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High doses of L-carnitine in acute myocardial infarction: metabolic and antiarrhythmic effects

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Fatty acids accumulate in the muscle cells in some carnitine deficiency syndromes due to a variety of genetic defects in intermediary metabolism. L-Carnitine administration may relieve this excess by transporting acyl compounds out of the cell as acylcarnitine. Similar fatty acid accumulation occurs during myocardial ischaemia because of the decreased rate of β-oxidation, and this has been put forward as a cause of ventricular arrhythmias. This study was carried out to investigate whether administration of high doses of i.v. L-carnitine in patients with acute myocardial infarction could increase urinary excretion of acylcarnitine and reduce early ventricular arrhythmias.

Fifty-six patients suffering from acute myocardial infarction, admitted to the Coronary Unit between 3 and 12 h after the onset of symptoms, were included in the study.

The design of the study was double blind, parallel and placebo controlled. Allocation of treatment to patients was done randomly after stratification (time from onset of pain and site of infarction). The first group (28 patients) received intravenous L-carnitine at a dose of 100 mg kg^{-1} b.w. every 12 h for 36 h while the second group (28 patients) received placebo intravenously. Immediately before starting treatment two blood samples were taken (at 5-min intervals) and a further 16 samples were taken at regular intervals over the following 48 h. Patients' urine was collected over the same period of time. Concentrations of free carnitine, short chain acylcarnitine esters and long chain acylcarnitine esters in serum and urine were measured. On days 1 and 2, 24-h ECG monitoring (Holter) was carried out.

Analysis of results showed that: (1) in patients receiving placebo, free carnitine serum levels increased significantly during the first 48 h after infarction; (2) in the same group of patients, free and total urinary carnitine excretion was significantly higher than in normal subjects; (3) administration of high doses of L-carnitine considerably increased urinary excretion of long and short chain carnitine esters; (4) this metabolic effect might explain the reduction in premature ventricular beats on the second day of treatment.

Introduction

The physiological role of L-carnitine is to transfer acyl compounds (fatty acids) from the cytosol to the mitochondrial matrix where they are oxidized. When β-oxidation is impaired, for example when there are genetic defects of intermediary metabolism, L-carnitine may relieve the pathological accumulation of acyl-CoA esters by extracting the acyl compounds from coenzyme A. Excess acylcarnitine esters may then be transported out of the mitochondrion and the cell: if they are preferentially excreted, a relative deficiency of carnitine is created[1]. This peculiar detoxifying activity of L-carnitine is explained by the fact that fatty acids are only excreted in the urine as acylcarnitine esters. Abnormally high concentrations of acyl-CoA esters also occur in the myocardial cells during ischaemia and this is the consequence of reduced fatty acid oxidation due to lack of oxygen. The myocardial fatty acid accumulation has been put forward as a cause of both conduction disturbances[2] and ventricular arrhythmias[3–8]. Free carnitine deficit has

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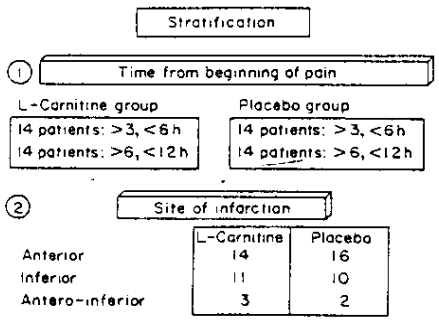
Table 1 Number and general characteristics of patients

	L-Carnitine	Placebo
Number	28	28
Age (years)	60±10	60±8
Sex	19(M)+9(F)	23(M)+5(F)
Weight (kg)	70±12	75±12
Height (cm)	166±9	168±6

Table 2 Stratification criteria and number of patients in each group



been repeatedly observed in the experimental ischaemic heart as well as in the myocardium of patients with cardiac infarction[9].

The present study was carried out to assess whether, and to what extent, administration of high doses of i.v. L-carnitine in patients with acute myocardial infarction could: (1) increase production and urinary excretion of acyl carnitine; (2) reduce early ventricular arrhythmias.

