Arch Pharm Res Vol 27, No 6, 676-681, 2004

Archives of Pharmacal Research
http://apr.psk.or.kr

HPLC Determination and Pharmacokinetics of Endogenous Acetyl-L-Carnitine (ALC) in Human Volunteers Orally Administered a Single Dose of ALC

Oh-Seung Kwon[1] and Youn Bok Chung

[1]Toxicology Lab., Korea Institute of Science and Technology, Seoul 136-791 and National Research Laboratory (NRL) of PK/PD, Biotechnology Research Institute, College of Pharmacy, Chungbuk National University, Cheongju, Chungbuk 361-763, Korea

(Received February 21, 2004)

Acetyl-L-carnitine (ALC), a naturally occurring endogenous compound, has been shown to improve the cognitive performance of patients with senile dementia Alzheimer's type, and to be involved in cholinergic neurotransmission. Because ALC is an endogenous compound, validation of the analytical methods of ALC in the biological fluids is very important and difficult. This study was presented validation and correction for plasma ALC concentrations and pharmacokinetics after oral administration of ALC to human volunteers. ALC concentrations in human plasma were corrected by subtracting the concentration of blank plasma from each sample. Precision and accuracy (bias %) for uncorrected ALC concentrations were below 2.6 and 6.5% for intra-days, and 4.0 and 9.4% for inter-days, respectively. Precision and accuracy (bias %) for corrected ALC concentrations were below 10.9 and 6.0% for intra-days, and 10.5 and 16.9% for inter-days, respectively. Quantitation limit was 0.1 μg/mL. After oral administration of a 500 mg ALC tablet to 8 healthy volunteers, the principle pharmacokinetic parameters were 4.2 h of the half-life ($t_{1/2,\beta}$), the area under the curve ($AUC_{0\to8}$) of 9.88 μg·h/mL, and 3.1 h of the time (T_{max}) to reach C_{max}. This study first describes the pharmacokinetic study after oral administration of a single dose of ALC in human volunteers.

Key words: Acetyl-L-carnitine, Endogenous compound, High-performance liquid chromatography (HPLC), Validation, Pharamcokinetics

INTRODUCTION

Acetyl-L-carnitine (ALC; 4-*N*-trimethylammonium-3-acetylbutyric acid), a derivative of L-carnitine, is a naturally occurring endogenous materials. L-Carnitine is a highly polar compound and plays an important role in oxidative metabolism and ketogenesis as a cofactor for the transfer of long-chain acyl groups by forming acyl-CoA derivatives across the inner mitochondrial membrane (Bremer, 1983; Fritz and Marquis, 1965). The entry of acyl carnitine into the mitochondrial matrix is mediated by a translocase. A defect in the translocase or a deficiency of carnitine is expected to impair oxidation of long-chain fatty acids (Fritz and Arrigoni-Martelli, 1993).

In clinical point of view, ALC has been shown to improve the cognitive performance of patients suffering from dementia of Alzheimer type (Passeri *et al.*, 1990; 1988), and several studies have supported its involvement in cholinergic neurotransmission (Piovesan *et al.*, 1995; Janiri *et al.*, 1991).

ALC was reported to be determined by high-performanc liquid chromatography (HPLC) and thermospray liquid chromatography/mass spectrometry (Millington *et al.*, 1989) in biological fluids. Among these instruments, there are found many HPLC methods, using UV or fluorescence detectors after the preparation of ALC derivatives. As derivatization agents, L-alanine-β-naphylamide (Kakawa *et al.*, 1999), 1-aminoanthracene with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride as a catalyst (Longo *et al.*, 1996), 4'-bromophenacyl trifluoromethanesulfonate or 2-(2,3-naphthalimino)ethyl-trifluoromethanesulfonate (Minkler *et al.*, 1995; Minkler and Hoppel, 1993),

Correspondence to: Youn Bok Chung, Ph.D., National Research Laboratory (NRL) of PK/PD, Biotechnology Research Institute, College of Pharmacy, Chungbuk National University, Cheongju, Chungbuk 361-763, Korea
Tel: 82-43-261-2824, Fax: 82-43-274-0752
E-mail: chungyb@chungbuk.ac.krs

3-bromomethyl-6,7-dimethoxy-1-methyl-2(1*H*)-quinoxalinone (Kamimori *et al.*, 1994), 4'-bromophenacyl bromide (Poorthuis *et al.*, 1993) were used to determine ALC or its analogues.

