Original Paper

Kidney & Blood Pressure Research

Kidney Blood Press Res 2006;29:100–107
DOI: 10.1159/000094363

Received: January 5, 2006
Accepted: May 18, 2006
Published online: June 30, 2006

Propionyl-L-Carnitine Therapy: Effects on Endothelin-1 and Homocysteine Levels in Patients with Peripheral Arterial Disease and End-Stage Renal Disease

Salvatore Santo Signorelli[a] Pasquale Fatuzzo[b] Francesco Rapisarda[b]
Sergio Neri[c] Margherita Ferrante[d] Gea Oliveri Conti[d] Roberto Fallico[d]
Luigi Di Pino[a] Giuseppe Pennisi[a] Gabriella Celotta[a] Massimiliano Anzaldi[a]

[a]Medical Angiology Section, [b]Kidney Disease Section and [c]Internal Medicine Section, Department of Internal Medicine and Systemic Pathology and [d]Department of Anatomy, Diagnostic Pathology, Legal Medicine and Public Health Faculty of Medicine, University of Catania, Catania, Italy

Key Words
L-Carnitine · Peripheral arterial disease · End-stage renal disease

Abstract
Background/Aims: Recent data have addressed the issue of higher levels of homocysteine (Hcy) and endothelin-1 (ET-1) in end-stage renal disease (ESRD) that may be considered an independent predictor for cardiovascular disease. The prevalence of peripheral arterial disease (PAD) in patients with ESRD has been reported to be relevant, highlighting its clinical importance. We aimed to explore the therapeutic role of propionyl-L-carnitine (PLC) in hemodialysis patients with PAD by measuring ankle/brachial index (ABI), ET-1 and Hcy. ***Design:*** Randomized, double-blind, placebo-controlled trial. ***Methods:*** Sixty-four patients on hemodialysis with chronic renal insufficiency and PAD were assigned to receive either intravenous PLC (600 mg) or placebo 3 times weekly for 12 months. The ABI and plasma levels of ET-1 and Hcy were measured at baseline, 6 and 12 months. ***Results:*** In the PLC-treated group, progressive increases in ABI were observed, while in the placebo group the reverse trend was seen. Highly significant and progressive reductions in plasma levels of ET-1 and Hcy, compared to baseline, were also seen in the PLC-treated group. ***Conclusions:*** Hemodynamic flow, endothelial profile and Hcy levels were ameliorated by the administration of PLC in hemodialysis patients with ESRD and PAD.

Introduction

In end-stage renal disease (ESRD) [1–5] there is an increased risk of cardiovascular disease (CVD) that could be related to metabolic disorders characterized by high levels of low density cholesterol and triglycerides and lower levels of high density lipoproteins [6]. The increased arterial resistance of blood vessels is probably one of the major reasons for arterial complications, such as arterial hypertension and CVD present in kidney disease [7–10], and raised levels of vasoconstrictor agents, such as angiotensin II and endothelin-1 (ET-1), are found in ESRD [10, 11].

Recent data from a meta-analysis have suggested that higher levels of homocysteine (Hcy) in ESRD may be an independent predictor for CVD [7], and Boushey et al.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1420–4096/06/0292–0100$23.50/0

Accessible online at:
www.karger.com/kbr

Prof. Salvatore Santo Signorelli
Medical Angiology Section
Department of Internal Medicine and Systemic Pathology
Via Firenze 123, IT–95123 Catania (Italy)
Tel. +39 095 759 4344, Fax +39 095 312 165, E-Mail ssignore@unict.it

[12] showed a close relationship between higher plasma concentrations of Hcy and peripheral arterial disease (PAD). Many researchers have shown that endothelial dysfunction plays a pivotal role in the development of atherosclerosis [13] and is associated with various other diseases such as arterial hypertension, diabetes, hypercholesterolemia, PAD, chronic renal insufficiency (CRI) and ESRD that are considered risk factors and/or markers for CVD [12–19].

Patients with ESRD have a high CVD mortality rate and it has been hypothesized that it may be a marker for CVD [19, 20]. The incidence of PAD depends on the diagnostic criteria and whether an ankle/brachial index (ABI) of $\leq$0.9 is used [21–23]; the prevalence of PAD in patients with CRI (creatinine clearance values <60 ml·min^{-1}·1.73 m^{-2}) has been reported to be 24%, highlighting its clinical importance [22, 24].

Various studies have suggested a beneficial role for antioxidant drugs in the clinical therapy of patients with either PAD or ESRD [25, 26]. L-Carnitine and its derivatives have received much attention for their potential utility as therapeutic interventions in PAD and CRI. Although the mechanism of action is not yet completely understood, carnitine derivatives appear to mediate the metabolism of monocytes according to studies in skeletal muscle [27]. A deficiency in muscular carnitine has been shown in patients with severe PAD [28, 29]; moreover, the carnitine derivative, propionyl-L-carnitine (PLC), has been reported to play a protective role during ischemia [30]. It has been established that PLC has a high affinity for muscular carnitine transferase, and through increasing cellular carnitine content plays a key role in the transfer of long-chain fatty acids into the mitochondria for β-oxidation [20, 31, 32].

