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Erythrocyte Metabolism and Renal Anemia in Hemodialyzed Patients Supplemented with L-Carnitine

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Abstract

Background and Aims: Potential role of L-carnitine in anemia adjuvant treatment is still a matter of concern. The aim of the study was to determine the influence of L-carnitine on renal anemia and erythrocyte metabolism in haemodialyzed (HD) patients.

Methods: We studied 26 patients (13 male and 13 female) aged from 13 to 65, (mean age 38) and 30 healthy volunteers (12 male, 18 female) aged from 25 to 65, (mean age 40). HD patients showed stable hemoglobin (Hb) levels and low plasma free carnitine (FC) levels. Laboratory markers of: carnitine metabolism, hematological status, iron stores as well as erythrocyte metabolism were measured. L-carnitine (500 mg/day) was administered orally for 12 months (dose from 5.3 to 16.7 mg/kg of body weight, mean dose 9 mg/kg).

Results: After six months of oral low doses administration of L-carnitine, plasma total (TC) and FC levels increased and reached a plateau (at almost two times the initial levels) being at 12 months significantly higher than in the controls. During L-carnitine treatment a significant increase of mean Hb concentration was observed. Patients who responded (14/26) to L-carnitine differed from those who did not respond, with significantly longer dialysis time and lower both initial FC concentrations and FC/TC ratio. The following changes in erythrocyte metabolism were noticed: 1) glycolytic enzymes: significant increase of erythrocyte sodium, potassium ATPase, hexokinase and lactate dehydrogenase activities, 2) glycolytic intermediates: increase of 2,3-diphosphoglycerate and decrease of adenosine triphosphate concentrations, 3) decrease of the glucose uptake and lactate production by erythrocytes.

Conclusions: 1. L-carnitine may ameliorate anemia in some hemodialysis patients. 2. L-carnitine induces metabolic changes in erythrocytes. 3. Long-term maintenance hemodialysis patients may benefit most from L-carnitine supplementation. 4. Low doses of L-carnitine are effective in restoring the plasma carnitine pool.

Keywords: L-carnitine, Anemia, Hemodialysis

Introduction

Chronic anemia in uremic patients on a long-term hemodialysis is characterised by both decreased bone marrow production rate and shortened survival time of red blood cells (RBC). The decreased rate of RBCs production is related to inadequate erythropoietin secretion but the shortened survival time of RBCs depends on several biophysical and

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biochemical RBC abnormalities (1, 2). Following factors of shortened RBC survival are reported in literature: increased RBC adenosine triphosphate (ATP) concentration (secondary to stimulated glycolysis and to decreased sodium, potassium adenosine triphosphatase's (Na,K-ATPase) activity), impaired pentose phosphate shunt and altered lipid and phospholipid RBC membrane components (3).

It has been reported that long term hemodialysis induces blood and tissue carnitine deficiency (4-6). L-carnitine supplementation in such patients corrects the depletion of free carnitine (FC) and total (TC) plasma carnitine concentrations (7). Before recombinant erythropoietin (epoetin) was available, few papers were published showing significant favourable effect of L-carnitine supplementation on hemoglobin (Hb) level (8-11). After 1989, the favourable effect of L-carnitine on RBCs was confirmed by analysing trials in which L-carnitine was administered concomitantly with epoetin (12-15). During L-carnitine administration both the improvement of anemia and changes in RBC metabolism were reported (8, 16-19). The pathophysiology of anemia improvement after L-carnitine therapy is not fully clarified. However, the body of evidence suggests that carnitine induces structural and metabolic changes in RBCs and it increases their survival (20, 21).

In the present study we provide some additional evidence on L-carnitine hemopoietic properties in anaemic hemodialysis patients and identify the patients who may benefit from L-carnitine supplementation.

Material and Methods

The studies were carried out at our Department of Nephrology in the group of 26 hemodialyzed patients (13 male, 13 female, aged from 13 to 65 years, mean age 38) and in 30 healthy volunteers (12 male, 18 female, aged from 25 to 65 years, mean age 40) in the years 1998-2002.

Chronic glomerulonephritis was the underlying renal disease in 17/26 cases, chronic interstitial nephritis in 2/26 cases, polycystic kidney disease in 1/26 case, diabetic nephropathy in 1/26 case and reflux nephropathy in 5/26 cases. The patients were dialysed for 6 to 174 months (mean dialysis time 63.9±42.5 months). They were qualified for the study on the basis of: a) low plasma FC levels (31.6±9 mol/L vs 46±11 mol/L in healthy controls) and b) stable Hb levels during 6 months before L-carnitine introduction. Before the study these patients did not receive epoetin and L-carnitine.

