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ORIGINAL ARTICLE

# Induction of apoptosis by L-carnitine through regulation of two main pathways in Hepa1c1c 7 cells

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**Abstract** This study shows the effects of L-carnitine treatment on cell proliferation with hepa1c1c7 mouse cancer cells and NCTC 1469 normal cells. In an MTT assay, L-carnitine increased the number of dead hepa1c1c7 cells, while there was no difference in the number of NCTC 1469 cells. mRNA and protein levels of TNF-α, Fas, and caspase-8, which are closely related to cell apoptosis by a death ligand/receptor-dependent apoptosis pathway, were increased by L-carnitine treatment. In addition, L-carnitine treatment regulated mitochondria-dependent apoptosis pathways by inducing the up-regulation of caspase-9 and caspase-3 and the down-regulation of Bcl-2 in hepa1c1c 7 cells. Taken together, the findings of this study have demonstrated that L-carnitine could induce apoptosis in hepa1c1c7 cells by regulating Fas ligands and inhibiting the expression of Bcl-2. These results suggest that L-carnitine treatment could be related to both a mitochondrion-dependent and a death ligand/receptor-dependent apoptosis pathway in hepa1c1c7 cells. Our results could give information for understanding the L-carnitine-induced apoptosis mechanism in some cancer cells.

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## Introduction

L-carnitine (β-hydroxy-γ-trimethyl-amino-butyrate), an amino acid derivative, is mainly distributed within mammalian skeletal and cardiac muscles. L-carnitine is supplied to the human body through dietary sources. Meat, poultry, fish, and dairy products are the richest sources of L-carnitine (Kenichiro et al. 2004; Tada et al. 1984; Leibovitz and Mueller 1993). Wheat, tempeh (fermented soybeans), and avocados contain some L-carnitine, whereas vegetables, fruits, and grain contribute relatively little L-carnitine to the diet.

L-carnitine plays an important role in energy production by chaperoning activated fatty acids (acyl-CoA) into the mitochondrial matrix for metabolism and chaperoning intermediate compounds out of the mitochondrial matrix to prevent their accumulation (Jogl et al. 2004; Calvani and Peluso 2003). Recent research has indicated that L-carnitine, besides its well-known functions in metabolic process, has other functions in programmed cell death (apoptosis). A number of studies have shown that L-carnitine has an effect on the induction of apoptosis in cancer cells. Interestingly, L-carnitine probably has different effects on apoptosis in normal cells compared to cancer cells. One study showed that L-carnitine could prevent apoptosis of skeletal muscle cells and plays a role in the treatment of congestive heart failure-associated myopathy (Vescovo et al. 2002). Other observations have suggested that apoptosis occurred and increased in cancer cells such as HT-29 cells, U937 leukemic cells for reasons that were related to the addition of L-carnitine or L-carnitine derivatives in vitro (Wenzel

et al. 2005; Ferrara et al. 2005) and inhibition of hepatocarcinogenesis by L-carnitine in vivo animal model (Chang et al. 2005). These results indicate that L-carnitine plays different roles depending on cell type, such as normal and cancer cells. To date, the behaviors of molecules related to apoptotic pathways are not completely understood. The induction of apoptosis is an important topic for the treatment of cancer. There are two main pathways in cell apoptosis; one is a mitochondrial-dependent pathway involving an apoptotic protease factor, such as caspase-9, and the other is a death receptor pathway that deals with caspase-8 through a death receptor signal such as tumor necrosis factor-alpha (TNF-alpha) or Fas. When caspase-3 is up-regulated by two pathways, it leads to cell apoptosis. Hepa 1c1c 7 cell, mouse hepatoma cell line, has been widely used for investigation of apoptosis induced by bioactive molecules such as antioxidant polyphenols from grape (Matito et al. 2003). NCTC 1469 cell, normal mouse liver cell line, has been also similarly used in vitro experiments such as chemopreventive effects of plant poly saccharides (Kim et al. 1999). In the present study, we estimate the different effect of L-carnitine on normal cell of NCTC 1469 and cancer cell of Hepa 1c1c 7, and elucidate the detailed mechanisms of inducing Hepa1c1c7 cell apoptosis in terms of messenger and protein levels.

## Materials and methods

### Cell cultures and reagents

Hepa1c1c 7 cells and NCTC 1469 cells were obtained from Korean Cell Line Bank. Hepa1c1c 7 cells and NCTC 1469 cells were cultured and passaged in minimum essential medium (MEM) and Dulbecco's modified Eagle's medium (DMEM), respectively, and were then supplemented with 10% fetal bovine serum (FBS) and 2 mM glutamine. One hundred kU/L penicillin was added to the media. All cultures were maintained in a humidified atmosphere of 95% air and 5% $CO_2$ at 37°C. Cells were detached for transfer with 0.1% trypsin. MEM, DMEM, FBS, and trypsin were obtained from Gibco. L-carnitine and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] were obtained from Sigma. All other chemicals used were of the purest grade available.

