Hepatobiliary physiological changes after Roux-en-Y cholecysto-colonic diversion

Yao Liu a,⁎, Wenyong Hou b, Long Li b,⁎⁎, Wei Cheng c,d,⁎⁎⁎

⁎ Department of Pediatric Cardiac Surgery Center, Fuwai Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, 100037 Beijing, P. R. China
⁎⁎ Department of Surgery, Capital Institute of Pediatrics, 100020 Beijing, P.R. China
⁎⁎⁎ Department of Pediatric Cardiac Surgery Center, Fuwai Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, 100037 Beijing, P. R. China
a Correspondence to: Yao Liu, Department of Pediatric Cardiac Surgery Center, Fuwai Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, P. R. China
b Correspondence to: Long Li, Department of Pediatric Surgery, Capital Institute of Pediatrics, Beijing, P. R. China
c Correspondence to: Wei Cheng, Departments of Paediatrics and Surgery, Faculty of Medicine, Nursing and Health Sciences, Monash University, Clayton, Victoria, Australia

ARTICLE INFO

Article history:
Received 4 June 2013
Received in revised form 11 February 2014
Accepted 12 February 2014

Key words:
Cholecysto-colonic bypass
PFIC
Biliary diversion

ABSTRACT

Background: We speculated that Roux-en-Y cholecysto-colonic diversion was as effective for treating children with progressive familial intrahepatic cholestasis (PFIC) as partial biliary diversion. The feasibility of the novel approach in bypassing bile was investigated in rabbits.

Methods: Twenty-four rabbits were randomly divided into three groups: sham operated group (Group 1), 30 cm limb group (Group 2), and 10 cm limb group (Group 3). Group 2 or 3 underwent a Roux-en-Y cholecysto-colonic anastomoses with a 30- or 10-cm-long Roux limb. 99mTcEHIDA dynamic biligraphy was used to detect alterations of bile flow among the three groups at 1 year postoperatively. TBA levels and histological changes were also evaluated.

Results: All animals survived and developed normally without clinical symptoms during 1 year follow-up. Bile was diverted into colon directly after cholecystocolonic anastomosis. In group 3, E20 and E35 values were (77.27 ± 6.15%) and (90.39 ± 1.49%) respectively. Gallbladder emptying was accelerated in 10 cm short limb group than in 30 cm long limb group. The ratio of bile shunt was (0.547 ± 0.182), which was also more than that in group 2 (p < 0.05). The activity-time curve for the gallbladder area in group 2 looks like a wave. A significant reduction in TBA level was observed in group 2 and 3 (p < 0.05).

Conclusions: Roux-en-Y cholecystocolonic bypass was safe and feasible. Its effectiveness is related to the length of Roux loop. Cholecystocolonic bypass led to a significant loss of bile acids in healthy rabbits and might be considered for bile diversion in pediatric patients with selected cholestatic diseases.

© 2014 Published by Elsevier Inc.
1. Materials and methods

1.1. Animals and surgical procedures

Twenty-four male New Zealand rabbits weighing 1.2–1.5 kg, aged 1–2 month, purchased from Vital River Laboratory Animal Technology Co. Ltd, Beijing were studied. The rabbits were housed under controlled conditions at a temperature of 21 ± 2 °C and a relative humidity of 30%–70% in a 12-h dark and light cycle. The animals were fasted, with free access to water 12 h prior to and after the operation. The study was conducted in accordance with the Helsinki Declaration for Scientific Experimentation on Animals. This study was approved by the Institutional Animal Care and Use Committee of Capital Institute of Pediatrics and all animal experiments were carried out at the premises of the Department of Experimental Surgery, Capital Institute of Pediatrics.

The animals were randomized into 3 groups (8 rabbits/group): sham-operated group (Group 1), 30 cm limb group (Group 2), and 10 cm limb group (Group 3). Group 2 or 3 underwent a Roux-en-Y cholecystocolonic anastomosis with a 30- or 10-cm-long Roux limb respectively.

Surgical procedures were performed under ether anesthesia. After laparotomy, 1 ml of bile from the gallbladder and 1 ml of blood from the portal vein (PV) were collected. For creation of Roux-en-Y loop, the colon was dissected free at the splenic and hepatic flexure. Transverse colon was transected proximal to splenic flexure (Fig. 1A). End-to-side anastomosis was established between the distal transverse colon and mid-descending colon with either 10 or 30 cm distal transverse colon loop (Fig. 1B). The gallbladder was incised longitudinally and end-to-end sutured to the Roux-en-Y loop. Vicryl 6-0 was used for anastomoses. Closure of abdomen was performed with Vicryl 3-0. In sham-operated animals the colon was dissected without bowel transection. All animals were reared for further 3–5 months before investigations were carried out.

