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Carnitine System in Uremic Patients: Molecular and Clinical Aspects

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Carnitine is a small water-soluble molecule that is present in almost all animal species. It plays an indispensable role in fatty acid metabolism, where it is involved in the transport of activated fatty acids between different cellular compartments. Uremic patients, as well as patients with chronic renal failure, appear to have abnormal renal handling of carnitine leading to dyslipidemia, lethargy, muscular weakness, hypotension, cardiac dysfunction and arrhythmias, and recurrent cramps. It often is difficult to distinguish these symptoms from similar ones related to uremia and dialysis. Many investigators have advocated L-carnitine supplementation in an attempt to alleviate carnitine deficiencies, and good results from this therapy have been reported. Moreover, several studies have shown that L-carnitine supplementation improves the response to erythropoietin. Chronic inflammation is another particular aspect affecting these patients. Anti-inflammatory properties of L-carnitine in hemodialysis patients have been shown by our group. Treatment with L-carnitine (20 mg/kg, given intravenously at the end of each dialysis session for 6 mo), significantly decreased serum C-reactive protein (CRP) levels, a proinflammatory cytokine known to inhibit erythropoiesis. Moreover, data from published literature are indicative of L-carnitine modulation of the immune system by the activation of glucocorticoid receptors and the modulation of the transcription of glucocorticoid-responsive genes. Our study showed that in these patients, treatment with L-carnitine has been able to improve their body mass index, likely by promoting a positive protein balance. This aspect is strictly correlated with the status of insulin resistance, which is well described in patients with renal diseases. Many studies showed that carnitine allowed mitochondrial fatty acid usage to link to the rate of glucose usage, thus improving insulin resistance. In conclusion, clinical beneficial effects of L-carnitine treatment on patients suffering from renal diseases are supported by molecular evidence involving both inflammatory and metabolic aspects of the disease.
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Carnitine is an essential molecule with indispensable physiologic actions in intermediary metabolism.[1,2] It is present in the cells of most, if not all, animal species. The liver and kidney represent the main sources of endogenous carnitine synthesis from lysine, methionine and ascorbate, niacin, pyridoxine, and Fe2+.[3] Carnitine is captured and stored by the muscle.

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Carnitine System Physiology

The intracellular carnitine system composed of free carnitine and acylcarnitines (ie, natural esters of carnitine) includes a family of membrane-bound carnitine acyltransferases able to synthesize acylcarnitines of different chain lengths for their use or elimination during cell metabolic processes.[4,5]

Carnitine, supplied principally in the diet, is absorbed in the intestine and transported to tissues by transporters. Recently, a new family of organic cation transporters, designated organic cation/carnitine transporter (OCTN), has been described.[6] OCTN2, the most important carnitine transporter, recently was cloned.[7] Mutations in this protein cause

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the autosomal-recessive inborn error, systemic carnitine deficiency.[8] OCTN2 is expressed in several tissues, such as kidney, and transports carnitine in an Na+-dependent manner, with high affinity and pH dependence.[7] OCTN2 also plays an important pharmacologic role because it mediates the transport of many drugs, including tetraethylammonium, pyrilamine, valproate, and verapamil.[7]

The recognized physiologic roles of the carnitine system are as follows: β-oxidation of fatty acids in mitochondria, β-oxidation of very long-chain fatty acids in peroxisomes, transfer of acetyl- and other short-chain acyl groups from peroxisomes to mitochondria, re-esterification of triacylglycerol in the endoplasmic reticulum before secretion as very low density lipoproteins, stimulation of pyruvate and branched-chain amino acid oxidative metabolism, scavenger system for acyl groups, deacylation and reacylation to remodel erythrocyte membrane phospholipids, partner in the pathway of phospholipid and triglyceride fatty acid turnover in neurons, synthesis and elongation of polyunsaturated fatty acids,[4,5,9] stabilization of proteins and membranes, and counteraction of denaturating solute effects, for example, ammonia.[10,11]

Carnitine Status in Uremic Patients

Carnitine regulation of fatty acids β-oxidation occurs through an adaptation of the fatty acids mitochondrial content from an exit of acyl and acetyl moieties, thus modifying the acyl-carnitine/free carnitine ratio.[12] The carnitine shuttle allows an adequate mitochondrial free fatty acid content and protects against a down-regulation of β-oxidation observed in certain conditions such as metabolic acidosis, hypoxemia, or impaired glucose metabolism.

