

ENDOCRINE-METABOLIC EFFECTS OF L-CARNITINE IN PATIENTS ON REGULAR DIALYSIS TREATMENT

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Summary

Twenty-three patients on regular dialysis treatment (RTD) were given l-carnitine orally or in dialysate for six months. All patients remained in a stable biochemical state; hyperlipidaemia was reduced with an increase in HDL-cholesterol. Hormonal pattern was unmodified. Serum and muscle carnitine and acetyl-carnitine constantly increased. L-carnitine in RDT, by restoring tissue reserves, improves metabolic alterations without any side-effects.

Introduction

Carnitine occurs in most tissues and is present at particularly high concentrations in skeletal muscles, heart, kidney, and lungs [1,2]. Its fundamental action is that of facilitating the transport of long-chain fatty acids from the cytoplasm to the mitochondrial matrix where such acids are oxidised resulting in the production of energy [1]. Plasma carnitine concentrations in uraemic patients on haemodialysis decrease at the end of each treatment by 70–80 per cent and rise again during the interdialysis interval by transfer from tissues with progressive cellular depletion [3,4]. Previous investigations have shown the benefit of dl-carnitine with improved performance of the skeletal muscles assessed electromyographically [5] and improvement in abnormalities of lipid metabolism [6]. The racemic form, however, may give rise to a myasthenia-like syndrome [5,7] probably related to progressive accumulation of the non-natural dextrorotary isomer in muscle cells. The present investigation evaluates the long-term endocrino-metabolic effects and the effects on muscle cells of l-carnitine administration to patients on intermittent haemodialysis.

Patients and methods

Twenty-three subjects (16 males and 7 females, aged between 17–73 years) were selected for the trial, after having obtained their informed consent. All the

patients were affected by end-stage chronic uraemia and had been on intermittent haemodialysis for 1–96 months (4hr thrice weekly, plate filters, cuprophane membrane, 1–1.4m² surface).

L-carnitine supplements were given orally to 12 patients (1g daily in 10% solution); the remaining 11 patients received l-carnitine in the dialysate at a concentration of approximately 100µmol/L. This treatment was given for six months. The parameters described below were determined under basal conditions, and at two, four and six months of treatment.

Blood tests

Residual GFR, BUN, creatinine, uric acid, Na, K, Ca, P, alkaline phosphatase, transaminase, glucose, full blood count.

Lipid pattern

Triglycerides (lipase-glyceraldehydrogenase enzymatic method, normal value 20–170mg/100ml); total cholesterol (cholesterol esterase – cholesterol oxidase enzymatic method, normal value 170–250mg/100ml); HDL-cholesterol (precipitation with heparin – MnCl₂; normal value >45mg/100ml); LD-cholesterol (Friederwal's formula: LDL = total cholesterol – HDL-cholesterol – triglycerides/5, normal value 70–150mg/100ml); VLDL-cholesterol (= total cholesterol – HDL-cholesterol – LDL-cholesterol, normal value 20–50mg/100ml); atherogenic risk index (total cholesterol/HDL-cholesterol); apoproteins A and B (nephelometric technique, normal values 163–291mg/100ml and 43–135mg/100ml respectively).

Hormonal pattern

Radioimmunological assay of PTH NH₂-terminal (normal value 0.3–0.9ng/ml), insulin (normal value 0–22µU/ml), C-peptide (normal value 0.5–2.3ng/ml), glucagon (normal value 50–250ng/ml), somatostatin (normal value 10–25pg/ml), and Growth Hormone (normal value 0–6ng/ml).

Serum carnitine

Serum carnitine (normal value 47.0 ± 6.0µmol/L) and acetyl-carnitine (normal value of carnitine: acetyl-carnitine = 5:1) using Seccombe's method [8].

Muscle biopsy of the musculus quadriceps femoris was performed with histological and histochemical examination in 11 of the 12 patients treated with oral l-carnitine (9 males and 2 females aged between 27 and 73 years on haemodialysis for 6–96 months). The techniques used for biopsy and staining (haematoxylin-eosin, ATPase at pH 4.3, 4.6, 9.4) were those of Dubowitz and Brooke [9]. The histological examination was repeated in eight patients at six months of treatment. Muscle carnitine (normal value 24.0 ± 1.4µmol/g non-

collagen protein (NCP) and acetyl-carnitine (normal value of carnitine: acetyl-carnitine = 5–10:1).

All the blood samples were drawn at the end of fasting and before mid-week haemodialysis treatment. Haemodialysis treatment and dietary habits were maintained throughout the study. The patients were closely supervised for possible side-effects due to l-carnitine. No therapy was used that could interfere with metabolic balance, no other drug was started during l-carnitine treatment.

Results

The data obtained from the trial were evaluated cumulatively since the increased serum carnitine values were comparable in both groups.

Biochemical pattern

Throughout the study all the patients exhibited perfect clinico-dialysis equilibrium and no change in the biochemical parameters occurred except for increased haematocrit ($23.09 \pm 5.23\%$ basal, $26.52 \pm 4.36\%$ at six months; $p < 0.02$).

