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ORIGINAL ARTICLE

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Hepatic reticuloendothelial function during parenteral nutrition including an MCT/LCT or LCT emulsion after liver transplantation – a double-blind study

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Abstract It has been demonstrated that total parenteral nutrition (TPN) modulates the function of the hepatic reticuloendothelial system (RES). The objective of this study was to evaluate the impact of two different TPN lipid emulsions on the recovery of allograft RES function after orthotopic liver transplantation (OLTx). In a prospective, double-blind study, OLTx patients were randomly assigned to two treatment groups. Group I ($n = 13$) received a TPN regimen that included long-chain triglycerides (LCT). Group II ($n = 9$) received a TPN regimen that included a fat emulsion consisting of both medium-chain triglycerides (MCT) and LCT. At baseline, i.e., on days 2 or 3 after OLTx ($t1$), before lipids for TPN were started, hepatic RES function was determined using the human serum albumin millimicrosphere technique (K-value, 1/min). A second measurement ($t2$) was obtained after 7 days of TPN, including one of the study's two fat emulsions. The mean (± SD) K-value (1/min) was 0.48 ± 0.16 in the LCT group and 0.55 ± 0.28 in the MCT/LCT group at $t1$, and it improved to 0.62 ± 0.21 in the LCT group and to 0.86 ± 0.32 in the MCT/LCT group at $t2$. RES function recovery was significantly better in the MCT/LCT group ($P \leq 0.05$). MCT/LCT emulsion appears to be the TPN fat emulsion of choice after OLTx as it seems to have less impact on hepatic RES recovery.

Keywords Liver transplantation · Reticuloendothelial system (RES) · Parenteral nutrition · Lipid emulsion · Medium-chain triglycerides · Long-chain triglycerides

Abbreviations MCT medium-chain triglycerides LCT long-chain triglycerides RES reticulo-endothelial system HSA-MM human serum albumin millimicrospheres SD standard deviation ns. not statistically significant

Introduction

Orthotopic liver transplantation (OLTx) is an extensive surgical intervention, necessitating postoperative nutritional support for most of the patients [25, 30]. The preoperative metabolic situation in hepatic cirrhosis is characterized by an impairment of glucose utilization. This involves a change in the organ distribution of free fatty acid metabolism and a reduction in glucose synthesis and intracellular uptake, which are associated with a glycogen storage disorder accompanied by increased lipid oxidation [16, 20, 21, 24]. As a consequence of this peripheral metabolic disorder, OLTx patients need an intake of 40–60% of their non-protein calories as lipids to fulfill their energy requirements during postoperative total parenteral nutrition (TPN) [18].

There is evidence to suggest that fat emulsions consisting of long-chain triglycerides (LCT) may compro-

mise reticuloendothelial system (RES) activity [12, 22, 31, 32]. Infection is still the leading cause of death in OLTx recipients, and allograft RES function is compromised in the donor and during ischemia until reperfusion. The lipid component of TPN regimens, therefore, must not further compromise RES function or recovery.

The purpose of this study was to investigate whether there is a difference in hepatic RES function recovery after OLTx in the early postoperative period using different lipid emulsions for TPN.

Patients and methods

Following the approval of the ethical committee, 32 liver transplant recipients were enrolled and prospectively randomly assigned to one of the two groups. The fat emulsion component of the TPN regime (for details, see below) was administered in a double-blinded manner. All patients received parenteral nutrition support for 10 days. Patients experiencing any of the following were excluded: fasting serum triglycerides ≥3.5 mol/l (determined daily), acute rejection, sepsis and open abdomen during the study.

The TPN regime shown in Table 1 was administered by continuous infusion via a central venous catheter 24 h a day. This regime is used routinely after hepatic transplantation at our center and is based on indirect calorimetry-estimated energy requirements.

Hepatic RES function was determined by ^{99m}Tc-labeled human serum albumin millimicrospheres (HSA-MMs, Rotop, Dresden), a tracer known to be taken up exclusively by RES and suitable for the estimation of human RES activity [2, 3, 28]. RES activity was estimated twice – first postoperatively, immediately before starting TPN including one or the other of the study's fat emulsions, and again 7 days later.

