

L-Carnitine has a liver-protective effect through inhibition of inducible nitric oxide synthase induction in primary cultured rat hepatocytes

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ABSTRACT

Background: L-Carnitine has protective effects on various injured organs. However, it has not been reported whether L-carnitine influences the induction of inducible nitric oxide synthase (iNOS) expression during inflammation. Nitric oxide (NO) produced by iNOS is an inflammatory indicator in organs which become inflamed, including the liver.

Objective: This study aimed to examine whether L-carnitine influences the induction of iNOS gene expression in inflammatory cytokine-stimulated hepatocytes and the mechanisms involved in the action.

Methods: L-Carnitine was added into the primary cultures of rat hepatocytes stimulated by interleukin-1 β (an *in vitro* liver injury model). The production of NO and induction of iNOS and its signaling pathway were analyzed.

Results: Transfection experiments with iNOS promoter-luciferase constructs revealed how L-carnitine inhibited iNOS mRNA synthesis activity and reduced its stability. In support of this observation, L-carnitine reduced iNOS mRNA and iNOS protein expression levels, resulting in reduced NO production. L-Carnitine blocked two essential pathways for iNOS induction: I κ B kinase (I κ B degradation/NF- κ B activation) and phosphatidylinositol 3-kinase/Akt (type I IL-1 receptor upregulation).

Conclusions: L-Carnitine inhibited the induction of inflammatory mediator iNOS, partially through inhibition of NF- κ B activation, which demonstrated L-carnitine has protective effects in an *in vitro* liver injury model. L-Carnitine may have therapeutic potential for organ injuries, including the liver.

Keywords: L-carnitine, hepatic encephalopathy, inducible nitric oxide synthase, liver injury, primary cultured hepatocytes, nuclear factor- κ B, type I interleukin-1 receptor

INTRODUCTION

L-Carnitine is mostly (75%) derived exogenously from foods in the diet. However, it is also synthesized in the body from the essential amino acids lysine and methionine. The liver is a main site for endogenous L-carnitine synthesis [1, 2]. It is well known that L-carnitine is involved in long-chain fatty acid transport into the mitochondrial matrix via the action of acyltransferases. As a result, within L-carnitine deficiency, fat oxidation and energy production from fatty acids are markedly impaired [3]. Additionally, animal model experiments have demonstrated that L-carnitine prevented oxidative stress and protected against hepatic damage including liver fibrosis induced by carbon tetrachloride (CCl₄) [4-9]. L-Carnitine has hepatoprotective effects in acute acetaminophen toxicity [10] and enhanced liver regeneration after partial hepatectomy in rats [11].

Clinically, long-term tube feeding is often necessary for pediatric patients with severe physical and mental disabilities because of various factors such as the severity of underlying diseases, impaired swallowing function, and respiratory failure [12]. These patients have a high risk of hypocarnitinemia that results from low L-carnitine intake, changes in their physique, and long-term use of antiepileptic and antibacterial drugs. Recently, increasing evidence has indicated that L-carnitine has a therapeutic effect in cirrhosis patients with hepatic encephalopathy and hyperammonemia [13-15].

Ringseis et al. reported that L-carnitine supplementation beneficially influences critical mechanisms involved in pathologic skeletal muscle loss [16]. L-Carnitine attenuated oxidative stress responses in patients with renal disease [17]. Malaguarnera et al. also reported that L-carnitine supplementation was useful for treating patients with non-alcoholic steatohepatitis [18]. However, there are few scientific reports about its action and mechanisms that are involved in the protective effects of L-carnitine on organ damage, including the liver.

In the inflamed liver, there is an increased production of nitric oxide (NO) by inducible nitric oxide synthase (iNOS), and inflammatory cytokines such as tumor necrosis factor (TNF)- α and interleukin (IL)-1 β , which have been implicated as factors in liver injury [19, 20]. We previously reported that the proinflammatory cytokine IL-1 β induced iNOS, TNF- α , and cytokine-induced neutrophil chemoattractant (CINC)-1/chemokine (C-X-C motif) ligand 1 (CXCL1) (human IL-8 analogue) gene expression in primary rat hepatocyte cultures (*in vitro* liver injury model), which was followed by excess NO production [21]. Using the *in vitro* liver injury model, a liver-protective effect of clinical drugs, traditional medicines, and functional foods may be determined. Prevention of iNOS induction and NO production should be considered indicators of liver protection.

In the current study, we examined whether L-carnitine inhibits iNOS induction and NO production in IL-1 β -stimulated hepatocytes and the mechanisms underlying the NO-inhibiting action (liver-protective effect) of L-carnitine.

MATERIALS AND METHODS

Materials

L-Carnitine (kindly provided from Otsuka Pharmaceutical Ltd, Tokyo, Japan) was dissolved in the culture medium (10–30 mg/mL) before use. Recombinant human IL-1 β (2×10^7 U/mg protein) was purchased from MyBioSource (San Diego, CA, USA). Male Wistar rats (200–250 g and 6–7 weeks old) were purchased from Charles River (Tokyo, Japan) and kept at 22°C under a 12:12 h light:dark cycle with *ad libitum* access to food and water. All experiments on rats were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health. The experiments were approved by the Animal Care Committee of Kansai Medical University.

