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LABORATORY INVESTIGATION

Percutaneous Vein Occlusion with Small Intestinal Submucosa: An Experimental Pilot Study in Swine and Sheep

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Abstract
Purpose The objective of this study was to investigate the feasibility, outcomes, and amount of small intestinal submucosa (SIS) material needed for embolization of jugular vein (JV) in a swine and sheep model. Our hypothesis was that SIS would cause vein occlusion.
Materials and Methods The external JVs (EJV) in swine ($n = 6$) and JVs in sheep ($n = 6$) were occluded with SIS fan-folded compressed strips. After percutaneous puncture of the peripheral portion of the EJV or JV, a TIPS set was used to exit their lumen centrally through the skin. The SIS strips were delivered into the isolated venous segment with a pull-through technique via a 10-Fr sheath. Follow-up venograms were done immediately after placement and at the time of sacrifice at 1 or 3 months. Gross examinations focused on the EJV or JV and their surrounding structures. Specimens were evaluated by histology.
Results SIS strip(s) placement was successful in all cases, with immediate vein occlusion seen in 23 of 24 veins (95.8%). All EJVs treated with two strips and all JVs treated with three or four strips remained closed on 1- and 3-month follow-up venograms. Two EJVs treated with one strip and one JV treated with two strips were partially patent on venograms at 1 and 3 months. There has been one skin inflammatory reaction. Necropsies revealed excluded EJV or JV segments with SIS incorporation into the vein wall. Histology demonstrated various stages of SIS remodeling with fibrocytes, fibroblasts, endothelial cells, capillaries, and inflammatory cells.
Conclusion We conclude that EJV and JV ablation with SIS strips using percutaneous exit catheterization is feasible and effective in animal models. Further exploration of SIS as vein ablation material is recommended.

Keywords Animal model · Ablation · Biomaterial · Embolization · Varicose veins

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Symptomatic lower extremity venous insufficiency develops in approximately 15% of men and 25% of women [1]. About half of these patients have varicose veins causing aching pain, night cramps, fatigue, heaviness, and restlessness. Great saphenous vein (GSV) reflux is the most common underlying cause of clinically significant varicose veins. The approximate size of an enlarged human GSV ranges from 8 to 12 mm in diameter. Surgical ligation and stripping have been the traditional treatment for saphenofemoral junction (SFJ) incompetence with GSV reflux but has been associated with significant perioperative morbidity, a prolonged recovery period, and occasional postoperative recurrences [2–4].

Recently, minimally invasive techniques such as radiofrequency ablation, endovenous laser therapy, and foam sclerotherapy have achieved growing acceptance as alternative options for treatment of varicose veins. Although encouraging results after radiofrequency ablation and endovenous laser therapy have been reported in several clinical studies, those techniques have occasionally been complicated by heat-related adverse effects such as paresthesias or skin burns and superficial phlebitis [5–8].

Foam sclerotherapy can cause complications similar to those with liquid sclerotherapy such as phlebitis and skin necrosis [9]. Recently, a stroke has also been reported, due to foam crossing the patent foramen ovale [10].

Small intestinal submucosa (SIS), a collagen-based acellular biomaterial, has recently been reported as an embolic material for management of endoleaks after endovascular abdominal aortic aneurysm (AAA) repair [11]. The objective of this study was to investigate the feasibility, outcomes, and amount of SIS material needed for embolization of the external jugular vein (EJV) in swine and the jugular vein (JV) in sheep models. The EJV in swine and the JV in sheep were chosen for the study because their diameters are similar to that of the enlarged human GSV. There are no animal models with GSV incompetence.

Materials and Methods

All experiments were approved by the institutional animal care and use committee.

Animals

Six adult female sheep weighing from 48 to 70 kg (mean, 57.2 kg) and six domestic swine, each weighing from 27 to 52 kg (mean, 37.5 kg), were used for the study. Animals had been fasted overnight with water available and were tranquilized intravenously with 10–20 mg (0.05 mg/lb) of diazepam (Midazolam; Ben Venue Labs, Bedford, OH) and 400–800 mg (2.0 mg/lb) of ketamine (Ketaset; Fort Dodge Animal Health, Fort Doge IA). Animals were then intubated. Inhalation anesthesia was maintained with 2%–2.5% isoflurane (IsoFlo; Abbott Laboratories, North Chicago, IL) and 2 L/min of oxygen.

