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Pharmacokinetics of IV and oral acetyl-L-carnitine in a multiple dose regimen in patients with senile dementia of Alzheimer type

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Summary. Acetyl-L-carnitine (ALC), a physiological component of the L-carnitine family, has been proposed for treating Alzheimer's disease in pharmacological doses. As this condition requires prolonged therapy, its kinetics has been examined after a multiple dose regimen, involving different routes of administration, in 11 patients suffering from Senile Dementia of Alzheimer Type.

The study design comprised a 3-day basal observation period, sham treatment with repeated blood sampling; treatment with 30 $mg \cdot kg^{-1}$ i.v. given twice for 10 days (plasma kinetics was studied on the 7th day), and 50 days of 2.0 g/day p.o. given in three daily doses. Total acid soluble L-carnitine, L-carnitine and acetyl-L-carnitine in plasma and CSF were evaluated using an enantioselective radioenzyme assay. Short chain L-carnitine esters were calculated as the difference between total and free-L-carnitine.

The plasma concentrations of individual components of the L-carnitine family did not change during the three days of the basal period, nor were they affected during the sham therapy period.

Following the i.v. bolus injections, the plasma concentrations showed a biphasic curve, with average $t_{1/2}$ of 0.073 h and 1.73 h, respectively.

At the end of oral treatment, plasma acetyl-L-carnitine and L-carnitine short chain esters were significantly higher than during the run-in phase.

The CSF concentrations paralleled those in plasma, suggesting that ALC easily crosses the blood-brain barrier.

It is concluded that i.v. and oral administration of multiple doses of ALC can increase its plasma and CSF concentration in patients suffering from Alzheimer's disease.

Key words: Acetyl-L-carnitine, Senile Dementia of Alzheimer Type; pharmacokinetics, plasma concentration, cerebrospinal fluid concentration, carnitine metabolism.

Acetyl-L-carnitine (ALC) is a naturally occurring molecule synthesized from L-carnitine (LC) by carnitine acetyl transferase [Hosein et al., 1966]. From the clinical point of view ALC has been shown to improve the cognitive performance of patients suffering from dementia of Alzheimer type (SDAT) [Hiersemenzel et al., 1988; Cucinotta et al., 1988]. Several studies have supported its involvement in cholinergic neurotransmission [Fritz, 1963; Falchetto et al., 1971; Onofri et al. 1983]. Other recent studies have indicated a possible neuronotrophic mechanism of ALC, since it is able to increase hippocampal binding of glucocorticoids [Angelucci et al., 1986] and of nerve growth factor (NGF) [Perez Polo et al., 1988]. This would support the existence of a modulatory effect of ALC on the physical and biochemical mechanisms of stress [Angelucci et al., 1988; Parnetti et al., 1990]. In animals treated with ALC reduced formation of lipofuscin and free radicals has been documented [Fariello et al., 1988], and it seems that these "anti-aging" properties of the molecule are particularly evident in hippocampus [Calvani and Carta, 1991], which is a very important integrative area for the systems involved in memory processes and in neuroendocrine regulation [Nappi et al., 1988; Petraglia et al., 1988].

The pharmacokinetics of ALC has been investigated in healthy volunteers and in dogs after single i.v. and oral doses [Marzo et al., 1988, 1989], and in patients suffering from renal failure [Kelly et al., 1990]. The studies demonstrated that after administration the plasma ALC concentrations and L-carnitine reached an equilibrium via the action of carnitine acetyl-transferase, and that both were excreted in urine by a process which involved a tubular reabsorption with a threshold higher for L-carnitine than for ALC. This means that the renal clearance of these substances is increased as the plasma concentration is increased.

The present report describes an investigation into the plasma and CSF concentrations of ALC, total acid soluble L-carnitine (TC) and free L-carnitine (LC) in patients suffering from senile dementia of Alzheimer type treated with multiple i.v. and oral doses of ALC.

