RENAL FAILURE, 22, (1), 73-80 (2000)

N. DOC. 27355

CLINICAL STUDY

EFFECT OF L-CARNITINE SUPPLEMENTATION ON RED BLOOD CELLS DEFORMABILITY IN HEMODIALYSIS PATIENTS

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ABSTRACT

Anemia is a serious problem in hemodialysis patients, the main cause of which is erythropoietin deficiency. After the discovery of recombinant human erythropoietin (rHuEpo) at the end of the last decade, the hematological profile of hemodialysis patients improved significantly but at considerable expense. The deformability of red blood cells (RBC) influences their microcirculation and tissue oxygen delivery along with their life span.

We investigated the deformabilty of RBCs in 15 hemodialysis patients before and after three months on L-carnitine supplementation (30 mg/Kg body wt/dialysis session). We excluded from the study all patients who received blood transfusions three months before or during the study, patients who had hemorrhagic episodes, those with hyperparathyroidism or infections, and any who required surgical intervention during the study. The serum iron, folic acid and vitamin B-12 levels were kept normal during the duration of the study. The erythropoietin dose taken before the beginning of L-cartnitine supplementation was not changed. The deformability of RBCs before and after dialysis, prior to and following three months on L-carnitine was determined and compared

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to the deformability of RBCs from a control group. Hematocrit levels were measured before entry into the study and every month for three months.

We found that the deformability of RBCs before the dialysis session was significantly greater than that found in the control group (t-test, $p < 0.00001$), and that there was a further increase after the end of the dialysis session. Three months following L-carnitine supplementation, we found a significant reduction of RBCs deformability (paired t-test, $p < 0.004$), and a significant increase in the henatocrit (ANOVA, $p < 0.0001$).

We concluded that abnormalities in the deformability of RBCs improved after L-carnitine and that this was responsible for the increase in the hematocrit. This may allow a substantial reduction in rHuEpo dose.

Key Words: Hematocrit, Erythropoeitin, L-carnitine, Hemodialysis.

INTRODUCTION

Anemia is one of the major problems in hemodialysis patients. It's etiology is multifactorial (1) with the main causes being a) Erythropoietin deficiency (2), b) blood losses from the gastrointestinal tract, in dialyzers and dialyzer lines and from blood drawings for laboratory testing (3), c) reduced levels of hemopoiesis compounds (iron, folic acid, vitamin B-12), and d) a reduced life span of erythrocytes. The latter may be influenced by such things as hyperparathyroidism, uremic toxins, osmotic trauma, hypersplenism, mechanical trauma, and carnitine deficiency. Moreover, various biochemical and biophysical changes of the red blood cells (RBCs) that alter the lipid and phospholipid components of their membrane may lead to reduced erythrocyte deformability and in this way lead to a reduced erythrocyte life span.

Carnitine, a quaternary ammonium molecule, is synthesized in the kidney. This molecule facilitates the entry of long chain fatty acids into mitochondria. Without this the inner mitochondrial membrane is impermeable to the long chain fatty acids. Thus, carnitine is absolutely necessary for normal lipid metabolism. However, carnitine is dialyzable and in each dialysis session, a small amount is lost. Indeed, tissue carnitine levels seem to be correlated with the duration of dialysis (4).

In a controlled, double blind study of L-carnitine (LC) supplementation versus a placebo, a significant increase in hematocrit (Hct) levels was observer and later confirmed by others (5). The proposed mechanism of this effect is the stabilization of the erythrocyte membrane by facilitating lipid uptake. It was also found that LC increases Na^+/K^+-ATPase activity and reduces abnormal ATP concentrations (6,7). The present study was designed to evaluate the effect of LC supplementation on RBC deformability in hemodialysis patients and the impact of this alternation on their Hct levels.

