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A NEW PERIOPERATIVE THERAPEUTIC AGENT FOR LIVER CANCER: L-CARNITINE MAY RESTORE AMMONIA METABOLISM AND IMPROVE LIVER FUNCTION IN HEPATECTOMIZED PATIENTS

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ABSTRACT
Background: One of the major goals in hepatic resection is the reduction of postoperative liver failure due to metabolic disturbances in the liver. A new therapeutic agent is needed in order to achieve the goal of improved safety in liver surgery. *Methods:* Twenty-four patients treated with L-carnitine (carnitine group) were compared with a propensity score-matched cohort of 50 patients who had not received L-carnitine (control group). *Results:* Ammonia levels increased immediately after the operation on the first postoperative day in both groups and the increase was more pronounced in the control group compared with the carnitine group. On the third postoperative day, ammonia levels restored faster to the preoperative levels in the carnitine group than the control group. Furthermore, patients in the carnitine group required significantly shorter hospitalization than those in the control group ($p < 0.05$). The results of the present study indicate that perioperative oral supplementation with L-carnitine in patients with liver malignancy undergoing curative hepatic resection was clinically associated with preserved postoperative ammonia metabolism and an immediate postoperative recovery of the liver function. *Conclusions:* L-carnitine may serve as a master regulator of liver injury and repair in metabolic disturbances of the liver following hepatic resection and may reduce the length of postoperative hospitalization.

KEY WORDS: liver, surgery, L-carnitine, ammonia, oxidative stress.

INTRODUCTION
Liver resection is a potentially curative treatment for primary and secondary liver tumors. Recent advances in surgical technique and perioperative management have made hepatic resection a safe procedure and the mainstay of curative treatment for liver malignancies.[1,2] However, hepatic surgery still carries a significant risk of postoperative morbidity; a deterioration of liver function might result from the inevitable reduction in functional liver mass. Disturbance of the liver's metabolic function is a commonly encountered obstacle in the postoperative management of hepatic surgery that contributes to the deterioration of liver function.[3,4] Furthermore, oxidative stress caused by metabolic disturbances in the liver following hepatic resection has recently been associated with a progression of liver injury and failure.[5] Oxidative stress results from an imbalance in the generation of reactive oxygen species triggered by environmental factors or mitochondrial dysfunction.

Over the past decade, we have increasingly begun to appreciate the clinical benefits of a perioperative treatment with branched-chain amino acids. We now know that the oral administration of branched-chain amino acids in patients undergoing hepatic resection can reduce postoperative morbidity and improve postoperative nutritional status and quality of life.[6-8] One of the major goals in hepatic resection is the reduction of postoperative liver failure due to metabolic disturbances in the liver. Therefore, we aimed to discover a new therapeutic agent to improve the safety of liver surgery. In recent years, promising therapeutic effects can be attributed to the interaction of L-carnitine and its derivatives with cellular membranes. Several studies of the therapeutic use of L-carnitine thus far have focused on the prevention and treatment of ischemic heart disease, ischemic kidney disease, hypertrophic heart disease, and peripheral arterial disease. L-carnitine use in these conditions was well tolerated without significant side effects.[9-11] The exploration of the

question of whether L-carnitine could be a master regulator of liver repair in liver malignancies treated with resection presented a logical extension to above studies.

However, no studies have addressed the potential effects of perioperative administration of L-carnitine on the postoperative outcomes in patients with liver malignancies undergoing liver surgery. In this study, we report that perioperative treatment using L-carnitine in these patients may restore ammonia metabolism and improve postoperative liver dysfunction. These selective rescue mechanisms point the way towards a new therapeutic target for the preservation of postoperative liver function in patients undergoing hepatic resection.

