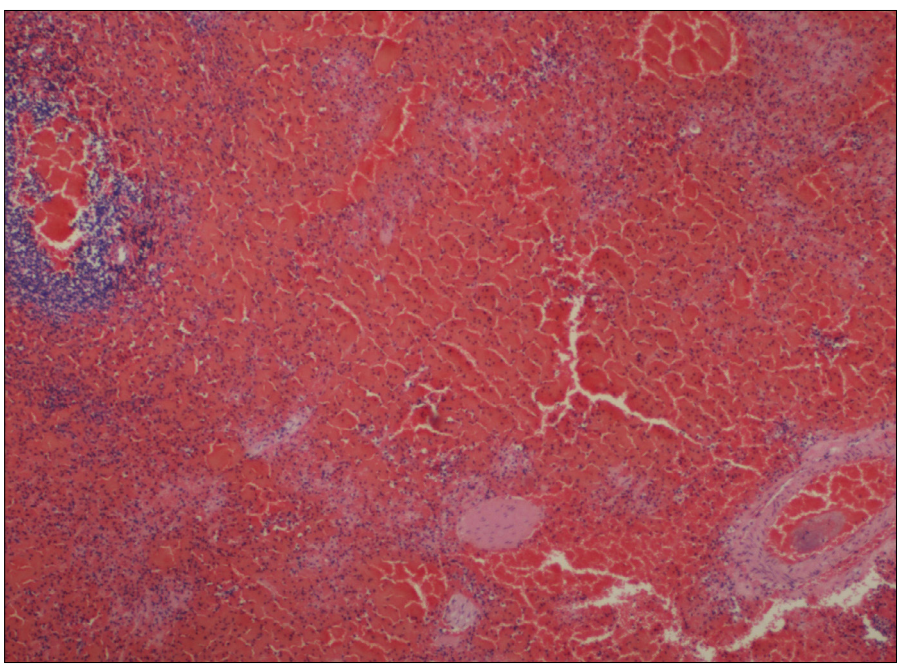

Figure 2. Diffuse sinusoidal necrosis was evident in the specimen of embolized spleen (hematoxylin-eosin staining, ×40).

plications may occur in cases of nontarget embolization (22–24). Different from other embolizing agents, sclerosing agents lead to tissue necrosis. Absolute alcohol is an effective embolizing agent but has several important adverse effects (13).

ABS is a novel hemostatic agent with a new mechanism of action independent of known clotting factors. The levels of coagulation factors II, V, VII, VIII, XI, X, XI, and XIII have been shown not to change by ABS application. Thus, ABS-induced formation of a protein network could be effectively used in both primary and secondary hemostatic defects. ABS has been shown not to be associated with acute mucosal toxicity, hematotoxicity, nephrotoxicity, or biochemical toxicity after oral administration in rabbits (25).

Some differences were noted in the splenic artery embolization of the sheep compared with splenic artery embolization in humans. First, splenic embolization could be difficult to some extent because of the smaller diameter of the splenic artery in the sheep compared with human splenic artery. The second difference was that exaggerated movements of stomach secondary to breathing in the animals led to motion artifacts in digital subtraction angiography. Despite the presence of these factors making the whole procedure more difficult, technical success rate was 100%.

In conclusion, endovascular splenic artery embolization is an effective treatment for diseases of the spleen, and this experimental study showed that ABS is a safe and effective agent for splenic artery embolization in a sheep model. In addition to its significantly low cost compared with other embolic agents, this study suggests that ABS is a very effective and rapid embolic agent. ABS could be an alternative embolic agent in splenic artery embolization, if our findings are confirmed in further large experimental and clinical studies.

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Conflict of interest disclosure

The authors declared no conflicts of interest.

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