Serum bile acid fractions in neonates on total parenteral nutrition — is lithocholic acid responsible for the occurrence of cholestasis?

Akio Kubota, Kenji Imura, Akira Okada*, Shinkichi Kamata*, Riichiro Nezu* and Hisayoshi Kawahara*.

*Department of Pediatric Surgery, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan and *Department of Pediatric Surgery, Osaka University Medical School, Osaka, Japan.*

In order to determine whether lithocholic acid (LCA) contributes to the occurrence of total parenteral nutrition (TPN)-associated intrahepatic cholestasis (IHC) in neonates, we investigated the serum bile acid fractions of neonates on TPN. Twenty-five surgical neonates, receiving TPN for more than 2 weeks were studied. TPN-associated IHC was defined as serum direct bilirubin greater than 2.0 mg/dl. Serum bile acid fractions were examined by HPLC using 3α-hydroxy steroid dehydrogenase. Eight patients (32%; IHC group) developed TPN-associated IHC. Serum direct bilirubin concentrations in the non-IHC and IHC groups were 0.99 and 3.31 mg/dl respectively. Serum total bile acid levels in both groups were 14.4 and 71.6 nmol/ml respectively. Glycine- and taurine-conjugated cholic and chenodeoxycholic acids could be detected, and unconjugated and secondary (deoxycholic and lithocholic) bile acid were detected in trace levels in both the IHC and non-IHC groups. In conclusion, LCA is unlikely to be a causative factor in TPN-associated IHC in neonates.

Introduction

Intrahepatic cholestasis especially in neonates frequently develops during the course of total parenteral nutrition. Despite the vast number of investigations dealing with its aetiology, the cause of this TPN-associated liver dysfunction remains unclear. This is now considered to be related to various factors, including immaturity, early fasting, surgical operations, underlying diseases, overloading or imbalance of macro-nutrients, deficiency of trace elements, and infection[1,2,3]. Our previous study revealed that in addition to energy overloading, coexistence of infection and intestinal stasis play major roles in IHC in neonates[4].

Capron et al. assumed that intestinal anaerobic bacterial overgrowth could be a significant contributing factor to the occurrence of IHC associated with TPN, and they showed that metronidazole, a drug which suppresses anaerobic intestinal organisms, prevented the occurrence of liver dysfunction during TPN in patients with chronic inflammatory bowel disease (CIBD)[5,6]. We also demonstrated a beneficial effect of metronidazole on TPN-associated liver dysfunction in surgical neonates[7]. The results of these studies suggest that intestinal overgrowth of anaerobic bacteria implicated in the occurrence of hepatic dysfunction associated with TPN via certain mediators. Fouin-Fortunet et al., in 1982, suggested a role of lithocholic acid (LCA) in the IHC associated with TPN in patients with CIBD[8]. In this study, in order to determine whether LCA contributes to the occurrence of TPN-associated IHC in neonates, we investigated the serum bile acid fractions.

Correspondence: Akio Kubota, 840 Murodo-cho, Izumi, Osaka 590–02, Japan.

Subjects and methods

Twenty-five surgical neonates receiving TPN for more than 2 weeks at our institutions between 1984 and 1987 were studied. Their age ranged from 14 to 24 days, with a mean age of 17.4±2.6 days. Their underlying diseases are shown in Table 1. The nutritional regimen contained 21% glucose, 2.5% amino acid, and electrolytes, vitamins and trace elements. The solution was delivered

Table 1. Underlying diseases.

Esophageal atresia	6
Duodenal atresia	5
Diaphragmatic hernia	5
Omphal ocele/gastroschisis	3
Midgut volvulus	3
Jejunal/Ileal atresia	2
Hirschsprung's disease	2
Necrotizing enterocolitis	1
CIIPS*1	1
Total	25

*1CIIPS: chronic idiopathic intestinal pseudo-obstruction syndrome.

