

Zinc and human immunodeficiency virus infection

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Abstract

The importance of zinc in normal immune function - and of its deficiency in immune dysfunction - has led to increased interest in its role in nutrition and immunity in human immunodeficiency virus type 1 (HIV) infection. Zinc deficiency produces reversible immune dysfunction, particularly of T-lymphocyte cell-mediated immunity. In developing countries where zinc deficiency is prevalent, zinc supplementation has been shown to reduce morbidity from respiratory and diarrheal illnesses. The relationship between zinc and HIV infection has not been well delineated. Malabsorption, repeated concurrent infections and increased losses probably increase zinc requirements in HIV infection. In some studies, low plasma zinc levels occur frequently in HIV infection and have predicted disease progression and mortality, suggesting supplementation might be beneficial. However, the finding that zinc binding to specific HIV proteins is essential for HIV function and replication and the association in one observational study of zinc intake with higher rates of HIV progression have raised questions regarding the safety of zinc supplementation among HIV-infected individuals. To date, no randomized, placebo-controlled study of moderate zinc supplementation in HIV infection has been published. We believe that available evidence supports the need for and acceptability of such a trial. © 2002 Elsevier Science Inc. All rights reserved.

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1. Introduction

The role of zinc in immune function and the benefits of zinc supplementation in reducing morbidity associated with diarrheal and respiratory illnesses have been well described. Far

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less is known about the interaction between zinc and HIV infection. In developing countries with high prevalences of zinc deficiency and of HIV infection, the potentially different effects of zinc supplementation in HIV-infected people compared to known benefits in those without HIV infection must be considered before undertaking population-based zinc supplementation programs for reduction of diarrheal and respiratory illness. This review will consider available *in vitro* evidence of zinc and HIV interaction, data on zinc status in HIV-infected people and the effects of zinc supplementation on HIV infection. The final section will summarize what is known about the interaction of HIV infection and zinc status and what questions remain to be answered.

1.1. *In vitro* evidence for interaction of HIV infection and zinc

1.1.1. Direct effects of zinc on immune function

The role of zinc in immune function has been well described by Prasad [1]. Zinc deficiency, like HIV infection, most prominently impairs T-lymphocyte cell mediated immunity (CMI). Zinc deficiency causes an imbalance between Th1 and Th2 functions with proportionally greater impairment of CMI and prevents regeneration of new CD4+ T-lymphocytes. HIV infection, similarly, results in dysregulated cytokine balance with shift from Th1 systemic cellular host defense cytokines (IL-2, IFN-gamma) to Th2 humoral response cytokines (IL-4, IL-5, IL-6, IL-10), even before loss of CD4+ lymphocytes [2]. Thus, in examining the interaction of zinc status and HIV-related immunologic impairment, as well as the potential consequences of zinc supplementation in HIV-infected people, it may be difficult to determine whether immunologic improvement is due to direct benefits of improved zinc status, an effect on HIV immunosuppression by higher zinc intakes, or an additive or even synergistic combination of these two effects.

1.1.2. Direct effects of zinc on HIV

The HIV nucleocapsid protein contains two highly conserved zinc fingers, crucial for proper core assembly and viral replication [3–5]. Zinc binding of the HIV integrase zinc finger is necessary to maintain form and optimal activity, allowing viral DNA to integrate into the host genome. While zinc is essential for HIV replication, there is no published evidence that increasing zinc concentration *in vitro* stimulates HIV replication. In fact, *in vitro* experiments show that zinc concentration of 100 $\mu\text{g/mL}$ (100-fold usual plasma concentrations) inhibits HIV RNA transcription without being toxic to the host cell [6]. Furthermore, zinc directly inhibits HIV protease by binding at the active site, a catalytic aspartate [7].

There is considerable interest in compounds with inhibitory activity against HIV nucleocapsid zinc fingers. These compounds have been associated with inactivation of cell-free virus and inhibition of infectious virus production from chronically infected cells [8]. Such compounds also appear to act synergistically with other anti-retroviral agents [9]. Several of these compounds (disulfide benzamides, dithiaheterocyclic compounds, alpha-carbonyl azoic compounds) are active against HIV zinc fingers, but not against host cellular zinc fingers [8], potentially permitting selective inhibition with limited host cell toxicity.

