Suppression of Human Immunodeficiency Virus Type 1 Viral Load With Selenium Supplementation

A Randomized Controlled Trial

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Background: Despite findings that selenium supplementation may improve immune functioning, definitive evidence of its impact on human immunodeficiency virus (HIV) disease severity is lacking.

Methods: High selenium yeast supplementation (200 µg/d) was evaluated in a double-blind, randomized, placebo-controlled trial. Intention-to-treat analyses assessed the effect on HIV-1 viral load and CD4 count after 9 months of treatment. Unless otherwise indicated, values are presented as mean±SD.

Results: Of the 450 HIV-1–seropositive men and women who underwent screening, 262 initiated treatment and 174 completed the 9-month follow-up assessment. Mean adherence to study treatment was good (73.0%±24.7%) with no related adverse events. The intention-to-treat analyses indicated that the mean change in serum selenium concentration increased significantly in the selenium-treated group and not the placebo-treated group (Δ=32.2±24.5 vs 0.5±8.8 µg/L; P<.001), and greater levels predicted decreased HIV-1 viral load (Δ=−0.29±1.1 log 10 units), and decreased CD4 count (Δ=−25.8±147.4 cells/µL). In contrast, selenium-treated subjects whose serum selenium increase was greater than 26.1 µg/L evidenced excellent treatment adherence (86.2%±13.0%), no change in HIV-1 viral load (Δ=−0.04±0.7 log 10 units), and an increase in CD4 count (Δ=+27.9±150.2 cells/µL).

Conclusions: Daily selenium supplementation can suppress the progression of HIV-1 viral burden and provide indirect improvement of CD4 count. The results support the use of selenium as a simple, inexpensive, and safe adjunct therapy in HIV spectrum disease.

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Selenium is an essential trace mineral and, when in vivo levels are deficient, syndromes involving myopathy, immune dysfunction, and cardiomyopathy occur, especially in regions with soil deficiencies, limited resources, and poverty.1-4 Selenium deficiency has been observed in human immunodeficiency virus (HIV) spectrum disease.5 Lower serum concentrations predict mortality for infected adults and children6,7 and have been linked to enhanced viral virulence, diminished natural killer cell cytotoxicity, increased mycobacterial disease risk, and progression of HIV disease.8-11 In contrast, in vitro incubation of HIV-1–infected monocytes with selenium suppresses HIV-1 replication.12 Moreover, clinical trials of selenium supplementation (200 µg/d) have resulted in lower incidence of various cancer types with no adverse effects.13 Patients with HIV now have an extended life expectancy, largely as a result of pharmaceutical advances in antiretroviral therapy (ART); nevertheless, clinicians are still faced with maintaining a delicate balance between virology and ART pharmacology in the context of patient-related factors.14,15 Strict adherence to ART is required to achieve adequate and sustained viral suppression and prevent the emergence of drug-resistant viral strains.16

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Even with adequate regimen adherence, there is a significant risk of ART-induced toxic effects and metabolic dysfunction. Thus, complete control of HIV over time using ART is unlikely, and pharmacotherapeutic limitations leave a significant void in the treatment arsenal. Despite promising findings that selenium may improve immune functioning, definitive evidence of its impact as an adjunct treatment on the severity of HIV disease is lacking. The present study evaluated the effect of selenium supplementation on serum selenium levels and the subsequent impact on HIV-1 viral load and helper T cell (CD4) count.

