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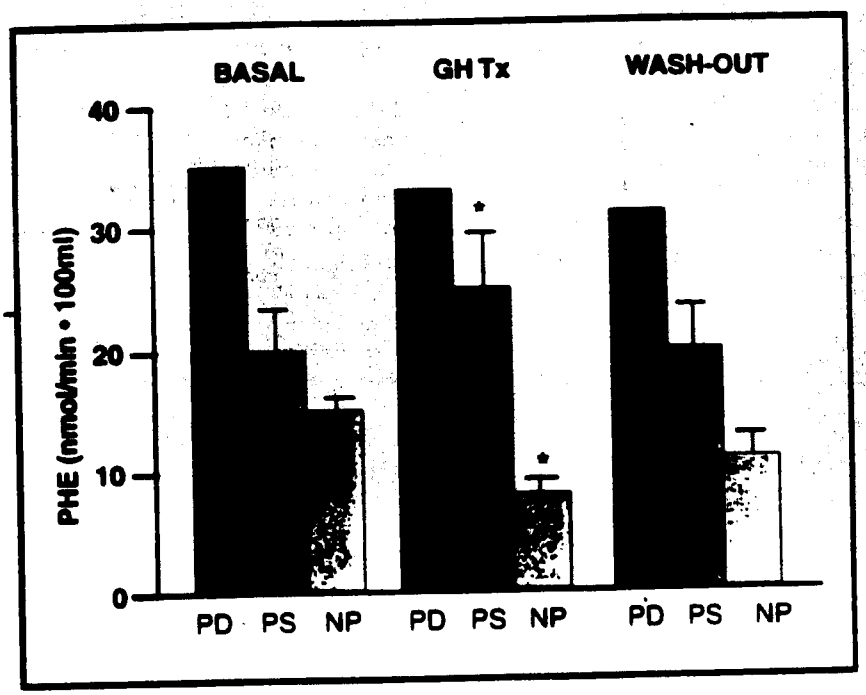

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The influence of L-Carnitine and simultaneous erythropoietin and L-Carnitine administration on erythrocyte metabolism in hemodialysis patients

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ABSTRACT

The aim of the study was to determine the influence of r-Epo and L-CAR administered separately and simultaneously in anemic hemodialysis patients on: hemopoietic answer, erythrocyte transmembrane transport and intraerythrocytic metabolism.

The studies were carried out on following groups: GROUP I (EPO) - 21 HD pts. (16M, 5W) receiving r-Epo for 6 months with initial dose 2000u 3x weekly. GROUP II (CAR)- 20 HD pts. (13M, 7W) receiving 0,5 g/day of L-CAR for 6 months (mean 5-7,5 mg/kg b.w.). GROUP III (EPO+CAR) - 9 HD pts (2M, 7W) receiving r-Epo for 39 ± 12 months and CAR during the last 6 months in the doses of r-Epo and L-CAR similar to used in the GROUP I and II. 30 healthy volunteers (12M, 18W) served as the controls (CONTR).

Significant increase of RBC in EPO and EPO+CAR groups was found. In RBC of all groups significant increase of Na,K- and Mg-ATPase activities were noticed. In EPO group significant increase of ATP, 2,3-DPG and decrease of glucose uptake and lactate production in RBC were observed. In CAR only ATP was decreased but the other parameters changed like in EPO. In EPO+CAR significant decrease of ATP and glucose utilization were found. Conclusions: 1. Both r-Epo and L-CAR improve hematological status of anemic hemodialyzed patients. 2. R- Epo acts mostly on the way of rejuvenation of RBC population; L-CAR by the stabilization of RBC structure. 3. Simultaneous r-Epo and L-CAR administration approach the metabolic profile of RBC to that observed in healthy population

Key words: Erythropoietin, L-carnitine, hemodialysis, RBC metabolism, anemia

INTRODUCTION

Chronic anaemia in uremic patients on long-term maintenance hemodialysis (HD) is characterized both by decreased bone marrow production rate and shortened survival of red blood cells (RBC) [1]. The decreased rate of RBC production is related to inadequate erythropoietin secretion but their shortened survival is dependent on several biophysical and biochemical RBC abnormalities. As factors of shortened RBC survival have been suggested: increased RBC adenosine-5-triphosphate (ATP) concentration (secondary to stimulated glycolysis and decreased membrane sodium potassium ATPase activity), impaired pentose phosphate shunt and altered lipid and phospholipid membrane components [2, 3, 4].

