Micronutrient Supplementation Increases CD4 Count in HIV-Infected Individuals on Highly Active Antiretroviral Therapy: A Prospective, Double-Blinded, Placebo-Controlled Trial

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Objective: To examine the immunologic, metabolic, and clinical effects of broad spectrum micronutrient supplementation in HIV-infected patients taking highly active antiretroviral therapy (HAART).

Design: A prospective, randomized, double-blinded, placebo-controlled trial.

Methods: Forty HIV-infected patients taking a stavudine and/or didanosine-based HAART regimen were prospectively randomized to receive micronutrients or placebo twice daily for 12 weeks. Data were collected at 4-week intervals including immunologic, metabolic, and clinical measurements. The study examined the effect of micronutrient supplementation on immunologic parameters as the primary end point. The secondary end points were metabolic and clinical effects and distal symmetrical polyneuropathy.

Results: The mean absolute CD4 count increased by an average of 65 cells in the micronutrient group versus a 6-cell decline in the placebo group at 12 weeks ($P = 0.029$). The absolute CD4 count increased by an average of 24% in the micronutrient group versus a 0% change in the placebo group ($P = 0.01$). The mean HIV-1 RNA decreased in the micronutrient supplementation group, although not significantly. Neuropathy scores improved in the micronutrient group by 42% compared with a 33% improvement in the placebo arm. This difference did not reach statistical significance. Fasting serum glucose, insulin, and lipids were not adversely affected in the patients taking the micronutrients.

Conclusions: Micronutrient supplementation can significantly improve CD4 cell count reconstitution in HIV-infected patients taking HAART. The micronutrient supplement tested was well tolerated and may hold promise as an adjuvant therapy in the treatment of HIV. Further investigation is warranted.

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In the era of highly active antiretroviral therapy (HAART), viral suppression is not always accompanied by complete immune reconstitution.[1] After viral control is achieved, immune recovery is frequently slow and incomplete with an initial increase in memory CD4 cells followed by an increase in naive CD4 cells.[2] The relationship between viral suppression and immune recovery is dynamic and complex and involves multiple factors,[3] of which nutritional status is believed to play a pivotal role.

The relationship between immune function and nutritional supplementation is a well-described phenomenon.[4–7] Numerous studies have reported a high prevalence of nutrient deficiencies early in the course of HIV infection.[8–10] These deficiencies have been shown to be associated with more frequent opportunistic infections, faster disease progression, and a greater incidence of HIV-related mortality.[11–15] Possible mechanisms include increased intracellular oxidative stress, enhanced viral replication, and a reduction in the number of circulating CD4 lymphocytes associated with individual or accumulated nutrient deficiencies.[14–18] These mechanisms, alone or in part, may contribute to the increased morbidity, more rapid disease progression, and the higher mortality seen in HIV-infected patients with nutrient deficiencies.[18–21]

Several prospective, randomized clinical trials now suggest that HIV-infected patients who take micronutrient supplements have improved clinical outcomes. Fawzi et al[22] showed that daily doses of a micronutrient supplement taken by HIV-infected Tanzanian women, in multiples of the recommended dietary allowances (including B-complex, vitamin C, and vitamin E), produced a significant increase in CD4 and CD8 cell counts when compared with those given placebo. In this prospective, double-blinded trial, patients taking the micronutrient supplement were also significantly less likely to die or progress to WHO stage 4 HIV disease classification.[23] Jiampton and colleagues have also demonstrated that by administering a micronutrient supplement to

HIV-infected men and women living in Thailand, overall HIV-associated mortality decreased by 50% compared with the placebo arm. This effect was most pronounced in patients with lower CD4 cell counts (<200 cells/μL).[24]

The occurrence of mitochondrial dysfunction due to drug toxicity is believed to promote increased levels of oxidative stress in a large number of cell lines. Peripheral neurons and adipocytes seem to be significantly affected.[25–28] The dideoxynucleosides stavudine and didanosine have been shown to produce significant depletion of mitochondrial DNA in both of these tissues.[29,30]

The cohort we chose to study consisted of HIV-infected patients exhibiting neurologic toxicity from either stavudine and/or didanosine so that we might examine the effects of micronutrient supplementation on HIV disease progression and on one clinical manifestation of mitochondrial toxicity: distal symmetrical polyneuropathy (DSP).