Primary hepatocyte culture

Hepatocytes were isolated from rats by perfusion with collagenase (Wako Pure Chemicals) [22], suspended in culture medium at 6×10^5 cells/mL, seeded into 35-mm plastic dishes (2 mL/dish; Falcon Plastic, Oxnard, CA, USA), and cultured at 37°C in a CO₂ incubator under a humidified atmosphere of 5% CO₂ in air. The culture medium was Williams' E (WE) medium supplemented with 10% newborn calf serum, HEPES (5 mM), penicillin (100 U/mL), streptomycin (100 μ g/mL), fungizone (0.25 μ g/mL), aprotinin (0.1 μ g/mL; Roche, Mannheim, Germany), 10 nM dexamethasone, and 10 nM insulin. After 2 h, the medium (1.5 mL/ dish) was replaced with fresh serum-free and hormone-containing WE medium (first medium change). After incubation for 5 h, the medium was replaced with fresh serum and hormone-free WE medium (second medium change), and the cells were cultured overnight. The number of cells attached to the dishes was calculated by counting the number of nuclei [23] and using a ratio of 1.37 ± 0.04 nuclei/cell (mean \pm SE, n = 7 experiments).

Treatment of cells with L-carnitine

On day 1, the cells were washed with fresh serum and hormone-free WE medium and incubated with IL-1 β (1 nM) in the same medium in the presence or absence of L-carnitine. The doses of L-carnitine used are indicated in the appropriate Figures and their legends.

Transfection and luciferase assay

Transfection of cultured hepatocytes was performed as previously described [24]. Briefly, hepatocytes were cultured at 4×10^5 cells/dish (35×10 mm) in WE medium supplemented with serum, dexamethasone, and insulin for 7 h, before being subjected to magnet-assisted transfection (MATra). The reporter plasmid pRiNOS-Luc-SVpA or pRiNOS-Luc-3'UTR (1 μ g) and the CMV promoter-driven β -galactosidase plasmid pCMV-LacZ (1 ng) as an internal control were mixed with the MATra-A reagent (1 μ L; IBA GmbH, Göttingen, Germany). After incubation for 15 min on a magnetic plate at room temperature, the medium was replaced with fresh WE medium containing serum. Cells were cultured overnight, and then treated with IL-1 β in the presence or absence of L-carnitine. Luciferase and β -galactosidase activities of cell extracts were measured using PicaGene (Wako Pure Chemicals) and Beta-Glo (Promega, Wisconsin, USA) kits respectively. The sequence of *iNOS* gene promoter was deposited in the DNA Data Bank of Japan (DDBJ)/European Bioinformatics Institute (EMBL-EBI)/GenBank under the accession number AB290142.

Determination of NO production and lactate dehydrogenase activity

Culture medium was used for measurements of nitrite (a stable metabolite of NO) to reflect NO production by the Griess method [25]. Culture medium was also used for measurements of lactate dehydrogenase (LDH) activity to reflect cell viability using a commercial kit (Roche Diagnostics, Mannheim, Germany).

Western blot analysis

Total cell lysates were obtained from cultured cells, as described previously [26] with minor modifications. Briefly, cells (1×10^6 cells/35 mm dish) were lysed with sample buffer for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (final: 125 mM Tris-HCl, pH 6.8; containing 5% glycerol, 2% SDS, and 1% 2-mercaptoethanol), subjected to SDS-PAGE, and electroblotted onto a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA, USA). Immunostaining was performed using primary antibodies against mouse *iNOS* (Thermo Fisher Scientific (PA1-036), Rockford, IL, USA), human $\text{I}\kappa\text{B}\alpha$, mouse type I IL-1 receptor (IL-1RI) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and rat β -tubulin (internal control; Clone TUB2.1; Sigma Chemical Co., St. Louis, MO, USA). This was followed by visualization with an enhanced chemiluminescence blotting detection reagent (GE Healthcare Biosciences Corp.,

Piscataway, NJ, USA).

For Akt, total cell lysates prepared from 100-mm dishes (5×10^6 cells/dish) were pre-cleared with protein A (Sigma Chemical Co.) and then mixed with a mouse monoclonal antibody against human Akt1 (Akt5G3; Cell Signaling, Beverly, MA, USA) and protein G-Sepharose (Pharmacia LKB Biotech, Uppsala, Sweden). After incubation for 2 h at 4°C, immunocomplexes were centrifuged (16,000 g for 5 min). The beads were washed with solubilizing buffer, dissolved in SDS-PAGE sample buffer, and analyzed by western blotting using rabbit polyclonal antibodies against human Akt and phospho-Akt (Ser473) (Cell Signaling) as primary antibodies.

Reverse transcriptase-polymerase chain reaction

Total RNA was extracted from cultured hepatocytes using the guanidinium-phenol-chloroform method [27]. For strand-specific reverse transcriptase-polymerase chain reaction (RT-PCR) analysis, cDNA was synthesized from total RNA using strand-specific primers and step-down PCR was performed using PC708 (Astec, Fukuoka, Japan), as previously described [28], with minor modifications. For iNOS (257 bp), TNF- α (275 bp), CINC-1 (231 bp), IL-1RI (327 bp), and elongation factor-1 α (EF; internal control) (335 bp) mRNA, an oligo(dT) primer, was used for RT and the indicated primers sets were used for PCR (Table 1). For the iNOS antisense transcript (211 bp), the sense primer (5'-TGCCCCTCCCCACATTCTCT-3') was used for RT and the indicated primer set was used for PCR (Table 1). These mRNAs and antisense transcript levels were measured by real-time PCR using the Rotor-Gene Q 2plex HRM (Qiagen, Tokyo, Japan). The Rotor-Gene SYBR Green PCR Kit (Qiagen) was included in the reaction mixture, and the following touchdown protocol was applied: one cycle at 95°C for 5 min and 45 cycles at 95°C for 5 s and 60°C for 10 s. The cDNAs for the rat iNOS mRNA and antisense transcript were deposited in the DDBJ/EMBL-EBI/GenBank under the accession numbers AB250951 and AB250952 respectively.