To reduce salivation, 5 mg atropine sulfate (American Regent Laboratories, Shirley, NY) was administered intravenously. Swines were initially sedated with an intramuscular injection of 3–6 mg/kg telazol (tiletamine HCl and zolazepam HCl; Fort Dodge Animal Health) and 1 mg atropine sulfate. Antibiotics (10 mg/kg cefazolin) were administered intramuscularly as single doses at the beginning of procedures. Intravenous volume substitution of saline (500–1000 ml) was administered, and respiratory rhythm and carbon dioxide saturation were monitored during the procedure. After the procedure, the access wounds were repaired and the animals were returned to the Department of Comparative Medicine, where veterinarians checked them on a daily basis.

Device

SIS occlusive strips were constructed out of a single 20- or 30-cm-long, 6-cm-wide sheet of lyophilized SIS (Cook Biotech, Bloomington, IN). Twenty-centimeter-long devices were used for swine, and 30-cm-long for sheep. The SIS sheet was fan-folded and compressed in order to reduce its profile, allowing delivery through a 10-Fr sheath. One or two compressed SIS strips were then pulled with a wire loop into a 10-Fr perforated cartridge and gas-sterilized. The cartridge was perforated to facilitate sterilization (Fig. 1).

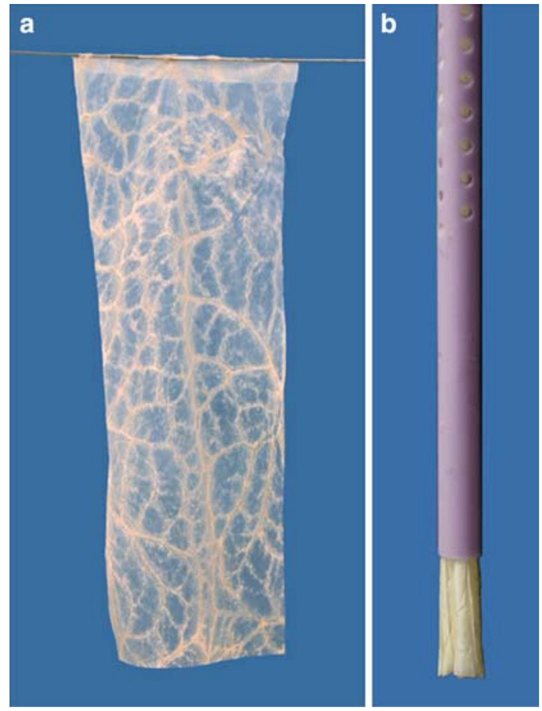

Fig. 1 The occlusive device. Sheet of porcine SIS, 20 × 6 cm, before (**a**) and after (**b**) it was fan-folded, compressed, and loaded into the perforated sheath (magnified view)

Procedure

After percutaneous puncture of the peripheral portions of JVs or EJVs, venograms were done and diameters of jugular veins were measured. According to the diameter of the vein, one, two, three, or four SIS strips were used for implantation. One SIS strip was used for veins 7–8 mm in diameter, two strips for 9- to 10-mm veins, and three or four strips for 11- to 12-mm veins.

A Rösch-Uchida transjugular access set (RUPS 100; Cook Inc.) with increased terminal curve of the cannula was then introduced and used to exit the central EJV or JV lumen. The cannula curve was oriented against the ventral venous wall and the trocar stylet was advanced through the venous wall into subcutaneous tissue (Fig. 2a).