METHODS

PARTICIPANTS

Participants in the Miami Selenium for Heart and Immune Health Trial consisted of a convenience sample from the Miami-Dade, Broward, and Palm Beach counties of Florida. Participants were recruited from June 5, 2001, through July 14, 2005, via newspaper advertisement, flyer distribution at HIV-AIDS clinics and support groups, and physician and chain referrals. Included subjects (1) provided informed consent; (2) presented HIV-1 infection documentation; (3) were aged 18 to 55 years; (4) were not being treated pharmacologically for cardiovascular (eg, with β-blockers, calcium antagonists, and angiotensin-converting enzyme inhibitors), diabetic (eg, with hypoglycemics and insulin sensitizers), psychiatric (eg, with antipsychotics and antidepressives), or endocrine (eg, with estrogen therapy) conditions; (5) had no history of diabetes or cardiovascular disorder or any other major systemic diagnosis unrelated to HIV; (6) presented no electrocardiographic evidence of myocardial infarction or atrioventricular conduction arrhythmias; (7) had no gross neurocognitive dysfunction; (8) had no surgery within 3 months of study entry; (9) were premenopausal and not pregnant and had no intent to become pregnant (for women); (10) were not in another clinical trial; and (11) discontinued use of any supplement containing more than 50 µg of selenium per pill. Participants with serum selenium levels below 75 µg/L, indicative of a biochemical deficiency, were excluded for scientific and ethical reasons. Informed consent was obtained at the initial screening and at study randomization. The trial was approved by the institutional review board of the University of Miami.

PROCEDURES

The study was a double-blind, randomized, placebo-controlled trial consisting of a pretreatment phase followed by an 18-month treatment protocol. The pretreatment phase included a screening visit, 2 run-in visits, and a baseline protocol. The treatment protocol included monthly contact for pill delivery, compliance and safety checks, blood draw for selenium assay, and follow-up cardiovascular, metabolic, and immune assessment sessions at 9 and 18 months after treatment onset. After a telephone interview, subjects who met study entry criteria were invited for the screening visit, where study eligibility was confirmed. These procedures included receipt of HIV serostatus documentation, physical examination by a physician, urinalysis (screening for alcohol, barbiturates, benzodiazepines, cannabinoids, hallucinogens, morphine, and amphetamines), and pregnancy screening. In addition, fasting samples were collected for standard blood chemistry analysis and a serum selenium assay. A history of substance use and abuse was assessed by the Structured Clinical Interview for DSM-IV Axis I Disorders, version 2.0. Gross neurocognitive dysfunction was ruled out by a Folstein Mini-Mental State Exam score of less than 26. Sociodemographic variables; use of alcohol, cigarettes, and other drugs; CD4 count history; time since HIV diagnosis; and personal medical history were documented and adherence to the ART regimen was assessed. Casual blood pressure was measured and 12-lead electrocardiography and anthropometric measures were performed. Qualifying subjects were then randomized into treatment with selenium or placebo, using a computer-blocked randomization technique. Treatment masking was implemented in a double-blind fashion. Study supplements were dispensed into capsules (Nutrition 21 Inc, Purchase, NY) and placed in a bottle for distribution by the study medical staff. The bottle was equipped with a computerized electronic medication-monitoring cap (eDEM; Aardex, Union City, Calif) that stored use information for subsequent downloading. The eDEM cap had a display that indicated the number of cap openings per day. Subjects were instructed to take only 1 capsule per day, which they could confirm from the cap display. Prior to the treatment phase, all qualifying subjects were provided with placebo supplements for the run-in protocol, which included 2 checkup visits at 2-week intervals. The purpose of the run-in visits was to identify for potential exclusion individuals who were noncompliant or who were no longer interested or study eligible. Ways to overcome obstacles to supplement adherence were discussed. During all sessions, adherence was assessed by pill count and by reviewing eDEM cap information. The pretreatment assessment included urinalysis and pregnancy screening, fasting blood sampling to measure serum selenium concentration, metabolic and hematologic indexes, CD4 count, HIV-1 viral load, and hepatitis virus serostatus. Although other immune, metabolic, and cardiovascular assessments were performed, these measures are not reported herein. Study supplements as per the randomization assignment were administered after completion of the pretreatment assessment. Placebo-treated subjects received 200-µg capsules with inactive yeast and dicalcium phosphate filler (Nutrition 21 Inc). The yeast was included in the placebo to provide a similar odor as the selenium capsules; the selenium and placebo capsules had the same white opaque appearance. Selenium-treated subjects received 200-µg capsules consisting of high selenium yeast (Selenomax; Nutrition 21 Inc), a dose that was independently validated by Vanguard Scientific Inc, Hatboro, Pa. The 18-month treatment phase included monitoring supplement adherence, treatment safety, and serum selenium concentration. Changes in health status, ART regimen, and other prescribed medications were documented. At the 9- and 18-month assessments, a physical examination and evaluation of the study outcomes were performed. The study data and safety monitoring board met biannually.

