Original Article
Effects of L-carnitine on liver injury in rats and its impact on blood lipids

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Abstract: Objective: To explore mechanisms of anti-oxidant activity of L-carnitine on liver injury and the impact of L-carnitine on serum lipid levels in rats. Methods: Ninty normal male Wister rats were selected and randomly classified into the blank control group (n=30), the placebo group (n=30) and the L-carnitine group (n=30). The rats in the L-carnitine group and the placebo group were intraperitoneally injected with endotoxin lipopolysaccharide at 0.1 mg/kg. Apart from that, those in the L-carnitine group also received concomitant L-carnitine at 1 g/kg by an intragastric route, while those in the placebo group were given equal amounts of normal saline by an intragastric route. Those in the blank control group were administered equal amounts of normal saline. The rats in the three groups were compared in the liver injury indicators, antioxidant-related indicators, inflammatory cytokines, and serum lipid content. Results: Compared with the blank control group, the serum aspartate aminotransferase (AST), alanine transaminase (ALT), malonialdehyde (MDA), low-density lipoprotein cholesterol (LDL-C), tetracycline (TC) and triglyceride (TG) levels in rats of the L-carnitine group and the placebo group were significantly elevated. Serum high-density lipoprotein cholesterol (HDL-C), superoxide dismutase (SOD), and and glutathione peroxidase (GSH-Px) levels were reduced. Nitric oxide (NO) content, nitric oxide synthase (NOS) activity, interleukine-6 (IL-6), interleukine-1β (IL-1β), and tumor necrosis factor-α (TNF-α) levels in hepatic tissue were markedly enhanced in rats of the L-carnitine group and the placebo group (all P<0.001). Compared to the rats in the placebo group, those in the L-carnitine group showed significantly lower serum AST, ALT, MDA, LDL-C, TC, and TG levels, considrably elevated serum HDL-C, SOD, and GSH-px levels, and reduced NO contents, NOS activity, IL-6, IL-1β, and TNF-α levels in hepatic tissue (all P<0.001). Conclusion: L-carnitine exerts a protective effect on endotoxin lipopolysaccharide-induced liver injury, and its protective mechanisms are associated with its anti-oxidant activity and reduced levels of inflammatory cytokines.

Keywords: L-carnitine, liver injury, antioxidation, blood lipid, inflammatory cytokine

Introduction

Liver injury is a complex pathophysiological process mediated by multiple factors. Increasing evidence shows that the pathogenesis of most liver disorders is related to involvement of reactive oxygen species (ROS) [1, 2]. Endotoxin acts on the body to produce a broad range of active molecules (such as ROS) which induce membrane damage and mitochondrial structure destruction of hepatocytes, and decreased liver metabolism, eventually leading to apoptosis or necrosis of hepatocytes in rats [3, 4]. Multiple studies indicate that botulinum toxin can specifically bind to liver macrophages, transmit cell signals and irritate cells to gener-

ate a sea of inflammatory cytokines, resulting in pathological changes in the liver. Additionally, destruction of liver structure and function contributes to decreased endotoxin phagocytosis in the body, which forms a vicious cycle, ultimately leading to succesive damage in the body [5, 6]. Patients with liver injury have a paucity of carnitine which may give rise to fatty acid accumulation, adverse reactions to cell function, and intracellular energy deficiency, with the manifestation of dyslipidemia. Increased free fatty acids in the blood circulation system contribute to lipid peroxidation, changes in lysosome cell membrane, mitochondrial dysfunction, protein kinase activation, generation of ROS in the endoplasmic reticulum, and activa-
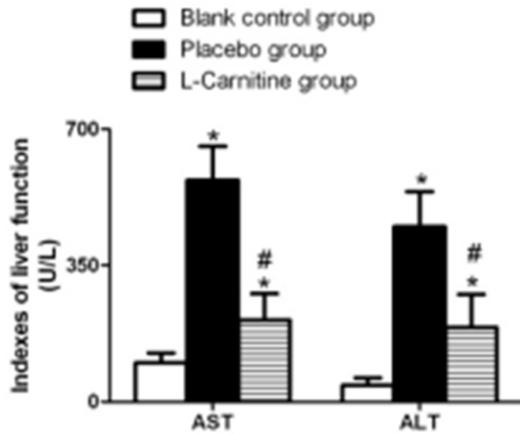
Figure 1. Effect of L-carnitine on the serum ALT and AST levels in rats with liver injury. *P<0.001, compared with the blank control group; #P<0.001, compared with the placebo group.

tion of inflammatory pathways, ultimately leading to liver injury.

