Int Surg 2016;**101**:241–248
DOI: 10.9738/INTSURG-D-16-00058.1

The Histopathologic Effects of L-Carnitine in a Sodium Taurocholate–Induced Severe Pancreatitis Model

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This study aims to evaluate the histopathologic effects of L-carnitine (LC) in an experimental severe pancreatitis (SP) model induced with sodium taurocholate. LC is an amino acid–like molecule that plays an active role in transporting fatty acids and producing acetyl CoA in mitochondrial matrix for β-oxidation to provide energy that is needed for metabolism. It has ameliorative effects on cell injury, as has been demonstrated in many studies. The present study focuses on evaluating the histopathologic effects of LC in an experimental SP model. A total of 32 Sprague-Dawley male rats were divided into 4 groups in a randomized fashion: control (C) group, L-carnitine (LC) group, pancreatitis (P) group, and pancreatitis and L-carnitine (P + LC) group. Pancreatitis was induced by a retrograde pancreatic duct injection of 4% sodium taurocholate, and LC was administered 200 mg/kg/d in the treatment group. Rats were euthanized with cardiac puncture under anesthesia at the 48th hour of the experiment for biochemical and histopathologic examination. In the P + LC group, the histopathologic findings of the pancreatitis were markedly reduced. Acinar cell degeneration was rarely seen. Interlobular and intralobular inflammation and edema

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was generally mild. The pancreatic damage score of the P + LC group was significantly lower than that of the P group ($P < 0.05$). This study revealed that LC has a significant histopathologic protective effect on acinar cell degeneration in a sodium taurocholate–induced SP model in rats.

Key words: L-carnitine – Sodium taurocholate – Severe pancreatitis

Severe acute pancreatitis (SP) is a life-threatening condition with a mortality rate as high as 10% to 30%.[1] Although an increasing number of treatment modalities have been developed, there is no effective treatment for SP.[2,3] In most of the cases, mortality from SP is a result of multiorgan dysfunction syndrome, which is related to mitochondrial dysfunction at the cellular level.[4,5] Recent studies have focused on identifying the pathophysiology behind the mitochondrial dysfunction, and highlight the need for an early mitochondrial-specific therapy in acute pancreatitis.[6–8]

L-carnitine (LC) has an important role in the transportation of long-chain fatty acids to the mitochondrial matrix for β-oxidation to provide energy. It also takes place in the transportation of intermediate toxic compounds out of mitochondria and the prevention of lipid peroxidation, which provides the stability of mitochondria under severe oxidative stress.[9–11] The protective effects on mitochondria and the anti-inflammatory and antioxidant efficacy of LC suggest that it could also be a therapeutic option in SP.

Acute pancreatitis induced by sodium taurocholate (STC) is the most often used model of acute severe necrotizing pancreatitis in rats. To our knowledge, the protective effects of LC on pancreatic tissue in the STC-induced SP model have not been demonstrated before in the literature. The aim of the present study is to determine whether LC is a protective agent from necrosis in an STC-induced experimental SP model in rats.

Materials and Methods

Animals

This study was held at the Inonu University Experimental Animals Research Unit with the approval of Experimental Animals Ethics Committee of the Inonu University Faculty of Medicine. A total of 32 Sprague-Dawley male rats with an average weight of 250 to 350 g were used in the study. The animals were kept in stainless steel cages under standard conditions (temperature 25°C ± 2°C and humidity 45%, with a 12-hour light/dark cycle) and were fed *ad libitum* with normal pellet. All the rats in both test and control groups were allowed free access to food and water during the experimental period. All of the animal experiments were conducted in conformity with the International Guiding Principles for Biomedical Research Involving Animals.