A pharmacokinetic study of ALC after intravenous and oral administration of multiple dose of ALC in patients with senile dementia of Alzheimer type was reported that the plasma concentrations showed a biphasic curve with average terminal half-life of 1.73 h, and ALC easily crosses the blood-brain barrier (Parnetti *et al.*, 1992). In patients undergoing long-term hemodialysis, repeated intravenous administration of L-carnitine resulted in increased plasma concentrations and finally in reaching apparent steady state after about 8 weeks (Evans *et al.*, 2000). And intravenous administration of propionyl-L-carnitine caused significant increases in the renal excretory clearances of propionyl-L-carnitine, L-carnitine, and ALC due to saturation of the renal tubular reabsorption process (Pace *et al.*, 2000). The determination of plasma ALC concentration is relatively difficult and there are no reports relating ALC concentrations after a single oral dose of ALC in human, even if multiple dosage of oral or intravenous administration studies for ALC were reported (Parnetti *et al.* 1992; Marzo *et al.*, 1989).

Because ALC is an endogenous compound, validation of the analytical methods of ALC concentrations in the biological fluids is very important. In this work, we presented validation and correction for analytical methods of plasma ALC concentrations and pharmacokinetics after oral administration of ALC to human volunteers.

MATERIALS AND METHODS

Chemicals

Authentic ALC was provided by Sigma (St. Louis, MO, USA). Acetonitrile and methanol were obtained from J. T. Baker (Phillipsburg, NJ, USA). Methanesulfonyl-L-carnitine, commercially unavailable, used as internal standard, was specially ordered to Sigma for synthesis. The other agents used for ALC analysis were of analytical grade.

Oral administration of an ALC tablet to human volunteers

A 21-gauge scalp-vein set was established on the arm vein of the volunteers, and 8 mL of ALC-free blood for blank were collected. According to the prescription directed by the doctor, a tablet (500 mg ALC) was orally taken to 8 male volunteers with 150 mL of water at Chungbuk National University Hospital (Cheongju, Chungbuk, Korea), according to the procedure approved by the Institutional Review Board for ethics. No food was allowed until 4 h after a single dose of ALC administration. Lunch and dinner were provided to volunteers according to a time schedule. Beverages and caffeine were not allowed during the study. Blood was taken into heparin-treated Vacutainer tube (Becton Dikinson, Rutherford, NJ, USA) at 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, and 12 h after the oral administration. The time interval of blood sampling between volunteers was 2 min to consider blood collection time. Blood was centrifuged to obtain plasma at 4°C. The plasma was stored at -70°C until analyzed.

HPLC equipments

ALC concentrations in the plasma were determined by using a HPLC system (Jasco Co., Tokyo, Japan) equipped with the pump (model PU-980), detector (F-1050 fluorescence), control borwin integrator (LC-Net II) and an auto liquid sampler (AS-950-10). ALC analysis was conducted at excitation 248 nm and emission 418 nm using the fluorescence detector. The separation of ALC was performed by using a Capcell Pak C_{18} column (4.6×250 mm, 5 μm particles, 120 Å pore size; Shiseido, Tokyo, Japan). The flow rate of the column was 1.3 mL/min. Mobile phase was consisted of 0.1 M ammonium acetate (pH 3.5)/acetonitrile (70:30, v/v, %).

Preparation of the calibration curve of ALC

The method for determination of ALC in human plasma was followed as described in Longo *et al.* (1996), with a little modification. To a 15 mL of centrifuging tube, 0.5 mL of the thawed blank plasma was added. And the various concentrations of ALC were spiked to make the final concentration of 0.1, 0.2, 0.5, 1, 2, and 4 μg/mL. Methanesulfonyl-L-carnitine (5 μg/mL, 100 μL) was added as internal standard. To the tube 2.4 mL of distilled water was added. After the tube was mechanically mixed on a vortex-mixer (Maxi Mix II, Thermolyne Co., Dubuque, IA, USA), the mixture was loaded on a pre-activated strong anion exchange cartridge (LC-SAX, 3 mL, Supelco, Bellefonte, PA, USA) in a vaccum manifold. After the mixture solution was removed from the catridge, 1.5 mL of 0.01 M phosphate buffer (pH 3.5) was loaded for elution. To the eluted solution, 60 of 1 M HCl, 300 of 1-aminoanthracene (16 mg/mL acetone), 300 of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (160 mg/mL $NaH_2PO_4{\cdot}H_2O$ (0.01 M, pH 3.5)) were sequentially added. The tube was maintained at room temperature for 20 min, and washed twice with 5 mL of diethyl ether. The aqueous layer of 900 was transferred to a new tube and pH was adjusted to about 7.0 with 2.8 mL of $Na_2HPO_4{\cdot}2H_2O$ (0.01 M, pH 9.1). And 5 mL of chloroform was added to wash the solution and this step was repeated. Aqueous solution of 1.5 mL was transferred to new tubes and diluted with 1.5 mL of $NaH_2PO_4{\cdot}H_2O$ (0.01 M, pH 3.5). This solution (100 μL) was applied to the HPLC system. Calibration curve was prepared from the peak area ratios of ALC to methane-

