Invited Review

Regeneration of extrahepatic bile duct
—possibility to clinical application by recognition of the regenerative process

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Abstract

The biliary epithelium is continuously exposed to highly cytotoxic bile acids and pathogens and thus is at persistent risk for injury. The monolayer mucosal epithelium protects the body from these dangers and once injured, it needs to be repaired without delay as is the case with other parts of the gastrointestinal tract (Okamoto et al., 2002). The bile duct repair process essentially involves reconstruction of the bile duct with migrating cells, in which some cytokines and monokines are shown to have a role (Kollet et al., 2003; Isse et al., 2005; Harada et al., 2006). There are many questions about the process by which bile ducts, especially extrahepatic bile ducts, regenerate to form a new tissue, and most of them remain unanswered. This is because many of the models available for in vivo studies of extrahepatic bile duct regeneration

Key words: artificial bile duct, tissue-engineering, bioabsorbable polymer, bone marrow transplantation

Introduction

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induce obstructive jaundice by ligating the extrahepatic bile duct to observe intrahepatic bile duct proliferation, and would not allow us to observe the extrahepatic bile duct till the healing process is triggered after injury and till the duct repairs, regenerates and grows mature (Holterman et al., 2002). We have reported that implantation of a bioabsorbable polymer tube (referred to as “artificial bile duct”) as a bypass graft into the extrahepatic bile duct resulted in bile duct regeneration in the graft site after the artificial duct had been degraded and absorbed (Miyazawa et al., 2005). This is an experimental model for artificial bile duct implantation that enables us to observe the injured extrahepatic bile duct up to completion of regeneration, and we have been performing histological studies on the extrahepatic bile duct regeneration process using this model.

We briefly describe our findings on extrahepatic biliary tissue regeneration with the possibility for clinical applications in mind.

Extrahepatic bile duct regeneration in the artificial bile duct implantation model

Bile ducts with the lumen lined with epithelial cells have a variety of functions, the most important of which is to carry bile out of the liver to the duodenum. Attempts have been made to develop a substitute for bile duct that can fulfill this function (Ishizaki et al., 1995; Rosen et al., 2002). However, the substitutes so far tested failed to form epithelium on the lumen of the duct where bile flows or on the anastomosis with the native duct, causing obstruction in the end, and none of them proved suitable for long-term use. Utilizing tissue engineering techniques rapidly evolving in recent years, we began trials for bile duct regeneration with a tube made of bioabsorbable polymer and seeded with autologous bone marrow cells. Briefly, bone marrow cells taken from the swine sternum were seeded onto the interior of a tubular scaffold of bioabsorbable polymer. The scaffold was made of a copolymer of polylactic acid and polycaprolactone and reinforced with polyglycolic acid fibers. It was designed to be absorbed in six to eight weeks by the body (Watanabe et al., 2001) (Fig. 1). Hybrid pigs served as recipients of the artificial bile duct fabricated by tissue engineering. One hour after seeding the scaffold with bone marrow cells, the artificial duct was implanted into each of the pigs from which bone marrow cells had been collected. The common bile duct was cut around the confluence with the cystic duct. The duodenal end of the common bile duct was ligated, while the hepatic end was anastomosed to the artificial bile duct. Then, a hole, 5 mm in diameter, was made in the descending duodenum, to which was sutured the remaining end of the artificial bile duct (Fig. 2). The graft site was recovered six months after implantation and subjected to macroscopy and histology. Recipient pigs survived up to the sixth month after implantation when they were sacrificed. They gained weight after operation with no evidence of jaundice. On histology as well as on macroscopy, the neo-bile duct was almost similar to the native common bile duct in morphology (Figs. 3a and 3b), and its portion possibly corresponding to bile duct epithelial cells was positive for CK19 just as the native duct was. The tubular artificial bile duct of bioabsorbable polymer seeded with autologous bone marrow cells effectively functioned as a bile duct for six months. The results showed that the tubular artificial bile duct, a substitute for extrahepatic bile duct, could carry bile to the duodenum without any leakage in the peritoneal
Regeneration of extrahepatic bile duct cavity while maintaining its tubular form in the short term following implantation, and that once the artificial bile duct had been degraded and absorbed by the body, a new extrahepatic bile duct similar to the native duct developed in the graft site and functioned as a bile duct without showing stenosis.

