Exogenous Secretin Improves Parenteral Nutrition-associated Liver Disease in Rats

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ABSTRACT

Objectives: Intestinal failure-associated liver disease (IFALD) is a feared and life-threatening complication in neonates with intestinal failure (IF) receiving long-term total parenteral nutrition (TPN). This study aims to investigate the effect of exogenous secretin on liver pathology and hepatic function in a rat model of PN-associated liver disease (PNALD).

Methods: Male Sprague-Dawley rats underwent right jugular venous catheterization to receive 14-day continuous TPN therapy. All rats were allocated into 3 groups: the Control group (n = 8) did not have surgery or TPN and was fed standard rat chow ad libitum; the TPN group (n = 8) underwent catheter insertion and TPN treatment; and the TPN/S group (n = 8) also underwent catheter insertion, TPN treatment, and exogenous secretin treatment (2.5 nmol·kg⁻¹·day⁻¹) daily. Fourteen days after initial surgery, we collected the animals’ liver and blood samples for further test.

Results: The TPN/S group had diminished direct bilirubin (TPN, 2.1 ± 0.7 μmol/L; TPN/S, 1.5 ± 0.2 μmol/L) and liver total bile acid levels (TPN, 144.5 ± 21.2 μmol/L; TPN/S, 123.4 ± 10.4 μmol/L) and improved histological outcomes compared with those in the TPN group. Exogenous secretin also enhanced the canalicular transporter (BSEP, 0.5-fold, P = 0.011) and inhibited the basolateral transporter (OSTA, −0.48-fold, P = 0.002; OSTB, −0.6-fold, P = 0.013) of liver bile acid.

Conclusions: In this animal model of PNALD, secretin may improve cholestasis by enhancing canalicular transport, inhibiting the basolateral export of bile acid, and eventually decreasing the total bile acid level in the liver. Exogenous secretin treatment may potentially prevent and treat IFALD in IF patients relying on long-term TPN therapy.

Key Words: intestinal failure associated liver disease, liver disease, secretin

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What Is Known

• Long term Parenteral nutrition therapy in intestinal failure is associated with hepatobiliary complications, such as cholestasis and steatosis, which are collectively described as intestinal failure-associated liver disease.

• Secretin has been shown to produce a remarkable choleretic effect in patients with intrahepatic cholestasis.

What Is New

• Exogenous secretin significantly improves canalicular transport and inhibits basolateral export of bile acid and eventually decreases total bile acid levels in the liver.

Parenteral nutrition (PN) was first described in the 1960s and has been regarded as the primary treatment and life-saving measure for patients with intestinal failure (IF) (1). Although PN increases the life expectancy of these patients, the feared and life-threatening complication of intestinal failure-associated liver disease (IFALD) follows prolonged PN therapy. IFALD is considered as a consequence of medical and surgical management strategies for IF and the old terminology of ‘‘parenteral-nutrition-associated liver disease (PNALD)’’ has been decreasingly adapted in reports after advances in the management of PN (2). The animal model in this study has been, however, made using 14-day continuous PN therapy without small bowel resection. Therefore, we use the novel terminology of ‘‘IFALD’’ when describing data in mankind and the old terminology of ‘‘PNALD’’ when describing our animal model. The incidence of IFALD differs considerably among studies and is approximately 40 to 60% in infants and up to 85% in neonates who rely on prolonged PN therapy (3,4). Moreover, a prolonged duration of PN often results in a higher probability of IFALD (15.7% for PN ≤1 month and 60.9% for PN ≥2 months) (5). Infants and neonates with severe IF and extended reliance on PN therapy are markedly vulnerable to IFALD.