Materials and methods

PATIENTS

Fifty-six patients with acute myocardial infarction (AMI) were enrolled in the study. Inclusion criteria were: transmural myocardial infarction, sinus rhythm, interval between beginning of pain and hospital admission of 3–12 h, age < 75 years. Exclusion criteria were: previous myocardial infarction; atrio-ventricular conduction defects, complete bundle branch blocks, Killip class III, IV.

The experimental design was double blind with two parallel groups. Patients were allocated at random to treatment with L-carnitine* or placebo after stratification based on two relevant factors: time between onset of pain and treatment (3–6 or 6–12 h), and site of infarction (anterior, inferior or antero-inferior) (Tables 1 and 2).

Patients arriving in the Coronary Unit before the 3rd hour were not included in the study since they received thrombolytic treatment. Patients in one group received 100 mg kg^{-1} b.w. of i.v. L-carnitine every 12 h for 36 h (four administrations), while patients in the other group received placebo. The protocol did not allow for antiarrhythmic prophylaxis, though antiarrhythmic therapy could be given in cases of threatening arrhythmias or arrhythmias which might cause haemodynamic impairment.

*The vials of L-carnitine (1 g) were supplied by Sigma-Tau S.p.A., Pomezia, Rome, Italy.

SERUM AND URINARY CARNITINE

Blood samples to determine free carnitine (FC), short chain acylcarnitine esters (SCACE) and long chain acyl carnitine esters (LCACE)[9–13] were taken 5 min before and immediately before starting treatment and then 2, 4, 8, 12, 14, 16, 20, 24, 26, 28, 32, 36, 38, 40, 44 and 48 h after starting. In order to determine urinary carnitine levels[9–13], urine samples (10 ml) were collected at each urination during the 48 h of the study. These samples, stored at −20 °C, were then analysed for their FC, SCACE and LCACE content. Results were expressed in μmol $(24\ h)^{-1}$, by adding together the quantities measured in each sample, and correcting for the volume of each single urination.

Blood samples for carnitine assays were also taken from 28 healthy control subjects (18 M and 10 F, aged 45 ± 12 years). The urine of 11 of these subjects was also collected over a 24-h period to measure urinary carnitine excretion.

ANALYSIS OF VENTRICULAR ARRHYTHMIAS

All patients underwent a continuous electrocardiographic (Holter) recording during the first 48 h after admission to the CCU. Recordings were made on an Avionics 445-B twin channel instrument, using V_2 and V_5 leads. The analysis of tapes was carried out on an Avionics-Trendsetter with computerized reports of heart rate, frequency of premature ventricular beats (PVB), paired PVBs and ventricular tachycardia (VT) defined as ⩾3 consecutive PVBs at a rate of ⩾100 min^{-1}.

STATISTICAL ANALYSIS

The following tests were used: the chi-squared test; the Mann-Whitney U test for data measured

