

Letters and Replies

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Effect of L-carnitine administration on erythropoietin use in thalassaemic minor haemodialysis patients

Sir,

We read with great interest the recent letter by Arduini *et al.* [1], on the effect of L-carnitine (LC) on erythrocyte survival in haemodialysis patients. In the same issue, there is a paper by Hothi *et al.* [2] showing that plasma free-carnitine (FC) levels fell from 26.54 ± 2.99 to 15.6 ± 2.34 $\mu\text{mol/l}$ ($P < 0.000.1$) in nocturnal haemodialysis (NHD). A similar reduction in plasma acyl-carnitine (AC) levels was observed (from 13.22 ± 1.34 to 6.24 ± 1.20 $\mu\text{mol/l}$ ($P < 0.001$)). The AC: FC ratio improved from 0.51 ± 0.03 to 0.39 ± 0.03 ($P < 0.005$) (normal < 0.25). They conclude that NHD is associated with an improvement in AC: FC ratio.

Anaemia is a common finding in patients on chronic haemodialysis (HD), and represents one of the leading causes of increased cardiovascular morbidity and mortality in these patients. While the main defect responsible for anaemia in chronic renal failure patients is inadequate production of erythropoietin, there is evidence that other factors may also be involved. Several studies have shown that LC supplementation may have a beneficial effect on renal anaemia [3].

Arduini *et al.* [1] report the first randomized controlled trial of the effects of LC on erythrocyte survival in HD patients. Red blood cells (RBC) survival was evaluated by the ^{51}Cr labelling procedure, a gold standard methodology in RBC survival studies [4]. There was a strong trend towards improved RBC survival in the treated group ($P = 0.058$), and this mechanism might explain in part the reported benefit of LC supplements on anaemia control in HD patients [1].

Several authors suggest various fundamental mechanisms of carnitine deficiency that may contribute to anaemia in patients with chronic uraemia [5–8].

The biophysical properties of the erythrocyte membrane and its cytoskeletal network are fundamental for RBC survival in the blood stream [5]. Indeed, RBCs must survive a variety of chemical and physical insults during their life span, and the loss of their elastic properties may severely compromise tissue oxygenation. Erythrocyte deformability has been found to be impaired in uraemia [5] and to correlate with shortened RBC survival time [9–11]. LC treatment of HD patients seems to alleviate their anaemic condition. Recently, Duranay *et al.* [12] showed that there was a significant benefit of L-carnitine on C-reactive protein (CRP), transferrin, total protein and albumin levels of haemodialysis patients.

LC treatment of HD patients seems to alleviate their anaemic condition. A recent meta-analysis of the randomized LC trials performed before and after the advent of erythropoietin therapy shows a beneficial effect of LC supplements on anaemia control in maintenance HD patients [1]. This conclusion is consistent with the concept that LC may favourably affect the impaired rheological and metabolic properties of erythrocytes in HD patients.

Both thalassaemia and carnitine deficiency represent causes of either anaemia or erythropoietin (EPO) resistance in uraemia, even though the effect of the combined presence of these conditions has never been evaluated [13,14].

We would like to present our prospective study, which involved 12 β -thalassaemia minor (β -thal-m) dialysis patients (eight males and four females) previously treated for a 1-year period by administering progressively higher EPO doses in order to reach haemoglobin (Hb) target levels (11–12 g/dl); subsequently, adequate control of anaemia was maintained for a further additional year [14].