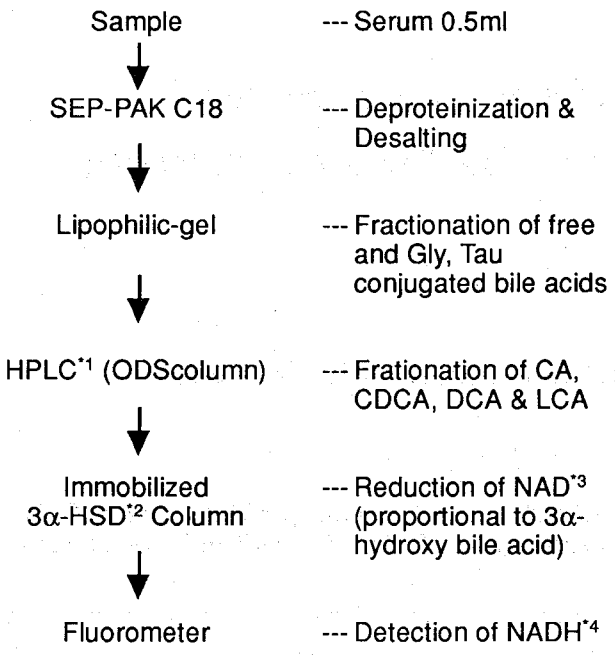

Figure 1. Procedure of serum bile acid analysis. *1HPLC: high-performance liquid chromatography *23α-HSD: 3α-hydroxy steroid dehydrogenase *3NAD: nicotinamide adenine dinucleotide. *4NADH: dihydro-NAD.

through a catheter placed in a central vein, and provided 70–100 kcal/kg/day continuously. The amino acid mixture consisted of a formula devised for paediatric use[9]. Blood samples for bile acid analysis were collected in the morning under complete fasting with continuous TPN infusion. Serum was collected by centrifugation immediately after sampling of venous blood, and was stored at –80°C. The serum samples were deproteinized and desalted with SEP-PAK C18[10], and fractionated into three groups using lipophilic gel chromatography[11]. Each fraction was then applied to a high-performance liquid chromatography system using an ODS column, and the fractionated cholic (CA), chenodeoxy-cholic (CDCA), deoxy-cholic (DCA) and lithocholic acids (LCA) were applied to the 3α-hydroxy steroid dehydrogenase-immobilized column[12]. NADH that was generated in proportion to 3α-hydroxy bile acids was measured by fluorometer (Figure 1). The external standard of unconjugated, glycine conjugated and taurine conjugated CA, CDCA, DCA and LCA were purchased from P-L Biochemicals, Inc, Milwaukee, Wis. In this system using 3α-HSD, sulfated and glucuronidated bile acids were not detected, since sulphate and glucuronide are conjugated with bile acid at the position of 3α-OH.

All results are expressed as mean±SD. Statistical analysis was performed by Student's t-test, and a probability of 5% or less was considered significant.

Results

Liver function tests

Table 2 shows the results of routine liver function tests. Out of 25 patients, 8 (32%) developed cholestasis with a serum concentration of direct bilirubin greater than 2.0 mg/dl during the first month of life. These 8 patients were included in the IHC group, and the other 17 in the non-IHC group. The mean serum concentrations of direct bilirubin in the non-IHC and IHC groups were 0.99 and 2.60 mg/dl, respectively, and those of total bile acid were 14.4 and 71.7 nmol/ml, respectively. Thus, total bile acid concentration was significantly higher in the IHC group. There was no significant difference in glutamic oxaloacetic transaminase, glutamic-pyruvic transaminase, γ-glutamyl transpeptidase and alkaline phosphatase between the non-IHC and IHC groups.

Serum bile acid fractions

In the non-IHC group, the mean glycine- and taurine-conjugated cholic acid were 4.9 and 3.1 nmol/ml, respectively (Table 3). Glycine- and taurine-conjugated chenodeoxycholic acids were 3.1 and 2.3 nmol/ml, respectively. However unconjugated bile acids or secondary bile acids, such as deoxycholic or lithocholic acid were not detected or were detected only in trace levels. In the IHC group, these fractions were detected in high amounts; glycine-conjugated bile acids were approximately four times higher than in the non-IHC group, and taurine-conjugated bile acids were approximately seven times higher than in the non-IHC group. However, in the IHC group, unconjugated and secondary bile acids were not detected or were detected only in trace levels. There was no marked increase in any single fraction in the IHC group.

Discussion

Certain bile acids, in particular the monohydroxylated secondary bile acid, LCA, are known to be cholestatic in a variety of animal species[13–19]. The histologic changes produced in LCA-treated animals[13,15,16] are similar to the changes observed in neonates and infants with TPN-associated IHC[4,20–24]. This suggests that the histologic changes seen in patients receiving TPN could be attributed to LCA. Intrinsic LCA is normally pro-

Table 2. Liver function tests.

	DB*1 (mg/dl)	TBA*2 (nmol/ml)	GOT*3 (iu/l)	GPT*4 (iu/l)	γ-GTP*5 (iu/l)	AL-P*6 (iu/l)
non-IHC*7	0.99±0.59	14.4±4.5	36.0±55.0	14.6±19.4	95.0±66.1	302.4±162.0
IHC	3.60±1.69	71.7±34.9	107.6±166.6	23.8±12.1	108.3±77.4	239.1±105.7
P values	*P*<0.01	*P*<0..01	NS	NS	NS	NS

*1DB: direct bilirubin. *2TBA: total bile acid. *3GOT: glutamic ozaloacetic transaminase. *4GPT: glutamic-pyruvic transaminase *5γ-GTP: γ-glutamyl transpeptidase. *6AL-P: alkaline phosphatase. *7IHC: intrahepatic cholestasis

Table 3. Serum bile acid fractions.