L-carnitine is a water-soluble amino acid derivative that is widely found in the tissues of the body. It primarily functions to promote β-oxidation of long-chain fatty acids, and scavenge free radicals as an anti-oxidant activity [7, 8]. If reactive oxygen species (ROS) production exceeds the scavenging capacity of the anti-oxidant system in the body, it may induce lipid peroxidation, protein denaturation, and gene mutation, eventually leading to oxidative damage to the cells. Previous evidence indicates the major mechanisms for carnitine to reduce liver injury are reducing the production and transport of tumor necrosis factor (TNF), preventing cell membrane damage, and improving cell permeability while blocking the activation of platelet activity factors, inhibiting the synthesis and release of vasoconstrictor substances and reducing portal hypertension [9-12]. However, mechanisms for carnitine antioxidant protection in endotoxin-induced liver injury are rarely reported. In this study, rat models of endotoxin-induced liver injury were constructed and assigned to receive L-carnitine and placebo interventions, respectively; meanwhile, additional healthy rats were taken as blank controls to study the effect of L-carnitine on blood lipids and the mechanisms of anti-oxidation in rats with liver injury, with an aim to provide experimental evidence for the use of L-carnitine in treating liver injury.

Materials and methods

Experimental methods

A total of 90 normal male Wister rats with 12 weeks of age and a weight of 180-220 grams were enrolled and randomly divided into the blank control group, the placebo group, and the L-carnitine group. The rats in the L-carnitine group and the placebo group were intraperitoneally injected with endotoxin lipopolysaccharide at 0.1 mg/kg. Additionally, the rats in the L-carnitine group also received cocomitant L-carnitine at 1 g/kg by intragastric route, whereas those in the placebo group were intragastrically given equal amounts of normal saline. Those in the blank control group received equal amounts of normal saline. All the rats were administered once daily for 3 weeks.

Outcome measures

Determination of serological indicators: Three weeks after intervention, 2 ml of blood was drawn from the tail vein of each rat in all the groups after 12-hour fasting, placed in an anticoagulant tube, and centrifuged at 3000 r/min for 10 min. Subsequently, the serum was separated, and stored at -20°C for testing. The alanine transaminase (ALT), aspartate aminotransferase (AST), and blood lipid levels were detected with an automatic biochemical analyzer (Olympus, Japan), while superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione peroxidase (GSH-Px) levels were determined by the biochemical enzyme assays. Assay kits were purchased from Sigma, USA, and were used strictly following the instructions.

Detection of nitric oxide (NO) content, nitric oxide synthase (NOS) activity, and inflammatory cytokines in liver tissue: Three weeks after intervention, rats were sacrificed under anesthesia. Hepatic tissue samples were harvested after laparotomy, weighed, homogenized at low temperature, and centrifuged at 4000 r/min for 10 minutes. Supernatant was removed and stored at -20°C for testing. The nitrate reductase-based colorimetric assay was employed for detection of the NO contents. The NO kits were purchased from R&D Science, USA, and the testing procedures were strictly followed the instructions on the kits.
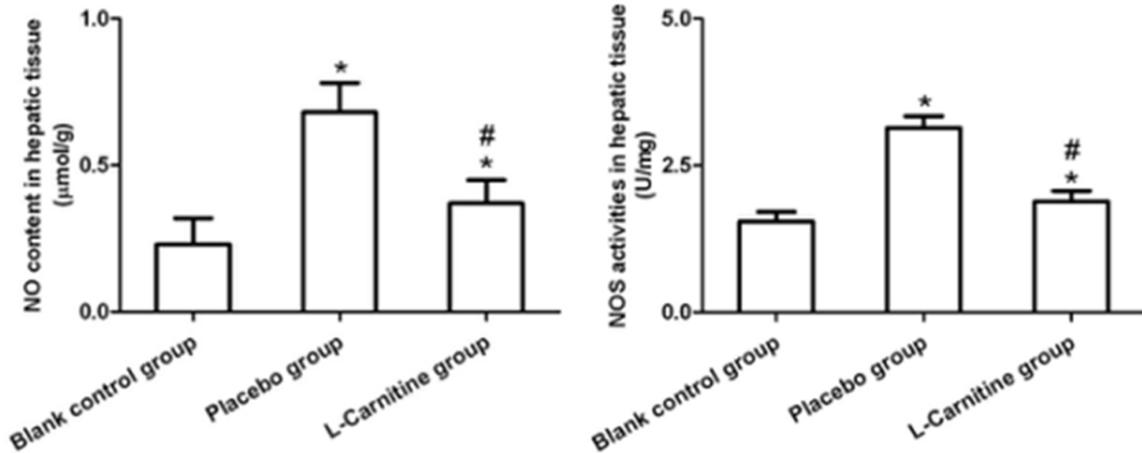
Figure 2. Comparison of the NO contents and NOS activity in the hepatic tissue of rats of all groups. $^*P<0.001$, compared to the blank control group; $^#P<0.001$, compared to the placebo group.