A small skin nick was then done with a scalpel to advance the set through the skin. So-called ''exit catheterization,'' a through-and-through catheterization of the isolated EJV or JV segment by the 10-Fr sheath, was thus

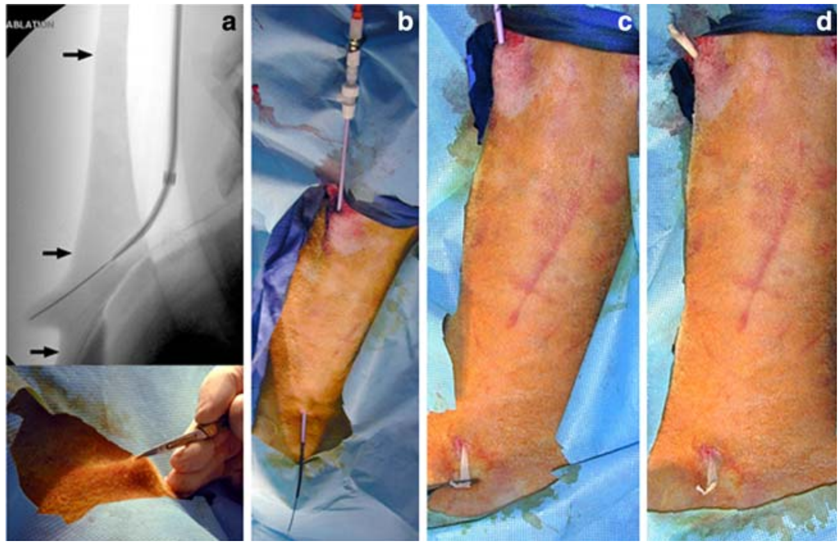

Fig. 2 Exit catheterization and ablation of the jugular vein in sheep. **a** Radiograph of the RUPS set after exit puncture from the jugular vein lumen toward the protruding skin and photography of a small skin nick (arrows on the radiograph indicate skin outline). **b** Exit catheterization of the jugular vein segment with RUPS set. **c** Fixation of the central end of the SIS strip during removal of the delivery sheath. **d** SIS strip ends prior to their cutting and subcutaneous burial

achieved [12] (Fig. 2b). Before SIS strip placement, a stainless-steel wire loop was attached to the strip end and the cartridge was soaked in saline to rehydrate the SIS. For vein ablation, one or two SIS strips were pulled into the 10-Fr RUPS sheath and then delivered into the vein by retracting the sheath while holding onto the SIS strip with a hemostat (Fig. 2c). In the case of three or four SIS strip implantations, a stiff safety guide wire was passed through the 10-Fr RUPS sheath before insertion of the first two SIS strips in order to preserve the tract access. After the first SIS strip placement the RUPS sheath was reintroduced and advanced over the wire for additional implantation. After deployment, both ends of the SIS strip(s) extended several centimeters beyond the skin surface (Fig. 2d). The free ends of the SIS strip(s) were then trimmed and held in subcutaneous tissue and the skin nick was closed with a suture. Immediate venograms through the ear vein were done to evaluate the degree of venous obstruction.

Follow-up Examinations

All animals were evaluated with ear vein venographies at 1 month. Six swine and one sheep were then sacrificed. Five sheep had follow-up ear venographies for 3 months before their termination. Animals were sacrificed with an intravenous application of 0.2 ml/kg pentobarbital and phenytoin sodium (Euthasol; Delmarva Lab, Midlothian, VA).

Gross and Histopathologic Studies

Necropsy with gross examinations focused on the ablated veins and surrounding structures. Ablated veins were photographed and harvested en bloc and specimens were preserved in 10% buffered formalin phosphate. After tissue fixation, vessel segments were cut into cross-sectional specimens and stained with hematoxylin/eosin (H&E).

Table 1 Outcomes of external jugular vein (EJV) ablations with SIS strips in six swine

No. of treated EJVs	12
Mean diameter(cm)	8.1 ± 0.7
Length of ablation (cm)	12.1 ± 2.7
Immediate occlusion (%)	12 (100)
1-month follow-up	
Occlusion (%)	10 (83.3)
Partial patency	2 (16.7)

Results

Bilateral exit catheterization of EJVs and JVs with modified RUPS was successfully accomplished in all animals. EJV and JV diameters and venographic results of MCD placement are summarized in Table 1 for the swine and in Table 2 for the sheep model.

The success rate of venous ablation related to the number of SIS strips used is summarized in Table 3 The length of isolated EJV and JV segments ranged from 5 to 13 cm in swine and from 14 to 24 cm in sheep, respectively.