Via a central venous catheter, 100 MBq of ^{99m}Tc-labeled HSA-MMs (0.03 mg per study; particle size, 1.0 μm) was administered in a volume of 2 ml, and the catheter was flushed with 10 ml of normal saline. At the same time, the liver was scanned for activity changes, and these were recorded using the Engypan portable scintillation camera instrument (Kernforschungszentrum Karlsruhe, Germany). This instrument features four probes held in place with adhesive tape over the patient's lung (no. 1), heart (no. 2) and liver (no. 3 and no. 4) (see Fig. 1). Scintiscans of the activity changes were obtained at 5-s intervals from 30 s before, until 10 min after tracer injection. Using the regions-of-interest (ROI) technique or individual probes, scintillation cameras can determine the kinetics of activity changes over isolated regions. Given that intravenously administered millimicrospheres are phagocytosed exclusively by RES [28], and that hepatic RES accounts for over 90% of total body RES, the combined activities in the liver and blood can be considered by approximation.

The accumulation of labeled colloids in the liver is a measure of hepatic blood flow and RES activity. Hepatic accumulation was earlier considered to depend primarily on blood flow. However, using a compartment model to describe this process – as for the quantification of regional blood flow in the human heart using ^{13}N-ammonia [11] – will register the time-variable input function of the radiotracer as well as of the non-extractable, or fractional, blood volume (fbv), allowing a separate calculation of the hepatic blood flow (K1) and rate of phagocytosis (K2). Applied to the hepatic activity probe curves, this means that K1 defines the amplitude and K2 the shape of the liver curve (formula 1). According to Reske et al. [26], activity output from the liver as a consequence of particle degradation takes about 20 min to start.

$$C_a \underset{K_2}{\overset{K_1}{\rightleftarrows}} C_g$$

$$C_g(t) = K_1 \times e^{-K2t} \times \int_0^t C_a(t') \times e^{K2t} \times dt'$$

$$C_g = K_1 \times C_a - K_2 \times C_g$$

$$C_{probe} = (1 - fbv)C_g + fbv \times C_a$$

C_a = activity (counts) in blood; C_g = activity (counts) in tissue; t = time; K1 = tissue blood flow; K2 = rate of phagocytosis, fbv = fractional blood volume.

Serum activities of glutamic-oxaloacetic transaminase (AST) and glutamate dehydrogenase (GLDH) were determined daily postoperatively for assessment of ischemic injury.

Two patients of the LCT group were withdrawn during the course of the trial, because they developed hypertriglyceridemia (defined as an exclusion criterion). Another patient died from cardiac complications. Thus, a total of 13 patients (three men and ten women) with a mean age of 44 years (range, 20–60 years) were assessed for hepatic RES function. The diagnoses resulting in the need for transplantation are listed in Table 2. In the MCT/LCT group, four patients were excluded during the course of the trial for hypertriglyceridemia, two patients for sepsis and one patient for acute rejection (all of these had been defined as exclusion criteria). Thus, a total of nine patients (five men and four women) with a mean age of 51 years (range, 36–66 years) were assessed for hepatic RES function. The diagnoses resulting in the need for transplantation in patients who could be evaluated are listed in Table 2. The two groups were comparable both in age and cause of end-stage liver disease. To suppress the immune systems, patients were given CsA (Sandimmun, Novartis) and steroids. The dosage for the steroids was 500 mg methylprednisolone intravenously at the time of reperfusion. On postoperative days 1 and 2, patients were treated intravenously with prednisolone at a dosage of 1.0 mg/kg of body weight. From day 3 to day 10, 0.5 mg/kg of body weight of prednisolone was given. The dosage of the steroids was then tapered down in weekly intervals to an absolute dose of 5 to 7.5 mg of prednisolone (Table 2).