1.1.3. Influence of zinc on HIV-infected host immune cells

Harrer *et al* [10] demonstrated that zinc is a mitogen for peripheral blood mononuclear cells (PBMC), exhibiting optimal activity at 0.05mmol/L (327 μ g/dL), a concentration several fold higher than normal serum levels (but lower than concentrations of zinc that are toxic to cell cultures). Zinc-induced PBMC proliferation was somewhat lower among 30 HIV-infected patients compared to 16 healthy donors. HIV-infected individuals with low zinc-induced lymphoproliferative responses (LPR) were significantly more likely to have lower CD4+ lymphocyte counts and lower serum zinc levels.

In a more recent study by Neves *et al*, [11] zinc alone at 0.1mmol/L (654 μ g/dL) induced *in vitro* LPRs in PBMCs from HIV-negative controls and asymptomatic HIV-infected patients, but no such mitogenic effect was seen in symptomatic AIDS patients or AIDS patients with CD4+ lymphocyte count $<200 \times 10^6/L$. In addition to its direct mitogenic effect, zinc amplified LPRs to phytohemagglutinin (PHA) in healthy controls, and this LPR-amplifying effect was preserved in symptomatic and asymptomatic HIV-infected individuals. Finally, while PHA enhanced apoptosis of PBMC from HIV-infected individuals with CD4+ lymphocyte counts of $200\text{--}500 \times 10^6/L$, addition of zinc at 0.1mmol/L (654 μ g/dL) inhibited such enhancement. The addition of PHA did not affect apoptosis when CD4+ lymphocyte counts were $<200 \times 10^6/L$; the authors speculated that this was due to a high proportion of cells being already apoptotic at very low CD4+ lymphocyte counts.

Deficits of antioxidants and free radical excess are associated with depressed T-lymphocyte cell mediated immunity (CMI), and Sappey *et al* speculate that such deficiency may increase HIV expression [12]. CMI impairment may occur via oxidative stress leading to increased T-lymphocyte apoptosis [13,14]. Antioxidants may also directly affect HIV replication. Kalebic *et al* [15] showed that HIV replication in cell culture was stimulated by oxygen free radicals (via activation of nuclear transcription factor) and inhibited by antioxidants. Deficiency of zinc, as well as of other antioxidants, may exacerbate already impaired CMI in HIV infection, while simultaneously permitting or stimulating increased HIV replication.

In summary, *in vitro* studies of the relationship between HIV and zinc are still quite limited. Available evidence indicates that HIV replication and assembly require zinc at levels also necessary for host cell survival. Selective blockade of active zinc finger sites of HIV may inhibit HIV while sparing the host immune cell; however, elimination of intracellular zinc could compromise the host cell (by enhancing apoptosis), while allowing protease-mediated production of infectious particles. Furthermore, reduction of oxidants and free radicals by zinc may help limit HIV replication. Avoiding zinc deficiency may not only allow normal or improved immune function particularly of CMI, but also reduce the susceptibility to apoptosis of T lymphocytes in HIV-infected patients. Finally, there are limited data suggesting that high zinc concentrations may inhibit HIV replication and no published *in vitro* evidence of high zinc concentrations stimulating HIV replication.

1.2. Associations of zinc with HIV infections in humans

1.2.1. Prevalence of plasma zinc deficiency in HIV infection

Determination of zinc deficiency is limited by a number of factors. While plasma or serum zinc concentration is easily measured, it may not reflect true body zinc status. Plasma zinc

Table 1
Prevalence of zinc deficiency among HIV-infected individuals

| Study | Number and Type of Participants | Definition of Zn Deficiency | Zn Deficient |
|----------------|--|-----------------------------|---|
| Baum [16] | 108 homosexual men | <75 µg/dL | 25–26% ^a |
| Bogden [17] | 30 outpatients (26 men) | <70 µg/dL | 30% |
| Beach [18] | 100 asymptomatic ^b homosexual men | <75 µg/dL | 26% |
| Bogden [33] | 106 outpatient adults (79 men) | <70 µg/dL | By CDC Stage: A: 14.7% B: 30.2% C: 36% |
| Baum [20] | 114 injection drug users (75 men) - all outpatients | <75 µg/dL | 57% of women, 45% of men |
| Coodley [19] | 47 adults (45 men) | <75 µg/dL | 4% ^c |
| Skurnick [45] | 64 heterosexual adults (43 men) during 116 outpatient visits | <70 µg/dL | 4% ^d |
| Koch [21] | 228 hospitalized adults | ≤65 µg/dL | 51% |
| Henderson [22] | 24 children | <55 µg/dL ^e | 0% |