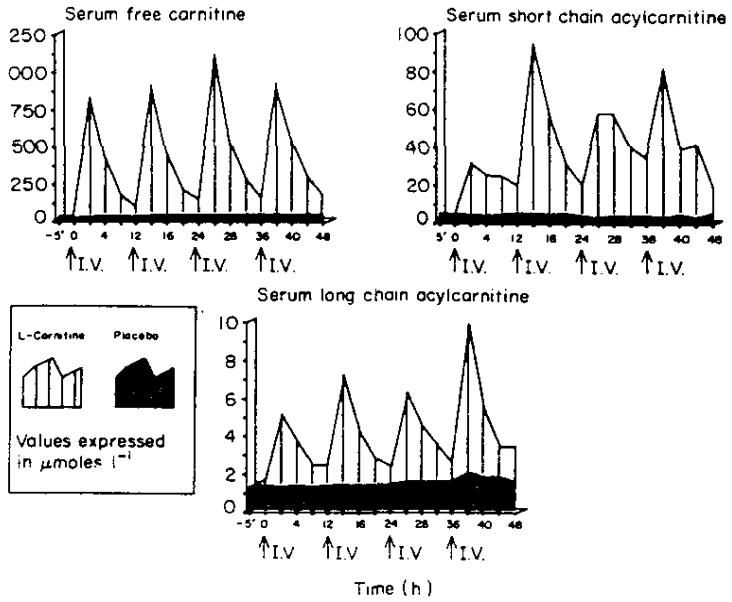


Figure 1 Serum carnitine (free, short and long chain esters) during the first 48 h after admission to the Coronary Unit in the two groups of patients — AMI treated with either L-carnitine or placebo. After starting treatment, the levels measured in the L-carnitine group were always significantly different with respect to the placebo group ($P<0{\cdot}001$). Normal values (28 subjects): Free carnitine 43 ± 8 μmol l^{-1}; SCACE $3{\cdot}6\pm2{\cdot}6$ μmol l^{-1}; LCACE $2{\cdot}3\pm1{\cdot}1$ μmol l^{-1}.

on a nominal scale; Student's two-tailed *t*-test for paired and unpaired data; and two-way variance analysis (ANOVA) for data which could be measured on a continuous or interval scale. The data from the last two tests were expressed as mean ± standard deviation. Student's *t*-test was corrected using the approximation to Behrens and Fisher's test proposed by Cochran and Cox[14] whenever the data seemed to suggest that the samples of the populations to be compared had different variances. The data were analysed separately for the first and second days of treatment, the aim being to study both the difference between the two treatments on each day and the difference between the days with regard to the same treatment. In this way, four separate comparisons were made. The chi-squared test was used to compare the two treatments on each of the study days, to analyse the hourly average of premature ventricular beats by dividing it into two classes: PVB $< 10\ h^{-1}$ and PVB $> 10\ h^{-1}$.

Mann Whitney's U test was used to compare urinary carnitine concentrations between the group treated with L-carnitine and the healthy control group.

The corrected *t*-test was used for the analysis of PVBs and VT and to compare urinary carnitine levels in the placebo and healthy control groups.

Analysis of variance according to randomized block design was used to compare treatments on the two study days and to see whether the interval between onset of pain and admittance to hospital (3–6 or 6–12 h) had any effect, either absolute or in terms of interaction with treatment.

Differences with $P<0{\cdot}05$ were considered significant.

Results

SERUM CARNITINE

Variations in serum concentrations of FC, SCACE and LCACE found in the two groups (L-carnitine or placebo) of patients with acute myocardial infarction are presented in Fig. 1. They show that whereas before treatment the serum concentrations of FC, SCACE and LCACE in both

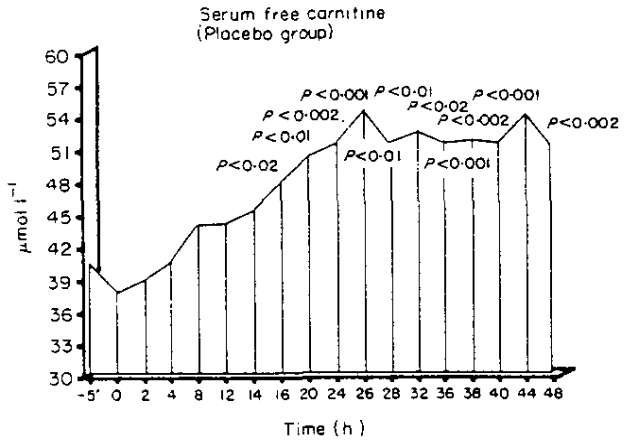


Figure 2 Free serum carnitine during the first 48 h after admission to the Coronary Unit in patients with AMI receiving placebo.