Current studies focus on a fish oil (FO)-containing lipid emulsion, which has several theoretic advantages over traditional soy oil (SO) and may lead to a resolution and reverse of cholestasis condition in children with IF on long-term PN (>4 weeks); however, there is no strong clinical evidence of FO-containing lipid emulsion changing direct bilirubin (DB), total bilirubin (TB), and diagnostic liver enzymes in short-term use compared with the SO group (6,7). Results from a meta-analysis by the ESPGHAN Committee on Nutrition also supported this conclusion (8).
FO-containing lipid emulsions may be beneficial for IF patients in short-term use and can be tested in further research; however, the point is that a novel strategy other than FO to prevent and treat IFALD is also possible and potentially effective. Lack of enteral feeding could result in several profound metabolic and endocrine alterations that may play an important role in the development of IFALD. Therefore, the prevention and treatment strategy for patients with IFALD should not be focused exclusively on lipid emulsion.

Secretin, as a well-studied gastrointestinal hormone, has been shown to produce a remarkable choleretic effect in patients with intrahepatic cholestasis (9–12). Considering the cholestatic condition in the setting of IFALD and the choleretic effect of secretin, we hypothesized that exogenous secretin may alter the gene expression of bile acid transporters and improve the impaired bile secretion in our experimental animal models on total PN (TPN).

MATERIALS AND METHODS

All procedures in this study were approved by the Ethics Committee in our hospital. Male Sprague-Dawley rats (age, 6–7 weeks, body weight, 220–240 g) used in this experiment were obtained from the Laboratory Animal Center of our local medical university. Male Sprague-Dawley rats underwent right jugular venous catheterization to receive 14-day continuous TPN therapy. No small bowel resection was performed during the surgery. All rats were allocated into 3 groups: the Control group (n = 8) did not have surgery or TPN and was fed standard rat chow ad libitum; the TPN group (n = 8) underwent catheter insertion and TPN treatment; and the TPN/S group (n = 8) also underwent catheter insertion, TPN treatment, and exogenous secretin treatment (2.5 nmol·kg⁻¹·day⁻¹) daily. Fourteen days after initial surgery, we collected the animals’ liver and blood samples for further test.

Postoperative Care

Rats were housed in individual cages at a room temperature of 25°C and a 12-hour light-dark cycle. TPN was infused continuously for 14 days via the central venous catheter at a volume of 205 kcal·kg⁻¹·day⁻¹. The TPN solution contained 450 mL of an amino acid mixture (Huarun Shuanghe Pharmaceutical Company, China), 360 mL of 50% glucose (Huarun Shuanghe Pharmaceutical Company), and 140 mL of SO-based lipids (Belang Medical Company, China) per liter. Trace elements, vitamins, and electrolytes were also added. TPN solution was delivered just after surgery at a beginning rate of 50% of the full rate for 24 hours and changed to the full rate for the rest of the experiment period. Energy intake was designed according to the study by Caroline et al (13). In the TPN/S group, exogenous secretin (TOCRIS, Bio-Techne China Co. Ltd., Shanghai, China) was given at a dosage of 2.5 nmol·kg⁻¹·day⁻¹ in 15 min daily. All rats relying on TPN had unlimited access to water.

Data Collection

On postoperative day 14, rats were fasting for 6 hours, after which they were anesthetized to obtain their liver specimens and blood samples by cardiac puncture. Tissue samples were fixed and stored in 4% paraformaldehyde (BL539A, Biosharp, China) for 24 hours for paraffin embedment; some samples were stored in RNA preservation solution (DP408, TIANGEN BIOTECH Company, China). Blood samples were centrifuged at a speed of 12,000 rpm for 15 min to obtain plasma.

Chemistry

Liver chemistry included TB, DB, serum albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutamyl transpeptidase (GGT), and alkaline phosphatase (ALP). Serum total bile acid (TBA) levels were measured using a bile acid enzyme-linked immunosorbent assay (ELISA) test kit (E003, Nanjing Jiancheng Bio Engineering Institute, China). Other parameters were analyzed by an automatic biochemical testing instrument (Cobas 6000, Roche, Switzerland). Liver TBA was also measured with this ELISA test kit by homogenizing the tissue in normal saline according to the manufacturer’s protocol. The concentration of bile acid per unit of liver weight was calculated by dividing the current concentration by the weight of liver sample.

