ORIGINAL ARTICLE

Immunoelectron Microscopic Study on IgA, Secretory Component and Complement Component C3 in the Liver of Children Undergoing Total Parenteral Nutrition through Neonatal Period

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Abstract. To study the mechanism of the intrahepatic cholestasis observed during total parenteral nutrition (TPN) in neonates, we examined the localization of IgA, secretory component (SC) and complement component C3 (C3) in the liver in 4 patients by light and electron microscopic immunohistochemistry. In the patients who received TPN for 1 month, IgA- and SC-positive cholestasis was limited to bile canaliculi, but the integrity of both the canalicular wall and intercellular tight junction was maintained. These findings suggest that obstruction in the biliary tract develops at the canalis of Hering, causing reflux of IgA and SC into the bile canaliculi. When the duration of TPN extended beyond 6 months, the cholestasis in bile canaliculi progressed further, and degeneration of hepatocytes became more marked. Bile retained in hepatocytes occasionally contained IgA and SC. IgA- and SC-positive cholestasis also developed in the interlobular bile ducts, where no cholestasis had been observed 1 month after the beginning of TPN. SC production and SC-mediated transport of IgA, which are important functions of bile duct epithelial cells in the local immune mechanism, were impaired in association with the injury of those cells. C3 was localized not only in the hepatocellular organelles where C3 is normally observed, but also in the lumen of dilated bile canaliculi, suggesting that C3 is released from hepatocytes into bile in neonates receiving TPN and that C3 may be involved in some local immune mechanism of biliary system.


Key words: Parenteral nutrition, neonate, intrahepatic cholestasis, SC-mediated transport of IgA

Introduction

Total parenteral nutrition (TPN) is a technique indispensable for alimentary support also in pediatric surgery. However, this essentially unphysiologic procedure has been reported to have problems concerning its technical aspects and its management, as well as various metabolic and nutritional complications. In particular, prolonged TPN in neonates is likely to induce cholestatic liver injury, and cases dependent on TPN eventually develop often fatal hepatic failure.

We have performed TPN in 66 neonates who underwent surgery and observed liver dysfunction accompanied by hyperbilirubinemia in 29 cases (44%). Five of these patients could not be weaned from TPN and died of hepatic failure. The mechanism of intrahepatic cholestasis associated with TPN remains largely obscure and its management is still difficult.

In the present study, we investigated the mechanism of intrahepatic cholestasis in neonates undergoing TPN by examining intrahepatic localization of IgA, SC and C3.

Materials and Methods

We performed TPN in 66 (11%) of 412 surgical neonates encountered during the past 13 years. The procedure was administered for 1 month or longer in 33, and liver dysfunction accompanied by hyperbilirubinemia was observed in 25 (75%) of these patients. The following evaluations were carried out in 4 of these patients.

TPN consisted of water (100-120 ml/kg/day), amino acid (2 g/kg/day, E/N ratio = 1), and glucose and fat (1-2 g/kg/day). The patients were 2 male and 2 female full-term neonates. Their primary diseases were ileal...
atresia in Case 1 (age 1 day), megacystis microcolon intestinal hypoperistasis syndrome (MMIHS) in Case 2 (age 3 days), extensive aganglionosis in Case 3 (age 3 days), and MMIHS in Case 4 (age 2 days). The duration of TPN until collection of the liver tissue sample was 1 month, 6 months, 9 months, and 28 months, respectively. The bilirubin, GOT, and GPT levels were normal in Case 1, who received TPN for 1 month, but hyperbilirubinemia and moderate elevations of the transaminase levels were observed in the other 3 patients, who received TPN for 6 months or longer. These 3 patients could not be weaned from TPN and died of hepatic failure.