Electrophoretic mobility shift assay

Nuclear extracts were prepared and an electrophoretic mobility shift assay (EMSA) was performed, as previously described [29]. Briefly, nuclear extracts from hepatocytes (4 μ g) were mixed with 1 μ g of poly(dI-dC) and a probe for 20 min at room temperature (total mixture, 15 μ L). To prepare a double-stranded DNA probe, annealed oligonucleotides harboring a κ B site (5'-AGTTGAGGGGACTTTCCCAGGC-3'; only the sense strand is shown) were labelled with [γ - 32 P]-adenosine-5'-triphosphate (ATP; DuPont-New England Nuclear Japan, Tokyo, Japan) and T4 polynucleotide kinase (Takara Bio Inc., Shiga, Japan). Samples were resolved on a 4.8% polyacrylamide gel, followed by drying and autoradiography. Protein concentrations were measured using the method of Bradford [30] with a binding assay kit (Bio-Rad), using bovine serum albumin as a standard.

Statistical analysis

The results shown are representative of three to four independent experiments yielding similar findings. All data are expressed as the mean \pm standard deviation (SD). Differences were analyzed by the Bonferroni–Dunn test. A value of $P < 0.05$ was considered to indicate a significant difference.

| Primer name | Nucleotide sequence |
|---------------|---|
| iNOS | 5' CCAACCTGCAGGTCTTCGATG 3'/5' GTCGATGCACAACCTGGGTGAAC 3' |
| F/R | |
| as iNOS | 5' CCTTTGCCTCATACTTCCTCAGA 3'/5' ATCTTCATCAAGGAATTATACACGG 3' |
| F/R | |
| TNF- α | 5' TCCCAACAAGGAGGAGAAGTTCC 3'/5' GGCAGCCTTGTCCTTGAAGAGA 3' |
| F/R | |
| CINC-1 | 5' GCCAAGCCACAGGGGCGCCCGT 3'/5' ACTTGGGGACACCCTTTAGCATC 3' |
| F/R | |
| IL-1RI | 5'-CGAAGACTATCAGTTTTTGG AAC-3'/5'-GTCTTCCATCTGAAGCTTTTGG-3' |
| F/R | |
| EF F/R | 5' TCTGGTTGGAATGGTGACAACATGC 3'/5' CCAGGAAGAGCTTCACTCAAAGCTT 3' |

Table 1. Primers and nucleotide sequences. iNOS, inducible nitric oxide synthase; as, antisense transcript; TNF- α , tumor necrosis factor-alpha; CINC-1, cytokine-induced neutrophil chemoattractant-1; IL-1RI, type I IL-1 receptor; EF, elongation factor-1 α ; F/R, forward/reverse

RESULTS***L-Carnitine inhibits iNOS mRNA synthesis and its stability in IL-1 β -stimulated hepatocytes***

iNOS mRNA expression is known to be regulated by iNOS promoter transactivation with transcription factors, such as NF- κ B, and by post-transcriptional modifications like mRNA stabilization [31]. First, we examined whether L-carnitine influences iNOS mRNA levels through the transcriptional and/or post-transcriptional steps. Transfection experiments were performed using two constructs containing firefly luciferase controlled by the iNOS promoter: pRiNOS-Luc-SVpA and pRiNOS-Luc-3'UTR, which detects iNOS promoter transactivation (mRNA synthesis) and mRNA stabilization (Fig. 1A) [32, 33], respectively. IL-1 β increased the luciferase activities of the two constructs, and simultaneous addition of L-carnitine and IL-1 β decreased the activities of both constructs (Fig. 1B and 1C). In support of the latter, iNOS antisense transcript (AST) analysis using real-time RT-PCR revealed that IL-1 β increased iNOS AST expression in a time-dependent manner, and that L-carnitine markedly inhibited this effect (Fig. 1D).

