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Protective effects of nitric oxide on hepatic steatosis induced by total parenteral nutrition in rats

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KEY WORDS parenteral nutrition; nitric oxide; hepatic steatosis; rats

ABSTRACT

AIM: To investigate the effects of nitric oxide (NO) on hepatic steatosis induced by total parenteral nutrition (TPN) in rats. **METHODS:** Thirty normal Wistar rats were randomly divided into five groups: group A, free access to food and water; group B, TPN; group C, TPN plus arginine; group D, TPN plus N^G-nitrio-*L*-arginine methyl ester (*L*-NAME, NO synthase inhibitor); group E, TPN plus arginine and *L*-NAME. At the seventh day, liver function tests were examined, lipid content, nitric oxide level, and nitric oxide synthase (NOS) activity were measured, and histology was examined. **RESULTS:** The hepatic lipid content [triglyceride (mmol·g^{-1} tissue), cholesterol (mmol·g^{-1} tissue)] in group B was increased compared with group A (39.3±2.4 and 13.1±1.1 *vs* 6.9±0.8 and 5.6±0.6) (P<0.05). It was higher in group D (50±6 and 14.1±1.7) than in group B (P<0.05), whereas lower in group C (18±4 and 7±3) than in group B (P<0.05). The activity and distribution of NOS in the liver were associated with the content and distribution of hepatic lipid. **CONCLUSION:** These results suggest that nitric oxide produced by the liver may protect hepatic steatosis induced by total parenteral nutrition in rats.

INTRODUCTION

Long-term total parenteral nutrition (TPN) can frequently lead to the development of hepatic steatosis. Although its incidence has decreased with modifications of the TPN regimen, such as increasing content of lipid and decreasing content of glucose, hepatic steatosis still remains the most common complication of TPN[1]. The exact pathogenesis of TPN-associated hepatic steatosis is still unclear. Nitric oxide (NO) produced by hepatic cells has many cytoprotective effects such as modulation of vascular smooth muscle tone, inhibition of leukocyte adhesion and platelet aggregation, and reduction of free radicals, and it may play an important pathophysiological role in hepatic diseases[2,3]. However, the prophylactic effects of nitric oxide on hepatic steatosis have not been documented. In this present study, we investigated the effects of nitric oxide on TPN-associated hepatic steatosis in rats and its possible mechanism.

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MATERIALS AND METHODS

Animals Thirty heathy male Wistar rats with a body weight of 250-300 g (Grade II, Certificate № 26-98A011), bred by the Experimental Animal Center of Sun Yat-Sen University of Medical Sciences, were randomly divided into five groups: group A (n=6), free access to food and water; group B (n=6), received TPN; group C (n=6), recieved TPN plus arginine; group D (n=6), recieved TPN plus N^G-nitrio-*L*-arginine methyl ester (*L*-NAME, NO synthase inhibitor); group E (n=6), recieved TPN plus arginine and *L*-NAME.

A 0.9-mm silicon cannula was placed into the internal jugular vein of rat under ether anesthesia. The cannula was coursed subcutaneously, and was brought outside and fixed at the back neck. Rats were kept in cages without limiting activity after cannulation with swivel, and TPN solution was infused continuously *via* the cannula up to 24 h per day.

Implementation of TPN TPN solution was prepared according to physiological needs of growing rats. It contained glucose, lipofundin (MCT/LCT lipid emulsion), amino acid, vitamin, trace elements, and electrolytes. The rats were provided approximately 840 $kJ \cdot kg^{-1} \cdot d^{-1}$ of nonprotein energy and 1.4 $g \cdot kg^{-1} \cdot d^{-1}$ of nitrogen. Source of nitrogen was provided with compound amino acids and 35 % of nonprotein energy was provided with lipofundin. Arginine was added to TPN solution at a dose of 500 mg/d in group C, and *L*-NAME added at a dose of 30 mg/d in group B. In group E, 500 mg of arginine and 30 mg of *L*-NAME were added to TPN solution per day.

The infusion was maitained at 3 mL/h by a rollerpump. The infusion apparatus was sterile and refilled daily. The cages were maintained at 25 ℃ with a 12-h light/dark cycle.