The InStat statistical packages (GraphPad Software, San Diego) was used to analyze the experimental data. The calculated number of patients was 16 for each group, as the maximum standard deviation of the K-values was expected to be 0.2 or

Table 1. Parenteral nutrition regime for the study population

	Day of surgery	Day 1	Day 2	Days 3–10
Normal saline, electrolytes, albumin	As needed	As needed	As needed	As needed
Glucose		1.50 g/kg bw/day	3.0 g/kg bw/day	3–5 g/kg bw/day
Amino acids		0.75 g/kg bw/day	1.5 g/kg bw/day	1.5 g/kg bw/day
Fat emulsions (depending on treatment group, see text)			0.5–1 g/kg bw/day	1–2 g/kg bw/day

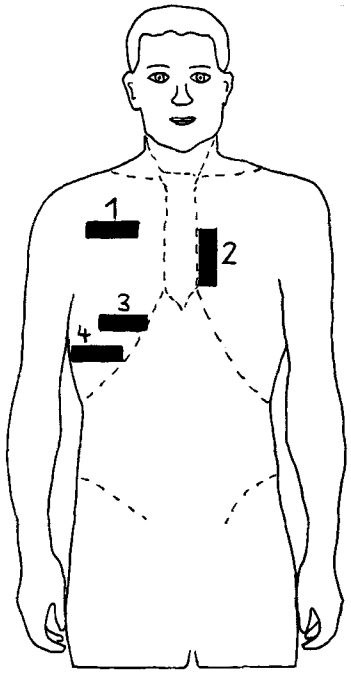

Fig. 1. Placement of the four probes for registration of ^{99m}technetium activity

less, and the minimum difference of means accepted as significant was 0.2 as well [14]. The level of significance for α was set at 0.05 and for β at 0.2. The test of David, Pearson and Stephens was used to determine whether or not the data showed normal distribution. The *F*-test was used to rule out heterogeneity of variance, and the *t*-test then was used to test mean values for comparability. In addition, the Kolmogoroff-Smirnoff test was used to assess the sum frequencies of the differences found.

Results

Amount of fat infused

The mean (±SD) amount of fat administered was 1.33±0.51 g/kg per day in the LCT group versus 1.45±0.66 g/kg per day in the MCT/LCT group.

Assessment of ischemic injury

AST and serum GLDH activities were determined to assess the extent of allograft cell injury. Both before and after 7 days of fat infusion, the MCT/LCT group showed higher activities of these liver enzymes than did the LCT group (Fig. 2), but the differences fell short of statistical significance. The cold ischemia time was 13 h, 15 min (±4 h, 12 min) for the grafts of the MCT/LCT-group and 12 h, 45 min (±5 h, 50 min) for the grafts of the LCT group. The median donor age was 46 years (±26) for the grafts of the MCT group and 44 years (±21) in the LCT group (Fig. 2).

Table 2. Underlying diseases leading to liver transplantation

	LCT-group (*n*)	MCT-group (*n*)
Cirrhosis	8	6
Malignancy of the liver	3	2
Acute hepatic failure	2	–
Chronic rejection	–	1

Hepatic RES activity

Both groups showed significant increases in hepatic RES activity. In the LCT group, the mean (±SD) K-value (1/min.) increased from 0.48±0.16 before LCT infusion to 0.62±0.21 after LCT ($P<0.05$, two-tailed Student's *t*-test for paired observations). In the MCT/LCT group, the mean K-value increased from