Design of the study

The rats were divided into 4 groups in a randomized fashion: control (C) group (8 rats), L-carnitine (LC) group (8 rats), pancreatitis (P) group (8 rats), and pancreatitis and L-carnitine (P + LC) group (8 rats). Anesthesia was provided by intramuscular administration of 5 mg/kg xylazine hydrochloride (Rompun, 2% injectable solution 20 mg/mL, Bayer, Ontario, Canada) and 20 mg/kg ketamine hydrochloride (Ketalar, injectable solution 100 mg/mL, Pfizer, Kent, United Kingdom), and the abdomen was shaved and cleaned with betadine.

C group

A 1.5-cm midline laparotomy incision was made and duodenum was exposured; the duodenum was returned to the peritoneal cavity and the abdominal wall and skin was sutured with 3-0 silk.

LC group

The rats of this group received LC (Carnitene, 5-mL injectable solution 200 mg/mL LC, Santa Farma, Istanbul, Turkey) with a dosage of 200 mg/kg/d intraperitoneally without laparotomy.

P group

In this group, a 1.5 cm-midline laparotomy incision was made and common pancreatic duct of rat was cannulated with a 28-gauge angiocath passed transduodenally through the ampulla of Vater and infused with STC 4% at 0.2 mL/min for 4 minutes. During this procedure, the common hepatic duct was occluded at the hilum of the liver with a vascular clip to prevent STC reflux.

P + LC group

In this group, SP was induced with STC as the procedure in the P group, and animals received LC with a dosage of 200 mg/kg/d intraperitoneally for 2 days. Initial dose of LC was administered synchronously with the induction of SP.

Normal saline solution (10 mL/kg of 0.9% NaCl) was injected subcutaneously in all groups for fluid resuscitation after surgery. They were provided free access to food and water placed on the bottom of the cage. Rats were monitored every 12 hours for survival and were euthanized with cardiac puncture under anesthesia (5 mg/kg xylazine hydrochloride, Rompun) and 20 mg/kg ketamine hydrochloride, Ketalar) at the 48th hour of the experiment (beginning of the experiment was accepted as the induction of acute pancreatitis with STC) for biochemical and histopathologic examination.

Serum amylase activity

Serum amylase activity was measured in blood samples with commercial kits in an auto analyzer operating with CNPG3 substrate methodology to confirm the pancreatitis model (Abbott Architect C8000, Chicago, Illinois). Amylase levels were recorded in units per liter (U/L).[12]

Histopathologic examinations

All of the tissue samples were assessed by the same histologist, who was unaware of the groups. Pieces from the central part of the pancreas were placed in 10% buffered formalin and prepared for routine paraffin embedding. Paraffin-embedded specimens were cut into 5-μm sections and stained with hematoxylin and eosin. Sections were examined using a Leica DFC 280 light microscope and Leica Q Win Plus Image Analysis System (Leica Micros Imaging Solutions Ltd, Cambridge, United Kingdom).

Histopathologic pancreatic damage scores were calculated by grading acinar cell degeneration, interstitial inflammation, edema, and hemorrhage, with a maximum score of 12. The standards of Schmidt *et al*[13] were modified as follows:

1. Acinar cell degeneration: 0, absent; 1, focal (<5%); 2, and/or sublobular (<20%); 3, and/or lobular (>20%).
2. Inflammation was scored as: 0, absent; 1, mild; 2, moderate; 3, severe.
3. Grading for edema was scaled as 0, absent; 1, edema in the interlobular space; 2, edema in the intralobular space; 3, the isolated-island shape of pancreatic acini.
4. Parenchymal hemorrhage was scored as: 0, absent; 1, mild; 2, moderate; 3, severe.

Statistical analysis

All of the data obtained from the study were analyzed by SPSS for Windows version 20.0 (SPSS Inc, Chicago, Illinois). Statistical analysis was performed using 1-way analysis of variance. The experimental values were expressed as the mean ± SD. The statistical significances and comparisons between amylase groups were evaluated using a post hoc test, Tukey test. Intergroup differences of histologic data were analyzed by Mann-Whitney *U* nonparametric test. $P < 0.05$ was considered as the level of significance for all analyzes.