^a 25% in individuals with CD4+ lymphocyte count <500, 26% in individuals with higher CD4+ lymphocyte counts.

^b Asymptomatic except some patients with persistent generalized lymphadenopathy.

^c Of 47 subjects, 25 had plasma zinc measurements, of which 1 (4%) was ≤75 µg/dL, not 3% as reported in the paper.

^d Calculated as number of measurements <70 µg/dL from total of 116 measurements from 64 subjects.

^e To convert mmol zinc/L to µg zinc/dL, multiply mmol by 6.54. To convert µg/dL to mmol/L, multiply µg/dL by 0.153.

concentration decreases as a non-specific acute phase response even in the absence of true zinc deficiency [23]. This may be particularly confounding in the setting of HIV infection where concurrent illnesses are common. Mean plasma zinc concentration may however be a useful indicator of population zinc status [24]. Cellular zinc concentration may more accurately reflect body zinc status than plasma zinc concentration [1]. Other proposed measures of zinc status include the fraction of biologically active serum thymulin and serum 5-nucleotidase, both of which decrease with zinc deficiency [25,26].

In spite of potential limitations, the vast majority of studies evaluating zinc status have measured plasma or serum levels. Varying rates of low plasma zinc concentration have been detected in HIV-infected patients (Table 1). Among 108 homosexual HIV-infected men, 25% of those with CD4+ lymphocyte counts <500 × 10⁶/L and 26% of those with higher counts had plasma zinc <75 µg/dL [16]. Similar low zinc concentrations were noted in 17% of uninfected controls. Plasma zinc concentrations decreased among HIV-infected individuals during 18 months of follow-up and decreases were particularly striking among individuals with lower CD4+ lymphocyte counts. In another study, 30% of 30 HIV-infected outpatient adults with a range of CD4+ lymphocyte counts and disease stages had plasma zinc concentrations <70 µg/dL [17]. Beach et al [18] found that 26% and 24% of 100 HIV-infected asymptomatic (other than generalized lymphadenopathy in some) homosexual men vs 17% and 31% of 42 uninfected controls had overt (<75 µg/dL) or marginal (75–84 µg/dL) deficiency as assessed by serum zinc concentration. Among HIV-infected

injection drug users, 57% of women and 45% of men had zinc concentrations of $\leq 75 \mu\text{g/dL}$ [20]. In contrast, a small study found only 1 of 25 (4%) HIV-infected adults had low plasma zinc concentrations ($\leq 75 \mu\text{g/dL}$) [19]. Skurnick et al [45] also detected low ($< 70 \mu\text{g/dL}$) plasma zinc levels in 64 heterosexual men and women during 116 outpatient visits only 4% of the time. The reason for the wide range in proportion of HIV-infected mostly outpatient adults with low serum or plasma zinc is not clear.

Among 228 hospitalized HIV-infected adults identified by chart review, 50% of those with plasma zinc measurements had low zinc levels, of whom 29% had very low ($< 55 \mu\text{g/dL}$) and 21% had low (55–65 $\mu\text{g/dL}$) serum zinc concentrations [21]. Few pediatric data are available, but among 24 HIV-infected children, none had a low plasma zinc concentration despite low mean zinc intake in the group [22].

