

Single Dose Administration of L-Carnitine Improves Antioxidant Activities in Healthy Subjects

Yu Cao,¹ Hai-jun Qu,¹ Ping Li,¹ Chun-bo Wang,² Le-xin Wang³ and Zhi-wu Han¹

¹Department of Pharmacy, the Affiliated Hospital of Medical College, Qingdao University, Qingdao, China

²Department of Pharmacology, Medical College, Qingdao University, Qingdao, China

³School of Biomedical Sciences, Charles Sturt University, Wagga Wagga, NSW, Australia

L-carnitine has been used as a supplement to treat cardiovascular or liver disease. However, there has been little information about the effect of L-carnitine on anti-oxidation capability in healthy human subjects. This study was designed to investigate the correlation between plasma L-carnitine concentration and antioxidant activity. Liquid L-carnitine (2.0 g) was administered orally as a single dose in 12 healthy subjects. Plasma concentration of L-carnitine was detected by HPLC. The baseline concentration of L-carnitine was $39.14 \pm 5.65 \mu\text{mol/L}$. After single oral administration, the maximum plasma concentration (C_{max}) and area under the curve ($\text{AUC}_{0-\infty}$) were $84.7 \pm 25.2 \mu\text{mol/L}$ and $2,676.4 \pm 708.3 \mu\text{mol/L}\cdot\text{h}$, respectively. The half-life and the time required to reach the C_{max} was $60.3 \pm 15.0 \text{ min}$ and $3.4 \pm 0.46 \text{ h}$, respectively. There was a gradual increase in plasma concentrations of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase and total antioxidative capacity (T-AOC) in the first 3.5 h following L-carnitine administration. The plasma concentrations of SOD, GSH-Px, catalase and T-AOC returned to baseline levels within 24 h. A positive correlation was found between L-carnitine concentration and the antioxidant index of SOD ($r = 0.992$, $P < 0.01$), GSH-Px ($r = 0.932$, $P < 0.01$), catalase ($r = 0.972$, $P < 0.01$) or T-AOC ($r = 0.934$, $P < 0.01$). In conclusion, L-carnitine increases activities of antioxidant enzymes and the total antioxidant capacity in healthy subjects. It may be useful as a supplementary therapy for chronic illnesses involving excessive oxidative stress.

Keywords: catalase; glutathione peroxidase; L-carnitine; superoxide dismutase; total antioxidative capacity
Tohoku J. Exp. Med., 2011, 224 (3), 209-213. © 2011 Tohoku University Medical Press

L-carnitine (3-hydroxy-4-N-trimethylammonium butyrate) is an endogenous compound derived from the diet, such as meat and dairy products, or being synthesized in the liver or kidneys from the essential amino acids lysine and methionine (Vaz and Wanders 2002; Bain et al. 2006; Tsakiris et al. 2008). L-carnitine plays an important role in intermediary metabolism, including the transport of long chain fatty acids across the mitochondrial inner membrane (Vaz and Wanders 2002; Bain et al. 2006). L-carnitine is an essential cofactor in the β -oxidation of long-chain fatty acids. It is also believed to be important for acting as an acyl group acceptor in order to maintain sufficient cellular levels of free coenzyme A (CoA) and may act as an osmo protectant in organs such as the kidney and as a general cell membrane stabilizer (Lahjouji et al. 2004; Biolo et al. 2008).

L-carnitine has received attention as a pharmacological agent in the treatment of a range of cardiovascular disorders, hemodialysis, liver disease, nervous system disease,

endocrine system disease, parenteral alimentation, anorexia, chronic fatigue and male infertility. Gulcin (2006) investigated the antioxidant activity of L-carnitine *in vitro* and found that L-carnitine was effective in scavenging superoxide anion radical, hydrogen peroxide, and in metal chelating on ferrous ions. L-carnitine can also help to prevent and reduce ischemia-reperfusion injury (Onem et al. 2006; Akin et al. 2007). However, studies related to the anti-oxidation capability in healthy human subjects have been scarce. Therefore, the aim of this study was to investigate the plasma antioxidant status through the measurement of plasma superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase and total antioxidative capacity (T-AOC) activities after a single oral administration of L-carnitine solution in healthy volunteers, and evaluate the relationship between L-carnitine concentration and antioxidant activities.