### Treatment of cultures

The Hepa1c1c 7 cells and NCTC 1469 cells were incubated for 48 h. Treatments of cultures with different concentrations of L-carnitine (1, 5 mM) were maintained for 24 h. The control group was created by adding only the vehicle each time.

### MTT cell viability assay

MTT was measured as described previously (Studzinski 1995). In brief, Hepa1c1c7 cells and NCTC 1469 cells were grown in 96-well plates, and were then treated with different concentrations of L-carnitine. A 5×stock (-2 μg/ml) of MTT in phosphate-buffered saline (PBS) was also prepared. The final concentration was typically about 0.4 μg/ml, but was individually optimized for each cell line. Twenty microliter of MTT stock was added to the 200 μl of medium in each well. Cultures were incubated at 37°C for a typical period of 4 h in length, but were individually determined for each cell line. Medium was aspirated off from each well, and 200 μl of DMSO was added to each well and incubated for 10 min while vibrating on a plate shaker to solubilize the formazan crystals. Absorbance was read at 540 nm. This wavelength is critical, and could not be changed.

### DNA fragmentation assay

Hepa1c1c 7 cells ($1 \times 10^6$ cells/ml) were exposed or were not exposed to L-carnitine (1, 5 mM) for 24 or 48 h, respectively. For DNA extractions, Hepa1c1c 7 cells were harvested and lysed with a lysis buffer (10 mM Tris–HCl, 10 mM EDTA, 0.5% Triton X-100). After incubation at 4°C for 10 min, lysates were centrifuged at 10,000*g* for 15 min. The supernatants were transferred to a new Eppendorf tube and incubated with Rnase A at 37°C for an hour, followed by proteinase K for an additional hour at 37°C. DNA was extracted at a 1/10 volume of 3 M NaCl and 1 volume of isopropanol at 4°C for 12 h. Finally, DNA samples were electrophoresed on 1.8% agarose gel and visualized under ultraviolet (UV) light and photographed.

### Reverse transcription-PCR assay

Semiquantitative reverse transcription-PCR (RT-PCR) with total RNA of Hepa1c1c 7 cells was performed according to a previously described method (Han et al. 2003). In brief, Hepa1c1c7 cells were treated with different concentrations of L-carnitine for 24 h, and total RNAs were then extracted with TRIzol reagent. The amount of RNA was estimated by spectrophotometry at 260 nm. The cDNAs were obtained from reverse transcription of 5 μl of RNA sample in a 10 μl reaction volume containing 10 mM dNTPmix and 0.5 μg/μl of ologo(dt). PCR amplifications were performed in a 50 μl reaction volume containing 1.5 μl of cDNA, 10× PCR buffer, 2.5 mM $MgCl_2$, 2 mM dNTPmix, Taq polymerase, and primers. The primers were designed according to the published sequences (Table 1.) Five microliter of each

**Table 1** PCR primers used in this study

| Probe | Sense primer antisense primer (5′ to 3′)[a] | Temp (°C) | Size (bp) | Reference |
|---|---|---|---|---|
| Caspase-3 | CCT CAG TGG ATT CAA AAT CC GAG TAA GCA TAC AGG AAG TC | 54 | 566 | NM 009810 |
| Caspase-8 | CTC AAG TTC CTG TGC TTG GAC GCA TGG TCC TCT TCT CCA TTT C | 57 | 391 | NM 009812 |
| Caspase-9 | CAT CTC CTG CTT AGA GGA CAC CCA GGG TGT ATG CCA TAT CTG | 57 | 361 | NM 015733 |
| Fas | GAG AAT TGC TGA AGA CAT GAC GTA GTT TTC ACT CCA GAC ATT | 55 | 313 | NM 007987 |
| TNF-a | CAC CAC GCT CTT CTG TCT ACT AGA TAG CAA ATC GGC TGA CGG | 52 | 359 | NM 013693 |
| P53 | CTA TCA GGA CAT AGC GTT GG TAT ACT CAG AGC CGG CCT | 55 | 483 | AB 020317 |
| Bcl-2 | TCC GTG CCT GAC TTT AGC AAG GGA ATC CCA ACC AGA GAT CTC | 58 | 412 | NM 009741 |
| GAPDH | CTC TAC CCA CGG CAA GTT CAA GGA TGA CCT TGC CCA CAG C | 60 | 515 | (Han et al. 2003) |

*GAPDH* Glyceraldehyde-3-phosphate dehydrogenase

[a] Primers for mouse

PCR product was electrophoresed in a 1.0% agarose gel containing 0.1 μl/ml ethidium bromide. The band intensity was determined by image analysis using the Bio Doc-It System (UVP, Inc. USA).