The medication consisted mainly of antihypertensive drugs (calcium antagonists, angiotensin converting enzyme inhibitors) and phosphate binders (calcium carbonate). Before and during the study the patients also received: folic acid (5 mg per week), C vitamin (500 mg per week) and B6 vitamin (50 mg per week) (a routine procedure in our department). While being qualified none of the patients suffered from any infection, inflammation, malnutrition, malignancy, blood loss or severe hyperparathyroidism. Before and during L-carnitine administration all the patients were treated with intravenous iron and they had sufficient iron stores (transferin saturation (TSAT) > 20% and plasma ferritin >200 g/l). All the patients were dialysed using bicarbonate dialysis fluid and capillary hollow filter three times a week, for 3 to 5 hours per session (mean Kt/V 1.2±0.05).

L-carnitine (Carnitine, Sigma – Tau or Carnivit Polfa S.A. - Kutno) was administered orally, 500 mg/day for 12 months (dose from 5.3 to 16.7, mean 9 ± 3 mg/kg b.w.). 4/26 patients did not complete the study asbetween 6th and 9th month of observation they underwent kidney transplantation. Other patients did not suffer from any serious clinical condition during the study.

Blood samples in hemodialysis patients were collected from dialysis fistula, after an overnight fast, before the initiation of L-carnitine supplementation and after 3, 6, 9 and 12 months of the study. Blood samples in the control group were taken once only

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– at the beginning of the study. The samples were obtained via venipuncture from anterocubital vein after an overnight fast.

The following laboratory parameters were tested:

1) ***red blood count:***

We used Technicon 1 autoanalyser

2) ***iron status parameters:***

Transferin and ferritin tests were performed by immunoturbidimetric methods using Boehringer-Mannheim/Hitachi 704 system. Serum iron was assessed by colorimetric methods using Roche/Hitachi 704 system. All measurements were performed according to the manufacturers' instructions.

3) ***RBC metabolism markers:***

Na,K-ATPase activity was determined by Geudeney method (22). The final volume of the incubation mixture was 1.1 ml. The mixture contained: 40 mM Tris HCl (pH 7.40), 150 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$. It also contained isolated in 0.9% NaCl erythrocytes corresponding to about 12 mg Hb/sample and ±0.75 mM ouabain. The reaction was triggered at 37C^o by the addition of 4 mM ATP and the reaction was terminated 15 minutes later by the addition of 1.0 ml of 10% TCA.

Glycolytic intermediates: ATP and 2,3-diphosphoglycerate (2,3-DPG) were measured by enzymatic method (Sigma Diagnostics; USA) and all measurements were obtained according to the manufacturers' instructions.

Glycolytic enzymes: hexokinase (Hx) and lactate dehydrogenase (LDH) were measured by kinetic methods using LKB spectrophotometer at 30 C^o (23).

Glycolytic rate was measured by evaluating the glucose uptake and lactate production by RBCs. Those measurements were performed after separation of RBCs in a medium by the Wallas method (24); glucose and lactate concentration were determined by means of kits from Human GmbH (Germany) on LKB spectrophotometer. Intracellular potassium was measured by ISE-method after separation and lysis of RBCs (25).

4) ***plasma carnitine profile***

TC and FC plasma measurements were performed by means of enzymatic method given by Cederblad (26) with Sałek modification (27). Acylcarnitines (AC) levels were calculated as the difference between TC and FC.

The results are expressed as mean values (± SD). The following tests were applied for the analysis of significance: for related data – paired t-Student`s and Wilcoxon's tests; for unrelated data unpaired t-Student`s or Cochran–Cox and Mann -Whitney tests. More than three data points during treatment were estimated using analysis of covariance and Newman-Keuls or ANOVA Friedman tests. Pearson`s or R Spearman`s coefficients were calculated for data correlation. Data were evaluated using STATISTICA PL (version 5.1, StatSoft Inc).

The study protocol was approved by the Local Ethical Commission.

Results

Effects of oral L-carnitine supplementation on haematological parameters

Table 1 shows changes of haematological parameters in the examined group. In comparison to the beginning of the therapy a significant increase of Hb levels was observed (Wilcoxon's test $p<0.05$ vs month 0) and analysis of covariance revealed significant increase of Hb levels in a time dependent manner (ANOVA Friedman's test – Kendall coefficient = 0.55, $p<0.05$). In 14/26 patients increment in Hb concentration was ≥ 1 g/dl. These patients were significantly longer treated with maintenance dialysis time (76 vs 46 months; $p<0.05$). RBC count increased gradually and reached its significance after twelve months of treatment (Wilcoxon's test, $p<0.05$). Analysis of covariance revealed significant increase of RBCs in a time dependent manner (ANOVA Friedman test – Kendall's coefficient = 0.29, $p<0.05$). No significant changes in mean corpuscular Hb concentration (MCHC) and in mean corpuscular

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Hb content (MCH) were noticed during the time of L-carnitine administration.

