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L-Carnitine, Immunomodulation, and Human Immunodeficiency Virus (HIV)-related Disorders

Salvatore Alesci[1,*], **Mariana Gerschenson**[2], and **Ioannis Ilias**[3]

[1] Clinical Neuroendocrinology Branch, National Institute of Mental Health, National Institutes of Health, 20892-1284 Bethesda, Maryland, USA
[2] Hawaii AIDS Clinical Research Program, Department of Medicine, John A. Burns School of Medicine, University of Hawaii, 96816 Honolulu, Hawaii, USA
[3] Department of Pharmacology, Medical School, University of Patras, Rion-Patras, GR-26504, Greece

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Summary. The use of pharmacologic doses of the conditionally-essential nutrient *L*-carnitine (*LC*) has been associated with positive effects on the immune system. We have recently suggested that this property of *LC* could be mediated through activation of the glucocorticoid receptor alpha. Human immunodeficiency virus (HIV)-infected individuals, especially those on antiretroviral therapy, may become *LC*-deficient. This evidence, together with the immunomodulatory properties of *LC*, its known major role in lipid and energy metabolisms, and its proposed antiapoptotic and neuroprotective actions, have encouraged the use of *LC* supplementation as a potential treatment for HIV-related disorders, such as lipodystrophy and peripheral neuropathy. Preliminary results, mostly from small-scale uncontrolled studies are conflicting, whilst larger controlled trials are warranted.

Keywords. Carnitine; Immunity; Glucocorticoid receptor; HIV.

Introduction

The essential amino acids lysine and methionine combine to synthesize the quaternary amine *L*-carnitine (4-(trimethylammonium)-3-hydroxybutyric acid; Fig. 1, top) [1]. *L*-Carnitine (*LC*) circulates in the body in both nonesterified (free) form and as esters of organic and fatty acids [1, 2], with acetyl-*LC* (gamma-trimethyl-beta-acetylbutyrobetaine, Fig. 1, bottom) being the predominant ester.

The major source of *LC* in humans is the consumption of meat and dairy products, whereas the minor source is the biosynthesis of endogenous *LC* in the brain, liver, and kidney. *LC* is absorbed by simple diffusion in the jejunum and

* Corresponding author. E-mail: alescisa@mail.nih.gov

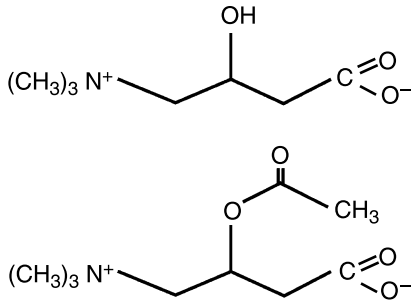

Fig. 1. Chemical structure of *L*-carnitine (4-(trimethylammonium)-3-hydroxybutyric acid, *top*) and acetyl-*L*-carnitine (gamma-trimethyl-beta-acetylbutyrobetaine, *bottom*)

circulates at levels of ~0.05 m*M*, while tissue concentrations are much higher (~1–100 m*M*) [3, 4]. *LC* is minimally metabolized, and around 5% is excreted by the kidneys [2]. Short-, medium-, and long-chain free fatty acids are transported