During chronic renal failure and before maintenance hemodialysis, total carnitine accumulates in response to a decreased renal clearance of acyl-carnitine moieties.[13] Furthermore, there is an increased need for free carnitine in response to hypoxemia or acidosis and often patients undergoing maintenance hemodialysis not uncommonly present with serum carnitine deficiency.[14] Indeed, the serum carnitine level rapidly decreases to 40% of the baseline level during the dialysis session.[15,16]

It is more difficult to assess muscle carnitine levels for technical reasons and because of a great variability of muscle carnitine levels in healthy volunteers. In addition, biopsy examinations have been performed at nonstandardized time points from the beginning of the dialysis session, preventing any comparison between studies.[17,18]

The role of dialysis membranes on carnitine loss into the dialysate also is uncertain. It should be emphasized, however, that carnitine cofactors and precursors may be lost throughout the dialysis session (ie, vitamin B_6, niacin, vitamin C, lysine, and methionine). Because of a molecular weight gradient, the acyl-carnitine moieties are less likely to be filtered by the membrane than the free carnitine, further impairing the abnormal serum acyl-carnitine/free carnitine ratio already present before end-stage renal disease.

Molecular Aspects

The preservation of an individual patient's life in the presence of kidney failure and the restoration of optimal quality of life are the main goals of maintenance hemodialysis therapy. In this purpose, the knowledge of molecular aspects of the carnitine system in uremic patients is crucial to understand some aspects of the disease. Three aspects of the uremic state particularly are linked to the carnitine system: muscle weakness, inflammation, and insulin resistance.

Muscle Weakness

Muscle tissue is highly dependent on the energy generated by β-oxidation of fatty acids and glycogen; therefore, it is important for muscle tissue to have adequate levels of carnitine.[19] Based on the evidence that skeletal muscle weakness in uremic patients may be associated with altered acyl-carnitine/free carnitine ratio, histologic and morphometric alterations were evaluated by Spagnoli et al.[20] Intravenous L-carnitine (2 g) was administered after each dialysis treatment to 22 patients for 12 months; treatment was suspended for 4 months, then continued for another 4 months with the addition of L-carnitine to the dialysate. Muscle biopsy examinations were performed at the end of the 12-month period (biopsy specimen 1), 4 months after cessation of treatment for use as a control (biopsy specimen 2), and at the end of 4 months of L-carnitine dialysate therapy (biopsy specimen 3). The study showed a direct correlation between carnitine level and mean diameter of type 1 fibers when comparing muscle biopsy specimens 1 and 3. There was a statistically significant reduction in mean diameter and the coefficient of hypertrophy of type 1 fibers. There were no morphometric parameter changes when biopsy specimens 1 and 2 were compared, which suggests that muscle fibers adapt slowly to variations in carnitine levels and relative metabolic conditions.