Table 1. Serum SOD, MDA and GSH-px levels in rats of all groups

Groups	SOD (U/mL)	GSH-Px (U/mL)	MDA (U/mL)
Blank control group	281.21±13.82	405.36±15.82	3.73±0.72
Placebo group	184.25±11.36*	318.3±13.19*	7.51±1.03*
L-carnitine group	228.53±12.27*,#	376.9±12.45*,#	4.64±0.85*,#

Note: SOD denotes superoxide dismutase; GSH-Px, glutathione peroxidase; MDA malonialdehyde.*P<0.001, compared with the Blank control group; #P<0.001, compared with the placebo group.

Table 2. Inflammatory cytokines levels in the hepatic tissue of rats of all groups

Group	IL-6 (ng/g)	IL-1β (ng/g)	TNF-α (ng/g)
Blank control group	164.25±10.36	20.81±2.2	165.72±17.82
Placebo group	413.15±12.74*	75.34±3.8*	361.38±14.77*
L-carnitine group	343.9±10.8*,#	37.8±2.6*,#	230.27±18.24*,#

Note: IL-6 denotes interleukine-6; IL-1β, interleukine-1β; TNF-α, tumor necrosis factor-α. *P<0.001, compared with the Blank control group; #P<0.001, compared with the placebo group.

The NOS activity was detected with the use of a colorimetric assay, and the NO synthase kits were purchased from Sigma, USA. The procedures were performed according to the instructions on the kits. The absorbance was measured at a wavelength of 530 nm, and NOS activity was calculated on the base of the absorbance values. The levels of inflammatory cytokines were detected by the enzyme-linkedimmunosorbent assay (EL-ISA). Kits for interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) detection were purchased from R&D Science, USA.

Statistical analysis

All the data were analyzed using the SPSS software, version 18.0. Quantitative data with normal distribution are presented as mean ± sd, and the differences across the three groups were analyzed by oneway analysis of variance (ANOVA) with post-hoc Bonferroni test. P<0.05 was deemed as significant.

Results

Effect of L-carnitine on serum ALT and AST levels in rats with liver injury

Serum AST and ALT levels in rats of the L-carnitine group and the placebo group were markedly higher than those of the rats of the blank control group (all P<0.001), and the serum ALT and AST levels in the L-carnitine group was remarakbly lower than those in the placebo group (P<0.001), as illustrated in **Figure 1**.

NO contents and NOS activity in hepatic tissue of rats

Greater improvements in the NO contents and NOS activity in hepatic tissue of rats were noted in the L-carnitine group and the placebo group than the blank control group (all P< 0.001). Compared with the placebo group, the

Table 3. Serum lipid levels in rats of all groups

Groups	LDL-C (mmol/L)	HDL-C (mmol/L)	TC (mmol/L)	TG (mmol/L)
Blank control group	1.38±0.15	0.95±0.04	1.93±0.13	0.71±0.08
Placebo group	3.85±0.22*	0.25±0.03*	7.62±0.32*	1.27±0.11*
L-carnitine group	2.41±0.18*,#	0.59±0.05*,#	4.23±0.25*,#	0.92±0.09*,#

Note: LDL-C denotes low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, tetracycline; TG, triglyceride. *P<0.001, compared to the Blank control group; #P<0.001, compared to the placebo group.

L-carnitine groups could significantly inhibit the increases in the NO contents and NOS activity (P<0.001; **Figure 2**).

Serum SOD, MDA and GSH-px levels of rats

Lower SOD and GSH-px levels, but higher MDA levels were observed in the placebo group than those in the blank control group and the L-carnitine group (all P<0.001). The rats in the L-carnitine group showed significant higher MDA levels than those in the blank control group, as well as lower SOD and GSH-px levels (all P<0.001; **Table 1**).

Levels of inflammatory cytokines in the hepatic tissue of rats

The levels of IL-6, IL-1β and TNF-α in hepatic tissue of rats in the placebo group were considerably higher than those in the blank control group (all P<0.001). The levels of IL-6, IL-1β and TNF-α were profoundly lower in the L-carnitine group than in the placebo group (all P<0.001; **Table 2**).