BLOOD ASSAYS

Serum selenium levels were measured using a standardized fluorometric method with some modifications, quality assurance for each assay used standard reference material 1577b bovine liver (National Institute of Standards and Technology, Gaithersburg, Md). The usable range is 0.02 to 10 ppm or greater (4-ng detection limit); intra-assay and interassay coefficients of variance were 1.3% and 3.8%, respectively. The HIV-1 viral load was determined using an in vitro nucleic acid amplification test (Ampliscreen HIV-1 monitor test, version 1.5; Roche Diagnostics, Branchburg, NJ) with ultrasensitive and standard methods (detection range, 50-750 000 HIV-1 RNA copies/mL). The mean CD3/CD4 helper T-lymphocyte count was determined from duplicate samples using flow cytometry. To obtain hepatitis C virus (HCV) serostatus, testing for HCV an-
Of the 972 telephone-screened subjects, 450 participated in the screening assessment; of these, 310 were randomized to treatment (Figure 1). After the run-in phase, an additional 48 subjects dropped out or were excluded. Therefore, 262 subjects completed pretreatment assessment (placebo group, n=121; selenium group, n=141). Of these subjects, 174 completed the 9-month assessment (placebo group, n=83; selenium group, n=91). There were no significant differences between the treatment groups on any of the demographic or other characteristics (Table). Unless otherwise indicated, values are presented as mean±SD.

The intervention resulted in no adverse events related to the study supplement. The 2 groups did not differ in serum selenium concentration at pretreatment (Table), but the selenium group evidenced significantly greater change in serum selenium concentration at the 9-month assessment (P<.001). The mean selenium change for the placebo group was 0.5±8.8 µg/L and for the selenium group was 32.2±24.5 µg/L. Treatment adherence did not differ between groups (eDEM, 72.9%±24.4% [placebo group] vs 73.1%±25.0% [selenium group]; pill count, 80.1%±20.6% [placebo group] vs 81.8%±20.1% [selenium group]). The eDEM adherence correlated with mean selenium concentration change in the selenium group (r=0.59; P<.001), but not in the placebo group (r=-0.06).

The ITT analyses examined whether the effect of treatment on the serum selenium concentration had an effect on HIV-1 viral load and CD4 count at the 9-month assessment, controlling for the pretreatment viral load and CD4 count (Figure 2). The model had good fit (P=.38). In this model, the path from the treatment group to the mean change in serum selenium concentration was significant (β=0.65). The paths from the serum selenium concentration change to the HIV-1 viral load and CD4 count at the 9-month assessment were significant (β=-0.16 and β=0.10, respectively). The analysis was repeated to examine the effect on CD4 count as being partially mediated by the viral load change. This model also had good fit (P=.45). The direct path between the serum selenium concentration change and CD4 count change became nonsignificant (β=0.06), as indicated in Figure 2. This finding indicates that the treatment effect on the CD4 count was indirect.

The final model (Figure 2) included the covariates of age, sex, ethnicity, income, education, current and past use of cocaine and other recreational drugs, HIV disease severity (HIV-1 viral load and CD4 count), independent of pretreatment levels. The SEM analyses also incorporated covariates (ie, subject demographics and characteristics) to increase precision due to heterogeneity. The SEM method offers numerous advantages relative to more commonly used statistical methods, permitting an investigation of the specific mediational model. Acceptable SEM goodness of fit was indicated by nonsignificant χ² test finding and other fit indexes. Individual variable estimates (path coefficients) were tested for significance using z=1.96 and α=.05 (2-tailed). To illustrate the treatment impact accounting for serum selenium levels, a follow-up subgroup analysis using mixed modeling (SPSS, version 12.0.1; SPSS Inc, Chicago, Ill) assessed change in viral load and CD4 count comparing the placebo group with 2 groups of selenium-treated subjects (responders vs nonresponders).