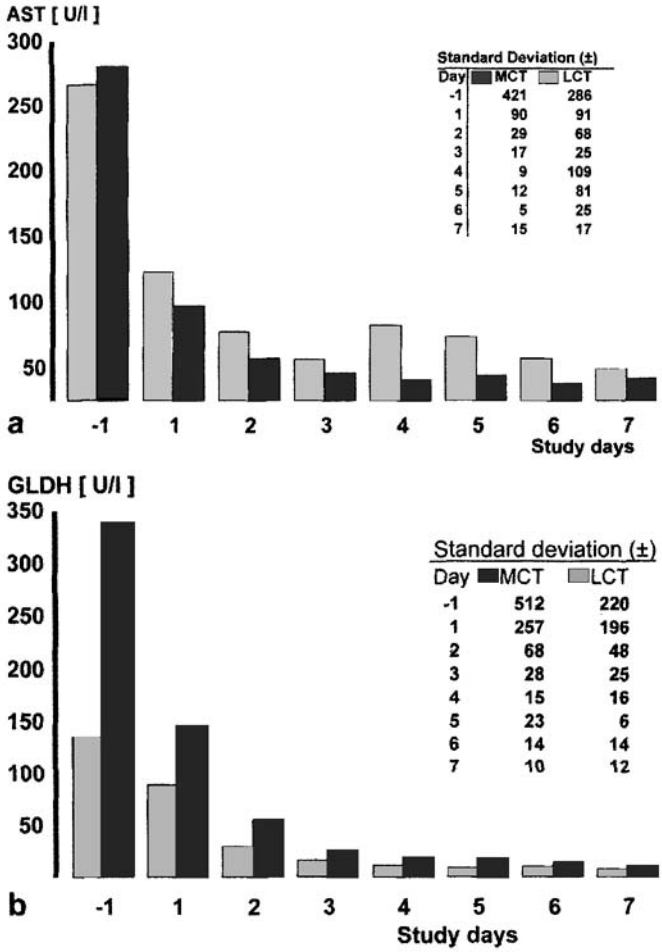

Fig. 2. **a** Comparison of ischemic injury in the two treatment groups in terms of AST release (U/l, mean, ±SD). **b** Comparison of ischemic injury in the two treatment groups in terms of GLDH release (U/l, mean, ± SD)

0.55 ± 0.28 before MCT/LCT to 0.86 ± 0.32 after MCT/LCT ($P < 0.05$). Individual data are shown in Fig. 3 and Fig. 4. There was no difference in the mean baseline K-value between the two groups (two-tailed Student's t-test for unpaired observations). After 7 days of TPN with fat emulsions, the MCT/LCT group attained a statistically significant higher K-value than the LCT group ($P < 0.05$, two-tailed Student's t-test for unpaired observations) (see Fig. 5). Using the Kolmogoroff-Smirnoff test to assess the sum frequencies of the differences found confirms the significantly greater improvement in hepatic RES function in the MCT/LCT group ($P < 0.05$) (Fig. 3, Fig. 4 and Fig. 5).

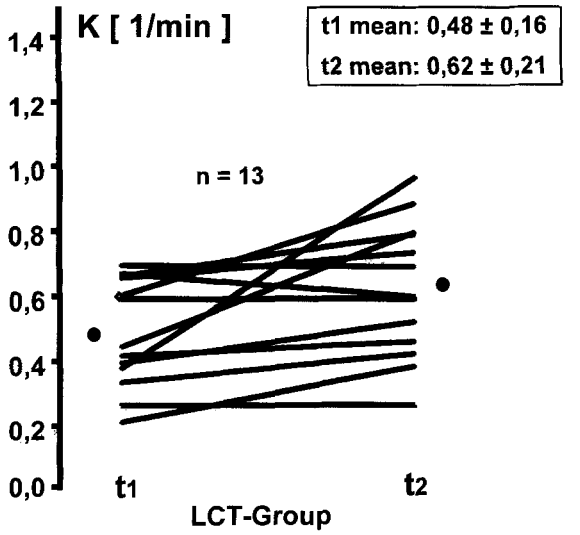

Fig. 3. Measurement of the hepatic RES function in the patients of the LCT group as described. Each *line* represents a patient, first measurement at t_1, second measurement at t_2 ($n = 13$)