Immunohistochemistry was performed according to Nakane's direct enzyme antibody technique. Liver tissues were obtained either during the operation (Case 1), by needle biopsy (Case 4), or by autopsy (Cases 2 and 3) and fixed with PLP (periodate-lysine-paraformaldehyde). The tissues were washed in increasing concentrations of sucrose-PBS and finally placed in 20% sucrose in PBS containing 10% glycerol. The fixed tissues were embedded in OCT (Lab-Tek) and frozen sections (6 to 12 μm) were cut in a cryostat. The sections, air-dried on glass slides coated with egg albumin, were reacted with 10% rabbit serum and then with the peroxidase-labeled anti-IgA, anti-SC or anti-C3 antibodies. For light microscopy, the antibody-treated sections were washed in PBS, reacted with Karnovsky's diaminobenzidine solution, and dehydrated in graded alcohol. For electronmicroscopy, the sections were postfixed in 2.5% glutaraldehyde in PBS, washed in PBS, immersed in incomplete Karnovsky's solution containing dimethyl sulfoxide, and immersed in complete Karnovsky's solution. The sections were washed and immersed in 2% osmium tetroxide in PBS, dehydrated in graded alcohol, and embedded in Epon-Araldite. Ultrathin sections were prepared and examined in a JEM 100-C electron microscope.

For labeling, anti-human IgA, SC, and C3 rabbit antibodies (Dako) were broken down to Fab's and treated with HRP by the method of Wilson & Nakane.

Results

In Case 1 receiving TPN for 1 month, the serum bilirubin and transaminase levels were normal, but IgA-and SC-positive bile was already found in dilated bile canaliculi (Fig 1). There was no difference in the localization of IgA and SC. Microvilli were degenerated or lost in some of the markedly dilated bile canaliculi, and their walls were slightly thickened (Fig 2). Both IgA and SC were also observed in bile canaliculi showing little dilatation (Fig 3). No reaction product was found in hepatocytes, and no marked changes were observed in organelles. Interlobular bile ducts showed normal localization of IgA and SC, and no cholestasis was noted in their lumens. C3 was localized in dilated bile canaliculi, in addition to the endoplasmic reticulum, Golgi apparatus, and vesicles of hepatocytes. However, the integrity of the bile canicular wall, including tight junction, remained almost normal (Fig 4).

In case 4 receiving TPN for 28 months, fatty degeneration of hepatocytes, and fibrosis and cell infiltration of the portal area were observed. IgA- and SC-positive cholestasis developed extensively from hepatocytes to interlobular bile ducts (Fig 5). Many of the bile canaliculi that could be examined showed remarkable dilatation and thickening of the wall and contained IgA and SC-positive substances in their lumens, but these findings were essentially the same as in case 1 (Fig 6). In hepatocytes...
cytes, swelling, dissociation, and loss of organelles were notable, and the cell membrane was obscured. Some hepatocytes contained IgA- and SC-positive substances in the cytoplasm. Interlobular bile ducts also showed remarkable stagnation of IgA- and SC-positive bile. Their lumens were irregularly narrowed, and microvilli were nearly obliterated (Fig 7). SC was observed in the
Table 1  Localization of IgA, SC and C3

<table>
<thead>
<tr>
<th>TPN Duration</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1 mo</td>
<td>6 mo</td>
<td>9 mo</td>
<td>1 yr 4 mo</td>
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<tr>
<td>Hepatocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>SC</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>C3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Bile Canaliculi</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>SC</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>C3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Interlobular Bile Ducts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>SC</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>C3</td>
<td>-</td>
<td>+</td>
<td>+</td>
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</table>

+, mild; ++, moderate; ++++, severe

Table 2  Injury of Hepatocytes, Bile Canaliculi and Interlobular Bile Ducts

<table>
<thead>
<tr>
<th>TPN Duration</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
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<tbody>
<tr>
<td></td>
<td>1 mo</td>
<td>6 mo</td>
<td>9 mo</td>
<td>1 yr 4 mo</td>
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<tr>
<td>Injury of Hepatocytes Organelles</td>
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<td></td>
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<tr>
<td>Dilatation of Bile Canaliculi</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Thickening of Bile Canaliculi Wall</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Injury of Interlobular Bile Ducts</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+ + +</td>
</tr>
</tbody>
</table>

+, mild; ++, moderate; ++++, severe

Discussion

Since Peden et al. first reported hepatopathy accompanied by cholestasis and hepatic fibrosis in premature neonates receiving TPN, increasing interest has been directed to hepatopathy as a major complication of TPN in neonates.