Fraction	non-IHC group	IHC-group	*P* values
G*[1] -CA*[7]	4.9±2.5	22.8±16.6	<0.05
T*[2] -CA	3.1±2.0	22.6±12.2	<0.05
U*[3] -CA	trace	trace	–
G-CDCA*[8]	3.1±1.1	12.7±7.7	<0.05
T-CDCA	2.3±1.2	16.6±10.2	<0.05
U-CDCA	trace	trace	–
G- DCA*[9]	trace	trace	–
T- DCA	trace	trace	–
U- DCA	trace	trace	–
G- LCA*[10]	trace	trace	–
T- LCA	trace	trace	–
U- LCA	trace	trace	–
Total CA	8.5±4.1	46.3±23.2	<0.05
Total CDCA	5.4±1.6	24.7±16.6	<0.05
Total DCA	trace	trace	–
Total LCA	trace	trace	–
Total Cly*[4]	8.0±3.1	35.5±23.4	<0.05
Total Tau*[5]	5.9±3.0	39.5±20.4	<0.05
Total Unc*[6]	trace	trace	–
TBA	14.4±4.5	76.1±34.4	<0.05

*[1]G: Glycine conjugated. *[2]: taurine conjugated. *[3]: Unconjugated *[4]Gly: Glycine conjugated bile acids. *[5]:Taurine conjugated bile acid. *[6]Unc: Unconjugated bile acids. *[7]CA: cholic acid. *[8]CDCA: Chenodeoxycholic acid. *[9]DCA: deoxycholic acid. *[10]LCA: lithocholic acid.

duced in the small intestine and colon by anaerobic bacterial dehydroxylation of chenodeoxycholic acid[25]. Recently, several investigators have reported the beneficial effects of metronidazole, a drug which suppresses intestinal anaerobic organisms[26], on TPN-associated liver dysfunction in adult patients with CIBD[5,6], in surgical neonates[7], and in animals[27]. These reports suggest that hepatotoxic substances produced by anaerobic intestinal bacteria contribute to the occurrence of liver dysfunction during TPN. Fouin-Fortunet et al. showed that in TPN patients with CIBD, the biliary concentration of LCA was significantly higher in patients with hepatic dysfunction than in patients with normal liver function[8]. Balistreri et al. found increased serum levels of sulfated LCA in infants receiving TPN who developed IHC[28,29]. Above studies suggest that intrinsic LCA may also cause liver dysfunction. However, no reports have yet shown that intrinsic LCA causes TPN-associated liver dysfunction in infants. We therefore investigated the serum bile acid fraction in neonates on TPN in this study, and attempted to determine whether LCA contributes to the occurrence of TPN-associated liver dysfunction in neonates. Sulfate and glucuronide conjugated bile acid were not analysed; however, Stiehl demonstrated that approximately 20% of serum LCA in infants with cholestasis was non-sulphated and non-glucuronated LCA[30], indicating that if sulphated or glucuronated LCA is increased, nonsulphated or glucuronated LCA is also increased. The present study showed that no single fraction, including LCA, was increased, even in patients with IHC, suggesting that LCA is unlikely to be a causative factor in TPN-associated IHC in neonates. In the previous studies reported by Stiehl or Farrell et al., serum LCA concentrations were as high as 2–4 μg/ml (approximately 4–8 nmol/ml). As reported by Stiehl et al., these concentrations were much lower than the amount given to animals to induce cholestasis, 120–240 μmol/kg intravenous infusion[14,17,18,19], and 0.1–1% in oral feeding[13,15]. And he concluded that it unlikely that such concentrations of monohydroxy bile acids measured in their patients were responsible for the cholestasis[30]. Furthermore, Cano et al. reported that in 8 non-CIBD patients with TPN-induced cholestasis, biliary LCA was normal; and in 6 cases with normal enterohepatic cycle where bile acid composition was normal, LCA represented less than 1% of total bile acids[31]. They concluded that LCA could not account for the occurrence of cholestasis in their patients.