Reticulocyte (Ret) count in L-carnitine treated patients was similar to controls and it was stable during the period of observation.

Table 1 also presents changes in iron parameters during L-carnitine treatment. Iron status in patients treated with L-carnitine was monitored by most widely used methods (28). Initially, our patients showed the following: plasma ferritin levels >200g/l and TSAT > 20%. Generally, these data reflect sufficient total body iron stores and the appropriate availability of iron to the bone marrow for erythropoiesis. During the whole period of L-carnitine treatment, TSAT and ferritin levels remained at the above mentioned levels and did not change significantly.

Effects of oral L-carnitine treatment on plasma carnitine profile (FC, TC and AC)

Plasma carnitine profile during L-carnitine supplementation is presented in Table 2. In the group of hemodialysis patients the initial level of plasma TC did not differ from that in controls. On the other hand, plasma FC and both FC/TC and FC/AC ratios were significantly lower in comparison to the controls (t-Student's, $p<0.05$). A negative linear correlation of hemodialysis duration time and initial levels of FC/TC and FC/AC (rxy - 0.5 and rxy - 0.46, respectively; $p<0.05$) were noticed. Plasma TC and FC increased significantly during the course of L-carnitine treatment (paired t-Student`s or Wilcoxon's test, $p<0.05$ vs month 0; ANOVA Friedman's test – Kendall's coefficient = 0.45, $p<0.05$). After six months of oral low doses administration of L-carnitine, plasma TC and FC levels increased and reached a plateau (at almost two times the baseline levels) being at 12 months significantly higher than in the controls. The FC/TC ratio increased significantly but was still significantly lower than in the controls. L-carnitine dose calculated per kilogram of body weight (from 5.3 to 16.7 mg/kg b.w.) did not correlate with plasma FC and TC levels.

Initial mean FC level and both FC/TC and FC/AC ratios were significantly lower in patients who presented increase in Hb concentrations as compare to those whose Hb concentrations were stable during the study (FC 28.5 vs 35.3 µmol/l; FC/TC 0.53 vs 0.66; FC/AC 1.26 vs 2.01 respectively) and what is more, their TC and FC levels got significantly higher after respectively 6 and 9 months of L-carnitine administration (t-Student's, $p<0.05$).

Table 1. Changes in hematological parameters: (hemoglobin (Hb), erythrocyte (RBC), reticulocyte (RET) count, mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin content (MCH) and in iron status parameters in L-carnitine treated patients during observation period

Parameters	Months of observation							
	-6	-3	-1	0	3	6	9	12
Hb (g/dl)	9.6±1.5	10.1±1.7	10.2±1.4	10.1±1.1	10.2±1.3	10.6±1.5*	10.8±1.8*	10.9±1.7*
RBC (T/l)	3.3±0.6	3.34±0.7	3.44±0.6	3.32±0.6	3.34±0.5	3.51±0.6	3.58±0.7	3.72±0.6*
RET (%)				9±6	9±7	10±6	10±6	8±4
MCHC(g/dl)				32.0±1.3	33.0±0.9	32.9±1.1	33.1±1.0	34.0±1.5
MCH (pg)				33.2±1.3	33.0±1.0	33.0±1.2	33.0±1.0	33.0±1.0
Ferritin (µg/l)				272±55	284±80	280±70	260±55	270±60
TSAT (%)				28±3	26±5	28±6	28±5	27±3

***p<0.05 vs month 0**

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Table 2. Changes in plasma carnitine profile: total (TC), free (FC), acylcarnitine (AC), FC/TC and FC/AC ratios in L-carnitine treated group of patients during observation period. The difference between the group of patients whose haemoglobin increased ≥ 1 g/dl and the rest of the group is presented