Long-term hemodialysis treatment is associated with a significant reduction in endogenous plasma and muscle L-carnitine levels [33, 34] and serum carnitine concentrations may even be correlated with clinical outcome parameters. While therapy with L-carnitine is not yet recommended as a standard protocol for hemodialysis patients, it has been suggested that L-carnitine increases plasma carnitine, reduces fatigue, and may preserve exercise capacity in hemodialysis patients [33, 34]. In addition, researchers have stated that L-carnitine therapy has been shown to correct EPO resistance in hemodialyzed patients, thereby reducing the need for r-HuEPO [34–38]. The above considerations prompted us to explore the therapeutic role of PLC in hemodialysis patients with PAD. We measured the effect on the two endothelial markers, ET-1 and Hcy. To our knowledge the effects of L-carnitine administration have, to date, not been examined in this group of patients. Herein, we present the results of a randomized, placebo-controlled clinical trial exploring the utility of intravenous PLC therapy in patients with both CRI and PAD, but not CVD.

Methods

Patient Population

Primary inclusion criteria were severe CRI requiring hemodialysis and a clinical diagnosis of PAD at the second stage according to the Leriche-Fontaine classification. An ABI index of <0.9 was used to diagnose PAD. The ABI is the ratio of ankle systolic to brachial systolic blood pressure while a person is at rest (normal resting ABI = 1 or 1.1). The arterial pressure is measured in the lower leg where possible, but another leg artery with a satisfactory acoustic signal can be used to obtain an image with good color flow. A continuous Doppler pulse wave (7–10 mHz) using a pencil probe (Apogée CX 800 ATL-Philips) was utilized.

Exclusion criteria included coronary ischemic disease (myocardial infarction in the previous 12 months and/or angina) and congestive heart failure. Additionally, patients with active hepatic disease or with active inflammatory diseases were excluded. Patients suffering from arterial hypertension were also excluded. The patients included in our trial were part of a cohort (n = 150) with CRI enrolled in the kidney disease section of the Department of Internal Medicine at the University of Catania. For this trial, only patients with PAD concomitant to CRI or complicating CRI were selected.

Study Design

Patients were randomly assigned, according to a simple scheme, to receive either intravenous PLC (600 mg in 100 ml physiological saline; Dromos®, Sigma-Tau Pharmaceuticals, Pomezia, Italy) or placebo (100 ml physiological saline alone) at the end of each dialysis session (3 times weekly) for a period of 12 months. The study was double-blinded and the groups were standardized with regard to dialysis schedules and times. The duration of the dialysis sessions was 4 h, with a mean ultrafiltration volume of 3 liters per 4 h; no patients received aspirin.

Follow-up visits were carried out at 6 and 12 months, at which time clinical and instrumental examinations were performed. Peripheral blood was drawn at baseline, and at the 6- and 12-month follow-up visits for evaluation of ET-1 and Hcy at the end of hemodialysis.

The study received approval from the local ethics committee and all patients gave informed consent to participate.

Determination of ET-1

The plasma sample was obtained from the EDTA-treated blood by centrifugation at 2,000 *g* for 10 min at 4°C. A 2-ml sample of the plasma was acidified with 0.25 ml of 2 M HCl, centrifuged at 10,000 *g* for 5 min at room temperature and loaded onto a column.

ET-1 was purified from these plasma samples by loading onto Amersham Pharmacia Biotech's C2 columns (Amprep 500 mg, code RPN 1913) connected to a Varion vacuum system. The col-

umns were equilibrated with 2 ml of methanol followed by 2 ml of ultrapure water. The flow rate for these and subsequent washes was maintained at 5 ml/min.

The column was washed with 5 ml of water plus 0.1% trifluoroacetic acid (TFA) and finally with 2 ml of a solution containing 80% methanol and 0.1% TFA.

The eluate collected was dried under nitrogen without drying the pellet, which was reconstituted in 500 μl of buffer from the Endothelin-1 EIA Kit (Cayman Chemical Company; Ann Arbor, Mich., USA). ET-1 concentration was determined using this ELISA kit as described below.

All reagents in the kit were prepared using deionized water and were free of all trace organic contaminants using the following procedure:

(1) The contents of the EIA buffer vial were diluted with 90 ml of ultrapure water, ensuring the complete dissolution of all salts in the vial.

(2) The contents of the wash buffer concentrate vial were diluted from 5 ml to a volume of 2 liters with ultrapure water and 1 ml of Tween 20 was added (care should be taken when measuring Tween 20 with a pipette as it is a very viscous liquid).

(3) The ET-1 standard was diluted with 1 ml of EIA buffer. A serial dilution was made from the 5 ng/ml mother solution, using 8 numbered tubes; 200 μl of the mother solution was added to tube 1 and diluted with 1 ml of EIA buffer. When mixed, this gave the highest concentration of the standard curve, 1,000 pg/ml. Five hundred microliters of EIA buffer was added to tubes 2–8 and 500 μl ET-1 solution was taken from tube 1 and added to tube 2, and mixed to give a concentration of 500 pg/ml. Then 500 μl was taken from tube 2 and added to tube 3 giving 250 pg/ml. These serial dilutions resulted in the lowest concentration of 15.6 pg/ml in tube 7. Tube 8 had only EIA buffer and was the control.