Table 1. Treatment scheme

Periods	Days	Treatment	Blood and CSF Sampling
A	T-3/T-2/T-1	None	8.00 a.m.
B	T0	0.9% saline i.v. at 8.00 h (= time zero)	0, 5, 15, 30, 45 min; 1, 2, 4, 12, 24 h[a]
C	T1→T10	ALC.HCl 15 mg/kg i.v. in 50 ml of 0.9% saline solution infused over 5 min. On the 7th day only one dose of 30 mg/kg was given at 08.00 h.	On the 7th day: 0, 5, 15, 30, 45 min, 1, 2, 4, 12, 24 h
D	T11→T60	ALC.HCl 2000 mg/day p.o. divided in three daily doses	T11, T12, T35, T60 at 08.00 h

[a] Note: Time 0 is before the start of the infusion
Time 5 min is 5 min from completion of the infusion etc, as are the other samples

Table 2. Mean (SD) plasma concentrations ($nmol \cdot ml^{-1}$) of L-carnitine (LC), total acid soluble L-carnitine (TC), acetyl-L-carnitine (ALC) and short chain L-carnitine esters (SCCE) relative to Period A in 11 SDAT patients

	T-3	T-2	T-1
LC	43.38 (6.63)	44.12 (6.17)	42.80 (6.49)
TC	50.88 (7.06)	52.32 (6.41)	50.70 (7.12)
ALC	6.90 (2.26)	7.49 (2.01)	7.19 (2.68)
SCCE	7.50 (2.50)	8.18 (1.89)	7.90 (2.90)

Subjects and methods

Eleven in-patients (3 m, 8 f, aged 70–86 y) suffering from probable Alzheimer's disease according to the NINCDS-ADRDA criteria [McKhann et al., 1984] were enrolled in the study, after informed consent had been obtained from the patient and his/her care-giver. The degree of mental deterioration was severe. The clinical onset of dementia was after 65 y. Each patient had a cerebral CT scan, in which focal lesions were not seen.

The patients underwent a wash-out period of at least 14 days in case they had been on cerebro-active treatment. They were treated with ALC intravenously and orally as shown in Table 1.

The study was divided into four periods (A-D) as shown in Table 1.

For the i.v. injection vials from lots 238 FI, 67/6409 and 18/7671 were used. The solution was prepared immediately before administration by dissolving the ALC.HCl powder in a sterile solution, diluting it with 0.9% saline 50 ml and infusing it over 5 min. The dose was calculated on the basis of body weight and is expressed as ALC inner salt.

Tablets for oral administration contained an amount of ALC.HCl corresponding to 500 mg ALC inner salt. The daily dose of 2.0 g was given as follows: 500 mg at 08.00 h, 1.0 g at 12.00 h and 500 mg at 16.00 h.

Clinical examination including ECG and routine blood analyses was carried out before treatment, and at the end of the i.v. treatment (Day 11) and the oral treatment (Day 60) in order to ascertain the tolerability of the therapy.

Blood samples (7 ml) collected in heparin were centrifuged at $2500 \times g$ for 10 min, and the plasma was stored at $-20\,^{\circ}C$ until assayed. After the patients and/or their relatives had given informed consent, a lumbar puncture was performed between 08.00 and 09.00 h, after overnight bedrest. The method was always the same: while the patient was still in bed the puncture was performed using a needle of I.D. 0.7 mm, after skin sterilization and local anaesthesia with 2 ml lidocaine. CSF 12 ml was collected in a tube on ice and was immediately stored at $-80\,^{\circ}C$ until assayed.

Total acid soluble L-carnitine, (TC), free L-carnitine (LC) and acetyl-L-carnitine (ALC) in plasma and CSF samples were evaluated by highly sensitive, enantioselective radioenzyme assays [Marzo et al., 1990].

The difference between TC and free LC was taken as the value of short chain L-carnitine esters (SCCE).