sulfonyl-L-carnitine. ALC concentrations were corrected by subtracting the concentration of blank plasma from that of the sample in each volunteer. Inter- and intra-day precisions and accuracy were obtained.

Preparation of plasma samples

One half ml of thawed plasma obtained from healthy human volunteers was added to the 15 mL centrifuging tubes, followed by addition of methanesulfonyl-L-carnitine as internal standard. The tube was treated as described above. Based on the calibration curve of ALC, the plasma concentrations of ALC were determined from peak area ratios of ALC to methanesulfonyl-L-carnitine.

Pharmacokinetic analysis

Pharmacokinetic parameters were determined from the time-plasma concentration curves of ALC by non-compartmental analysis by using WinNonlin software (Scientific Consulting Inc., Cary, NC, USA). The highest concentration (C_{max}) and the time to reach the highest concentration (T_{max}) were read directly from the time-plasma concentration curves of ALC. The area under the curve of time-plasma concentrations of ALC until the last sampling time ($AUC_{0\rightarrow last}$) was determined by the equation of $AUC_{0\rightarrow inf} = AUC_{0\rightarrow last} + C_{last/\beta}$, where β is the slope of the terminal phase of the time-log plasma concentration curve and C_{last} is the concentration at the last sampling time (Shargel and Yu, 1993).

RESULTS

HPLC chromatograms

ALC and internal standard methanesulfonyl-L-carnitine were with high resolution separated from interfering peaks by a HPLC/fluorescence detector. Retention times of ALC and methanesulfonyl-L-carnitine were about 10.4 and 12.3 min, respectively (Fig. 1).

Validation for the determination of plasma ALC concentrations

Precision and accuracy data were shown in Table I and Table II as in uncorrected or corrected value, respectively. Precision of intra- and inter-day for uncorrected ALC concentrations was below 2.55 and 4.04%, respectively, showing that the HPLC method of ALC has very high reproducibility. Accuracy of intra- and inter-day for uncorrected ALC concentrations was also very good as below 7.99 and 9.40% (Table I). Although precision and accuracy % for corrected values were increased, compared to those of uncorrected value, these values were still located within ±15% in all concentrations except for the lowest concentration, which was located within ±20% (Table II).

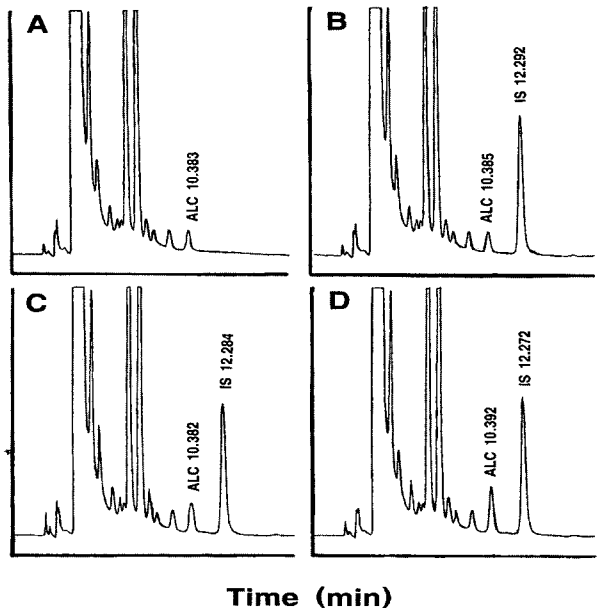

Fig. 1. HPLC chromatograms obtained from blank (**A**), the blank plasma spiked methanesulfonyl-L-carnitine as internal standard (IS; **B**), the blank plasma spiked the internal standard and 200 ng/mL of acetyl-L-carnitine (ALC; **C**), and the sample obtained 3 hr after oral administration of a acetyl-L-carnitine tablet (500 mg) to a human volunteer (**D**).

