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Use of a fluorescent bile acid to enhance visualization of the biliary tract and bile leaks during laparoscopic surgery in rabbits

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Abstract

Background: We set out to determine whether intravenously administered cholylglycylaminofluorescein (CGF), a fluorescent bile acid, would enhance the visualization of the biliary tract and bile leaks in rabbits undergoing laparoscopic cholecystectomy (LC).

Methods: CGF was infused at doses of 1, 5, and 10 mg/kg b.w. Biliary recovery was determined spectrophotometrically (six rabbits). For LC (seven rabbits), a blue (fluorescein) filter was attached to the light source, and a fluorescein-emission filter was attached to the charge coupled device (CCD) camera. The biliary tract and bile leak (made by incising the gallbladder) was observed under standard and fluorescent illumination.

Results: Apple-green fluorescence appeared in 2 min and persisted for 30–60 min, enhancing visualization of bile duct anatomy as well as the bile leak. Biliary recovery of CGF at 90 min was high (86–96% of the infused dose).

Conclusion: In rabbits, CGF is secreted quantitatively in bile, induces biliary fluorescence, and enhances visualization of the bile ducts and bile leaks when viewed with appropriate filters.

Key words: Fluorescence — Laparoscopic surgery — Fluorescent bile acids — Cholylglycylaminofluorescein — Laparoscopic cholecystectomy — Rabbits

Iatrogenic biliary tract injury represents the single greatest problem in laparoscopic cholecystectomy (LC). Major bile duct injuries have been reported in 0.3–0.6% of operations. If all biliary tract injuries and bile leaks are considered, the total incidence is likely to be still higher [15]. At present, there is no reliable laparoscopic method for the prevention or diagnosis of intraoperative biliary tract injury. Intraoperative cholangiography can be used to detect an injury that has already occurred [6]. However, its use has not been reported to decrease injury rates; and at present, most surgeons do not perform intraoperative cholangiography routinely since the benefit is small in relation to the effort required.

Attempts have been made to find a method for improved identification of the biliary tract during LC. In 1992, Araki et al. [1] described a method for enhanced visualization of the biliary tract using indocyanine green (ICG) and reported its use in 54 patients undergoing LC. ICG was chosen because of its rapid hepatic uptake, efficient biliary excretion, lack of toxicity, and prompt fecal elimination [9]. In 1993, Pertsemlidis et al. [10] reported that ICG enhanced visualization of the extrahepatic bile duct in 14 of 18 patients undergoing LC.

Herein we report a series of experiments aimed at testing whether an intravenously administered fluorescent bile acid would enhance visualization of the biliary tract and experimental bile leaks in rabbits undergoing LC. We chose cholylglycylaminofluorescein (CGF), because previous studies [5, 6] have shown that this bile acid is not toxic, it resembles natural conjugated bile acids in its hepatic transport properties, and it does not undergo an enterohepatic circulation. The experiments were performed in rabbits because this species has a gallbladder, and the laparoscopic equipment available for small animal surgery is of the appropriate size for this species.

Materials and methods

Chemicals

Cholylglycylaminofluorescein (CGF) was synthesized as by conjugation of commercially available 5-aminofluorescein (Sigma Chemical Co., St. Louis, MO, USA) with the carboxylic group of the natural bile acid cholylglycine (glycocholate) (CG), as described previously [13]. The chemical structure of CGF was confirmed by nuclear magnetic resonance and is given in Fig. 1. The compound was purified by adsorption chromatography using silica gel column chromatography, as previously described [2], and

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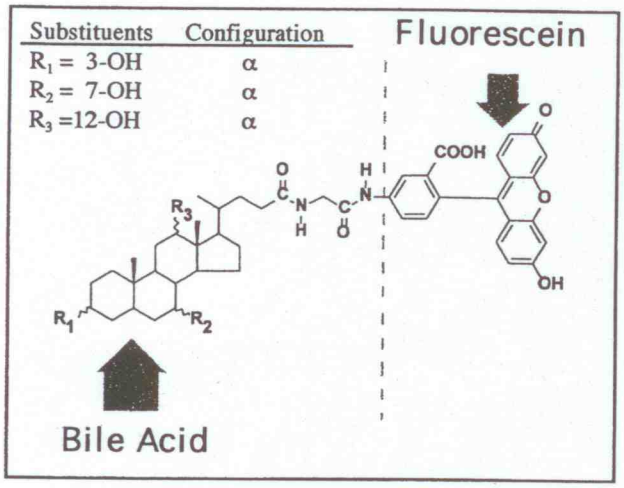

Fig. 1. Chemical structure of the fluorescent bile acid CGF. CGF was synthesized by conjugation of 5-aminofluorescein with the carboxylic group of cholylglycine (also called glycocholic acid or glycocholate).

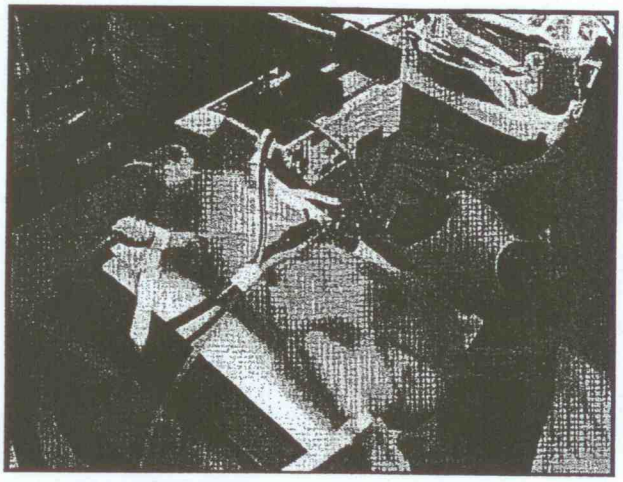

Fig. 2. Experimental technique for laparoscopic surgery in rabbits.

the final compound was 98% pure by high-pressure liquid chromatography (HPLC) [11]. The fluorescence properties of CGF are essentially those of fluorescein; the wave length for maximum excitation is 492 nm, whereas that for maximum emission is 512 nm [5, 6].