PATIENTS AND METHODS

We studied the RBC deformability in 15 hemodialysis patients (12 males and 3 females), aged 30 to 65 years (median = 55 years), suffering from various renal diseases, undergoing hemodialysis from 24 to 120 months (mean ± SD = 59.2 ± 30.2) as well in a control group of 15 normal individuals. Patients who were transfused three months before or during the study, those who underwent a surgical operation during the study or had blood losses, hyperparathyroidism or infections were excluded from the study. Also excluded were diabetic patients along with those who were in the hemodialysis program for less than 12 months. Serum iron levels were normal (as was shown by serum ferritin and TIBC levels), as was the serum vitamin B-12 levels. The patients continued to take the same erythropoietin doses as they took before the study.

RBC deformability was determined before and after a hemodialysis session, as well as before LC supplementation (30 mg/Kg body wt intravenously after each session) over a period of 3 months. Hct levels were also determined before LC administration as well as 1, 2 and 3 months afterwards.

RBC deformability was determined using an hemorheometer (Filter method) that estimates the time that RBCs need to pass one by one through the fibers holes, under constant and stable conditions including pH, Hct, osmolality and temperature. The final result using this method is referred to at the Indice de rigidite (IR) (8) and the higher the value is found to be, the more rigid are the RBCs. The Hct was determined by an automatic hematological analyzer.

ANOVA, *t*-test and paired *t*-test were employed to evaluate statistical significance. Differences more than 0.05 were significant.

RESULTS

The IR of hemodialysis patients was significantly higher (more rigid, reduced deformability) compared to that of the controls (*t*-test, $p < 0.00001$) (Table 1). We also observed an increase in IR after the hemodialysis session (paired *t*-test, $p < 0.00001$). After a three months period of LC administration, the IR prior to dialysis was significantly reduced, when compared to the pre-treatment values (paired *t*-test, $p < 0.004$) (Table 1). We also found a significant increase in Hct levels after this period (ANOVA, $p < 0.00001$) (Table 2). After three months on LC, Hct levels were more than 35% (target level) in four out of 15 patients.

Table 1. Deformability of RBC In Hemodialysis Patients Before and After the Dialysis Session, as Well as in Normal Controls

	Patients		
n	Before HD	After HD	Before HD (after LC)
1	17.99	27.92	10.4
2	15.68	16.44	14.53
3	11.90	21.25	11.02
4	15.78	24.11	14.62
5	10.08	19.29	10.03
6	11.80	22.81	11.02
7	15.78	34.67	10.00
8	12.34	19.73	11.90
9	15.27	16.33	15.44
10	19.71	20.01	10.29
11	13.19	13.49	9.50
12	20.58	22.63	10.40
13	17.77	25.65	11.76
14	13.88	25.00	6.60
15	13.12	16.71	14.70
Mean ± SD	15.0 ± 3.1	21.7 ± 5.4	11.5 ± 2.4

(before HO vs normal controls $p < 0.00001$),
(before HD vs after HD $p < 0.00001$),
(before HD vs before HD after 3 months LC supplementation, $p < 0.004$),
(before HD after 3 months LC supplementation vs normal control, $p < 0.02$).

DISCUSSION

Erythropoietin and carnitine, are synthesized in the kidney. It is known that during dialysis sessions, there is a continuous loss of carnitine because it can pass through the dialysis membrane (9,10). As a result, it might be expected that their levels would be affected in end stage renal failure patients undergoing hemodialysis and that this would influence hemopoiesis. However, it has been suggested that carnitine levels are not affected because dietary intake balances the losses (11). Nevertheless, in clinical practice many physicians have observed a positive effect of LC supplementation in various clinical and metabolical disorders (12,13).

The relationship between carnitine and erythropoietin was described by Kooistra et al. (13) who observed that patients with severe anemia due to renal failure had lower carnitine levels than patients who were non-anemic or who had only mild anemia. This view has recently been supported by others (14). Specifically, Matsumura et al. in a study of 26 hemodialysis patients, found that mean hemolysis end point has a negative correlation with serum