Results

Serum amylase activity

Serum amylase activities of the C and LC groups were similar (mean ± SD, 641.25 ± 209 and 522.37 ± 91.42, respectively), and there was no statistical significance between the two groups ($P = 0.937$). Amylase activities were significantly increased in both the P and P + LC groups compared with control animals ($P < 0.05$). When amylase activities in the P + LC group and the P group were compared (mean ± SD, 1557.12 ± 601.10 and 1780.62 ± 505.52, respectively), there was no statistical significance between groups ($P = 0.697$). The amylase activities of the groups are shown in Fig. 1.

Histopathologic findings

The pancreatic tissue of the control and carnitine groups' rats showed normal histology with light microscopy (Fig. 2). Most of the organ was made of tubuloacinar exocrine pancreas. Islets of Langerhans were placed as isolated islands within the lobules.

The pancreas specimens of the pancreatitis group showed prominent histopathologic changes. The histologic structure of the exocrine pancreas was damaged. Acinar degeneration, inflammatory cell infiltration, parenchymal hemorrhage, and edema in the interlobular and intralobular interstitium and fat necrosis were detected (Fig. 2). Histopathologic scores of the groups are summarized in Table 1. Pancreatic damage was significantly increased in the P group compared with the C group ($P < 0.05$).

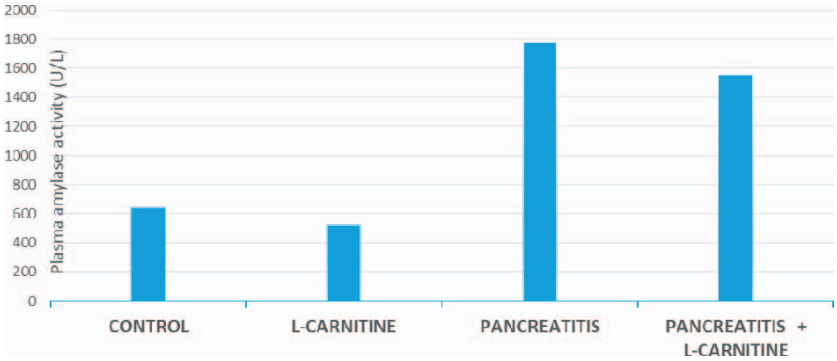

Fig. 1 Serum amylase activities of the groups.

In the P + LC group, the histopathologic findings of the pancreatitis were markedly reduced. Focal acinar cell degeneration was rarely seen. Parenchymal hemorrhage was also rare. Interlobular and intralobular inflammation and edema were generally mild. Few apoptotic bodies were detected (Fig. 2). The pancreatic damage score of the P + LC group was significantly lower than that of P group, but it was significantly higher those that of the C and LC groups ($P < 0.05$).