Serum lipid levels in rats of all groups

Higher serum low-density lipoprotein cholesterol (LDL-C), tetracycline (TC), and triglyceride (TG) levels, and lower high-density lipoprotein cholesterol (HDL-C) levels were seen in the placebo group than in the blank control group (all P<0.001). The L-carnitine group had significantly reduced serum LDL-C, TC and TG levels and elevated serum HDL-C levels compared to the placebo group (all P<0.001; **Table 3**).

Discussion

When various factors induce hepatocyte necrosis, the morphology and physiological functions of the liver membrane are destroyed, and various enzymes in the cells appear in the serum [13, 14]. It has been reported that the AST and ALT levels are enzyme markers for detecting liver injury, and elevated serum AST and ALT levels indicate more severe damage to the cell membrane and mitochondria of hepatocytes [15]. The results of the present study indicate that ASL and ALT levels are significantly elevated in the placebo group, indicating that rat models of endotoxin-induced liver injury were successfully constructed. Abnormal blood lipid metabolism is associated with liver injury [16]. Oxidative stress mediated by free fatty acids at high concentrations has shown to be closely linked to lipoapoptosis of liver cells [17]. In our current study, the TG, TC and LDL-C levels were significantly elevated and the HDL-C level was remarkably decreased in the placebo group, implying that impairment of liver function affects the lipid metabolism in the body. Three weeks after L-carnitine medication by intragastric route, elevation of AST, ALT, TG, TC and LDL-C levels were significantly inhibited and the HDL-C levels were remarkably elevated in the rats of the L-carnitine group, implying that L-carnitine can protect the liver from damage, improve the lipid levels, and reduce lipotoxicity.

Recent studies indicate that antioxidant mechanisms play a crucial role in the protection of L-carnitine against tissue damage [18]. NO, a highly reactive free radical, acts as both a neurotransmitter and a second messenger to mediate endotoxin, TNF and other cytokines. NOS, a synthase that catalyzes NO production by L-arginine and O_2, is extensively present in tissues including the liver. Evidence shows that endotoxin is the major cause for stimulating NO synthesis in liver cells [19]. In the current study, NO and NOS levels in rats with liver injury in the placebo group were remarkably higher than those in the blank control group, suggesting that NO and NOS were implicated in liver injury. Nevertheless, the NO and NOS levels of rats in the L-carnitine group were substantially lower than those in the placebo group, implying a protective effect of L-carnitine on rats with liver injury might be related to the NO and NOS levels,

which is in line with the results reported by Babicova et al. [20].

Under normal conditions, the scavenging and production of free radicals maintain homeostasis in the body. Changes in the SOD activity, MDA contents, and GSH-Px activity can reflect the capacities of anti-oxidation and free radical scavenging [21, 22]. SOD, one of the most important antioxidant enzymes in the defense system against oxidative damage, can reflect the body's ability to scavenge oxygen free radicals. MDA, an essential end product of lipid peroxidation, reflects the severity of damage to the tissues and cells by free radicals. GSH-Px, a dominant enzyme that catalyzes the decomposition of hydrogen peroxide, can specifically catalyze the reduction of hydrogen peroxide by reduced glutathione, thereby playing a role in protecting the structure and functions of the cell membrane. In this study, rats in the L-carnitine group had significantly lower MDA levels than those in the blank control group, as well as markedly higher SOD and GSH-px levels than those in the placebo group, suggesting that L-carnitine confers anti-oxidant protection in endotoxin-induced liver injury, which is similar to the result reported in previous literature [23].

The liver is the most vulnerable organ in endotoxemia patients. Endotoxins going into the liver along with the blood circulation can directly induce the liver Kupffer cells to release a variety of inflammatory mediators and cytokines which include IL-6, IL-1β, TNF-α and free radicals. Inflammatory mediators and cytokines play decisive roles in the pathogenesis of the liver, and act together with endotoxin to induce hepatic stellate cells and vascular endothelial cells to further release more inflammatory mediators, leading to cascade amplification effect [24]. The results of the current study reveal that the use of L-carnitine considerably can reduce elevation of IL-6, IL-1β and TNF-α levels in endotoxin-induced liver injury, downregulate over-expression of inflammatory cytokines, and reduce the inflammatory response *in vivo*.

In summary, L-carnitine intervention for endotoxin lipopolysaccharide-induced liver injury in rats, significantly improved the liver antioxidant contents, enhanced SOD content and GSH-PX activity, and reduced MDA, NO and NOS expression. Moreover, it also reduced expression of inflammation cytokines, providing more experimental evidence for the clinical treatment of liver injury.

Disclosure of conflict of interest

None.

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