**Histology of extrahepatic bile duct regeneration**

It is of interest to know how the injured extrahepatic bile duct heals. In the early stages of
study, we seeded the interior of the artificial duct with bone marrow cells prior to implantation. This was to prevent bile leakage, since the scaffold we used was in a tubular form and made of porous spongy polymer, and to investigate the possibility that bone marrow cells might accelerate biliary epithelization as was reported for blood vessels (Noishiki et al., 1996). Neo-bile ducts arising from the scaffold not seeded with bone marrow cells did not leak bile in the early post-implantation phase. The rate and extent of biliary epithelization were comparable.
whether bone marrow cells had been seeded or not. To seed bone marrow cells made no contribution to regeneration in this model. The observation that the polymer graft implanted without cell seeding gave rise to biliary epithelium demonstrated that migrating cells from outside the graft site formed biliary epithelium. The cell source for biliary epithelial regeneration was sought using this model. Findings at seven weeks post-implantation showed that epithelial regeneration proceeded in no directed manner, starting neither from the hepatic nor duodenal part of the neo-bile duct, but that the graft site was uniformly re-epithelialized at about the same rate (Fig. 4). Re-epithelization may not result from continuous division, proliferation and differentiation of cells in the hepatic or duodenal part of the graft but from differentiation of stem/progenitor cells for biliary epithelial cells that have uniformly adhered to the scaffold. Bone marrow cells are reported to migrate to injured liver via peripheral circulation and to differentiate into hepatocytes there (Jang et al., 2004; Oh et al., 2007). This may be the case with our model and we are investigating the possibility that juvenile cells like bone marrow cells may adhere onto the scaffold and differentiate to regenerate the bile duct. In the subepithelium of the extrahepatic bile duct is smooth muscle. Whether smooth muscle regenerated with the epithelium was assessed using our bile duct implantation model. Desmin positive cells were detected in the neo-bile duct at seven weeks post-implantation (Fig. 5), giving an evidence for regenerating smooth muscle in the subepithelium, though scant compared with the native extrahepatic bile duct. These results demonstrated the potential of this artificial bile duct as a scaffold for regeneration of the bile duct epithelium and smooth muscle, and thus regeneration of the entire bile duct tissue.

**Development of novel therapies for biliary disease**

The outcomes of current treatment strategies for biliary disease are not always favorable. This may reflect poor knowledge of the biliary system. To cite balloon dilatation of the bile duct, effects of dilatation on epithelial cells, adventitial connective tissue and smooth muscle remain unclear; criterion on which to define the extent of dilatation has not been established; mechanism and process by which the bile duct severely injured during balloon dilatation heals and repairs remain to be elucidated. Treatment modalities in disregard of bile duct regeneration
may partly be responsible for the unfavorable outcome (Tsujino et al., 2006; Grader et al., 2007). We have proposed to use our method of inducing bile duct regeneration as the following treatment aids in clinical practice:

1) **Bioabsorbable biliary stent** (Fig. 6)

To reduce troubles with tubular stents, metal stents and balloon dilatation for biliary stenosis, a bioabsorbable stent is being developed as one of treatment aids especially for benign biliary stenosis (Miyazawa et al., 2007). The stent is characterized by that it is degraded and

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**Fig. 5.** Regenerating bile duct seven weeks after artificial bile duct implantation (desmin staining). Desmin positive cells were sparse in the subepithelium.

**Fig. 6.** Bioabsorbable bile duct stent. Made of a copolymer of polycaprolactone and polylactic acid (25:75), the stent gets fragile within the body in about three to six months.
absorbed by the body after it has ensured patency wide enough for free passage of bile for a certain period of time and after it has induced epithelial regeneration.

2) Novel surgical procedures making use of artificial bile ducts and patches

Surgery in the 21st century seeks to preserve biological function to the utmost. The papilla of Vater is one example. Retrograde infection following loss of papilla function causes narrowing and chronic inflammation at the anastomosis, which have recently been reported to promote the development of carcinomas (Farazi et al., 2006; Malhi and Gores, 2006). We have been developing artificial bile ducts and bile duct patches made of bioabsorbable polymer as tools for a new therapeutic approach that resects/replaces only the affected portion of bile duct and preserves the papilla of Vater (Aikawa, M., unpublished observation).

3) Potential for cell replacement therapy for biliary disease

In our artificial bile duct implantation model, extrahepatic bile duct regeneration proceeded in no directed manner, suggesting that undifferentiated cells in the peripheral circulation might migrate into the graft site to differentiate and grow into a mature bile duct. If peripheral circulating cells contribute to bile duct regeneration, cell replacement therapy with bone marrow cells containing stem/progenitor cells for bile duct epithelial cells will be feasible just as bone marrow transplantation considered for liver cirrhosis (Fausto, 2004; Kisseleva et al., 2006). We are making experimental approaches to study the feasibility using our model.

Conclusions

Tissue engineering techniques were successfully applied to extrahepatic bile duct regeneration. The results encourage us to work out a method for inducing bile duct regeneration that is effective and can be used in clinical settings. The method is based on quite a new concept and evidence of its feasibility may still be inadequate. Progress in the understanding of regeneration factors in bile will foster development of therapeutic strategies aimed at preservation of biological function and built on tissue engineering techniques.

References


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