Statistical analysis

Means and standard deviations were calculated by standard procedures [Kirk, 1982]. Data were compared by randomized block analysis of variance (ANOVA).

Final versus basal plasma concentrations were compared both by randomized block ANOVA and Student's "t" test for paired samples; in the latter case the mean value observed during the three days of Period A was compared with that on the last day of treatment.

The plasma concentrations of ALC on the 7th day of Period C were fitted to an open two-compartment model for i.v. injection [Gibaldi and Perrier, 1982]:

$$Cp\,(t) = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t}$$

where
A and B are the intercepts on the concentration axis of the two exponential slopes
α and β are the angular parameters of the two exponential slopes
Cp (t) is the plasma concentration at the generic time "t".

Results

Period A

The plasma concentration of LC, TC, ALC and SCCE during the three days of baseline observation, are given in Table 2.

Period B

The plasma concentration-time behaviour of LC, TC, ALC and SCCE in the sham-treated patients showed only marginal fluctuations, which did not show any time-related significance according to the randomized block ANOVA (Table 3).

Period C

Similarly, the plasma concentration-time behaviour of LC, TC, ALC, and SCCE in patients treated with i.v. ALC 30 $mg \cdot kg^{-1}$ is presented in Table 4.

Table 3. Mean (SD) plasma concentrations of l-carnitine (LC), total acid soluble L-carnitine (TC), acetyl-L-carnitine (ALC) and short chain carnitine esters (SCCE) relative to Period B (nmol · ml^{-1})

	0	5 min	15 min	30 min	45 min	1 h	2 h	4 h	12 h	24 h
LC	38.9 (5.13)	38.6 (5.00)	39.3 (6.50)	39.6 (6.20)	39.0 (5.14)	39.6 (5.41)	37.2 (5.19)	37.9 (5.77)	39.4 (8.96)	41.3 (9.75)
TC	45.6 (6.12)	46.2 (5.93)	46.5 (7.43)	46.4 (7.53)	45.1 (6.40)	46.0 (6.92)	43.2 (6.44)	44.6 (6.43)	46.4 (9.88)	48.9 (11.1)
ALC	6.39 (1.77)	6.85 (2.12)	6.67 (2.14)	6.36 (1.96)	5.87 (1.70)	5.74 (1.72)	5.30 (1.66)	5.99 (2.70)	6.25 (2.51)	7.13 (2.85)
SCCE	6.79 (1.73)	7.56 (1.88)	7.22 (2.33)	6.85 (1.91)	6.19 (1.68)	6.40 (1.78)	5.94 (1.48)	6.71 (2.51)	6.96 (2.38)	7.59 (2.87)

Table 4. Mean (SD) plasma concentration-time behavior of L-carnitine (LC), total acid soluble L-carnitine (TC), acetyl-L-carnitine (ALC) and short chain L-carnitine esters (SCCE) relative to Period C (nmol · ml^{-1})

	0	5 min	15 min	30 min	45 min	1 h	2 h	4 h	12 h	24 h
LC	55.7 (9.55)	185 (56.8)	131 (28.1)	127 (24.0)	124 (25.3)	126 (22.9)	117 (24.4)	104 (18.8)	67.4 (13.9)	53.4 (8.63)
TC	66.0 (10.0)	1270 (600)	660 (123)	548 (135)	497 (99.1)	458 (81.4)	332 (71.6)	210 (46.2)	84.1 (18.1)	64.2 (10.5)
ALC	9.70 (2.97)	1000 (476)	496 (118)	394 (110)	330 (79.9)	305 (67.3)	201 (58.3)	102 (32.4)	15.6 (5.68)	9.84 (2.99)
SCCE	10.3 (3.17)	1080 (549)	529 (115)	422 (120)	373 (88.0)	332 (70.1)	215 (58.3)	106 (31.7)	16.6 (6.18)	10.9 (2.98)