We performed a 6 month run-in period in order to verify the stability of Hb levels (coefficient of variation $< 3\%$). L-carnitine was then administered at the end of each of the three HD session of the week, at a dose of 2 g/haemodialysis for a 1 year period-time in controls and in β -thal-m dialysis patients. At the end of carnitine administration, 6 month follow-up was performed. Patients and controls were comparable at the start of study. In controls and β -thal-m subjects serum iron levels were 78 ± 30 and 89 ± 28 mg/l ($P = \text{NS}$), transferrin saturation 30 ± 4 and $29 \pm 5\%$ (NS), ferritin 245 ± 56 and 256 ± 60 mcg/l ($P = \text{NS}$), respectively. PTH_s levels were 205 ± 78 in controls and 187 ± 98 pg/ml in β -thal-m ($P = \text{NS}$); serum aluminium measured 45 ± 12 and 38 ± 16 mcg/ml, respectively in control and β -thal-m groups ($P = \text{NS}$). Also, groups were similar for age, dialysis age and renal disease (Table 1). Patients did not differ in body weight (60.2 ± 9.9 vs 57.9 ± 14.2 kg), serum albumin levels (3.96 ± 0.08 vs 3.95 ± 0.07 g/dl) and nutrient intake as indicated by protein nitrogen appearance (1.21 ± 0.04 vs 1.19 ± 0.06 g/kg/day). During the study BW, dialysis UF rate, and intradialytic Hb change were stable in the two groups). Controls showed both significant Hb increase and EPO decrease from the third month. β -thal-m patients also demonstrated these changes, but they were registered only after 8 months of carnitine administration. In Thal-m the EPO dose at time zero is 280 UI/kg/dialysis (in controls is 100 UI), and at the peak of the response (at the 12th month) the EPO dose is 170 UI/kg/dialysis (in controls is 60 UI, from third to sixth month); of note, the EPO dose decreases by 39%. Hb levels were unchanged (Figure 1). The study first demonstrates the lowering of EPO need in β -thal-m by a mean of 33% after 1 year carnitine administration, as compared with a 40% reduction that occurred in controls also after 3–6 months. Such a reduction of EPO need was also associated with haemoglobin target.

In summary, carnitine has been shown to be effective in many patients for the adjunctive treatment of anaemia associated with kidney disease [15,16]. Our data suggest that a very long-term carnitine administration would be necessary for uraemic patients on chronic dialysis affected by β -thalassaemia with unresponsive anaemia, or requiring large doses of erythropoietin.

Conflict of interest statement. None declared.

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Reply: potential mode of action of L-carnitine on uraemic anaemia

Sir,

We thank Di Iorio *et al.* [1] for their interest in our paper. The findings of their study provide another piece of evidence to support the notion that L-carnitine (LC) treatment may improve the anaemic status of haemodialysis patients irrespective of EPO sensitivity. A more in-depth discussion of the potential mode of action of LC on uraemic anaemia may thus be worthwhile.

Uraemic red blood cells (RBCs) must survive a variety of chemical and physical insults (i.e. oxidative stress, haemodialysis (HD) sessions), which severely affect the biophysical properties of RBC membrane and, hence, contribute to a significant reduction of RBC life span [2]. LC has been shown to favourably affect key biophysical properties of normal erythrocytes [3]. In keeping with these actions, LC might improve renal anaemia by alleviating the deteriorated rheological properties of uraemic RBCs [4]. In addition to the biophysical intervention, LC is able to exert a favourable metabolic action in a cellular environment deprived of any sub-cellular organelle. LC is known for its role in facilitating the transport of long-chain fatty acids across the mitochondrial membrane. However, LC also plays a pivotal role in the membrane phospholipid fatty acid turnover [3], a metabolic pathway involved in the repair process of oxidatively injured membrane phospholipids. A large body of evidence indicates that uraemic RBCs are continuously exposed to oxidative stress, which may oxidatively damage their lipid and protein components [2]. Since oxidized phospholipids may severely impair membrane properties, repairing them could improve membrane integrity and potentially reduce haemolysis. Thus, despite the lack of mitochondria, evidence for a role of LC in red cell metabolism is suggested by the presence of LC and carnitine palmitoyl-transferase (CPT) in the RBC, and their involvement in membrane phospholipid fatty acid turnover. That this may be occurring in RBCs of HD patients is suggested by the observation that LC treatment of these patients partially restored the alteration of the long-chain acyl-CoA/free CoA ratio associated with significant reduction of key enzymes involved in membrane phospholipid fatty acid turnover [5].

If the above discussion argues that an important LC target is the mature, circulating RBC (peripheral action), one may not exclude the fact that the ameliorative action of LC on uraemic anaemia may be present at the level of erythropoiesis (central action). Recent evidence seem to support the latter hypothesis. Matsumura *et al.* [6] demonstrated that the addition of high amounts of LC (>200 µM) to erythroid colonies in cell cultures from fetal mouse liver, a major location of erythropoiesis during the embryonic period, resulted in a significant increase of such erythroid colonies. Some reports have also indicated that the elevation in inflammatory cytokines and oxidative parameters correlated with rhEPO resistance, and that LC treatment improved these impairments [7].