METHODS

Patients

The diagnoses of liver malignancies were made by the use of imaging and confirmed by pathological analysis. The surgical pathology database of the Kochi Health Sciences Center was used to identify patients who were planning to undergo resection for malignant neoplasms of the liver during the periods of March 2005 to December 2015. Information retrieved from the surgical pathology database included age, gender, part of the tumor, size of the tumor, operative procedures, and pathological data. A complete physical examination was performed and the clinical history was recorded for all patients in the study. Laboratory tests were performed to measure serum levels of albumin, total bilirubin, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, ammonia, and creatinine. Furthermore, peripheral thrombocyte counts, prothrombin time, and the retention rate of indocyanine green at 15 minutes (ICG 15) were measured. The infection status for hepatitis B and C virus (HBV and HCV) was assessed in all patients by testing for the presence of the HBV antigen and HCV antibodies respectively. Where the status was positive, the viral nucleic acid in the sera of patients was examined by PCR methodology. Each patient was followed-up to obtain information on clinical outcomes. The enrolled patients were diagnosed with hepatocellular carcinoma, intrahepatic cholangiocarcinoma, and secondary metastatic liver tumors. This study was approved by the Ethics Committee of the Kochi Health Sciences Center and all patients provided written informed consent.

Assessment

The study data were prospectively collected. A propensity score-matched analysis was performed to match the patients treated with L-carnitine (carnitine group) to the patients treated with usual intake (control group) on gender, age, diagnosis of liver tumors, pathological background of the liver, and type of surgery, all of which are factors that may impact the outcome of radical surgery. All patients in the carnitine group were orally administered fixed doses of L-carnitine (30 mg/kg of body weight) for two weeks prior to liver resection. The primary endpoints of this study included clinical outcomes of postoperative liver function, comprising of various laboratory tests including the changing levels of ammonia, in order to test whether the postoperative liver function was affected by the perioperative administration of L-carnitine. The secondary endpoint was a comparison of the length of hospitalization between the two groups. Subgroup analyses for the primary endpoints were performed using an unadjusted test of interaction in a logistic model to evaluate which patients would benefit from perioperative L-carnitine supplementation.

Statistical analysis

Patients alive in October 2015 were right-censored at the time of follow-up. Qualitative variables were compared using the chi-square test of Fisher's test, while quantitative variables were analyzed using *t* test or a nonparametric test. All tests were two-sided, with a *p* value of less than 0.05 considered to indicate statistical significance. All analyses were performed using SPSS® (SPSS; Chicago, IL).

RESULTS

Among 403 patients with liver malignant neoplasms who underwent surgical resection at the Kochi Health Sciences Center, only 24 patients (14 males and 10 females) received oral administration of L-carnitine. Comparison of these 24 patients treated with L-carnitine in the carnitine group to 50 patients receiving ordinary care in the control group, after matching for gender, age, diagnosis of the liver tumors, pathological background of the liver, and type of surgery, revealed no difference in body mass index between the groups. Table 1 shows the perioperative clinical profiles of patients in both groups. There was no significant difference between the two groups in the baseline hepatic function parameters including serum albumin, total bilirubin, prothrombin time, the retention rate of indocyanine green at 15 minutes (Table 1). All patients underwent a hepatectomy involving the curative resection of hepatic tissue for the removal of a tumor. Ten patients underwent a hemi-hepatectomy, 23 patients underwent segmentectomy, and 41 patients underwent a partial hepatectomy. Surgical duration and estimated blood loss volumes did not differ significantly between the two groups. The final diagnoses of liver malignancies in the control group included hepatocellular carcinoma in 29 patients, intrahepatic cholangiocellular carcinoma in 12 patients, and metastatic adenocarcinoma in 9 patients. The carnitine group diagnoses revealed 14 patients with hepatocellular carcinoma, 8 with intrahepatic cholangiocellular carcinoma, and 2 with metastatic adenocarcinoma. Finally, there were no significant pathological differences in the background liver for each resected specimen between the two groups, as was expected.

Baseline preoperative values of all laboratory parameters were comparable between the control and carnitine groups. However, significant differences were observed

between the two groups for several parameters during the postoperative period. Ammonia levels increased immediately after the operation on the first postoperative day in both groups (Figure 1); however, the increase was more pronounced in the control group compared with the carnitine group ($p < 0.05$). It is noteworthy that on the third postoperative day, ammonia levels restored faster to the preoperative levels in the carnitine group than the control group. Furthermore, aspartate transaminase and lactate dehydrogenase levels increased immediately after surgery in both group (Figure 2), but the decrease was more evident in the carnitine group than in the control group. The current study revealed that several parameters were restored more quickly to pre-surgery levels in the carnitine than the control group ($p < 0.05$). It is of note that patients in the carnitine group required significantly shorter hospitalization than those in the control group ($p < 0.05$). There was no operative mortality and no operation-related readmission of patients in the present study.