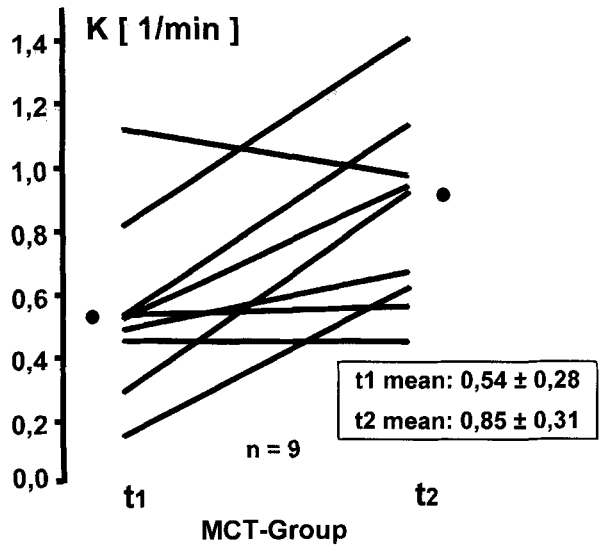

Fig. 4. Measurement of the hepatic RES function in the patients of the MCT group as described. Each *line* represents a patient, first measurement at t_1, second measurement at t_2 ($n = 9$)

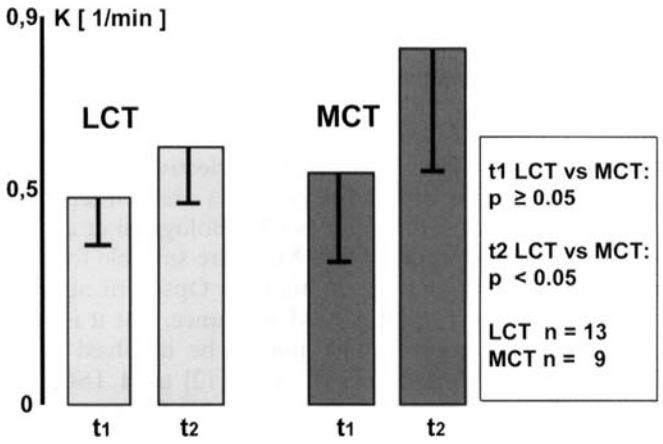

Fig. 5. Comparison of means and standard deviations for the K-values of both groups. (t_1 first measurements, t_2 the repeated measurements after 7 days of parenteral nutrition with fat emulsions)

Discussion

Fat emulsions are an established part of TPN regimens. However, there is still some controversy as to whether intravenous fat emulsions may compromise RES function.

While various methods have been developed to study the relation between RES activity and pathologic conditions, their utility is still a matter of debate and there is, as yet, no standardized methodological approach. All methods are based on the ability of RES cells to remove certain substances from the bloodstream. Carbon particles, gold, ^{99m}Tc-labeled sulfur colloids (TSCs), labeled bacteria (animal studies) and microaggregated human serum albumin were among the indicators of immunononspecific phagocytosis used in the past, and IgG- or IgM-labeled red blood cells were once employed as tracers of immunospecific phagocytosis [1, 9, 13]. Bacteria are the natural choice in terms of clinical relevance and therefore are often used in animal studies [10, 22, 31]. However, they are unacceptable for in vivo studies in humans.

Efforts for identifying the most suitable tracer for in vivo studies in humans have defined the following essential criteria: RES specificity, rapid clearance from the vascular system by RES and saturable kinetics, particle size of about 1 μm, controlled particle size (low variability), being an organic, nonantigenic substance, being a biotransformable substance and having the ability to be tagged with short-lived radionuclides [7, 9, 28].

Two tracers have become established for in vivo studies: ^{99m}Tc-labeled sulfur colloids (TSCs) [12, 29] and ^{99m}Tc-labeled human serum albumin millimicrospheres (HSA-MMs) [2, 3, 17].

