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DOSE-DEPENDENT EFFECTS OF L-CARNITINE ON BLOOD SIALIC ACID, MDA AND GSH CONCENTRATIONS IN BALB/C MICE

YAPAR K, KART A, KARAPEHLIVAN M and CITIL M

Department of Pharmacology and Toxicology, College of Veterinary Medicine, University of Kafkas, Kars, Turkey

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L-carnitine is an essential quarternary amine having an important role in the β-oxidation of fatty acids. Although L-carnitine was shown to be protective against toxic effects of some chemicals the dose-effect relationship with respect to its antioxidant action and protection from lipid peroxidation is unknown. To evaluate the dose-response profile of L-carnitine on blood sialic acid, glutathione and malondialdehyde concentrations, 40 mice were randomly allocated to 4 groups. Experimental mice were treated with intraperitoneal saline for 5 days (Group 1), L-carnitine at 100 mg/kg for 5 days (Group 2), L-carnitine at 250 mg/kg for 5 days (Group 3), L-carnitine at 500 mg/kg for 5 days (Group 4). Following the treatments, blood samples were collected, and blood glutathione, malondialdehyde and sialic acid concentrations were determined. L-carnitine provided an antioxidant action at doses of 100, 250 and 500 mg/kg with the strongest antioxidant action observed at 500 mg/kg dose. There was a significant increase in malondialdehyde and sialic acid concentrations at all doses of L-carnitine with the highest effect seen at 500 mg/kg dose. In addition, L-carnitine caused a dose-dependent elevation in glutathione level.

These results suggest that L-carnitine applied at 500 mg/kg dose provides strong antioxidant action by increasing glutathione, malondialdehyde and sialic acid in BALB/c mice.

Key words: L-carnitine, lipid peroxidation, oxidative stress, aialic acid

INTRODUCTION

L-Carnitine is an important cofactor in the fatty acid metabolism and is known to have antioxidant properties (Arduni, 1992; Rani and Panneerselvam, 2001). It plays an important role in β-Oxidation of long chain fatty acids in the mitochondria (Brass, 2000). L-Carnitine is a naturally occurring methylated compound which is responsible for the conversion of long chain fatty acids into acylcarnitine and the transport of acyl groups across the mitochondrial membranes (Kelly, 1998; Szilagyi, 1998). Carnitine also serves as a buffer for

excess acyl-Co A which could accumulate and be deleterious to the cells (Brass, 2000). Antioxidant effects of L-Carnitine has been reported by several studies where L-carnitine is protective against lipid peroxidation, and it could improve the antioxidant status in rats. In addition, it is associated with scavenging of free radicals in cellular sites (Kalaiselvi and Panneerselvam, 1998; Rani and Panneerselvam, 2002; Sener *et al*., 2004). Sialic acid is a name referring to the acetylated derivatives of neuraminic acid. They are part of terminal residues of oligosaccharide chains of mucins, glycoproteins and glycolipids on cell membranes (Sillanaukee *et al*., 1999; Wang and Brand-Miller, 2003). Increased sialic acid concentration of serum was reported to be an indicator of early signs of some disorders. For example, plasma sialic acid concentration was increased in cardiovascular disorders, cancer, nephropathies, pneumonia and various other diseases (Lindberg *et al*., 1991; Shamberger, 1984; Sillanaukee *et al*., 1999). Therefore, measuring sialic acid has a diagnostic value in several disease states.

As far as we are aware, there are currently no reports associated with the dose-response study on L-carnitine. Therefore, we attempted to evaluate the effect of 3 different doses of L-Carnitine (100, 250 and 500 mg/kg for 5 days) on blood glutathione (GSH), malondialdehyde (MDA), total sialic acid (TSA) as well as lipid bound (LBSA) and protein bound sialic acid (PBSA) levels in BALB/c mice.