Inflammation

In recent years, several reports have suggested that inflammation, alone or in combination with a low protein intake, plays a significant role in uremic patients.[21,22] This is not unexpected because both serum albumin and C-reactive protein (CRP) participates reciprocally in the same acute-phase process. It has been established recently that increased plasma concentrations of CRP are associated with an increased risk for cardiovascular disease in dialysis patients.[23]

Serum levels of CRP appear to reflect generation of proinflammatory cytokines (interleukin-1, interleukin-6, and tumor necrosis factor-α), which also have been reported to be increased in chronic renal failure patients.[24] It is well documented that high levels of proinflammatory cytokines may cause muscle wasting by stimulating protein catabolism via the ubiquitin-proteosome pathway.[25]

Moreover, it is described that high levels of tumor necrosis

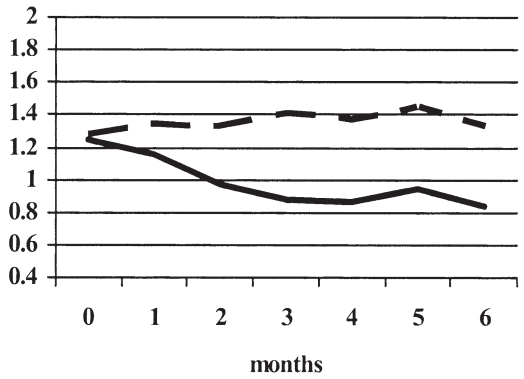

Figure 1 Serum CRP level during L-carnitine treatment (n.v.= 0-1 mg/dL). - - -, Placebo; —, carnitine. $P < .002$.

factor-α directly result in insulin insensitivity by decreasing insulin receptor tyrosine phosphorylation.[26]

Carnitine relationships and functions in cells of the immune system have been investigated extensively in both healthy and diseased patients. Further preclinical and clinical studies have shown the effect of carnitine congeners on the modulation of cytokine production. Evidence exists that carnitine can improve the course of infections, sepsis, or septic shock because these are all processes in which large amounts of cytokines are produced. In particular, carnitine can decrease the production of tumor necrosis factor-α, interleukin-1, and interleukin-6 to which several pathogenic processes have been attributed.[27-30]

Recently, our group showed that treatment of hemodialyzed patients with L-carnitine significantly decreased serum CRP levels, a proinflammatory cytokine known to inhibit erythropoiesis (Fig. 1).[31]

Moreover, data from published literature are indicative of L-carnitine modulation of the immune system by the activation of glucocorticoid receptors and modulation of the transcription of glucocorticoid responsive genes.[32]

Insulin Resistance

Increasing numbers of patients are being treated with dialysis therapy and atherosclerotic cardiovascular disorders have been found to have a great impact on mortality in these patients.[33] It has been shown that insulin resistance may contribute to the pathogenesis of atherosclerotic cardiovascular disease,[34] and if the prognosis of chronic dialysis patients is to be improved, we should devote more attention to insulin resistance in uremic patients. It is widely known that hypertension and hyperlipidemia play important roles in the progression of renal disease[35] and that insulin resistance may be involved in the pathogenesis of hypertension.[36] Furthermore, nutritional, metabolic, and cardiovascular complications of renal disease may be consequences of abnormal insulin action.[37] Therefore, long-standing renal insufficiency may cause atherosclerosis before the initiation of dialysis therapy. It has been known for the past 80 years that patients with end-stage renal disease exhibit glucose intolerance,[38] which is caused by insulin resistance, as evident from their reduced peripheral sensitivity to the hypoglycemic action of insulin.[39] DeFronzo et al[40] investigated glucose intolerance in uremic patients by using a glucose clamp technique and showed that hemodialysis 3 times/wk for 10 weeks improved insulin resistance in patients with end-stage renal disease.

Hyperinsulinemia, by increasing malonyl-coenzyme A (CoA), inhibits carnitine palmitoyl-transferase 1 activity and shunts long-chain fatty acids away from oxidation and toward storage in human muscle as triglycerides.[41]