(4) The vial containing the endothelin Fab conjugate was reconstituted with 10 ml of EIA buffer. The sample plate was covered with a plastic film and incubated overnight at 4°C.

The next day, fresh Ellman's reagent was prepared with 50 ml water and protected from the light. The plate was washed 6 times with wash buffer and 200 μl of Ellman's reagent was added to each well. The plate was then covered with plastic film and allowed to react in the dark for 30 min. At the end of the incubation, the plate was read on a Plate Reader (DAS automatic 8 channel) at 405 nm and sample ET-1 concentrations were determined from the standard curve.

Determination of Hcy

Hcy concentration in the plasma samples was determined using ion-exchange chromatography. An HPLC system comprising a C-18 reverse phase column, 15 × 0.46 cm, a series 200 LC pump (Perkin Elmer) and a fluorescence detector (Varian Prostar) was used.

The plasma sample was obtained from blood containing EDTA and separated by centrifugation at 3,000 rpm for 15 min at 4°C, and maintained at –20°C until analysis. This was carried out using the kit for the determination of Hcy in plasma and serum from Chromosystems (distributed by Polytechne Srl, Livorno, Italy).

The test for Hcy was performed in the following way: a 20-μl plasma sample was injected into the system with a run time of 4–5 min. The HPLC pump was set at a flow rate of 1.5 ml/min, with the column maintained at a constant room temperature (20–25°C). The HPLC was equipped with a fluorescence detector set at wavelengths of EX: 385 nm and EM: 515 nm. To reconstitute the plasma calibration standard lyophilisate, 1 ml of distilled water was added to the vial. The derivatization mix was obtained by reconstituting the derivatization reagent 1 with 2 ml of derivatization reagent 2, and was ready for use after about 10 min. The preparation of the standard was identical to that of the samples.

Statistical Analysis

A comparison between the two treatment groups was performed using a one-way variance test (ANOVA), Friedman's test, and Kendall's concordance coefficient. A p value of <0.05 was considered significant. The SPSS (version 10.1 for Windows) statistics package was used. Univariate correlations were performed between Hcy and ABI and between ET-1 and ABI.

Results

A total of 64 patients with CRI and PAD on hemodialysis were enrolled in the study and randomized to PLC treatment or placebo (32 per arm). Patient characteristics at baseline are detailed in table 1. Patients were well matched with respect to baseline characteristics, with the exception of triglyceride levels which were significantly lower in the PLC group (179.3 vs. 209.2 mg/dl). The average duration of CRI was 10.2 ± 5.8 years. All the patients enrolled for the study were also receiving ACE inhibitors, diuretics and r-HuEPO.

Ankle/Brachial Index

In the PLC-treated group, statistically significant, progressive increases in ABI were observed, from 0.71 ± 0.06 at baseline to 0.76 ± 0.08 and 0.78 ± 0.08 at 6 and 12 months, respectively ($p < 0.001$) (fig. 1). In the placebo group, the reverse trend was seen, where progressive and significant reductions in ABI values were obtained, starting at 0.75 ± 0.08 at baseline and falling to 0.73 ± 0.06 and 0.72 ± 0.01 at 6 and 12 months, respectively ($p < 0.001$ vs. baseline). The between-group differences were also statistically significant at both 6 and 12 months ($p < 0.0001$).

ET-1 and Hcy

In patients receiving PLC, there was a highly significant decrease from baseline in plasma ET-1 and Hcy levels. Mean ET-1 levels (fig. 2) decreased from 21.53 ± 6.8 pg/ml at baseline to 16.78 ± 4.8 pg/ml ($p < 0.0001$) and 12.31 ± 3.4 pg/ml ($p < 0.0001$) at 6 and 12 months, respectively. In the placebo group, the decrease in mean ET-1 concentration from baseline was significant (fig. 3), but less so than in than in the PLC-treated group ($p < 0.009$). Hcy plasma levels in the PLC-treated group (fig. 4)

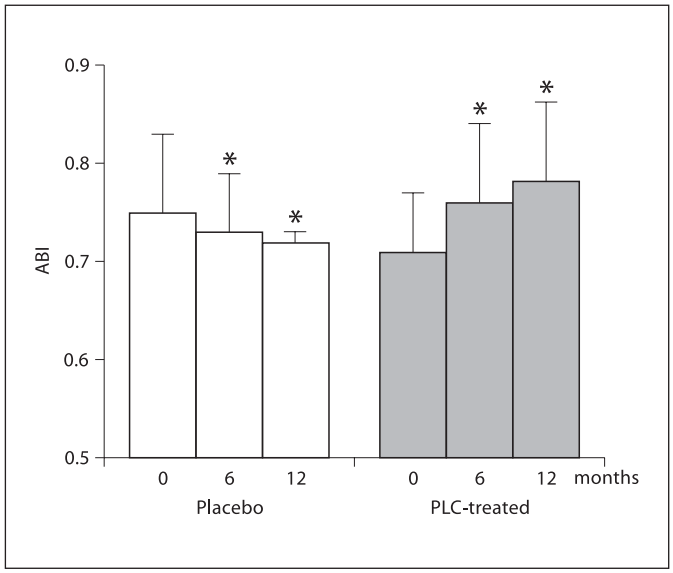

Fig. 1. Changes in ABI index at baseline, 6 and 12 months after administration of intravenous PLC or placebo. * $p < 0.001$ vs. baseline. The between-group difference was statistically significant at both 6 and 12 months ($p < 0.0001$).