Swine

The EJV diameters in six swine ranged from 7.0 to 8.9 mm. Successful EJV closure, defined by venogram as absence of blood flow inside the excluded EJV, was seen in 12 of 12 EJVs (100%) after SIS strip placements. Four EJVs were occluded with one SIS strip, and eight with two SIS strips.

Table 2 Outcomes of jugular vein (JV) ablations with SIS strips in six sheep

No. of treated JVs	12
Mean diameter (cm)	11.4 ± 0.6
Length of ablation (cm)	21.8 ± 1.3
Immediate occlusion (%)	11 (91.7)
1-month follow-up (*n* = 1)	
Occlusion (%)	2 JV (100%)
3-month follow-up (*n* = 5)	
Occlusion (%)	8 JV (80%)
Partial patency	2 JV (20%)

Table 3 Success rate of venous ablation related to number of SIS strips used

	Swine (*N* = 6)	Sheep (*N* = 6)
No. treated veins	12	12
No. immediate occlusions	12 (100%)	11 (91.7%)
Midterm occlusions based on no.of SIS strip		
	1-month follow-up: EJV (N = 12)	3-months follow-up: JV (N = 10)
1 strip	2/4 (50%)	—
2 strips	8/8 (100%)	2/5 (50%)
3 strips	—	3/3 (100%)
4 strips	—	2/2 (100%)

On follow-up venograms at 1 month, two of four EJVs treated with one SIS strip in growing pigs that doubled their size and weight were partially patent (50%). The other two EJVs treated with one SIS strip and eight of eight EJVs treated with two SIS strips remained closed (Table 3 and Fig. 3).

No progression of thrombosis in the deep venous system was found. Necropsies revealed 10 completely excluded EJVs and 2 partially patent EJV segments with partial incorporation of the SIS into the vein wall.

Sheep

The JV diameters in six sheep ranged from 10.4 to 12.1 mm. Successful closure of the JV was found on initial venograms in 11 of 12 JVs after SIS strip(s) placements. One JV treated with two SIS strips was partially patent. The other 11 JVs, treated with two to four SIS strips, were occluded. One sheep with two occluded JVs was sacrificed at 1 month for gross and histologic evaluation. Five animals with nine excluded JVs and one partially patent JV were followed for 3 months. Follow-up venograms in these animals showed partial patency in two of five JVs treated with two SIS strips. The rest of the five JVs treated with three or four SIS strips remained occluded (Table 3 and Fig. 4).

Necropsies revealed nine occluded veins completely filled with SIS. No progression of thrombosis into the deep vein system was found. In three cases of partially patent JVs, SIS was eccentrically located at the vein wall with partial incorporation into the wall. There has been one skin inflammatory reaction due to skin surface contact with implanted SIS.

Histologic Findings

At 1 month, histology in six swine and an early sacrificed sheep showed incomplete SIS remodeling with host cells. At 3 months, histologic evaluation in five sheep demonstrated almost-complete SIS remodeling and its penetration from the surface to the center, with variable fibrocytes, fibroblasts, endothelial cells, capillaries, and inflammatory cells (Fig. 3).

Discussion

SIS is a type I collagen-based biomaterial obtained from swine small intestine. In addition to the absence of adverse immunologic response and resistance to infection, SIS allows host tissue migration and works as a resorbable scaffold for remodeling by surrounding tissue. SIS has been experimentally investigated for various uses, including abdominal wall and urethral repairs, vascular grafts, and venous valve [13–18]. Recently, Schoder et al. successfully used SIS as an embolic material for management of type II endoleak after endovascular AAA repair. They reported that SIS had been remodeled by extensive scar tissue with an organized thrombus inside the aneurysmal sac [11].