We have carried out TPN in 66 neonates who underwent surgery and observed cholestatic liver disorder in 25 patients (75%) of 33 who were maintained by TPN for 1 month or longer. Three of these patients could not be weaned to enteric alimentation and died of hepatic failure. Prematurity,6-9 respiratory distress syndrome,10 sepsis,6,8,9 asphyxia,10 drug therapy,6,10 early fasting,7,8,10-12 and malnutrition6 have been suggested as clinical background of hepatopathy associated with TPN, and hyperammonemia,6,10,11 endotoxemia,7,12,13 impaired bile secretion and bile salt formation,10,11 protein hydrolysate toxicity,10,11 essential fatty acid deficiency,10,11 and amino acid imbalance6,10,11 have been speculated as pathogenic mechanisms.

Many electron microscopic evaluations of liver damage caused by TPN have been reported: Dilatation of the lumen,14-17 cholestasis,14-17 degeneration or loss of microvilli,14-17 and thickening of the wall in bile canaliculi; and injuries and degeneration of endoplasmic reticulum,6,14-16,18 mitochondria14,16 and lysosomes,14 appearance of fat droplets,17,18 vortical materials,15 bile salts and pigments,6 increase or decrease in glycogen granules,14,15,18 and thickening of the cell membrane6,17 in hepatocytes. In the present study, we noted the enterohepatic circulation of dimer IgA and SC-mediated transport of IgA from bile duct epithelial cells into the lumen,19,16 and carried out an immunoelectron microscopic study of intrahepatic localization of IgA and SC in children receiving TPN through the neonatal period. Localization of the complement component C3 was also examined.
In the patients who received TPN for 1 month, IgA- and SC-positive bile stasis was limited to bile canaliculi, while the serum bilirubin and transaminase levels were still normal. These findings suggest that obstruction develops in the biliary tract about the canalis of Hering partially formed by bile epithelial cells within 1 month after the beginning of TPN, causing reflux of IgA and SC into bile canaliculi. However, impairment of bile secretion appeared to be still mild, and few damaged organelles were observed in hepatocytes at this stage. The integrity of both bile canalicular and hepatocellular walls was maintained, and no influx of IgA or SC into hepatocytes was noted.

When the duration of TPN was extended beyond 6 months, cholestasis in bile canaliculi further progressed and degeneration of hepatocytes, including the bile canalicular wall, became more notable. Bile retained in hepatocytes occasionally contained IgA and SC. Although the route of its entry could not be determined, it was considered to have entered the cells via the damaged bile canalicular wall. Injury of hepatocyte organelles was also marked, and few cells appeared to be capable of functioning as hepatocytes. IgA- and SC-positive cholestasis developed also in the interlobular bile ducts, where no cholestasis was observed 1 month after the beginning of TPN. SC production and SC-mediated transport of IgA, which are important functions of bile duct epithelial cells, were impaired with the injury and degeneration of those cells. Since the bile ducts were scarcely dilated, cholestasis in the portal region is unlikely to be due to obstruction of distal bile ducts. Although the causative relationship between cholestasis and injury of bile duct epithelial cells must still be clarified, our findings suggest a vicious circle in which extensive functional impairment of bile duct epithelial cells first induces cholestasis, and toxic factors in the retained bile, such as bile acids, then damage epithelial cells.

The complement component C₃ is produced primarily in the hepatocyte, and the serum C₃ level is reported to increase in patients with obstructive jaundice. The behavior of intrahepatic C₃ during cholestasis due to TPN is of profound interest. In addition to the normal localization of C₃ in the organelles, it was observed also in the bile of dilated bile canaliculi. Oshio et al ascribed the increase of C₃ in bile in patients with obstructive jaundice to damaged hepatocytes. Our results appear to partly support this view. Among complement components, C₃ is considered to be released from hepatocytes into bile in neonates receiving TPN and to be involved in some local immune mechanism of the biliary system.

References