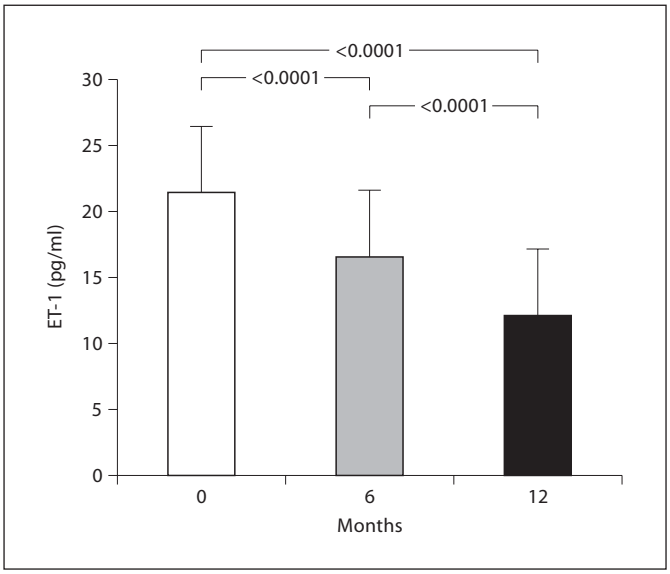

Fig. 2. Mean plasma ET-1 concentrations in the PLC group at baseline, 6 and 12 months.

Table 1. Baseline characteristics of patients and controls

	PLC	Placebo	p value
Age, years	66.7 ± 6.6	66.0 ± 2.7	NS
Duration of chronic renal insufficiency, months	66 ± 4.1	66.6 ± 5.6	NS
Urea, mg/l	139.97 ± 37.3	147.1 ± 37.7	NS
Creatininemia, mg/l	9.00 ± 2.7	9.18 ± 3.4	NS
Total cholesterol, mg/dl	168.5 ± 39.8	170.7 ± 46.6	NS
Triglycerides, mg/dl	179.3 ± 12.3	209.2 ± 15.4	<0.02
Fasting blood glucose, mg/dl	108.6 ± 43.6	108.1 ± 36.4	NS
Na, mEq/l	139.6 ± 3.4	141.6 ± 3.2	NS
K, mEq/l	5.9 ± 0.8	6.0 ± 0.8	NS

NS = Not significant.

also decreased significantly, from 68.89 ± 4.31 μmol/ml at baseline to 49.14 ± 2.26 and 29.61 ± 2.73 μmol/ml at 6 and 12 months ($p < 0.0006$), respectively. In the placebo group, Hcy levels decreased from 42.59 ± 23.77 μmol/ml at baseline to 40.62 ± 28.62 and 30.41 ± 12.95 μmol/ml (fig. 5), although this was not statistically significant at 6 or 12 months ($p < 0.3$ for both). Reductions in ET-1 and Hcy were significantly greater in the active treatment group compared with placebo ($p < 0.0001$). The univariate analysis concerning the group of patients assigned to the active drug (PLC) showed a significant correlation between ET-1 and ABI and between Hcy levels and ABI at 6 months and between ET-1 and ABI at 12 months (table 2). There was a close and inverse relationship between the lowering of these parameters and ABI. The correlations with ABI were statistically of greater significance (i.e. lower p value) for ET-1 than for Hcy.

Tolerability Profile

The product was well tolerated and no adverse reactions were observed.

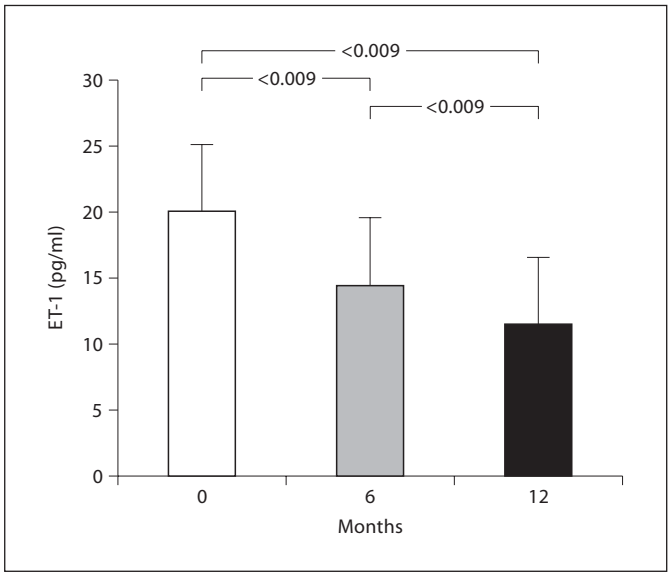

Fig. 3. Mean plasma ET-1 in the placebo group at baseline, 6 and 12 months.