MATERIALS AND METHODS

Forty BALB/c mice (obtained from the University of Kafkas, Animal Research Farm) weighing 25-35 g were randomly divided into 4 groups containing 10 mice each. Group 1 (control) received intraperitoneal (i.p.) injections of isotonic saline solution daily for 5 days. Group 2 was treated with daily i.p. injection of 100 mg/kg L-Carnitine (CARNITENE®, Sigma–Tau Industrie Farmaceutiche, Pomezia-Italy) for 5 days. Group 3 was treated with 250 mg/kg of L-carnitine for 5 days. Group 4 was injected with 500 mg/kg of L-carnitine (i.p.) for 5 days. Blood samples from the treatment groups were collected from the heart via cardiac puncture under light ether anesthesia on the 6^{th} day. For GSH analysis, blood was taken into the tube without EDTA and for MDA and sialic acids measurements, blood samples were taken into the EDTA tubes, and were allowed to stand for 1 hour at room temperature. Then, all tubes were centrifuged at 3000 rpm for 10 minutes to separate the plasma. The samples were kept at -25 ^{o}C until analyzed. Analyses were carried out by the methods of Beutler (1963) and Yoshoiko (1979) for GSH and MDA levels, respectively. Plasma total sialic acid (TSA) and lipid bound sialic acid (LSA) levels were measured colorimetrically using a spectrophotometer (UV-1201, Shimadzu, Japan) by the method of Sydow (1985). The concentration of protein bound sialic acid (PBSA) was calculated by subtracting LBSA from TSA. For statistical analysis, differences between the groups were tested by analysis of variance (ANOVA) followed by Duncan test using SPSS for Windows version 6.0. Data were presented as mean ± SEM, and p values less than 0.05 were considered significant.

RESULTS

Plasma TSA, LBSA and PBSA concentrations in mice treated with L-carnitine at doses of 100, 250 and 500 mg/kg were lower than in the control samples. However, there was a dose dependent reduction in these parameters in L-carnitine treated mice. As the dose of L-carnitine increased, TSA, LSA and PBSA concentrations decreased (Table 1). In addition, while serum GSH concentration in the control was significantly lower compared to mice receiving 100, 250 and 500 mg/kg of L-carnitine, MDA concentration appeared to decrease in a dose dependent manner (Table 1). Moreover, serum GSH concentration in group 2 (L-carnitine: 100 mg/kg), 3 (L-carnitine: 250 mg/kg) and 4 (L-carnitine: 500 mg/kg) was significantly higher than in the control group. The highest GSH concentration observed was in group 4 (receiving 500 mg/kg of L-carnitine).

Table 1. Plasma total sialic acid (TSA), lipid bound sialic acid (LBSA), protein bound sialic acid (PBSA), malondialdehyde (MDA) and glutathione (GSH) concentrations in response to L-carnitine administration according to treatment. Group 1 (control: saline for 5 days), group 2 (L-carnitine: 100 mg/kg for 5 days), group 3 (L-carnitine: 250 mg/kg for 5 days) and group 4 (L-carnitine: 500 mg/kg for 5 days)

Parameters	Treatments			
	Control (Saline)	L-carnitine (100 mg/kg)	L-carnitine (250 mg/kg)	L-carnitine (500 mg/kg)
TSA (mg/L)	574.16±3.62 ^{a}	527.07±3.27 ^{b}	470.27±2.41 ^{c}	316.54±3.81 ^{d}
LBSA (mg/L)	209.00±2.26 ^{a}	185.27±2.28 ^{b}	144.91±2.48 ^{c}	108.61±2.19 ^{d}
PBSA (mg/L)	365.15±4.49 ^{a}	341.80±3.06 ^{b}	325.36±3.41 ^{c}	207.98±4.38 ^{d}
GSH (mg/L)	71.40±0.97 ^{d}	74.67±0.81 ^{c}	80.21±0.66 ^{b}	95.81±0.96 ^{a}
MDA (μmol/L)	14.35±0.43 ^{a}	13.4±0.22 ^{b}	12.34±0.19 ^{c}	10.56±0.18 ^{d}

$P<0.05$, different letters within the same row indicate significant difference between values; (TSA: total sialic acid, LBSA: lipid bound sialic acid, PBSA: protein bound sialic acid, MDA: malondialdehyde, GSH: glutathione)