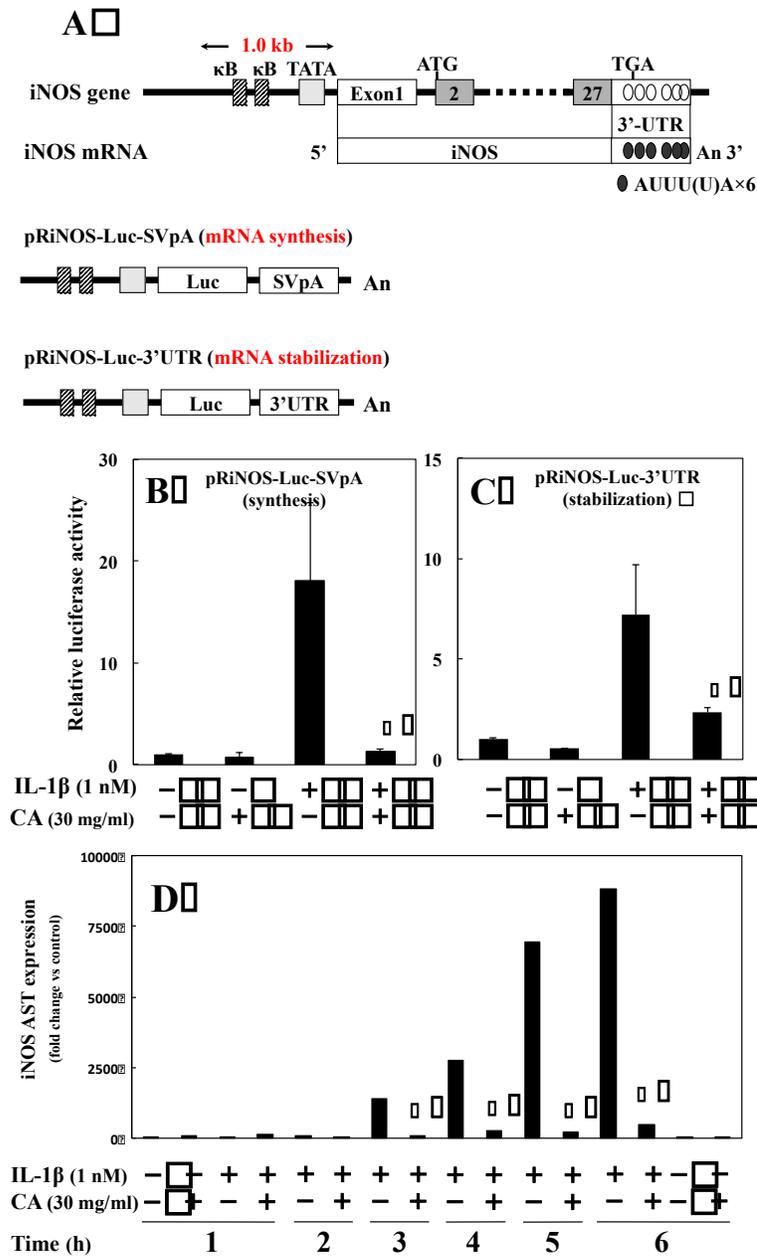


Fig. 1. Effects of L-carnitine on iNOS promoter transactivation (iNOS mRNA synthesis and its stability) and iNOS gene antisense transcript expression in hepatocytes. (A) Schematic representation of the iNOS gene promoter region. Two reporter constructs are shown beneath the iNOS gene and mRNA. The constructs consist of the rat iNOS promoter (1.0 kb), a luciferase gene, and the SV40 poly(A) region (pRiNOS-Luc-SVpA) or iNOS 3'-UTR (pRiNOS-Luc-3'UTR). "An" indicates the presence of a poly(A) tail. The iNOS 3'-UTR contains AREs (AUUU(U)A × 6), which contribute to mRNA stabilization. (B, C) Each construct was introduced into hepatocytes, and the cells were treated with IL-1β (1 nM) in the presence or absence of L-carnitine (CA, 30 mg/mL) for 7 h for pRiNOS-Luc-SVpA (B) and 3.5 h for pRiNOS-Luc-3'UTR (C). Luciferase activity was normalized to β-galactosidase activity. Fold activation was calculated by dividing luciferase activity by control activity (without IL-1β and L-carnitine). Data are presented as the mean ± SD for n = 4 dishes. (D) The cells were treated with IL-1β (1 nM) in the presence or absence of L-carnitine (30 mg/mL) for the indicated times. Total RNA was analyzed using strand-specific RT-PCR to detect the iNOS gene antisense transcript (AST). *P < 0.05 versus IL-1β alone.

L-Carnitine inhibits NO production and iNOS induction

Then, we discovered that the simultaneous addition of L-carnitine and IL-1 β reduced the levels of nitrite (a stable metabolite of NO) in a time- and dose-dependent manner in the culture medium (Fig. 2A and 2B; upper). L-Carnitine demonstrated more than 80% inhibition at 30 mg/ml. Western blot analysis revealed that L-carnitine reduced iNOS protein expression in a time- and dose-dependent manner (Fig. 2A; middle and 2B; lower), showing its maximal effect at 30 mg/ml (186 mM). L-Carnitine demonstrated no cellular cytotoxicity with the various concentrations used, as evaluated by the release of LDH into the culture medium (Fig. 3) and Trypan blue exclusion by hepatocytes (data not shown).