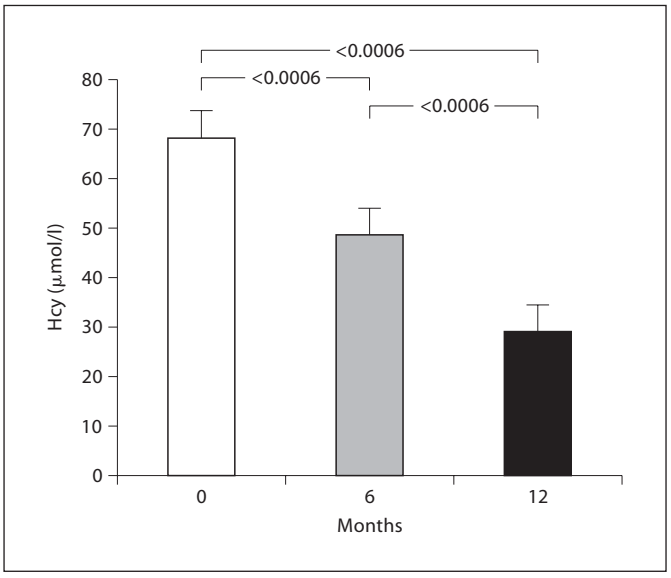

Fig. 4. Mean plasma Hcy concentrations in the PLC group at baseline, 6 and 12 months.

Discussion

The results of the present study suggest that intravenous administration of PLC to hemodialysis patients with ESRD and PAD leads to improvements in clinical and endothelial parameters. Significant changes were evident in the endothelial markers, as shown by statistically significant decreases in ET-1 and Hcy. Importantly, the changes in both outcome measures were progressively improved at both the 6- and 12-month follow-up visits. No safety considerations were raised in this study.

In patients with PAD, it has been amply demonstrated that local hemodynamic perturbations determine a reduced hematic flow with respect to muscular requirements, leading to a reduction in oxygenation of smooth muscle and reduced muscular levels of carnitine [27]. Consequently, significant alterations in cellular respiration occurs that leads to a reduction in the capacity of the muscle to work in anaerobic conditions [24].

Hyperhomocysteinemia has been described in patients suffering from PAD, more so than in patients with other clinical profiles of atherothrombotic diseases such as coronary heart disease and stroke [29], and an association between Hcy and PAD has been shown after adjustment for so-called known risk factors for CVD [37]. Results of other studies have stated that the metabolism of Hcy may influence the development of PAD in a high percentage of patients with atherothrombotic disease and finally, Hcy can be used as a marker to monitor clinical evaluation of certain risk groups [14, 38].

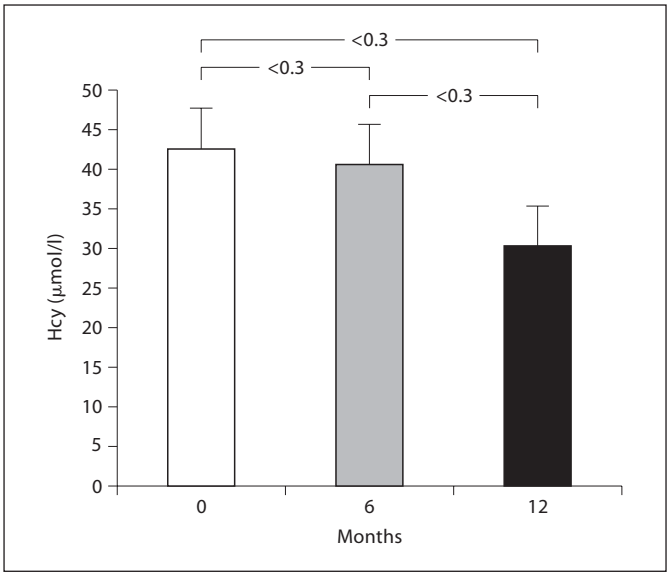

Fig. 5. Mean plasma Hcy concentration in the placebo group at baseline, 6- and 12-month follow-up.