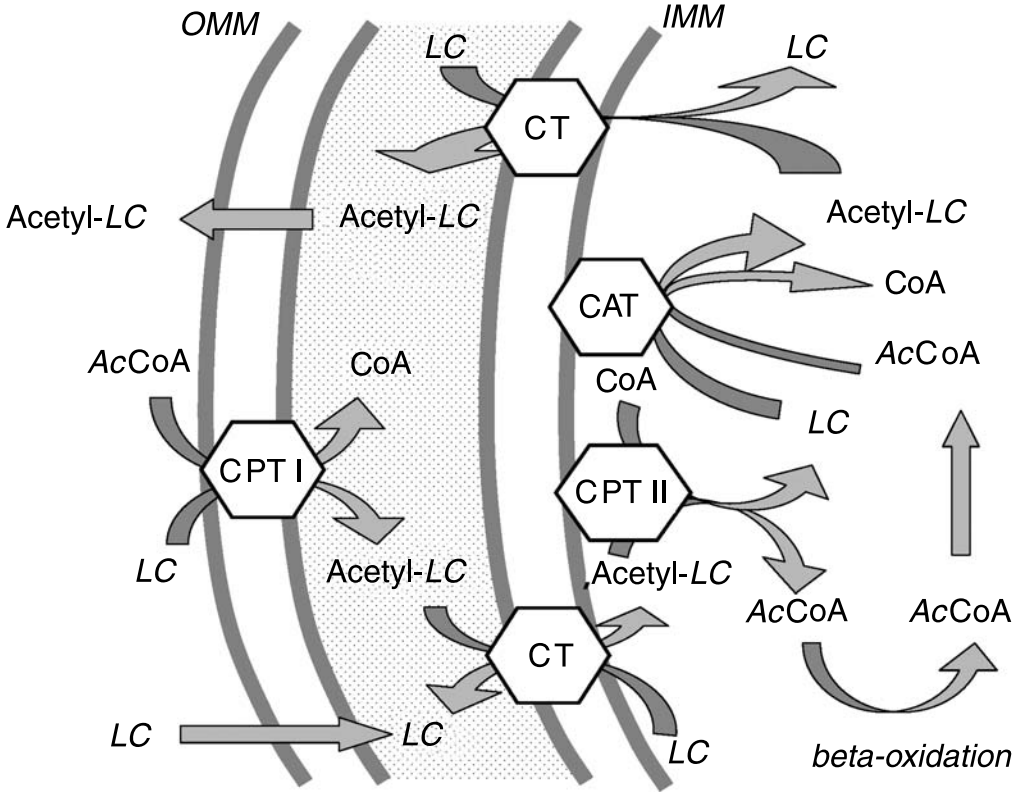

Fig. 2. *L*-Carnitine (*LC*) plays a major role in the entry of activated long-chain fatty acids from the cytosol into mitochondria and is also involved in the transport of activated medium- and short-chain organic acids from peroxisomes to mitochondria; the ratio of acetyl-coenzyme A to coenzyme A is maintained by *LC*, which functions as a pool of activated acetyl units; the toxic effects of poorly metabolized acetyl groups are lowered through transesterification from CoA and excretion of acetyl-*LC* esters by carnitine palmitoyltransferases (CPT I & CPT II) and carnitine acetyltransferase (CAT); carnitine-acylcarnitine translocase (CT) permits the entry and exit of short-chain acetyl-*LC* esters in and out of mitochondria; *OMM: outer mitochondrial membrane; IMM inner mitochondrial membrane*

through cell compartments as acyl-*LC* esters (Fig. 2). Transfer of the latter from peroxisomes to mitochondria is essential for cell energy production and membrane stabilization [5, 6].

At pharmacologic doses (up to 600 mg/kg body weight/day), *LC* is used in the treatment of primary and secondary *LC* deficiencies [7–11]. Additionally, *LC* is also available as a nutritional supplement. Potentially beneficial effects of *LC* and/or acetyl-*LC* supplementation include neuroprotection and positive effects on oxidative stress and cognition [12].

Human immunodeficiency virus (HIV)-infected individuals on antiretroviral therapy may become deficient in *LC*. This deficiency limits mitochondrial fat metabolism, facilitates apoptosis, and contributes to lipodystrophy and peripheral neuropathy.

Carnitine and Immunomodulation: A Role for the Glucocorticoid Receptor

Immunomodulatory Properties of LC

Animal and human studies suggest that *LC* may mimic some properties of glucocorticoids (GCs), including their immunomodulatory effect. *LC* given to rodents at doses of 50–100 mg/kg body weight markedly suppressed lipopolysaccharide (LPS)-induced cytokine production, and improved their survival during cachexia and septic shock [13, 14]. Moreover, *LC* reduced the *ex vivo* release of tumor necrosis factor-alpha (TNFα) by *S. Aureus*-stimulated human polymorphonuclear white blood cells [15]. This corroborates the finding of profoundly decreased TNFα serum levels following *LC* supplementation in patients that had undergone surgery (8 g *i.v.* at the end of surgery and 24 h afterwards), and in patients with HIV (6 g/day for 2 weeks) [16, 17].