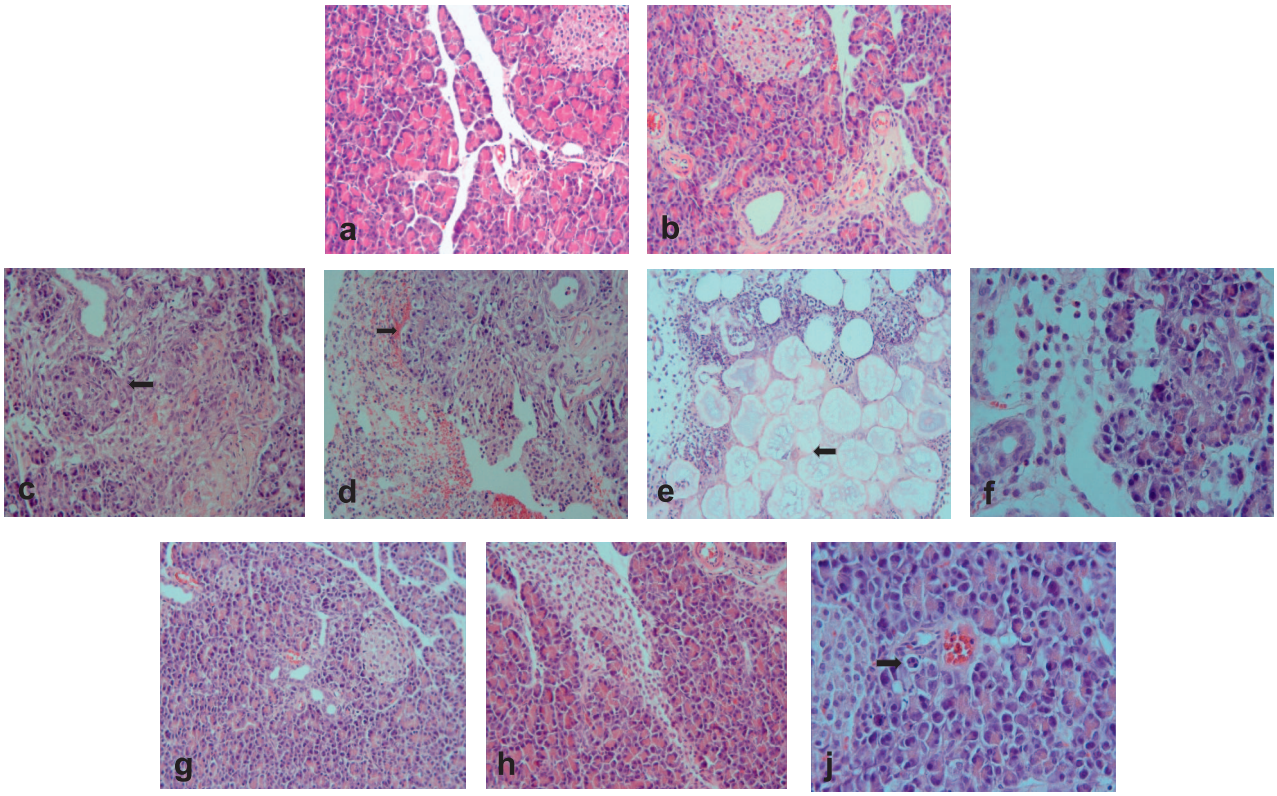

Fig. 2 (a) Control group. Normal histologic appearance [hematoxylin-eosin (H&E), ×20]. (b) LC group. Normal histologic appearance (H&E, ×20). (c) Pancreatitis group. Acinar cell degeneration and inflammation (H&E, ×20). (d) Pancreatitis group. Acinar cell degeneration, inflammatory cell infiltration, and parenchymal hemorrhage (H&E, ×20). (e) Pancreatitis group. Fat necrosis and inflammatory infiltration (H&E, ×20). (f) Pancreatitis group. Acinar cell degeneration, inflammatory cell infiltration, and interstitial edema (H&E, ×40). (g) Pancreatitis + LC group. Normal histologic appearance (H&E, ×20). (h) Pancreatitis + LC group. Mild interlobular and intralobular inflammation and edema (H&E, ×20). (j) Pancreatitis + LC group. Apoptotic bodies in pancreatic parenchyma (H&E, ×40).

Table 1 Total pancreatic damage scores of the groups[a]

Groups	Inflammation	Acinar cell degeneration	Edema	Hemorrhage	Total pancreatic damage score
C	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
LC	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
P	2.37 ± 0.91[b]	2.12 ± 0.64[b]	2.00 ± 0.75[a]	1.00 ± 0.75[b]	8.00 ± 2.13[b]
P + LC	1.00 ± 0.75[b,c]	0.37 ± 0.51[c]	0.87 ± 0.35[b,c]	0.12 ± 0.35[c]	2.37 ± 1.50[b,c]

[a]Data are presented as means ± SD.

[b]Significantly different from control group.