1.2.2. Dietary zinc intake in HIV-infected persons

Consumption of less than the recommended dietary allowance (RDA) of 15 mg zinc daily is prevalent among HIV-infected and -uninfected adults [18,27]. [Revised RDA for Zinc (Institute of Medicine, 2001) is 11mg/day for men and 8mg/day for women.] Baum et al found that 25% of HIV-infected and 50% of HIV-uninfected controls consumed less than the RDA of zinc [27]. In a Baltimore-Washington cohort study, nearly 50% of HIV-infected men had a zinc intake below the RDA prior to learning of their HIV infection status [28]. Among HIV-infected and-uninfected children, the mean zinc intake was two-thirds of the RDA and almost none of the children received zinc supplementation [22]. HIV-infected adults, on the other hand, commonly self-prescribed dietary supplements, including zinc [29,30]; in one study 64% of HIV-seropositive men and 25% of HIV-seronegative controls took supplements containing zinc [27].

Dietary zinc intake correlated with plasma zinc concentration in (mostly asymptomatic) HIV-infected subjects [18,27]. However, intake of the RDA of zinc was still associated with low plasma zinc concentration in 28% of HIV-infected and 18% of HIV-uninfected men [27]. No seronegative controls taking 2–5 times the RDA of zinc had low plasma zinc concentrations; however, 25% of HIV-infected individuals taking that amount of zinc had low plasma zinc concentrations. Among those men, only supplementation with 6–10 times the RDA of zinc was associated with normal or above normal plasma zinc concentrations.

1.2.3. Altered zinc homeostasis in HIV infection

Several factors appear to contribute to low plasma zinc concentration and/or zinc deficiency in HIV infection. Dietary insufficiency, poor appetite, reduced total food intake and frequent nausea and vomiting associated with HIV infection are likely to directly increase the risk of inadequate zinc intake [31]. Increased plasma tumor necrosis factor (TNF) levels as well as inflammatory mucosal reactions may compromise gastrointestinal absorption in HIV infection [2,31], while overproduction of cytokines may increase renal zinc wasting [32].

1.2.4. Zinc and illness associated with HIV infection

In some studies, more advanced disease stage and lower CD4+ lymphocyte counts have been associated with lower zinc concentrations [12,20,23,33], though it is not clear if low

plasma zinc concentrations are a consequence, confounder or contributing cause of more severe HIV-related disease. Concurrent illnesses in HIV-infected individuals may also affect zinc levels. In a retrospective study of hospitalized HIV-infected adults, the incidence of bacterial infections diagnosed during hospitalization was inversely correlated in dose-response fashion with serum zinc concentration during the first week of hospitalization [21]. This finding may represent an association between the acute phase response during bacterial infection and decreased serum zinc concentration or a more causal link between low body zinc and infection susceptibility.

1.2.5. Zinc and antiretroviral treatment

In a prospective study of 108 homosexual men, CD4+ lymphocyte counts increased in patients whose plasma zinc concentration normalized during the study and decreased in those whose plasma zinc concentration became subnormal during the study (borderline significance for latter finding); however, this relationship did not persist when (non-randomized) zidovudine (ZDV) use was taken into account [16]. In an observational study of HIV-infected asymptomatic men, of whom 15 received ZDV and 22 did not, there was an increased prevalence of low plasma zinc concentration among the ZDV recipients (67% v 24%); this difference persisted after controlling for dietary intake, illness, CD4+ lymphocyte count, or albumin [34]. However, baseline low plasma zinc concentration was more common in the ZDV group (35%) than in the non-ZDV group (15%), and the physicians may have prescribed ZDV preferentially for patients who were sicker or different in some way than untreated counterparts. In the same study, PHA and pokeweed lymphocyte responses decreased in most of the untreated patients (regardless of serum zinc concentration), decreased or remained stable in ZDV-treated but hypozincemic patients, and were enhanced in ZDV-treated normozincemic patients. Since zinc is required for intracellular activation of ZDV, zinc depletion could be postulated to limit the beneficial effect of ZDV in preserving mitogen responsiveness [35]. An additional study in which HIV-infected adults were treated with highly active antiretroviral therapy (HAART) showed significantly increased plasma zinc concentrations (mean increased from 78 to 96 $\mu\text{g/dL}$), increased CD4+ lymphocyte counts, reduced total/active thymulin ratio and decreased quantitative RNA over a 4 month period without zinc supplementation [36]. These findings indicate the importance of controlling for antiretroviral therapy in assessing the relationship of zinc status and HIV infection. Perhaps improved control of HIV infection allows for greater zinc absorption/reabsorption or alters other aspects of zinc regulation. Alternatively, if plasma zinc concentration reflects illness status, then better treatment of HIV infection probably leads to higher plasma zinc concentration.