1.2. General conditions and sample obtaining

The general conditions and body weights of rabbits were carefully observed. The cause of death of rabbit, if any, was examined by autopsy. Overall survival rates of the animals were calculated and recorded. Isotope cholangiography was performed under general anesthesia on day 360 (D360) in all 24 animals. After the radioisotope investigation, laparotomy was performed. One milliliter of bile was collected from the common bile duct using a 16-G catheter and 1 ml of blood was collected from the portal vein. Gallbladder, liver and Roux limb were sampled for histology. The animals were then euthanized by cervical dislocation. Blood was collected. Plasma was extracted from blood by centrifugation. Plasma and bile samples were frozen at −80 °C pending processing.

1.3. TBA and histological examination

Serum and bile concentrations of total bile acid (TBA) were measured using a fully-automatic biochemical analyzer type 7180 (Hitachi, Tokyo, Japan). Tissue samples (liver, gallbladder, and colon) were fixed in 10% formalin, embedded in paraffin, and sectioned into 5-μm thick slides. They were then stained with hematoxylin and eosin (HE). Qualitative analysis of histology was performed using the light microscopy.

1.4. Radioisotope investigations

In isotope cholangiography, 99mTcEHIDA (volume 1 ml, radioactivity 3 mCi) was injected intravenously at time 0. Imaging was carried out using a large field of view gamma camera (Siemens ZLC 750, Siemens, Germany) attached to a dedicated computer system. Data were recorded with the subject supine under the gamma camera as a continuous series of 60 frames per minute in 64 × 64 matrix and stored on magnetic disk for later analysis.

Our methods of computer processing of the clearance curve were as follows:

A. Bile diversion ratio. Two regions of interest (ROI) were chosen: one over the Roux loop, a second over the abdomen (excluding the liver, gallbladder, and urinary bladder). The time-activity curves, for the entire 60 min, were generated for the two regions. To study the bile diversion ratio, these two curves were compared.

B. Gallbladder emptying. Two regions of interest were chosen: the first over the gallbladder (GB), the second (background) over the superiolateral aspect of the liver. The liver background counts were subtracted from the gallbladder to obtain the net gallbladder counts. Time-activity curves were normalized, setting the maximum count rate to 100%. Results were expressed as (Fig. 2): (1) Tmax (time in minutes from zero to maximum counts) reflecting gallbladder filling status. (2) E20 (% gallbladder emptying at 20 min) i.e. activity cleared at 20 min as a percentage of the maximum counts in the ROI. (3) E35 (% gallbladder emptying at 35 min) i.e. activity cleared at 35 min as a percentage of the maximum counts in the ROI.

C. Liver function. Region of interest (ROI) was drawn around the entire liver. The liver time-activity curve was used to represent hepatocyte activity. Exl5 (% hepatic excretion at 15 min) is the activity cleared by liver cell during 15 min.

Fig. 1. Surgical procedures. The Roux-en-Y cholecystocolonic anastomosis and the length of the Roux limbs are indicated. A, Transverse colon was divided proximal to splenic flexure. B, Y-type colono-colonostomy and cholecystocolostomy.
1.5. Statistical analysis

Statistical analysis was conducted using the SPSS 15.0 for Windows (SPSS, Chicago, IL). All results are expressed as mean ± SD and the one-way ANOVA test was used to compare the differences in the 3 groups. The paired t-test was used to compare paired data (D360 vs. D0). Differences of p < 0.05 were considered statistically significant.

2. Results

2.1. General conditions and survival rate of animals

All the animals survived the experiments. The survival rate was therefore 100%. During the follow-up period, no fever, bile leakage, jaundice, diarrhea, infection, cholangitis, peritonitis, or other postoperative complications were encountered. The animals were maintained on their usual diet, gained weight gradually and remained in a good condition till euthanasia.

2.2. TBA levels in bile and portal vein

Bile acid measurements in bile and portal vein at D0 and D360 are shown in Table 1. Baseline bile and serum TBA profiles at D0 were comparable among the three groups. In the short loop group, mean TBA concentration in bile dropped from 792.46 ± 22.56 umol/l at D0 to 213.93 ± 13.76 umol/l at D360. Similar declining trend could be found in portal vein, i.e., from 86.45 ± 3.12 umol/l at D0 to 23.99 ± 3.43 umol/l at D360. The TBA level was decreased by 73% in bile and 72% in portal vein. A significant decrease in TBA levels also occurred in long loop group, by less than 70% drop in both bile and portal venous blood. In control group, the levels of TBA in bile were similar at D0 and D360 (796.67 ± 15.29 vs. 791.50 ± 21.02 umol/l), so are the TBA levels in the portal vein. When the three groups were compared at D360, TBA levels in bile and portal vein were significantly decreased in the two biliary diversion groups than that in the control group (p < 0.05).

