Small intestinal submucosa as a bioscaffold for biliary tract regeneration

Michael Rosen, MD, Jeffrey Ponsky, MD, Robert Petras, MD, Alicia Fanning, MD, Fred Brody, MD, and Frank Duperier, MD, Cleveland, Ohio

Background. Porcine small intestinal submucosa (SIS) biograft is used as a bioscaffold for regeneration of a variety of tissues. To date, SIS has not been used as a biliary tract graft. The purpose of this study was to evaluate the feasibility of using SIS as a scaffold for bile duct tissue regeneration in a canine model.

Methods. Fifteen, 25- to 35-kg mongrel dogs underwent midline laparotomy and exposure of the common bile duct. Nine dogs had a longitudinal choledochotomy and a 2- × 1-cm elliptical patch of 4-ply SIS placed using 6-0 polypropylene suture. Six dogs had the anterior two thirds of the bile duct resected and a 2- to 3-cm tubularized 4-ply SIS interposition graft placed. Dogs were killed at intervals ranging from 2 weeks to 5 months. Before killing, liver function tests (alkaline phosphatase [U/L] and total bilirubin [mg/dL]) were evaluated, cholangiograms were performed, and the bile duct was examined histologically.

Results. Fourteen out of 15 dogs survived and were healthy at the time of killing. The one failure was a result of a bile leak in a patched animal. The SIS showed signs of incorporation with infiltration of native fibroblasts, blood vessels, and biliary mucosa within 2 weeks. Within 3 months the SIS graft was replaced with native collagen covered with a biliary epithelium. No changes occurred at 5-month follow-up. One animal with an interposition graft developed a stricture at the proximal anastomosis within 2 months. In the remaining dogs, liver enzymes were normal, and the caliber of the common bile duct remained normal.

Conclusions. SIS can be used for regeneration of bile duct tissue in a canine model. In 13 of 15 dogs SIS resulted in regeneration of canine common bile duct when used as a patch or as an interposition graft. The potential for the use of SIS as a patch for biliary stricturoplasty, or as an interposition graft for repair of complex biliary injuries is encouraging. (Surgery 2002;132:480-6.)

From the Department of General Surgery, Cleveland Clinic Foundation, and Ameripath Corporation, Cleveland, Ohio
ingrowth and appears to foster cellular differentiation. This study analyzes SIS as a scaffold for regeneration of biliary ductal tissue in a canine model.

METHODS

Fifteen male adult mongrel dogs weighing 25 to 35 kg each were studied. The Cleveland Clinic Animal Research Protocol Committee reviewed and approved the study. Each animal was restricted from solid food 12 hours before the operation. All animals were anesthetized with intravenous sodium pentothal (20 mg/kg) and were maintained in a surgical plane of anesthesia with inhaled isoflurane (1%-2%) throughout the procedure. One gram of intravenous cefazolin was administered preoperatively.

An upper midline incision was made, and the extrahepatic biliary tree was exposed. In 9 animals, a 2-cm longitudinal choledochotomy was made on the anterior surface of the common bile duct. After soaking the SIS in normal saline solution for 10 minutes before implantation, a 2 × 1-cm elliptical patch of 4-ply SIS was sutured full thickness to the mucosal edges of the incised bile duct with interrupted 6-0 polypropylene. An additional 6 animals had 2 to 3 cm of the anterior two-thirds of their bile duct resected (Fig 1). Tubular SIS interposition grafts were made by wrapping a sheet of 4-ply SIS over an appropriately sized (16-18F) stent and sewing the edges of the SIS together with 6-0 polypropylene suture. The proximal and distal anastomoses were completed in an end-to-end fashion approximating the SIS to biliary mucosa using a running 6-0 polypropylene suture. Neither a T-tube nor an external drain was used. Oral ampicillin was given for 5 postoperative days. The animals were fed a standard chow diet beginning the first postoperative day.

Serum bilirubin and alkaline phosphatase were measured before killing each animal. The initial 9 dogs undergoing patch choledochoplasty were killed at the following intervals; 2 weeks (N = 3), 1 month (N = 3), 2 months (N = 2), and 5 months (N = 1). The 6 dogs with SIS interposition grafts were killed at 1 month (N = 1), 2 months (N = 2), 3 months (N = 2), and 5 months (N = 1). Before being killed, the animals were again placed under general anesthesia as previously described and reexplored through their midline incision. A 19G butterfly angiocatheter was placed in the gallbladder, and cholangiograms were performed under fluoroscopy. The dogs were killed with intravenous potassium chloride to effect. The common bile duct was excised and opened longitudinally on its posterior aspect. After photographs were taken, tissues were fixed with 4% formaldehyde solution. After fixation, each specimen was sectioned in a transverse fashion and tissue samples submitted for light microscopy. Segments of proximal normal common bile duct were studied as controls. The tissue sections were processed, imbedded in paraffin wax, cut at 6 μm, and stained with hematoxylin and eosin for microscopic analysis.