Depletion of free carnitine induced by hemodialysis may aggravate the RBC abnormalities [5, 6]. Improvement of anemia and changes in RBC metabolism during L-CAR administration have been previously reported [5, 6].

The aim of the study was to determine the influence of r-Epo and L-CAR administered separately and simultaneously to anemic hemodialysis patients on: hemopoietic answer, erythrocyte transmembrane transport and intraerythrocytic metabolism.

PATIENTS AND METHODS

The studies were carried out in three groups of patients

and in the controls. GROUP I (EPO) consisted of 21 HD patients (16M, 5W) at the age 35 ± 13 years dialyzed for 35 ± 14 mts. They received r-Epo (Eprex, Cilag-Janssen) for 6 months with initial dose 2000u three times per week. GROUP II (CAR) was created of 20 HD patients (13 M, 7W) at the age 33 ± 10 years being dialyzed for 30 ± 14 mts. They received 0,5 g/day of L-CAR (L-Carnitene, Sigma - Tau) for 6 months. Mean dose of of CAR was 5-7,5 mg/kg b.w. GROUP III (EPO+CAR) included 9 HD patients (2M, 7W) at the age 40 ± 8 years dialyzed for 46 ± 12 mts. They received r-Epo for 39 ± 12 months and CAR during the last 6 months in doses similar to those used in GROUP I and II respectively.

30 healthy volunteers (12M, 18W) aged 40 ± 10 years served as the controls (CONTR). R-Epo dose during the the study was similar in EPO and EPO+CAR groups and was stable during the time of observation. Presented patients had not received blood transfusion.

HD was performed 3 times per week, 3-5 hours per session using cuprophane or polysulphone filters and bicarbonate dialysis fluid. The serum levels of predialysis Ca, P and K were similar in presented group.

Blood samples were collected by venipuncture without stasis before hemodialysis after overnight fasting. Hematological parameters (RBC, Hb) were measured using Technicon HI autoanalyser. Na,K- and Mg-ATPase activities were determined after Geudeney [7]; Incubation mixture contained in final volume of 1.1 ml: 40 mM Tris HCl pH 7.40, 150 mM NaCl, 20 mM KCl, 5mM $MgCl_2$; and isolated in 0.9% NaCl erythrocytes corresponding to about 12 mg Hb/sample and ± 0.75 mM ouabain. Reaction at 37°C was started by the addition of 4 mM ATP and terminated after next 15 minutes by addition of 1 ml of 10% TCA. Pi was determined in supernatant by the method of Fiske and Subba Row. For estimation of the ATP and 2,3-diphosphoglycerate (2,3-DPG) Sigma - (St. Louis, USA) kits were used. Enzymes of glycolysis: hexokinase and lactate dehydrogenase (LDH) were measured by kinetic methods on LKB spectrophotometer. Glucose uptake and lactate production by erythrocytes were performed after separation of RBC in medium by the Wallas method [8]; glucose and lactate were determined by the kits from Human GmbM (Germany). Intracellular K was done by ISE- method after separation and lysis of RBC. Carnitine was estimated by enzymatic method [9].

Statistical analysis was done using Students paired and unpaired t-tests.

RESULTS

Particular data concerning parameters described below are contained in the table 1 and 2 and on figures 1A and 1B.

The initial level of total carnitine (TC) in EPO and CAR groups was similar to the CONTR, but in EPO+CAR was significantly lower ($p<0,05$). Before L-CAR administration the free (FC) to total carnitine ratio in EPO, CAR and EPO+CAR groups was significantly lower than in the CONTR (respectively 61%, 58%, 56% vs 68% in CONTR; $p < 0.001$). Oral administration of low doses of L-CAR caused 60% increase of TC in CAR and 70% in CAR+EPO; FC/AC increased but was still significantly lower than in the CONTR.

Significant increase of RBC in EPO and EPO+CAR groups and no changes in this parameter in CAR group were observed during 6 months of treatment. Reticulocytes count (RET) increased significantly only in EPO group; In EPO+CAR this parameter was higher than in the CONTR but had not changed during the period of observation.

