Combined therapy with L-carnitine and erythropoietin of anemia in chronic kidney failure patients undergoing hemodialysis

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Abstract: Objectives. The aim of this study was to analyze the influence of combined therapy with L-carnitine and erythropoietin on selected blood morphology parameters in patients treated with hemodialysis and to assess whether combined therapy could decrease the requirement for exogenous erythropoietin. **Patients and methods.** The results of anemia treatment were compared in three groups of patients: 27 patients treated with L-carnitine and erythropoietin, 15 patients treated with erythropoietin and 9 patients treated only with L-carnitine. The patients were treated for 6 months. L-carnitine was given orally at a dose of 4 x 250 mg daily. Erythropoietin was administered intravenously after each hemodialysis session and the mean dose of erythropoietin at the beginning of observation was 5642 ±2134 units/week. Before treatment serum concentrations of free and total carnitine, parathormone (PTH), aluminium, lead were determined and basic laboratory examinations were performed. The blood morphology was evaluated once a month. **Results**. Combined therapy resulted in the improvement of blood morphology parameters (hemoglobin [Hb] before treatment 9.9 ±1.4 g/dl, during treatment 10.7 ±1.6 g/dl), compared to treatment with erythropoietin (Hb before treatment 9.5 ±1.2 g/dl, during treatment 9.9 ±1.4 g/dl) or L-carnitine alone (Hb before treatment 11.3 ±1.0 g/dl, during treatment 12.0 ±1.1 g/dl). Combined therapy was associated with the reduction of erythropoietin dosage during treatment from 6287 ±1987 units/week to 2286 ±1684 units/week. The correlation between serum carnitine concentration and erythrocyte osmotic resistance indicates indirectly the beneficial effect of L-carnitine administration on erythrocyte cell membrane stabilization.

Key words: anemia, chronic renal failure, L-carnitine and erythropoietin treatment

INTRODUCTION

During last years there is growing interest in the impact of L-carnitine deficiency on anemia of chronic renal failure (CRF) that a complex and poorly understood phenomenon. As shown in experimental studies, L-carnitine does not influence erythroid progenitors in the bone marrow, while its palmitic acid (palmitoyl-l-carnitine) ester stimulates erythropoiesis [1,2]. Available evidence indicates that L- carnitine deficiency destabilizes erythrocyte membrane and its metabolism, thus causing survival time reduction and in consequence leads to anemia development and progression. In CRF patients hemolysis, decreased erythrocyte osmotic fragility and reduced erythrocyte survival time by 1/4 was observed [1-3]. Cheng et al. suggested that one of the reasons of this phenomenon may be the incorrect function of erythrocyte sodium-potassium pump (Na^+ – K^+ ATPase). Several factors that deteriorate sodium-potassium pump function have been identified. One candidate is the excess of free fatty acids and long-chain carnitine esters, not oxidized in the mitochondria in CRF patients as a result of L-carnitine deficiency [2]. A key role of L-carnitine in sodium- potassium pump activity improvement consists in binding, transport and incorporating fatty acids in erythrocyte cell membrane structure and, as a result, its stabilization. L-carnitine and enzymic carnitine-palmitic transferase structures affect cell membrane phospholipids metabolism and consequently reduce the effect of oxidative stress on CRF and hemodialyzed patients [5].

Witko-Sarsat et al. [6] suggested dysfunction of anaerobic glycolysis in erythrocytes in CRF patients, in whom red blood cells are unable to produce a sufficient amount of NADPH (Nicotinamide adenine dinucleotide phosphate), which is essential for regeneration of reduced glutation (GSH) from oxidized glutation (GSSG). These factors limit red blood cells H_2O_2 (Hydrogen Peroxide) and oxygen radicals inactivation

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Table 1. Clinical characteristic of 51 CRF hemodialyzed patients enrolled to study