### Western blot analysis

The cytosolic proteins were isolated from Hepa1c1c 7 cells after treatment with 1 mM or 5 mM L-carnitine for 24 h, and the total proteins were extracted by 1× SDS sample buffer to the cell pellets on ice, followed by heating at 100°C for 3 min and cooling on ice for 10 min. The cytosol proteins were separated by 10% SDS-PAGE, and were then transferred to immobilon polyvinylidene difluoride membrane (PVDF) with transfer buffer by Trans-Blot. After blocking with skim milk, strips of the membranes were exposed to the primary antibodies, following caspase-8 antibody detecting endogenous levels of active caspase-8 protein, TNF-α antibody (Cell Signaling Technology Inc., Camarillo, CA, USA), Fas (Santa Cruz Biotechnology, Inc.), and β-actin antibody (Biolegend San Diego, CA, USA). After incubation overnight, the membranes were washed three times, and were then incubated with alkaline phosphatase-conjugated goat anti-rabbit IgG (Chemicon International Inc. Temecula, CA, USA). The reaction was developed with an alkaline phosphatase conjugate substrate kit (5-bromo-4-chloro-3-indolil phosphate p-toluidine salt/nitroblue tetrazolium; Bio-Rad Laboratories, Hercules, CA, USA). Densitometric measurements of the bands in Western blot analysis were performed using Biocapt software (version 99.01, vilber lourmat, Cedex, France).

### Statistical analysis

Data were evaluated using SPSS 10.0, and were presented as means ± SD. Testing used one-way analysis of variance (ANOVA). Differences are considered significant with $P$ values $<0.01$.

## Results

### Effects of L-carnitine treatment on viability of normal and mouse cancer cells

The cell viability after treatment with L-carnitine was determined by MTT assay. The NCTC 1469 cell line and the Hepa1c1c 7 cell line were used in this study as a mouse normal cell line and a mouse cancer cell line, respectively. L-carnitine was administered to both NCTC 1469 cells and Hepa1c1c 7 cells at two different concentrations, 1 and 5 mM. As shown in Fig. 1a, there were no morphological changes in NCTC 1469 normal cells even after treatment with 1 and 5 mM L-carnitine, and these morphological results were supported by the results of the MTT assay (95.62 and 98.82% in 1 and 5 mM, respectively). In contrast, treatment of Hepa1c1c 7 cells with L-carnitine significantly changed the morphological cell patterns as shown in Fig. 1a, and the MTT assay also showed significant reductions of viability, 58.79 and 74.65% in 1 and 5 mM, respectively, compared to the control ($P < 0.01$) (Fig. 1b). These results raise the hypothesis that L-carnitine acts differently depending on cell type.

### DNA fragmentation found at 48 h following L-carnitine treatment

The decreased cell number was confirmed by DNA fragmentation assay. Hepa1c1c 7 cells were treated with 1 and 5 mM L-carnitine for 24 and 48 h. As shown in Fig. 2, at an early time of 24 h after treatment with L-carnitine, DNA fragment bands were not found in the control or L-carnitine

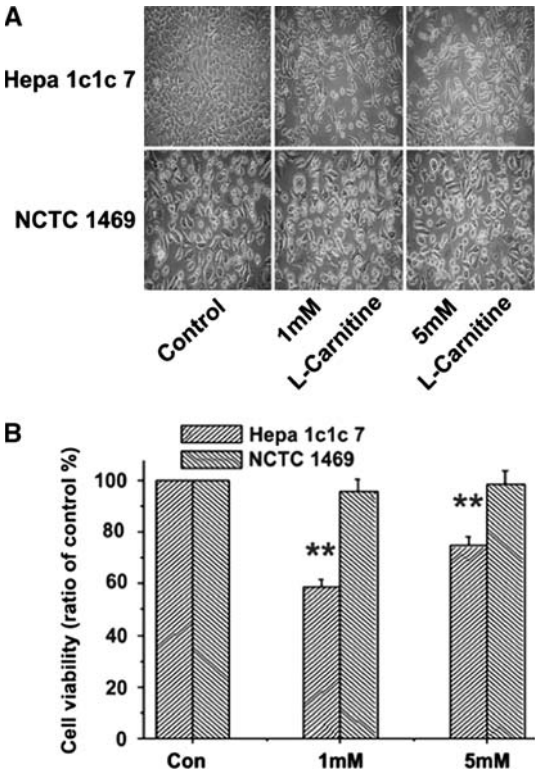


**Fig. 1** Effects of L-carnitine treatment on the viability of normal and mouse cancer cells. **a** Morphological changes of Hepa1c1c 7 cells and NCTC 1469 cells after L-carnitine treatment for 24 h. Magnification; ×20. **b** Viabilities of NCTC 1469 normal cells and Hepa1c1c 7 mouse cancer cells were determined by an MTT assay after 24 h treatment with L-carnitine. Data represent the mean ± SD of three experiments. Statistically significant differences from the control are indicated as $^{**}P < 0.01$