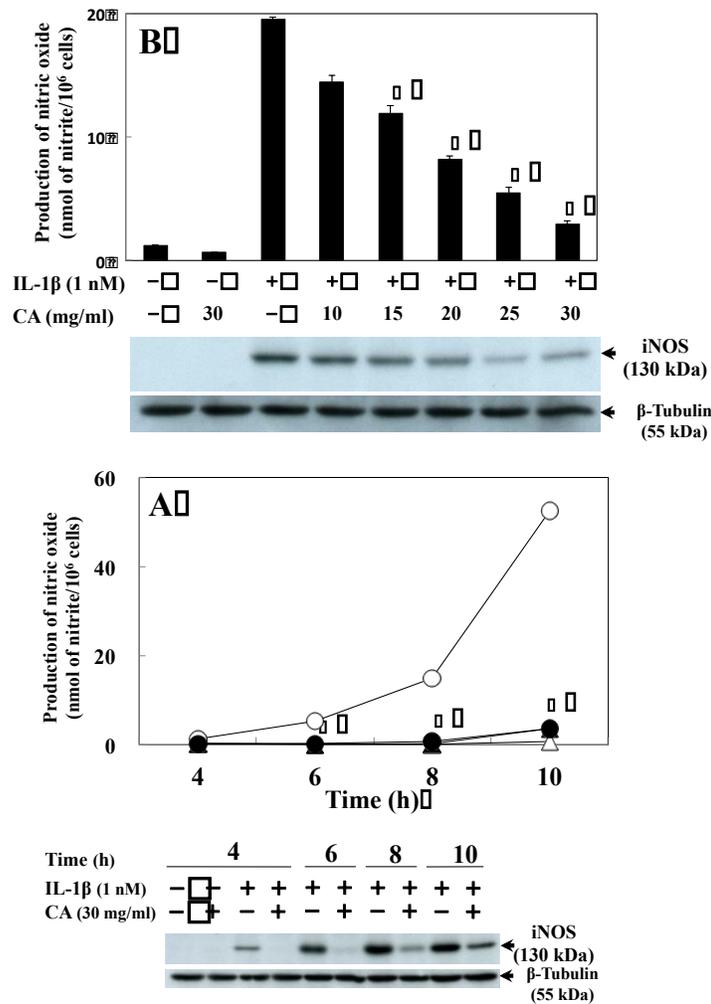


Fig. 2. Effects of L-carnitine on induction of NO production and iNOS in IL-1 β -stimulated hepatocytes. Hepatocytes were treated with interleukin (IL)-1 β (1 nM) in the presence or absence of L-carnitine (CA). w(A) Effect of L-carnitine treatment (30 mg/mL) for the indicated times on nitric oxide (NO) production (IL-1 β , open circles); IL-1 β + L-carnitine, filled circles; L-carnitine, filled triangles; controls (without IL-1 β and L-carnitine), open triangles) and inducible nitric oxide synthase (iNOS) protein (lower). (B) Effects of treatment with various doses of L-carnitine (10–30 mg/mL) for 8 h on NO production (upper) and iNOS protein (medium). Nitrite levels were measured in culture medium. Data are presented as the mean \pm SD for n = 3 dishes/point. *P < 0.05 versus IL-1 β alone. In the western blot panels, cell lysates (20 μ g of protein) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 7.5% gel, and immunoblotted an anti-iNOS or anti- β -tubulin antibody.

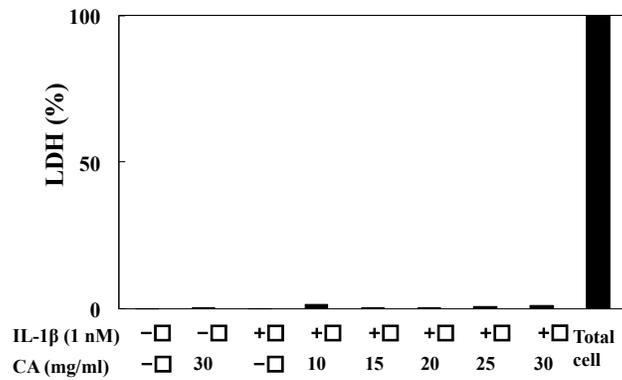


Fig. 3. Effects of L-carnitine on cellular cytotoxicity. Cells were treated with IL-1β (1 nM) in the presence or absence of L-carnitine (10–30 mg/mL) for 8 h. Lactate dehydrogenase (LDH) activity was measured in the culture medium (data are presented as the mean ± SD, n = 3 dishes/point).

RT-PCR analysis revealed how L-carnitine decreased iNOS mRNA expression in a time-dependent manner (Fig. 4A). In addition to the iNOS mRNA, IL-1β increased proinflammatory cytokine mRNA expression, such as that of TNF-α and CINC-1 [34, 35]. L-Carnitine decreased TNF-α mRNA levels (Fig. 4B), but had no effect on CINC-1 mRNA (Fig. 4C).