Parameters	Months of observation					
	0	3	6	9	12	Control
TC whole group (µmol/L)	54.7±11	84.4±30*	85.4±27*	86.5±28*	86.6±35*#	54.7±13#
TC in responders	53.9±6.7	91.3±32.2*	99.7±22.8*^	99.9±28*^	106.5±35.8*^#	
TC rest of the group	55.9±14.5	76.4±25.2*	69.8±21.9*^	72.0±20*^	69.1±20.3*^	
FC whole group (µmol/L)	31.6±9#	46.7±16*	52.5±16*	57.5±18*	58.9±25*#	46±11#
FC in responders	28.5±7.2#^	49.4±15.6*	57.9±15.2*	64.3±15.9*^	67.7±24.4*^#	
FC rest of the group	35.3±7.99#^	43.1±19.9*	45.6±14.4*	49.4±16.4*^	49.6±22.3*^	
FC/TC	0.58±0.1#	0.57±0.2	0.60±0.1	0.66±0.1*	0.66±0.1*#	0.85±0.1#
FC/AC	1.61±0.9#	1.87±1.5	1.63±0.5	2.29±1.3*	1.89±0.6*#	8.68±7.9#
AC (µmol/L)	23.9±8#	38.3±29*	34.6±14*	31±13*	40.6±18*#	8±5#

***p**<0.05 vs month 0;

#p<0.05 between tested groups and controls before and after 12 months of treatment

^p<0.05 between responders and non-responders in corresponding periods of observation

Changes in RBC enzymes during L-carnitine administration (Na,K-ATPase, Hx, LDH).

Table 3 presents some changes in the levels of RBC enzymes during L-carnitine administration. Significantly lower Na, K-ATPase activity was found in RBCs of hemodialysis patients before L-carnitine administration in comparison to the healthy group ($p<0.05$). Our prospective study revealed significant increase of Na, K-ATPase activity that achieved the range of the controls after 6 months of L-carnitine treatment (paired t-Student`s, $p<0.05$ vs month 0; Newman-Keuls, test $p< 0.05$).

Initial RBC Hx and LDH activities were similar to the controls. A significant increase of Hx and LDH levels was noticed during L-carnitine administration and in the third month it exceeded the level observed in the controls (paired t-Student`s, $p<0.05$ vs month 0; Newman-Keuls test $p< 0.05$). The highest increment of Hx and LDH activities was noticed during the first three months of L-carnitine supplementation.

Changes in Embden-Meyerhof intermediates duringL-carnitine administration (ATP, 2,3-DPG)

Changes in Embden-Meyerhof intermediates during L-carnitine administration are presented in Table 3. ATP level in RBCs was higher than in the control group, before L-carnitine supplementation. 2,3-DPG level was similar to the level in the control group. These two facts resulted in lower than in the controls 2,3-DPG/ATP ratio (3.24 vs 3.8). ATP levels decreased significantly during the time of observation (paired t-Student`s, $p<0.05$ vs month 0; Newman-Keuls test $p< 0.05$), while the 2,3-DPG level increased significantly (paired t-Student`s, $p<0.05$ vs 0; ANOVA Friedman's test – Kendall's coefficient = 0.22, $p<0.05$). The last two changes raised the 2,3-DPG/ATP ratio to 4.01.

Changes in RBC K and Pi during L-carnitine administration

Table 4 presents changes in intracellular concentration of K and Pi in the examined group of patients.

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Initial intracellular K concentration was similar to the controls. It slightly increased during the first two periods of observation but the value did not reach any statistical significance.

In HD patients intracellular Pi concentration was significantly higher both before and during the studies in comparison to the controls. Pi concentration did not change significantly during the observation except for the transient significant increase in the third month of L-carnitine administration (paired t-Student`s, $p<0.05$ vs month 0).

Changes in glucose uptake and lactate production by RBCs

Table 4 presents changes in glucose uptake and lactate production by RBCs during L-carnitine administration. The values of both glucose uptake and lactate production by RBCs were significantly higher than in the controls, before the L-carnitine supplementation. However, the ratio of lactate production versus glucose uptake was significantly lower than in the controls (1.44 vs 1.89) ($p<0.05$). Starting from the sixth month, up to the end of the observation, the levels of glucose uptake and lactate production decreased significantly (paired t-Student`s, $p<0.05$ vs month 0; Newman-Keuls test $p< 0.05$). These changes led to an increase of lactate production versus glucose uptake ratio which achieved the value of 1.67.

Table 3. Changes in RBC enzymes: hexokinase (Hx), lactate dehydrogenase (LDH) and sodium potassium ATPase (Na,K-ATPase) and Embden-Meyerhof intermediates: adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG) during L-carnitine administration

Parameters	Months of treatment					
	0	3	6	9	12	Control
Heksokinase (µmol/gHb/min)	1.49±0.4	1.75±0.4*	1.83±0.3*	1.83±0.3*	1.81±0.3*#	1.45±0.4#
LDH (µmol/gHb/min)	66.8±11	76.4±12*	82.1±16*	80.1±13*	78.1±10*#	63±6#
Na,K-ATPase (nmol Pi/gHb/min)	219±303#	250±31*	251±38*	250±29*	245±25*	249±38#
ATP (µmol/gHb)	3.95±0.5#	3.48±0.5*	3.58±0.4*	3.56±0.3*	3.59±0.4*#	3.3±0.3#
2,3-DPG (µmol/gHb)	12.7±1.8	14.0±1.5*	14.4±1.4*	14.6±0.9*	14.6±0.9*#	12.5±1.1#
2,3-DPG/ATP	3.24#	4.05*	4.06*	4.14*	4.01*	3.8#