On the other hand, diverse studies have stated that endothelial dysfunction plays a key role in the development of atherosclerotic plaques, and that vasoactive peptides

Table 2. Correlation between ET-1 and Hcy with ABI in patients with CRI and PAD receiving PLC or placebo

	ABI baseline		ABI 6 months		ABI 12 months	
	r	p value	r	p value	r	p value
ET-1, placebo, base.	–0.088	0.690	–0.137	0.534	–0.026	0.905
ET-1, placebo, 6	–0.127	0.565	–0.119	0.587	–0.028	0.901
ET-1, placebo, 12	–0.275	0.204	0.122	0.580	–0.035	0.874
Hcy, placebo, base.	–0.129	0.557	0.136	0.536	–0.029	0.896
Hcy, placebo, 6	–0.152	0.488	0.203	0.353	0.067	0.762
Hcy, placebo, 12	–0.168	0.584	0.528	0.064	0.403	0.172
ET-1, PLC, base.	–0.342	0.119	–0.143	0.527	–0.194	0.388
ET-1, PLC, 6	–0.199	0.375	–0.406	0.061	*–0.461*	*0.031*
ET-1, PLC, 12	–0.307	0.165	*–0.478*	*0.024*	*–0.510*	*0.015*
Hcy, PLC, base.	–0.006	0.980	–0.077	0.741	–0.192	0.403
Hcy, PLC, 6	–0.147	0.526	–0.179	*0.0437*	0.096	0.0680
Hcy, PLC, 12	0.137	0.553	0.179	*0.0438*	0.064	0.0783

Values are given for baseline and 6 and 12 months. The significant correlations are shown in italics.

such as ET-1 are crucial triggers of endothelial dysfunction [39]. Moreover, the more severe the damage to the endothelial barrier, the more significant the risk of cardiovascular events. ET-1 influences water homeostasis through its effects on the renin-angiotensin-aldosterone system, on vasopressin, and through its ability to stimulate the sympathetic nervous system [14, 38, 39]. All of these are involved in atherothrombotic disease, and also in chronic renal failure where this peptide plays a pivotal role in the pathophysiology of CVD, complications in renal failure and in hemodialyzed patients [18, 19]. Therapy with PLC is justified by its capacity to intervene in specific biochemical pathways in order to allow the recovery of efficient cellular respiration, in addition to reducing the damaging effects of free radicals produced by the hydrolysis of O_2 [26, 27]. Furthermore, studies have demonstrated that L-carnitine ameliorates oxidative damage via its antioxidant properties, free radical scavenging [40], and via its capacity to antagonize the suppression of the activity of antioxidant enzymes [41]. Our results demonstrate that PLC therapy leads to progressive improvements in metabolic and hemodynamic performance in patients with PAD and ESRD. Similar results on improvements in walking performance and functional status in patients with PAD alone have been previously observed [25, 42–44]. Previous publications of studies have discussed the positive effects of positive L-carnitine therapy and have stated that these effects could be brought about by an increase in blood flow velocity, reduction of PAI-1 activity, and an increase in red blood cell deformability [45, 46].

It is likely that at least some of the beneficial effects of PLC can be attributed to its role as an antioxidant [47, 48]. To this end, our results also showed improvements in the endothelial balance and shed further light on its mechanism of action. The progressive and significant reduction in all measured parameters confirms that PLC affects endothelial disruption. It is possible that this is due to the reestablishment of efficient mitochondrial activity and improved efficiency in the utilization of O_2, in addition to inhibiting the production of free radicals and their negative effects on endothelial function. This increased capacity is further reflected in improved hemodynamic function in peripheral areas and an increase in the ABI. It is well known that patients on hemodialysis have reduced concentrations of carnitine in plasma and muscular tissue [39], which led to the study of L-carnitine supplementation in the clinical context. Indeed, several reports have indicated that administration of L-carnitine reduces fatigue and may maintain exercise capacity in hemodialysis patients [33, 34, 43, 44]. CRI is often associated with PAD. Therefore, the possibility of therapy with PLC in hemodialysis patients with PAD is extremely attractive, given the close clinical association between these two pathologies. However, to our knowledge, the prospect of utilizing L-carnitine therapy in patients with ESRD as well as PAD has not been previously examined.

Both PAD and ESRD have grave effects on cardiovascular morbidity. The significant increase in the ABI after therapy with PLC is reflective of an improvement in he-

modynamic conditions, and is a prognostic factor in patients with PAD. Thus, even though more long-term clinical outcome studies have not been performed, a similar or even more pronounced effect on prognosis would be expected in patients with both PAD and ESRD.

In the present trial, long-term administration of PLC led to significant improvements in parameters of endothelial function in patients with PAD and ESRD. The improved clinical parameters, reflected in hemodynamic parameters such as ABI, suggest that the endothelial profile also undergoes statistically significant changes following intravenous administration of PLC. Thus, adjunctive therapy with PLC may be warranted for treatment of PAD in patients with ESRD on hemodialysis.

In conclusion, our results indicate that administration of PLC to patients with both ESRD and PAD leads to progressive improvements in clinical measurements of ET-1 and Hcy after 6 and 12 months' administration. Moreover, this clinical benefit is likely to be related to changes that concern the reduction of vasoconstrictor activity as shown by lower levels of ET-1, and may also concern antithrombotic activity as demonstrated by lower levels of Hcy at follow-up. Our findings suggest that therapy with PLC may be of clinical value for the treatment of hemodialysis patients with PAD.

References

1 Coresh J, Longenecker JC, Miller ER 3rd, Young HJ, Klag MJ: Epidemiology of cardiovascular risk factors in chronic renal disease. J Am Soc Nephrol 1998;9(suppl 12):S24–S30.