Table 5. Main pharmacokinetic parameters obtained by fitting plasma ALC concentrations from the 11 patients on the 7th day of Period C, i.e. after 30 mg · kg^{-1} i. v. Data fitting was carried out on the net values obtained subtracting the basal concentrations

Patients	AUC nmol · ml^{-1}	$t_{1/2\,\alpha}$ (h)	$t_{1/2\,\beta}$ (h)	A nmol · ml^{-1}	α (h^{-1})	B nmol · ml^{-1}	β (h^{-1})	last t[a] (h)
1	1800	0.014	1.47	2150	48.4	395	0.469	4.0
2	802	0.076	1.47	737	9.03	311	0.468	4.0
4	1710	0.058	1.56	2720	11.9	548	0.443	12.0
5	1520	0.045	1.74	3210	15.2	424	0.396	12.0
6	776	0.111	1.41	413	6.20	262	0.489	12.0
7	1120	0.047	1.69	893	14.4	416	0.407	4.0
8	1850	0.052	1.72	1170	19.1	589	0.401	12.0
9	1880	0.122	2.18	695	5.64	492	0.316	12.0
10	1520	0.168	1.77	355	4.10	463	0.389	12.0
11	2110	0.039	2.26	6640	17.5	448	0.306	12.0
Mean	1510	0.073	1.73	1900	14.6	434	0.408	
(SD)	(463)	(0.047)	(0.29)	(1940)	(12.7)	(98)	(0.62)	

[a] last T = the last time used in data fitting
Plasma concentration of Patient 3 did not fit the relationship

Plasma of ALC at the first sampling time after administration (5 min) averaged 1000 nmol · ml^{-1}, which was more than 100-times the basal value of 9.70 nmol · ml^{-1}.

ALC levels then decreased biexponentially. The plasma concentration-time data were analysed by the open two compartment model (Table 5). The two phases had average $t_{1/2}$ of 0.073 and 1.73 h, respectively. A particular problem in the kinetic analysis arose from the need to subtract the baseline concentration, which fluctuated to a certain extent. The plasma level had usually been restored to normal within a 4–12 h period.

Plasma concentrations of LC, TC and SCCE also showed the highest values at the first sampling time, 5 min, and then decreased like ALC in the case of SCCE, but showing more sustained levels of LC and TC.

Plasma concentrations during the multiple dose regimen (T8, T9, T10 of the Periods C and D up to) (T60)

Plasma levels of the endogenous components of the L-carnitine family in the period T8–T60 are shown in Table 6.

The plasma concentrations of all the substances evaluated were decreased when i. v. was replaced by oral administration. The lowest value occurred on the last day at T60. In the case of ALC and SCCE, the concentrations at T60 were higher than those observed at T-3, T-2 and T-1 in the preliminary period (significant by both randomized block ANOVA and Student's "t" test for paired samples; Table 7).

Table 6. Mean (SD) plasma concentration (nmol · ml^{-1}) of L-carnitine (LC), total acid soluble L-carnitine (TC), acetyl-L-carnitine (ALC) and short chain L-carnitine esters (SCCE) during multiple dose therapy. (T8–T10 i.v.; T11–T60 oral route)

	Period C			Period D			
	T8	T9	T10	T11	T12	T35	T60
LC	178 (83.3)	132 (45.9)	166 (50.7)	58.0 (12.0)	55.7 (10.7)	46.3 (8.82)	43.9 (10.5)
TC	928 (509)	612 (219)	965 (452)	71.9 (13.1)	66.4 (11.1)	57.4 (9.04)	55.5 (10.9)
ALC	722 (425)	445 (154)	737 (360)	12.4 (3.57)	9.77 (2.24)	9.72 (2.94)	10.3 (3.72)
SCCE	750 (430)	480 (176)	799 (409)	13.9 (4.26)	10.7 (2.38)	10.9 (3.14)	11.6 (3.74)