The results of our previous study showing that coexistence of infection and intestinal stasis were two major contributing factors in the occurrence of IHC during TPN in neonates[4], and the beneficial effects of metronidazole on this TPN-associated liver dysfunction[5,6,7,27] indicate the role of another sepsis-mediated mechanism such as endotoxin release or portal

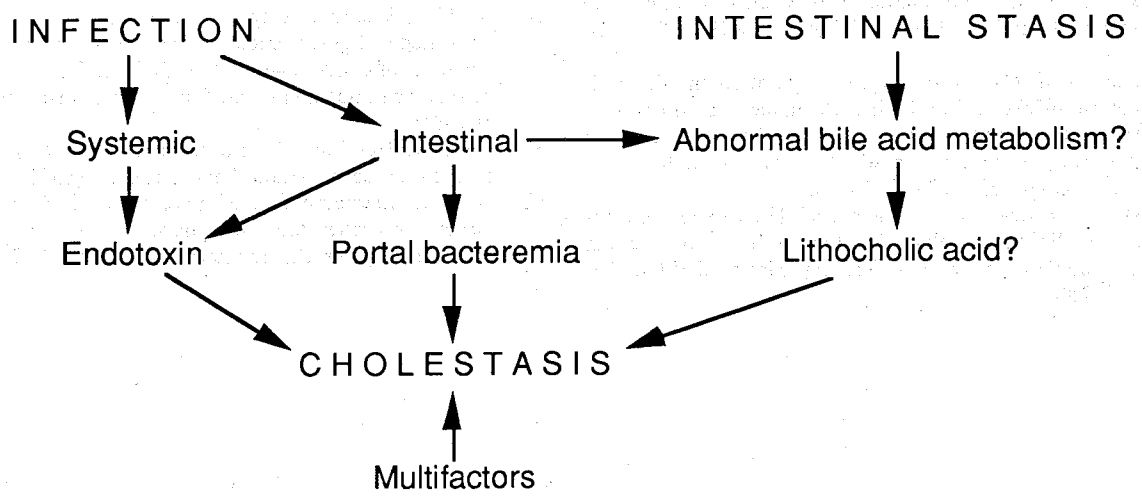

Figure 2. Mechanisims of TPN-associated intrahepatic cholestasis.

bacteremia (Figure 2). Cholestatic effects of endotoxin have been demonstrated in isolated perfused rat liver by Utili et al.[32]. Because young infants are highly susceptible to endotoxin, it is reasonable to presume that endotoxin may induce liver dysfunction in neonates on TPN. The observation of pericholangitis in cases of TPN-associated liver dysfunction[4,21,22], supports the possibility that ascending cholangitis is caused by protal bacteremia secondary to intestinal bacterial overgrowth, and results in intrahepatic cholestasis. Thus, portal bacteremia is another possible route for sepsis-mediated mechanism. Further investigations are necessary to clarify the relationship between intestinal or systemic bacterial proliferation and TPN-associated liver dysfunction in infants.

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要旨：新生児期TPN施行に伴う肝内胆汁うっ滞の発生に内因性リトコール酸が関与しているか否かを検討する目的で、血清胆汁酸分画の測定を行った。症例は手術後、非経腸栄養下に2週間以上TPNを受けている25症例で、日齢14～24日の新生児であった。胆汁酸分析は3α-HSD固定化酵素を用いたHPLCによって行った。25例中8例に胆汁うっ滞（血清直接ビリルビン値>2mg/dl）を認め、その血清総胆汁酸値は76.1nmol/mlで、非うっ滞群の14.4nmol/mlに比し高値を示した。分画ではタウリンおよびグリシン抱合型CAおよびCDCAのみが高値を示し、遊離型およびLCAなどの二次胆汁酸は殆ど検出されなかった。これより、新生児期TPN施行に伴う胆汁うっ滞の発生に内因性LCAが関与している可能性は少ないと考えられた。

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摘 要

爲了確定石膽酸是否引起全胃腸外營養（TPN）新生兒的肝内膽汁鬱滯（IHC），作者研究了全胃腸外營養新生兒的血清膽汁酸組分。他們選擇了２５位手術後並接受全胃腸外營養２周以上的新生兒爲研究對象。把血清直接膽紅質大於２．０毫克%定爲全胃腸外營養合併肝内膽汁鬱滯。血清膽汁酸組分用高壓液層析檢驗（３α一羥膽固醇脱氫 每法）。８個病人（３２%，肝内膽汁鬱滯組）有全胃腸外營養合併肝内膽汁鬱滯。血清直接膽紅質濃度在非肝内膽汁鬱滯組與肝内膽汁鬱滯組分別爲 ０．９９和３．３１毫克%。血清總膽汁酸水平兩組分別爲１４．４與７１．６微微克分子量/毫升(N MOL/ML)。該法可以測出甘氨膽酸，牛磺膽酸和鵝膽酸，在肝内膽汁鬱滯組與非肝内膽汁鬱滯組中均可測出微量的非結合膽酸，脱氧膽酸和石膽酸。從上述結果，作者認爲石膽酸不太可能是引起全胃腸外營養新生兒合併肝内膽汁鬱滯的因素。

關鍵詞：全胃腸外營養，肝内膽汁鬱滯，石膽酸，膽汁酸，新生兒。