Modulatory Effects of LC on Glucocorticoid Receptor Functions

Glucocorticoids interact with ubiquitous intracellular receptors: when they bind to the glucocorticoid receptor-alpha (GRα) the complex hormone-receptor is activated and translocates to the cell nucleus, regulating the transcription of several responsive genes by binding to specific DNA-associated GC-responsive elements (GREs) in the promoters of these genes [18, 19].

We recently demonstrated that millimolar concentrations of *LC* (not cytotoxic *in vitro*), reduced significantly the whole-cell binding of [^{3}H]-dexamethasone (*Dex*) to GRα in HeLa cells. A significant increase in the K_d values of GRα for *Dex* was noted, although B_{max} values remained unchanged, pointing to a decrease in the affinity of this receptor for its steroid ligand [20]. At the same concentrations, *LC* was able to trigger nuclear translocation of green fluorescent protein (GFP)-fused human GRα and to transactivate the GC-responsive mouse mammary tumor virus (MMTV) and TAT3 promoters in a dose-dependent fashion. All these effects were dependent on the presence of GREs on the promoter, and on the expression of functional GRα by the cell [20].

The secretion of TNFα and interleukin-12 (IL-12, an ''immunomodulatory'' cytokine) is reduced by GCs, both *in vitro* and *ex vivo* [21–23]. In an analogous

fashion, *LC* (at concentrations that had maximally stimulated the transcription of GC-responsive promoters) also suppressed the *ex vivo* release of TNFα and IL-12 by IFN-γ primed and/or LPS stimulated human primary monocytes, mimicking *Dex* [20]. RU 486 (a GRα-antagonist) annulled the transactivation of GRα by *LC* and the GRα-mediated cytokine suppression [20]. Our data suggest that *LC* may act as a partial agonist/antagonist of the GRα, which may explain its immunomodulatory properties [24, 25].

Carnitine and HIV-related Disorders

Carnitine Deficiency and Replacement in HIV Patients

HIV-positive patients are at increased risk for *LC* deficiency due to malabsorption, renal disease, antibiotic and antiviral treatment, and loss of adipose tissue, which increases fatty acid availability [26]. Serum carnitine was low in 29 of 79 HIV-infected children receiving antiretroviral treatment, a finding that was attributed to malabsorption or defective synthesis [27]. Exposure of peripheral blood lymphocytes from subjects with symptomatic HIV-1 primary infection to *LC* reduces their tendency to undergo spontaneous apoptosis, probably by protecting mitochondria [28].

Adjunctive therapy with *LC* and/or acetyl-*LC* has been studied, albeit in a limited fashion, in patients with HIV infection. In lymphocytes from HIV-positive patients receiving *LC*, Fas and caspase-1 are down-regulated, whereas p35 is over-expressed [29]. In 11 HIV-infected patients that were given *LC* intravenously (6.0 g/day) for 4 months, an absolute increase in CD4 lymphocytes, and a lower frequency of apoptotic CD4 and CD8 lymphocytes were noted at the end of the study [30]. In a follow-up study, acetyl-*LC* (3 g/day for 5 months) decreased lymphocytic apoptosis. These effects of acetyl-*LC* were attributed to reduced ceramide generation (ceramide, a membrane-bound phospholipid, has been shown to induce Fas ligand-mediated apoptosis, which increases in experimental models of HIV infection) and increased serum levels of insulin-like growth factor-1 [31].

Approximately 30% of patients with HIV infection suffer from distal symmetric polyneuropathy (DSP) [32]. The exact etiology of DSP is elusive, although *LC* and/or acetyl-*LC* deficiency has been implicated in its pathogenesis [33, 34]. Despite a lack of relevant controlled clinical trials, supplementation with *LC* is frequently recommended to patients with HIV infection, especially to those with wasting or diarrhea syndromes. Ten out of 16 HIV-infected patients with DSP that received acetyl-*LC* (*i.m.* or *i.v.* at doses ranging from 0.5 to 1.0 g/day) for 3 weeks reported an improvement in symptoms, whereas 5 patients did not report any change, and in one patient the symptoms worsened [35].