Table 2. Hct Levels In Hemodialysis Patients Before and After LC Supplementation

Patients	Hct before LC	Hct after 1 month on LC	Hct after 2 months on LC	Hct after 3 months on LC
1	30.0	29.5	32.0	32.0
2	30.0	33.3	34.1	39.1
3	31.5	30.9	30.7	30.9
4	30.5	32.0	32.5	34.0
5	31.0	31.3	31.0	31.9
6	34.0	32.0	31.5	33.5
7	32.0	32.0	34.0	35.2
8	32.0	34.0	33.5	34.5
9	27.0	28.0	34.0	36.0
10	28.5	29.8	33.8	32.8
11	32.9	32.8	32.8	33.2
12	30.0	33.0	34.0	39.0
13	34.0	34.0	33.5	34.0
14	29.0	31.5	33.4	32.9
15	30.0	32.0	34.0	34.0
Mean ± SD	30.8 ± 1.9	31.8 ± 1.7	33.0 ± 1.9	34.2 ± 2.4

(ANOVA, $p < 0.00001$)

triglyceride concentrations, as well with acyl-carnitine concentrations. The hemolysis maximum point has a significantly negative correlation with serum triglyceride and free carnitine concentrations. Dose requirement of recombinant human erythropietin (rHuEpo) maintaining target hematocrit correlated with serum triglyceride and free carnitine levels. These data support that low serum free carnitine levels accelerate erythrocyte osmotic fragility. Carnitine may contribute to the metabolism of erythrocyte membrane and have an impact on the efficacy of rHuEpo in renal failure anemia (14). In addition, Berard and Lordache observed a significant increase in Hct levels in two young hemodialysis patients with low plasma carnitine levels, after three months of LC supplementation (15). More recently, other investigators demonstrated that LC supplementation decreases the need for rHuEpo (16). This is in agreement with our results in this study. Moreover, emphasis must be given to that in four patients in whom Hct levels became very high (upper the target level). The Hct surpassed the target levels, making possible the reduction of rHuEpo requirements (reduction of anemia cost therapy).

Also, Jendryczko et al. studied the cholesterol content of the erythrocyte membrane in hemodialysis patients in order to estimate if the blood rheological disturbances are related to the erythrocyte lipid composition. They investigate 40 patients with chronic renal insufficiency, 34 of whom had hypertension and proteinuria. The cholesterol/phospholipid ratio was higher in the patients after hemodialysis while a significant correlation was

found between membrane cholesterol/phospholipid ratio and serum levels of LDL-cholesterol (17). In addition, Lapshina et al. studied the changes in the structure and properties of erythrocyte membranes that are induced by free fatty acids, and found that fatty acids perturb the lipid bilayer and disturb the protein complementarily of the erythrocyte membrane (18). Finally, Ahmad et al. determined the serum fatty acid profiles in eight hemodialysis patients and their data suggested depletion of essential fatty acids (linoleic, linolenic, arachidonic acids) which is partially corrected by treatment with LC (6 to 18 months intravenous therapy) (19).

The proposed mechanism by which LC improves Hct levels of hemodialysis patients are the favorable action on membrane phospholipid fatty acid turnover and the stabilization of the erythrocyte membrane (20). Specifically, Labonia et al. hypothesized that LC supplementation in hemodialysis patients (1 gr/dialysis session intravenously for 6 months period) might act by removing the toxic acyl residues that might impair erythropoiesis. They excluded any effects on RBC membrane which might increase the RBC half life because they did not find any change in the osmotic fragility (16). Arduini et al., however, demonstrated that LC prolongs the in vitro life span of RBCs (21), while Berard et al. showed that LC improved the erythrocyte fragility in 12 out of 18 hemodialysis patients after four months of treatment with LC (22). Our results are in agreement with these findings (improvement of RBCs deformability and the effectiveness of rHuEpo), since we found a significant increase in Hct levels after a 3 months LC supplementation. This is a very important benefit for patients with insufficient response to rHuEpo and for the a reduction in the cost to treat anemia. In at least in 4 out of our 15 patients, the rHuEpo dose were reduced at least in half per dialysis session. That also has been demonstrated by others (16).

We concluded that a) there are disturbances in the deformability of RBC in hemodialysis patients that are improved with LC supplementation and that b) LC improves significantly the Hct levels and reduces the needs for rHuEpo and by so doing, reduces the cost of anemia therapy.

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