Effect of S-adenosylmethionine on total parenteral nutrition-associated cholestasis

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Background: Total parenteral nutrition (TPN) has been used clinically for more than 30 years, but hepatobiliary complications associated with TPN remain to be solved. The aim of this study was to investigate the effect of S-adenosylmethionine (SAMe) on TPN-induced cholestasis and hepatocytic apoptosis.

Methods: Twenty-four newborn rabbits were randomly divided into 3 groups: normal control group receiving breast milk, TPN group receiving TPN at a dose of 200 kcal/kg per day, and SAMe group receiving TPN plus SAMe at an intravenous dose of 100 mg/kg per day. Blood and liver samples were collected one week later. The levels of serum bile acid, alanine aminotransferase (ALT), alkaline phosphatase (AKP), total bilirubin, direct reaction bilirubin, albumin and globulin levels were detected by an automatic biochemical analyzer. Hepatic pathological changes were observed under the light microscope, and apoptosis of hepatocytes was determined with the TUNEL method.

Results: There were no significant differences in the levels of serum bile acid, ALT, AKP, total bilirubin, albumin and globulin between the SAMe group and control group (P>0.05). The level of direct reaction bilirubin in the SAMe group was obviously higher than that in the control group (P<0.01), but significantly lower than that in the TPN group (P<0.01). Cholestatic changes and mild hepatic steatosis were observed in the TPN group, while no such changes were found in the SAMe and control groups. The apoptotic cell counts were 0.263%±0.041% in the control group, 1.060%±0.217% in the TPN group, and 0.467%±0.182% in the SAMe group. The apoptotic cells were much more in the TPN group than in the control (P<0.01) and SAMe groups (P<0.05).

Conclusions: TPN can cause cholestasis and increase apoptosis of hepatocytes in newborn rabbits. SAMe can prevent TPN-induced cholestasis effectively, and the inhibition of hepatocytic apoptosis may be one of its mechanisms.

Key words: S-adenosylmethionine; total parenteral nutrition; apoptosis; cholestasis; steatosis; newborn

Introduction

Total parenteral nutrition (TPN) can induce hepatobiliary complication, which is characterized by intrahepatic cholestasis in newborns and infants. Its mechanism is obscure to the present. There is no effective means of protection and treatment except enteral feeding instead of TPN administration. Cholestasis can cause obstruction of bile acid excretion. Accumulation of toxic bile acid within hepatocytes can induce apoptosis of hepatocytes, which plays an important role in cholestatic hepatic injury. S-adenosylmethionine (SAMe) serves as a methyl donor and a precursor of glutathione in numerous metabolic reactions. An in vitro study has shown that SAMe can inhibit apoptosis of hepatocytes induced by bile acid, but there is no experiment in vivo. The effect of SAMe on TPN-associated cholestasis and its relation with apoptosis of hepatocytes require further research.

Methods

Animals

Twenty-four newborn New Zealand white rabbits were randomly divided into three groups (8 rabbits in each group). Rabbits in the control group received breast milk for one week, those in the TPN group received TPN at a dose of 200 kcal/kg per day, and those in the SAMe group received TPN plus SAMe
at an intravenous dose of 100 mg/kg per day for one week.

**TPN formula**
All-in-one TPN solution (87.0 kcal/100 ml) was prepared at the Clinical Nutrition Center of the hospital. The formula is shown in Table 1.

**Newborn rabbit TPN model**
Newborn rabbits in the TPN group had a silastic catheter inserted through the right jugular vein into the superior vena cava after body weighing and anesthesia. Cannulated animals were given TPN solution (230 ml/kg per day) continuously via an infusion pump and were housed in individual cages at a temperature of 33ºC-35ºC. The volume infused was regulated according to the body weight every day. Animals in the SAMe group received isonitrogenous and isocaloric TPN plus 100 mg/kg intravenous SAMe every day. Seven days later, blood and liver tissue samples were collected for measurement.

**Items of measurement**

**Serum biochemistry**
Serum was separated by centrifugation. The levels of serum bile acid, alanine aminotransferase (ALT), alkaline phosphatase (AKP), total bilirubin, direct reaction bilirubin, albumin and globulin were detected with an automatic biochemical analyzer.

**Liver pathology**
Samples of the liver tissue at the same site were collected for dehydration, embedment, slicing and HE staining. Pathological changes of cholestasis were observed under the light microscope.

**Apoptosis of hepatocytes**
Apoptosis of hepatocytes was determined with the terminal deoxynucleotidyl transferase-medicated dUTP nick end labeling (TUNEL) method. Biotin-11-dUTP was used to label the terminal of DNA fragment on paraffin sections. The nuclei of apoptotic cells were stained brown. The percentage of apoptotic cells was calculated in 10 fields of vision selected randomly at 400 magnification under the light microscope.