Table I. Precision and accuracy of the plasma acetyl-L-carnitine concentrations (Uncorrected)

Concentrations (μg/mL)	Precision (CV%)		Accuracy (bias %)	
	Within days	Between days	Within days	Between days
0.1	2.55	4.04	3.28	5.92
0.2	1.17	2.59	6.51	3.97
0.5	0.54	0.55	7.99	9.40
1	2.23	0.40	4.00	3.82
2	0.47	1.22	0.54	1.58
4	0.92	0.12	1.23	1.20

Each value was obtained from three different experiments.

Table II. Precision and accuracy of the plasma acetyl-L-carnitine concentration (Corrected*)

Concentrations (μg/mL)	Precision (CV%)		Accuracy (bias %)	
	Within days	Between days	Within days	Between days
0.1	10.9	14.6	13.3	16.9
0.2	6.26	10.5	1.50	7.44
0.5	7.31	12.0	5.98	4.83
1	2.40	6.73	5.00	6.10
2	1.61	2.86	1.04	2.73
4	0.62	1.55	1.48	1.77

Each value was obtained from three different experiments.
*Corrected plasma concentrations were obtained by subtracting the peak area ratios of acetyl-L-carnitine to the internal standard from plasma total concentrations of acetyl-L-carnitine at each time.

Table III. Peak area ratios for endogenous acetyl-L-carnitine concentrations at blank plasma

Blank	Within days			Between days			Mean (n=6)	S.D[b] (n=6)	CV[c] (%)
	1	2	3	1	2	3			
Ratios[a]	0.201	0.199	0.208	0.201	0.215	0.198	0.204	0.007	3.28
Concentrations (μg/mL)	1.289	1.275	1.332	1.289	1.379	1.265	1.305	0.043	3.28

[a] The peak area ratios of acetyl-L-carnitine to methanesulfonyl-L-carnitine
[b] Standard deviation
[c] Coefficient of varinace

Baseline levels of ALC and quantitation limit

The endogenous concentrations of ALC in the blank plasma were determined to 1.30±0.04 μg/mL (n=6, CV= 3.29%), which is corresponded to 0.204 ± 0.007 as peak area ratios of ALC to methanesulfonyl-L-carnitine (Table III). Based on the criteria of precision and accuracy %, quantitation limit was determined to 100 ng/mL of ALC in plasma samples.

Calibartion curves

Calibration curves of ALC for either uncorrected or corrected ALC concentrations in the plasma showed high linearity (r^2>0.9998) at concentrations ranged from 0.1 to 4 μg/mL (Fig. 2). Correction for this calibration curve was made by subtracting peak area ratios of ALC to internal standard for blank plasma from each concentration.

Pharmacokinetic parameters of ALC

This method was applied to the pharmacokinetic studies of ALC after single oral administration of a 500 mg ALC tablet to 8 human male volunteers. The principle pharmacokinetic parameters were determined based on non-compartment model. The half-life is about 4.2 h. The area under the curve ($AUC_{0\rightarrow8}$) was 9.88 μg·h/mL. The maximal concentration (C_{max}) and the time to reach C_{max} (T_{max}) were determined to be 1.19 μg/mL and 3.1 h, respectively. The plasma concentration-time curves and their curve fitting lines were shown in Fig. 3 at a semi-logarithmic scale for both uncorrected or corrected plasma concentration of ALC. The results for curve-fitting were showed that a great bias in uncorrected data exists, compared to the corrected data.