[c]Significantly different from pancreatitis group ($P < 0.05$).

Discussion

Acute pancreatitis is an inflammatory disorder of the pancreas with a high mortality, up to 30%. It is one of the leading causes of gastrointestinal disease requiring hospitalization and intensive care, with an annual incidence of 13 to 45 cases per 100,000 persons in the United States.[14] On the other hand, there is no specific treatment, except for symptomatic management including fluid resuscitation, gastric decompression, enteral feeding, and intravenous antibiotics. Moreover, patients with infected necrosis need surgical, radiologic, or endoscopic drainage procedures.[15] Because the incidence of acute pancreatitis is increasing worldwide, many researchers are focusing on effective treatment modalities.

According to the revised Atlanta classification, severity of pancreatitis is classified as mild, moderate, or severe. Mild acute pancreatitis has no organ failure, local complications, or systemic complications. Moderately severe acute pancreatitis is defined by the presence of transient organ failure (<2 days) and local complications, and severe acute pancreatitis is defined by the presence of persistent organ failure (>2 days). This classification also identifies 2 phases of the disease, early and late.[16] Patients suffering from early and persistent multiorgan dysfunction syndrome have a high risk of mortality. About 20% of patients with SP develop organ dysfunction in first 3 days, and almost half of these patients do not respond to intensive treatment.[17,18] Therefore, the present study focused on the early phase of the disease.

The experimental model of STC-induced pancreatitis in rats demonstrates acute severe hemorrhagic pancreatitis in humans.[19] There are other frequently used experimental models that produce mild pancreatitis induced by different chemicals, including cerulein, L-arginine, and L-aspariginase.[20–22] However, we decided to work on an STC-induced SP model in our study because the management of SP remains a challenging problem at present. Although SP is associated with a mortality rate of 30%, mild pancreatitis is associated with a mortality rate that is less than 1%.[1]

An STC pancreatitis model is a commonly used experimental model that is thought to be the model that most similarly demonstrates biliary pancreatitis clinically and histologically in humans. Histopathologic changes in the STC model, including edema, hemorrhage, inflammation, and acinar cell necrosis, can be observed within 48 hours after induction of pancreatitis. However, it has several limitations, such as long procedure time, technical difficulty of surgery, and the high death rates of experimental animals.[23] Wang *et al*[24] reported that pancreatic injection of 3.8% STC could establish an SP model with multiple organ dysfunction but with a high mortality rate of 90% in 7 days in rats. So we defined the end point time of the study as the 48th hour after induction of SP, which is a long enough period to observe the histopathologic changes but also a short enough period to observe during the experiment the rat mortality from multiorgan failure. All of the rats in our study survived until the end of the experiment.

LC is a conditionally essential nutrient; 75% of its amount in the organism is from dietary sources, and 25% is derived from *de novo* synthesis from lysine and methionine amino acids in liver and kidneys.[25] LC is an amino acid–like molecule that serves in transporting fatty acids and producing acetyl CoA in mitochondrial matrix for β-oxidation to provide energy that is needed for metabolism. In addition, it plays a critical role in transporting intermediate toxic compounds out of mitochondria and preventing lipid peroxidation, which provides mitochondrial membrane integrity and stability under severe oxidative stress.[9–11] A recent study demonstrated that the mitochondrial damage and the disturbance of mitochondrial function in pancreas occurs after 6

hours of induction of SP with STC.[26] The disturbance of mitochondrial energy metabolism in pancreas is very important in the development and progression of SP. In most cases, mortality from SP is a result of multiorgan dysfunction syndrome, which is related to mitochondrial dysfunction at the cellular level. Herein, LC may ameliorate this disturbance with its anti-inflammatory and antioxidant effects on mitochondria during SP. The protective effects of LC in different models of mild pancreatitis were demonstrated previously, but protective effects of LC on pancreatic tissue in the STC-induced SP model has not been demonstrated yet in the literature.[21,22]