***p**<0.05 vs month 0;

 # p<0.05 between tested group and controls before and after 12 months of treatment

Table 4. Changes in glycolytic rate measured by glucose uptake (GU) lactate production (LP) in L-carnitine treated group of patients during observation period

Parameters	Months of treatment					
	0	3	6	9	12	Control
Glucose uptake (µmol/gHb/h)	15.4±2.6#	12.6±2.0*	11.4±0.9*	11.4±0.8*	10.4±0.9*#	5.3±0.8#
Lactate production (µmol/gHb/h)	22.2±3.8#	18.5±1.7*	18.3±1.8*	16.9±1.0*	17.4±1.3*#	10.01±1.5#
LP/GU	1.44#	1.47	1.6*	1.48*	1.67*	1.89#

***p**<0.05 vs moth 0;

 # p<0.04 between tested group and controls before and after 12 months of treatment

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Table 5. Pearson`s or R Spearman`s linear correlation coefficients found in the group of patients supplemented with L-carnitine

Correlated parameters	Period of the study (month)	Correlation coefficient
Duration on dialysis vs FC/TC	0	-0.46
Duration on dialysis vs FC/AC	0	-0.5
FC/AC vs Hb	3, 9	0.71, 0.79
FC/TC vs Hb	9	0.85
FC/AC vs RET	9	0.62
FC/TC vs RET	9	0.64
FC/TC vs 2,3-DPG	9	0.78
FC/AC vs 2,3-DPG	9	0.64
Hb vs LDH	12	0.68
FC/TC vs Hx	12	0.9
FC/TC vs LDH	12	0.74
TC vs Hx	12	0.72
TC vs LDH	12	0.65

Significance accepted at $p< 0.05$

All significant linear correlations found in the group of patients supplemented with L-carnitine are given in Table 5.

Discussion

Anemia is a common finding in patients with chronic renal insufficiency and it is present in a vast majority of maintenance hemodialysis patients. The main reason for anemia in these patients is inadequate erythropoiesis caused by functional erythropoietin deficiency (28). Another reason of anemia in hemodialysis patients is reduced survival time of RBCs. It may be due to alterations in lipid's and phospholipid's membrane components as well as such metabolic changes as increased glycolysis and impairment of the pentose-phosphate shunt (1-3, 29). Disturbances in carnitine metabolism may contribute to the development of this kind of anemia (11). L-carnitine molecule plays a role of an important carrier in the transportation of long chain-fatty acids. The reversible transfer of long-chain fatty acids from CoA to FC is catalysed by RBC carnitine palmitoyl transferase (CPT). This reversible transfer modulates the acyl CoA/free CoA ratio, and this reaction is essential in regulation of the membrane phospholipid long-chain fatty acids turnover (30). Carnitine deficiency leads to accumulation of long-chain acyl carnitines (LCAC) and to the increase of LCAC/FC ratio. This increase, together with a reduced erythrocyte CPT activity, alters acyl trafficking in RBC membrane. Administration of L-carnitine, by inducing erythrocyte CPT activity, restores the equilibrium between acyl CoA pools in RBC, thus allowing normal acyl transportation and proper restoration of the RBC membrane phospholipids. In consequence, L-carnitine administration improves metabolism of RBCs (31). Aforementioned hypothesis is supported by the Bayon et al and Farrell et al studies (21, 32). Additionally a decrease in abnormally high erythrocyte ATP content is also reported (16) and Labonia et al (33) and Albertazzi et al (9) noticed an increase of erythrocyte Na,K-ATPase activity during L-carnitine administration. Many studies suggest that simultaneous L-carnitine and epoetin supplementation may have the epoetin

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saving effect (15, 19, 34-39).

