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Improvement of graft function and animal survival by fat emulsion in liver transplant rats

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Abstract

Nutritional supports are required for liver transplant patients. However, no systematical assessment has been made of the optimal composition of energy yielding substrates in these patients. This study is to evaluate whether mixed energy system consisting of carbohydrate and lipid emulsions is more advantageous over single energy source of glucose for nutritional support in liver transplant recipients and whether structured lipid emulsion (STG) is superior to medium-chain triglyceride/long-chain triglycerides (MCT/LCT) and long-chain triglycerides (LCT) using a total parenteral nutrition model. Liver transplant rats were randomly divided to four groups according to the energy source, i.e. glucose (GLU), MCT/LCT, STG and LCT groups. Sham operated rats served as control. Hepatic function and lipid profile were determined to investigate the roles of lipid emulsion in hepatic function and lipid metabolism. Morphological changes of liver were observed, and nitrogen balance was determined. The results showed that infusion of lipid emulsion was well tolerated. The 1-week survival rate in the lipid emulsion groups was significantly higher than in the GLU group (100% versus 50%, $P < 0.05$); compared with the GLU group, hepatic function recovered quickly and returned to normal level, and morphological alterations were less severer in the lipid emulsion groups, especially in the STG group; the lipid emulsions groups had normal serum TG and TC levels, especially STG and MCT/LCT groups; the lipid emulsions groups achieved a positive nitrogen balance on day 7 compared with the GLU group, and the STG group had the highest nitrogen balance. In conclusion, lipid emulsion is beneficial in improving hepatic function and the recipients' survival and does not influence the lipid metabolism. Mixed energy system consisting of carbohydrate and lipid is more advantageous over single energy source of glucose after liver transplantation, and STG is superior to MCT/LCT and LCT.

Keywords: Lipid emulsion; Liver transplantation; Hepatic function

1. Introduction

Liver transplantation is the only method for the treatment of end stage liver diseases. However, these patients always present nutritional abnormalities and metabolic disturbances which can decrease their survival [1,2]. Although liver transplantation allows resolution of metabolic dysfunction, nutritional supports are required for these patients because their nutritional status can deteriorate rapidly in the postoperative period [2,3]. However, no systematical assessment has been made of the optimal composition of energy yielding substrates (carbohydrate and fat) in these patients.

Lipid emulsions, products of emulsion system with phospholipids as emulsifiers [4], can provide condensed energy source and play positive roles in the prognosis in many critically ill patients. However, whether lipid emulsion can improve the recipient's survival after liver transplantation remains unclear. Conflicting results have been obtained about the influence of lipid emulsion on hepatic function in patients [5–7].

Three kinds of lipid emulsions are available now, i.e. LCT, a physical mixture of MCT and LCT (MCT/LCT) and STG. It has been proposed that a combination of MCT and LCT may be of advantage compared with either MCT or LCT alone. STG with a random distribution of medium-chain and long-chain fatty acids to glycerol demonstrates beneficial effects compared with LCT in patients on home parenteral nutrition with Crohn's disease or

Abbreviations: AKBR, arterial ketone body ratio; ALT, alanine transaminase; FFA, free fatty acids; GLU, glucose; HDL-C, high density lipoprotein cholesterol; LCT, long-chain triglycerides; LDL-C, low density lipoprotein cholesterol; MCT, medium-chain triglycerides; MCT/LCT, medium-chain triglyceride/long-chain triglycerides; SO, sham operation; STG, structured lipid emulsion; TBIL, total bilirubin; TC, total serum cholesterol; TG, serum triglyceride; TPN, total parenteral nutrition

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short bowel syndrome and has some advantages over MCT/LCT in patients with abdominal surgery [8,9]. Unfortunately, in liver transplantation recipients, little information is available about the roles of STG and whether STG is superior to MCT/LCT and LCT needs to be clarified.

To systematically evaluate the roles of lipid emulsions for nutritional support in liver transplant recipients, we observed the effects of lipid emulsion on animal survival, hepatic function and morphological changes, lipid metabolism and nitrogen balance in liver transplant rats by a TPN model.

2. Materials and methods

2.1. Experimental animals and surgical procedure

One hundred and twenty male SD rats weighing from 220 to 240 g were purchased from Sino-British Sippr/Bk Lab Animal Ltd. (Shanghai), and fed in an animal room with a temperature of 20–23 °C for 2 weeks before experiment. This project was approved by the Animal Care and Use Committee of Third Military Medial University, and the procedures were carried out according to the routine animal-care guidelines.