Table 3 Urinary excretion of free carnitine (FC), short chain acylcarnitine esters (SCACE), long chain acylcarnitine esters (LCACE) and total carnitine in 11 healthy subjects (0–24 h) and in the two groups with AMI treated with placebo or L-carnitine during the first two days in the Coronary Unit

	Healthy subjects		AMI (Placebo)		AMI (L-Carnitine)	
	0–24 h	24–48 h	0–24 h	24–48 h	0–24 h	24–48 h
FC (μmoles)	126±51	-	547±631	576±608	30991±18070 $(•••)	26954±11934
SCACE (μmoles)	146±123	-	219±257	154±150	2676±4224 $(•••)	1818±2151
LCACE (μmoles)	6·7±4·3	-	8·2±4·9	9·5±8·2	§(•••) 230±192 $(•••)	§(•••) 200±142
TOTAL (μmoles)	279±148	-	775±880	738±650	§(•••) 33734±19520 $(•••)	§(••) 28992±12069

* = $P<0·05$; ** = $P<0·01$; *** = $P<0·001$.

L-carnitine and placebo groups were virtually the same, L-carnitine treatment induced, throughout treatment, a significant increase ($P<0·001$) in these compounds whose maximum values, as expected, were found in the first blood sample taken after each L-carnitine administration.

It is worthwhile to note that in the placebo group (Fig. 2) serum FC increased progressively from 38 ± 13 μmol l^{-1}, recorded at 'time 0', to 55 ± 17 μmol l^{-1} ($P<0·001$) after 26 h. Thereafter until 48 h, mean values ranged between 52 and 55 μmol l^{-1}. Statistical significance was reached at 16 h ($P<0·02$).

URINARY CARNITINE (TABLE 3)

Urinary excretion of free and total carnitine in the placebo group was significantly higher than in the 11 healthy control subjects. On both days of treatment L-carnitine induced a similar and remarkable increase in the elimination of FC, SCACE, LCACE and total carnitine compared with the placebo group ($P<0·001$).

HOLTER ANALYSIS

Mean heart rate $(24\text{ h})^{-1}$ was 85 ± 17 beats min^{-1} in the L-carnitine group and 77 ± 13 beats min^{-1} ($P<0·05$) in the placebo group during the first 24 h

Table 4 Premature ventricular beats and ventricular tachycardia in the placebo and L-carnitine groups

	1st day		2nd day		
	Placebo	L-Carnitine	Placebo	L-Carnitine	
Recording time (h)	22·4±3·4	22·5±5	20·4±7·2	20·4±7·6	
PVBs h^{-1}	93±272	60±115	20±36	4±7	*
Time with multiform PVBs (h)	11·1±9	8·9±8	7·3±8	2·9±4	*
Time with paired PVBs (h)	6·6±7·6	5·6±6·8	2·8±4·4	0·6±1·2	**
Number of paired PVBs (24 h)	23±54	23±46	7±16	0·7±1·5	
Time with ventricular tachycardia	4·1±5·9	3±4	0·7±1·1	0·2±0·4	*
Number of ventricular tachycardiac episodes	17±43	23±97	1±2	0·2±0·5	*

*: $P < 0.05$; **: $P < 0.02$

Table 5 Number of patients with premature ventricular beats (PVBs $< 10\,h^{-1}$ and PVBs $> 10\,h^{-1}$) during the first and second day of treatment (placebo or L-carnitine)

	1st day		2nd day	
	<10	>10	<10	>10
Placebo	10	18	15	13
L-Carnitine	11	17	23	2
	n.s.		$P<0.01$	

of recording, and 91 ± 19 beats min^{-1} (L-carnitine) and 84 ± 16 beats min^{-1} (placebo) during the following 24 h (P = n.s.). No significant differences in maximum heart rate were observed between or within the groups on the first day (L-carnitine: 123 ± 16 beats min^{-1}; placebo 125 ± 24 beats min^{-1}) nor on the second day (L-carnitine: 123 ± 19 beats min^{-1}; placebo 118 ± 18 beats min^{-1}). PVB frequency is shown in Table 4. The L-carnitine group, compared with the placebo one, showed a significantly lower incidence on the second day in: PVBs h^{-1} ($P<0.05$); hours with multiform PVBs ($P<0.05$); hours with paired PVBs ($P<0.02$); hours with VT ($P<0.05$) and number of VT episodes ($P<0.05$). A significant difference ($P<0.01$) in favour of the L-carnitine group was also observed on the second day of treatment when the number of patients with PVBs < or > 10 h^{-1} was considered (Table 5).