2 Levin A: Anemia and left ventricular hypertrophy in chronic kidney disease populations: a review of the current state of knowledge. Kidney Int Suppl 2002;80:35–38.

3 London GM: Cardiovascular disease in chronic renal failure: pathophysiologic aspects. Semin Dial 2003;16:85–94.

4 Parfrey PS, Foley RN: The clinical epidemiology of cardiac disease in chronic renal failure. J Am Soc Nephrol 1999;10:1606–1615.

5 Sarnak MJ, Levey AS: Cardiovascular disease and chronic renal disease: a new paradigm. Am J Kidney Dis 2000;35(suppl 1): S117–S131.

6 Landray MJ, Thambyrajah J, McGlynn FJ, Jones HJ, Baigent C, Kendall MJ, Townend JN, Wheeler DC: Epidemiological evaluation of known and suspected cardiovascular risk factors in chronic renal impairment. Am J Kidney Dis 2001;38:537–546.

7 Homocysteine Studies Collaboration: Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. JAMA 2002;288:2015–2022.

8 Agapitov AV, Haynes WG: Role of endothelin in cardiovascular disease. J Renin Angiotensin Aldosterone Syst 2002;3:1–15.

9 Lariviere R, Lebel M: Endothelin-1 in chronic renal failure and hypertension. Can J Physiol Pharmacol 2003;81:607–621.

10 Rohmeiss P, Birck R, Braun C, Kirchengast M, van der Woude FJ: Targets for endothelin in the diseased kidney: clues for therapeutic intervention. Exp Nephrol 1999;7:1–10.

11 Demuth K, Blacher J, Guerin AP, Benoit MO, Moatti N, Safar ME, London GM: Endothelin and cardiovascular remodelling in end-stage renal disease. Nephrol Dial Transplant 1998;13:375–383.

12 Boushey CJ, Beresford SA, Omenn GS, Motulsky AG: A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA 1995;274:1049–1057.

13 Harrison DG: Endothelial dysfunction in atherosclerosis. Basic Res Cardiol 1994; 89(suppl 1):87–102.

14 Darius H, Pittrow D, Haberl R, Trampisch HJ, Schuster A, Lange S, Tepohl HG, Allenberg JR, Diehm C: Are elevated homocysteine plasma levels related to peripheral arterial disease? Results from a cross-sectional study of 6880 primary care patients. Eur J Clin Invest 2003;33:751–757.

15 Foley RN, Parfrey PS, Sarnak MJ: Clinical epidemiology of cardiovascular disease in chronic renal disease. Am J Kidney Dis 1998; 32(suppl 3):S112–S119.

16 Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T: Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. Circulation 2001;104:2673–2678.

17 Kang SS, Wong PW, Malinow MR: Hyperhomocyst(e)inemia as a risk factor for occlusive vascular disease. Annu Rev Nutr 1992;12:279–298.

18 Klahr S: Mechanisms of progression of chronic renal damage. J Nephrol 1999; 12(suppl 2):S53–S62.

19 Levey AS, Eknoyan G: Cardiovascular disease in chronic renal disease. Nephrol Dial Transplant 1999;14:828–833.

20 Kundhal K, Lok CE: Clinical epidemiology of cardiovascular disease in chronic kidney disease. Nephron Clin Pract 2005;101:c47–c52.

21 Lamar Welch VL, Casper M, Greenlund K, Zheng ZJ, Giles W, Rith-Najarian S: Prevalence of lower extremity arterial disease defined by the ankle-brachial index among American Indians: the Inter-Tribal Heart Project. Ethn Dis 2002;12:S1-63–67.

22 O'Hare AM, Glidden DV, Fox CS, Hsu CY: High prevalence of peripheral arterial disease in persons with renal insufficiency: results from the National Health and Nutrition Examination Survey 1999–2000. Circulation 2004;109:320–323.

23 Rose GA: The diagnosis of ischaemic heart pain and intermittent claudication in field surveys. Bull World Health Organ 1962;27: 645–658.

24 Leskinen Y, Salenius JP, Lehtimaki T, Huhtala H, Saha H: The prevalence of peripheral arterial disease and medial arterial calcification in patients with chronic renal failure: requirements for diagnostics. Am J Kidney Dis 2002;40:472–479.

25 Ikizler TA: Role of nutrition for cardiovascular risk reduction in chronic kidney disease patients. Adv Chronic Kidney Dis 2004;11: 162–171.

26 Violi F, Loffredo L, Mancini A, Marcoccia A: Antioxidants in peripheral arterial disease. Curr Drug Targets 2003;4:651–655.

27 Cittanti C, Colamussi P, Giganti M, Orlandi C, Uccelli L, Manfrini S, Azzena G, Piffanelli A: Technetium-99m sestamibi leg scintigraphy for non-invasive assessment of propionyl-L-carnitine induced changes in skeletal muscle metabolism. Eur J Nucl Med 1997;24: 762–766.

28 Brevetti G, Angelini C, Rosa M, Carrozzo R, Perna S, Corsi M, Matarazzo A, Marcialis A: Muscle carnitine deficiency in patients with severe peripheral vascular disease. Circulation 1991;84:1490–1495.

