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THE EFFECT OF L-CARNITINE ON NEUTROPHIL CHEMILUMINESCENCE RESPONSE IN CHRONIC RENAL FAILURE PATIENTS

KRONİK BÖBREK YETMEZLİĞİ HASTALARINDA L-KARNİTİN KULLANIMININ NÖTROFİL KEMİLÜMİNESANS CEVABINA ETKİSİ

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ÖZET

Nötrofil fonksiyon bozuklukları Kronik Böbrek Hastalarında bildirilmiştir. Bu çalışmada biz hemodiyaliz tedavisi gören hastalarda L-karnitinin nötrofil kemilüminesans cevabına (in vitro ve in vivo) etkisini araştırdık.

Çalışmaya 15 hemodiyaliz hastası ve 15 sağlıklı kontrol alınmıştır. Nötrofillerin zimozanla uyarılmış oksidatif metabolizması, luminole bağlı kemilüminesans yöntemiyle bakılmıştır. Hasta ve kontrol nötrofilleri izole edildikten sonra 0, 25, 50, 100 nmol/ml L-karnitinle 10 dk inkübasyondan sonra kemilüminesçırts değerleri saptanmıştır. Hasta grubunun nötrofil kemilüminesans cevabı (2933±393) sağlıklı kontrollere göre (4496±717, p<0.001) anlamlı derecede düşük bulunmuştur. 25, 50, 100 nmol/ml L-karnitin konsantrasyonlarında anlamlı derecede (p<0.001) artış gözlendi (3527±459, 4372 ±561, 4322±636). Ancak L-karnitin kontrollerde benzer konsantrasyonlarda artış göstermemiştir. Hastaların nötrofil kemilüminesans cevapları kontrollerin seviyesine 50 nmol/ml L-karnitinle inkübe edildiğinde ulaşmıştır. Çalışmanın in vivo bölümünde bu hastalara 2x1g/gün olacak şekilde 30 gün süreyle L-karnitin verilmiştir. Bu sürenin sonunda nötrofil kemilüminesans cevapları tekrar çalışılarak (4562±560) anlamlı bir artış olduğu gözlenmiştir, (p<0.001) Bulduğumuz bu değerler sağlıklı kontrollerden farklı değildir.

Bulgularımız göstermiştir ki, kronik böbrek yetmezliği olan hastalarda nötrofil kemilüminesans cevapları anlamlı derecede artmıştır.

Anahtar Kelimeler: Nötrofil, L-karnitin, Kemilüminesans, Kronik Böbrek Yetmezliği

ABSTRACT

Neutrophile abnormalities have been reported in chronic renal failure patients. In this study, the effect of l-carnitine on neutrophile chemiluminescence (CL) response (invitro and invivo) was investigated in patients on hemodialysis. Fifteen hemodialized patients and 15 healthy age matched controls were studied. The zymosan induced oxidative metabolism of phagocytes was assessed by means of luminol dependent CL. Control and patients' neutrophils were isolated and CL were determined after 10 minutes incubation with 0, 25, 50 and 100 nmol/ml l-carnitine. The CL response of neutrophils in patient group (2933+393) was significantly lower than the healthy controls (4496+717, p<0.001). Significant increases were found in the neutrophil CL response with 25, 50, 100 nmol/ml l-carnitine concentrations (p<0.001) (3527+459, 4372+561, 4322+636, respectively), but same concentrations of l-carnitine did not cause any change in controls' neutrophils. Patients' neutrophil CL responses were reached to controls' levels when incubated with 50nmol/ml l-carnitine. After the in vitro study, 2xlg/day P.O. l-carnitine were given to these patients for 30 days. At the end of this period, CL response was restudied (4562+560) and found to be significantly increased when compared with the before l-carnitine values(p<0.001).

These values were not different from the healthy controls. Our findings indicate that neutrophil CL response is significantly increased by l-carnitine in chronic renal failure patients.

Key words: Neutrophil, L-carnitine, Chemiluminescence, Chronic Renal Failure

INTRODUCTION

Phagocytic cells play important role in antibacterial host defence [1-3]. Several abnormalities of neutrophil function have been reported in patients with chronic renal failure (CRF), and thought to be responsible for the increased susceptibility of these patients to bacterial infections [4-5]. After phagocytosis, a marked increase in energy expenditure and oxidative metabolism (respiratory burst) occurs which is very important in bactericidal activity of neutrophils, neutrophil chemihjminescence assay (CL) reflects this activity. Decrease in 1-carnitine levels were reported in patients on hemodialysis[6]. This compound has important effects on energy metabolism of cells[7]. Effect of 1-carnitine (in vitro and in vivo) on neutrophil CL response, in patients on hemodialysis, were investigated in this study.