STATISTICAL ANALYSIS

Based on the intention-to-treat (ITT) approach, all randomized participants were included in the primary analysis in their assigned treatment groups, regardless of adherence to the assigned treatment. All variables were screened for missing data and distributional assumptions and transformed where necessary. Data were missing for no more than 1% of subjects on any single variable, except for income (with 5% missing). The ITT analyses of the treatment effect on primary outcomes at the 9-month assessment using structural equation modeling (SEM) analyses were conducted with Mplus, version 3.1 (Muthen & Muthen, Los Angeles, Calif), where variables were estimated in the presence of missing data using full information maximum likelihood. The SEM analyses tested the hypothesized treatment effect (0 for placebo, 1 for selenium) on mean serum selenium concentration change and the corresponding effect on HIV disease severity (HIV-1 viral load and CD4 count), independent of pretreatment levels. The SEM analyses also incorporated covariates (ie, subject demographics and characteristics) to increase precision due to heterogeneity. The SEM method offers numerous advantages relative to more commonly used statistical methods, permitting an investigation of the specific mediational model. Acceptable SEM goodness of fit was indicated by nonsignificant χ² test finding and other fit indexes. Individual variable estimates (path coefficients) were tested for significance using z=1.96 and α=.05 (2-tailed). To illustrate the treatment impact accounting for serum selenium levels, a follow-up subgroup analysis using mixed modeling (SPSS, version 12.0.1; SPSS Inc, Chicago, Ill) assessed change in viral load and CD4 count comparing the placebo group with 2 groups of selenium-treated subjects (responders vs nonresponders).
Therefore, it may be concluded that the selenium treatment effect occurred over 9-month viral load and 78.1% of the variance in the 9-month CD4 count.

Specifically, the path from serum selenium change restricted to include only those receiving ART (independent of all covariates, treatment effect remained significant when the intention-to-treat analysis was placebo-treated than for selenium-treated subjects (ie, mean ± SD change in for those persons receiving ART was more than 2-fold greater for indication that the increase in viral load during the 9 months of study treatment indicated that the selenium responders had significantly greater increases in serum selenium concentration than did the nonresponders and the placebo subjects (results, placebo group, 72.9%±24.5%; responders, 86.2%±13.0%; nonresponders, 56.8%±29.8%; significance. Overall the model was of good fit (P=.81) and explained 40.9% of the variance in the 9-month viral load and 78.1% of the variance in the 9-month CD4 count. Therefore, it may be concluded that the selenium treatment effect occurred over and above any effect due to ART or the lack thereof.

Figure 2. The final structural equation model predicts human immunodeficiency virus type 1 (HIV-1) viral load and CD4 count at the 9-month analysis. The model included the covariates age, sex, ethnicity, income, education level, current and past cocaine use, current and past use of other illicit drugs, HIV disease stage (asymptomatic, symptomatic, or AIDS), hepatitis C virus coinfection, antiretroviral therapy (ART) regimen (with protease inhibitors, without protease inhibitors, or no ART), ART adherence, and time since HIV diagnosis. The pathways (straight arrows) between indicators reflect direct effects, and the pathway coefficients indicate the relationship strength. In sum, a greater increase in selenium concentration predicted more diminished viral load (β =−0.14); the indirect path from treatment assignment to viral load change was significant (β=−2.7; P<.03; β=0.09). In addition, a greater decrease in viral load predicted a greater increase in CD4 count (β =−0.29; selenium levels and viral load mediated the effect of treatment on CD4 count (β=2.3; P<.03; β=0.06). The total variance (R²) in a factor accounted for by predictors is provided for each indicator and includes the direct and indirect effects through other model components. The model had good fit. NS indicates nonsignificant.