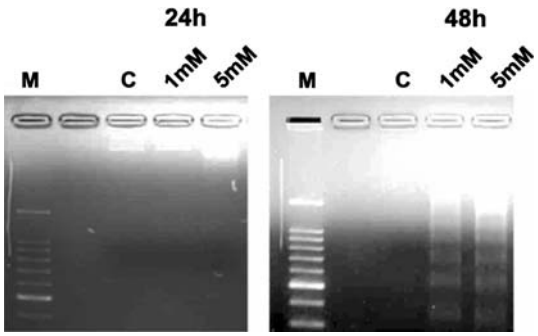


**Fig. 2** Induction of DNA fragmentation of Hepa1c1c 7 cells by L-carnitine treatment. Cells were treated with L-carnitine at concentrations of 1 and 5 mM for the indicated periods of time, and DNA fragmentations were assayed by electrophoresis in 1.8% agarose gel. *M* DNA ladder marker; *C* control

treatment groups. In contrast, DNA fragment bands were clearly observed the L-carnitine treatment groups following treatment with 1 mM and 5 mM L-carnitine for 48 h.

## Regulation of Bcl-2 and capspase-9 expression on the mitochondrion-dependent pathway by L-carnitine in Hepa1c1c 7 cell

The mRNA expression of caspase-9 and Bcl-2, central molecules related to the mitochondrion-dependent apoptosis pathway in Hepa1c1c7 cells, were significantly changed compared to the levels in control cells ($P < 0.01$) (Fig. 3). The levels of aspartic acid-specific protease caspase-9 is up-regulated during apoptosis. Induction of the JNK/SAPK stress signaling pathways causes the release of cytochrome c from mitochondria, as well as apaf-1, which regulate caspase-9. The Bcl-2 protein is an anti-apoptotic protein that resides in the outer mitochondrial membrane and the membrane of the endoplasmic reticulum. Overexpression of Bcl-2 is known to block the release of cytochrome c, possibly through the inhibition of Bax and Bak. Because apoptosis was positively induced, the expression of caspase-9 increased and the expression of Bcl-2 decreased. These patterns are clearly shown in Fig. 3, which indicates that regulation by L-carnitine positively induced apoptosis of Hepa1c1c 7 mouse cancer cells.

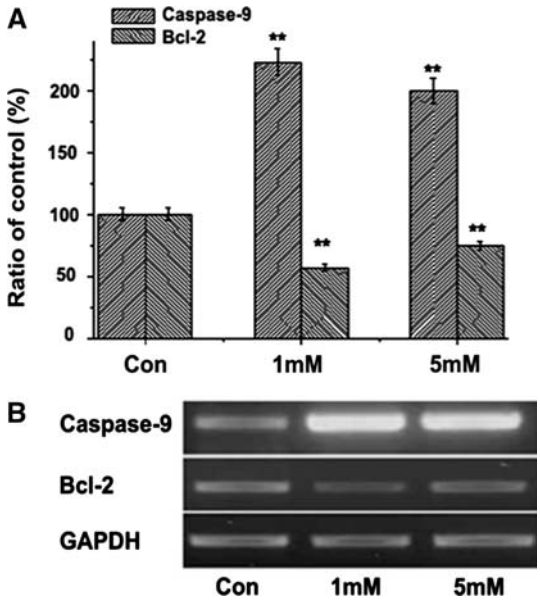


**Fig. 3** Effect of L-carnitine treatment on the expression of molecules related to the mitochondria-dependent pathway in Hepa1c1c 7 cells. **a** mRNA expression for caspase-9 and Bcl-2 in Hepa1c1c 7 cells was semiquantified by RT-PCR. **b** Representative agarose gel electrophoretic patterns of PCR products are shown. The ratios of the densitometric signals of molecules to that of the internal control (glyceraldehydes-3-phosphate dehydrogenase, GAPDH) were analyzed. Data are shown as ratios (%) relative to the control group, and are expressed as the mean ± SD of three independent experiments ($^{**}P < 0.01$ compared with the control group)

### The death ligand/receptor pathway is regulated by L-carnitine in Hepa1c1c 7 cells

When L-carnitine was applied to Hepa1c1c 7 cells, the mRNA levels of TNF-α and Fas were significantly increased (Fig. 4) compared with those of control cells ($P < 0.01$). These parameters are closely linked to the death ligand/receptor pathway. The expression of caspase-8, which is induced by Fas and various apoptotic stimuli, was increased by the administration of L-carnitine to Hepa1c1c 7 cells. The expression of caspase-3, which has been shown to play a pivotal role in the terminal execution phase of apoptosis induced by diverse stimuli, was dramatically increased. P53 can initiate apoptosis if the DNA damage by an exogenous stimulus proves to be irreparable In our study, p53 was slightly decreased but not to a significant degree, which indicates that L-carnitine did not directly damage the cancer cells (Fig. 4). Changes in the expression of TNF-a, Fas, and caspase-8 by L-carnitine treatment were also confirmed by Western blot analysis (Fig. 5).