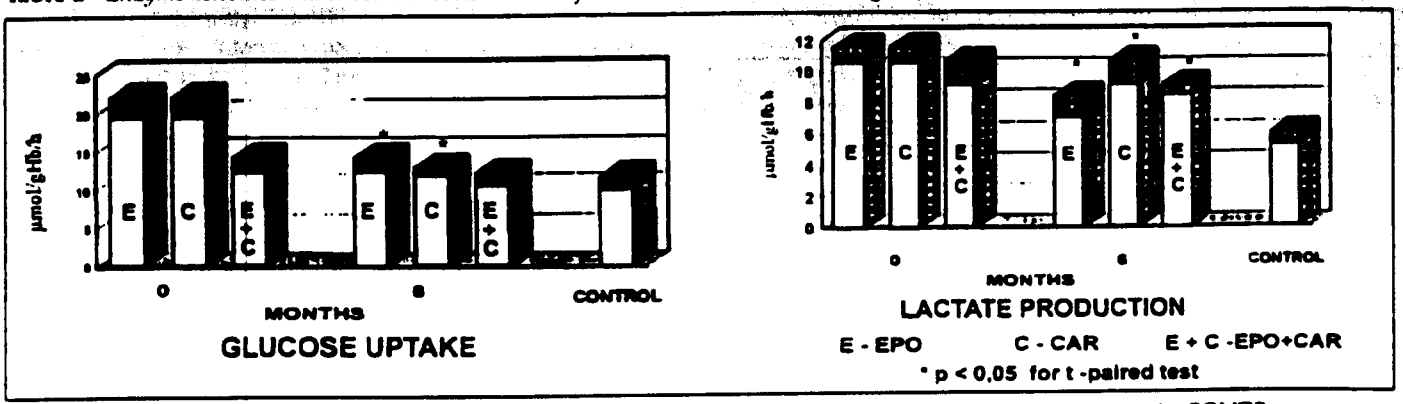
Significantly lower Na, K-ATPase and Mg-ATPase activities were found in RBC of dialysis patients before r-Epo and CAR administration in comparison to the healthy group ($p<0.01$) (Table 2). The prospective study revealed significant increase of the activity of these enzymes in EPO and CAR groups. However the highest significant increase of Na,K-ATPase was found in EPO + CAR group after 6 months of treatment. Na, K-ATPase

mts of treatment		0			3			6		
GROUPS	CONTR	EPO	CAR	EPO + CAR	EPO	CAR	EPO+ CAR	EPO	CAR	EPO+ CAR
r-Epo dose u/kg/week	—	—	—	81 ± 42	75 ± 8	—	93 ± 31	76 ± 48	—	88 ± 38
Total carnitine µmol/L	53 ± 9	56 ± 9	60 ± 9	46 ± 10		98 ± 33*	79 ± 33*		97 ± 26*	82 ± 32*
Free carnitin µmol/L	36 ± 6	34 ± 7	35 ± 8	26 ± 8		64 ± 23*	45 ± 18*		57 ± 17*	47 ± 17*
RBC T/L	4,4 ± 0,9	2,5 ± 0,3	3,2 ± 0,2	2,9 ± 0,3	3,2 ± 0,4*	3,1 ± 0,3	3,2 ± 0,4*	3,2 ± 0,4*	3,3 ± 0,4	3,3 ± 0,3*
RET promilles	8 ± 5	9,2 ± 5,6	7,4 ± 3,6	11 ± 4	16 ± 9,7*	8,8 ± 4,6*	11 ± 6	15 ± 7,1*	7,1 ± 5,9	11 ± 5,6
X ± SD *p<0.05 for t-paired test										

Table 1 - *R-Epo dose, carnitine levels, RBC and RET counts in EPO, CAR, EPO + CAR groups and in the CONTR*

mts of treatment		0			3			6		
GROUPS	CONTR	EPO	CAR	EPO + CAR	EPO	CAR	EPO+ CAR	EPO	CAR	EPO+ CAR
Na,K-ATPase nMPi/min/gHb	252 ± 34	205 ± 33	215 ± 33	233 ± 36	224 ± 38	251 ± 30*	257 ± 34	254 ± 45*	244 ± 40*	264 ± 40*
Mg-ATPase nMPi/min/gHb	194 ± 31	156 ± 26	148 ± 26	163 ± 25	167 ± 23	168 ± 24*	176 ± 11	177 ± 22*	170 ± 17*	171 ± 17
Hexokinase µmol/gHb/min	1,4 ± 0,4	1,5 ± 0,2	1,3 ± 0,23	1,8 ± 0,4	1,7 ± 0,3*	1,56 ± 0,2*	1,7 ± 0,3	1,8 ± 0,2*	1,7 ± 0,2*	1,7 ± 0,2
LDH µmol/gHb/min	63 ± 3	65 ± 10	63 ± 7,5	83 ± 13	73 ± 12	72 ± 6	78 ± 8	80 ± 11*	78 ± 15*	77 ± 8,7
ATP µmol/gHb	3,3 ± 0,3	4,0 ± 0,2	3,9 ± 0,4	4,4 ± 0,4	4,3 ± 0,3*	3,6 ± 0,3*	4,1 ± 0,2*	4,4 ± 0,3*	3,6 ± 0,3*	4,0 ± 0,2*
2,3-DPG µmol/gHb	12,6 ± 1,0	13,2 ± 1.0	13,2 ± 1,3	14,4 ± 1,1	13,5 ± 0,98	13,8 ± 1,4	13,7 ± 0,7	14,6 ± 0,7*	14,6 ± 1,5*	13,8 ± 1,2
K intracellular µmol/gHb	308 ± 15	297 ± 18	297 ± 12	313 ± 18				313 ± 18	316 ± 19	327 ± 22
Pi intracellular µmol/gHb	103 ± 11	121 ± 17	121 ± 17	124 ± 23				124 ± 17	117 ± 10	125 ± 21
X ± SD, *p<0.05 for t-paired test										