In post-hoc subgroup analysis, we evaluated the association between the treatment with L-carnitine and both the improvement of liver injury and the repair of metabolic disturbances in the liver. Significant differences were observed between the two groups for several postoperative parameters in patients with chronic liver diseases. The ammonia, aspartate transaminase, lactate dehydrogenase, and creatinine levels increased immediately after the operation in both groups, and this was more pronounced in the control group compared to the carnitine group (Figure 3). It is noteworthy that these parameters restored faster to the preoperative levels in the carnitine group than the control group. The prothrombin time levels decreased immediately after the operation in both groups and were better preserved in the carnitine group compared to the control group (Figure 3).

Table
The baseline characteristics of the patients and operation-related parameter

Characteristics	Control (n = 50)	Carnitine (n = 24)	*P* value
Gender (male/female)	35/15	14/10	matching
Age	71 ± 8	73 ± 9	matching
Body mass index	24.2 ± 2.9	23.3 ± 3.0	0.246
Liver background			
HBV/HCV/Alcohol	5/17/15	5/4/5	0.403
Preoperative labo data			
Albumin	4.1 ± 0.5	4.1 ± 0.5	0.820
Total bilirubin	0.6 ± 0.4	0.7 ± 0.5	0.550
Prothrombin time (%)	86.3 ± 14.6	93.6 ± 20.5	0.402
ICG-R15	12.5 ± 7.0	14.3 ± 7.2	0.925
Ammonia	39.5 ± 13.3	33.7 ± 14.8	0.198
Pathologic findings			
HCC/ICC/LM	29/12/9	14/8/2	matching
LC/CH/NL	16/22/12	10/11/3	matching
Operative procedure			
Hemi/Seg/PR	7/16/27	3/7/14	matching
Time (min)	191 ± 88	145 ± 71	0.132
Blood loss (mL)	250 ± 846	240 ± 449	0.229
Transfusion (%)	22.0	25.0	0.994
Hospitalization (days)	11 ± 19	9 ± 2	< 0.001

HBV, hepatitis B virus; HCV, hepatitis C virus; ICG15, % retention of indocyanine green at 15 minutes; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocellular carcinoma; LM, liver metastases; LC, liver cirrhosis; CH, chronic hepatitis; NL, normal liver; Hemi, hemihepatectomy; Seg, segmentectomy of the liver; PR, partial resection of the liver.