Received May 6, 2011; revision accepted for publication June 6, 2011. doi: 10.1620/tjem.224.209

Correspondence: Le-xin Wang, School of Biomedical Sciences, Charles Sturt University, Wagga Wagga, NSW 2678, Australia.

e-mail: lwang@csu.edu.au

Zhi-wu Han, the Affiliated Hospital of Medical College Qingdao University, Qingdao 266003, P.R. China.

e-mail: jidi1767@126.com

Materials and Methods

Drugs, reagents and apparatus

Standard preparations of L-carnitine (purity 99%) and L-carnitine solution (Batch No.060708, 10 ml:1 g) were obtained from Northeast Pharmaceutical Group Co., LTD, China. Tetrabutylammonium hydroxide (TBA) and 2,4'-Dibromoacetophenone (PBPB) were supplied by Sigma, USA. Acetonitrile and methanol (HPLC grade reagent) was purchased from Honeywell international INC, USA. Other reagents (ammonium acetate, hydrochloric acid, acetone, glacial acetic acid, ether, chloroform) were of analytical grade. Water used in this assay was doubly distilled.

Citric acid buffer (6.4×10^{-3} mol/L; pH 5.0) was prepared by dissolving 1.35 g of citric acid ($C_6H_8O_7 \cdot H_2O$) in water, and then 1.70 mL of triethylamine was added to the solution and diluting to 1000 mL with water. Derivatizing reagent was prepared by dissolving 40 mg of PBPB in 1 ml acetonitrile, and then 100 μ L of 10% aqueous tetrabutylammonium hydroxide was added to this solution. The reagent appears to be stable for at least 24 h. Precipitating reagent was prepared by mixing 9 volume of acetonitrile and 1 volume of methanol.

The following apparatus were used in this study: Waters 2690 HPLC equipment system and Waters 2,487 UV detector (USA), Hypersil SiO₂ column (4.6 mm \times 200 mm, 5 μ m), Biochemistry analyzer (SYNCHRON LX20, BECKMAN, USA), Hemodialyzer (Haidylena, Egypt).

Study participants

This study was approved by the Institutional Review Board of Qingdao University. Informed written consent was obtained from all participants. Healthy volunteers were invited from the staff working at the hospital clinics and those who attended the clinics for annual health check-ups. All subjects were free from chronic or acute diseases. They underwent a thorough medical examination, including blood cell counts, biochemistry profile, liver and renal function tests and electrocardiogram. The volunteers were not permitted to consume alcohol for 72 h before or during the study, and were asked to abstain from any medications for at least 1 week before and during the study. Those who had a history of drug or alcohol abuse or allergy to the components of L-carnitine, and those who had concomitant drug therapy of any kind were excluded. All subjects were prescribed a similar diet commencing two weeks before the study.

Twelve healthy Chinese volunteers were recruited from 15 volunteers, and three were excluded, because one consumed alcohol before the study and two had a medical history. There were 6 males and 6 females with a mean age 27.7 ± 4.7 years. Their mean weight was 62.9 ± 8.8 kg and the mean height was 167.0 ± 6.15 cm. Assignment of subject numbers and dosing groups were randomly determined in advance.

Study design

Immediately before (0 h) and at 0.5, 1.0, 1.5, 2, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 12.0 and 24.0 h after oral administration of L-carnitine (2.0 g in 200 ml warm water) (Sahajwalla et al. 1995), 3 ml venous blood was collected and transferred into a chilled tube, which was centrifuged (2,300 g for 5 min) within 10 min to separate the plasma. The plasma was transferred into a polypropylene tube, which was kept at -20°C for analysis.

Chromatographic conditions and extraction procedure

The UV derivatives were separated on a Hypersil SiO₂ column, and the mobile phase consisted of acetonitrile: Citric acid buffer in the ratio of (10:90), the flow rate was 1.2 ml/min. The derivatives were monitored with a UV detector set at 260 nm (Li et al. 2007).