RESULTS

All 15 dogs survived the operation, and 14 out of 15 dogs were healthy at the time of killing. One of
the patched animals was alive at 2 weeks with a decreased appetite, diarrhea, and weight loss. Liver function tests revealed an alkaline phosphatase 980 U/L, total bilirubin 1.6 mg/dL (laboratory normal values; alkaline phosphatase 20-120 U/L; total bilirubin 0.0-1.5 mg/dL). The animal was killed. At autopsy, a large bile leak was noted. It appeared that a technical suturing error resulted in disruption of the distal end of the suture line. Microscopically, the patch was covered with a biliary mucosa, and native blood vessels were identified infiltrating the patch. No signs of necrosis were evident on the native common bile duct. While we acknowledge the possibility of patch or bile duct necrosis causing this leak, on the basis of the aforementioned reasons we considered this animal to be a technical failure, and it was excluded from further analysis.

One animal in the patch group had a slightly elevated alkaline phosphatase activity (127 U/L) at 2 months despite having a normal cholangiogram and no biliary dilatation. One of the interposition graft animals had an abnormal alkaline phosphatase (200 U/L) at 2 months. This animal also had a narrowing at the proximal anastomosis secondary to stricture formation. The other studied liver enzyme activities were normal throughout the study.

On gross inspection at 2 weeks, there were moderate adhesions of the omentum and liver edge to the SIS. The SIS was visible macroscopically and bile stained. At 1 month, filmy adhesions to the SIS remained, and only the central area of the SIS was visible grossly. By 2 months, the SIS was barely discernible from within the lumen and only minimal adhesions were present. By 5 months, minimal adhesions to the surgical area were apparent, and

Fig 2. Cholangiogram performed at 3 months. Arrows indicate area of SIS interposition graft. There is no evidence of luminal narrowing or stricture formation. There is no proximal dilatation and contrast passes freely into the duodenum.
the area of SIS could only be verified by identification of the remaining polypropylene sutures. Importantly, bile crystals did not form within the duct or on the SIS.

We detected neither biliary leaks nor biliary fistulas on any cholangiogram; however, 1 stricture occurred in the 6 dogs in the interposition group. This animal appeared clinically well at killing of 2 months. However, on cholangiogram a discrete narrowing at the proximal common bile duct anastomosis was noted. Contrast passed into the duodenum, and moderate proximal dilatation was present. No other animals developed narrowing or strictures during the 5-month follow-up period. The lumen remained of normal caliber. There was no proximal biliary dilatation, and contrast passed freely into the duodenum (Fig 2).

The microscopic appearance of both the patch and interposition graft animals was similar. At 2 weeks the predominate cells included macrophages, histiocytes, fibroblasts, and polymorphonuclear cells. The edge of the patch showed signs of tissue incorporation with deposition of native collagen and infiltration of native blood vessels. Within 2 weeks, biliary epithelium was evident at the edges of the SIS patch (Fig 3). By 1 month, the SIS showed increasing signs of incorporation. The inflammatory response lessened with the cellular infiltrate comprised predominantly of fibroblasts and scant macrophages. The bile duct wall was composed of fibrous tissue similar to the normal canine common bile duct (Fig 4, A). Within 2 months, the SIS was almost completely replaced with native collagen, and only tiny amounts of residual SIS were identified. The area was covered with biliary epithelium and a surrounding fibrous wall. At 5 months, the SIS was replaced with native collagen covered with biliary epithelium (Fig 4, B).

DISCUSSION

This study suggests that SIS can be placed successfully as a patch or as an interposition graft in the canine common bile duct. It may be possible to use a SIS patch to treat bile duct strictures. After opening the stricture longitudinally, the anterior wall could be reconstituted with a SIS patch. This would avoid the necessity of a biliary-enteric bypass. Furthermore, if part of the common duct wall is accidentally excised during a biliary operation, our results support that a noncircumferential defect can be bypassed with a SIS interposition graft. On the basis of this study, it is probable that sufficient regeneration of the biliary tract will occur to provide an adequate and functioning biliary channel.

SIS is a xenogenic, relatively acellular collagen-rich membrane. SIS is a trilaminar derivative of the porcine small intestine composed of the stratum compactum layer of the tunica mucosa, the tunica

---

Fig 3. The lumen is at the top of the image. Small arrow depicts new blood vessels growing in the patched area by 2 weeks. Large arrow identifies the biliary mucosa covering the SIS patch by 2 weeks.
muscularis mucosae, and the tunica submucosa. It is prepared from slaughterhouse pigs by mechanical abrasion removing the mucosal and serosal layers. In vitro, SIS supports the growth of various cell types including keratinocytes, endothelial cells, smooth muscle cells, and bone cells. In vivo studies with SIS document neovascularization, epithelial cell growth, and connective tissue deposition without immune rejection. Several animal models have studied the use of SIS as a dural replacement, a wound dressing, a ureteral replacement, and a diaphragmatic prosthesis. SIS has been used experimentally for vascular grafts, bladder replacement, tendon and articular cartilage repair, and for correction of abdominal wall and corneoscleral defects.