Liver Histology

The paraformaldehyde-preserved specimen was placed in a cassette and embedded in paraffin according to standard procedures. Then, hematoxylin and eosin (HE)-stained 5-μm sections were prepared for histological analysis by a senior hepatopathologist at our hospital who was blinded to the study design. The HE sections were examined at 200× and 400× for evidence of PNALD, such as hepatocyte vacuolation and cholestasis.

Real-time Polymerase Chain Reaction

Total RNA was extracted from 20 mg frozen liver samples in an RNA preservation solution using an RNA extraction kit (DP431, TIANGEN BIOTECH Company) according to the manufacturer’s protocol. The OD 260/280 value of the RNA sample was measured using a Nanodrop2000 device and was normalized to 10 ng of cDNA. The sequences of rat-specific forward and reverse primers are provided in Supplemental Table 1 (Supplemental Digital Content, http://links.lww.com/MPG/B778). DNA amplification was performed in an automatic RT-PCR machine (CFX96, BIO-RAD, Singapore) under the following conditions: 95°C for 15 seconds, 40 cycles of 95°C for 30 seconds, and 400°C for 30 seconds. Internal controls included HPRT1, RPLP-0, and GAPDH.

Data Analysis and Statistics

Our results are presented as the mean ± standard deviation. Normality was determined by the Kolmogorov-Smirnov test, and data were regarded as normal at P > 0.1. Comparisons between groups were analyzed using 1-way analysis of variance and least significant difference. Relative gene expression was calculated using the ΔΔct relative quantification model. Data were analyzed using SPSS 22.0 software, and statistical significance was set at P < 0.05.

RESULTS

Liver Pathology

Representative sections are shown in Figure 1. These findings were recorded and analyzed descriptively because of the absence of a validated scoring system for neonatal IFALD. According to an expert review, control rats had normal histology, and TPN-fed rats had evidence of PNALD, such as hepatocyte pigment and vacuolation consistent with steatosis. In the TPN/S group, liver specimens also developed hepatocyte vacuolation and cholestasis.
Liver Function

Table 1 provides liver biochemistry results for all groups. TPN resulted in multiple abnormalities in serum biochemistry, characterized by conjugated hyperbilirubinemia and hypoalbuminemia, and secretin remarkably improved PNALD by decreasing serum DB and liver TBA levels. Serum DB serves as a major marker in the diagnosis and prognosis of PNALD and was significantly increased after administration of 14-day TPN (control, 1.6 ± 0.2 μmol/L; TPN, 2.1 ± 0.7 μmol/L, P = 0.049). After administration of exogenous secretin, serum DB levels declined significantly (TPN/S, 1.5 ± 0.2 μmol/L). Serum TB levels changed similarly to DB; the TPN group had the highest TB levels (TPN, 2.5 ± 0.9 μmol/L) among groups, and the TPN/S group had lower

**FIGURE 1.** Liver histology. P = pigmentation; V = vacuolation; D = dividing hepatocytes; M = megakaryocytes. (A) The Control group had normal liver histology and architecture (200× magnification). (B) The TPN group had liver sections that showed evidence of PNALD, such as hepatocyte pigmentation (P) suggestive of cholestasis and vacuolation (V) consistent with steatosis (200× magnification). (C) The TPN/S group developed hepatocyte pigmentation and vacuolation but with a decreased number compared with that in the TPN group (200× magnification). (D and E) The TPN/S group showed evidence indicating liver regeneration, including the presence of hepatocyte cellular division (D) and megakaryocytes (M) (400× magnification). PNALD = parenteral nutrition-associated liver disease; TPN/S, total parenteral nutrition/secretin.
TABLE 1. Liver biochemistry