The carnitine dose of our experiment was determined according to the previous studies with LC in order to compare the results and maintain a standardization. There are several studies evaluating the effects of LC not only in pancreatic tissue but also in other organs, with a dose of 200 mg/kg intraperitoneally.[22,27–29] In addition, intraperitoneal injection is a preferred route in many studies with experimental animals. It is commonly used in rats and mice, intraperitoneal injection administration results in a faster absorption into the systemic circulation than subcutaneous administration, it is almost equal to intravenous administration, and anesthesia is generally not required.[30]

There was a decrease in the serum amylase activity of the LC group compared with the C group, but there was no statistical significance between the 2 groups in our study (amylase levels in the C and LC groups were 641.25 ± 209 and 522.37 ± 91.42, respectively; $P = 0.937$). In a study of the cerulein-induced pancreatitis model of Arafa *et al*[22] (this study is one of the two studies that evaluate the protective role of carnitine in an experimental pancreatitis model), in parallel with our study, the amylase levels of the control and carnitine groups were similar. Also, in Kaya *et al*[21] (a model of an L-aspariginase–induced pancreatitis model) there was no difference between these groups (amylase levels in the C and LC groups were 1314.9 ± 36.9 and 1333.0 ± 105.3, respectively). These results are reasonable because a significant decrease in basal amylase level after carnitine administration in a healthy individual is not an expected effect of LC.

In the present study, amylase activity was significantly increased as a result of pancreatitis in both the P and P + LC groups compared with control animals ($P < 0.05$). This result is in accordance with those of previous studies. When amylase activities in the P + LC group and the P group were compared, there was no statistical significance between groups. This result is in contrast with the study of Arafa *et al*.[22] They found decreased amylase activity by 64% in LC treatment group (200 mg/kg/d for 7 days). In addition, Kaya *et al*[21] reported lower amylase activities in the LC treatment group (500 mg/kg/d for 5 days), nearly approaching those of the control group. This contrast may originate from the difference in severity of the pancreatitis models between the studies and the administration of LC at a lower dose (200 mg/kg/d) for a shorter period (48 hours) in our study.

The histopathologic examination of the pancreatic specimens of the P group revealed prominent changes; thus, pancreatic damage score was significantly higher in the P group compared with the C group ($P < 0.05$). The histopathologic changes showing the damage of exocrine pancreas in the P group are well matched with the histopathologic findings of previous studies in the STC-induced SP model.[19] In the treatment group, the histopathologic findings of the pancreatitis were markedly reduced. Focal acinar cell degeneration and parenchymal hemorrhage were rarely seen. Interlobular and intralobular inflammation and edema were generally mild. Bordalo *et al*[31] demonstrated that acinar cell degeneration is the precursor of the pancreatic necrosis in the pathogenesis of pancreatitis. Therefore, it is obvious that a specific treatment of pancreatitis independent from the etiology has to prevent acinar cell degeneration. The ameliorating effect of LC on acinar cell degeneration during STC-induced SP was significant in the present study. LC improved the inflammatory injury histologically, even with a short period of administration (48 hours).

One limitation of our study was the unavailability of defining the pathophysiologic mechanism that underlies the ameliorating effect of LC on acinar cell degeneration. The present study focused on histopathologic changes only. The role of LC in mitochondrial dysfunction during SP remains unclear. From this point of view, our future research will investigate the potential healing mechanisms of LC therapy and demonstrate the biochemical and ultrastructural changes of the mitochondria of acinar cells during the course of SP.

Conclusion

This experimental study revealed that LC has a significant histopathologic protective effect on acinar cell degeneration in an STC-induced SP model in rats. This result suggests that LC administration may be an adjunctive therapy in the management of SP.

Acknowledgments

Financial support for this experimental study (drugs, equipment, etc) was provided by the authors (A total of $2150). The authors have nothing to disclose. The authors have no conflict of interest.

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