OTHER VARIABLES

Complications occurring during hospital stay were: left ventricular wall rupture (L-carnitine one case; placebo one case), shock (L-carnitine one case; placebo one case), heart failure (L-carnitine one case; placebo five cases), complete heart block (L-carnitine one case; placebo no cases), cardiac aneurysm (L-carnitine no cases; placebo one case) and pericarditis (L-carnitine four cases; placebo two cases).

Two patients died in the L-carnitine group, both on the first day: one because of left ventricular wall rupture 4 h after admission and the other of complete heart block and shock 14 h after admission. Two patients died in the placebo group, both on the second day: one patient because of shock 35 h after admission and the other of left ventricular wall rupture 28 h after admission.

Concomitant therapies used during the first two days after admission are shown in Table 6. The two groups were balanced with respect to drugs most frequently used; antiarrhythmic drugs were used in very few cases and could not influence the analysis of the results.

Discussion

The results of this study show that: (1) in the placebo group the serum level of free carnitine increases significantly in the first 48 h after AMI; (2) in the same group, urinary excretion of free and total carnitine during the first 48 h after AMI is significantly higher than in normal subjects; (3) administration of L-carnitine induces a remarkable and significant increase in carnitine esters in both serum and urine. In the placebo patients, this could be accounted for by loss of carnitine from damaged heart tissue. This assumption would be in agreement with previous results showing a significant release of carnitine from ischaemic and anoxic heart[15–17]. However, if carnitine is not also provided by other tissues the extent of the observed increase of carnitine in both plasma and urine would require almost half of the carnitine contained in the heart. Considering that the rate of carnitine excretion continues for at least two days, it is likely

Table 6 Concomitant therapies during the first and second day of the study. The numbers shown in the table indicate how many patients received each particular treatment

	1st day		2nd day	
	L-Carnitine	Placebo	L-Carnitine	Placebo
Ca-antagonists:				
nifedipine	14	14	25	23
Nitroderivatives:				
isosorbide dinitrate	1	3	4	6
Nitroglycerine	–	1	–	2
Diuretics:				
Furosemide	1	3	2	4
K^+Canreonate	–	–	–	1
Antiarrhythmics				
Lidocaine	2	–	1	–
Amiodarone	–	1	–	1
Mexiletine	–	1	–	1
Bunaftine	–	–	–	1
Cardiac glycosides:				
3-methyl-digoxine	–	–	–	1

that carnitine is also released by other tissues, probably skeletal muscle, as a result of stress.

Increased synthesis of carnitine in liver and kidney cannot be excluded, especially if one considers the very probable stimulation of proteolysis occurring in AMI, and consequently the large availability of carnitine precursors, methionine and trimethyllysine. All these events might concur to different extents to explain the observed increase in carnitine.

Another point which needs discussing is the dramatic increase in serum and urinary levels of carnitine esters found after L-carnitine treatment. The serum increase may be explained as a consequence of the esterification between exogenous carnitine and the acyl groups within the cells, catalysed by CoA: carnitine acyl transferase, an enzyme well represented in myocardial tissue. This esterification allows the 'wash-out' of acyl groups into the blood and, consequently, into the urine. In other words, an increased availability of FC can react with acyl CoA to form acyl carnitine, enabling these compounds to diffuse through membranes. This constitutes a useful mechanism which, by eliminating acyl compounds in excess, exercises a protective function during cardiac ischaemia and may account for the reduction in ventricular arrhythmias observed on the second day in the treated group compared with the placebo one.

These data confirm previous results on laboratory animals demonstrating the antiarrhythmic activity of carnitine[18–22], especially in ischaemic conditions.

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References

[1] Cowan JC, Vaughan-Williams EM. The effect of various fatty acids on action potential shortening during sequential periods of ischemia and reperfusion. J Mol Cell Cardiol 1980; 12: 347–51.

[2] Kurien VA, Yates PA. The role of free fatty acids in the production of ventricular arrhythmias after acute coronary artery occlusion. Eur J Clin Invest 1971; 1: 225–41.