Follow-up analyses explored the treatment effect, wherein the selenium-treated responders (n=50) were defined as those whose mean serum selenium concentration change was more than 3 SDs above the mean serum selenium concentration change of the placebo group, a value (ie, 26.1 µg/L) exceeding the serum selenium concentration variation observed in all of the placebo-treated subjects. Thus, selenium-treated nonresponders were defined as displaying a mean serum selenium concentration change of no more than 26.1 µg/L (n=41). The selenium-responder group displayed greater adherence than did the nonresponders and the placebo subjects (responders, 86.2%±13.0%; nonresponders, 56.8%±29.8%; placebo group, 72.9%±24.5%; P<.001). Figure 3 illustrates the serum selenium concentration change across the 9 treatment months (A) and the change in HIV-1 viral load and CD4 count (B) at the 9-month assessment relative to pretreatment levels. In sum, these analyses indicated that the selenium responders had significantly greater increases in serum selenium concentration (P<.001), less viral load increase (P<.02), and greater CD4 count increase (P<.02) than did the placebo and nonresponder groups, who did not differ. The group differences in viral load change remained significant if the responder group criterion was lowered to 15 µg/L but not...
This study is, to our knowledge, the first double-blind, randomized, placebo-controlled trial in a community-based cohort of HIV-infected men and women to demonstrate that daily supplementation with 200 µg of selenium for 9 months elevates the serum selenium level and suppresses the progression in HIV-1 viral load. The selenium supplement resulted in no adverse events, suggesting that it may be administered safely at the dosage used—a finding consonant with that of previous oncological clinical trials. The treatment effects were independent of subject-related factors, including age, sex, ethnicity, income, education, and past and current drug use. In addition, the findings remained significant after correcting for the effects of disease-related factors, including ART regimen and adherence, HIV disease stage and duration, and HCV coinfection. The study showed that the induced change in serum selenium concentration significantly predicted change in the HIV-1 viral load. Moreover, there is strong evidence that the primary selenium effect was on the viral burden. In contrast, the observed benefit of treatment on the CD4 cell count was indirect via the treatment effect on the viral load. Although no previous studies have examined the relationship of serum selenium level with HIV-1 viral load, previous HIV studies have shown a relationship between a lower serum selenium level and a lower CD4 count, more opportunistic infections, faster disease progression, and greater HIV-related mortality. The only other randomized controlled trial of selenium supplementation in HIV-infected individuals found a significant decrease in hospital admission rates and CD4 counts declining below 50 cells/µL for those treated with selenium. Thus, selenium supplementation appears to have beneficial effects on HIV disease severity and progression.

The mean pretreatment selenium level of the present cohort was 111.9 ± 12.9 µg/L (range, 78.5-158.7 µg/L), which reflects a slightly depressed value but still a nutritionally adequate level relative to healthy US residents. Of the cohort, about 97% had serum selenium values greater than 90 µg/L, a level considered minimally adequate for optimal selenoenzyme activity and selenoprotein synthesis. In the present trial, selenium-enriched yeast was selected as a vehicle because it contains high concentrations of organic, bioavailable forms of selenium. The selenium-treated responders had good treatment adherence (86.2%) and serum selenium concentration elevated on average 44.5% compared with pretreatment levels, whereas the serum selenium concentration of the selenium-treated nonresponders failed to substantially increase, likely a consequence of deficient treatment adherence (56.8%). However, 6 subjects (15%) in the nonresponder group had adherence to the supplement regimen of greater than 80% but showed little or no serum selenium concentration change. Supplement misadministration in these cases was ruled out by the inspection of returned capsule contents. The literature indicates that, throughout HIV disease progression, micronutrient and trace element deficiencies are prevalent and may result from malabsorption, altered metabolism, gastrointestinal tract infection, and altered gut barrier function. Of the 6 nonresponders who failed to display a change in serum selenium concentration, 2 had chronic diarrhea, 1 had ulcerative colitis, and 1 was diagnosed as having a benign colon tumor just before the 9-month examination. For the remaining 2 subjects, there was no clinical evidence of gastrointestinal tract complications. Recent evidence in an HIV-infected cohort has shown that malabsorption may not necessarily be associated with wasting or with current or chronic diarrhea. Moreover, it is possible that polymorphisms in the selenoprotein genes due to selenium deficiency or another etiology may deleteriously influence selenium intracellular trafficking and incorporation.