Investigation By the end of one week feeding, rats were weighed and then sacrificed under ether anesthesia. Blood sample was collected for determining total bilirubin (TBIL), aspartate transaminase (AST), alanine transaminase (ALT), and albumin (ALB). The liver was excised for histologic and biochemical study. One part of the liver was prepared for Sudan staining and histochemical staining with nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase. The remainder of the liver was prepared for measurement of lipid content, nitric oxide (NO) level, and nitric oxide synthase (NOS) activity. Level of the stable end products of NO[nitrite (NO_2^-)/nitrate (NO_3^-)] in the liver was measured using a colorimetric procedure based on the Griess reaction. Triglycerides and cholesterol in the liver were determined using solvent extraction method[4]. The sections were stained with Sudan for histopathological examination. Histochemical staining with NADPH- diaphorase was used to evaluate NOS activity[5]. The severity of hepatic steatosis was evaluated semi-quantitatively according to the following grading system suggested by Ruwart *et al*[6]:

-: absence of steatosis.

+: mild steatosis with presence of lipid, mainly macrovesicular, in no more than 1/3 hepatocytes,.

++: moderate steatosis with presence of both micro- and macro-vesicular lipid in 1/3 to 2/3 of hepatocytes.

+++: severe steatosis with mixed micro- and macro-vesicular lipid in more than 2/3 of hepatocytes.

++++: severe steatosis with presence of macrovesicular lipid in all or almost all hepatocytes.

Statistical analysis Results were expressed as mean±SD. Statistical analysis was performed using commercially available SPSS package, and one-way ANOVA and Student's *t*-test were employed to compare the differences between groups. Statistical significance was considered as $P<0.05$.

RESULTS

Survive condition All the rats survived in the study. The weight of the rats increased about 2 g per day. There was no statistically significant difference in the change of body weight among all the groups (F=0.21, $P>0.05$).

Liver function With respect to liver function, the total bilirubin level was not elevated in any group and remained below normal levels. There was no significant difference in TBIL, AST, ALT, and ALB among the groups (F was 0.47, 0.72, 0.41, and 0.88

respectively, $P>0.05$).

Hepatic lipid content (triglycerides and cholesterol) Hepatic lipid content was remarkably elevated in TPN-treated rats in comparison with the free feeding rats ($P<0.05$), but lower in TPN+arginine-treated rats and significantly higher in TPN+*L*-NAME-treated rats when compared with TPN-treated rats ($P<0.05$). There was no significant difference between TPN-treated rats and TPN+arginine+*L*-NAME-treated rats ($P>0.05$). Compared with TPN+*L*-NAME -treated rats, hepatic lipid content was lower in TPN+arginine+*L*-NAME-treated rats ($P<0.05$, Tab 1).

Tab 1. Hepatic lipid content in various groups. *n*=6. Mean±SD. [a]*P*>0.05, [b]*P*<0.05 *vs* TPN group. [e]*P*<0.05 *vs* TPN+*L*-NAME group.

Group	Triglycerides/ μmol·g^{-1} wet tissue	Cholesterol/ μmol·g^{-1} wet tissue
Control	6.9±0.8 [b]	5.6±0.6[b]
TPN	39.3±2.4	13.1±1.1
TPN+arginine	18±4[b]	7±3[b]
TPN+*L*-NAME	50±6[b]	14.1±1.7
TPN+arginine+*L*-NAME	36±9[abe]	10.0±2.4 [abe]

Hepatic NO_2^-/NO_3^- level and NOS activity Hepatic NO_2^-/NO_3^- level was remarkably reduced in TPN-treated rats in comparison with the free feeding rats ($P<0.05$), but it was higher in TPN+arginine-treated rats, and was lower in TPN+*L*-NAME-treated rats when compared with TPN-treated rats ($P<0.05$). There was no significant difference between TPN-treated rats and TPN+arginine+*L*-NAME-treated rats ($P>0.05$). Hepatic NO_2^-/NO_3^- level was not different between TPN+*L*-NAME -treated rats and TPN+arginine+*L*-NAME-treated rats ($P>0.05$).

