Photodynamic therapy for biliary tract organ via a novel ultra-small composite optical fiberscope

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Abstract. Photodynamic therapy (PDT) is a method used treat tumors via utilizing photodynamic reactions between photosensitive substances with tumor affinity and lasers. For overall bile duct cancers, PDT has been demonstrated to resolve stenosis and improve prognosis; however, when limited to intrahepatic bile duct cancers, modifications to the laser irradiation are necessary as surrounding hepatocytes incorporate a large amount of photosensitive substances. Furthermore, the intrahepatic bile duct is thin, and a guide sheath and thin fiber are necessary to transport laser irradiation probes to the target region. In the present study, a parallel-type ultra-small composite optical fiberscope (COF) with an outer diameter of 1 mm or smaller was developed to target a thin intrahepatic bile duct. PDT was performed using an animal model and talaporfin sodium (Laserphyrin), which is rapidly excreted by hepatocytes and is suitable for use with a long-wavelength laser due to its high tissue penetrating ability. The results demonstrated that Laserphyrin does not cause necrotic changes in the normal biliary tract mucosa. In addition, COF images of sufficient quality were acquired. The present results suggest that COF may be used for the treatment of deep bile duct lesions.

Introduction

Bile duct cancers are characterized by horizontal advancement along the bile duct wall. When a lesion extends toward the liver side and reaches the intrahepatic region, hepatectomy is necessary for resection (1). However, the resection of intrahepatic bile duct has limitations. Further resection is not possible if cancer cells are still present (1). Furthermore, residual cancer may be observed in the bile duct stump on postoperative histopathological examination, and postoperative adjuvant therapy may be required (2).

Photodynamic therapy (PDT) is a method used to treat tumors by utilizing photodynamic reactions between photosensitive substances with tumor affinity and lasers (3-5). For inoperable bile duct cancer, PDT has been demonstrated to effectively resolve cancer-associated stenosis and improve patient prognosis (3-5). However, the effects of PDT on bile duct cancer that have advanced into the intrahepatic bile duct have not been reported. This is due to two difficulties; the first is that it remains unclear whether PDT may be safely applied in the liver, as photosensitive substances accumulate there. When PDT impairs normal bile duct epithelial cells, outflow obstruction of bile occurs in a thin intrahepatic bile duct, causing segmental cholangitis and biloma (6,7). Furthermore, impairment of endothelial cells in the hepatic artery and portal vein, which are parallel to the bile duct, may cause thrombus formation and vascular breakage, inducing hepatic infarction and hemorrhage (6,7). To overcome this, a novel second-generation photosensitive substance, talaporfin sodium, was used in the present study. Talaporfin sodium accumulates very rapidly in tumors compared with conventional photosensitive substances, and is excreted by normal tissues (8-10). The second difficulty is that the charge-coupled device (CCD) camera-equipped cholangioscope typically used for treatment has a large diameter and cannot be advanced into intrahepatic bile ducts. To overcome this, a parallel-type ultra-small composite optical fiberscope (COF) was developed, through which transpapillary PDT was able to be applied to peripheral intrahepatic bile duct cancer (11,12). The COF used in the present study was equipped with a camera for observation with ordinary light and a laser irradiation hole, with a diameter <1 mm. By advancing the COF upstream of the stenoic region using the conventional duodenoscope as a parent scope, observation of and laser irradiation to intrahepatic bile ducts becomes possible. The aim of the present study was to investigate the efficacy of talaporfin sodium and COF.

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Abbreviations: PDT, photodynamic therapy; COF, parallel-type ultra-small composite optical fiberscope

Key words: photodynamic therapy, biliary tract, ultra-small, fiberscope

Materials and methods

Animals. A total of 2 female Japanese white rabbits (mean body weight, 2.57 kg; age, 13 weeks old) were obtained from Sankyo Labo Service Co., Inc., (Tokyo Japan). Rabbits were fed with standard feed (CR-1; CLEA Japan, Inc., Tokyo, Japan) and adminsitered 150 g/day and rabbits had free access to water. A total of 2 female miniature swines (mean body weight, 8.4 kg; age, 5 months old) were obtained from Orienttal Yeast Co., Ltd. (Tokyo, Japan). Their feed (MP-A; Orienttal Yeast Co., Ltd.) was given *ad libitum* following high-pressure steam sterilization and swines had free access to water. Animals were individually housed in cages in a temperature-controlled room (22-24˚C) with a relative humidity of 60-65% and subjected to a 12-h light/dark cycle at Tokyo Medical University Hospital (Tokyo, Japan). All experiments were approved by the Animal Care and Ethics Committee of Tokyo Medical University.