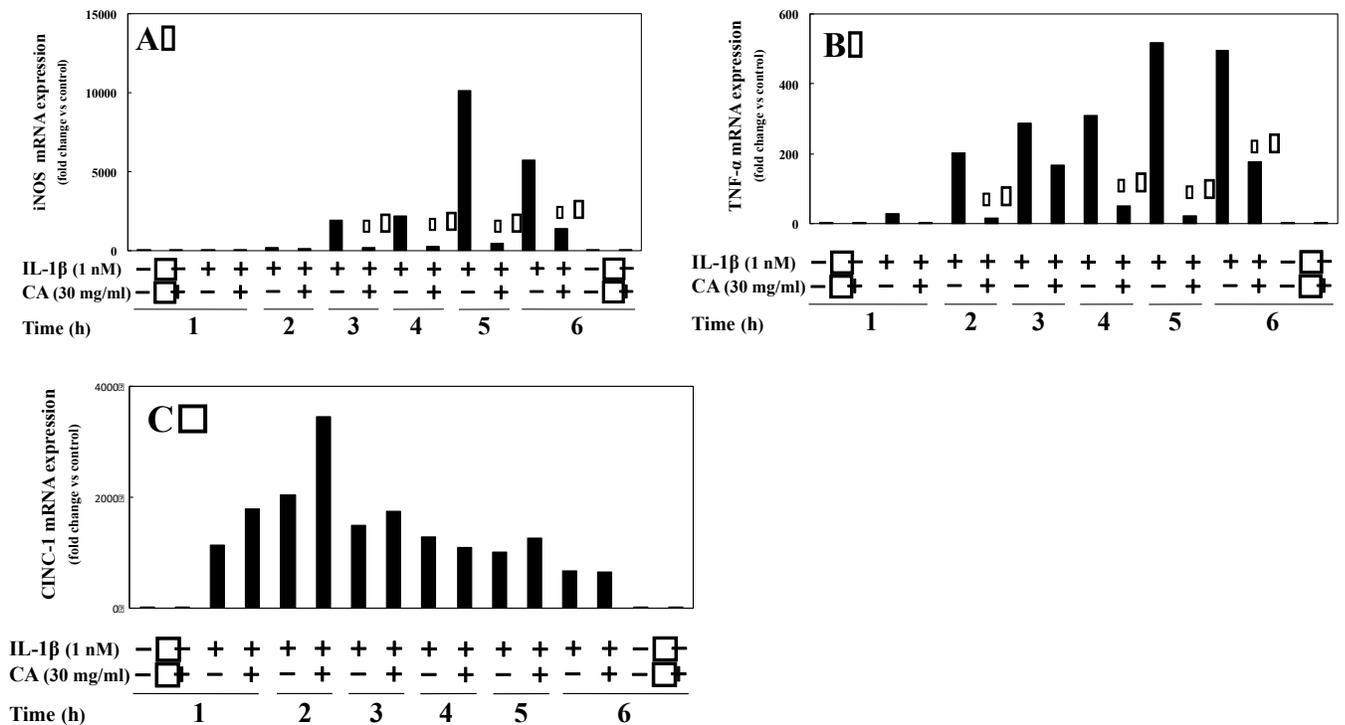


Fig. 4. Effects of L-carnitine on iNOS, TNF-α, and CINC-1 mRNA expression. Cells were treated with IL-1β (1 nM) in the presence or absence of L-carnitine (30 mg/mL) for the indicated times. Total RNA was analyzed using strand-specific RT-PCR to detect (A) iNOS, (B) TNF-α, and (C) CINC-1, using EF mRNA as an internal control. *P < 0.05 versus IL-1β alone.

L-Carnitine inhibits activation of NF-κB and upregulation of IL-1RI

There are two essential signaling pathways for iNOS induction, called the IκB kinase and phosphatidylinositol 3-kinase (PI3K)/Akt pathways. In the IκB kinase pathway, IL-1β stimulated IκB protein phosphorylation, followed by IκB protein degradation and NF-κB activation (i.e., translocation from the cytoplasm to the nucleus and DNA binding). L-Carnitine partially inhibited IκBα degradation at 15 min after IL-1β stimulation, but had no effect at 30 min and afterwards enhanced degradation (Fig. 5A). However, an electrophoretic mobility shift assay with nuclear extracts demonstrated how L-carnitine inhibited NF-κB activation at 1 and 2 h, indicating the blockade of NF-κB nuclear translocation (Fig. 5B).

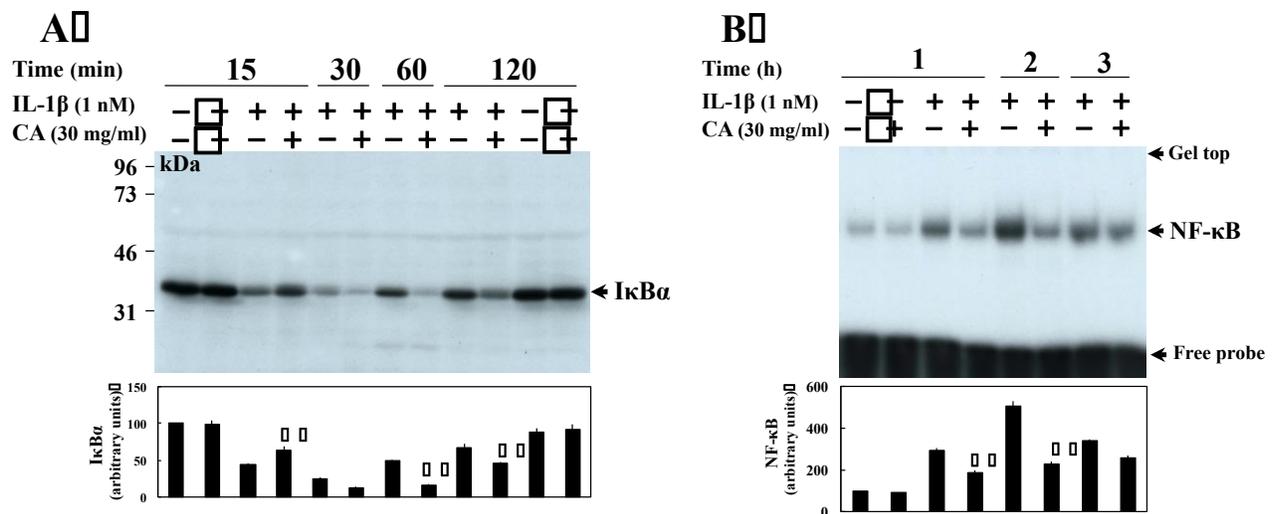
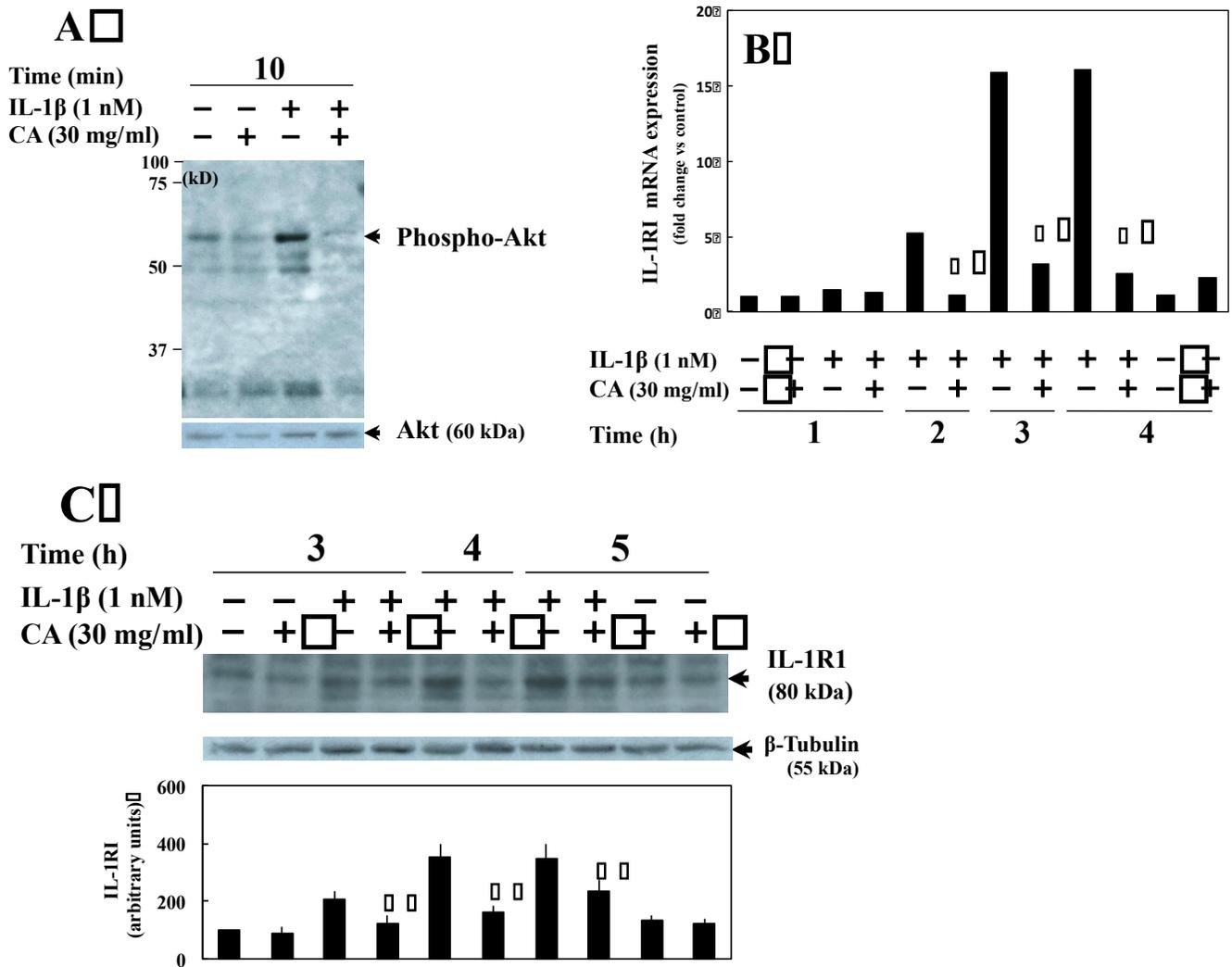


