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ORIGINAL ARTICLE

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Oral L-carnitine does not decrease erythropoietin requirement in pediatric dialysis

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Abstract The use of recombinant human erythropoietin (rhEPO) has greatly facilitated the treatment of anemia in children with chronic renal failure, but is expensive. Several reports on adult patients have shown that supplementation with L-carnitine can decrease the requirement for rhEPO. The objective of this study was to investigate the effect of oral supplementation with L-carnitine on the rhEPO requirement in children on dialysis. We investigated 16 children on dialysis (11 hemodialysis, 5 peritoneal dialysis) with a median age of 10.2 years. All children were stable on rhEPO treatment at least 3 months before study entrance. After obtaining baseline data, all children were supplemented with L-carnitine 20 mg/kg/day. Data were collected for 26 weeks. Follow-up was completed for 12 patients (8 hemodialysis, 4 peritoneal dialysis). At baseline free carnitine (32±18 µmol/l) and total carnitine levels (54±37 µmol/l) were normal. At the end of the study free carnitine levels had increased to 97±56 µmol/l ($P<0.05$) and total carnitine levels to 163±90 µmol/l ($P<0.05$). There was no significant change in rhEPO requirement. Hemoglobin level or hematocrit did not change significantly during the study. In conclusion we could not demonstrate a beneficial effect of supplementation with L-carnitine on rhEPO requirement in children on dialysis.

Key words L-Carnitine · Erythropoietin · Anemia · Peritoneal dialysis · Hemodialysis

Introduction

Anemia is a major complication of chronic renal failure. Diminished production of erythropoietin by the diseased kidneys is the major cause of this anemia, and it can be treated effectively with the use of recombinant human erythropoietin (rhEPO). This therapy is expensive, however, and several different strategies have been employed to decrease the requirement for rhEPO in patients with renal failure. Studies in adult dialysis patients have shown that supplementation with L-carnitine decreases the dose of rhEPO required to maintain target hemoglobin levels [1–3]. Data on this effect of L-carnitine supplementation in children on dialysis are scarce [4].

The objective of this study was to investigate whether oral supplementation with L-carnitine in pediatric dialysis patients can decrease the dose of rhEPO needed to maintain target hemoglobin levels.

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Patients and methods

We investigated 16 children on dialysis (11 hemodialysis, 5 peritoneal dialysis) with a median age of 10.2 years (range 0.6–18.9 years), of whom 8 were male. All children were on dialysis for at least 3 months prior to start of the study and had a stable rhEPO requirement. All children but one were prescribed oral iron supplementation, and five children on hemodialysis (HD) received intravenous iron during the study. All children on HD received rhEPO intravenously. Of the children on peritoneal dialysis (PD) the route of rhEPO administration was intraperitoneal in four and subcutaneous in one. After obtaining baseline data, oral supplementation with 20 mg/kg/day of L-carnitine (Carnitene, Sigma Tau Ethifarma, The Netherlands) in two divided daily dosages was started. To enable comparison between children on peritoneal dialysis and children on hemodialysis, the same route of administration was used in all patients. As intravenous administration 3 times a week is not feasible in the children on peritoneal dialysis, we chose an oral route of administration. The choice of dose was based on previously published data on oral supplementation with L-carnitine in children on peritoneal dialysis [5]. During the study rhEPO dose was adjusted to maintain postdialysis hematocrit (Ht) levels between 0.30 and 0.35 in children on HD. The same target levels were used for children on PD.

Data were collected at 4, 8, 12, and 26 weeks after starting L-carnitine supplementation. Hemoglobin, hematocrit and cell indices were determined postdialysis in children on HD. Serum iron, ferritin and transferrin concentrations were determined by standard analytical procedures, parathyroid hormone concentration was determined by RIA, and plasma total L-carnitine and free car-

nitine levels were determined by spectrophotometry with DTNB [5,5'-dithiobis-(2-nitrobenzoic acid)] [6]. Short-chain acylcarnitine values were calculated from total carnitine minus free carnitine values.

All children participated after informed consent from their parents and from themselves when appropriate.