Photosensitizer. Talaporfin sodium (Laserphyrin; Meji Seika, Ltd., Tokyo, Japan) is composed of aspartic acid conjugated to the D ring of the chlorine structure with an absorption peak at 664 nm (8,13). Laserphyrin was reconstituted as a 1.0 mg/ml solution to prevent degradation by light.

Laser. A high-power red laser diode system (Panasonic Healthcare Co., Ltd., Tokyo, Japan), was used in the present study. The laser wavelength was adjusted to 664 nm to match the absorption bands of Laserphyrin, and the system has a power output of 10-500 mW/cm^2 at the fiber tip in a continuous wave mode. The delivered energy was adjustable from 50-1,000 J/cm^2.

Laser probe one. Radial probes (ZH-L5041HJP; Panasonic Healthcare Co., Ltd.) were used for laser radiation to the Japanese white rabbits.

Laser probe two. COF (OK Fiber Technology Co., Ltd., Kyoto, Japan) was used for laser radiation to miniature swine. This COF was independently developed as an endoscope by co-author Dr. Oka for this study as previously described (11,12), but with a smaller outer diameter. The outer diameter of the COF is <1 mm, and the scope has a light guide, holes for observation (10,000 pixels) and a photodynamic laser with a diameter of 0.4 mm (Fig. 1A). The COF was used with the transpapillary approach. Firstly, the target peripheral bile duct was identified by endoscopic retrograde cholangiopancreatography and a guide wire was inserted. A guide sheath (disposal guide sheath SG-200C; Olympus Corp., Tokyo, Japan; outer diameter, 1.95 mm; translucent with a slightly bent tip and high bile duct selectivity) was applied into which the COF was inserted. The guide sheath was used to avoid excess bending of the COF and saline was perfused into the sheath in order to secure a visual field in the bile duct. The COF is able to be directly inserted through the forceps hole of a duodenoscope, cholangioscope, SpyScope and next-generation direct cholangioscope (Fig. 1B).

Evaluation of Glisson's capsule ensheathing the hepatic artery, portal vein, and bile ducts and hepatocytes disorder by PDT (experiment one). This experiment was performed to evaluate bile duct and hepatic disorders associated with laser irradiation inside the biliary ductal lumen. Japanese white rabbits were intraperitoneally anesthetized with pentobarbital sodium (Kyoritsuseiyaku Corp., Tokyo, Japan) and underwent laparotomy 4 h following the intravenous administration of Laserphyrin at 63 mg/kg, as previously described (9,10). An incision was made in the duodenum wall, and a 6 Fr sheath was inserted trans-transpapillary. Laser probe one was inserted toward an intrahepatic bile duct using the lumen of the sheath. Intraluminal laser irradiation (100 J/cm^2) was performed with continuous application of physiological saline into the lumen of the sheath. Following irradiation, the fiber and sheath were removed and the duodenum wall was closed with sutures. Rabbits were administered with Evans blue stain solution via an ear vein and were stained blue at 48 h after PDT. The liver and the biliary tract were harvested and fixed for 2 days in 10% neutral buffered formaldehyde (Wako Pure Chemical Industries, Ltd., Osaka, Japan) at room temperature and embedded in paraffin. Sections (2-3 μm thickness) were stained using hematoxylin-eosin for histological diagnosis at a magnificaiton x400 using an optical microscope (Olympus Corp.) following sacrifice.

Evaluation of normal gallbladder epithelial damage by PDT (experiment two). A miniature swine was laparotomized under general anesthesia, the gallbladder wall was incised and the guide sheath was inserted. The COF was inserted and advanced to near the sheath, and the gallbladder mucosa was observed while physiological saline was applied. Laserphyrin was then intravenously administered at a dosage of 10 mg/kg. A total of 4 h following Laserphyrin administration, a laser was applied to the gallbladder mucosa at the fundic and neck area of the gallbladder (100 J/cm^2). The irradiation region was marked with non-absorbable surgical suture. The fundic area of the gallbladder alone was excised immediately following irradiation. The lumen of the remnant gallbladder was subsequently closed. Subsequently, the swine was sacrificed and the remnant gallbladder was harvested at 4 weeks post irradiation. The excised specimen was processed through the standard course described above (n=2).