29 Hiatt WR, Wolfel EE, Regensteiner JG, Brass EP: Skeletal muscle carnitine metabolism in patients with unilateral peripheral arterial disease. J Appl Physiol 1992;73:346–353.

30 Signorelli SS, Malaponte G, Di Pino L, Digrandi D, Pennisi G, Mazzarino MC: Effects of ischaemic stress on leukocyte activation processes in patients with chronic peripheral occlusive arterial disease: role of L-propionyl carnitine administration. Pharmacol Res 2001;44:305–309.

31 Ferrari R, Merli E, Cicchitelli G, Mele D, Fucili A, Ceconi C: Therapeutic effects of L-carnitine and propionyl-L-carnitine on cardiovascular diseases: a review. Ann NY Acad Sci 2004;1033:79–91.

32 Sayed-Ahmed MM, Shouman SA, Rezk BM, Khalifa MH, Osman AM, El-Merzabani MM: Propionyl-L-carnitine as potential protective agent against Adriamycin-induced impairment of fatty acid beta-oxidation in isolated heart mitochondria. Pharmacol Res 2000;41:143–150.

33 Brass EP, Adler S, Sietsema KE, Hiatt WR, Orlando AM, Amato A: Intravenous L-carnitine increases plasma carnitine, reduces fatigue, and may preserve exercise capacity in hemodialysis patients. Am J Kidney Dis 2001;37:1018–1028.

34 Hurot JM, Cucherat M, Haugh M, Fouque D: Effects of L-carnitine supplementation in maintenance hemodialysis patients: a systematic review. J Am Soc Nephrol 2002;13:708–714.

35 Labonia WD: L-Carnitine effects on anemia in hemodialyzed patients treated with erythropoietin. Am J Kidney Dis 1995;26:757–764.

36 Vlassopoulos DA, Hadjiyannakos DK, Anogiatis AG, Evageliou AE, Santikou AV, Noussias CV, Papandreou PT, Hadjiconstantinou VE: Carnitine action on red blood cell osmotic resistance in hemodialysis patients. J Nephrol 2002;15:68–73.

37 Taute BM, Taute R, Heins S, Behrmann C, Podhaisky H: Hyperhomocysteinemia: marker of systemic atherosclerosis in peripheral arterial disease. Int Angiol 2004;23:35–40.

38 Loncar R, Hrboka V, Tabakovic-Loncar V, Ostojic Z, Deussen A: Screening of plasma homocysteine in peripheral arterial disease. Ann Med 2001;33:48–54.

39 Dashwood MR, Jagroop IA, Gorog DA, Bagger JP: A potential role for endothelin-1 in peripheral vascular disease. J Cardiovasc Pharmacol 2000;36(suppl 1):S93–S94.

40 Sener G, Eksioglu-Demiralp E, Cetiner M, Ercan F, Sirvanci S, Gedik N, Yegen BC: L-Carnitine ameliorates methotrexate-induced oxidative organ injury and inhibits leukocyte death. Cell Biol Toxicol 2006;22:47–60.

41 Sener G, Paskaloglu K, Satiroglu H, Alican I, Kacmaz A, Sakarcan A: L-Carnitine ameliorates oxidative damage due to chronic renal failure in rats. J Cardiovasc Pharmacol 2004;43:698–705.

42 Hiatt WR, Regensteiner JG, Creager MA, Hirsch AT, Cooke JP, Olin JW, Gorbunov GN, Isner J, Lukjanov YV, Tsitsiashvili MS, Zabelskaya TF, Amato A: Propionyl-L-carnitine improves exercise performance and functional status in patients with claudication. Am J Med 2001;110:616–622.

43 Miller B, Ahmad S: A review of the impact of L-carnitine therapy on patient functionality in maintenance hemodialysis. Am J Kidney Dis 2003;41(suppl 4):S44–S48.

44 Steiber AL, Weatherspoon LJ, Spry L, Davis AT: Serum carnitine concentrations correlated to clinical outcome parameters in chronic hemodialysis patients. Clin Nutr 2004;23:27–34.

45 Pola P, De Martini D, Gerardino L, De Rossi S, Tondi P: The action of propionyl-L-carnitine on the vasal endothelium: increased t-PA synthesis and a decrease in the activity of PAI-1. A preliminary study. Drugs Exp Clin Res 1992;18:343–348.

46 Pola P, Flore R, Tondi P, Nolfe G: Rheological activity of propionyl-L-carnitine. Drugs Exp Clin Res 1991;17:191–196.

47 Hulsmann WC, Dubelaar ML: Carnitine requirement of vascular endothelial and smooth muscle cells in imminent ischemia. Mol Cell Biochem 1992;116:125–129.

48 Spagnoli LG, Orlandi A, Marino B, Mauriello A, De Angelis C, Ramacci MT: Propionyl-L-carnitine prevents the progression of atherosclerotic lesions in aged hyperlipemic rabbits. Atherosclerosis 1995;114:29–44.