Table 7. Comparison of basal concentrations (T-3, T-2, T-1) versus T60 concentration of L-carnitine family compounds according to ANOVA and Student's "t" test for paired samples (nmol · ml^{-1})

	T-3	T-2	T-1	Mean	T60	ANOVA	"t" Student
LC	43.4	44.1	42.8	43.4	43.9		
TC	50.9	52.3	50.7	51.3	55.5		
ALC	6.90	7.49	7.19	7.19	10.3	$P < 0.01$	$P < 0.05$
SCCE	7.50	8.18	7.90	7.86	11.6	$P < 0.01$	$P < 0.05$

Table 8. Mean (SD) CSF concentrations (nmol · ml^{-1}) of L-carnitine (LC), total acid soluble L-carnitine (TC), acetyl-L-carnitine (ALC) and short chain L-carnitine esters (SCCE) in patients in the trial under basal condition (T-3), on the last day of i.v. treatment (T10) and on the last day of oral treatment (T60) with acetyl-l-carnitine

		T-3 ($n = 9$)	T10 ($n = 9$)	T60 ($n = 7$)
LC	Mean (SD)	1.48 (0.35)	3.66 (0.79)	1.48 (0.19)
TC	Mean (SD)	2.50 (0.52)	7.03[a] (1.89)	2.81 (0.41)
ALC	Mean (SD)	0.93 (0.30)	3.55[a] (1.47)	1.23[b] (0.37)
SCCE	Mean (SD)	1.02 (0.27)	3.64 (1.27)	1.33 (0.34)

[a] $P < 0.01$, [b] $P < 0.05$ compared to T-3 (Student's "t" test for paired samples)

CSF concentrations

The CSF concentrations of the substances in the L-carnitine family at T-3, T10 and T60 are listed in Table 8.

The CSF levels of LC, TC, ALC and SCCE were increased at T10 and were higher at T60 than at T-3 but to a lesser extent than at T10.

Discussion

Acetyl-L-carnitine is an endogenous component of the L-carnitine family. If the pool is altered, the body itself re-establishes homeostatic equilibrium of the L-carnitine family by two mechanisms which operate simultaneously. L-carnitine becomes equilibrated with its endogenous esters via tissue transferases. L-carnitine and the esters are then excreted by the kidney, with a saturable tubular reabsorption process, which has different thresholds for each component. This was described by Marzo et al. in a study of the pharmacokinetics and metabolism of ALC in the dog [Marzo et al., 1988], and in healthy volunteers [Marzo et al., 1989]. The present report shows that the mechanism described in healthy volunteers also operates in patients suffering from dementia of Alzheimer type, in which plasma carnitine is rapidly equilibrated with injected ALC, as shown by the detection of the peak plasma LC 5 min after the i.v. dose. As carnitine-acetyl-transferase operates in tissues, mainly in the skeletal muscle and myocardium, the equilibrium observed between ALC and LC in the CNS must be considered a rapid process.

As the patients here were severely demented, cumulative collection of urine was not possible, so data on the amount excreted and clearance could not be obtained. However, the fact that after the multiple dose regimen, plasma ALC and SCCE were significantly increased is note worthy.

An important problem is whether an increase in plasma ALC levels may also affect its CSF concentration. The present results demonstrate that the administration of ALC caused an increase of ALC in SDAT patients and a parallel increase in the CSF level, thus showing that ALC readily crosses the blood-brain barrier. This is important because ALC acts as a cholinergic agonist in the CNS.