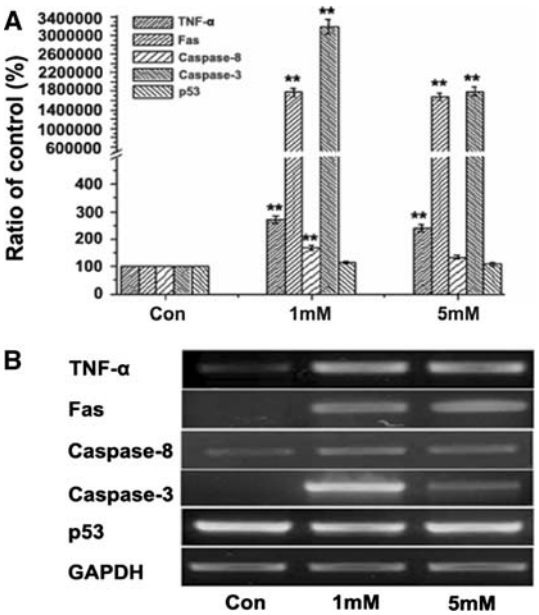


**Fig. 4** Effect of L-carnitine treatment on the expression of molecules related to the death receptor-dependent pathway in Hepa1c1c 7 cells. **a** mRNA expression of TNF-a, Fas, and caspase-8, and mRNA expression for caspase-3 and p53 in the Hepa1c1c 7 cell line was semiquantified by RT-PCR. **b** Representative agarose gel electrophoretic patterns of PCR products are shown. Data are shown as ratios (%) relative to the control group, and are expressed as the mean ± SD of three independent experiments (**$P < 0.01$ compared with the control group)

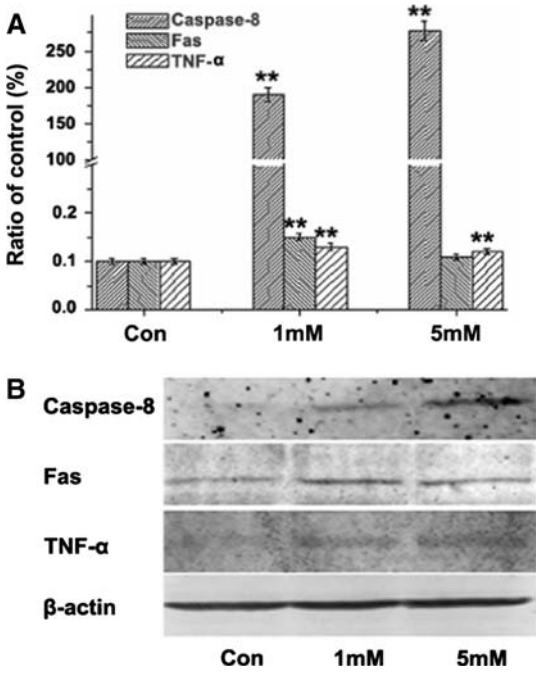


**Fig. 5** Western blot analyses for TNF-α, Fas, and caspase-8 in Hepa1c1c 7 cells following L-carnitine treatment. **a** Changes of protein levels for TNF-α, Fas, and caspase-8 after L-carnitine treatment were determined by Western blotting. **b** Representative patterns of PVDF membranes for the molecules are shown. The ratios of the densitometric signals of molecules to that of the internal control, β-actin, were analyzed. Data are shown as ratios (%) relative to the control group, and are expressed as the mean ± SD of three independent experiments (**$P < 0.01$ compared with the control group)

## Discussion

L-carnitine is a naturally occurring quaternary amine zwitterion that facilitates the transport of fatty acids into mitochondria for beta-oxidation. Recently studies on the effects of L-carnitine on cell apoptosis have been evaluated in cardiac myocyte, Jurkat cells, skeletal-muscle myocytes (Andrien-Abadie et al. 1999; Mutomba et al. 2000; Vescovo et al. 2002). Concentration of L-carnitine treatment varies from 0.1 mM to a few mM, depending on the tissue (Mutomba et al. 2000). The doses of L-carnitine applied in this study were based on the physiological concentration in plasma (Nemoto et al. 2004). Previously, Kong et al. (2002) reported 1 and 5 mM L-carnitine have no significant effect on myocytes cell viability by MTT assay whereas a high concentration (30 mM) carnitine induced a 4.1 ± 0.8-fold increase in apoptotic nuclei compared with control. Xie et al. (2007) showed 0.1–10 mM of L-carnitine protected MC3T3-E1 cells against serum deprivation induced apoptosis. To date, although there were some observations that demonstrated that L-carnitine is a cofactor with palmitate or that L-carnitine derivatives significantly increased the induction of some cancer cells apoptosis in vitro, few study reported that L-carnitine alone was directly able to significantly increase cancer cell apoptosis. Also, there have been no report about the pro-apoptotic effects of L-carnitine on Hepa1c1c 7 mouse liver cancer cells, and new data are required in order