In an attempt to gain more definitive information on the potential action of LC on erythropoiesis, Kitamura *et al.* [8] investigated the erythropoietic effect of LC using cells from mouse bone marrow, where erythropoiesis takes place only after birth. The authors have clearly shown that in the presence of 0.5 or 1.0 IU/ml of rhEPO, LC at concentrations

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>200 μ M significantly enhanced CFU-E colony formation in mouse bone marrow cell cultures.

Although these observations are still preliminary, some conjecture may be offered regarding the potential effect of LC on differentiation during RBC maturation. Programmed cell death is known to occur during the differentiation of progenitor cells. Erythropoietin contributes to RBC maturation by retarding apoptosis, thus allowing erythroid progenitors to complete their differentiation programmes. Indeed, a major negative regulation of erythropoiesis is the caspase-mediated cleavage of GATA-1 or other erythropoietic factors [9]. Mutomba *et al.* [10] reported that LC at millimolar levels inhibits the activation of caspases at various point in the Fas ligation pathway in Jurkat cells. Collectively, these reports suggest that LC influences erythropoiesis, possibly by inhibiting the apoptosis of progenitor cells.

A better understanding of the mechanisms involved in LC-mediated effect on uraemic anaemia, peripheral and/or central action, will help to clarify the role of LC in the treatment of renal anaemia.

Conflict of interest statement: A.A. is currently the Director of the Research and Development Department of Iperboreal Pharma Srl.

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Impact of calcium and vitamin D therapy on arterial and cardiac disease in young adults with childhood-onset end stage renal disease

Sir,

In the discussion of their well-documented study on cardiovascular disease in young adults with childhood-onset of end-stage renal disease (ESRD), the Berlin paediatricians Briese *et al.* [1] pointed out interesting differences with a quite similar study reported 4 years ago by their colleagues of Heidelberg [2]: the prevalence of coronary calcifications (10 vs 92%) and of cardiac valve calcifications (0 vs 32%) was quite lower in the Berlin study than in the Heidelberg one, while the technique of evaluation was comparable (Table 1). This difference was all the more remarkable in that the population characteristics were quite similar regarding the proportion of transplanted patients. However, the age was slightly younger (23 vs 27 years) while time on dialysis was shorter (2.9 vs 5 years) and that of transplantation longer (9.2 vs 7.8 years). In spite of these differences, at the time of cardiovascular evaluation, the classical cardiovascular risks [body mass index (BMI), smoking, low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol] were comparable (with the exception of mean blood pressure which was 11 mmHg lower), as well as the non-classic risk factors such as albumin and homocysteine, with the exception of CRP that was 11 mg/l higher in the Berlin study. Regarding the serum mineral parameters, although their evaluation in the Berlin study was only punctual and not time-integrated as in the Heidelberg one, it is remarkable to note that the Ca \times PO₄ product was similar while the serum parathyroid hormone (PTH) levels were twice lower in Berlin, despite the fact that the cumulative doses of both calcium oral phosphate binder (Ca-OPB) and 'active' vitamin D (alfacalcidol or calcitriol) were much lower (respectively by 7- and 35- folds). Since the Berlin authors did not comment on such differences in calcium and vitamin D therapy and clinical results, we would like to propose the following comments.

We have noted that in contrast to the Heidelberg group, the Berlin group mentioned the use of cholecalciferol i.e. plain vitamin D at a dose of 2.1–10⁶ IU per year i.e. 5.750 IU (or 143 μ g) per day i.e. a dose that would certainly secure a serum 25 OH vitamin D level well above the recommended thresholds of 30–40 ng/ml [3]. Even though the 3 year difference in age and the 2 years less time on dialysis may have contributed to the 2-fold difference in PTH suppression, we think that a causal relationship between lower PTH levels and lower prevalence of cardiovascular calcifications with cholecalciferol use cannot be excluded. Indeed, there is a rationale to relate this better PTH suppression to cholecalciferol use since the Slatopolsky group [4] recently evidenced in bovine parathyroid cell cultures that at a concentration of 40 ng/ml 25 OH vitamin OH D was as efficient as calcitriol at a maximally PTH suppressive dose (40–80 ng/ml), for suppressing PTH. The reason for this efficiency is not only that the concentration of 25 OH vitamin D is about 10³ higher, but that provided calcidiol concentration is sufficient, it can be taken up by the LR2-megalin receptor present in the parathyroid cells and presented to their mitochondrial 25 OH vitamin D-1 α hydroxylase, in order to synthesize 1.25(OH)₂ vitamin D [5]. This *in situ* synthesized calcitriol can then suppress the