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References

Angelucci L, Patacchioli FR, Tagliatela G et al (1986) Brain glucocorticoid receptor and adrenocortical activity are sensitive markers of senescence-retarding treatments in the rat. In: Biggio G,

Spano PF, Toffano G et al (eds) Modulation of central and peripheral transmitter function. Fidia Research Series, Symposia in Neuroscience. Padova, Liviana Press/Berlin, Springer, 3: 338–343
Angelucci L, Ramacci MT, Amenta F et al (1988) Acetyl-L-carnitine in the rat's hippocampus aging. Morphological, endocrine and behavioural correlates. In: Gorio A, Perez Polo JR, De Velis J et al (eds) Neural Development and regeneration. NATO ASI Series. Springer, Berlin, H22: 57–66
Calvani M, Carta A (1991) Clues to the mechanism of action of acetyl-L-carnitine in the central nervous system. Dementia 2: 1–6
Cucinotta D, Passeri M, Ventura S et al (1988) Multicenter clinical placebo-controlled study with acetyl-L-carnitine in the treatment of mildly demented elderly patients. Drug Dev Res 14: 213–216
Falchetto S, Kato G, Provini L (1971) The action of carnitines in cortical neurons. Can J Physiol Pharmacol 49: 1–7
Fariello RG, Ferraro TN, Golden GT et al (1988) Systemic acetyl-L-carnitine normalizes pituitary-adrenocortical hyperactivity in pathological ageing brain. Med Sci Res 16: 291–292
Fritz JB (1963) Carnitine and its role in fatty acids metabolism. Adv Lipid Res 1: 285–334
Gibaldi M, Perrier D (1982) Pharmacokinetics, 2ed. Dekker, New York
Hiersemenzel R, Dietrich B, Hermann WM (1988) Therapeutic and EEG-effects of acetyl-L-carnitine in elderly outpatients with mild to moderate cognitive decline. Results of two double-blind placebo-controlled studies. In: Agnoli A, Cahn J, Lassen N et al (eds) Senile dementias. 2nd Int Symp J Libbey, Paris, pp 427–431
Hosein EA, Both SJ, Gasoi I et al (1966) Neuromuscular blocking activity and other pharmacological properties of various carnitine derivatives. J Pharmacol Exp Ther 156: 565–572
Kelly JG, Hunt S, Doyle GD et al (1990) Pharmacokinetics of oral acetyl-L-carnitine in renal impairment. Eur J Clin Pharmacol 38: 309–312
Kirk RE (1982) Experimental design. Procedures for behavioural sciences. Brooks-Cole, Publ Co, 2nd Edition
Marzo A, Arrigoni Martelli E, Cardace G et al (1988) Pharmacokinetics of acetyl-L-carnitine after i.v. administration in healthy volunteers and dogs. Troisième Journées Mediterranéennes de Pharmacocinétique. Cap D'Adge: 19–21
Marzo A, Arrigoni Martelli E, Urso R et al (1989) Metabolism and disposition of intravenously administered acetyl-L-carnitine in healthy volunteers. Eur J Clin Pharmacol 37: 59–63
Marzo A, Cardace G, Monti N et al (1990) Chromatographic and non chromatographic assay of L-carnitine family components. J Chromatogr 527: 247–258
McKhann, Drachman D, Folstein M et al (1984) Clinical diagnosis of Alzheimer's disease. Report of the NINCDS-ADRDA Work Group under the auspices of the Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34: 939–944
Nappi G, Martignoni E, Sinforiani E et al (1988) Acetyl-L-carnitine normalizes pituitary-adrenocortical hyperactivity in pathological ageing brain. Med Sci Res 16: 291–292
Onofri M, Bodis-Wollner I, Pola P et al (1983) Central cholinergic effects of levo-acetylcarnitine. Drugs Exp Clin Res 9: 161–169
Parnetti L, Gaiti A, Mecocci P et al (1990) Effect of acetyl-L-carnitine on serum levels of cortisol and adrenocorticotropic hormone and its clinical effect in patients with senile dementia of Alzheimer Type. Dementia 1: 165–168
Perez Polo JR, Werrbach-Perez K, Ramacci MT et al (1988) Role of nerve growth factors in neurological disease. In: Agnoli A, Cahn J, Lassen N et al (eds) Senile dementias. 2nd. International Symposium. Libbey, Paris, pp 15–25

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