The present study has shown the influence of low oral doses of L-carnitine not only on plasma carnitine profile, but also on haematological parameters and some metabolic properties of RBCs. The interrelationship among many different factors may result in carnitine deficiency in hemodialysis patients (5, 7, 11, 40). In our study the initial mean level of TC was similar to that in the controls. However, the levels of FC and both FC/TC and FC/AC ratios were significantly lower than in the control. It means that the study was conducted in carnitine deficient patients. In our material, the increase of mean Hb levels and RBC count during L-carnitine supplementation was noticed. These changes were not accompanied by an increase of Ret count. The same was observed by Donatelli et al and Trovato et al in their studies (8, 16). The increase of RBC count and Hb concentration was noticed after quite a long period of L-carnitine supplementation (6-9 months), which is in concordance with some other observations (11, 41). We noticed that the group of patients who experienced increase of Hb concentration (14/26) had significantly lower initial FC concentration and both FC/TC and FC/AC ratios and were dialysed for longer period of time (76 vs 46 months; $p<0.05$). A negative correlation between plasma carnitine bioavailability markers and the duration of hemodialysis treatment was noticed. It shows that long-term hemodialysis patients with deep carnitine deficiency may benefit from L-carnitine supplementation. It should also be taken into account that the haematological response to L-carnitine supplementation might depend on the muscle pool of carnitine. Human muscles (skeletal and heart) contain over 95% of carnitine, and only 0.6% of carnitine is localised the extracellular fluid (7). Our previous results show lack of any relationship between muscle and serum carnitine concentrations in hemodialysis patients (5). This lack of any relationship suggests that concentration of carnitine in blood does not always represent the real carnitine status in these patients. Therefore, the non-homogenous response to L-carnitine administration may depend on the degree of muscle carnitine deficiency, independently from plasma carnitine levels. Patients deprived most of the muscle carnitine are those on long-term haemodialysis (5).

Doses of L-carnitine administered in our study were significantly lower (500 mg/daily; mean dose 9 mg) than usually previously used orally (1000-3000 mg/daily) (19). Nevertheless, approximately a double increase of TC and FC was observed in the studied group of patients. Similar findings, related to the increment of serum carnitine levels after administration of low doses of L-carnitine, were observed by Wanner et al (7). It should be mentioned that actually recommended intravenous dose of L-carnitine is 1g (10-20 mg/kg) after each hemodialysis in selected patients with anemia and/or very large epoetin requirements (42).

RBCs from patients with chronic renal disease are hypermetabolic (43, 44). Preferential loss of older RBCs results in a residual RBCs population of younger mean age, and a younger enzyme pattern and also intrinsic hypermetabolism. Some of these changes in RBC metabolism may produce a rightward shift in the oxygen dissociation curve providing a greater oxygen release to the tissues. It partially compensates the reduced RBC mass in the uremic patients. The presence of younger RBC population was probably responsible for some RBC metabolic changes observed in our group of patients before L-carnitine administration. We found higher than in the controls, the initial concentrations of both Hx and ATP. Na,K-ATPase concentration was significantly lower which corresponded with most other studies focused on Na,K-ATPase activity in uremic patients (45-47). Glucose utilization and lactate production were increased perhaps as the result of an increased glycolysis rate. During L-carnitine supplementation we noticed the increase of the activity of such RBC enzymes as Na,K-ATPase, Hx and LDH. The

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levels of 2,3-DPG and 2,3-DPG/ATP ratio were also increased while ATP concentration of RBCs got significantly decreased. The increase of 2,3-DPG levels and 2,3-DPG/ATP ratio and also positive correlation between markers of carnitine bioavailability (FC/TC, FC/AC) and 2,3-DPG levels may suggest the influence of L-carnitine onto 2,3-DPG production in Rapoport-Luebering pathway. The increase of 2,3-DPG concentration during L-carnitine supplementation means that the tissue oxygenation gets improved not only as a consequence of improvement of anemia, but also as a result of the increase of 2,3-DPG concentration because the latter increase shifts the Hb dissociation curve to the right. Similar changes in intracellular Na,K-ATPase and ATP activities during L-carnitine administration were previously reported by Donatelli et al and Labonia et al (16, 33) and it was also mentioned in our previous short communication (47). In the ninth month and the twelfth month of L-carnitine administration we also observed some positive correlations between the markers of carnitine bioavailability and the enzymes and intermediates of Embden-Meyerhof pathway (Table 5). This may explain the increased efficiency of glycolysis during L-carnitine supplementation.

Conclusion

We want to underline that this study has shown that oral L-carnitine could ameliorate anemia in a subgroup of hemodialysis patients. L-carnitine induces metabolic changes in RBCs that lead to better RBC transportation properties, improved oxygen delivery and reduced utilisation of glucose. Long-term maintenance hemodialysis patients may benefit most from L-carnitine supplementation. Low doses of L-carnitine are effective in restoring plasma carnitine pool.

Conflict of interest

None of the authors have any conflict of interest to declare.