Results

Experiment one. Following PDT via the biliary ductal lumen, tissue damage in the liver and biliary duct of Japanese white rabbits (n=2) were assessed. All animals survived without apparent disorder 48 h following treatment. All organs were stained blue, and the blood flow of the whole liver was confirmed macroscopically. Histologically, the epithelium of the extrahepatic bile duct and adjacent tissue and blood vessels were normal. No disorder or damage was observed in the intrahepatic bile ducts, portal vein, intrahepatic veins, hepatic artery or intrahepatic arteries around the first branch of the right intrahepatic Glisson (Fig. 2A). Focal ischemic necrosis was observed in hepatic cells around the extrahepatic Grison and those at the most marginal site in the irradiation area (Fig. 2B).

Experiment two. The mucosal folds of the gallbladder were observed using the COF and saline circulation (Fig. 3). Congestion occurred immediately following irradiation, and

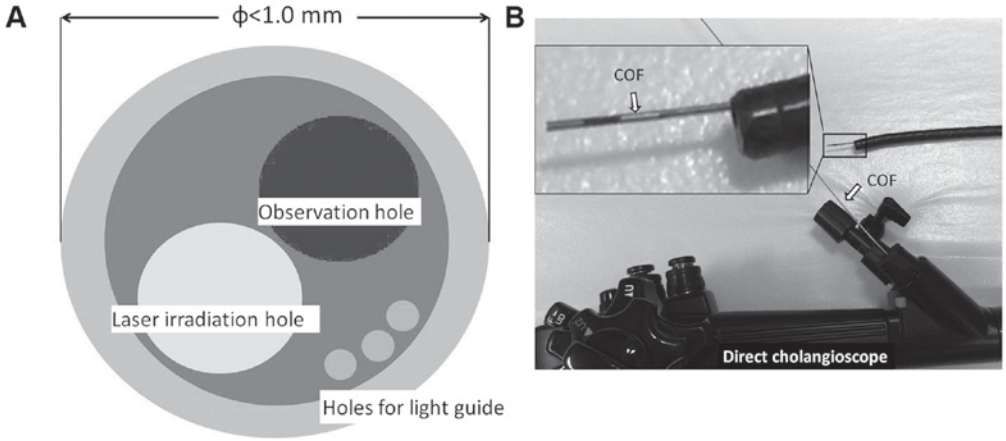

Figure 1. Schematic and photographic representations of COF. (A) Tip image of COF (front view). The outer diameter of the COF is <1 mm, and the scope has holes for observation and a photodynamic laser with a diameter of 0.4 mm in parallel. (B) The COF may be directly inserted via the forceps channel of a direct cholangioscope. COF, parallel-type ultra-small composite optical fiberscope.

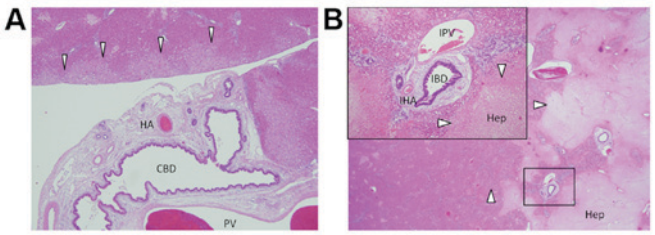

Figure 2. Histological features of the extra- and intrahepatic bile duct and adjacent tissue. (A) No damage was observed to the bile duct epithelium, portal vein or hepatic artery. In contrast, mild degeneration was observed in liver cells adjacent to the irradiation field (white arrow heads). (B) Damage to intrahepatic Glissons capsule was low compared with the surrounding hepatocytes (white arrow heads). HA, hepatic artery; CBD, common bile duct; PV, portal vein; IBD, intrahepatic bile duct; IPV, intrahepatic portal vein; IHA, intrahepatic hepatic artery; Hep, hepatocytes. (magnification, x40).