The exact mechanism by which selenium exerts its effect on HIV-1 viral replication is not known, although the literature suggests several possibilities. One prominent hy-
These findings support the notion that selenium may served to diminish oxidative stress and HIV-1 viral burden; antioxidant multivitamin supplementation has been observed to diminish oxidative stress and HIV-1 viral burden.45 These findings support the notion that selenium may act on the HIV virus indirectly.

The cellular actions of selenium are also linked to the redox regulation of genes. Others have provided evidence that the HIV-1 virion encodes homologues of selenoproteins that influence immune-related genes that regulate cytokine production, cellular proliferation, and apoptosis.47,48 Therefore, the selenoprotein is posited to act directly on the HIV-1 virion to suppress its replication. However, supporting evidence for this hypothesis remains to be obtained. Therefore, benefits derived from selenium supplementation may be due to its indirect and direct effects, but may also be related to another, as yet unidentified, chemopreventive activity.

A major study limitation is that the analysis involved only 2 time points during 9 months of treatment, and therefore it is not known whether the effect on viral load may be sustained with continued treatment. On completion, the trial will provide an assessment after 18 months of treatment. The study cohort was heterogeneous with regard to subject variables such as race or ethnicity, HIV disease stage, ART regimen, history of drug use, and HCV coinfection, hence increasing the generalizability of the findings. Although these subject variables and others were included as covariates, they were not analyzed as specific subgroups, which might be of clinical interest. Any model derivation must balance system complexity with power constraints, and the present study model adhered to accepted statistical modeling standards. The principal study strength is that the analysis was based on participants obtained in a thorough and consistent manner, randomized to treatment and blinded to treatment group. Moreover, this study has extended previous research by evaluating the concomitant effect of selenium treatment on HIV-1 viral load and CD4 count.

The study findings indicate that 9 months of selenium supplementation appears efficacious in elevating serum selenium concentration, suppressing the progression of HIV-1 viral burden, and providing indirect improvement of CD4 count in adult HIV-infected men and women. Future research is necessary to confirm the directional relationships observed. For example, it is of interest to determine whether the indirect effect of selenium on CD4 count is restricted to the mediational role of HIV-1 viral load. An investigation of the mechanisms driving the effects of selenium on HIV-1 replication and other potential aspects of immunocellular expression and function is also indicated. Given the challenges of using conventional pharmacotherapy to achieve and maintain virologic suppression in HIV-spectrum disease, our results support the use of selenium as a simple, inexpensive, and safe adjunct therapy.

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Author Contributions: Dr Hurwitz had full access to all of the data and had final responsibility for the decision to submit for publication. Dr Hurwitz coordinated the trial and designed the data collection instruments; Mr Lawrence provided database management; Dr Maher performed and supervised immune assays; and Dr Skyler was the study physician. Study concept and design: Hurwitz, Llabre, Baum, Shor-Posner, and Schneiderman. Acquisition of data: Hurwitz, Klaus, Gonzalez, Lawrence, and Greeson. Analysis and interpretation of data: Hurwitz, Klaus, Llabre, and Lawrence. Drafting of the manuscript: Hurwitz, Klaus, Llabre, and Lawrence. Critical revision of the manuscript for important intellectual content: Hurwitz, Klaus, Llabre, Maher, Greeson, Baum, Shor-Posner, Skyler, and Schneiderman. Statistical analysis: Hurwitz, Klaus, Llabre, and Lawrence. Obtained funding: Hurwitz, Baum, Shor-Posner, Skyler, and Schneiderman. Study supervision: Hurwitz and Gonzalez.

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