	Group I	Group II	Group III
Age (years)	48.14 ±7.72	49.87 ±12.6	51.2 ±14.5
Period of dialyses (months)	31.8 ±18.41	35.51 ±12.5	30.78 ±15.4
Urea (mmol/l)	20.18 ±3.25	22.5 ±5.1	19.6 ±12.6
Creatinine (μmol/l)	843.36 ±156.34	872.11 ±122.27	789.16 ±135.4
Parathormone (pg/ml)	276.12 ±25.95	278.00 ±25.12	301.11 ±38.5
Aluminum (μg/l)	1.68 ±0.86	1.72 ±0.96	1.71 ±1.1
Lead (μg/l)	0.76 ±0.51	0.80 ±0.47	0.79 ±0.54
Data shown as mean value ± SD			

capacity thus generate the SH protein group oxygenation and then peroxidation cell membrane lipids and also enhance erythrocyte lysis susceptibility. It has been revealed that L-carnitine protects cells against oxidative stress in the mechanism involving iron chelation [7]. Some authors [5] presented thus justified view that combined supplementation of L-carnitine, iron and vitamin C in CRF patients can protect against oxidative stress.

A number of authors emphasized a role of combined L-carnitine and epoietin therapy via the synergistic effect on anemic patients. Moreover, due to insufficient funding from the National Health Fund (NFZ), acquirement of hemoglobin target level sometimes turned out to be impossible thus attempts aimed at improved therapy effectiveness through combining drugs seems justified.

The purpose of this study was to analyze the influence of combined L-carnitine and epoietin therapy on basic hematological peripheral blood parameters in hemodialyzed anemic patients and to investigate whether combined L-carnitine and epoietin therapy may decrease need of epoietin.

PATIENTS AND METHODS

The study was performed in chronic hemodialyzed patients in Nephrology, Transplantation and Internal Medicine Clinic of Medical Academy in Poznań. A local Ethics Committee consent was obtained.

Fifty-one patients, aged on average 49.5 ±9.7 years chronically dialyzed from 33.8 ±20.4 months participated in the study. Hemodialysis was performed three times a week with an average procedure time of 5 hours. Procedures were carried out typically with single use capillary dialysators by Fresenius with polyacrylnitrile or polysulphone membrane filled with 0,9% sodium chloride. Dialysis fluid flow averaged 500 ml/min, blood flow 200 ml/min. Dialysis fluid with bicarbonate buffer 35 mEq/l was used. A mean value Kt/v was 1,38 ±0,19.

Forty-two patients were treated with epoietin alpha intravenously after each dialysis session ensuring appropriate values of iron metabolism. Despite the average weekly epoitetin dose of 5642 ±2134 U (hospital financial limit), without any chronic inflammation symptoms, the recommended hemoglobin blood level was not achieved (mean hemoglobin [Hb] level 10.3 ±1.4 g/dl).

Patients were divided into 3 groups:

- Group I, 27 including patients (10 with glomerulonephritis, 3 diabetic nephropathy, 4 hypertension nephropathy, 3 with renal failure from unknown reason), treated with L-carnitine (Carnivit, Polfa Kutno) 4 x 250 mg/d *p.o.* in addition to a previous epoietin therapy.
- Group II including 15 patients (6 with glomerulonephritis, 7 pyelonephritis, 2 diabetic nephropathy) continued treatment with epoietin alone.
- Group III including 9 patients (6 with polycystic kidney disease, 2 diabetic nephropathy, 1 Alport syndrome) not treated with epoietin because of satisfactory Hb concentration. This group was treated with L-carnitine alone with the same doses as in Group I.

Basic patients' characteristics were shown in table 1.

The study time was 6 months. Basic peripheral blood parameters were controlled each month as well as one month before the study onset and two months after the end of the study. Epoietin dose was individually modified with dose reduction when hemoglobin level higher than 11–12 g/dl was achieved. Before and 6 months after treatment serum free and total carnitine levels were estimated (diagnostic tests by Boehringer Mannheim) as well as erythrocyte osmotic fragility (macroscopic method) in all patients.

Blood was collected before dialysis from venous part of arteriovenous fistula. Before study treatment started basic laboratory assays with commercial diagnostic tests were performed. Parathormone (PTH) serum level was estimated by a radioimmunological method and aluminum and lead level by inverse voltamperometry.