This experimental pilot study showed that SIS can also be used for vein ablation without external compression and without need for tumescent anesthesia either. Its efficacy in this indication depends on the amount of SIS placed inside the targeted vein. The characteristics of SIS provide advantages for its use as an embolic material. In its lyophilized form, SIS is thin and compressed and, thus, can be delivered into the target vein through a relatively small-diameter introducer. In the target vein, it hydrates, expands, and occupies space. As this study showed, the targeted vein needs to be fully filled with SIS strips to achieve its durable obliteration-ablation. In our bench tests, we found that a SIS strip formed by fan-folding of a lyophilized SIS sheet and measuring about 2–3 mm in its compressed form expands to about 5–6 mm in diameter after immersion into saline. This expansion occurs in about 15–20 min. There-

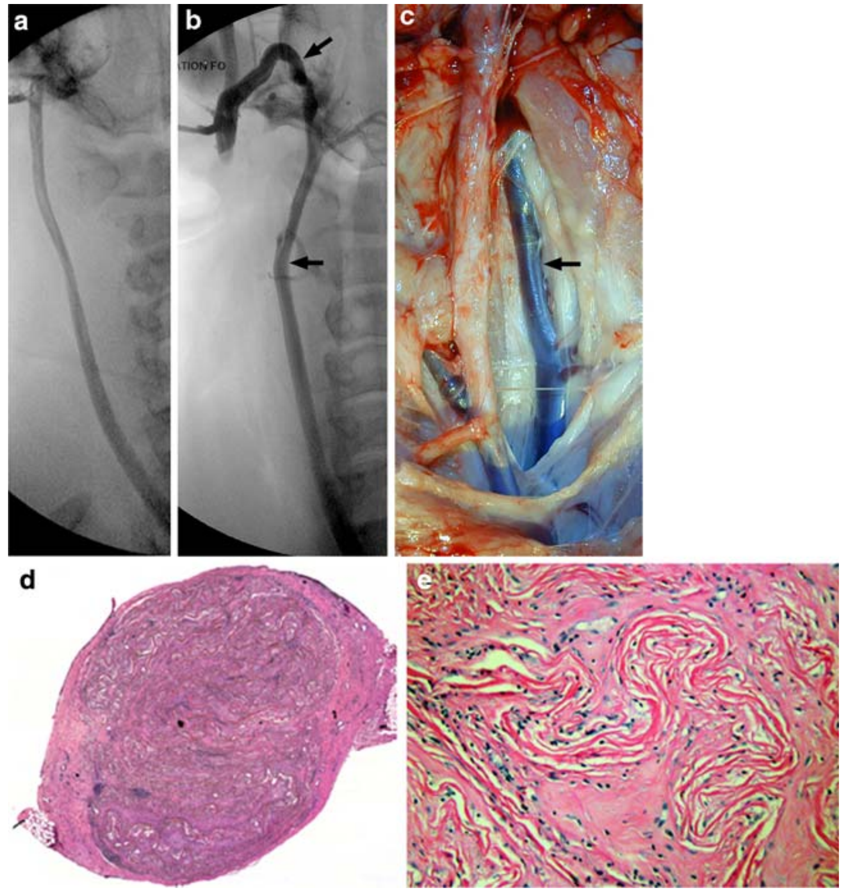

Fig. 3 Ablation of the external jugular vein (EJV) in swine with two SIS strips. **a** Baseline EJV venogram prior to ablation shows an 8.5-mm-diameter vein. **b** Follow-up ear vein venogram 1 month after ablation demonstrates exclusion of the EJV with opacification of the right internal jugular vein via a perforator (arrows). **c** Open-neck photography shows ablation of the EJV with a patent internal jugular vein (arrow). **d** Cross section of the occluded EJV. (H&E; original magnification, × 15.) **e** Magnified view shows SIS partially remodeled by fibrocytes, endothelial cells, lymphocytes, and plasma cells. (H&E; original magnification, × 200)

fore, we did not achieve durable ablation in two EJVs with diameters of 7 and 8 mm using one SIS strip.

Two SIS strips also were not sufficient for permanent occlusion in two JVs 11 and 12 mm in diameter. Immediate follow-up venograms showed occlusion of these veins, probably due to additional spasm after two punctures and RUPS set manipulation.