Fig. 5. Effects of L-carnitine on degradation of IκBα and activation of NF-κB. Cells were treated with IL-1β (1 nM) in the presence or absence of L-carnitine (30 mg/mL) for the indicated times. (A) Cell lysates (20 μg of protein) were subjected to SDS-PAGE in a 12.5% gel, followed by immunoblotting with an anti-IκBα antibody. (B) NF-κB activation. Nuclear extracts (4 μg of protein) were analyzed using an electrophoretic mobility shift assay. Representative results of three independent experiments are shown. The bands corresponding to IκBα or NF-κB were quantitated using densitometry (mean ± SD; n= 3 experiments). *P < 0.05 versus IL-1β alone.

In the PI3K/Akt pathway, IL-1β stimulated IL-1RI upregulation by Akt phosphorylation [36]. Immunoprecipitation-western blotting analysis showed that L-carnitine inhibited phosphorylation (activation) of Akt, a downstream kinase of PI3K (Fig. 6A). Real-time RT-PCR and western blotting analyses demonstrated how L-carnitine reduced IL-1RI mRNA and protein expression levels (Fig. 6B and 6C).



6. Effects of L-carnitine on upregulation of type I IL-1 receptor. Cells were treated with IL-1 β (1 nM) in the presence or absence of L-carnitine (30 mg/mL) for the indicated times. (A) Phosphorylation of Akt. Total cell lysates were immunoprecipitated with an anti-Akt antibody, followed by immunoblotting (SDS-PAGE in a gel with a gradient of 6–9%) with an anti-phospho-Akt or anti-Akt antibody. (B) Total RNA was analyzed using strand-specific RT-PCR to detect type I IL-1 receptor (IL-1RI) mRNA, using EF mRNA as an internal control. (C) Cell lysates (40 μ g of protein) were subjected to SDS-PAGE in a 7.5% gel and immunoblotted with an anti-IL-1RI or anti- β -tubulin antibody. *P < 0.05 versus IL-1 β alone

Delayed administration of L-carnitine inhibits iNOS induction

We examined whether delayed administration of L-carnitine affects iNOS induction. L-Carnitine was added to the medium 0–5 h after the addition of IL-1 β . Delayed administration of L-carnitine up to 5 h after IL-1 β addition still markedly inhibited NO production (Fig. 7).