For comparison serum carnitine concentration was determined once in 30 healthy subjects at an average age of 45.2 ±12.6 years. Serum urea, creatinine and PTH levels averaged 2.5 ±1.1 mmol/l, 72.1 ±22.,3 umol/l and 78.0 ±56.7 pg/ml, respectively.

Table 2. Average hemoglobin and hematocrite level, red blood cells count, white blood cells count and platelet count before, during and after treatment with L-carnitine and epoietin (group I), epoietin alone (group II) and L-carnitine alone (group III)

	L-carnitine+epoietin			Epoietin			L-carnitine		
	Before treatment	During treatment	After treatment	Before treatment	During treatment	After treatment	Before treatment	During treatment	After treatment
Hb (g/dl)	9.9 ±1.4	10.7 ±1.6**	10.5 ±1.6	9.5 ±1.2	9.9 ±1.4	10.2 ±1.3	11.3 ±1.0	12.0 ±1.1	10.7 ±1.3
Hct (%)	28.7 ±3.8	31.5 ±4.2**	30.6 ±4.6	27.0 ±3.3	28.7 ±3.8	30.4 ±3.6	32.7 ±3.4	33.8 ±2.8	31.6 ±4.0
RBC (T/l)	2.9 ±0.6	3.3 ±0.5*	3.1 ±0.4	3.0 ±0.4	3.1 ±0.5	3.1 ±0.4	3.9 ±0.7	3.8 ±0.4	3.4 ±0.7
WBC (G/l)	6.4 ±3.1	6.3 ±1.5	6.6 ±1.4	7.3 ±1.7	6.4 ±2.2	6.4 ±1.3	6.9 ±1.3	6.2 ±1.3	6.8 ±1.4
PLT (G/l)	207.0 ±71.3	227.2 ±59.8	214.5 ±61.4	224.2 ±59.4	212.7 ±51.6	183.2 ±42.3	234.3 ±36.1	232.3 ±22.0	230.3 ±58.7

Data shown as mean value ± SD. * p <0.01, ** p <0.0006 comparing with before treatment value

Statistical analysis involved a comparison and relationship between analyzed features. Comparison of the variables studied was performed in three groups. Mean values before, during and after treatment were assessed with the variance analysis. After finding differences in groups analyzed altogether, the Newman-Keuls test was used for comparison in pairs and t-Student test was used to asses where the mean values of features are lower. In the results, the differences that were statistically significant for assumed probability p = 0,05 were noted. Variance analysis has been preceded by verifying its theoretical assumption (normality was verifying by Shapiro-Wilk test). Feature correlation was tested with Pearson correlation factor for assumed confidence level of p = 0.05. Calculations were performed using an excel spreadsheet and the Statictica software [8].

RESULTS

Mean hemoglobin concentrations, red blood cells, white blood cells, platelets are and hematocrit before, during and after study in 3 groups of patients showed in table 2.

Using univariate analysis, mean hemoglobin concentration, hematocrite, erythrocyte, leukocyte and platelet count during treatment period were compared among 3 groups.

The average erythrocyte count during treatment period in group I was 3.3 ±0.4 T/l, in group II 3.1 ±0.5 T/l, in group III 3.8 ±0.4 T/l. Using Newman-Keuls test statistically significant differences between groups I and III (p < 0.03) and I and II (p<0.01) were showed.

Average hemoglobin concentration during treatment period in group I was 10.7 ±1.6 g/dl, in group II 9.9 ±1.4 g/dl, in group III 12.0 ±1.1 g/dl. Using the Newman-Keuls test, statistically significant differences between groups I and III (p <0,03), I and II (p <0,001) and II and III (p <0,03) were showed.

Average hematocrite during treatment period in group I was 31.5 ±4.2% , in group II 28.7 ±3.8%, in group III 33.8 ±2.8%. Using the Newman-Keuls test, statistically significant differences between groups I and II (p <0,004) and II and III (p <0,001) were showed.