An aliquot of plasma sample (100 μ L) was extracted by protein precipitation with 1 mL volume of precipitating reagent (acetonitrile: methanol 9:1), and vortex-mixed for 1 min. About 400 mg of a mixture of 9 parts NaH₂PO₄ (i.e., anhydrous), 1 part Ag₂O, and about 400 mg of KH₂PO₄ were added to the solution, with sonic oscillation for 30 min. After centrifugation for 5 min at 3,000 g, the supernate (700 μ L) was added in 300 mg of a mixture of 9 parts NaH₂PO₄, 1 part Ag₂O, and about 300 mg of KH₂PO₄ was added to the solution again, sonic oscillation for 30 min, and centrifuged for 5 min at 3,000 g, an aliquot of 320 μ L supernatant sample and 40 μ L of the derivatizing reagent were transferred to a glass tube. After vortex-mixed well the mixture was heated at 70°C for 1.5 h in a heating bath, and then cooled rapidly under running water. Sample solution of 20 μ L was injected into the HPLC system.

Determination of antioxidant index

Plasma levels of SOD, GSH-Px, catalase, T-AOC were measured by spectrophotometric methods, following specific procedures outlined by the manufactures of the assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Date analysis

The pharmacokinetic parameters of L-carnitine were calculated by Drug and Statistic program (DAS, version 2.0, Statistical Solutions, Sun Ru Yuan, China). The pharmacokinetic parameters derived from these calculations were maximum plasma concentration (C_{\max}), half-life ($t_{1/2}$), area under the curve (AUC), and time to reach the C_{\max} (T_{\max}).

Data were expressed as means \pm s.d. SPSS15.1 software was used for data analysis. Numerical data were analyzed with one-way ANOVA. Categorical data were analyzed with Chi-square test. $P < 0.05$ was considered statistically significant.

Results

There were no side effects, such as gastrointestinal symptoms or skin rashes, reported from the study participants after the administration of L-carnitine. Blood biochemistry results, such electrolytes, plasma urea and creatinine remained unchanged 24 h after the drug administration. The serum levels of aspartate aminotransferase (AST, 21.2 ± 6.9 vs. 20.5 ± 7.2 IU/L, $P > 0.05$) and alanine aminotransferase (ALT, 29.9 ± 4.4 vs. 29.5 ± 6.3 IU/L, $P > 0.05$) also remained unchanged.

Mean plasma concentration-time curve and pharmacokinetic parameters of L-carnitine

The mean plasma concentration-time curve is shown in Fig. 1. After single oral administration, the maximum plasma concentration (C_{\max}) and area under the curve ($AUC_{0-\infty}$) was 84.7 ± 25.2 μ mol/L and $2,676.4 \pm 708.3$ μ mol/L \cdot h, respectively. The half-life ($t_{1/2\beta}$) and the time required to reach the C_{\max} (T_{\max}) was 60.3 ± 15.0 min and 3.4 ± 0.46 h, respectively.

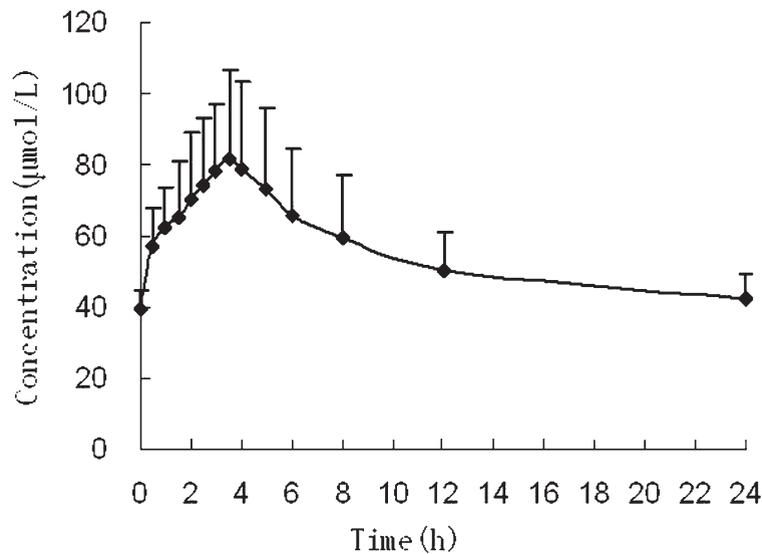


Fig. 1. Mean plasma concentration-time curve of L-carnitine after single oral administration.

Table 1. The mean values of L-carnitine, SOD, GSH-Px, catalase, and T-AOC after oral administration of L-carnitine.