DISCUSSION

This paper describes a HPLC method for the determination of endogenously occurring ALC in human plasma after derivatization of ALC with 1-aminoanthracene and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, and its application to the pharmacokinetic study of ALC. This method gives good reproducibility and accuracy, indicating that it is suitable to determine plasma ALC concentrations for

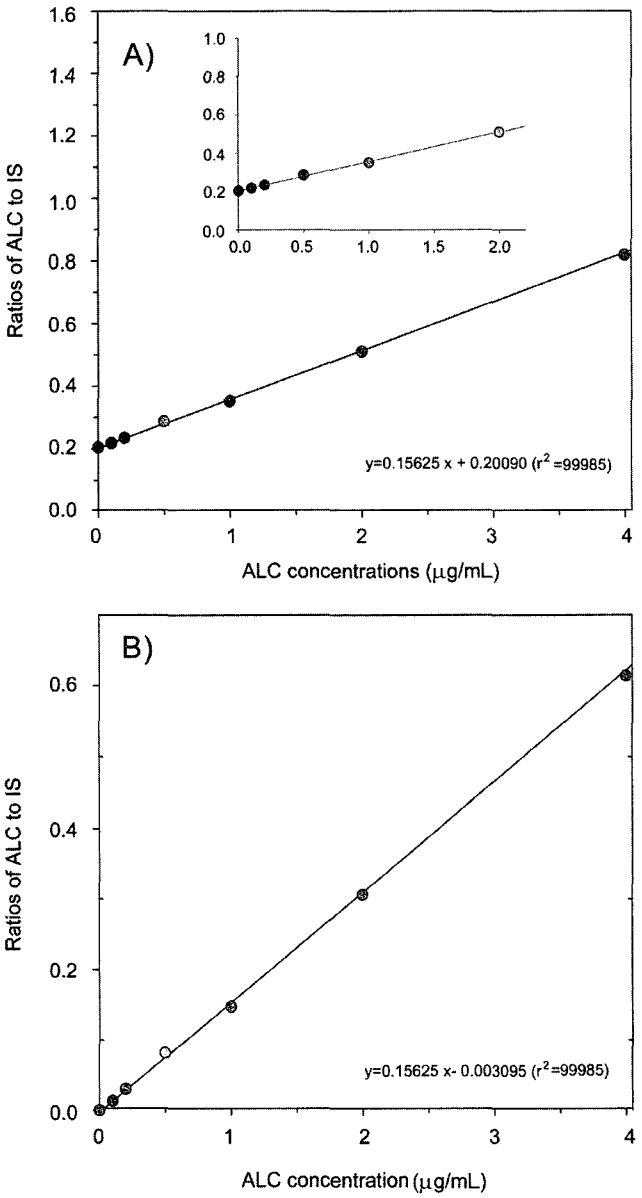

Fig. 2. Acetyl-L-carnitine (ALC) calibration curves showing uncorrected (**A**) and corrected (**B**) plasma acetyl-L-carnitine concentrations. Peak area ratios of acetyl-L-carnitine to internal standard (IS) were indicated in Y-axis and the concentrations in X-axis. Each concentration ranging from 0.1 to 8 μg/mL was presented mean value of 6 different experiments.

Table IV. Pharamcokinetic parameters of acetyl-L-carnitine (ALC) after oral administration of a single 500 mg ALC tablet to human volunteers

Parameters	500 mg ALC Volunteers (n=8)
$AUC_{0?8}$, /mL·h	9.879 ± 3.757
$AUC_{0?12hr}$, /mL·h	6.611 ± 1.316
C_{max}, /mL	1.188 ± 0.148
MRT, h	4.5 ± 0.3
$t_{1/2,â}$, h	4.2 ± 1.6
T_{max}, h.	3.1 ± 0.2

Definitions: $AUC_{0?8}$, the area under the concentration-time curve; C_{max}, the maximal concentration; MRT, mean residence time; T_{max}, the time to reach C_{max}; $t_{1/2,â}$, the elimination half-life.

pharmacokinetic studies. By using this method, we can make routine analysis of many samples per day, and reaction mixtures have advantages of preparing in aqueous medium. The linearity, reproducibility, and accuracy are enough to study pharmacokinetics of ALC in humans.

The baseline level of ALC in the blank plasma was 1.30 ± 0.04 μg/mL. And it maintained considerably high concentrations and was constant among human volunteers. Parnetti *et al.* (1992) also reported that the plasma concentrations of individual components of the L-carnitine family were not changed during the 3 days of the basal observation period, as 1.43-1.53 ng/mL (6.9-7.49 nmol/mL). While ALC levels showed a wide range of variation as 1.63-15.1 ug/mL (8-74 nmol/mL) according to the report of Lambert *et al.* (1998). Cugini *et al.* (2002) reported that L-carnitine showed peak levels in the afternoon, suggesting variability by the circardian rhythm. However, in another report of Rebuzzi *et al.* (1989), the diurnal serum ALC levels at the morning were not significantly different from those at the afternoon. The most important data were showed that ALC and L-carnitine levels were relatively constant during a 24 h period after HPLC analysis of the sample collected at every 2 h interval until 12 h and then 24 h (Longo *et al.*, 1996). Our study was designed, in many parts, based on the data of Longo *et al.* (1996). Even though the homeostatic equilibrium between ALC and L-carnitine is maintained in the body, the circardian rhythmic phenomena of blood ALC levels were greatly ambiguous yet, and remained to be clear.