Table 2 - *Enzyme activities and substrate levels in RBC of EPO, CAR and EPO+CAR groups and in the CONTR*



Figures 1A and 1B - *Glucose uptake and lactate production by RBC in EPO, CAR and EPO+CAR groups and in the CONTR*

activity in all groups after 6 months of treatment achieved the range of the control. Positive correlation between Na, K- and Mg-ATPases and RET measured in 6th month of r-Epo treatment (EPO group) was found ($r = 0.6414$ Na, K-ATPase; $r = 0.5805$ Mg-ATPase; $p < 0.05$).

Intraerythrocytic K and Pi have not changed significantly but the level of K was the highest in EPO+CAR group.

Hexokinase and LDH activities were similar in CONTR, EPO and CAR groups but in CAR+EPO were higher than in CONTR by about 30%. Treatment with r-Epo or L-CAR alone resulted in an increase in the activity of the above enzymes except in the case of EPO+CAR group where the LDH and hexokinase activities had not changed during observation.

At the "0" time ATP and 2,3-DPG levels in observed groups were higher than in CONTR but the 2,3-DPG/ATP ratio was lower (3,3 vs 3,8 in CONTR). In EPO group significant increase of ATP and 2,3-DPG with unchanged ATP/2,3-DPG ratio was noticed. In the case of CAR group ATP levels decreased significantly while the 2,3-DPG increased significantly what raised

the ratio to 4.0. Simultaneous L-CAR and r-Epo administration decreased both ATP and 2,3-DPG and the ATP/2,3-DPG remained unchanged.

On the figures 1A and 1B the influence of given treatment on glucose utilization and lactate production by isolated RBC is presented. After 6 month of observation the glucose uptake achieved 160% in Epo, 170% in CAR and 130% in EPO+CAR in comparison with CONTR. What concerns lactate production it was respectively 124%, 119% and 106% of the lactate production in the CONTR. Calculated lactate production/glucose uptake ratio in the CONTR was 1,9 and 1,4-1,5 in the other groups.

DISCUSSION

For ten years r-Epo has been administered to anemic, hemodialysis patients and the improvement of anemia was achieved almost in any treated patient [10, 11]. Our study also confirms this opinion since we got statistically significant increase of RBC count in r-Epo treated patients. L-CAR supplementation in patients receiving r-Epo ameliorated the hemopoietic response without changes in r-Epo dose and rise in RET. Although separate L-CAR administration has not resulted in an increase in RBC count during 6 months of treatment. It may be surprising because there are data showing favorable influence of L-CAR supplementation on anemia in HD patients [6]. Supposedly it happened because patients from CAR group had higher TC and FC serum concentration, than patients from EPO+CAR where the additional L-CAR supplementation increased RBC count. This confirms reports on higher r- Epo dose requirements in patients with low serum carnitine levels [12]. Other the explanation of this phenomenon could be a very low dose of L-CAR we used in comparison to the other study [5], however after 12 months of observation we were also able to notice an increase in RBC count (unpublished data). The mechanism of L-CAR action in anemia of HD patients is unclear. It rather influences RBC metabolism and produces changes in RBC membrane structure than stimulates bone marrow to RBC production. R-Epo stimulates bone marrow to new RBC production [1, 10]. Although r-Epo therapy evokes also qualitative alterations of RBC [13, 14]. There are reports on decreased RBC membrane fluidity in HD patients studied by means of electron spin resonance and on positive influence of r-Epo treatment on the structure of RBC lipid bilayer [2, 13, 14]. Membrane fluidity is closely involved in various important membrane functions such as permeability; transport of ions, glucose or oxygen and membrane - associated enzymes. Transmembrane transport seems to be one of the most valid properties of RBC. Although RBC has several well characterized Na transport mechanisms, the effect of renal failure on all these pathways has not been precisely established. Most studies focused on Na,K-ATPase found that uremia suppresses its activity suggesting the influence of uremic toxins on the pump [3,4]. In our study we also found the lower activity of Na,K and MgATPases in hemodialysis patients in comparison to healthy population. During separate r-Epo and L-CAR administration continuous increase of the ATPases activity has been observed. In the case of r-Epo it could have been partially dependent on the generation of a new, younger population of RBC since the positive correlation between RET and ATPases was found. Although after longer r-Epo therapy the correlation disappeared (after 9-10 mts; unpublished data).