In group III treated with L-carnitine there were no differences between mean values of the parameters before, during and after treatment.

In group II treated with epoietin mean values of estimated parameters did not differ significantly in consecutive study periods.

A mean epoietin dose during 6 months of study treatment was 5642 ±2134 U/week and was unchanged.

In group I treated with L-carnitine and epoietin a statistically significant increase of Hb concentration level (p <0,0005), hematocrite value (p <0,0006) and erythrocyte count (p <0,01) was observed during the study compared to the values before the treatment.

An initial epoietin dose in this group was 6287 ±1987 U/week, final dose 2286 ±1684 U/week. A difference between the average initial and final epoietin dose was 4000 ±2417.88 U/week. Reduction in epoietin dose was statistically significant (p <0.0001).

Maximal and minimal values of erythrocytes osmotic fragility before and after treatment in three groups of patients were shown in table 3.

As a result of variation analysis for comparison of erythrocytes osmotic fragility before and after treatment statistically significant differences for maximal value after treatment p <0.0008) were found. A comparison using the Newman-Keuls test demonstrated statistical significance (p <0.01) of differences between group I and III and also between and II.

Table 3. Erythrocyte osmotic fragility before and after combined L-carnitine and epoietin treatment and epoietin or L-carnitine alone

Dane	L-carnitine+epoietin		Epoietin		L-carnitine	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Minimal osmotic fragility (mmol NaCl/l)	102.0 ±7.9	69.3 ±4.8**	95.0 ±9.2	84.4 ±8.9*	100.0 ±4.7	89.0 ±6.7**
Maximal osmotic fragility (mmol NaCl/l)	81.3 ±7.9	46.0 ±6.3*	58.8 ±9.2	44.4 ±12.0*	71.0 ±8.8	48.0 ±6.3**

Data shown as mean value ± SD. * $p < 0.05$ comparing with before treatment value, ** $p < 0.0002$ comparing with before treatment value

Table 4. Free and total carnitine serum level before and after treatment in three groups of patients

Parameter	L-carnitine+epoietin		Epoietin		L-carnitine	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Free carnitine (μmol/l)	24.45 ±3.47	48.78 ±5.9*	20.2 ±2.14	19.8 ±3.15	22.2 ±2.47	45.0 ±5.22*
Total carnitine (μmol/l)	43.2 ±5.13	68.15 ±8.1*	39.1 ±2.13	38.2 ±2.68	41.0 ±3.03	65.5 ±6.29*

Data shown as mean value ± SD. * $p < 0.05$ comparing with before treatment value

Reference values: maximal fragility 30–50 mmol NaCl/l, minimal 89–90 mmol NaCl/l.

Serum free and total carnitine levels in three groups were shown in table 4.

There were no statistically significant differences between serum free and total carnitine level in three groups before treatment. After treatment in groups I and III free and total serum carnitine level was significantly higher ($p < 0.05$) than before treatment.

A negative correlation between total carnitine concentration after treatment and maximal erythrocyte osmotic fragility was demonstrated ($r = -0.945$, $p < 0.001$).

DISCUSSION

The results of previous studies published by the authors confirmed their observations of beneficial effect of L-carnitine and epoietin combined treatment in CRF patients. Effectiveness of combined therapy was demonstrated by statistically significant increases in hematocrit, hemoglobin concentration and erythrocyte count after treatment. However, epoietin or L-carnitine when administered alone did not induce a statistically significant increase in hematocrit, hemoglobin concentration and erythrocyte count.

Our previous studies showed also that combined treatment allowed statistically significant epoietin dose reduction. Observation is consistent with our previous report of L-carnitine and epoietin combined treatment predominance in significant improvement in peripheral blood parameters leading in consequence to reduction in epoietin required dose [1-3,8-9].

In our study we observed significant improvement of erythrocyte osmotic stability after 6 months of treatment when L-carnitine was administered. The correlation between total carnitine concentration and osmotic fragility of erythrocytes after treatment showed indirectly favourable influence of higher L-carnitine concentration on erythrocyte cell membrane stabilization.

