Choledocho-choledochostomy: the natural history of healing in pigs

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Abstract

Background/Purpose. Aiming to investigate the natural history of the healing of choledocho-choledochostomies.

Methods. Fifty-five female pigs of 57 kg median weight were used for the experiments. The gallbladder was removed and the common bile duct transected. Continuity was re-established by standardized single-line, interrupted, and inverted sutures. The pigs had a planned postoperative survival of up to 14 days with a subsequent laparotomy for evaluation. Blood samples were drawn prior to the first and the final operations. During laparotomy the animals were investigated for signs of cholascos, and an intraoperative cholangiography was performed. The excised anastomosis was examined for breaking strength and collagen content.

Results. Standard liver parameters were not significantly affected by the surgery, and cholangiography showed no signs of extrahepatic stenosis or intrahepatic dilatation. Breaking strength showed a decrease for the initial 3 postoperative days (PODs), then an increase to a stable level on PODs 6 to 14. Collagen content per volume showed a rise on PODs 0 to 1, then no change until POD 4, followed by a gradual rise until day 6. Subsequently a stable level was reached until POD 14. Two pigs were excluded due to minor cholascos.

Conclusions. The present study on pigs shows that choledocho-choledochostomies, judged by breaking strength and collagen content, regain a stable level of strength 6 days after operation.

Key words Choledocho-choledochostomy · Healing · Natural history

Introduction

Iatrogenic injury during cholecystectomy is by far the most common lesion requiring reconstruction of the extrahepatic biliary tree. Benign biliary stricture is another condition that requires reconstruction. End-to-end duct repair is sometimes possible in these patients. This procedure, however, is often accompanied by complications in the form of leakage or stricture. One possible explanation is that dissection in the hepatoduodenal ligament results in an impaired blood supply to the common bile duct (CBD)/hepatic duct, which, in turn, results in poor healing.1 Due to this, most surgeons would, a priori, opt for a Roux-en-Y hepaticojejunostomy, although this is a vastly greater surgical procedure with a somewhat high inherent risk of complications.

Despite these facts and considerations very little research with regard to the healing of anastomoses of the extrahepatic bile ducts has actually been done.

Methods

Animals

Fifty-five female pigs (Danish Land Race/Yorkshire) with a median weight of 57 kg were used for the experiments. The animals were kept under standard conditions under a 12-h day/night cycle and fed a standard diet. All pigs received care according to ethical standards and Danish laws on animal experimentation. Approval was given by the Board on Animal Experimentation in Denmark.

Anesthesia

The animals were premedicated with intramuscular injection of midazolam 0.5 mg/kg, azaperone 4 mg/kg, and etomidate 0.5 mg/kg. After intubation they were anesthetized with isoflurane gas (2%–3%) and fentanyl 500 µg/h intravenously, and mechanically ventilated with 41 of oxygen/min and 41 of N2O/min.
Medication
Preoperatively the pigs were treated with antibiotics, comprising 2 g Pentrexyl (Ampicillin; Bristol-Myers Squibb, New York, USA). Immediately after surgery an intramuscular injection of 5 ml of a nonsteroidal anti-inflammatory (NSAID), flunixin meglumine (50 mg/ml; Norbrook Laboratories; Station Works, Newry, County Down, Northern Ireland) was administered for analgesic purposes.

Procedure
All operations were performed by the same surgeon. Under sterile conditions an oblique incision in the right subcostal area was used. The cystic duct and artery were identified, transected, and ligated.

After gallbladder removal, the hepatoduodenal ligament was dissected and the CBD was isolated for a distance of 1 cm and transected. Continuity was re-established by standardized single-line, interrupted, and inverted sutures (Biosyn 4-0 CV-23; Surgalloy; United States Surgical, Norwalk, USA), evenly spaced at 2 mm. A silicone drain Charriere 18 (Silkomed; Rüsch, Kernen-Rommelshausen, Germany) was placed near the choledocho-choledochostomy and brought through the abdominal wall in the rear part of the right side of the pig. The abdominal wall was closed with a loop suture (MAXON 0, Syneture; United States Surgical, Norwalk, USA). Finally the skin was closed with a Vicryl 3-0 (Ethicon, Johnson & Johnson International) running suture.

Evaluation
The pigs had a planned postoperative survival ranging from 1 to 14 days and a laparotomy was subsequently performed. Weight was determined before the primary and the final surgeries. The integrity of the anastomosis was evaluated at laparotomy. The peritoneal cavity was inspected for signs of cholascos, bilomas, and abscesses. An intraoperative cholangiography was done.

The biomechanical properties of the choledocho-choledochostomy specimens were investigated in an Alwetron TCT-5 materials testing machine (Lorentzen & Wettre, Stockholm, Sweden). Strips of 7.5-mm width were cut from the anastomosis, holding a length of 10 mm on both sides of this. The strips were mounted on two opposed jaws with a space of 3 mm, and each strip was stretched until rupture at a constant deformation rate of 20 mm/min.²

While testing, load and deformation values were successively recorded, and the breaking strength was defined as the load required to break the strip.

The remaining part of the circumference of the anastomosis was cut 5 mm from the fusing point on both sides. These specimens were dried in an oven at 80°C and defatted in acetone for 72 h to measure the defatted dry weight. The content of hydroxyproline (OH-proline), as a measurement of collagen, was then determined after acid hydrolysis.³ All analyses for OH-proline were done at the same time on frozen samples.

Four pigs, referenced as “postoperative day (POD) 0”, had no anastomotic repair, but to establish the physiologic properties in the unmodified CBD, this was removed for biomechanical and biochemical analysis only.

At the start of the study we planned to evaluate three pigs for breaking strength every day from POD 0 until POD 10. After having analyzed breaking strength in these pigs, more animals were included in the study and evaluated on POD 0, POD 1, POD 3, POD 4, POD 5, POD 6, POD 8, POD 12, POD 13, and POD 14 in accordance with the results.

Blood samples were drawn prior to the first and the final operations for the determination of standard liver parameters and leukocyte count.

Statistics
The nonparametric Mann-Whitney two-sample test was used for comparison between groups, and probability values of less than 5% were considered significant.

Results
No significant change was found on comparing initial weight and weight at final laparotomy. Two pigs were excluded due to minor cholascos. No other animals showed signs of leakage. No abscesses were encountered. Macroscopic examination of all anastomoses showed no signs of stenosis, and standard liver parameters were without indications of bile stasis (Fig. 1). In fact, a decrease was seen in alkaline phosphatase. Cholangiographies were without signs of extrahepatic stenosis or intrahepatic dilatation (Fig. 2).

Breaking strength showed a decrease the first three postoperative days, then an increase to a stable level on postoperative day six to fourteen. Collagen content per volume showed no change the first four postoperative days, then a rise to day six.

Two pigs were excluded due to cholascos.

Breaking strength showed a decrease for the initial 3 PODs, then a gradual increase until POD 6 (P = 0.004; Fig. 3A, B). Subsequently breaking strength was stable until POD 14 (Fig. 3A).

Collagen content per volume showed a rise from POD 0 to POD 1, then no change until POD 4, followed by a gradual rise until POD 6 (P = 0.008; Fig. 4A, B). Subsequently a stable level was reached until POD 14.