2.3. Bile diversion ratio

Hepatobiliary scintigram at 2 min showed good liver uptake and some renal (K) excretion of 99mTcEHIDA. Gallbladder, intestine, and Roux limb were seen, with kidneys invisible within 1 min. By 30 min most of radioactivity enters colon and intestine. Thus, the animal model was successful in diverting bile into colon. Bile diversion ratio in Group 3 was higher than that in Group 2, its positive rate was 0.547 ± 0.182 vs. 0.289 ± 0.024 (t = 7.241, p = 0.005).

2.4. Gallbladder emptying and liver function

Results for Tmax, E20, E35 and Ex15 for Group1, 2, and 3 are shown in Table 2. Tmax for Group 3 was increased compared with normal control group, but no significant difference could be found (p > 0.05). Yet, when comparing Group 1 with Group 2, it was significantly decreased (F = 29.624, p = 0.000 < 0.05). E20 and E35 were also significantly increased in Group 3 compared with either Group 1 or Group 2 (E20: F = 252.629, p = 0.000 < 0.05; E35: F = 520.848, p = 0.000 < 0.05). Group 2 animals thus exhibited characteristic 99mTcEHIDA emptying curves with prolonged time to peak counts and slow gallbladder emptying compared with either group 1 or 3 (Fig. 2). As for Ex15, no significant difference was observed between any of the groups (F = 1.765, p = 0.196 > 0.05).

2.5. Histological examination

One year postoperatively, the gallbladder was well-filled and the integration between the gallbladder and the Roux-en-Y colonic limb was perfect. The gallbladder and colon healed well. No stones or stenosis was found.

---

**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>D0 (umol/l)</th>
<th>D360 (umol/l)</th>
<th>Ratio</th>
<th>PV (umol/l)</th>
<th>D0 (umol/l)</th>
<th>D360 (umol/l)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>796.67 ± 15.29</td>
<td>791.50 ± 21.02</td>
<td>0.99</td>
<td>88.76 ± 3.74</td>
<td>88.49 ± 3.01</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>806.42 ± 24.94</td>
<td>285.07 ± 3.21</td>
<td>0.35</td>
<td>84.49 ± 4.88</td>
<td>27.74 ± 6.47</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>792.46 ± 22.56</td>
<td>213.93 ± 13.76</td>
<td>0.27</td>
<td>86.45 ± 3.12</td>
<td>23.99 ± 3.43</td>
<td>0.28</td>
<td></td>
</tr>
</tbody>
</table>

* Paired t-test, p < 0.05.

---

**Fig. 2.** Gallbladder activity/time curve and major parameters. Tmax = time in minutes from zero to maximum counts. E20 = % gallbladder emptying at 20 min and E35 = % gallbladder emptying at 35 min.

**Fig. 3.** Photograph of a longitudinally opened specimen containing liver, gallbladder and the Roux-en-Y colonic limb. The gallbladder and colon healed well. No stones or stenosis was found.
was also well-established. After removal of the specimen containing the liver, gallbladder and Roux limb, the specimen was longitudinally opened (Fig. 3). Good healing of anastomosed structures was observed and there was no sign of stenosis. With light microscopy, we found that the colonic histology of the Roux limb was indistinguishable from that of the normal controls, except for some evidence of increased goblet cell numbers. In addition, liver and gallbladder histology did not differ between groups nor was any apparent disruption of the normal structure observed.

3. Discussion

Several operations have been described for the treatment of progressive familial intrahepatic cholestasis (PFIC). Partial external biliary diversion (PEBD), first defined by Whitington [7,8] in 1990s, had a reasonably good track record of success and is the standard treatment at some centers. In this operation, an external biliary fistula was created to divert excess bile acids.

To avoid a permanent stoma, partial internal biliary diversion (PIBD), was created by Bustorff-Silva et al. in 2007 [2]. They prepared a jejunal conduit between the terminalolateral aspect of gallbladder and ascending colon distally. PIBD as a stoma free procedure could eliminate excess bile acids more conveniently than PEBD [2,3,9]. However, the process also had some disadvantages such as multiple anastomoses, impairment of the normal passage of intestinal contents, motility difference between the isolated jejunal conduit and the ascending colon.

Because of the proximity of gallbladder to hepatic flexure of colon, we tried to develop a novel approach, Roux-en-Y cholecystocolonic anastomosis, to divert excess bile into colon directly. Obviously, this procedure simplified the operation step by omitting one anastomosis, kept the integrity of jejunum, and did not disturb the normal absorption for nutrients and fluids.