SUBJECTS AND METHODS

Fifteen CRF patients on hemodialysis for more than 6 months and 15 healthy adult controls were enrolled in the study with ages ranging from 23 to 43 years. All patients received the same, bicarbonate dialysis three times a week for 4h with polysulfon hollow-fiber filter [F-Series Low-Flux (F5-8), Fresenius Medical Care AG], and heparin as anticoagulant. Blood flow varied from 250-350 ml/min, and dialysate flow was set at 500 ml/min. Patients with active infection, DM, SLE, and vasculitis and patients who had received alcohol or drugs which were known to affect neutrophil function (e.g. anti-inflammatory drugs, antibiotics) were not included. All patients were receiving erythropoietin.

Blood samples were obtained from patient just before morning dialysis session. In invitro part of study, CL assays were performed after neutrophils, which were isolated from controls' and patients' peripheral blood, were incubated with 1-carnitine for 10 minutes in the concentrations of 25, 50, 100 nmol/ml (normal serum levels of 1-carnitine were reported as 51.6(26-76) nmol/ml)[8]. We obtained the best results at ten minutes in our previous studies. In in vivo part of study, 1-carnitine (2g/day/P.O. in two divided doses, for 30 days) was administered to the same patients whose CL were evaluated previously. At the end of the medication period CL was determined again.

Chemiluminescence assay: CL generation was determined according to the method described by Leino et al. [9]. Briefly, neutrophils were isolated by density gradient separation and adjusted to 2xlO6/ml. Zymosan activated serum (ZAS) was prepared using five AB Rh (+) pooled sera which were incubated for 30 minutes with zymosan A (Sigma chemical Co., St. Louis, MO.) at a concentration of 10 mg/ml in 37C0 water bath. The 1/3 dilution of this ZAS was used throughout the study as standard stimulus. Luminol.(1.5 niM) was used enhanced for chemiluminescence response. CL generation was determined by using beta counter in both stimulated and nonstimulated vials and seven counts were taken at ten minutes intervals.

CL response was given as stimulation index (Stimulation index(SI)= Stimulated peak value-non stimulated peak value)

Statistical analysis:

Results were expressed as meanSD. Comparison of groups were performed by variance analysis in in vitro study. Paired t test was used to compare the pre and post 1-carnitine treatment in in vivo study (Crunch Statistical Package version 4.04; 1992(402024) Crunch Software Corporation Oakland, California, MA). P value less than 0.05 was accepted statistically significant. Informed consent was obtained from patients and controls.

RESULTS

In in vitro study, baseline CL mean values of patients (2933393) were significantly lower than that of controls (4496717) ($p< 0.05$). In nine of 15 patients (60%), CL values were less than two standard deviation of controls.

Neutrophil CL SI values after ten minutes incubation with L-carnitine in the concentrations of 25, 50, 100 nmol/ml were found as 3527459, 4372561, 4322636 CPM/min respectively in the patient's group. These values were significantly higher than the baseline ($p<0.001$) **(Tablel).** Control group studies showed that there was no statistically significant correlation between concentrations and CL response ($p>0.05$) **(Table II).**

In in vivo study, 30 days after L-carnitine administration, baseline neutrophil CL values (2933 393) increased to (4562560) ($p<0.001$) **(Table I),** and this value was not statistically different from healthy controls ($p>0.05$).

DISCUSSION

Neutrophils play important roles in host defence against bacterial and fungal infections. An abrupt increase in energy expenditure and oxidative metabolism (respiratory burst) occurs after phagocytosis [1,2]. Our method reflects this respiratory burst activity which is very important in bactericidal activity.

Table I: Effect of 1-carnitine to the chemiluminescence response of neutrophils in the patients group.

	Baseline	In vitro(L-carnitine)			In vivo#
		25nmol/ml	50nmol/ml	lOOnmol/ml	2g/day
1	3190*	3400	3610	4290	4982
2	2950	2450	3465	2870	3330
3	3294	3448	4336	3828	5022
4	3208	3328	4897	5252	4342
5	2742	3170	4356	4107	5166
6	3610	4148	5307	4560	5475
7	2860	3565	3768	4448	4932
8	2710	3611	4973	3580	4468
9	2447	3468	4809	5346	4346
10	2106	3480	4990	4844	3993
11	3078	4583	4450	4677	4811
12	2810	3511	3999	4533	4690
13	2661	3369	4521	4398	4217
14	3506	3632	3749	3790	3877
15	2867	3752	4358	4311	4726
Mean±lSD	2933±393	3527±459	4372±561	4322±636	4562±560

* CPM/min
# After 2g/day P.O. L-camitine for thirty days

Table II: Effect of 1-carnitine to the chemiluminescence response of neutrophils in the controls group.