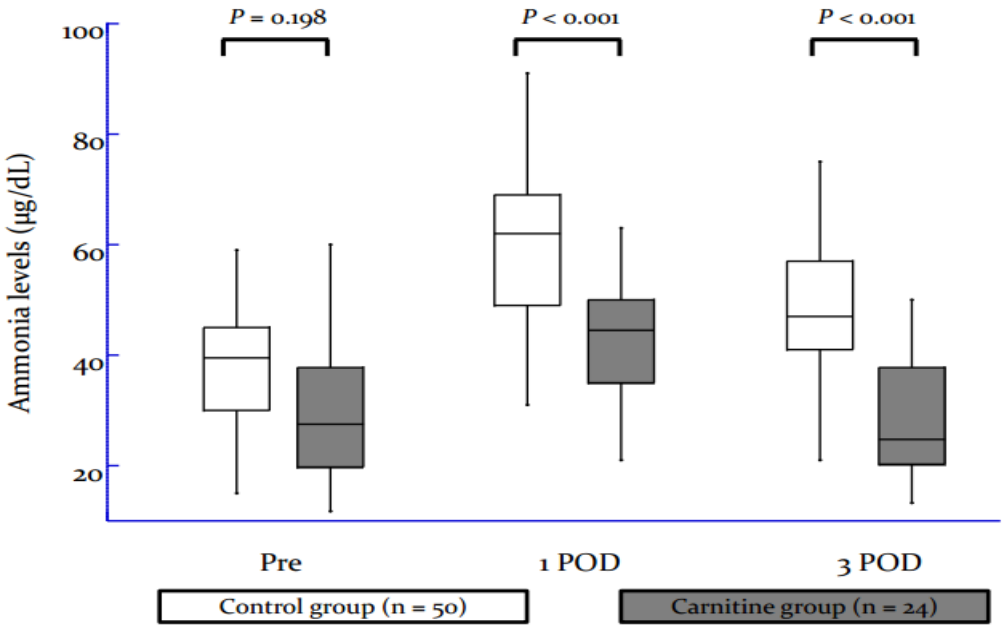

Figure 1: Changing trends of perioperative ammonia levels

Ammonia levels increased immediately after the operation (at first postoperative day) in both groups, which was more obviously seen in the ordinary group compared to the carnitine group ($P < 0.05$). It is noteworthy that, at postoperative 3 days, ammonia levels restored faster to the comparable levels before the operation in the carnitine group than the ordinary group.

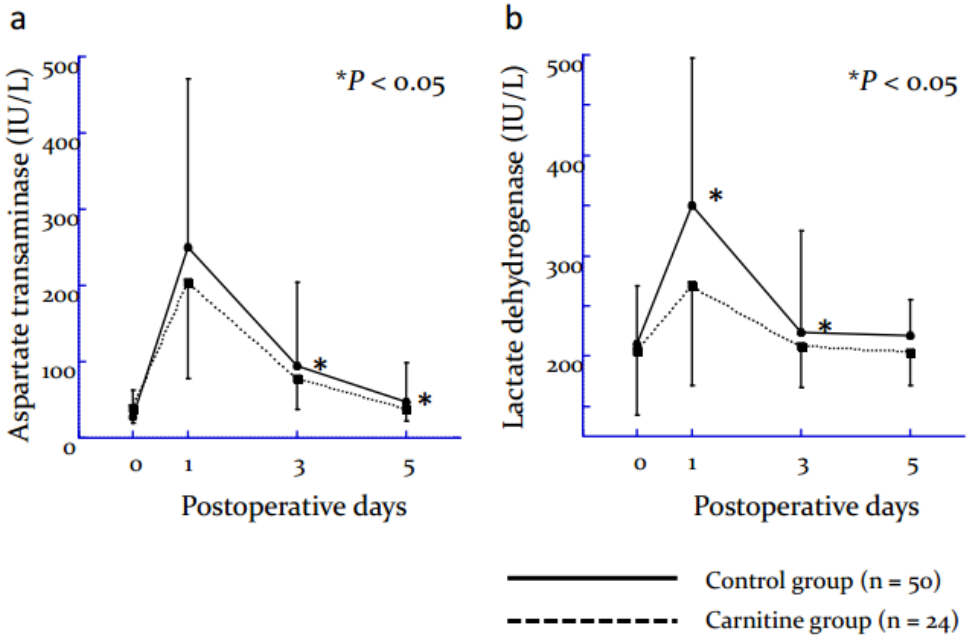

Figure 2: Perioperative laboratory data in patients receiving perioperative supplementation with L-carnitine and in the ordinary group. Data are the mean ± SD. *$P < 0.05$ compared with the ordinary group.

Aspartate transaminase (Fig. 2a) and lactate dehydrogenase levels (Fig. 2b) increased immediately after surgery in both groups, but the decrease was more evident in the carnitine group than in the ordinary group. The current study revealed that these parameters were restored more quickly to comparable presurgery levels in the carnitine than the ordinary group ($P < 0.05$).