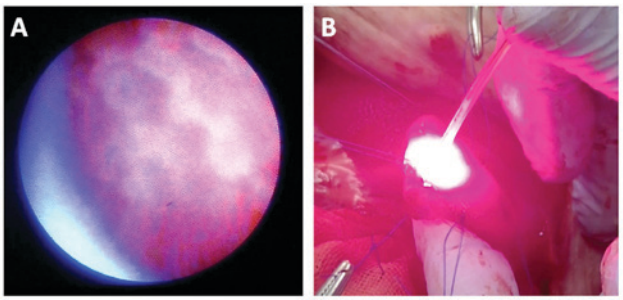

Figure 3. Images acquired using COF. (A) Endoscopic images acquired using COF. The quality of the image obtained allows the shape of the mucous membrane to be observed. (B) Radiation field expands with the distance between the COF tip and the target. COF, parallel-type ultra-small composite optical fiberscope.

intra- and submucosal vasodilatation occurred (Fig. 4A). At 4 weeks post-irradiation, the mucosa appeared mostly normal (Fig. 4B).

Discussion

Transhepatic PDT was applied to 7 patients with non-resected bile duct cancer (locally advanced cancer in 5 patients, and

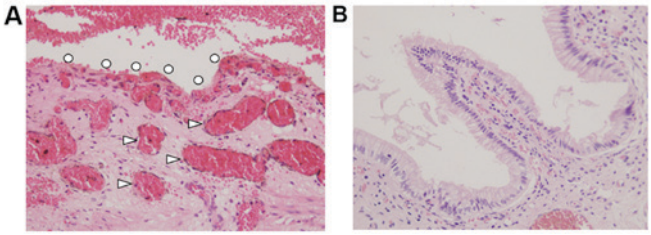

Figure 4. Histological features of the gallbladder mucosa. (A) Mucosa defects (white circles) and marked vasodilatation (white arrow heads) were observed immediately following laser irradiation. (B) Complete repair and maturation of the mucosa was observed 4 weeks following laser irradiation. (magnification, x200).

lesions were localized in the wall but not resected due to another reason in 2 patients) at Tokyo Medical University in 1999, resulting in sufficient tumor control in the bile duct lumen and conversion to cancer negativity on biopsy (6). In 2006, median survival time was compared between patients treated with multidisciplinary treatment including PDT (n=34) and untreated (n=23) (7). In 2006, a study by Witzigmann *et al* (5) reported the 10-year outcomes of PDT-treated patients (n=60) with unresectable bile duct cancer. The 1-, 3- and 5-year survival rates were 69, 30 and 22%, respectively, whereas the 5-year survival rates of patients surgically treated with R0, R1 and R2 resections were 27, 10 and 0%, respectively. These results demonstrated that the 5-year survival rate of patients treated with PDT exceeded those of patients treated with R1 and R2 resections (5).

The objective of the present study was to investigate local treatment with PDT as a surgical resection-associated neo-adjuvant or postoperative adjuvant therapy. Although there is no specification of PDT as adjuvant therapy, PDT was applied to stump-positive cases predicted to have residual cancer on the cut surface of the bile duct as adjuvant. In 2000, a study by Berr *et al* (14) applied PDT to a tumor of the common bile duct at 250 J/cm^2, and the bilateral bile ducts at 250 J/cm^2, and performed a hepatectomy 23 days later (14). It was observed that the tumor at a 4-mm depth was completely necrotized, hepatic duct-jejunum anastomosis formation

completed, and no stenosis of the anastomosed region occurred even at 18 months post-treatment. This was the initial report on neo-adjuvant PDT for bile duct cancer (14). In 2003, a study by Wiedmann *et al* (15) performed surgery following PDT at 242 J/cm^2 and achieved complete necrosis of the tumor at a 4-mm depth, with only mild inflammation of the tumor-free bile duct and mild complications of hepatocholangiojejunostomy in 4 patients (15). Based on these reports, surgery performed following PDT does not seriously impair hepatic duct-jejunum anastomosis. In the present study, PDT applied to the gallbladder epithelium in miniature swine caused congestion and edema immediately following irradiation; however, the epithelium recovered to almost normal after 4 weeks. A study by Nanashima *et al* (16) applied Photofrin-PDT to the bile duct in 8 patients following non-curative surgical resection. The recurrence-free period was 17.6 months, significantly longer compared with the patients who were not treated with PDT (8 months). A total of 4 stump-positive patients were subsequently treated with Laserphyrin-PDT. Liver metastasis occurred in 1 patient (without local recurrence), however 6-13-month recurrence-free survival was confirmed in the remaining 3 patients (16).