The follow-up venograms at 1 and 3 months, however, showed partial vein patency, with incomplete SIS filling of venous lumens. With the use of two SIS strips in swine and three or four SIS strips in sheep, their EJV and JVs, respectively, remain ablated in midterm follow-up studies. The ablated veins formed fibrotic cords and their lumens were packed with partially remodeled SIS at 1 month and completely remodeled SIS at 3 months, which contained fibroblasts, fibrocytes, lymphocytes, and endothelial and plasma cells. Technically, the SIS venous ablation was easy to achieve in both swine and sheep with exit catheterization. With the increased terminal curve of the RUPS, the anterior venous wall did not offer much resistance to puncture and a small scalpel skin nick allowed through-and-through catheterization. The puncture distances were not long, averaging about 12 cm in swine and 22 cm in sheep. Application of the SIS strips, even when four strips were used, was smooth with the pull-through technique, which did not offer much resistance. The residual ends of the SIS strips needed to be trimmed and tucked under the subcutaneous tissue.

The SIS venous ablation was safe in this study. The excluded venous segments formed a cord and there was no perivenous inflammation and no reaction or damage to the surrounding structures. No extension of thrombosis in the surrounding veins was found. There was only one slight inflammatory reaction at the puncture site when the SIS strip end was insufficiently trimmed and extended out of the skin.

The positive results of the study justify further exploration of SIS as material for venous ablation. Several routes need to be investigated. Long-term follow-ups need to be done to determine whether the ablated vein will remain occluded or undergo recanalization by new formed veins. The technique of a single instead of a double puncture should be explored. Exit catheterization is suitable for experimental animals, but for potential use in humans, with a 30- 50-cm-long GSV, a single puncture would be preferred. Other SIS forms should be explored, particularly those oriented on bigger SIS expansion such as a SIS sponge or SIS cohesive foam. These would require changes in application techniques, but if successful, SIS venous ablation could become an attractive alternative to the

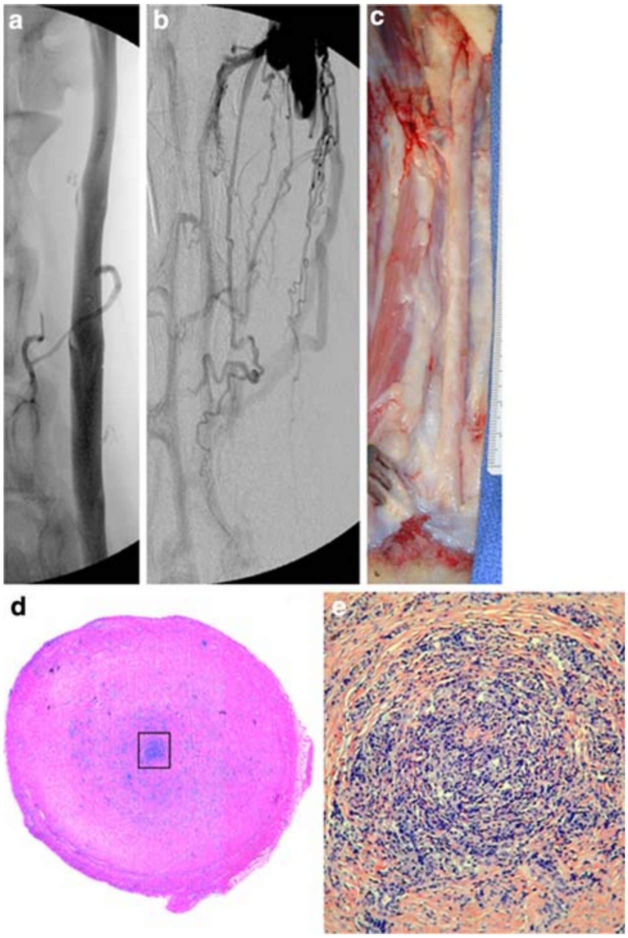

Fig. 4 Ablation of the left jugular vein (JV) in sheep with three SIS strips. **a** Baseline venogram prior to ablation shows a 12-mm-diameter vein. **b** Follow-up 3-month venogram shows exclusion of JV and collaterals to vertebral vein. **c** Open-neck photography shows smooth shiny surface of the cord like excluded JV. **d** Cross section of the occluded vein. (H&E; original magnification, × 15.) **e** Magnified view of the SIS remodeled with new collagen, fibrocytes, lymphocytes, and endothelial cells. (H&E; original magnification, × 200)

present surgical and minimally invasive treatments of varicose veins.

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