Statistical analysis

Differences in proportion between groups were tested by Fisher's exact test. Differences of mean values between respective time points were analyzed by Student's *t*-test for paired samples, and differences of mean values between groups were analyzed by Student's *t*-test for independent samples. Correlations between variables were analyzed by linear regression analysis and by multiple linear regression analysis. A *P* value of less than 0.05 was considered significant.

Results

During the study four children dropped out (three HD and one PD); reasons for dropout were: transplantation in two cases, transfer to another dialysis center and patient refusal after 4 weeks in one case each.

Baseline data are presented in Table 1. Serum total carnitine level was below the normal range (30–65 µmol/l) in one PD patient, and serum free carnitine level was below the normal range (25–60 µmol/l) in four patients (two HD and two PD). No patient had hyperparathyroidism (data not shown). Of all patients four had an iron status with ferritin above 100 µg/l and transferrin saturation (TSAT) above 20% (three HD and one PD). There were no significant differences at baseline between the HD and PD groups for carnitine levels, free carnitine to total carnitine ratio, iron status, hemoglobin level or rhEPO dosage.

Data after 26 weeks of L-carnitine supplementation are presented in Table 2. During the study none of the patients reported diarrhea, a possible side-effect of L-carnitine ingestion. Plasma total carnitine, free carnitine and acylcarnitine levels had increased significantly in the whole group of patients and the increase was also significant for total and free carnitine when analyzed for HD and PD subgroups. There was no change in free carnitine to total carnitine ratio. Carnitine levels tended to increase more in the HD group than in the PD group, but this did not reach statistical significance.

There was no change in hemoglobin level or hematocrit, nor was there a significant change in rhEPO dose.

At 26 weeks two patients had an iron status with ferritin above 100 µg/l and TSAT above 20% (one HD and one PD). Iron status was not different between the HD and PD groups. Ferritin levels and TSAT had not changed significantly from baseline.

There was no significant correlation between hemoglobin level and total carnitine level, free carnitine level or acylcarnitine level, nor was there a significant correlation between rhEPO dose and total carnitine level, free carnitine level or acylcarnitine level by linear regression analysis.

When hemoglobin was introduced as the dependent variable and rhEPO dose, ferritin level, TSAT, total carnitine level, free carnitine level, and acylcarnitine level as the independent variables in multiple linear regression analysis, only rhEPO dose showed a significant negative correlation with hemoglobin level (P=0.03). When rhEPO dose was introduced as the dependent variable and ferritin level, TSAT, total carnitine level, free carnitine level, and acylcarnitine level as the independent variables, none of the variables showed a significant correlation with rhEPO dose.

Table 1 Baseline data of all patients and subdivided into dialysis modality

	All patients *n*=16	Hemodialysis *n*=11	Peritoneal dialysis *n*=5	*P* value
Total carnitine (µmol/l)	54±37	60±42	43±16	NS
Free carnitine (µmol/l)	32±18	36±19	22±9	NS
Acylcarnitine (µmol/l)	23±20	24±24	20±9	NS
Ferritin (µmol/l)	102±84	109±95	88±56	NS
TSAT (%)	18±7	19±7	16±8	NS
hemoglobin (mmol/l)	6.8±1.3	6.8±1.5	6.8±1.1	NS
rhEPO dose (U/kg/week)	206±132	207±133	205±145	NS

Table 2 Data in patients who completed 26 weeks of L-carnitine supplementation

	All patients *n*=12		Hemodialysis *n*=8		Peritoneal dialysis *n*=4	
	Baseline	26 weeks	Baseline	26 weeks	Baseline	26 weeks
Total carnitine (µmol/l)	58±41	163±90*	64±49	191±98*	43±16	108±38*
Free carnitine (µmol/l)	33±20	97±56*	38±22	113±62*	24±10	65±23*
Acylcarnitine (µmol/l)	25±23	66±37*	26±28	78±40*	23±8	43±18
Ferritin (µmol/l)	101±95	92±66	107±111	85±67	91±64	105±73
TSAT (%)	19±8	21±13	20±7	18±11	15±9	27±17
Hemoglobin (mmol/l)	7.0±1.4	6.7±1.5	7.0±1.6	7.3±1.4	6.9±1.2	5.3±0.9
rhEPO (U/kg/week)	191±124	222±150	173±112	213±158	228±156	241±155