Surgical procedures

Surgical preparation

Animal studies were approved by the Animal Subjects Committee of the University of Bern. Thirteen Burgundian rabbits (Tierstall Inselspital, Bern, Switzerland) weighing 2.8±0.4 kg were fasted overnight before the experiment. Anesthesia was induced by intramuscular injection of ketamine (Narketan 10, 45 mg/kg b.w.; Chassot AG, Bern, Switzerland) and xylazine-hydrochloride (Xylapan, 6.5 mg/kg b.w.; Chassot AG). Anesthesia was maintained by repeated intramuscular doses of 0.5 ml of a 1:1 (by volume) mixture of ketamine and xylazine-hydrochloride every 30–45 min. After the induction of anesthesia, the animals were shaved in the abdominal region, placed on a small animal operating table (Karl Kaps, Asslar, Germany), and secured in a supine position. An electric warming pad (Solis Electronics AG, Glattbrug, Switzerland) had been placed on the operating table to prevent development of hypothermia during the surgical procedure. The jugular vein was cannulated using PE-10 polyethylene tubing (Clay Adams, Parsippany, NJ, USA). A solution of 0.9% sodium chloride (Bichsel AG, Interlaken, Switzerland) was infused at a rate of 6 ml/h using a Harvard syringe pump (Harvard Apparatus Co., Millis, MA, USA). Two sets of experimental models were used.

Biliary recovery of CGF in the biliary fistula animal

In the first set of experiments, the abdomen was opened by a midline incision, and an external biliary fistula was constructed by cannulating the common bile duct using a 14-gauge intravenous catheter (Abbocath-T; Abbot, Sligo, Ireland) connected to a Combidyn polyethylene tube (B. Braun Melsungen AG, Melsungen, Germany). Bile was collected every 5 min in preweighed plastic vials.

After a 30-min control period, CGF (dissolved in 0.9% sodium chloride, pH adjusted to 8.0 with 0.05 N NaOH) was infused into the Jugular vein over a 5-min period in doses of 0.25, 1.25, or 2.5 μmol/kg min. These 5-min infusions resulted in a total input dose of 1, 5, and 10 mg CGF/kg b.w., respectively. At the end of the infusion period of CGF, saline was infused for a subsequent 90 min. Biliary output, recovery, and dose-related time curves of CGF fluorescence were measured.

To test for the renal excretion of CGF and its metabolites, the urinary bladder was cannulated at the end of each experiment, and urine was collected for thin-layer chromatography (TLC). At the end of each experiment, the biliary fistula was removed, and the common bile duct was closed with a running suture using Vicryl 6/0 (Ethicon, Norderstedt, Germany). The laparotomy was closed with a two-layer running suture.

Laparoscopic cholecystectomy

In the second set of experiments, a 5-mm skin incision was made about one-third the distance between the xiphoid and the os pubis. Under visual control, the camera trocar (Aesculap EJ 562, Tuttlingen, Germany) was introduced into the abdominal cavity using a blunt obturator and attached to a flexible arm. A pursestring suture was applied to prevent gas leakage. CO_2 was insufflated (1 L/min) to establish a pneumoperitoneum with a maximal intraabdominal pressure of 5 mmHg. The 4-mm 30° wide-angle laparoscope (Aesculap PE 504A, Tuttlingen, Germany) was introduced into the peritoneal cavity. All laparoscopic instruments (micro-scissors, straight and angled forceps) were 2.7 mm in diameter (Wolf, Knittlingen, Germany). No electric coagulation device was needed.

Following a diagnostic overview of the abdominal cavity, additional working 3-mm and 10-mm trocars were introduced into the upper right and left hemi-abdomen (Fig. 2). The gallbladder and extrahepatic biliary tract were exposed and observed under standard and fluorescence conditions before and after intravenous infusion of 1, 5, and 10 mg CGF/kg b.w., respectively. Before completing LC, simulated biliary tract injury was induced by making a small incision with the micro-scissors into the gallbladder neck.

After we had observed the fluorescence of the bile leak using fluorescence illumination, a cholecystectomy was performed using conventional illumination. The cystic duct was ligated using a 5-mm Endo Clip Disposable Applier (Auto Suture, Elancourt, France). The gallbladder was resected using micro-scissors and extracted through the left-side 10-mm trocar. At the end of the laparoscopic experiment, the trocars were removed, and the trocar sites were closed with a two-layer technique. The whole operative procedure was recorded simultaneously on videotape with time measurement.

Visualization of biliary fluorescence by selective optical filters

In order to eliminate autofluorescence and to obtain a high signal/noise ratio of CGF fluorescence, optical filters (Carl Storz GmbH, Tuttlingen, Germany) were introduced into the lighting system of the laparoscopic equipment. A fluorescein-blue filter (maximal transmission at 497 nm) was attached to the light source and used for excitation of CGF. A fluorescein emission filter (transmission peak at 530 nm) was attached in front of the CCD camera and used to detect the fluorescent signal emitted by CGF. The filter system was connected in such a way as to permit instantaneous