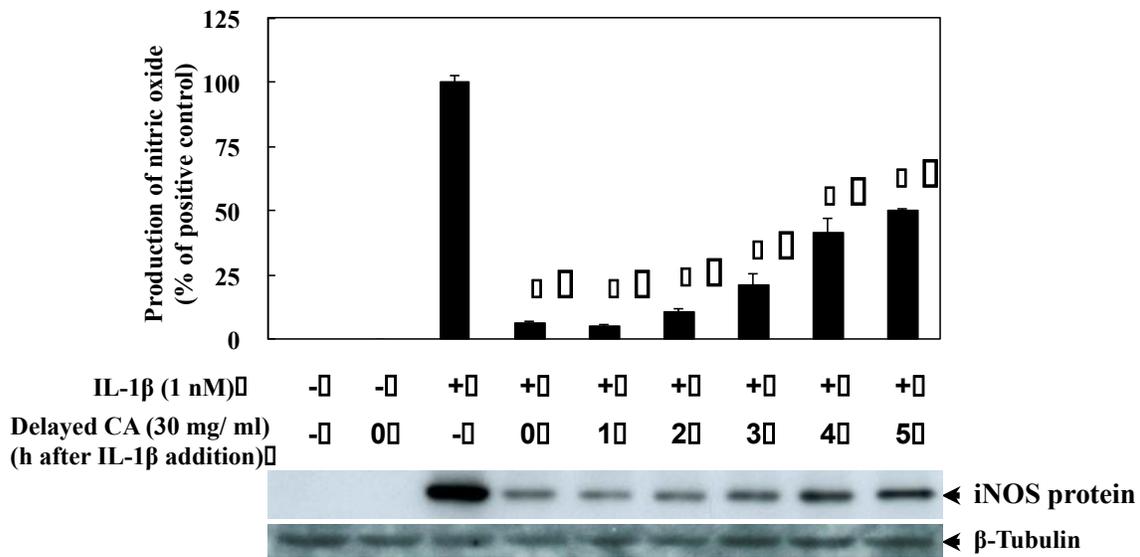


Fig. 7. Effects of delayed L-carnitine administration on iNOS induction in hepatocytes. Cells were treated with L-carnitine (30 mg/mL) at 0–4 h after addition of IL-1 β (1 nM). The effects of L-carnitine (30 mg/mL) on NO production (upper panel) and iNOS protein (lower panel) were analyzed at 8 h after the addition of IL-1 β . Nitrite levels were measured in the culture medium. Data are presented as the mean \pm SD, $n = 3$ dishes/point; * $P < 0.05$ versus IL-1 β alone. In the western blot panels, cell lysates (20 μ g of protein) were subjected to SDS-PAGE in a 7.5% gel, and immunoblotted with an anti-iNOS or anti- β -tubulin antibody.

DISCUSSION

In the current study, we discovered L-carnitine inhibited the induction of iNOS gene expression and reduced NO production in the primary culture of rat hepatocytes (Figs. 2 and 4A), which suggests L-carnitine has liver-protective effects. Transfection experiments with iNOS-luciferase constructs revealed that L-carnitine could decrease iNOS mRNA expression at both the transcriptional and post-transcriptional steps (Fig. 1B and 1C). For the latter, we reported that iNOS antisense transcript (AST) is transcribed in hepatocytes, and interacts and stabilizes iNOS mRNA [32]. In the present study, L-carnitine decreased iNOS AST expression (Fig. 1D).

Furthermore, our results using an *in vitro* liver injury model demonstrated that L-carnitine blocked two essential signaling pathways of iNOS induction: activation of I κ B α /NF- κ B by I κ B kinase and upregulation of IL-1RI by PI3K/Akt (Figs. 5 and 6). L-Carnitine markedly inhibited the activation of NF- κ B (nuclear translocation and DNA binding) and the upregulation of IL-1RI expression through inactivation of Akt phosphorylation, although I κ B α degradation was not blocked at 30 min or at subsequent time points.

Hepatic encephalopathy (impaired mental status and impaired neuromotor function) is a common complication of hepatic cirrhosis, although the pathogenesis of hepatic encephalopathy has not been clearly defined. The general consensus is that elevated ammonia levels and an

inflammatory response work. L-Carnitine or acetyl-L-carnitine administration was shown to reverse various neuropsychological activities and induce ureagenesis, leading to decreased blood and brain ammonia levels [15]. As a result, L-carnitine or acetyl-L-carnitine improved cognitive functions in severe hepatic encephalopathy [37, 38]. Administration of L-carnitine and/or branched-chain amino acids during invasive treatment for hepatocellular carcinoma reduced ammonia concentration and suppressed decreases in albumin [39]. L-Carnitine supplementation was effective in attenuating oxidative stress responses in patients with renal disease [17]. L-Carnitine was useful for reducing TNF- α and C-reactive protein, and for improving liver function, glucose plasma levels, and the lipid profile in patients with nonalcoholic steatohepatitis [18]. Our current results may support the published evidence described above.

These previous reports, in addition to our study, prompted us to examine whether L-carnitine supplementation in the diet is useful in patients with various diseases including liver diseases. Future research should address the relationship between supplement dosage, changes and maintenance of tissue L-carnitine concentration, and metabolic and functional changes and outcomes.

CONCLUSION

L-Carnitine prevented iNOS inhibition in IL-1 β -stimulated hepatocytes, partially through inhibiting NF- κ B activation. L-Carnitine may have therapeutic potential for various organ injuries including liver injury.

List of abbreviations: iNOS, inducible nitric oxide synthase; NO, nitric oxide; IL-1 β , interleukin-1 β ; NF- κ B, nuclear factor-kappa B; IL-1RI, type I interleukin-1 receptor; TNF- α , tumor necrosis factor-alpha; CINC-1, cytokine-induced neutrophil chemoattractant-1

Competing Interest: The authors declare that they have no conflicts of interest.

Authors' Contributions: YN, HI, and TS participated in data collection, statistical analysis, and drafting of the manuscript. TO, RN, MK, YH, and TD assisted in the design of the study, participated in supervision, and provided oversight when the manuscript was being drafted. TO and TD provided advice regarding development of the protocol for the study.

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