The increase of ATPase activity after long term presence of erythropoietin can be explained as rejuvenation of the RBC population. The rejuvenation in our mind means that after the achievement of steady state during long term r-Epo treatment the ratio of young age RBC on whole RBC population is higher than in not treated patients. With respect to L-CAR it is known that this substance is the natural carrier of long-chain fatty acid esters to sides of beta-oxidation in the mitochondrial matrix [15]. The depletion of serum free carnitine induced by HD might facilitate the RBC accumulation of carnitine esters that have been reported to posses a powerful inhibitory activity on membrane Na,K-ATPase and lytic properties on RBC membranes [6, 16]. Therefore, L-CAR administration may reverse the depletion of serum and cellular store of free carnitine and prevent the accumulation of long chain acyl carnitine esters and their inhibitory effect on ATPase [6]. It has to be mentioned that carnitine and carnitine-palmityl-transferase (CPT) may cooperate with the reacylation process of membrane phospholipids by modulating the ratio of acylCoA/free CoA in RBC and plays similar role as in mitochondria [17, 18]. Phospholipids determine the RBC membrane structure and survival of RBC.

After simultaneous r-Epo and L-CAR administration the activity of Na,K-ATPase additionally increased and the intracellular K was the highest what suggest more active transmembrane transport. The effect of r-Epo was intensified by L-CAR which acts on different way. In dialyzed patients before r-Epo and L-CAR administration we observed higher ATP, 2,3-DPG concentrations and increased glucose utilization and lactate production then in healthy control what could suggest increased glycolysis rate. Some of these changes may produce a rightward shift in the oxygen dissociation curve providing an enhanced amount of oxygen release to the tissues and partially compensating

the reduced RBC mass in the uremic subject. The influence of higher phosphate concentration on the RBC glycolytic rate also is possible [20]. Increase or decrease of ATP level and 2,3-DPG/ ATP ratio reflects differences between generation and the ATP consuming processes including ion and oxygen transport and processes connected with cell shape, deformability and normal life-span [6, 21]. In RBC, generation of ATP only depends on the flux through glycolysis, regulated by the enzyme activities of this pathway especially hexokinase and fosfofruktokinase. Hexokinase is strongly cell age -dependent [22]. After r-Epo treatment the increase of ATP, 2,3-DPG, hexokinase, LDH was noticed. Probable some of this changes are dependent on the appearance of young age RBC and rejuvenation of RBC population [19, 21]. Hence, the predominant effect of increased RBC level of 2,3-DPG and ATP after r-Epo treatment is increased oxygen delivery. After L-CAR the increase of 2,3-DPG, LDH and decrease ATP concentration were found (Table 1). The decrease of ATP concentration after L-CAR administration in CAR and in CAR+EPO groups may be related to it increased consumption rate dependent on increased Na,K-ATPase activity and profitable changes in the structure of RBC membrane. During r-Epo and L- CAR given separately or together the decrease glucose utilization and lactate production were observed. Although the reduction of glucose uptake was higher during simultaneous r-Epo and L-CAR administration. The ratio glucose uptake /lactate production suggests that greater part than in healthy condition of glucose utilization goes through other than glycolytic pathway.

It seems that RBC after r-Epo and L-CAR treatment have better transport properties, improved oxygen delivery and reduced glucose utilization what reminds normal conditions. Simultaneous r-Epo and L-CAR administration particularly in patients dialyzed for many years seems to be a way of optimization of anemia treatment in HD patients.

CONCLUSIONS

1. Both r-Epo and L-CAR improve hematological status of anemic hemodialyzed patients.
2. R-Epo acts mostly on the way of rejuvenation of RBC population; L-CAR by the stabilization of RBC structure.
3. Simultaneous r-Epo and L-CAR administration approach the metabolic profile of RBC to observed in healthy population.

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