1.2.6. Predictive value of plasma zinc concentration in HIV-related disease

Low plasma zinc concentrations have been associated with disease progression [23] and mortality [37] among HIV-infected adults. In a nested case-control study of 54 patients who progressed from asymptomatic HIV infection to AIDS compared to 54 non-progressors, low serum zinc concentration (and high serum copper concentration), independent of CD4+ lymphocyte count, age, zinc intake or copper intake, predicted progression to AIDS [23]. Neither serum zinc concentration nor progression to AIDS correlated with dietary zinc intake

or with toenail zinc concentration, suggesting that low serum zinc concentration acted as a marker (e.g., acute phase reactant) of disease activity instead of reflecting true zinc deficiency. This study did not control for the effect of antiretroviral therapy, although in 1985, many were probably not treated.

In a later report, Baum et al [37] found that low plasma zinc concentration, as well as low levels of vitamins A and B12, and selenium (Se), individually predicted mortality in 125 intravenous drug using HIV-infected patients, controlling for CD4+ lymphocyte counts. Only Se remained a predictor when all of these factors were assessed simultaneously. Campa et al [38] followed 24 symptomatic HIV-infected children over 5 years and found a similar prediction of mortality by low baseline serum Se concentration ($\leq 85 \mu\text{g/L}$), as well as by baseline CD4+ lymphocyte count $< 200 \times 10^6/\text{L}$, but not by low serum zinc concentration ($\leq 75 \mu\text{g/dL}$).

1.3. Zinc supplementation HIV infection

Few studies have evaluated the effect of zinc supplementation in HIV infection.

1.3.1. Observational studies

In an observational study, 281 HIV-infected gay men (Baltimore-Washington Cohort Study) were followed for up to 8 years for development of AIDS [39] and survival [28]. This study group represented a subsample of 1153 men recruited into the Baltimore/Washington, DC site of the Multicenter AIDS Cohort Study in 1984. Of this total cohort, HIV seropositive individuals (29%) who completed a nutrition questionnaire 6 months after enrollment (88%) and who did not develop AIDS during the 12 months after enrollment ultimately comprised the 281 subjects in these studies. Baseline data were obtained by questionnaire before patients knew their HIV status. Approximately fifty percent had zinc intakes below RDA levels. Mid-level vitamin A intake, higher niacin intake, higher vitamin C intake and higher zinc intake predicted progression to AIDS in a multinutrient model adjusted for age, symptoms, CD4+ lymphocyte count, energy intake, antiretroviral use and PCP prophylaxis [39]. Baseline CD4+ lymphocyte counts were lowest in the highest quartile of intake of zinc and of several other micronutrients [28]. In the multivariate analysis adjusted for age, CD4+ lymphocyte count, energy intake and antiretroviral treatment, increasing reported zinc intake was associated with decreasing survival in a dose response fashion [28]. In fact, consumption of zinc at any level above the RDA was associated with increased mortality. Higher survival, on the other hand, was associated with several B vitamins.

While these results raise questions about potential negative impact of increased zinc intake in persons with HIV infection, the study had several limitations. Analyses were based on self-reports of diet at baseline and did not account for changing intakes over time. The amount of zinc intake was not assigned in a controlled fashion. The finding that men with lower baseline CD4+ lymphocyte counts had higher zinc and other micronutrient intake suggests that sicker men may have been giving themselves disproportionately more zinc; having more advanced disease rather than consuming higher amounts of zinc may have been the primary reason these individuals had decreased survival. This study did not evaluate

other markers of disease progression such as HIV viral load, nor did it assess plasma zinc concentration or other measures of zinc status.

Isa et al [40] supplemented eleven HIV-infected Stage V male outpatients with zinc (1 mg/kg per day) for 10 weeks. 10 age-matched healthy subjects served as controls. Zinc concentration was measured in serum, platelets, mononuclear and polymorphonuclear cells, and erythrocytes. Prior to supplementation, serum zinc concentrations were not significantly lower among HIV-infected individuals compared to controls. Although supplementation produced a significant increase in serum zinc concentration, the level in blood cells remained unchanged. All patients showed progressive weight gain and a slight increase in CD4+ lymphocyte levels. No adverse side effects were noted. The small number of patients evaluated and lack of information regarding other interventions (e.g., antiretroviral treatment) limit conclusions that can be drawn from this study.