| Outcome measures | Control (n = 8) | TPN (n = 8) | TPN/S (n = 8) | P value | ps | pc | pb |
|------------------|----------------|-------------|---------------|---------|----|----|----|
| TB (μmol/L)      | 1.8 ± 0.3      | 2.5 ± 0.9   | 1.5 ± 0.6     | 0.038bc | 0.081 | 0.389 | 0.013 |
| DB (μmol/L)      | 1.6 ± 0.2      | 2.1 ± 0.7   | 1.5 ± 0.2     | 0.057bc | 0.049 | 0.808 | 0.029 |
| ALP (IU/L)       | 308.0 ± 57.9   | 209.5 ± 40.1| 137.9 ± 32.4  | <0.001abc | <0.001 | <0.001 | 0.007 |
| GLB (g/L)        | 39.8 ± 7.1     | 27.5 ± 2.2  | 29.3 ± 2.2    | <0.001abc | <0.001 | <0.001 | 0.129 |
| ALT (IU/L)       | 48.6 ± 7.1     | 13.3 ± 1.9  | 10.3 ± 1.0    | <0.001abc | <0.001 | <0.001 | 0.187 |
| AST (IU/L)       | 100.6 ± 10.6   | 102.7 ± 22.1| 105.8 ± 27.2  | 0.900    | 0.854 | 0.652 | 0.788 |
| GGT (IU/L)       | 2.7 ± 0.9      | 4.1 ± 1.0   | 2.5 ± 0.5     | 0.002abc | 0.003 | 0.576 | 0.001 |
| TBA (μmol/L)     | 14.8 ± 5.0     | 24.8 ± 7.8  | 54.2 ± 26.1   | <0.001abc | 0.258 | <0.001 | 0.002 |
| TBA<sub>1</sub> (μmol/L) | 134.5 ± 20.3 | 144.5 ± 21.2| 123.4 ± 10.4  | 0.086bc  | 0.275 | 0.233 | 0.029 |

Values represent “mean ± standard deviation” and superscripts refer to statistical difference. “TBA” without superscript means total bile acid in serum sample whereas “TBA<sub>1</sub>” with superscript means total bile acid in liver specimen. ALB = serum albumin; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; DB = direct bilirubin; TB = total bilirubin; TBA = total bile acid.

TB levels than those in the TPN group (TPN/S, 1.5 ± 0.6 μmol/L). TPN also caused hypoalbuminemia (Control, 39.8 ± 2.0 g/L; TPN, 27.5 ± 2.2 g/L) and did not improve after the administration of secretin (TPN/S, 29.3 ± 2.2 g/L), which indicated a compromised synthetic function of liver after 14-day TPN. ALP levels were significantly decreased after long-term TPN (Control, 308.0 ± 57.9 μmol/L; TPN, 209.5 ± 40.1 μmol/L, P < 0.001), and ALP levels continued to decline after the administration of secretin (TPN/S, 137.9 ± 32.4 μmol/L). ALT levels were significantly decreased after TPN (Control, 39.8 ± 7.1 IU/L; TPN, 10.3 ± 1.0 IU/L, P < 0.001), with a trend towards reduced ALT concentration in the TPN/S group compared with the TPN group (TPN/S, 10.3 ± 1.0 IU/L). AST levels were not different among all groups. Serum GGT, as an important indicator measuring the damage of bile canaliculus, was significantly increased after TPN (Control, 2.7 ± 0.9 IU/L; TPN, 4.1 ± 1.0 IU/L, P = 0.003). After administration of exogenous secretin, serum GGT levels reduced significantly (TPN/S, 2.5 ± 0.5 IU/L). Liver TBA levels were decreased in the TPN/S group compared with those in the TPN group (TPN, 144.5 ± 21.2 μmol/L; TPN/S, 123.4 ± 10.4 μmol/L, P = 0.029), which was the pathological evidence of secretin improving cholestasis in a PNALD model.