DISCUSSION

While administration of L-carnitine at doses of 100, 250 and 500 mg/kg to mice showed a dose-dependent increase in GSH and decrease in MDA, L-carnitine applied at 500 mg/kg provided a strong antioxidant action as evidenced by increased GSH and decreased MDA concentrations. Previous studies on the effects of L-carnitine on various types of chemically-induced toxicities indicate that L-carnitine a dose of 500 mg/kg ameliorated the adverse effects in rats (Sener *et al*., 2004; Sayed-Ahmed *et al*., 2004). It was reported that increases of MDA and reduced level of GSH in tissues and blood were reversed with intraperitoneal administration of 500 mg/kg L-carnitine given for 4 weeks in Wistar rats suffering

from chronic renal failure associated with oxidative stress (Sener *et al.*, 2004). Similarly, in Cisplatin-induced nephrotoxicity where the oxidative stress and lipid peroxidation are tought to play a major role in the pathophysiology of nephrotoxicity, administration of L-carnitine at 500 mg/kg for 10 days in Sprague-Dawley rats normalized kidney function as evidenced by histopathology, BUN and serum creatinine levels. In addition, L-carnitine at this dose attenuated the increased MDA and reduced GSH levels (Sayed-Ahmed et al, 2004). Other than nephrotoxicity, L-carnitine at 500 mg/kg was shown to prevent ethanol induced lesions in gastric mucosa and protected against lipid peroxidation as well as normalized GSH of the gastric mucosa in rats (Dokmeci et al. 2005). In an *in vitro* study, apoptosis induced by doxorubicin in cardiac myocytes is prevented by L-carnitine (Andrieu-Abadie *et al*., 1999). Methamphetamine neurotoxicity, mediated by peroxynitrite radicals, was protected by L-carnitine (Ashraf *et al*., 2002). In addition, brain injury associated with hypoxia and ischemia in neonates was ameliorated by L-carnitine. Production of reactive oxygen species is a common finding during ischemia-reperfusion cases (Pallor *et al*., 1984). Therefore, L-carnitine may be involved in scavenging of reactive species. L-carnitine could prevent oxidative damage by increasing oxygen utilization and protecting from ATP depletion. Another way of protection by L-carnitine could be associated with increasing the antioxidant level in tissues, since L-carnitine increases GSH levels (Di Giacomo *et al*., 1993). This effect has been also observed by an increase in GSH in our study at doses of 100, 250 and 500 mg/kg with the highest increase in serum GSH of mice injected with 500 mg/kg L-carnitine. Oxidative stress is one of the leading factors in the initiation of many disease processes (Galle, 2001). A balanced redox state of the cell is maintained by adequate antioxidant potential against reactive species, otherwise an overwhelmed antioxidant status results in oxidative stress leading to lipid peroxidation, protein and DNA injury (Tylicki *et al.*, 2003). Glutathione is an important part of antioxidant defense system which plays an important role in preventing the harmful effects of free radicals by scavenging hydroxyradicals and singlet oxygen (Diplock, 1994). Therefore, reduced GSH may contribute to the decreased level of antioxidant potential leading to oxidative stress which could consequently result in cytotoxicity. An important consequence of enhanced free radical formation due to oxidative stress is lipid peroxidation leading to the breakdown of cellular membranes.

Polyunsaturated fatty acids in membrane phospholipids are the major targets for free radicals which are capable of inducing a chain reaction of lipid peroxidation. These reactions in lipid membranes give rise to the formation of end products which are used to detect free radical damage. One of the important end products measured as an indicator of lipid peroxidation is known to be MDA (De Zwart *et al*., 1999). In addition to MDA the release of sialic acid located on the terminal residues of glycolipids of cell membranes could ensue as a result of the breakdown of cell membranes and/or lipid peroxidation. The released sialic acid reaches the plasma, and this could be reflected by the elevated plasma sialic acid concentracion. Elevation of TSA and LBSA concentrations is considered to be the reflection of altered structural integrity of glycolipids in the cellular membranes

(O'Kennedy *et al*., 1991; Shutter *et al*., 1992). Indeed, serum sialic acid level in various types of diseases was found to be increased in cancer patients and several types of inflammatory diseases such as arthritis, Crohn's disease and psoriasis (Shamberger, 1984; Silver *et al*., 1983). The increase in the level of sialic acid was also correlated with the rate of cardiovascular mortality (Lindberg *et al*., 1991). In addition, serum sialic acid concentrations are increased in chronic glomerulonephritis, chronic renal failure, chronic liver disease and pneumonia (Sillanaukee *et al*., 1999). Protection of cellular membranes through L-carnitine could be due also to the action of L-carnitine via detoxification of acetyl groups and free CoA (Fritz and Arrigoni-Martelli, 1993).