1.3.2. *Randomized trials*

Mocchegiani and colleagues [41,42] conducted a study in which 57 Stage III/IV-C1 adults on ZDV for at least 3 months and with CD4+ lymphocyte counts of $250-400 \times 10^6/L$ were stratified by stage of disease, and then randomized to either receive no supplementation or supplementation with 45.5mg elemental zinc (approximately 3 times RDA) per day for 1 month. Stage III subjects and Stage IV-C1 subjects were separately randomized and followed for 2 years. Body weight increased or stabilized in the supplemented groups compared to the control groups. Baseline plasma zinc concentrations were borderline or low for all groups (average 76–80 $\mu g/dL$ for all groups), declined further in the non-supplemented groups, and increased in the supplemented groups. CD4+ lymphocyte counts declined in the unsupplemented groups, but rose in the supplemented groups. While total thymulin levels were unaffected, the active thymulin fraction dramatically increased in the supplemented groups, but did not change in the non-supplemented groups. Zinc supplementation delayed the time to first opportunistic infection (OI) among Stage III individuals and decreased overall incidence of OI among the Stage IV-C1 groups. Important limitations of this study included the lack of a placebo, lack of blinding (certainly of subjects and seemingly also of researchers) regarding supplementation status, and exclusion from analysis of an unreported number of patients who, after assignment, interrupted zinc supplementation or pentamidine prophylaxis. If patients who became sicker were more likely to stop taking zinc or pentamidine, the results of the analysis could be significantly affected. Nonetheless, the apparent benefits of zinc supplementation on improving body weight, improving CD4+ lymphocyte counts and decreasing incidence of OIs together with documented plasma zinc concentrations and active thymulin fractions support the need for a larger study of zinc supplementation in HIV infection.

Kelly et al [43] evaluated 106 HIV-infected Zambian adults with persistent diarrhea and average CD4+ lymphocyte counts $<300 \times 10^6/L$ randomly assigned to receive albendazole/placebo or albendazole/micronutrient supplement (45.5mg elemental zinc as sulfate, plus vitamin A, vitamin C, vitamin E, and Se) for 14 days. Patients were followed for 3 months and there was no difference between groups in mortality, diarrhea remission or CD4+ lymphocyte counts. The high overall rate (52%) of diarrhea remission was attributed to albendazole treatment. It is difficult to assess the role of individual micronutrients in the

supplement. While no negative effects of supplementation were observed, the length of follow-up was limited and viral load and other makers of disease progression were not measured. It also should be noted that a nonstandard assay for CD4+ lymphocyte counts was used in this study. The authors claim that, based on extrapolations from a separate study population, most of this study group had very low CD4+ lymphocyte counts. Results may not be generalizable to HIV-infected patients with lesser degrees of immunosuppression.

More recently, investigators evaluated the effect of vitamin A and/or zinc supplementation on HIV disease markers and antibody responses to pneumococcal and *Haemophilus influenzae* vaccines [44]. Two weeks before being immunized, HIV seropositive injection drug users with CD4+ lymphocyte counts $> 200 \times 10^6/L$ were randomized to receive either 400,000 IU vitamin A, 300 mg Zinc (given as 50mg thrice weekly for 2 weeks), both, or a placebo. Study participants were subsequently followed for 7 months. None of the individuals experienced any serious adverse events and neither the vitamin A nor the zinc supplementation was associated with an increase in HIV viral load. Mean CD4+ lymphocyte counts transiently decreased in the group who received zinc, but the difference disappeared within 7 months. Zinc supplementation had no effect on antibody responses to the pneumococcal conjugate vaccine.