**Statistical analysis**
All parameters were expressed as mean±SD and were analyzed using one-way analysis of variance (ANOVA) or Student's t test with SPSS 11.5 statistic software package for Windows XP. P values less than 0.05 were considered statistically significant.

**Results**

**Serum biochemistry**
There were no significant differences in the levels of serum bile acid, ALT, AKP, total bilirubin, albumin and globulin between the SAMe group and control group (P>0.05). The level of direct reaction bilirubin in the SAMe group was obviously higher than that in the control group (P<0.01), but it was significantly lower than that in the TPN group (P<0.01) (Table 2).

**Pathological changes**
Cholestatic changes such as bile plugs in interlobular...
bile ducts, proliferation of bile ducts, bile pigments in Kupffer cells and hepatocytes, and mild hepatic steatosis were observed in the TPN group, while no such changes were found in the SAMe and control groups.

Apoptosis of hepatocytes
The apoptotic cell counts were 0.263%±0.041% in the control group, 1.060%±0.217% in the TPN group, and 0.467%±0.182% in the SAMe group. Apoptotic cells were much more in the TPN group than in the control (P<0.01) and SAMe groups (P<0.05); they were mainly distributed in the III area near the central vein of the hepatic lobe.

Discussion
SAMe, the essential metabolite of methionine in mammals, regulates the flowing property of hepatocytic membrane through phospholipid methylating of the plasma membrane, which plays an important role in the maintenance of normal liver function. In varied liver diseases, dysfunction of adenosylmethionine synthetase, which catalyzes methionine transforming to SAMe, can cause decrease of SAMe itself and its metabolites such as cysteine, taurine, glutathione and result in accumulation of methionine in the body. The imbalance of amino acids in TPN solution is one of the causes leading to intrahepatic cholestasis. Methione can cause cholestasis,[5] whereas its metabolites—cysteine, taurine and glutathione can attenuate TPN associated cholestasis.[6-8] Hence, dysfunction of adenosylmethionine synthetase and the resultant decrease of SAMe and its metabolites play an important role in the pathogenesis of TPN-associated cholestasis. It has been confirmed that SAMe can prevent liver injury caused by various diseases including cholestasis,[9,10] but the mechanism of the prevention has not been clarified. Restoring the exhausted glutathione in hepatocytes was regarded as one of its mechanisms.[9] But a recent study[11] showed that SAMe might have hepatoprotective effects which were independent of glutathione. SAMe is not only the precursor of glutathione but also the precursor of polyamine. 5'-methylthioadenosine (MTA) is the end product in polyamine synthesis. Another study[12] revealed that MTA similar to SAMe has the same protective effect for hepatocytes, but MTA is not the precursor of glutathione and does not involve in the methylation. Hence, the mechanism of SAMe needs further elucidation.

TPN may cause intrahepatic cholestasis and dysfunction of bile acid excretion. The accumulation of bile salts, especially hepatotoxic hydrophobic bile salts, can lead to injury of hepatocytes. The main pathological changes of cholestasis are the presence of atrophic small hepatocytes and eosinophilic bodies, which coincide with the pathological changes of apoptosis[14] We think that hepatocytic injury during cholestasis may be due to the apoptosis of hepatocytes induced by bile salts, because studies showed that bile salts could directly induce apoptosis of hepatocytes.[15,16] In our study, TPN-induced cholestasis was characterized by significant enhancement of apoptosis without necrosis of hepatocytes, illustrating that apoptosis of hepatocytes plays an important role in the development of TPN-associated cholestasis.

In vitro studies proved that SAMe can inhibit the bile salts-induced apoptosis of hepatocytes,[4,17] which was independent of glutathione. The inhibition of apoptosis may take place in the mitochondrial level. SAMe enters mitochondria through a specific carrier mediated system and inhibits the release of cytochrome C from mitochondria, which is the key step of apoptosis induced by varied inducers.[18] SAMe can sustain the normal ratio of lipid/protein and Na+, K+-ATP enzymes in the plasma membrane, thus stabilizing the membrane. SAMe inhibits the apoptosis of hepatocytes through the maintenance of the stability of mitochondrial membrane and the inhibition of cytochrome C release. In our study, SAMe given intravenously at a dose of 100 mg/kg per day during TPN administration significantly reduced the levels of serum bile acid and bilirubin and attenuated the pathological changes of cholestasis with reduced apoptosis of hepatocytes in newborn rabbits. These results indicate significant protective effect on TPN-induced cholestasis and hepatocytic apoptosis.

In conclusion, TPN may cause intrahepatic cholestasis with increased apoptosis of hepatocytes, which plays an important role in the development of cholestatic liver injury. SAMe can prevent cholestatic liver injury caused by TPN, and the inhibition of apoptosis is one of its mechanisms.

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