References

1. Komidori K, Kamada T, Yamashita T, Harada R, Otsuji Y. Erythrocyte membrane fluidity decreased in uremic hemodialyzed patients. Nephron. 1985;40:185-8.
2. Zannad F, Royer RJ, Kessler M, Huriet B, Robert J. Cation transport in erythrocytes of patients with renal failure. Nephron. 1982;32:347-50.
3. Corry DB, Tuck ML, Brickman AS, Yamagawa N, Lee DB. Sodium transport in red blood cells from dialyzed uremic patients. Kidney Int. 1986;29:1197-202.
4. Bartel LL, Hussey JL, Shrago E. Effect of dialysis on serum carnitine, free fatty acids and trigliceride levels in man and the rat. Metabolism. 1982;31:944-7.
5. Dębska-Ślizień A, Kawecka A, Wojnarowski K, et al. Carnitine content in different muscles of patients receiving maintenance hemodialysis. J Ren Nutr. 2007;17:275-81.
6. Mir S, Kantar M, Yalaz M, et al. Effect of hemodialysis on carnitine levels in children with chronic renal failure. Pediatr Int. 2002;44:70-3.
7. Wanner CH, Hörl W. Carnitine abnormalities in patients with renal insufficiency. Nephron. 1988;50:89-102.
8. Trovato G, Ginardi W, Marco W, Dell Aira E, Corsi M. Long -term L-carnitine treatment of chronic anemia of patients with end stage renal failure. Curr Ther Res. 1982;31:1042-9.
9. Albertazzi A, Capelli P, Di Paolo B, et al. Endocrine metabolic effect of L-carnitine in patients on regular dialysis treatment. Proc Eur Dial Transplant Assoc. 1982;19:302-7.
10. Ahmad S, Robertson HT, Golper TA, et al. Multicenter trial of L-carnitine in maintenance hemodialysis patients. II. Clinical and biochemical effects. Kidney Int. 1990;38:912-8.
11. Ahmad S. Carnitine, kidney and renal dialysis. In: Ferrari R, DiMauro S, Sherwood G eds. L-Carnitine and its role in medicine: From function to therapy, chap 2. Academic Press 1992:381-400.
12. Boran M, Dalva I, Gönenç F, Çetin S. Response to

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recombinant human erythropoietin (r-HuEPO) and L-Carnitine combination in patients with anemia of end –stage renal disease. Nephron. 1996;73:314-5.

13. Hedayati SS. Dialysis-related carnitine disorder. Semin Dial. 2006;19:323-8.
14. Kwack K, Balakrishnan VS. Managing Erythropoietin hyporesponsiveness. Semin Dial. 2006;19:146-151.
15. Berns JS, Mosenkis A. Pharmacologic adjuvants to epoetin in the treatment of anemia in patients on hemodialysis. Hemodial Int. 2005;9:9-22.
16. Donatelli M, Terrizzi C, Zummo G, et al. Effects of L-carnitine on chronic anemia and erythrocyte adenosine triphosphate concentration in hemodialyzed patients. Cur Ther Res. 1987;41:620-4.
17. Kleczmayr J, Mayer G, Legenstein E, et al. Anemia and carnitine supplementation in hemodialysed patients. Kidney Int. 1999;55:93-106.
18. Caruse U, Leone L, Cravotto E, et al. Effect of L-carnitine on anemia in aged hemodialysis patients treated with recombinant human erythropoietin: a pilot study. Dial transplant. 1998;27:498-506.
19. Hurot J-M, Cucherat M M, Huagh M, et al. Effect of L-carnitine supplementation in maintenance hemodialysis patients. A systemic review. J Am Soc Nephrol. 2002;13:708-14.
20. Matsumura M, Hattakeyama S, Koni I, Mabuchi H, Muramoto H. Correlation between serum carnitine levels and erythrocyte osmotic fragility in hemodialysis patients. Nephron. 1996;72:574-8.
21. Bayon JE, Alvrez AL, Barrio JP, Diez C, Prieti JG. Effect of stazolol and L-carnitine on erythrocyte osmotic fragility during aerobic exercise in rat. Comp Hematol Int. 1993;3:196-200.
22. Geudeney X, Chanez C, Grenier A, Scherrmann JM. Temperature dependent immunoreactive assay to screen for digoxin like immunoreactive factors. Clin Chem. 1991;37:1900-4.
23. Land J, Booth R, Berger R, Clark J. Development of mitochondrial energy metabolism in rat brain. Biochem J. 1977;164:339-48.
24. Wallas C H. Metabolic studies on red cells from patients with chronic renal disease on hemodialysis. Br J Hematol. 1974;26:71-8.
25. Fadeche A, Salako L. Abnormal cation composition on transport in erythrocytes from hypertensive patients. Europ J Clin Invest. 1979;9:369-75.
26. Cederblad G, Harper P, Lingren K: Spectrophotometry of carnitine in biological fluids and tissue with Cobas Bio centrifugal analyser. Clin Chem. 1986;32:342-6.
27. Sałek, Mierzewski P, Wąsewicz M, Uszycka-Karcz M, Łysiak-Szydłowska W. L-carnitine in serum and urine. The attempt to monitoring of carnitine treatment. Diagn Lab. 1989;25:109-18.
28. Revised European Best Practice Guidelines for the management of anemia in patients with chronic renal failure. Nephrol Dial Transplant. 2004;19:ii32-4.
29. Yawata Y, Jacob HS. Abnormal red cell metabolism in patients with chronic uremia: Nature of the defect and its persistence despite adequate hemodialysis. Blood. 1975;45:231-9.
30. Arduini A, Mancinelli G, Radatti G, et al. Role of carnitine and carnitine palmitoyltransferase as integral components of the pathway for membrane phospholipid fatty acid turnover in intact human erythrocytes. J Biol Chem. 1992;267:12673-81.
31. De los Reyes B, Perez-Garcia R, Liras A, Valderrabano F, Arenas J. L-carnitine normalizes the reduced carnitine palmitoyl transferase activity in red cells from hemodialysis patients [letter]. Nephrol Dial Transplant. 1994;12:1300-1.
32. Farrell S, Vogel J, Bieber LL. Entry of acetyl L-carnitine into biosynthetic pathways. Biochim Biophys Acta. 1986;876:175-7.
33. Labonia W D, Morelli O, Gimenez M, Freuler P. Effect of L-carnitine on sodium transport in erythrocytes from dialyzed uremic patients. Kidney Int. 1987;32:754-9.
34. Berard E, Barillon D, Iordache A, Bayle J, Cassuto-Viguier E. Low dose of L-carnitine impairs membrane fragility of erythrocytes in hemodialysis patients. Nephron. 1994;68:145.
35. Linde T, Sandhager B, Wikstrom B, Danielson BG. The required dose of erythropoietin during renal anemia treatment