The baseline levels of ALC in our results were very similar to those reported by Parnetti *et al.*(1992), even if they have used radioenzyme assays for the determination of plasma ALC.

The half-life at terminal phase was about 4.2 h, which was about 2 times longer than the reported value in patients with senile dementia of Alzheimer's type (1.73 ± 0.29 h; Parnetti *et al.*, 1992). It is interesting that the half-life in Alzheimer's patients is longer than that in healthy volunteers. This may be due to the difference of age, dosing (single *vs.* multiple) and renal function. Kelly *et al* (1990) reported that in patients with renal-impairment, the larger dose resulted in greater elimination. As in ALC concentration-time curves (Fig. 3), $AUC_{0\rightarrow8}$ determined from uncorrected plasma ALC concentrations (46.47 μg/mL·h) was 4.7 times higher than that of corrected plasma $ALC_{0\rightarrow8}$ concentrations (9.88 μg/mL·h). The maximal concentration (C_{max}) in uncorrected plasma ALC concentrations was twice higher than that of corrected results (1.19 vs. 2.17 μg/mL).

This data may be one of the example that corrected value of plasma ALC concentrations must be used for the determination of pharmacokinetic parameters because the baseline level of endogenous ALC is relatively very high. This study first describes the pharmacokinetic study after oral administration of a single dose of naturally occurring ALC in human volunteers. Therefore, these results indicate that the developed assay and the correction method are practically relevant in the bioequivalence study for ALC.

ACKNOWLEDGMENT

This study was supported by a National Research Laboratory (NRL) program (#M1-0302-00-0069) from the Ministry of Science and Technology of the Republic of Korea.

REFERENCES

Bremer, Carnitine-metabolism and functions. *Pharmacol. Rev.*, 63, 1420-1480 (1983).

Cugini, P., Danese, C., Colotto, M., Arceri, D., Angrisani, L., Colosi, L., and De Rosa, R., Circardian rhythm of serum levocarnitine: parameters. *Clin. Ther.*, 153, 237-242 (2002).

Evans, A. M., Faull, R., Fornasini, G., Lemanowicz, E. F., Longo, A., Pace, S., and Nation, R. L., Pharmacokinetics of L-carnitine in patients with end-stage renal disease undergoing long-term hemodialysis. *Clin. Pharmacol. Ther.*, 68, 238-249 (2000).

Falchetto, S., Kato, G., and Provini, L., The action of carnitine on cortical neurons. *Can. J. Physiol. Pharmacol.*, 49, 1-7 (1971).

Fritz, I. B. and Arrigoni-Martelli, E., Sites of action of carnitine and its derivatives on the cardiovascular system: interactions with membranes. *Trend Pharmacol. Sci.*, 14, 355-360 (1993).

Fritz, I. B. and Marquis, N. R., The role of acylcarnitine esters and carnitine palmityltransferase in the transport of fatty acyl groups across mitochondrial membranes. *Proc. Natl. Acad. Sci. U.S.A.*, 54,1226-1233 (1965).

Janiri, L., Falcone, M., Persico, A., and Tempesta, E., Activity of L-carnitine and L-acetylcarnitine on cholinoceptive neocorical

neurons of the rat *in vivo*. *J. Neural. transm.*, 86, 135-146 (1991).

Kagawa, M., Machida, Y., and Nishi, H., Enantiomeric purity determination of acetyl-L-carnitine by reversed-phase high-performance liquid chromatography using chiral derivatization. *J. Chromatogr. A.*, 857, 127-135 (1999).

Kamimori, H., Hamashima, Y., and Konishi, M., Determination of carnitine and saturated-acyl group carnitines in human urine by high-performance liquid chromatography with fluorescence detection. *Anal. Biochem.*, 218, 417-424 (1994).

