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

Yi-Sheng Liu, Wei Cai, Sheng-Mei Wu, Long-Hua Qian

Shanghai, China

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.**

World J Pediatr 2007;3(3):218-221

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.[1,2] 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.[3] 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,[4] 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

Author Affiliations: Xinhua Hospital Affiliated to Medical School, Shanghai Jiaotong University, Shanghai Institute for Pediatric Research, Shanghai 200092, China (Liu YS, Cai W, Wu SM, Qian LH)

Corresponding Author: Yi-Sheng Liu, MD, PhD, Xinhua Hospital Affiliated to Medical School, Shanghai Jiaotong University, Shanghai Institute for Pediatric Research, Shanghai 200092, China (Tel: 86-21-65790000 ext 3426; Fax: 86-21-65791316; Email: liuyisheng2002@yahoo.com.cn)

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.

Table 1. Formula of total parenteral nutrition solution

Contents	Volume (ml)	Calorie (kcal)	Caloric percentage (%)
20% long-chain fatty acid	17.5	35.0	40
11.4% amino acids	38.0	17.4	20
50% glucose	12.5	34.6	40
10% glucose	24.0	-	-
10% NaCl	2.1	-	-
10% KCl	1.4	-	-
10% calcium gluconate	0.5	-	-
25% $MgSO_4$	0.5	-	-
Glycerophosphate sodium	1.0	-	-
Water-soluble vitamin (soluvit)	1.0	-	-
Fat-soluble vitamin (vitalipid)	0.5	-	-
Multi-trace elements (addamel)	1.0	-	-
Total	100	87.0	100

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

Table 2. Results of serum biochemical analysis

Parameters	Control group	TPN group	SAMe group	*F*	*P*
Bile acid (mmol/L)	28.6±12.9	62.6±29.8*	41.7±18.9	4.864	0.018
ALT (U/L)	15.9±4.5	17.8±8.4	18.6±7.1	0.336	0.718
AKP (U/L)	322.5±37.2	336.8±31.3	289.9±65.7	2.086	0.149
Total bilirubin (mmol/L)	6.2±1.8	11.1±3.1*	8.6±2.7	7.090	0.004
Direct bilirubin (mmol/L)	0.4±0.3	5.5±2.0*	2.1±0.6*†	34.59	0.000
Total protein (g/L)	39.5±2.5	42.4±7.9	44.1±5.2	1.362	0.278
Albumin (g/L)	24.4±3.0	24.1±3.2	23.9±4.1	0.042	0.959

*: $P<0.01$, compared with the control group; †: $P<0.01$, compared with the TPN group. TPN: total parenteral nutrition; ALT: alanine aminotransferase; AKP: alkaline phosphatase.

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 glutatione 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.[13] 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.

Funding: None.
Ethical approval: Not needed.
Competing interest: None declared.
Contributors: LYS proposed the study, wrote the first draft and analyzed the data. All authors contributed to the design and interpretation of the study and to further drafts.

References

1 Postuma R, Trevenen CL. Liver disease in infants receiving total parenteral nutrition. Pediatrics 1979;63:110-115.

2 Pereira GR, Sherman MS, DiGiacomo J, Ziegler M, Roth K, Jacobowski D. Hyperalimentation-induced cholestasis: increased incidence and severity in premature infants. Am J Dis Child 1981;135:842-845.
3 Patel T, Bronk SF, Gores GJ. Increases of intracellular magnesium promote glycodeoxycholate-induced apoptosis in rat hepatocytes. J Clin Invest 1994;94:2183-2192.
4 Benz C, Angermuller S, Kloters-Plachky P, Sauer P, Stremmel W, Stiehl A. Effect of S-adenosylmethionine versus tauroursodeoxycholic acid on bile acid-induced apoptosis and cytolysis in rat hepatocytes. Eur J Clin Invest 1998;28:577-583.
5 Moss RL, Haynes AL, Pastuszyn A, Glew RH. Methionine infusion reproduces liver injury of parenteral nutrition cholestasis. Pediatr Res 1999;45:664-668.
6 Gomez MR, Benzick AE, Rogers LK, Heird WC, Smith CV. Attenuation of acetaminophen hepatotoxicity in mice as evidence for the bioavailability of the cysteine in D-glucose-L-cysteine *in vivo*. Toxicol Lett 1994;70:101-108.
7 Guertin F, Roy CC, Lepage G, Perea A, Giguere R, Yousef I, et al. Effect of the taurine on total parenteral nutrition-associated cholestasis. JPEN J Parenter Enteral Nutr 1991;15: 247-251.
8 Sokol RJ, Taylor SF, Devereaux MW, Khandwala R, Sondheimer NJ, Shikes RH, et al. Hepatic oxidant injury and glutathione depletion during total parenteral nutrition in weanling rats. Am J Physiol 1996;270(4 Pt 1):G691-700.
9 Mato JM, Alvarez L, Ortiz P, Pajares MA. S-adenosylmethionine synthesis: molecular mechanisms and clinical implications. Pharmacol Ther 1997;73:265-280.
10 Ming Z, Fan YJ, Yang X, Lautt WW. Synergistic protection by S-adenosylmethionine with vitamins C and E on liver injury induced by thioacetamide in rats. Free Radic Biol Med 2006;40:617-624.
11 Mcmillan JM, McMillan DC. S-adenosylmethionine but not glutathione protects against galactosamine-induced cytotoxicity in rat hepatocyte cultures. Toxicology 2006;222: 175-184.
12 Simile MM, Banni S, Angioni E, Carta G, De Miglio MR, Muroni MR, et al. 5'-Methylthioadenosine administration prevents lipid peroxidation and fibrogenesis induced in rat liver by carbon-tetrachloride intoxication. J Hepatol 2001;34:386-394.
13 Schmucker DL, Ohta M, Kanai S, Sato Y, Kitani K. Hepatic injury induced by bile salts: correlation between biochemical and morphological events. Hepatology 1990;12:1216-1121.
14 Patel T, Gores GJ. Apoptosis and hepatobiliary disease. Hepatology 1995;21:1725-1741.
15 Faubion WA, Guicciardi ME, Miyoshi H, Bronk SF, Roberts PJ, Svingen PA, et al. Toxic bile salts induce rodent hepatocyte apoptosis via direct activation of Fas. J Clin Invest 1999;103:137-145.
16 Li D, Sun JB, Sun HC, Li F, Liu FJ, Liu S. Mechanism of hepatocytic injury caused by obstructive jaudice—apoptosis of hepatocytes induced by bile salts. Zhonghua Waike Zazhi 1998;36:624-626.
17 Ansorena E, Garcia-Trevijano ER, Martinez-Chantar ML, Huang ZZ, Chen L, Mato JM, et al. S-adenosylmethionine and methylthioadenosine are antiapoptotic in cultured rat hepatocytes but proapoptotic in human hepatoma cells. Hepatology 2002;35:247-280.
18 Bossy-Wetzel E, Newmeyer DD, Green DR. Mitochondrial cytochrome C release in apoptosis occurs upstream of DEVD-specific caspase activation and independently of mitochondrial transmembrane depolarization. EMBO J 1998;17:37-49.

Received April 18, 2006
Accepted after revision March 26, 2007