Although L-carnitine applied at 3 different doses (100, 250 and 500 mg/kg) showed an improvement in the antioxidant and protective effect against lipid peroxidation, it appears that L-carnitine applied at 500 mg/kg for 5 days to mice had greater effects on these parameters than the other applied doses (100 and 250 mg/kg).

In conclusion, L-carnitine at 100, 250 and 500 g/kg doses is capable of improving selected blood parameters including MDA, GSH and sialic acid via increasing the antioxidant status and reducing lipid peroxidation, as well as by protecting cellular membranes, with the highest effect seen at 500 mg/kg administered for 5 days.

Address for correspondence:
Dr. Asim Kart
Department of Pharmacology and Toxicology,
College of Veterinary Medicine,
University of Kafkas, 36040, Kars,
Turkey
E-mail: asimkart@hotmail.com

REFERENCES

1. *Andrieu-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, 1501-10.
2. *Arduini A,* 1992, Carnitine and its acyl esters as secondary antioxidants? *Am Heart J*, 23, 1726-7.
3. *Ashraf V, Franco G, Syed I, Zbigniew B, Syed A,* 2002, The protective role of L-carnitine against neurotoxicity evoked by drug of abuse, methamphetamine, could be related to mitochondrial dysfunction, *Ann N Y Acad Sci*, 965, 225-32.
4. *Beutler E, Duran O, Kelley BM*, 1963, Improved method for determination of blood glutathione, *J Lab Clin Med*, 28, 882-8.
5. *Brass EM*, 2000, Supplemental carnitine and exercise, *Am J Clin Nutr*, 72, 618-23.
6. *De Zwart LL, Meerman JHN, Commandeur JNM, Vermeulen NPE,* 1999, Biomarkers of free radical damage applications in experimental animals and in humans, *Free Radic Biol Med*, 26, 202-26.
7. *Di Giacomo C, Latteri F, Fichera C, Sorrenti V, Campisi A, Castorina C et al*., 1993, Effect of acetyl-L-carnitine on lipid peroxidation and xanthine oxidase activity in rat skeletal muscle, *Neurochem Res*, 18, 1157-62.
8. *Diplock AT,* 1994, Antioxidants and free radical scavengers, In Rice-Evans CA, Burdon RH, editors, Free radical damage and its Control, Amsterdam: Elsevier, 113-30.
9. *Dokmeci D, Akpolat M, Aydogdu N, Doganay L, Turan FN*, 2005, L-carnitine inhibits ethanol-induced gastric mucosal injury in rats, *Pharmacol Rep*, 57, 481-8.