Hepatic NOS activity was remarkably increased in TPN-treated rats compared with the free feeding rats ($P<0.05$), but it was reduced in TPN+arginine-treated rats, and was higher in TPN+*L*-NAME-treated rats when compared with TPN-treated rats ($P<0.05$). There was no significant difference between TPN-treated rats and TPN+arginine+*L*-NAME-treated rats ($P>0.05$). Compared with TPN+*L*-NAME-treated rats, hepatic NOS activity was lower in TPN+arginine+*L*-NAME-treated rats ($P<0.05$, Tab 2).

Tab 2. Hepatic NO_2^-/NO_3^- level and NOS activity in various groups. *n*=6. Mean±SD. [a]*P*>0.05, [b]*P*<0.05 *vs* TPN group. [d]*P*>0.05, [e]*P*<0.05 *vs* TPN+*L*-NAME group.

Group	NO_2^-/NO_3^-	NOS activity/ (μmol·min^{-1}·g^{-1} protein)
Control	42±5[b]	7.9±1.8[b]
TPN	32±6	17.3±1.2
TPN+arginine	50±7[b]	11.8±1.2[b]
TPN+*L*-NAME	26±6[b]	24.4±1.4[b]
TPN+arginine+*L*-NAME	32±4[ad]	16.3±1.6[ae]

Morphologic studies Under Sudan staining, the degree of hepatic steatosis was negative in the free feeding rats; +~++ in TPN+arginine-treated rats; ++~+++ in both TPN-treated rats and TPN+arginine+*L*-NAME -treated rats; and +++~++++ in TPN+*L*-NAME -treated rats (Fig 1). Lipid droplets were major around the regions of centrilobular veins in the livers.

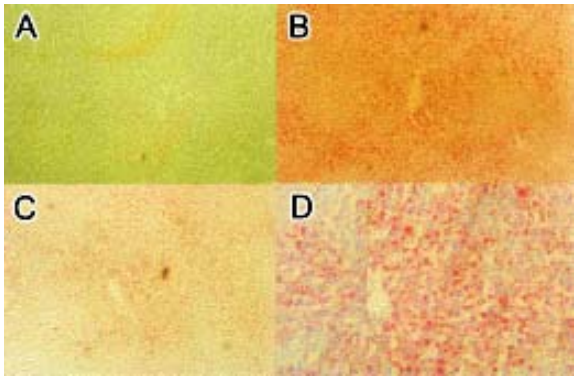

Fig 1. Pathological features of hepatic steatosis in various groups (Sudan staining, ×200). Negative in the free feeding rats (group A); ++~+++ in TPN-treated rats (group B); +~++ in TPN+arginine-treated rats (group C); +++~++++ in TPN+*L*-NAME-treated rats (group D).

Liver staining with NADPH-diaphorase showed

that the staining was not blue in the free feeding rats, blue in TPN-treated rats and TPN+arginine+*L*-NAME-treated rats, slight blue in TPN+arginine-treated rats, and heavy blue in TPN+*L*-NAME-treated rats (Fig 2). The intensity of NADPH-diaphorase staining was also major around the regions of centrilobular veins.

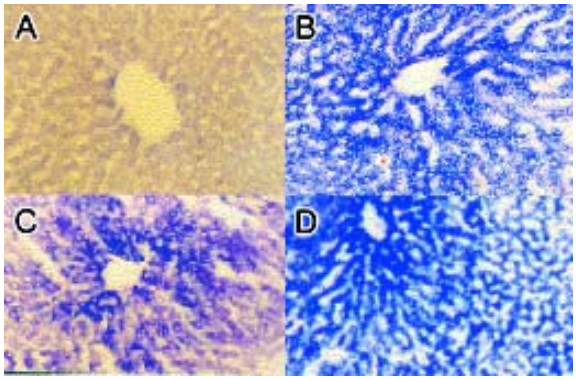

Fig 2. Hepatic histochemical staining with NADPH-diapherase in various groups (×200). The staining was not blue in the free feeding rats (group A), blue in TPN-treated rats (group B), slight blue in TPN+arginine-treated rats (group C), and heavy blue in TPN-*L*-NAME -treated rats (group D).