METHODS

Study Design

This was a prospective, randomized, double-blinded, placebo-controlled clinical trial designed to determine the effect of micronutrient supplementation on HIV-1 disease progression and on DSP in HIV-infected patients taking HAART. Four study centers serving HIV-infected patients in the USA were selected. The study design and protocol were approved by an independent institutional review of the Copernicus Group Institutional Review Board (Cary, NC). Written informed consent was obtained from all study participants before enrollment.

Study Subjects

Enrollment took place between January 2002 and May 2003. Forty HIV-infected patients taking a stable HAART regimen for at least 3 months and who developed symptoms of DSP after beginning either stavudine and/or didanosine were randomized at each study site using a random block design to receive either the micronutrient supplement or an identically appearing placebo taken twice daily for 12 weeks. Patients who were pregnant, receiving treatment of an active opportunistic infection or malignancy, had vitamin B_{12} deficiency at screening, or were already taking more than 1 micronutrient supplement pill per day were excluded from the study. All clinical and follow-up staff were unaware of the patient treatment assignments.

The micronutrient supplement tested included 33 ingredients (Table 1) and was consumed twice daily with food. Patients were allowed to take their antiretroviral medication at the same time as the micronutrient supplement. The micronutrient and placebo capsules were produced to our specifications by Thorne Research, Dover, ID.

Clinical and Laboratory Evaluations

Study patients visited their respective research centers for screening, which included the collection of demographic information, medication history, and laboratory tests. Eligible participants returned to the clinic for baseline and follow-up visits every 4 weeks for a total of 12 weeks. Each clinic visit consisted of the following evaluations: a laboratory-monitoring panel, a clinical assessment, and a neurological examination.

Laboratory testing was performed at baseline and every 4 weeks for a total of 12 weeks. It included a CBC, CD4 lymphocyte panel, HIV-1 RNA, fasting glucose, fasting lipids, fasting insulin, liver function tests (aspartate aminotransferase, alanine aminotransferase (ALT), total bilirubin), serum creatinine, and fasting venous lactate. The CD4 lymphocyte count was measured by conventional flow cytometry. Plasma HIV-1 RNA was measured using an ultraquantitative polymerase chain reaction assay with a lower limit of quantification to 20 copies/mL (serial diluted standards calibrated against an AIDS Clinical Trials Group reference). Fasting venous lactate levels were collected in the resting state, without the use of a tourniquet or fist clenching, and the blood sample was immediately immersed into a chilled water bath before centrifugation and freezing. All laboratory samples were shipped on dry ice to a central laboratory for processing (Immunodiagnostic Laboratories, San Leandro, CA).

The clinical assessment incorporated 2 additional self-administered patient questionnaires. The linear analogue self-assessment tool assessed the patient's energy level, ability to perform daily activities, and overall quality of life on a linear scale.

The neurological examination at baseline and follow-up was performed by a clinician using the neurological examination assessment tool. This tool consisted of the objective measurement of sensory function of the lower extremities by sharp pin and tuning fork methods and motor function of the toes and ankles by movement against resistance. Abnormal findings were rated on an increasing numerical scale (0–4) based on severity. An examination

TABLE 1. Micronutrient Supplement Tested

Micronutrient	Total Daily Dosage	Micronutrient	Total Daily Dosage
N-Acetyl cysteine (NAC)	1200 mg	Calcium	800 mg
Acetyl L-carnitine	1000 mg	Magnesium	400 mg
Alpha lipoic Acid	400 mg	Selenium	200 μg
Beta carotene	20,000 IU	Iodine	150 μg
Vitamin A	8000 IU	Zinc	30 mg
Vitamin C	1800 mg	Copper	2.0 mg
Vitamin B_1	60 mg	Boron	2.0 mg
Vitamin B_2	60 mg	Potassium	99 mg
Pantothenic acid	60 mg	Iron	18 mg
Niacinamide	60 mg	Manganese	10 mg
Inositol	60 mg	Biotin	50 μg
Vitamin B_6	260 mg	Chromium	100 μg
Vitamin B_{12}	2.5 mg	Molybdenum	300 μg
Vitamin D	400 IU	Choline	60 mg
Vitamin E	800 IU	Bioflavonoid complex	300 mg
Folic acid	800 μg	L-Glutamine	100 mg
		Betaine HCL	150 mg

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