Lipid pattern

Under basal conditions 14 patients (60.8%) displayed hypertriglyceridaemia and in five of these patients (21.7%) hypercholesterolaemia was present. At six months triglyceridaemia was still high in seven patients (30.4%), while cholesterol concentrations had returned to normal values in 100 per cent of the cases, triglyceride ($217.6 \pm 88.89\text{mg}/100\text{ml}$ basal, $171.43 \pm 61.77\text{mg}/100\text{ml}$ at six months; $p < 0.05$) and cholesterol ($223.57 \pm 47.96\text{mg}/100\text{ml}$ basal, $190.35 \pm 25.17\text{mg}/100\text{ml}$ at six months; $p < 0.01$). L-carnitine produced a decrease in LDL-cholesterol ($134.33 \pm 40.26\text{mg}/100\text{ml}$ basal, $97.93 \pm 22.91\text{mg}/100\text{ml}$ at six months; $p < 0.001$) and an increase in HDL-cholesterol ($48.39 \pm 6.35\text{mg}/100\text{ml}$ basal, $53.74 \pm 6.84\text{mg}/100\text{ml}$ at six months; $p < 0.01$). VLDL-cholesterol did not change ($40.93 \pm 15.11\text{mg}/100\text{ml}$ basal, $38.68 \pm 14.05\text{mg}/100\text{ml}$ at six months; $p = \text{NS}$), and the atherogenic risk index was reduced (4.67 ± 1.00 basal, 3.60 ± 0.74 at six months; $p < 0.001$).

Apoprotein showed particular changes: increased apoprotein A ($130.74 \pm 20.20\text{mg}/100\text{ml}$ basal, $192.48 \pm 21.32\text{mg}/100\text{ml}$ at six months; $p < 0.001$) contrasted with the reduction in apoprotein B ($102.17 \pm 15.46\text{mg}/100\text{ml}$ basal, $87.09 \pm 12.60\text{mg}/100\text{ml}$ at six months; $p < 0.001$).

Hormonal pattern

PTH ($4.76 \pm 2.67\text{ng}/\text{ml}$), insulin ($29.21 \pm 22.05\mu\text{U}/\text{ml}$), C-peptide ($10.37 \pm 5.59\text{ng}/\text{ml}$) and glucagon ($326.13 \pm 98.10\text{ng}/\text{ml}$) were increased under basal conditions. Somatostatin ($12.83 \pm 7.05\text{pg}/\text{ml}$) and GH ($1.21 \pm 0.95\text{ng}/\text{ml}$) were within the normal range. Carnitine treatment did not induce any change in the hormonal pattern.

Carnitine and acetyl-carnitine

Serum concentrations, on the average at the lower limits of normal range values under basal conditions (39.28 ± 10.96 and $9.10 \pm 2.46 \mu\text{mol/L}$) progressively increased (72.10 ± 11.80 and $20.21 \pm 5.63 \mu\text{mol/L}$ at six months, $p < 0.001$) maintaining a physiological ratio. There was no correlation between basal values and the dialytic age of patients. Basal muscle carnitine values were lower than normal in only four out of 11 patients (36.4%), and inversely correlated with dialytic age ($p < 0.001$). The muscle carnitine : acetyl-carnitine ratio (mean value 15:1) was altered since an inverse correlation was present ($p < 0.01$). Dialytic age correlated directly with muscle acetyl-carnitine ($p < 0.001$).

L-carnitine orally was associated with an increase in muscle concentration of carnitine ($22.80 \pm 5.79 \mu\text{mol/g}$ NCP basal, $28.08 \pm 4.14 \mu\text{mol/g}$ NCP at six months; $p < 0.05$) and acetyl-carnitine ($1.51 \pm 0.56 \mu\text{mol/g}$ NCP basal, $2.39 \pm 0.68 \mu\text{mol/g}$ NCP at six months; $p < 0.01$) although an inverse correlation persisted between the two ($p < 0.001$). There was no correlation between serum and muscle carnitine and acetyl-carnitine values either under basal conditions or at six months of treatment.

Muscle biopsy (Table I)

Under basal conditions three patients exhibited values within the normal range. The other eight patients had a selective reduction in the size of type 2 fibres: in four cases the diameter was less than 40μ (lowest limit considered as normal in man); in the remaining four cases the mean diameter was greater than 40μ ,