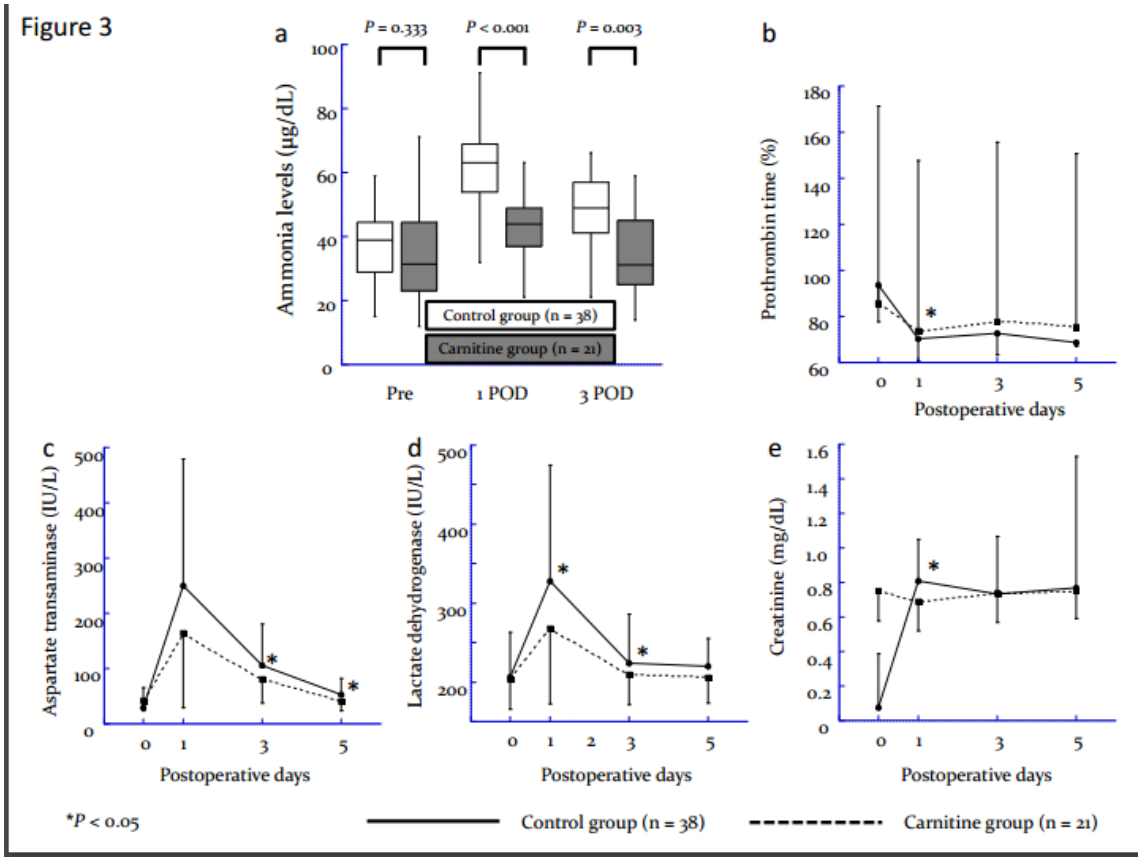

Figure 3: Subgroup analyses of perioperative laboratory data in patients with chronic liver diseases receiving perioperative supplementation with L-carnitine and in the ordinary group. Data are the mean ± SD. *P < 0.05 compared with the ordinary group.

(a) Ammonia levels, (b) prothrombin levels, (c) aspartate transaminase levels, (d) lactate dehydrogenase levels, (e) creatinine levels.

DISCUSSION

The results of the present study indicate that perioperative oral supplementation with L-carnitine in patients with liver cancer undergoing curative hepatic resection was associated with preserved ammonia metabolisms postoperatively and an immediate postoperative recovery of liver function. Overall, the results suggested that L-carnitine administered perioperatively could be used as a beneficial nutritional supplement for patients with liver cancer scheduled to undergo hepatic surgery.

Primary and secondary metastatic tumors of the liver are among the most common malignant neoplasms worldwide. Unfortunately, palliative treatment is the only option for most patients with liver tumors even after curative surgical resection, due to the advanced stage of disease on presentation or the presence of severe underlying chronic liver disease. According to recent advances in surgical methods and perioperative management, hepatic resection is often effective in prolonging patient survival.[12-15] However, due to poor hepatic reserve, regeneration dysfunction, portal hypertension, and metabolic disturbances of the liver, liver resection may involve considerable postoperative liver failure and the incidence of postoperative complications, thereby affecting postoperative outcomes. For this reason, one of the major goals in liver resection is the preservation of postoperative liver function.[7,16,17]