Sampling point (h)	Plasma L-carnitine (μmol/L)	SOD (U/ml)	GSH-Px (U/ml)	Catalase (U/ml)	T-AOC (U/ml)
0	39.14 ± 5.65	25.52 ± 4.01	204.77 ± 9.00	1.23 ± 0.22	11.79 ± 1.96
0.5	57.37 ± 10.75	31.57 ± 6.34	215.68 ± 11.24	1.38 ± 0.34	12.30 ± 2.33
1	62.20 ± 11.66	36.04 ± 6.13**	223.09 ± 10.85	1.52 ± 0.25*	13.17 ± 2.08
1.5	65.43 ± 15.46	38.92 ± 9.26**	230.02 ± 18.57	1.69 ± 0.57**	13.68 ± 3.14
2	70.53 ± 18.48	40.46 ± 8.14**	250.83 ± 26.22**	1.70 ± 0.36**	14.53 ± 2.51*
2.5	74.38 ± 19.02	43.81 ± 11.92**	274.31 ± 27.94**	1.81 ± 0.62**	15.36 ± 3.01*
3	78.04 ± 19.28	45.67 ± 10.34**	280.17 ± 31.09**	1.91 ± 0.42**	16.29 ± 2.87**
3.5	81.91 ± 24.70	46.88 ± 12.69**	297.32 ± 48.91**	2.03 ± 0.74**	17.88 ± 3.49**
4	78.75 ± 24.82	44.94 ± 11.52**	276.56 ± 44.03**	1.87 ± 0.39**	16.37 ± 3.16**
5	73.22 ± 22.95	41.87 ± 10.73**	262.44 ± 44.03**	1.75 ± 0.36**	15.36 ± 2.84**
6	65.93 ± 18.63	37.92 ± 9.19**	239.52 ± 32.97**	1.58 ± 0.30*	13.85 ± 2.55
8	59.32 ± 17.63	34.20 ± 7.21*	219.59 ± 20.11	1.44 ± 0.24	12.75 ± 2.06
12	50.11 ± 10.97	29.84 ± 5.39	211.85 ± 13.44	1.29 ± 0.21	12.07 ± 1.94
24	42.15 ± 7.02	25.65 ± 3.72	205.89 ± 9.64	1.23 ± 0.21	11.80 ± 1.93

SOD, superoxide dismutase activity; GSH-Px, glutathione peroxidase; T-AOC, total antioxidative capacity. * $P < 0.05$ and ** $P < 0.01$, compared with 0 h.

Baseline plasma concentrations and antioxidant activity

The baseline plasma concentration of L-carnitine was $39.14 \pm 5.65 \mu\text{mol/L}$. The plasma activities of SOD, GSH-Px, catalase and T-AOC were $25.52 \pm 4.01 \text{ U/ml}$, $204.77 \pm 9.00 \text{ U/ml}$, $1.23 \pm 0.22 \text{ U/ml}$, and $11.79 \pm 1.96 \text{ U/ml}$, respectively.

Plasma antioxidant status

As shown in Table 1, after single oral administration, the concentration of SOD, GSH-Px, catalase and T-AOC increased gradually from 0 h to 3.5 h point, reached the peak at 3.5 h, and returned to the 0 h level at the 24 h point. The mean concentration of SOD at 1-8 h following L-carnitine administration was higher than the baseline

value ($P < 0.05$ or $P < 0.01$). The mean concentrations of GSH-Px, catalase and T-AOC in the first 5-6 h following L-carnitine administration were also higher than their baseline values ($P < 0.05$ or $P < 0.01$).

Correlation analysis

A positive correlation was found between plasma concentration of L-carnitine and concentration of SOD ($Y = 0.5319X + 3.2429$, $r = 0.9922$, $P < 0.01$), GSH-Px ($Y = 0.1606X + 103.63$, $r = 0.9316$, $P < 0.01$), and catalase ($Y = 0.019X + 0.3845$, $r = 0.9720$, $P < 0.01$). A positive correlation was also found between L-carnitine concentration and T-AOC ($Y = 0.1346X + 5.4452$, $r = 0.9338$, $P < 0.01$).