However, available data concerning a role of L-carnitine in the treatment of anemia in CRF patients is not so consistent. Some authors considered that carnitine does not affect directly erythropoiesis and therefore, played a supportive role only [10]. However, Kitamura et al. [11] and Trovato et al. proved that L-carnitine and in particular its ester, palmitoyl-l-carnitine, stimulate erythropoiesis, which was expressed by a significant increase of erythroid progenitors in bone marrow (CFU-E) and reticulocyte count. A majority of authors do not confirm this observation, emphasizing a sole effect of L-carnitine on erythrocyte cell membrane metabolism and function. [8, 9].

Vlassoupoulos et al. [13] and others [1-3] suggested a significant role of L-carnitine in erythrocyte cell membrane stabilization in CRF patients. According to those authors hemolysis occurred exclusively in patients with low total carnitine serum level as well as erythrocyte osmotic fragility and erythrocyte survival time unsettled. Treatment with L-carnitine led to improvement of above-mentioned parameters, increase of hema-

tocrite and hemoglobin level. In the group of patients treated with epoietin alone those authors observed neither erythrocyte osmotic fragility improvement nor increasing erythrocyte survival time. They did not demonstrate a direct epoietin influence on red blood cell membrane function on the basis of the results achieved. Notwithstanding our study suggested that erythrocyte osmotic fragility changed during combined treatment with epoietin and L-carnitine as well as with epoietin alone. Presumably epoietin influences somehow red blood cell membrane or membrane metabolic pathways stabilizing cell membrane of blood cells.

A correlation between carnitine serum level and osmotic fragility improvement confirms the discussed carnitine favourable effect on erythrocyte cell membrane stabilization.

Matsumoto et al. [14], Sotirakopoulos et al. [15] and Kooistra et al. [16] showed different epoietin requirement in CRF patients, which is probably related with carnitine deficiency. When free and total carnitine serum level were low (free 23.0 ±3.0 μmol/l, total 28.5 ±8.9 μmol/l] and hematocrite is less than 30%, substitution with carnitine and epoietin was essential. Whereas in patients with normal carnitine level (free 43.4 ±8.2 μmol/l, total 52.3 ±1.4 μmol/l) and normal hematocrit, epoietin substitution was not indispensable.

Our studies did not fully confirm those observations. Average serum free and total carnitine levels were comparably low in the three groups of patients. Renal failure progression was comparable while hematocrit and hemoglobin values differed. It seems to be related to different epoietin serum level and different epoietin request in individual cases. Only in two patients who received up-front lower epoietin doses, carnitine serum level was significantly higher than in others. In those patients there was no longer need of epoietin administration after one month of treatment.

Analysis of other causes of anemia showed no clinically significant blood loss or aluminum or lead serum level increase. There was hyperparathyroidism diagnosed in all patients, whereas neither of their parathormone serum level exceeded 400 pg/ml.

L-carnitine treatment is safe; there was no side effect during treatment observed [17]. NKF DoQI guidelines propose l-carnitine implementation in patients requiring high epoietin doses, after severe hyperparathyroidism and aluminum or lead intoxication is excluded [17,18].

Results of our study are in agreement with available data indicating beneficial therapeutic effect of combined L-carnitine and epoietin usage in CRF anemic patients' treatment. This treatment scheme provides a measurable benefit from the economic point of view as well. A monthly cost of carnitine treatment is lower than cost of epoietin treatment. Moreover, we showed in our study that combined treatment allows to reduce epoietin dose significantly. At present when epoietin treatment of CRF patients' reimbursement is insufficient, a proposed treatment schedule is well-grounded.

CONCLUSIONS

Combined L-carnitine and epoietin treatment leads to significant improvement of hematological parameters in comparison to treatment with L-carnitine or epoietin alone. This therapeutic management allowed reducing epoietin doses.

The observed relationship between carnitine serum level and osmotic erythrocyte fragility suggests indirectly its beneficial role in erythrocyte membrane stabilization.