[3] Kurien VA, Oliver MF. A metabolic cause for arrhythmias during acute myocardial hypoxia. Lancet 1970; i: 813–5.

[4] Tansey MJB, Opie LH. Relation between plasma free fatty acids and arrhythmias within the first twelve hours of acute myocardial infarction. Lancet 1983; ii: 419–22.

[5] Katz AM, Messineo FC. Fatty acid effects on membranes: possible role in the pathogenesis of ischemic myocardial damage. J Mol Cell Card 1982; 14(3): 119–22.

[6] Liedtke AJ, Nellis S, Neely JR. Effects of excess free fatty acids on mechanical and metabolic function in normal and ischemic myocardium in swine. Circ Res 1978; 43: 652–61.

[7] Shug AL, Paulson DJ. Fatty acid and carnitine-linked abnormalities during ischemia and cardiomyopathy. In: Ferrari R, ed. Myocardial ischemia and lipid metabolism. New York: Plenum Press 1984: 203–24.

[8] Spagnoli LG, Corsi M, Villaschi R, Palmieri G, Maccari F. Myocardial carnitine deficiency in acute myocardial infarction. Lancet 1982; i: 1419–20.

[9] Bohmer T, Rydning A, Solberg HE. Carnitine levels in human serum in health and disease. Clin Chem Acta 1974; 57: 55–61.

[10] Cederblad G, Lindstedt S. A method for determination of carnitine in the picomole range. Clin Chim Acta 1972; 37: 235–43.

[11] Pace JA, Wannemacher RW, Neufeld HA. Improved radiochemical assay for carnitine and its derivatives in plasma and tissue extracts. Clin Chem 1978; 24: 32–5.
[12] McGarry JD, Foster DW. An improved and simplified radioisotopic assay for the determination of free and esterified carnitine. J Lipid Res 1976; 17: 277–81.
[13] Parvin R, Pande SV. Microdetermination of (−) carnitine and carnitine acetyltransferase activity. Anal Biochem 1977; 79: 190–201.
[14] Cochran WG, Cox GM. Completely randomized, randomized block, and latin square designs. In: Cochran WG, Cox GM, eds. Experimental designs. New York: J. Wiley & Sons; 1957: 95–148.
[15] Suzuki Y, Kamikawa T, Kobayashi A, Yamazaki N. Effects of L-carnitine on tissue levels of acyl carnitine, acyl coenzyme A and high energy phosphate in ischemic dog hearts. Jpn Circ J 1981; 45: 687–93.
[16] Liedtke AJ. Alteration of carbohydrate and lipid metabolism in the acutely ischemic heart. Prog Cardiovasc Dis 1981; 23: 321–36.
[17] Suzuki Y, Kamikawa T, Yamazaki N. Carnitine distribution in subepicardial and subendocardial regions in normal and ischemic dog hearts. Jpn Heart J 1981; 22: 377–85.
[18] Rebouche CJ, Engel AG. Kinetic compartmental analysis of carnitine metabolism in the dog. Arch Biochem Biophys 1983; 220: 60–70.
[19] Di Palma JR, Ritchie DM, McMichael RF. Cardiovascular and antiarrhythmic effects of carnitine. Arch Int Pharmacodyn 1975; 217: 246–50.
[20] Kobayashi A, Suzuki Y, Kamikawa T *et al.* Effects of L-carnitine on ventricular arrhythmias after coronary reperfusion. Jpn Circ J 1983; 47: 536–42.
[21] Imai S, Matsui K, Nakazawa M *et al.* Anti-arrhythmic effects of (−)-carnitine chloride and its acetyl analogue on canine late ventricular arrhythmia induced by ligation of the coronary artery as related to improvement of mitochondrial function. Br J Pharmacol 1984; 82: 533–42.
[22] Suzuki Y, Kamikawa T, Yamazaki N. Effects of L-carnitine on ventricular arrhythmias in dogs with acute myocardial ischemia and a supplement of excess free fatty acid. Jpn Circ J 1981; 45: 552–9.