www.SID.ir

Archive of SID

is related to the degree of impairment in erythrocyte deformability. Nephrol Dial Transplant. 1997;12:2375-9.

36. Labonia WD. L-carnitine effect on anemia in hemodialyzed patients treated with erythropoietin. Am J Kidney Dis. 1995;26:757-64.
37. Kooistra MP, Struyvenberg A, Van Es A. The response to recombinant human erythropoietin in patients with the anemia of end-stage renal disease is correlated with serum carnitine levels. Nephron. 1991;57:127-8.
38. Golper TA, Goral S, Becker BN, et al. L-carnitine treatment of anemia. Am J Kidney Dis. 2003;41:27-34.
39. Dębska-Ślizień A, Owczarzak A, Kunicka D, Łysiak-Szydłowska W, Rutkowski B. Plasma carnitine profile during chronic renal anemia treatment with recombinant human erythropoietin. Int J Artif Organs. 2003;26:33-8.
40. Calvani M, Banatti P, Mancinelli A, et al. Carnitine replacement in end-stage renal disease and hemodialysis. Ann NY Acad Sci. 2004;1033:52-66.
41. Golper TA, Ahmad S. L-carnitine administration to hemodialysis patients: Has its time come? Seminars Dial. 1992;2:94-8.
42. Clinical practice guidelines for nutrition in chronic renal failure. K/DOQI, National Kidney Foundation. Am J Kidney Dis. 2000;35:S1-40.
43. Wallas CH. Metabolic studies on the erythrocyte from patients with chronic renal disease on hemodialysis.II ATP metabolism. Br J Hematol. 1974;27:145-52.
44. Wallas CH. Metabolic studies on the erythrocyte from patients with chronic renal disease on hemodialysis. Br J Hematol. 1974;26:71-8.
45. Izumo H, Izumo S, DeLuise M, Flier JS. Erythrocyte Na,K Pump in uraemia. Acute correction of a transport defect by hemodialysis. J Clin Invest. 1984;74:581-8.
46. Corry DB, Tuck ML, Brickman AS, Yanagawa N, Lee DBN. Sodium transport in red blood cells from dialyzed uremic patients. Kidney Int. 1986;29:1197-202.
47. Dębska-Ślizień A, Owczarzak A, Krol E, et al. The influence of L-carnitine and simultaneous erythropoietin and L-carnitine on erythrocyte metabolism in hemodialysis patients. Adv Ren Nutr Metabol. 1997;1:139-44.

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