Because of the similarities of its hepatobiliary system with infant, young rabbits were chosen. They have been widely used for experimental modeling in preclinical studies. The colon of the New Zealand white rabbit is about 1 m long [10,11], which is the best option for creating cholecystocolonic anastomosis with Roux-en-Y loop. In addition, the rabbit ear marginal vein was easily accessible and was cannulated during scintigraphy. In the Roux Y-operated model, gallbladder was incised completely, a single-layer continuous suturing cholecystocolonic anastomosis was performed. We performed a 1 cm (equal to the diameter of colon) end-to-end anastomosis to ensure free flow of bile. There was no blind pouch (BP) at the cholecystocolonic anastomosis site. Otherwise, blind pouch may cause bile stasis in the BP itself as well as in the gallbladder, increasing the risk of stone formation and chronic biliary infection.

The cholecystocolonic bypass was well tolerated in rabbits once normal intestinal movements resumed. No complications were observed in our animals and the normal growth in study animals indicates that cholecystocolonic diversion did not cause malabsorption of nutrients. Hepatobiliary scintigraphy showed that gallbladder bile was diverted into colon directly and smoothly, no-reflux or stone formation was observed. In addition, the cholecystocolonic anastomosis sites were examined thoroughly one year postoperatively. No stone, bile leakage or stricture was observed. The extent of BA depletion in bile and the low BA levels in PV were confirmed by laboratory investigations in our study groups. The new animal model was successful in diverting bile acid and reducing BA level.

Dynamic cholangiography was a highly sensitive method in identifying partial bile duct obstruction after bypass surgery [12]. 99mTcEHIDA was predominantly trapped by the liver and excreted into the biliary tract. Detection of isotope in biliary tract permits continuous assessment of bile flow [13]. In the present study, the gallbladder bile was passed onto colon directly after cholecystocolonic anastomosis, and bile flow was faster in 10 cm short limb group than in 30 cm long group. Bile diversion ratio in the short limb group was also higher than that in long limb group. The shape of radio-activity-time curve for the GB area in 30 cm long limb group was wave-like, which indicated a degree of bile reservoir effect in gallbladder. No colon-gallbladder reflux was observed during the period of scintigraphy. Similar results could be found in a rat model conducted by Robson Azevedo Dutra et al. [14]. In their study, gastric emptying was accelerated in 7.5 cm short limb group than in 15 cm long limb group evaluated by scintigraphic methods, thus supporting the notion that the gastric emptying delay may be related to the long standard 40 cm Roux limb. A study of 234 adult subjects subjected to Roux gastrectomy concluded that patients presenting gastric stasis symptoms had long Roux-en-Y limbs of 41 cm of average length; moreover, it suggested that the shortening of such limbs must be needed to improve the symptoms [15]. Although our new approach was successful in diverting bile in rabbits, it had been rarely performed in children because of the alleged risks of adverse events such as ascending cholangitis, BA-induced diarrhea or colonic tumorigenesis. Cholangitis might result from reflux of colonic content up to the gallbladder. Actually, infectious risks had been correlated more strongly with hepatic disease than surgical intervention. The length of the loop was not considered a relevant factor in preventing cholangitis [8]. In the present study, there was, however, no apparent biliary infection occurred in either of the study groups.

Diarrhea and colorectal carcinogenesis were two side effects of elevated colonic BA concentration in colon. In our study bile acids directly enter the descending colon. This reduces the stimulation of water secretion and intestinal motility. Stools have become semisolid when moving to the descending colon, so no diarrhea occurred in animal model groups. Furthermore, our previous cholecystocolostomy clinical series had also found the influx of bile acid into the descending colon seems to have less effect on the formed feces. Neither our animal models nor the 20 patients reported developed diarrhea postoperatively [16]. Otherwise, diarrhea is a symptom in PFIC 1 patients that may be not directly induced by bile acids.

High bile acid concentration is one of the contributing factors of colon cancer, although the clinical concentrations of bile acids required are uncertain. Colorectal cancer is usually triggered by environmental factors in genetically susceptible individuals. Furthermore, BA-induced histological alterations preceding colorectal-cancer development were not observed during histological viewing of the rabbit colon samples. Consistent with our animal model study, in clinical practice, diarrhea had not been reported as a major complication, and no BA-induced colonic malignancy had been reported after PIBD [3,9,17] so far.

The average life span of a rabbit is between 7 and 12 years. Therefore, one year follow-up may be not long enough to assess lifetime cancer risk caused by bile bypass. Further long-term outcome studies would produce more convincing results. In addition, carcinoembryonic antigen (CEA) tests, fecal occult blood tests (FOBTs) and colonoscopies are warranted.

In conclusion, Roux-en-Y cholecystocolonic bypass is safe and feasible. In rabbit model, bile diversion ratio was affected by the length of Roux loop, an individualized 10 cm short limb may be better. Cholecystocolic bypass leads to a significant decrease in bile acid level in healthy rabbits. Cholecystocolostomy might be a viable alternative for patients with selected cholestatic diseases in the future.

References