DISCUSSION

Hepatic complications are common in both infants as well as adults receiving total parenteral nutrition (TPN) who have no underlying liver disease. In the pediatric age group the incidence was reported to reach as high as 84 % whereas in adults, a broad spectrum of various liver function derangements have been reported in 26 %-90 % of patients with all types and regimens of TPN[7]. The wide spectrum of clinical, biochemical, and pathological manifestations might include any combination from transient benign increases in liver enzymes in TPN patients to liver disease which is characterized histologically by centrilobular, intrahepatic cholestasis, portal fibrosis, reactive bile duct proliferation, nonspecific portal and periportal inflammation, and hepatic steatosis. With improvement of TPN solution and technology, the complications associated with TPN had been ameliorated obviously. However, hepatic steatosis was still common in adults. In our experiment, hepatic steatosis was the only morphologic derangement, and serum transaminase and bilirubin levels were normal.

Nitric oxide is synthesized from arginine by nitric oxide synthase. Nitric oxide produced by hepatic cells took part in modulation of vascular smooth muscle tone, inhibition of leukocyte adhesion and platelet aggregation, reduction of free radicals, increase in blood flow in the liver, and improvement of hepatic microcirculation in some liver injury[8]. Our results showed that the intensity of NADPH diaphorase staining corresponded with NOS activity. The highest activity of NOS in TPN+*L*-NAME-treated rats, the heaviest blue staining was. However, that was contrary in the free feeding rats. NADPH-diaphorase staining could reflect the activity and distribution of NOS[9].

Cantoni *et al* reported that treatment with LPS increased liver NOS expression, while treatment with LPS and exogenous arginine decreased NOS expression/activity in the liver[10]. Our results showed that hepatic NO synthesis was decreased, hepatic NOS activity was elevated, and hepatic steatosis was induced by TPN infusion. NOS, a site of superoxide synthesis, can catalyze nitric oxide synthesis, and also can generate superoxide[11]. The generation of superoxide would increase at suboptimal concentrations of arginine[12]. Sokol reported that hepatic oxidant injury and glutathione depletion occurred during total parenteral nutrition[13].

Moreover, hepatic NO synthesis could be increased and NOS activity could be reduced by arginine, and hepatic steatosis was also ameliorated. The exogenous arginine was effective in elevating hepatic membrane transport activity and increasing production of nitric oxide[14]. Arginine could promote hepatic NO synthesis, improve hepatic microcirculation, feedback to inhibit NOS activity, reduce generation of superoxide catalyzed by NOS, and decrease hepatic damage. Much more hepatic NO synthesis was decreased and much more severe hepatic steatosis was induced by *L*-NAME. The effects of *L*-NAME on hepatic NO synthesis, NOS activity, and hepatic steatosis were reversed by arginine.

In conclusion, adequate TPN infusion could still cause hepatic steatosis in rats. Hepatic NO synthesis could be increased by arginine to reduce hepatic damage. Nitric oxide may be protective for hepatic steatosis

caused by total parenteral nutrition in rats.

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一氧化氮对大鼠全胃肠外营养肝脂肪变性的防护作用

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关键词 全胃肠外营养; 一氧化氮; 肝脂肪变性; 大鼠

目的: 探讨一氧化氮(NO)在全胃肠外营养(TPN)引起的肝脂肪变性中的作用. **方法**: 30只正常Wistar大鼠随机分为5组: A组, 自由进食和水; B组, TPN; C组, TPN+精氨酸; D组, TPN+N^G-硝基-*L*-精氨酸甲酯(N^G-nitrio-*L*-arginine methyl ester, *L*-NAME); E组, TPN+精氨酸+*L*-NAME. 实验7天后测肝功能、肝内脂肪、肝脏NO含量及NOS活性并进行肝脏组织学检查. **结果**: B组肝内脂肪[甘油三酯、胆固醇(mmol·g^{-1})] (39.3±2.4和13.1±1.1)较A组(6.9±0.8和5.6±0.6)明显增加($P<0.05$), D组肝内脂肪(50±6和14.1±1.7)较B组增加更显著($P<0.05$), C组肝内脂肪(18±4和7±3)较B组明显减少($P<0.05$), 肝内NOS活性及分布与肝内脂肪含量及分布相平行. **结论**: 一氧化氮在TPN引起的肝脂肪变性中起防护作用.

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