1.4. Summary of current state of knowledge of zinc and HIV infection

Zinc deficiency produces reversible immune dysfunction, particularly of T-lymphocyte cell-mediated immunity; conversely, experimental high-dose zinc supplementation (20-fold RDA) can also produce similar immune dysfunction. Dermatologic, gastrointestinal and respiratory abnormalities and infections are clinical manifestations of zinc deficiency that can be ameliorated by zinc repletion. Inadequate zinc intake occurs frequently in developing countries, but even healthy subjects in developed countries commonly take in less than the RDA of zinc. Plasma zinc concentration may be an adequate measure of zinc status on a population basis, but it is an inaccurate measure of zinc status in individuals, particularly those with chronic infectious/inflammatory conditions like HIV infection.

Malabsorption and repeated infections occurring in HIV infection as well as enhanced zinc loss stimulated by HIV-induced cytokines likely cause zinc requirements in HIV infection to be several-fold above RDA. Dietary zinc intake is variably related to plasma zinc concentration in HIV infection. Low plasma zinc concentration occurs in HIV infection, especially with advancing illness, and may predict disease progression and mortality. Antiretroviral treatment is an important confounder or mediator of the relationship of plasma zinc and disease progression. The balance of evidence favors the view that low plasma zinc is a marker for disease progression and not that zinc deficiency is the proximate (or treatable) cause. Most studies have taken place in settings of low population prevalence of zinc deficiency.

HIV, like most organisms, requires zinc for replication. High or very low zinc concentrations may interfere with replication. Selective blockade of active zinc finger sites of HIV may inhibit the virus, while sparing host immune cells. Progressive depletion of intracellular zinc may more likely compromise the host cell (apoptosis) while potentially allowing HIV-protease-mediated production of infectious HIV particles. Avoiding deficient zinc states

may not only allow normal or improved immune function particularly of T-lymphocyte mediated cellular immunity, but also appears to inhibit susceptibility of T lymphocytes to apoptosis in HIV-infected patients.

To date, there has been no randomized, placebo-controlled study of moderate zinc supplementation in HIV infection. One observational US study suggested an adverse effect of increased zinc intake on HIV disease progression and mortality, while an Italian intervention study suggested a beneficial effect. A Zambian study in advanced stage HIV-infected people failed to demonstrate any clinical effect of micronutrient (including zinc) supplementation.

Available evidence supports the need for and acceptability of a randomized, placebo-controlled trial of moderate zinc supplementation in HIV infection. A number of studies suggest possible benefit and few data provide evidence of adverse clinical or immunologic effects due to moderate zinc supplementation. Several studies have shown that zinc supplementation decreases pediatric morbidity and mortality particularly due to respiratory and diarrheal diseases. Concerns regarding the possibility that zinc might enhance HIV replication and/or accelerate disease progression have limited use of zinc supplements in areas where HIV infection prevalence is high. At this time, the highest HIV prevalence rates are found in areas with high background child mortality rates, areas in which HIV-uninfected children would likely benefit from zinc supplementation. Therefore, it is essential to determine whether zinc supplementation can improve outcome among HIV-infected individuals and to establish its safety in such populations. If zinc supplementation is beneficial in the setting of HIV infection, it will offer a low cost intervention where few other treatment options exist. If supplementation is found to be safe among HIV-infected individuals, then widespread supplementation programs can be undertaken without concern for harm to the HIV-infected members of the population or need to exclude them from such programs.

2. Conclusion

A trial of moderate zinc supplementation and HIV infection should be conducted promptly in a population setting of high HIV prevalence and endemic zinc deficiency. Strong evidence exists for the benefits of correcting zinc deficiency in children. Most clinical and *in vitro* evidence suggests there is no harm and there may be benefit in improving zinc status in HIV-infected individuals. The proposed trial should follow HIV illness stage and opportunistic illnesses, respiratory and diarrheal illness incidence and morbidity, weight (and linear growth in children), antiretroviral and anti-OI treatment, CD4+ lymphocyte counts, HIV viral load, plasma zinc, serum thymulin, as well as possibly *in vitro* tests of mitogen responses, cytokine levels and apoptosis of subject PBMCs. Although such a trial could be conducted among HIV-infected individuals of any age, particular consideration should be given to the inclusion of preschool children, since this is the age group for which benefit of zinc supplementation has been demonstrated in regard to diarrhea, pneumonia and malaria.

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