Bile Acid Synthesis and Transport

To explore potential mechanisms for the secretin-mediated effect on PNALD, the expression of genes involved in bile acid homeostasis was analyzed. These results are shown in Figures 2 and 3. Farnesoid X receptor (FXR), a major regulator of bile acid homeostasis that represses cholesterol 7α-hydroxylase (CYP7A1) by interacting with short heterodimer partner (SHP) in the liver, was decreased in both groups receiving PN compared with the Control group (TPN, –0.4-fold; P = 0.001, TPN/S, –0.4-fold; P < 0.001). No significant difference was, however, found between the TPN and TPN/S groups. CYP7A1 is the rate-limiting enzyme of bile acid synthesis and is repressed by the FXR-induced pathway. A significant difference was found in CYP7A1 gene expression between groups (P < 0.001), and the Control group had the highest expression.
whereas the TPN and TPN/S groups had a similar low expression ($P < 0.001$ between the TPN and Control groups; $P < 0.001$ between the TPN/S and Control group), which is indicative of a decrease in hepatic concentration of bile acids and slight improvement of PNALD in the TPN/S group compared with the TPN group. SHP was an adaptor protein for FXR to inhibit CYP7A1 gene expression.

Although the expression of FXR was similar between the 2 groups receiving PN, SHP gene expression was significantly increased in the TPN/S group compared with that in the TPN group (3.3-fold; $P < 0.001$), in line with the alteration of CYP7A1 expression. Sterol 12a-hydroxylase (CYP8B1) was a downstream enzyme following CYP7A1 in bile acid synthesis and was also regulated by FXR. The expression of CYP8B1 was similar in all groups.

Bile acid was exported from the liver through 2 pathways: the canalicular pathway to the biliary system and the basolateral pathway to serum circulation. The canalicular bile acid transporters mainly included bile salt export pump (BSEP) and multidrug resistance-associated protein 2 (MRP2), which were significantly expressed at low levels in the setting of PN (BSEP, $-0.4$-fold, $P = 0.002$; MRP2, $-0.6$-fold, $P = 0.001$ between the TPN and Control groups). The expression of the BSEP gene, however, increased after the administration of secretin (0.5-fold, $P = 0.011$), with a trend towards enhanced expression of the MRP2 gene in the TPN/S group over the TPN group. Multidrug resistance-associated protein 3 (MRP3) was one of the basolateral bile acid transporters and was similar between all groups. Organic solute transporter α (OSTA) and organic solute transporter β (OSTB) formed a heterodimer and functioned as a basolateral bile acid transporter together. The expression of OSTB increased after 14 days of PN (2.9-fold, $P = 0.020$), and OSTA showed a slightly increasing trend. After using secretin, expression of both OSTA and OSTB significantly decreased in the TPN/S group compared with that in the TPN group (OSTA, $-0.5$-fold, $P = 0.002$; OSTB, $-0.6$-fold, $P = 0.013$).

**DISCUSSION**

Bile acid homeostasis involves several key genes and enzymes regulating various metabolisms, such as bile acid synthesis and transport. Regarding synthesis of bile acid, FXR is a key regulator that interacts with bile acid and regulates bile acid homeostasis by transcriptionally inhibiting CYP7A1 expression, the rate-limiting enzyme in bile acid synthesis, with the assistance of SHP. FXR also regulates the CYP8B1, which is a downstream enzyme in the bile acid synthesis pathway. Regarding the transport of bile acid, canalicular bile acid export involves 2 key enzymes: BSEP and MRP2. Basolateral bile acid export includes 3 key enzymes: MRP3, OSTA, and OSTB.

We have postulated that the administration of secretin may improve cholestasis by enhancing the transport of bile acid but not affecting its synthesis. To explore how the genes involved in bile salt homeostasis were affected in the setting of PN, we studied the different gene expressions between the TPN and TPN/S groups.

Regarding synthesis of bile acid, the gene expression levels of CYP7A1 (the rate-limiting enzyme of bile acid synthesis) and FXR (a critical regulator in bile salt synthesis) were similar between the TPN and TPN/S groups, which indicated that secretin did not significantly affect the process of bile salt synthesis in rats receiving PN. Regarding transport of bile acid, secretin altered bile acid-enhanced canalicular transport and inhibited the basolateral export of bile acid by increasing the gene expression of BSEP and decreasing the gene expression of OSTA/B. This is in line with a significantly decreased liver TBA level, suggesting alleviation of liver cholestasis.