Carnitine Supplementation for Treating Complications of Highly Active Antiretroviral Therapy

Large studies in HIV-infected subjects receiving highly active antiretroviral therapy (HAART) suggest that 13–62% of these patients develop lipodystrophy (LD) [36–43]. This complex syndrome is characterized by fat accumulation in

the dorsocervical region, by fat wasting of the face, subcutaneous region, abdomen, buttocks, and extremities, and by insulin resistance and hypertriglyceridemia. The etiology of LD is multifactorial. HIV drug therapy definitely contributes to the syndrome. Nucleoside reverse transcriptase inhibitors (NRTIs) and in particular stavudine (*d4T*) have been implicated in the peripheral fat wasting, whereas protease inhibitors (PIs) have been linked to insulin resistance and hypertrygliceridemia [33, 36, 37, 44–47]. Altered mitochondrial biogenesis and increased proinflammatory cytokines seem to play a major role in the pathogenesis of LD. Mitochondrial DNA depletion, mRNAs changes in uncoupling proteins and fatty acid metabolism enzymes, and increased apoptosis in subcutaneous adipocytes are common findings in patients with LD [48–51]. Furthermore, increased levels of the transcripts for fatty acid transport and binding proteins, interleukin-6, and CD45 (a common leukocyte marker) have been documented in patients with LD [49].

In the only study evaluating the effects of *LC* treatment for LD, 12 patients were treated twice daily with 1000 mg/day. After twelve weeks no changes in body fat amount or distribution were noted, although serum cholesterol levels did significantly decrease, while triglycerides were unchanged [52]. In a prospective open-label trial of 16 patients with HIV under HAART, *LC* at a dose of 3 g/day for nine months significantly decreased serum triglyceride levels (the result of HAART-related hypertriglyceridemia) after one and two months as well as at the end of the study [53].

Muscle tissue samples from HIV-positive patients receiving zidovudine (*AZT*) show decreased levels of *LC* [54]; moreover low serum levels of acetyl-*LC* have been reported in patients treated with didanosine (*ddI*), zalcitabine (*ddC*), or *d4T* [55, 56]. These findings have encouraged the use of acetyl-*LC* supplementation in patients with HIV, despite the lack of significant relationships between serum *LC* levels and HAART-related myopathy or neuropathy [32, 57]. In a randomized trial of 20 male patients with AIDS treated with *AZT*, administration of *LC* (6 g/day for 2 weeks) led to a significant decrease in triglycerides and TNFα [16]. The latter effect might be mediated by activation of the GRα by *LC* [20, 24, 25]. A prospective study of 21 HIV-positive patients with antiretroviral toxic neuropathy evaluated the effect of 1500 mg of acetyl-*LC* (given orally, twice a day for up to 33 months). The therapeutic effect was assessed with skin biopsy before acetyl-*LC* treatment and at 6 month intervals thereafter. Innervation improved after 6 months and continued improving after 24 months of treatment [58].

Clinical studies on acetyl-*LC* administration as a treatment for HIV-related LD or peripheral neuropathy are ongoing. The University of Hawaii is currently conducting a prospective study on 20 patients with HIV that are being given acetyl-*LC* supplementation for 6 months. Preliminary results from this study have shown improved serum levels of lactate (a marker of anaerobic metabolism) and alanine aminotransferase in HIV patients after 23 weeks of acetyl-*LC* supplementation, which may indicate improved mitochondrial function. On the other end, no statistically significant changes in body composition or metabolic parameters were observed in acetyl-*LC*-treated HIV patients compared to controls [59]. The Adult AIDS Clinical Trials Group has recently begun a multi-center open-label, 24-weeks pilot study with dose escalation of acetyl-*LC* for the treatment of distal symmetric

peripheral neuropathy (DSPN) associated with dideoxynucleosides in 36 HIV-infected subjects.

Conclusion

LC given at high doses can directly influence the various activities of the GRα *in vitro*, providing a molecular mechanism for previously reported immunomodulatory properties of *LC* supplementation. This and other potentially beneficial effects of *LC* and acetyl-*LC*, such as protection from mitochondrial damage, suggest that supplementation of these nutrients may be useful in patients with complications of HIV infection and/or HAART treatment. At present, the evidence supporting this therapeutic approach in HIV positive patients is mostly based on the results of small-scale uncontrolled trials, with conflicting outcomes. Encouraging findings need to be confirmed in larger randomized controlled studies, before the efficacy of this treatment can be universally accepted.

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