	Baseline	In vitro(L-carnitine)		
		25nmol/ml	50nmol/ml	I00nmol/ml
1	5526*	5311	5500	5440
2	5318	5274	5292	5476
3	5197	5380	5229	5171
4	4968	5057	4901	4807
5	4569	4623	4374	4326
6	5431	5240	5302	4969
7	4442	4570	4574	4500
8	4309	4390	4502	4406
9	4370	4355	4148	4274
10	4811	4724	4693	4947
11	4289	4043	4062	4135
12	3634	3603	3556	3876
13	3752	4073	3529	3550
14	3268	3446	3468	3403
15	3562	3500	3779	4020
Mean 1SD	4496717	4505600	4460693	4486695

* CPM/min

has been demonstrated that neutrophils from CRF patients exhibit low CL responses[10-ll]. We found significantly lower CL values in CRF patients than in healthy controls, which is in agreement with the previous reports[5,10-11].

The results of this study show that 1-carnitine can recover the defective CL response of these patients. There is only one report on the relationship between 1-carnitine and CL response, in this study Schinetti et al. [12], examined the effect of 1-carnitine on healthy adults' neutrophils and found that 1-carnitine had no effect on neutrophil CL response. This is consistent with our observation in healthy adults (Table II). The results of this study show that 1-carnitine can correct the defective CL response of these patients (table I).

Significant decreases in 1-carnitine levels in CRF patients were reported previously [6]. Although we could not determine the serum levels in these patients, providing normal serum levels of 1-carnitine (in vitro) significantly increased the CL in CRF patients and did not make any effect in normal controls. This observation suggests that low 1-carnitine levels may be responsible for the defective CL response in these patients and 1-carnitine supplementation can correct this disturbance. We do not know the exact mechanism of this positive effect of 1-carnitine on human CL response in CRF patients. L-carnitine's primary function is the transport of long chain fatty acids across the mitochondrial membrane to the site of beta-oxidation, resulting in the production of energy. Neutrophil energy expenditure increases two fold in chemotaxis and 5-10 fold in phagocytosis and these increased energy requirements are provided by aerobic and anaerobic glycolysis. It is not known if 1-carnitine level, which is important in cell energy production, effect this metabolism. Our observation of increase in CL response in CRF patients after 1-carnitine supplementation may be due to 1-carnitin's positive effect on cells' energy metabolism. The effect of lipid metabolism in neutrophil energy production is not known. We hypothesised that, during neutrophil activities (e.g. chemotaxis, phagocytosis), there is a sudden increase in energy requirement, additive energy supplementation by fatty acid oxidation may effect these activities positively.

In conclusion, patients with chronic renal failure have decreased neutrophil CL response which is markedly increased by 1-carnitine supplementation. This effect may be helpful to these patients by increasing these antibacterial defence. It remains to be seen, by clinical studies, if 1-carnitine supplementation helps these patients in the prevention of their increased susceptibility to infection. Further studies are needed to explain the possible effect of 1-carnitine on energy production, provided by fatty acid oxidation, in neutrophil metabolism.

REFERENCES

1. Malech HL, NauseefWM: Primary inherited defects in neutrophil function. Semin Hematol 1997;34:279-290
2. Yang KD, Hill HR: Neutrophil function disorders: Pathophysiology, prevention and therapy. J Pediatr 1991;119:343-353
3. Hill HR: Biochemical, structural and functional abnormalities of polymorphonuclear leukocytes in the neonate. Pediatr Res 1987;22:371-383
4. Haag-Weber M, Hörl W:Altered cellular host defence in malnutrition and uraemia. Contrib Nephrol 1992;98:105-111
5. Chatenoud L, Jungers P, Descamps-Latscha B: Immunological considerations of the uremic and dialysed patient. Kidney Int 1994;45:92-96
6. Bartell LL, Hussey JL, Shrago E: Perturbation of serum carnitine levels in human adults by chronic renal disease and dialysis therapy. Am J Clin Nutr 1981;34:1314-1320
7. Boehm K, Heims R, Chnstensen ML, Storm M:Carnitine: A review for the pharmacy. Clinician HospPharm 1993;28:847-850
8. Rebouche CJ, Engel AG: Primary systemic carnitine deficiency: I. Carnitine biosynthesis. Neurology 1980;31:813-818
9. Leino L, Nuutila J, Pelliniemi T, Rajamaki A: Human recombinant GM-CSF selectively primers receptor mediated respiratory burst of neutrophils in vitro. Immunol Lett 1993;38:26-31
10. Vanholder R, Ringoir S, Dhondt A, Hakim R: Phagocytosis in uremic and hemodialysis patients: A prospective and cross sectional study. Kidney Int 1991;39:320-327
11. Ritchey E, Walhn JD, Shah SV: Chemiluminescence and superoxide anion production by leukocytes from chronic hemodialysis patients. Kidney Int 1981;19:349-358
12. Shinetti ML, Mazzini A: Effect of L-carnitine on human neutrophil activity. Int J Tissue React 1986;8:199-203