Both donors and recipients were fasted overnight and consumed water ad libitum prior to operation. At the time of the experiment, the rats were anesthetized with ether, and liver transplantation was performed orthotopically between pairs of rats. The donor liver was flushed out with 20 ml of the lactated Ringer's solution at 4 °C and then immediately removed. After 15 min of preservation in the lactated Ringer's solution at 4 °C, liver transplantation was performed using cuffed anastomoses of portal vein and infrahepatic inferior vena cava without the reconstruction of hepatic artery. The mean during of anhepatic phase was 13.0 ± 1.6 min. In the sham operated rats, a midline incision was made, ligaments around the liver were dissociated, and no liver transplantation was performed.

TPN was given 6 h after liver transplantation. The right internal jugular vein was cannulated with a silastic catheter under sterile conditions. The catheter was tunneled subcutaneously to the back and exited through a spring that was attached to a swivel, allowing free mobility of the animal inside the individual metabolic cages. The cages were put in a 20–23 °C room with a 12 h-light/dark cycle. Rats were fasted during the entire experiment period. Penicillin was administered via intramuscular injection at a dose of 100,000 U day^{-1}. The total experimental period was 7 days.

2.2. Preparation of TPN solution and grouping of animals

Animals were randomly divided into five groups. Group I (SO group, $n = 24$) was sham operated rats without liver transplantation, and rats were fed standard rat chow. Group II (GLU group, $n = 24$) received a TPN regimen with glucose as energy source after liver transplantation. Group III (MCT/LCT group, $n = 24$) received a TPN regimen including 20% MCT/LCT (Sino-Swed Pharmaceutical Corp. Ltd.) and glucose as energy sources after liver transplantation. Group IV (STG group, $n = 24$) received a TPN regimen including 20% structolipid (Fresenius Kabi) and glucose as energy sources after liver transplantation. Group V (LCT group, $n = 24$) received a TPN regimen including 20% intralipid (Sino-Swed Pharmaceutical Corp. Ltd.) and glucose as energy sources after liver transplantation. The TPN program was redesigned on the basis of Tao's [10], prepared aseptically and kept at 4 °C. The solution provided caloric at 741.8 kJ kg^{-1} day^{-1} and nitrogen 1.5 g kg^{-1} day^{-1} with a non-protein caloric:nitrogen of 494.5 kJ:1 g. The TPN solution was infused at a speed of 8.9 ml kg^{-1} h^{-1} by an infusion pump during 24 h at room temperature, and was refilled daily. All four TPN solutions were isonitrogenous and isocaloric. The caloric ratio of lipid emulsion to glucose was 6:4 in the three lipid emulsion groups, which meant that 60% of non-protein caloric was supplied to the three lipid emulsion groups as the form of MCT/LCT, STG or LCT, respectively. No lipid emulsion was provided in the GLU group. The compositions of the TPN solutions are shown in Table 1.

Table 1
Compositions of TPN solution for liver transplant rats

Component (ml)	GLU	20% MCT/LCT	20% STG	20% LCT
50% glucose	440	266	264.3	263
10% glucose	14	0	8.7	14
Lipid emulsion	0	188	181	177
10% amino acid complex	536	536	536	536
10% sodium chloride	35	35	35	35
10% potassium chloride	15	15	15	15
10% calcium gluconate	10	10	10	10
Soluvit N	10	10	10	10
Vitalipid	10	10	10	10
Addamel N	10	10	10	10
Total	1070	1070	1070	1070

Six animals of each TPN group were used to observe animal survival and 1, 4 and 7 days animal mortality was recorded. The causes for death were clarified by thanatopsy and pathological examination.

After ceasing of infusion for 12 h in the TPN groups and fasting for 12 h in the SO group on days 1, 4 and 7, six animals in each group were anesthetized and weighed. A midline incision was made and the animals' abdominal cavities were opened. Arterial blood was drawn from the abdominal aorta. Serum samples were obtained after centrifugation at 2500 × g for 25 min at 4 °C and stored at −70 °C until assay. The entire liver was rapidly excised and weighed.

2.3. Biochemical analysis

ALT, AST, TBIL, TG, TC, HDL-C and LDL-C were determined by using commercially available reagents (Roche, Germany):

Twenty-four hours urine specimens were collected for determining nitrogen balance in the four TPN groups. Hepatic total cholesterol and TG were determined according to the method by Carr et al. [11] following extraction of hepatic lipids with chloroform–methanol (2:1).

Arterial ketone body ratio (AKBR), a marker of hepatic energy charge and function [12], was measured to assess hepatic energy levels in the experimental animals. Briefly, 1 ml of