10. *Fritz IB, Arrigoni-Martelli E*, 1993, Sites of action of carnitine and its derivatives on the cardiovascular system: interactions with membranes, *Trends Pharmacol Sci*, 14, 355-60.
11. *Galle J*, 2001, Oxidative stress in chronic renal failure, *Nephrol Dial Transplant*, 16, 2135 - 37.
12. *Kalaiselvi T, Panneerselvam C,* 1998, Effect of L-carnitine on the status of lipid peroxidation and antioxidants in aging rats, *J Nutr Biochem*, 9, 575-81.
13. *Kelly GS,* 1998, L-Carnitine: Therapeutic Applications of a Conditionally-Essential Amino Acid, *Altern Med Rev*, 3, 345-60.
14. *Lindberg G, Eklund GA, Gullberg B, Rastam L,* 1991, Serum sialic acid concentration and cardiovascular mortality, *Br Med J*, 302, 143-6.
15. *O'Kennedy R, Berns G, Moran E, Symth H, Carroll K, Thornes RD et al*, 1991, A critical analysis of the use of sialic acid determination in the diagnosis of malignancy, *Cancer Lett,* 58, 91-100.
16. *Pallor MS, Holdal JR, Ferris TF,* 1984, Oxygen free radicals in ischemi acute renal failure in the rat, *J Clin Invest*, 74, 1156-64.
17. *Rani PJ, Panneerselvam C,* 2001, Carnitine as a free radical scavenger in aging, *Exp Gerontol*, 36, 1713-26.
18. *Rani PJ, Panneerselvam C,* 2002, Effect of L-carnitine on brain lipid peroxidation and antioxidant enzymes in old rats, *J Gerontol A Biol Sci Med Sci*, 57, 134-7.
19. *Sayed-Ahmed MM, Eissa MA, Kenawy SA, Mostafa N, Calvani M, Osman AM,* 2004, Progression of cisplatin-induced nephrotoxicity in a carnitine-depleted rat model,*Chemotherapy*, 50, 162-70.
20. *Sener G, Paskaloglu K, Satiroglu H, Alican I, Kacmaz A, Sakarcan A,* 2004, L-carnitine ameliorates oxidative damage due to chronic renal failure in rats, *J Cardiovasc Pharmacol*, 43, 698-705.
21. *Sillanaukee P, Pönniö M, Jääskeläinen IP,* 1999, Occurrence of sialic acids in healthy humans and different disorders, *Eur J Clin Nutr*, 29, 413-25.
22. *Silver HKB, Murray RN, Worth AJ, Salinas FA, Spinelli JJ,* 1983, Prediction of malignant melanoma recurrence by serum N-acetylneuraminic acid, *Int J Cancer*, 31, 39-43.
23. *Shamberger RJ,* 1984, Serum sialic acid in normals and in cancer patients, *J Clin Chem Clin Biochem*, 22, 647-51.
24. *Shutter MJ, Visser JJ, Kamp GJ, Poully SM, Dijk W, Hilgers J et al*, 1992, The utility of lipid-associated sialic acid (LASA or LSA) as a serum marker for malignancy, *Tumor Biol*, 13, 121-32.
25. *Sydow G,* 1985, A simplified quick method for determination of sialic acid in serum, *Biomed Biochem Acta*, 44, 1721-3.
26. *Szilagyi M,* 1998, L-carnitine as essential methylated compound in animal metabolism, An Overview, *Acta Biol Hung*, 49, 209-18.
27. *Tylicki L, Rutkowski B, Hörl WH*, 2003, Antioxidants: A Possible role in kidney Protection, *Kidney Blood Press Res*, 26, 303-14.
28. *Wang B, Brand-Miller J,* 2003, The role and potential of sialic acid in human nutrition, *Eur J Clin Nutr*, 57, 1351-69.
29. *Yoshoiko T, Kawada K, Shimada T,* 1979, Lipid peroxidation in maternal and cord blood and protective mechanism aganist actived-oxygen toxicity in the blood, *Am J Obstet Gynecol*, 135, 372-6.

DOZNA ZAVISNOST L-KARNITINA I KONCENTRACIJA SIJALINSKE KISELINE, MALONDIALDEHIDA I GLUTATIONA KOD BALB/C MIŠEVA

YAPAR K, KART A, KARAPEHLIVAN M i CITIL M

SADRŽAJ

U cilju ispitivanja dozne zavisnosti efekata L-karnitina na koncentraciju sijalinske kiseline, malondialdehida i glutationa kod BALB/c miševa, ovo jedinjenje je intraperitonealno aplikovano grupama od po 10 životinja u dozama od 100, 250 i 500 mg/kg tokom pet dana. Kontrolna grupa miševa (10 jedinki) je u istom periodu i na isti način primala fiziološki rastvor. Nakon tretmana, životinje su žrtvovane i u njihovom serumu je određivana koncentracija sijalinske kiseline, malondialdehida i glutationa. L-karnitin je ispoljavao antioksidativno delovanje povećavanjem koncentracije ispitivanih supstanci i najizraženiji efekat je uočen pri dozi od 500 mg/kg.