Despite numerous clinical studies regarding insulin sensitivity of patients undergoing hemodialysis therapy, few data are available concerning the effect of carnitine treatment. Gunal et al[42] found that L-carnitine may improve insulin resistance in patients with chronic renal failure, possibly by regulating the cell energy metabolism or reducing free fatty acids and abnormalities in carnitine metabolism. However, the sample size in this study was too small to permit any firm conclusion about L-carnitine effects on insulin resistance. Panzetta et al[43] showed that glucose tolerance after dialysis was improved significantly in patients treated with L-carnitine when compared with untreated patients. Nevertheless, many studies support a link between mitochondrial long-chain fatty acid use and the rate of glucose use (oxidation and/or nonoxidative glucosal disposal, and glycogen and lipid synthesis).[44]

L-carnitine infusion improves insulin sensitivity in insulin-resistant diabetic patients; a significant effect on whole-body insulin-mediated glucose uptake also is observed in normal subjects. In patients with diabetes, glucose, taken up by the tissues, appears to be used promptly as fuel because glucose oxidation is increased during L-carnitine administration. The significantly decreased plasma levels of lactate suggest that this effect might be exerted through the activation of pyruvate dehydrogenase, whose activity is depressed in the insulin-resistant patient.[45,46] L-carnitine also is able to increase the use of glucose by peripheral tissues and sensitivity of the cells to insulin[47,48] by increasing oxidative glucose use by activating pyruvate dehydrogenase and decreasing intramitochondrial acetyl-CoA/CoA ratio.[49]

In obese insulin-resistant diabetic patients, the decreased fatty acid oxidation in skeletal muscle is correlated with low expression of the enzyme carnitine-acylcarnitine translocase, which is implicated in the transport of long-chain fatty acids into mitochondria as carnitine esters for energy-generating processes. The low level of carnitine-acylcarnitine translocase in insulin-resistant muscle may contribute to the increased muscle concentrations of triglycerides, diacylglycerol, and fatty acyl-CoA observed in these patients.[50]

L-Carnitine Treatment

The potential targets for administering L-carnitine in maintenance hemodialysis patients include muscle weakness because of a decreased muscle carnitine content; dyslipidemia, because carnitine increases mitochondrial transport of fatty acids and reduces fatty acid availability for triglyceride synthesis; cardiac symptoms, because the myocyte has one of the highest intracellular carnitine concentrations of the body and myocardial ischemia generates acylcarnitine products and

intracellular lactate production; and anemia, for correcting numerous metabolic abnormalities (ie, oxidative stress and impaired phospholipid turnover).

Since 1978, many trials have been conducted in humans to evaluate the effects of administering L-carnitine. Despite the fact that nearly 2,000 patients were included in more than 80 studies, it is still a matter of debate as to whether L-carnitine treatment can improve a patient's status and symptoms.

Available data indicate that postdialysis administration of L-carnitine is able to replace the carnitine removed from the rapidly equilibrating pool (ie, mainly the bloodstream) and to slow carnitine loss from the slow-equilibrating pool (ie, mainly skeletal muscle).[51]

Clinical benefits of L-carnitine supplementation in chronically uremic patients include improvement of insulin resistance, lipid metabolism, intradialytic symptoms, cardiac function, and muscle weakness.[52] Recent data showed that L-carnitine treatment is able to modify some particular aspects of the inflammatory status characterizing these patients. Indeed, the effects of L-carnitine on the reduction of proinflammatory cytokines may open new perspectives in the treatment of uremia.[31]

Diversity in experimental design may account for discrepancies among published studies. Two recent meta-analyses indicated that L-carnitine supplementation may improve: (1) hematologic status by increasing hematocrit level or allowing a reduction of erythropoietin dosage, (2) exercise tolerance by increasing the aerobic capacity and decreasing cramp occurrence, (3) plasma lipid profiles by decreasing cholesterol and possibly triglyceride levels, and (4) the patient's overall sense of well-being.[53]

These results, combined with the fact that L-carnitine exhibits a very positive risk/benefit ratio, contributed to support the recent approval by the US Food and Drug Administration of L-carnitine supplementation, not only for the treatment of, but also for the prevention of, carnitine deficiency in patients with end-stage renal disease who are undergoing hemodialysis.

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