Conjugated bilirubinemia is regarded as an early clinical sign of IFALD in patients depending on TPN (14). Conjugated hyperbilirubinemia occurred in rats receiving long-term TPN with elevated serum-conjugated bilirubin and a trend towards increased serum TB compared with that in the Control group, similar to results in mice receiving TPN (15). This phenomenon reflected damaged canalicular secretion of bilirubin glucuronides, and the fact that gene expression of canalicular transporter (BSEP and MRP2) was significantly decreased after TPN administration also supported this notion, although the underlying mechanism is still unknown. After administration of secretin, the gene expression of the canalicular transporter BSEP was markedly enhanced, and the basolateral transporter OSTA/B was significantly decreased, which indicated that secretin prompted bile acid secretion into the intestine and hindered its export to circulation. This result was in line with the
reduction in serum TB and DB after using secretin, which is an additional powerful evidence of potential secretin-induced improvement of IFALD.

A lack of enteral feeding results in several profound metabolic and endocrine alterations that may play an important role in the development of IFALD. Studies regarding the secretion of several gastrointestinal hormones in a fasted state found that the deprivation of enteral feeding inhibited the secretion of cholecystokinin, gastrin, and peptide YY, which are instrumental in adjusting bile acid secretion and gallbladder contractility (16,17). Moreover, the reduction in hormone levels may lead to a negative effect on the permeability of the intestinal barrier and subsequent bacterial translocation. Secretin participates in the regulation of many physiological functions, such as regulating the secretion of gastric acids and other hormones, affecting the motility of the small intestine, relaxing the lower esophageal sphincter and sphincter of Oddi, and delaying postprandial gastric emptying (10,11,12). Secretin also stimulates bile secretion from the liver and increases mesenteric blood flow, suggesting that secretin has trophic effects on the small intestine and may improve chronic liver disease during PN therapy.

Enhanced secretion of bile acid into the canalicular duct may play a critical role in treating IFALD. Previous studies have shown that bile and bile acid particularly stimulate the growth of the intestinal mucosa.ledo et al (18) reported that in cultured rat enterocytes, bile acid stimulates proliferation of intestinal epithelial cells and prevents these cells from undergoing apoptosis. Hwang and Henning (19) also found that bile acid administration can induce precocious intestinal maturation. Ajay et al also confirmed these results and proposed a novel mechanism for bile acid-induced intestinal mucosal growth (20). Collectively, these data demonstrated that bile acid has played an important beneficial role in regulating intestinal mucosal growth and repair. Additionally, reduced intestinal luminal exposure to bile acid, which occurs during PN, may have a negative effect on the integrity of intestinal mucosa.

The clinical transformation of exogenous secretin treatment can be performed in neonates and infants diagnosed with IF but not those who have progressed to IFALD. In patients already developing IFALD, the therapeutic effect of secretin has not been reported, and further studies are needed.

Although this study has shown an intriguing result regarding the role of secretin in TPN, there are several obvious limitations. First, in this study, we found an increased expression of genes regulating transport of bile acid but failed to measure the accurate bile acid content in the intestinal lumen. A second limitation would be the maldistributed condition in these experimental rats. Serum ALB levels in the TPN and TPN/S groups were significantly decreased compared with those in the Control group, probably because of nutrient deficiency in our TPN solution. A modified TPN solution with increased energy and nutrition should be considered in further study. A third limitation could be the animal model in this study was made by fasting and TPN therapy, which cannot represent the impact of long-term PN therapy in IF. Further experimental studies in rats with small bowel resection could help to increase knowledge in this field.

CONCLUSIONS

Exogenous secretin improves PNALD by enhancing the transport of bile acid and reducing TBA levels in the liver. For patients with IF receiving long-term TPN therapy, secretin may potentially play an important role in preventing and treating IFALD.

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