Discussion

Several methods of detecting L-carnitine in biological fluids have been previously reported. The most common methods are those employing enzymes (Takeyama et al. 1989) and those using HPLC with precolumn derivatization and UV or fluorimetric detection (Minker and Hoppel, 1993; Marzo and Curti 1997). HPLC methods based on pre-derivatization of L-carnitine have also been developed to analyze human plasma concentration of L-carnitine (van Kempen and Odle 1992; Longo et al. 1996). Although the HPLC methods for human plasma L-carnitine measurements are sensitive, the extraction processes were time-consuming and expensive (Longo et al. 1996), and the limit of quantitation was unsatisfactory in some cases (Cao et al. 2007). The present study describes a sensitive HPLC-UV method for the determination of L-carnitine in plasma. It involves the activation of carnitine carboxylic groups with TBA and subsequent reaction with BBPB. A good linearity of the assay was observed over the plasma concentration ranges, 5-320 $\mu\text{mol/L}$ for L-carnitine. The methods were proved to be stable, accurate and convenient to study the plasma concentrations of L-carnitine in a clinical setting.

To the best of our knowledge, this is the first study to investigate the pharmacokinetics of L-carnitine and its antioxidant activity in plasma after single oral administration in healthy Chinese volunteers. Before L-carnitine administration, the mean plasma concentration of L-carnitine was 39.1 $\mu\text{mol/L}$, which was lower than in the other population (Angelini et al. 1992; Vernez et al. 2006). The baseline SOD, GSH-Px, catalase and T-AOC concentration was 25.5, 204.8, 1.23, and 11.8 U/ml, respectively. In 1991, Rebouche (1991) published a paper that provided a quantitative estimation of the fate of an oral tracer dose of [*methyl-3H*] L-carnitine in five men who had previously received a high-carnitine diet and L-carnitine supplementation. It was found that the absorption of oral [*methyl-3H*] L-carnitine was slow and incomplete, with a maximum concentration of [*methyl-3H*] L-carnitine in serum occurred at 2.0 to 4.5 hours after administration of the tracer. They also found that urinary excretion of total carnitine was 16% to 23% of intake, and fecal excretion of total carnitine was negligible.

It is known that an increase in peroxidative damage is associated with the process of aging. The free radical theory of aging assumes that oxidative stress is one of the major causes of age-related cellular and molecular damage (Harman 1993). All organisms have enzymatic and non-enzymatic mechanisms to scavenge oxidants, or to repair damage caused by reactive oxygen species. Oxygen species are known to cause oxidative damages to macromolecules (Lahjouji et al. 2004). Chemical compounds and reactions capable of generating potential toxic oxygen species/free radicals are referred to as prooxidants. On the other hand, compounds and reactions disposing off these species, scavenging them, suppressing their formation or

opposing their actions are called antioxidants. Some of the important antioxidants include GSH, GSH-Px, SOD and catalase. In a normal cell, there is an appropriate prooxidant-antioxidant balance. However, this balance can be shifted towards the prooxidant when production of oxygen species is increased or when levels of antioxidants are diminished. This state is called oxidative stress and can result in serious cell damage if the stress is massive or prolonged. Oxidative stress is implicated in the pathogenesis of a variety of human diseases (Cross et al. 1987; Irshad and Chaudhuri 2002). Among the enzymatic defenses, the removal of damaging oxygen products is catalyzed by SOD, catalase and GSH-Px. SOD removes the superoxide anion in a dismutation reaction, producing hydrogen peroxide and molecular oxygen. The removal of hydrogen peroxide is catalyzed by either catalase or GSH-Px.

There has been little published information about the effect of L-carnitine on antioxidative status in plasma. In the present study, after a single oral administration of L-carnitine, there was a gradual increase in the plasma concentrations of SOD, GSH-Px, catalase and T-AOC. The peak concentrations of these enzymes were detected 3.5 h after L-carnitine administration. The mean concentrations of SOD, GSH-Px, catalase and T-AOC in the first 5-8 h were higher than their baseline values. Furthermore, a strong correlation was found between plasma L-carnitine concentration and concentrations of SOD, GSH-Px, catalase and T-AOC, suggesting that plasma L-carnitine was associated with the increase in these antioxidant enzymes.

In summary, this study in healthy volunteers has found that oral administration of L-carnitine is associated with a significant increase in the activities of antioxidant enzymes such as SOD, GSH-Px, catalase and T-AOC. These results suggest that oral administration L-carnitine may be useful as a supplementary therapy for chronic illnesses involving excessive oxidative stress.

Acknowledgments

This study was supported by a research grant from Chinese national key technology R&D programme and medical science technology programme of Shandong Province.

Conflict of Interest

The authors have no conflicts to declare.

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