to clarify the molecular mechanism of cancer cell apoptosis. In this study, our results suggest an interesting concept i.e., that L-carnitine induced death in Hepa1c1c 7 mouse liver cancer cells, but did not induce death in normal NCTC 1469 cells (Fig. 1). This could be explained by the different metabolic changes that occur between cancer cells and normal cells. Furthermore, some cancer cells have been shown to have lower levels of free L-carnitine than normal cells (Peluso et al. 2000). Malaguarnea et al. (2006) reported that gastro-intestinal cancer patients were lower 6.8 μmol/L in free carnitine than patients without cancer. Concentration ratios of free carnitine and carnitine esters also have been shown to be altered in cancer patients compared with healthy controls, suggesting a cancer-associated metabolic dysfunction related to carnitine availability (Yazdanpanah et al. 1997; Rössle et al. 1989). Previous studies showed that exogenous carnitine is readily transported into the cell across the sarcolemma membrane to increase intracellular carnitine concentration (Broderick et al. 1992; Vary et al. 1982). When supplementation of L-carnitine enters these cells, the homeostasis metabolisms in cancer cells were more greatly disturbed by extrinsic L-carnitine than were the homeostasis metabolisms in normal cells, finally leading to more deaths in cancer cells. Wenzel et al. (2005) showed 2 mM L-carnitine, as exogenous cofactor agent with palmitate, significantly increase the apoptosis in human colon cancer cells. On the other hand, L-carnitine is an essential substrate for and an important regulator of carnitine palmitoyltransferase 1A (CPT-1), and its intra-mitochondrial concentration governs the enzyme kinetics of CPT-1. A previous MTT assay reported that CPT-1 largely mediated cell apoptosis as L-carnitine was combined with palmitate (Kong et al. 2002).

The induction of cell apoptosis will activate endonuclease, which is involved in the cleavage of DNA into oligo-nucleosome size fragments, resulting in a prominent ladder in agarose gel electrophoresis. In the present study, the DNA fragmentations had not appeared at 24 h after L-carnitine treatment, but with an extended L-carnitine treatment time, DNA ladders were observed (Fig. 2). This finding suggests that the apoptosis-inducing effect of L-carnitine in Hepa1c1c 7 cells appeared in a time-dependent manner. However, L-carnitine treatment did not show any differences compared to other DNA fragmentation assays with NCTC 1469 cells (data not shown), which supports the result of the MTT assay mentioned above in Fig. 1.

To date, the L-carnitine-induced molecular mechanisms of apoptosis are incompletely understood. There are two independent and/or correlated pathways that lead to the induction of apoptosis. One of these is the intrinsic mitochondria-dependent pathway that is induced by irreparable DNA damage or external stresses such as UV light, radiation, and viral infection. The mitochondrion is the main site of action for members of the apoptosis-regulating molecules, which include the Bcl-2 family (pro-apoptotic factors of Bid, Bax and anti-apoptotic factor of Bcl-2). Previous studies have indicated that decreased expression of anti-apoptotic Bcl-2 resulted in the development of apoptosis (Gross et al. 1999), and overexpression of anti-apoptotic Bcl-2 protected cells from apoptosis by inhibiting cytochrome c, which is released by the mitochondria in response to pro-apoptotic stimuli (Rokhlin et al. 2001). Bcl-2 levels are decisive factors that play an important role in determining whether or not cells will undergo apoptosis (Yeh and Yen 2005). In our study, decreased Bcl-2 expression was observed in the L-carnitine-treated groups. Furthermore, the 1 mM L-carnitine treated group was significantly weaker than the control group (Fig. 3). The mitochondria contain a number of specific substances that can initiate apoptosis when released into the cytosol. One representative of these is the small electron transporter, cytochrome c. The release of cytochrome c from mitochondria promotes the formation of a caspase-activating complex of cytochrome c, APAF-1, and procaspase-9, which acts as a trigger to up-regulate caspase-9. The presence of up-regulated caspase-9 leads to increase the expression of caspase-3, followed by the execution of apoptotic events in a cell. The increased caspase-9 expression in Hepa1c1c 7 cells (Fig. 3) might be induced by higher degree of release of cytochrome c, which is associated with decreased expression of Bcl-2 by L-carnitine treatment. Our study demonstrated that L-carnitine induced apoptosis through the mitochondria pathway of decreasing the level of Bcl-2 and of increasing the level of caspase-9 in Hepa1c1c 7. This pathway was dramatically different from the levels of those molecules through L-carnitine inhibiting apoptosis on some cell type (Kashiwagi et al. 2001; Vescovo et al. 2002; Xie et al. 2007).