On the basis of microscopic studies, SIS does not appear to stimulate cellular immune rejection in animal models. SIS is essentially an acellular collagen. It is conjectured that the collagen molecule is so structurally conserved between species that it fails to be immunogenic across species and therefore elicits a minimal inflammatory reaction that could be a result of the trauma of operation, itself, during implantation. After undergoing extensive immunologic testing in more than 600 cross-species implants, rejection of SIS has never been documented. Thus, SIS acts as a nonimmunogenic, collagen-rich acellular material that could function as a universal tissue graft.

SIS-related site-specific regeneration of tissues has been observed consistently, however, the exact mechanism remains unclear. There is evidence that host cells proliferate and native site-specific tissues differentiate into and invade the SIS material. Operations and perhaps the SIS graft, itself, trigger regenerative processes including release of angiogenetic factors and various cytokines associated with cell migration, differentiation, and deposition of extracellular matrix. As a result, the reconstructed tissue is structurally and functionally similar to that of the target tissue. Candidate factors identified within SIS that could initiate this process include glycosaminoglycans, fibronectins, fibroblast growth factor, and transforming growth factor β-related compound. Several investigators hypothesize that these molecules and various other growth factors provide the appropriate signals to surrounding tissue to invoke a site-specific remodeling process.

The duration and cellular events of SIS tissue remodeling are well documented in the literature. In other animal models, SIS initially elicits a granulation tissue and foreign body giant cell response with moderate inflammation. In our biliary grafts, we did not observe this foreign body giant cell reaction. However, as time passes, the granulation tissue is replaced by fibroblasts with minimal inflammation. This process appears complete by 2 months with no evidence of change at 3 months. Using monoclonal antibodies to SIS, there is a gradual deterioration of the implanted native SIS. Kropp et al used SIS as a bladder wall substitute in bladder augmentation operation in rats. At 2 weeks postoperatively, the graft material was completely covered by urothelium, and by 48 weeks all 3 layers of the normal bladder (urothelium, smooth muscle, and serosa) were present and were grossly and microscopically indistinguishable from the normal rat urinary bladder. In our experimental model, we document a biliary epithelium covering the SIS graft as early as 2 weeks and completed by 1 month. By 2 months, the SIS graft was replaced by an organized deposition of collagen, and at 5 months the SIS was completely replaced with native collagen with a covering biliary epithelium that was normal caliber for canine common bile duct.

Historically, patch biliary strictureplasty using other substances induces excessive scar tissue in the reconstructed bile duct resulting in a recurrent stricture. It is postulated that the exuberant fibrosis is the direct result of the necrosing and sclerosing effect of bile on native mesenchymal tissues. Rutledge et al suggests, and we agree, that any successful repair of a noncircumferential bile duct injury should use readily available tissue, be impermeable to bile, and avoid the formation of excess scar tissue. On the basis of our experimental model, SIS could be an ideal type of graft material for the biliary tree. SIS is impermeable to bile, and the only leak in our series resulted from a technical suturing error. We detected no example of SIS necrosis throughout the study period, which is likely a result of the early ingrowth of capillaries into the patch by 2 weeks. SIS appears to adequately resist the sclerosing affects of bile as it is rapidly covered with a biliary epithelium. Furthermore, the SIS graft, itself, is replaced by organized collagen deposition without the excessive scar tissue. The 1 stricture occurring in the interposition graft animal might have been a result of native bile duct ischemia, which, however, may have important implications for the use of SIS in established ischemic biliary structures clinically. Because the native common bile duct consists of predominately collagen covered by a biliary mucosa, determining the exact histologic transition between native common bile duct and SIS is unclear. Despite the proximal anastomotic stricture in this animal, the
Fig 4. A, Normal canine common bile duct, with biliary mucosa and predominately fibrous wall with minimal smooth muscle. B, At 5 months the interposition graft is replaced with normal appearing canine common bile duct.
remaining SIS interposition graft was undergoing successful remodeling and was covered with a bilary mucosa and being infiltrated with native blood vessels. Thus, 8 of 9 patch grafts and 5 of 6 interposition grafts of SIS resulted in regeneration of normal canine common bile duct tissue.

In conclusion, a SIS biliary conduit is appealing because it is readily available, can be tailored to fit any bile duct defect, does not involve enteric anastomoses, does not result in patch dilation and resultant bile stasis, and does not require stenting during the period of tissue ingrowth.

REFERENCES

17. Leary HJ, Kelley GE, Michaels WL. The use of preserved bile duct homografts to bridge common duct defects. Surgery 1953;34:238-44.