Photofrin, a first-generation photosensitive substance, requires an ~4-week shading period in a dimly-lit room (100-300 lux) and sunlight and intensive light must be avoided following administration to the human body (7,17). Early discovery of intrahepatic bile duct cancer is difficult, and so lesions are typically already advanced cancer at the point of discovery (17). Photofrin-PDT cannot be additionally applied for a prolonged period following PDT, making it unsuitable for the treatment of intrahepatic bile duct cancer (17). In contrast, this shading period is reduced to 3-7 days with Laserphyrin. The 664-nm long wavelength laser used with Laserphyrin penetrates tissue well, for which a strong PDT effect is expected. Nanashima *et al* (16) initiated Photofrin-PDT in 2001 and subsequently adopted Laserphyrin-PDT. They also compared the cytocidal effects between the combinations of Laserphyrin-PDT with cisplatin, oxaliplatin, gemcitabine and fluorouracil, and PDT alone. All agents potentiated the effect. The excimer dye laser used in Photofrin-PDT is a large device, whereas the PD laser is much smaller (18).

The most important thing to consider when applying PDT through the inside of the bile duct is its influence on important organs, i.e., the portal vein, hepatic artery and bile duct. Destruction or damage to these blood vessels may cause hemorrhage following PDT, and damage to the thick bile duct may cause obstructions and cholangitis (7). In experiment one of the present study, PDT was applied via the intrahepatic bile duct lumen employing the transpapillary approach, and the intra- or extrahepatic Glisson's capsule (hepatic artery, portal vein and bile duct) was not affected, suggesting that Laserphyrin in the bile does not damage the bile duct epithelium. It was assumed that, although Laserphyrin is present in blood, excited Laserphyrin rapidly moves through the blood vessels and does not damage the vascular endothelium in the irradiated region. In contrast, hepatocytes that incorporated a large amount of Laserphyrin and bile components were impaired by Laserphyrin. However, this does not cause complications due to the regenerative ability of hepatocytes unless hemorrhage, abscess or bile congestion occurs (10). Based on the above basic experimental findings (10), liver disorders caused by Laserphyrin accumulating in hepatocytes were expected; however, based on clinical experience of radiofrequency irradiation, it was concluded that this may be avoided by adjusting the laser irradiation dose.

Transpapillary PDT is typically performed using a circumferential probe with a non-X-ray permeable marker under fluoroscopy (7). However, application under direct vision may be advantageous for the effect and safety if it is possible. In 2011, a study by Choi *et al* (19) inserted a nasal endoscope into the common bile duct using an auxiliary balloon-equipped over-tube (5 Fr) or 5.5-mm tube for insertion, and PDT was applied under direct vision with a success rate of 77.8% (7/9). With this endoscope, the limit of the application site is the lower bile duct, and a fiber with an extra-fine diameter is necessary for insertion into the liver side. For the fiber with a transpapillary insertable extra-fine diameter, SpyGlass system, which may be inserted through the conventional duodenoscope, has been clinically used since 2011 (20). This system is comprised of a child scope, SpyGlass (outer diameter, 0.8 mm; light source and fiber for observation, 6,000 pixels) and SpyScope (outer diameter, 3.3 mm; two holes for water supply, one hole for device water absorption and one hole for SpyGlass) serving as an outer frame. SpyScope is capable of transporting SpyGlass with an ultrafine diameter into a deep bile duct without damaging it, as well as sufficiently washing and removing bile in the bile duct through the independent water supply and absorption holes. In the present study, COF was applied instead of the SpyGlass. A study by Shigetomi *et al* (21) reported insertion and laser irradiation of COF in the uterus following hysterectomy. A guiding catheter was used to insert COF into the uterine cavity via an orifice in the uterus, similar to the present study. The uterine cavity was filled with saline and observed. It was reported that the image quality of COF was compatible compared with a conventional uteroscope. However, the obtained image was inferior compared with CCD (21). In the present study, the well-known normal fold-like protuberance of the reticular pattern was visually recognized on the gallbladder mucosa via the COF. It was decided that this level of accuracy was sufficient for viewing the mucosal lesions. In conclusion, Laserphyrin did not induce necrotic changes in the normal bile duct in the present study. In addition, the COF was capable of acquiring high quality images. PDT applied to the superficial layer of bile duct cancer under direct vision may be a suitable technique for the treatment of intractable tumors, including hilar and intrahepatic cholangiocarcinomas.

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