*P<0.05 baseline vs 26 weeks

Discussion

Anemia due to impaired synthesis of erythropoietin by the diseased kidneys is one of the major complications of chronic renal failure. Since the introduction of rhEPO it is possible to treat the anemia effectively but the cost of this therapy is considerable. Several strategies have been developed to decrease the dose of rhEPO required to maintain target hemoglobin levels. Studies in adult patients showed that plasma total carnitine levels are negatively correlated with required rhEPO dosage [7]. Moreover, it was shown that intravenous supplementation with L-carnitine reduces requirements for rhEPO by 38–50% [1–3]. This would make L-carnitine supplementation a cost-effective measure in the treatment of anemia in chronic renal failure.

Published data on the effect of L-carnitine supplementation on the treatment of anemia in children are very scarce. Bérard was able to increase the hematocrit by 34% in two children on hemodialysis with L-carnitine supplementation, without modification of rhEPO dosage [4]. Data suggest that children on hemodialysis have low plasma carnitine levels [8–10], whereas data on children on peritoneal dialysis are conflicting [5, 11].

In the present study mean plasma levels for total and free carnitine were well within the normal range at baseline, although one patient had a total carnitine level below normal and four patients had free carnitine levels below normal. There was no significant difference in carnitine levels in children on hemodialysis compared to children on peritoneal dialysis. Although the enteral resorption of L-carnitine is said to be slow, incomplete and unpredictable, oral supplementation with 20 mg/kg/day of L-carnitine led to a significant increase in plasma carnitine to supranormal levels within 4 weeks and was able to sustain these increased levels during 26 weeks of supplementation. In one study in which a fivefold higher oral dose was used in children on PD, plasma carnitine levels increased to 148±84 µmol/l after 2 months [5]. This value is higher than the levels we attained in our PD group. Although that study did not primarily address the issue of anemia, no change was found in hemoglobin levels after supplementation. Data on the use of rhEPO were not reported.

In contrast to most studies in adults we could not demonstrate an effect on the required dose of rhEPO after 26 weeks of L-carnitine supplementation. Data in adults suggest, however, that the beneficial effect of L-carnitine supplementation may be restricted to specific subpopulations, e.g., elderly patients [12, 13]. In those studies where L-carnitine levels were measured, much higher values were attained than in our study. It is possible that only a very high oral dosage of L-carnitine has an influence on rhEPO requirements but this would also increase the likelihood of gastrointestinal side-effects of L-carnitine supplementation. Again in contrast to studies in adults, we could not demonstrate a correlation between plasma carnitine levels and hemoglobin levels or rhEPO requirement. Therefore, we conclude that oral supplementation with L-carnitine does not result in a decreased rhEPO requirement in children on dialysis.

More aggressive treatment of body iron stores in our patients might have decreased the requirement for rhEPO, both at baseline and after 26 weeks of carnitine supplementation. Compared to the recently published DOQI guidelines, only a minority of our patients had adequate iron stores. These guidelines are based on data in adult patients, and specific pediatric parameters still have to be determined. The dose of rhEPO used in our patients, however, was comparable to recently published guidelines [14]. In our opinion the patients in this study did not show rhEPO resistance due to clinically important iron deficiency.

The average dose of rhEPO used intraperitoneally in the patients on PD was comparable to that reported for intravenous use in other pediatric studies and was not different from the dose used in the patients on HD [14, 15]. The mode of administration of rhEPO in individual patients was not changed during the study. Therefore, it is our opinion that the mode of administration did not influence the results of L-carnitine supplementation on rhEPO requirement.