The early outcomes of liver resection are not predicted accurately by prognostic models. Reasons include methodological deficits in the definition of endpoints and predictors; lack of intraoperative event indicators other than the extent of surgery and blood loss or transfusion; and lack of validation studies. Ammonia and bicarbonate are condensed in the liver mitochondria to yield carbamoylphosphate initiating the urea cycle, the major mechanism of ammonium removal in humans. Healthy kidney produces ammonium which may be released into the systemic circulation or excreted into the urine depending predominantly on acid-base status, so that metabolic acidosis increases urinary ammonium excretion while metabolic alkalosis induces the opposite effect.[18] High environmental ammonia induces oxidative stress and the antioxidant response; these processes have often been investigated in the liver. Because the prognostic value of ammonia has been reported widely in critical care setting such as in liver failure, this study sought to evaluate the changing levels

of perioperative ammonia in liver cancer patients undergoing hepatic resection.[19,20]

Oxidative stress is becoming recognized as a key factor in the progression of liver dysfunction.[5] The liver is an important metabolic organ and a major reservoir of mitochondria that serve as sources of reactive oxygen species responsible for the initiation of necroinflammation. Previous studies have reported that oxidative stress is increased by the generation of reactive oxygen species and because of defects in redox defense mechanisms involving glutathione, catalase or superoxide dismutase.[21] Since mitochondria comprise the most important and abundant source of intracellular reactive oxygen species, mitochondrial dysfunction therefore plays a central role in the pathological mechanisms of liver metabolic disturbances. L-carnitine is an essential nutrient that converts fat into energy in mitochondria and ameliorates liver damage. It acts as a fatty acid carrier across the mitochondrial membrane and it also exists in its free form or as acyl derivatives in plasma.[22] L-carnitine plays an important role in lipid metabolism as it is an essential co-factor for the β-oxidation of fatty acids facilitating the transport of long-chain fatty acids and activating carnitine palmitoyltransferase, the key enzyme in fatty acid oxidation.[23] L-carnitine has recently been reported as treatment for various diseases. In recent years, it has been reported that sirtuin-3 is protective against acute kidney injury and findings suggest that enhancing sirtuin-3 improve mitochondrial dynamics has potential use as a strategy for improving outcomes of renal injury. However, a few studies have shown that L-carnitine can ameliorate or prevent liver damage in various liver diseases. Previous authors reported that L-carnitine supplementation in patients with liver diseases greatly improved glucose plasm levels, lipid profiles, and histological manifestations.[24] Furthermore, L-carnitine ameliorated fatty liver in high-calorie diet/streptozotocin-induced type 2 diabetic mice by improving mitochondrial function.[25] It is suggested that L-carnitine may alter not only the uptake of long-chain fatty acids in mitochondria, but also the activity of the reactive oxygen species-scavenging antioxidant enzymes. In addition, studies showed that L-carnitine reinforced mitochondrial β-oxidation and the activity of key reactive oxygen species-scavenging antioxidant enzymes such as superoxide dismutase 2 and catalase without increasing oxidative stress. This is the first clinical interventional study to test whether L-carnitine restores ammonia metabolism associated with liver injury in hepatectomized patients.

This study has some limitations associated with errors and biases inherent in a small retrospective study design, where samples sizes are not large enough to performed analyses for each patient group. A major limitation of our study is the question of whether our results can be compared with previous reports of L-carnitine treatment of ischemic heart disease, ischemic kidney disease, hypertrophic heart disease, and peripheral arterial disease, since it has not been previously shown whether L-carnitine can be a master regulator of liver injury and repair in hepatectomized patients. Therefore, determining the best management strategy in patients undergoing scheduled liver surgery and improving the accuracy of postoperative outcomes for patients treated with L-carnitine will required collection and analysis of further epidemiological and pathological data. Another potential limitation is that the duration of L-carnitine administration in this study was relative short and the ideal duration of peri-surgical treatment of L-carnitine has not been established yet. However, we believe that our data will help guide the design of future randomized trials of L-carnitine therapy in patients undergoing liver resection.

In conclusion, perioperative treatment of L-carnitine was significantly better than ordinary treatment in reducing postoperative serum ammonia levels, suggesting L-carnitine may serve as a master regulator of liver injury and repair and result in shorter postoperative hospitalization. An important question that has arisen from this study is how to choose the ideal patients candidate to receive L-carnitine treatment; further studies will be required to address this issue.

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