The second main apoptosis pathway is an extrinsic apoptotic pathway that is induced by the binding of death ligand-receptor such as the FasL–Fas complex and TNF-α(-TNFR-I or TNFR-II complex (Bishopric et al. 2001; Jacobson 2002). These specific binding complexes induce receptor aggregation, leading to increase the mRNA and protein levels of caspase-8 that, in turn, up-regulate expression of caspase-3. Sequential regulation of caspases plays a central role in the execution phase of cell apoptosis.

L-carnitine treatment of Hepa1c1c 7 cells showed increased mRNA expression of TNF-α, Fas, and caspase-8 (Fig. 4). The increase in these protein levels was confirmed by Western blot analysis (Fig. 5), which indicated that L-carnitine regulated the extrinsic apoptotic pathway through the increase mRNA and protein expression levels of Fas ligands.

Caspase-3 is a cysteine protease that exists as an inactive zymogen in cells, and it becomes activated by sequential proteolytic events (Nicholson et al. 1995). The function of caspase-3 is to cleave key regulatory and structural proteins, thus enabling the collapse of the cell (Srinivasula et al. 1996). Figure 4 showed that L-carnitine treatment increased the expression of caspase-3, and the increase was more significant at 1 mM of L-carnitine than at 5 mM. These findings might be explained by the fact that the cell stage was proceeded to apoptosis to produce a messenger of the final apoptotic cascade caspase of caspase-3. The function of p53, which can activate DNA repair proteins when DNA has sustained damage, is often altered in cancer. It has been suggested that p53 could suppress tumor proliferation, and this specific action is exerted mainly through the triggering of apoptosis (Levine 1997). The expression of p53 increased when the cell encountered extrinsic stimuli such as toxic chemicals, radiation, and UV irradiation. In this study, p53 showed no significant changes after 24 h of L-carnitine treatment compared to that in control cells (Fig. 4), thus indicating that L-carnitine is different from toxic stimuli. The results of the DNA fragmentation assay showed that there is no DNA ladder in Hepa1c1c 7 cells at 24 h after L-carnitine treatment.

In conclusion, this study has demonstrated that L-carnitine could induce apoptosis in Hepa1c1c 7 cells through the regulating of Fas ligands and inhibiting Bcl-2 expression. These results suggest that L-carnitine treatment could be related to the mitochondrion-dependent and death ligand/receptor-dependent apoptosis pathways in Hepa1c1c 7 cells. Our results could help us to better understand the apoptosis mechanism in cancer cells induced by L-carnitine, and may raise the possibility of the use of L-carnitine as a bioactive material in functional foods.

In addition, further investigations will be conducted in an in vivo animal model in order to investigate the function of L-carnitine.

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## References

Andrien-Abadie N, Jaffrezou JP, Hatem S, Laurent G, Levade T, Mercadier JJ (1999) L-carnitine prevents doxorubicin-induced apoptosis of cardiac myocytes: role of inhibition of ceramide generation. FASEB J 13:1501–1510

Bishopric NH, Andreka P, Slepak T, Webster KA (2001) Molecular mechanisms of apoptosis in the cardiac myocyte. Curr Opin Pharmacol 1:141–150

Broderick TL, Quinney HA, Lopaschuk GD (1992) Carnitine stimulation of glucose oxidation in the fatty acid perfused isolated working rat heart. J Biol Chem 267:3758–3763

Calvani M, Peluso G (2003) The role of carnitine system in maintaining muscle homeostasis. Basic Appl Myol 13:105–120

Chang B, Nishikawa M, Nishiguchi S, Inoue M (2005) L-carnitine inhibits hepatocarcinogenesis via protection of mitochondria. Int J Cancer 5:719–729

Ferrara F, Bertelli A, Falchi M (2005) Evaluation of carnitine, acetylcarnitine and isovalerylcarnitine on immune function and apoptosis. Drugs Exp Clin Res 31:109–114

Gross A, Mcdonnell JM, Korsmeyer SJ (1999) Bcl-2 family members and the mitochondria in apoptosis. Genes Dev 13:1899–1911

Han GD, Koike H, Nakatsue T, Suzuki K, Yoneyama H, Narumi S, Kobayashi N, Mundel P, Shimizu F, Kawachi H (2003) IFN-inducible protein-10 has a differential role in podocyte during Thy 1.1 glomerulonephritis. J Am Soc Nephrol 14:3111–3126

Jacobson M (2002) Apoptosis. In: McCarthy NJ, Bennett MR (eds) Death signaling. Oxford University Press, New York, pp 200–209

Jogl G, Hsiao YS, Tong L (2004) Structure and function of carnitine acyltransferases. Ann N Y Acad Sci 1033:17–29

Kashiwagi A, Kanno T, Arita K, Ishisaka R, Utsumi T, Utsumi K (2001) Suppression of T(3)- and fatty acid-induced membrane permeability transition by L-carnitine. Comp Biochem Physiol B Biochem Mol Biol 13:411–418