Kelly, J. G., Hunt, S., Doyle, G. D., Laher, M. S., Carmody, M., Marzo, A., and Arrigoni Martelli, E., Pharmacokinetics of oral acetyl-L-carnitine in renal impairment. *Eur. J. Clin. Pharmacol.*, 38, 309-312 (1990).

Lambert, M. E., Shipley, K., Holbrook, I., Faragher, E. B., and Irving, M. H., Serum carnitine levels in normal individuals. *JPEN J. Parenter Enteral Nutr.*, 12, 143-146 (1988).

Longo, A., Bruno, G., Curti, S., Mancinelli, A., and Miotto, G., Determination of L-carnitine, acetyl-L-carnitine and propionyl-L-carnitine in human plasma by high-performance liquid chromatography after pre-column derivatization with 1-aminoanthracene. *J. Chromatogr. B. Biomed. Appl.*, 686, 129-139 (1996).

Marzo, A., Arrigoni Martelli, E., Urso, R., Rocchetti, M., Rizza, V., and Kelly, J. G., Metaboilsm and disposition of intravenously administered acetyl-L-carnitine in healthy volunteers. *Eur. J. Clin. Pharmacol.*, 37, 59-63 (1989).

Millington, D. S., Norwood, D. L., Kodo, N., Roe, C. R., and Inoue, F., Application of fast atom bombardment with tandem mass spectrometry and liquid chromatography/mass spectrometry to the analysis of acylcarnitines in human urine, blood, and tissue. *Anal. Biochem.*, 180, 331-339 (1989).

Minkler, P. E. and Hoppel, C. L., Quantitation of free carnitine, individual short- and medium-chain acylcarnitines, and total carnitine in plasma by high-performance liquid chromatography. *Anal. Biochem.*, 212, 510-518 (1993).

Minkler, P. E., Brass, E. P., Hiatt, W. R., Ingalls, S. T., and Hoppel, C. L., Quantitation of carnitine, acetylcarnitine, and total carnitine in tissues by high-performance liquid chromatography: the effect of exercise on carnitine homeostasis in man. *Anal. Biochem.*, 231, 315-322 (1995).

Pace, S., Longo, A., Toon, S., Rolan, P., and Evans, A. M., Pharmacokinetics of propionyl-L-carnitine in humans: evidence for saturable tubular reabsorption. *Br. J. Clin. Pharmacol.*, 50(5), 441-448 (2000).

Parnetti, L., Giati, A., Mecocci, P., Cadini, D., and Sinin, U., Pharmacokinetics of IV and oral acetyl-L-carnitine in a multiple dose regimen in patients with senile dementia of Alzheimer type. *Eur. J. Clin. Pharmacol.*, 42, 89-93 (1992).

Passeri, M., Crucinotta, D. Bonati, P. A., Iannuccelli, M., Parnetti, L., and Senin, U., Acetyl-L-carnitine in the treatment of mildly demented elderly patients. *Int. J. Clin. Pharmacol. Res.*, 10, 75-79 (1990).

Passeri, M., Iannuccelli, M., Ciotti, G., Bonati, P. A., Nolfe, G., and Crucinotta, D., Mental impairment in aging: selection of patients, methods of evaluation and therapeutic possibilities of acetyl-L-carnitine. *Int. J. Clin. Pharmacol. Res.*, 8, 367-376 (1988).

Piovesan P., Quatrini, G., Pacifici, L., Taglialatela, G., and Angelucci, L., Acetyl-L-carnitine restores choline acetyltransferase activity in the hippocampus of rats with partial unilateral fimbra-fornix transsection. *Int. J. Dev. Neurosci.*, 13, 13-19 (1995).

Poorthuis, B. J., Jille-Vlckova, T., and Onkenhout, W., Determination of acylcarnitines in urine of patients with inborn errors of metabolism using high-performance liquid chromatography after derivatization with 4'-bromphenacylbromide. *Clin. Chim. Acta*, 216 (1-2), 53-61 (1993).

Rebuzzi, A. G., Schiavoni, G., Lanza, G. A., Amico, C., Natale, A., Lucente M., and Manzoli, U., Rhythm of carnitine levels in serum and urine of normal subjects. *Drugs Exp. Clin. Res.*, 15, 43-46 (1989).

Shargel, L. and Yu, A. B. C., Chapter 9. Pharmacokinetics of drug absorption. In *Applied Biopharmacokinetics and Pharmacokinetics*, 3rd ed., Appleton & Lange, Nowwalk, USA, 169-192 (1993).