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LITERATURE ABSTRACTS

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cAMP stimulates the in vitro proliferation of renal cyst epithelial cells by activating the extracellular signal-regulated kinase pathway

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Background Cellular proliferation is a key factor in the enlargement of renal cysts in autosomal dominant polycystic kidney disease (ADPKD). We determined the extent to which adenosine 3′:5′-cyclic monophosphate (cAMP) may regulate the in vitro proliferation of cyst epithelial cells derived from human ADPKD cysts.
Methods Epithelial cells from cysts of individuals with ADPKD and from normal human kidney cortex (HKC) of individuals without ADPKD were cultured. The effects of agonists and inhibitors on the rate of cellular proliferation and the activation of extracellular signal-regulated kinase (ERK1/2) were determined.
Results 8-Br-cAMP (100 micromol/L) stimulated the proliferation of cells from eight different ADPKD subjects to 99.0% above baseline; proliferation was inhibited by protein kinase A (PKA) antagonists H-89 (97%) and Rp-cAMP (90%). Forskolin (10 micromol/L), which activates adenylyl cyclase, increased proliferation 124%, and receptor-mediated agonists arginine vasopressin, desmopressin, secretin, vasoactive intestinal polypeptide, and prostaglandin E2 stimulated proliferation 54.2, 56.3, 46.7, 37.1, and 48.3%, respectively. The mitogen extracellular kinase (MEK) inhibitor PD98059 completely inhibited ADPKD cell proliferation in response to cAMP agonists, but genistein, a receptor tyrosine kinase inhibitor, did not block cAMP-dependent proliferation. cAMP agonists increased the activity of ERK above control levels within five minutes. In contrast to ADPKD, proliferation and ERK activity of cells derived from normal HKC were not stimulated by cAMP agonists, although electrogenic Cl- secretion was increased by these agonists in both ADPKD and HKC cell monolayers.
Conclusions We conclude that cAMP agonists stimulate the proliferation of ADPKD but not HKC epithelial cells through PKA activation of the ERK pathway at a locus distal to receptor tyrosine kinase. We suggest that the adenylyl cyclase signaling pathway may have a unique role in determining the rate of cyst enlargement in ADPKD through its actions to stimulate cellular proliferation and transepithelial solute and fluid secretion.

Kyushu Pediatric Nephrology Study Group

Acquired cystic kidney disease in children undergoing continuous ambulatory peritoneal dialysis

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Our cross-sectional study aims to elucidate the prevalence of acquired cystic kidney disease (ACKD), time of occurrence of ACKD, relationship between the prevalence of ACKD and duration of dialysis, progression of cysts, and ACKD-related complications in 54 children undergoing continuous ambulatory peritoneal dialysis (CAPD). ACKD was defined as four or more cysts detected in each kidney by ultrasonography, computed tomography (CT), or magnetic resonance imaging. The presence of less than four cysts was diagnosed as solitary cysts (SCs). Noncystic primary disease was present as glomerulonephritis in 23 patients (42.6%); hypoplastic kidney, 7 patients (13.0%); reflux nephropathy, 6 patients (11.1%); and other, 18 patients (33.3%). ACKD was evident in 16 patients (29.6%) during the 57.9 +/– 39.8 months after the start of CAPD. Nine patients (16.7%) had SCs. SCs and ACKD were detected initially in patients with 1 and 3 years of CAPD, respectively. The mean duration of CAPD for patients with ACKD (96.1 +/–36.6 months) differed from that of patients with SCs (49.8 +/–29.9 months) and no cysts (38.3 +/–25.8 months). The groups were classified according to time after the start of CAPD: 0 to 4 years (n = 33), 5 to 9 years (n = 16), and longer than 10 years (n = 5). The prevalence of ACKD among these three groups was 9.1%, 50%, and 80%, respectively, and this prevalence increased significantly with increasing duration of CAPD. Of 15 patients examined two to four times by ultrasonography or CT, the number and size of cysts increased in 7 patients with ACKD and 2 patients with SC. Two patients with many and large cysts experienced gross hematuria, and one of those patients had intracystic and retroperitoneal bleeding caused by cyst rupture. No solid mass lesion was found by imaging diagnostic modalities in the 54 patients. In conclusion, the prevalence of ACKD in children undergoing CAPD is just as high as that in adults. The prevalence of ACKD and number and size of cysts increased with increasing duration of CAPD.