Kenichiro S, Yoshinori S, Junichi W, Michihiro F, Mitsuo S, Keigo K, Masayuki M (2004) Species and muscle differences in L-carnitine levels in skeletal muscles based on a new simple assay. Meat Sci 68:357–362

Kim HS, Kacew S, Lee BM (1999) In vitro chemopreventive effects of plant polysaccharides (*Aloe barbadensis* miller, *Lentinus edodes, Ganoderma lucidum* and *Coriolus versicolor*). Carcinogenesis 20:1637–1640

Kong JY, Rabkin SW (2002) Palmitate-induced cardiac apoptosis is mediated through CPT-1 but not influenced by glucose and insulin. Am J Physiol Heart Circ Physiol 282:717–725

Leibovitz B, Mueller J (1993) Carnitine. J Opt Nutr 2:90–109

Levine AJ (1997) p53, the cellular gatekeeper for growth and division. Cell 88:323–331

Malaguarnea M, Risino C, Gargante MP, Oreste G, Barone G, Tomasello AV, Costanzo M, Cannizzaro MA (2006) Decrease of serum carnitine levels in patients with or without gastrointestinal cancer cachexia. World J Gastroenterol 12:4541–4545

Matito C, Mastorakou F, Centelles JJ,Torres JL, Cascante M (2003) Antiproliferative effect of antioxidant polyphenols from grape in murine Hepa-1c1c7. Eur J Nutr 42:43–49

Mutomba MC, Yuan H, Konyavko M, Adachi S, Yokoyama CB, Esser V, McGarry JD, Babior BM, Gottlieb RA (2000) Regulation of the activity of caspases by L-carnitine and palmitoylcarnitine. FEBS Lett 478:19–25

Nemoto S, Yasuhara K, Nakamura K, Miyoshi Y, Sakai A (2004) Plasma carnitine concentrations in patients undergoing open heart surgery. Ann Thorac Cardiovasc Surg 1:19–22

Nicholson DW, Ali A, Thornberry NA, Vaillancourt JP, Ding CK, Gallant M, Gareau Y, Griffin PR, Labelle ML, Munday NA, Raju SM, Smulson ME, Yamin TT, Yu VL, Miller DK (1995) Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. Nature 376: 37–43

Peluso G, Nicolai R, Reda E, Benatti P, Barbarisi A, Calvani M (2000) Cancer and anticancer therapy-induced modifications on metabolism mediated by carnitine system. J Cell Physiol 182:339–350

Rokhlin OW, Guseva N, Tagiyev A, Knudson CM, Cohen MB (2001) Bcl-2 oncoprotein protects the human prostatic carcinoma cell

line PC3 from TRAIL-mediated apoptosis. Oncogene 20:2836–2843

Rössle C, Pichard C, Roulet M, Bergström J, Fürst P (1989) Muscle carnitine pools in cancer patients. Clin Nutr 8:341–346

Srinivasula SM, Fernandes-Alnemri T, Zangrilli J, Robertson N, Armstrong RC, Wang L, Trapani JA, Tomaselli KJ, Litwack G, Alnemri ES (1996) The Ced-3/interleukin 1beta converting enzyme-like homolog Mch6 and the lamin-cleaving enzyme Mch2alpha are substrates for the apoptotic mediator CPP32. J Biol Chem 271:27099–27106

Studzinski GP (1995) Cell growth and apoptosis. Oxford University Press, Oxford, pp 179–181

Tada M, Sugiyama S, Ozawa T (1984) Carnitine contents in various foods. J Jpn Soc Nutr Food Sci 37:13–17

Vary TC, Neely JR (1982) Characterization of carnitine transport in isolated perfused adult rat hearts. Am J Physiol 242:H585–592

Vescovo G., Ravara B, Gobbo V, Sandri M, Angelini A, Della BM, Dona M, Peluso G, Calvani M, Mosconi L, Dalla LL (2002) L-Carnitine: a potential treatment for blocking apoptosis and preventing skeletal muscle myopathy in heart failure. Am J Physiol Cell Physiol 283:802–810

Wenzel U, Nickel A, Daniel H (2005) Increased carnitine-dependent fatty acid uptake into mitochondria of human colon cancer cells induces apoptosis. J Nutr 135:1510–1514

Xie H, Tang SY, Li H, Luo XH, Yuan LQ, Wang D, Liao EY (2007) L-carnitine protects against apoptosis of murine MC3T3-E1 osteoblastic cells. Amino Acids. Epub ahead of print

Yazdanpanah M, Luo X, Lau R, Greenberg M, Fisher LJ, Lehotay DC (1997) Cytotoxic aldehydes as possible markers for childhood cancer. Free Radic Biol Med 23:870–878

Yeh CT, Yen GC (2005) Induction of apoptosis by the Anthocyanidins through regulation of Bcl-2 gene and activation of c-Jun N-terminal kinase cascade in hepatoma cells. J Agric Food Chem 53:1740–1749