TABLE I. Histological—histochemical examination of muscle biopsy

Name	Age	Sex	Mean fibre diameter				Atrophy factor				Per cent			
			Type 1		Type 2		Type 1		Type 2		Type 1		Type 2	
			B	6m	B	6m	B	6m	B	6m	B	6m	B	6m
DMA	73	M	54	52	44	47	59	61	369	298	36	38	64	62
DB	52	M	62		54		0		68		34		66	
PG	27	M	48	49	42	46	53	46	273	265	38	39	62	61
PB	36	F	54		41		0		126		38		62	
MS	59	M	52	52	36	40	40	55	572	441	36	37	64	63
FM	48	M	64		54		0		121		42		58	
CE	64	M	49	50	45	45	89	48	281	268	36	39	64	61
RN	62	M	49	51	38	40	86	59	550	447	35	36	65	64
GF	52	M	51	49	36	39	41	40	725	610	27	30	73	70
CR	61	F	50	51	41	40	35	22	210	251	40	40	60	60
ME	35	M	49	52	38	40	0	11	630	428	12	20	87	80
Normal values			M 40–80 μ				M 0–150				M 36		64	
			F 30–70 μ				F 0–100 0–200				F 39		61	

but atrophy was still present. No correlation was found between carnitine and/or acetyl-carnitine and the mean diameter and/or the atrophy factor of type 2 fibre. L-carnitine treatment had no evident effect on muscle tissue.

Side effects

No patient displayed side-effects attributable to l-carnitine treatment; 78 per cent reported a sensation of well-being and increased muscular strength.

Discussion

Our results confirm progressively decreased muscle carnitine concentrations related to dialytic age, though such a decline reached significantly pathological values in only four cases (36%). The direct relationship between dialytic age and muscle acetyl-carnitine is of interest: uraemic intoxication might interfere with carnitine-acetyl-transferase by inhibiting the acetyl-CoA + carnitine \rightarrow acetyl-carnitine + CoA reaction. The final outcome would be that of reduced intra-mitochondrial concentration of acetyl-carnitine and a diminished availability of CoA which plays an essential role in the citric acid cycle. Since haemodialysis treatment at least partly removes uraemic toxins, this would enable acetyl-carnitine concentrations to progressively increase. L-carnitine orally produces a significant mean increase in carnitine and muscle acetyl-carnitine levels.

Basal histological data appear to be in agreement with those described in the literature [10] and suggest muscle involvement secondary to damage to nerve fibres. No histological changes were attributable to carnitine depletion nor did l-carnitine orally alter the basic morphological picture. On the contrary, our previous observations [5], demonstrated significantly improved electromyographic findings in patients on haemodialysis after d,l-carnitine administration. It is, therefore, clear that muscle carnitine replenishment, particularly increased acetyl-carnitine concentrations, play a fundamental role in muscle function.

Lipid metabolism disorders, present in most patients, markedly improved. HDL-cholesterol, whose protective function against atherosclerosis is well-known, and vector apoproteins (ApoA) increased significantly in all the patients while LDL cholesterol which is involved in the pathogenesis of atherosclerotic lesions and respective vector proteins (ApoB) decreased. Hence, l-carnitine significantly reduces the atherosclerotic risk index in haemodialysis patients who are subject to vascular accidents. Total cholesterol and triglycerides were equally reduced, even if some patients continued to exhibit pathological values of the latter parameter. It is not possible to completely correct hyperlipidaemia in view of the multiplicity of the pathogenic factors in uraemia.

Throughout the study dialytic equilibrium conditions did not change and no side-effects attributable to l-carnitine occurred. Most of the patients reported a sensation of well-being and increased muscular strength. No patient, not even those who were completely anuric, displayed myasthenia-like symptoms. This supports the view that such symptoms are caused by the dextrorotary isomer. Increased haematocrit at six months of treatment is an important

result, but needs further confirmation. This improvement could be accounted for by lessened fragility of the erythrocyte membrane due to higher availability of energy substrate arising from a protective action of carnitine on the erythrocyte membranes. In conclusion, we believe that the administration of l-carnitine supplements to haemodialysis patients, by restoring tissue pools impoverished by haemodialysis treatment, helps to repair lipid metabolism disorders and reduce the vascular risk index without determining any undesirable effect.

Acknowledgments

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References

- 1 Bohmer T, Rydning A, Solberg HE. *Clin Chim Acta* 1974; 57: 55
- 2 Cederblad G, Lindstedt S, Lundholm K. *Clin Chim Acta* 1974; 53: 311
- 3 Bohmer T, Bergrem H, Eiklid K. *Lancet* 1978; i: 126
- 4 Bartel LL, Hussey JL, Shrago E. *Am J Clin Nutr* 1981; 34: 1314
- 5 Albertazzi A, Cappelli P, Di Paolo B et al. *Proc ESAO* 1980; VII: 122
- 6 Guarnieri GF, Ranieri F, Toigo G et al. *Am J Clin Nutrition* 1980; 33: 1489
- 7 Bazzato G, Mezzina C, Ciman M et al. *Lancet* 1979; i: 1041
- 8 Seccombe DW, Dodek P, Frohlick J et al. *Clin Chem* 1976; 22: 1589
- 9 Dubowitz V, Brooke MH. *Muscle biopsy. A Modern Approach* 1973. WB Saunders Company Ltd.
- 10 Savazzi GM, Cambi V, Migone L et al. *Proc EDTA* 1980; 17: 312

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