The HSA-MMs used in this study fulfill all of the above criteria. They are removed selectively by RES from the bloodstream, and about 90% are extracted in the liver with saturable kinetics [28]. Bolognesi et al. [3] therefore concluded that HSA-MMs are suitable for the estimation of RES activity in humans. Opsonins appear not to be involved in HSA-MM clearance, but it is still unclear which receptor may indeed be involved [17]. Seidner et al. [29] and Jensen et al. [12] used TSCs in their studies, but these appear unsuitable for macrophage function assessment. In studies by Hirschberg et al. [14], TSCs were cleared from the bloodstream with nonsaturable kinetics. Moreover, bacteria and TSC were presumed to be eliminated by different mechanisms. Other authors [6] merely found TSCs attached to the outside of cells rather than being phagocytosed. Hirschberg et al. [14] concluded that TSC uptake reflected hemodynamics and therefore was an unsuitable indicator of macrophage function. Given that TSCs are not subject to phagocytosis, substances that alter the phagocytic properties of cells will not necessarily produce a fall in TSC uptake, unlike substances that have an effect on blood flow, receptors or membrane properties.

The modified estimation method used in the present study registers the variable amounts of tracer in the blood. The time-activity curve recorded over the heart reflects the falling activity of tracer in the blood and simulates tracer disposition to the phagocytosing cells of the liver at any given time. This fact is taken into account when analyzing the accumulation curve recorded over the liver so that the net curve reflects only the gamma rays emitted by colloid taken up by hepatic RES.

Goyal et al. [7] compared the various RES activity estimation methods and found that the method used in our study provides the best estimate of RES function.

Before the start of TPN with fat emulsion infusion, the two treatment groups of this study were comparable in age, gender and ischemic injury to the transplanted livers. The two groups did not differ significantly in terms of hepatic RES activity prior to the administration of the fat emulsion. After 7 days of fat emulsion infusion, hepatic RES activity improved in both groups, but significantly better in the MCT/LCT group than in the LCT group. Unlike general surgical or medical patients, liver transplant recipients generally stand to experience an increase in hepatic RES activity. Allograft Kupffer cells are normally subject to ischemic damage and will be replaced by recipient monocytes maturing to Kupffer cells in the course of a dynamic transformation and adaptation process lasting several months [14, 33]. As a result, no further decrease in RES activity is likely to occur in this model. The question remains whether or not there is an impact on RES recovery. For MCT/LCT emulsions, such an impact on hepatic RES recovery after liver transplantation could previously be ruled out up to a dosage of 1.5 g/kg per day administered by continuous infusion [17]. While this dosage was not exceeded in our study, the patients receiving the MCT/LCT emulsion showed significantly better hepatic RES activity after 7 days than those receiving the LCT emulsion. Additional compromise of the graft in the course of the study could be ruled out. As the two groups showed no differences in terms of cell injury and synthetic performance during fat infusion, a compromise of the LCT emulsion-induced hepatic RES recovery is suspected. In fact, an adverse impact of pure LCT emulsions on RES activity has been suggested in earlier studies. After liver biopsy had revealed fat droplet accumulation in Kupffer cells in a 5-week-old infant after 12 days of parenteral administration of an LCT emulsion in the usual dosage [8], animal studies also found hepatosplenomegaly [31], accumulation of fat in Kupffer cells [5] and diminished bacterial clearance [5, 10, 22, 31] following administration of pure LCT emulsions. Comparative studies in animals [22, 31] and general surgical patients [12, 29] demonstrated the impact of dosage and could show that the compromise of RES function occurred with MCT/LCT emulsions only at significantly higher doses or not at all. For the newer lipid emulsions (fish oils, structured lipids), their influence on the immune system still is very rarely documented; especially when focusing on the hepatic RES, no such literature is available.

Diminished RES clearance compromises nonspecific immune defences, increasing susceptibility to systemic infections [10, 34]. Up to 89% of OLTx deaths occurring within 60 days of transplantation are related to infection [4, 19, 23]; hence, it is necessary to avoid all therapeutic interventions that may compromise RES function or recovery during this phase. The clinical significance of this issue has been established by studies on larger populations of cirrhotic patients [2, 27], confirming the clinical relevance of our data by the results reported by other authors. We conclude from the presented data that in patients after liver transplantation or major hepatic surgery as well as in clinical situations involving RES impairment, MCT lipid emulsions should be preferred for parenteral nutrition instead of LCT emulsions.

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