Short-Term Effects of Large-Dose Vitamin A Supplementation on Viral Load and Immune Response in HIV-Infected Women

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Summary:
Vitamin A supplementation has been suggested for treatment and prevention of HIV infection. However, some in vitro data indicate that vitamin A may activate HIV. Randomly, 40 HIV-seropositive women of reproductive age were allocated to receive a single oral dose of 9900 µmol (300,000 IU) vitamin A or placebo. Plasma HIV-1 RNA concentration, total lymphocytes, selected lymphocyte subsets and activation markers, and in vitro lymphocyte proliferation to phytohemagglutinin (PHA) and Candida were measured before dosing and at various time points over an 8-week follow-up period. No differences were found between treatment groups in the frequency of signs or symptoms of acute vitamin A toxicity, nor were differences evident in any lymphocyte subset or activation marker at any time during follow-up. Mean and median viral load concentration at each time point and change in viral load from baseline to each follow-up point did not differ between treatment groups. No difference was measured between treatment groups in the proportion of women who responded to PHA or Candida. This study provides no evidence that high dose vitamin A supplementation of HIV-infected women is associated with significant clinical or immunologic adverse effects.

During HIV-1 infection, vitamin A deficiency is common (1) and an independent risk factor for adult mortality (2,3). Modest increases in vitamin A intake by HIV-infected people are associated with improved vitamin A status (4) and slower progression to AIDS (5). Among HIV-infected pregnant women, low serum retinol concentration is a risk factor for infant mortality (6,7), mother-to-child HIV transmission (8), and a greater likelihood of detecting HIV DNA in colostrum (9). These
observations suggest that vitamin A supplementation of HIV-infected people may be clinically beneficial. Specifically, vitamin A supplementation of HIV-infected women during pregnancy with small daily doses, or in the immediate postpartum period with single large doses, may reduce mother-to-child HIV transmission (10).

Interaction of vitamin A with HIV, however, is complex. Some in vitro data indicate that vitamin A supplementation may activate HIV replication. Several groups have reported that pretreatment of human monocytes or monocyte-like cells with retinoic acid (the intracellular active form of vitamin A) before infection with HIV increases virus production (11-13). In one study, retinol (the form in circulation) or retinyl acetate (the form in food and supplements) produced similar effects, leading these authors to suggest that "use of retinoids in HIV-infected patients should be with caution" (13). Conversely, when retinoic acid treatment was begun concurrently with transfection, the upregulatory effect of retinoic acid treatment was greatly reduced (13) and when retinoid supplementation was begun several days after infection, it either had no effect (J. Turpin, personal communication, March 21, 1997) or dramatically downregulated viral expression (12). Thus, the effects of retinoids on HIV replication in vitro may vary dichotomously as a function of experimental conditions.

Several trials are currently underway, or are being planned, in which HIV-infected women receive small daily doses of vitamin A throughout pregnancy and/or single large doses of vitamin A during the immediate postpartum period to test the impact on vertical transmission. Furthermore, in some countries where HIV infection is prevalent among women of reproductive age, women are already routinely supplemented with a large dose of vitamin A at delivery, an intervention recommended by the World Health Organization to control vitamin A deficiency in breastfed infants (14).

To assess the risk-benefit ratio of vitamin A supplementation for HIV-infected women better, we conducted this study to monitor potentially adverse immune responses over close intervals following a single large dose of vitamin A or placebo among HIV-infected women of reproductive age. Because this was a safety study, we chose to conduct it in the United States within a clinical research center where we could most easily ensure clinical back-up should any patient suffer adverse effects. The effect of a single large dose of vitamin A was of particular interest because one-time maternal supplementation during the immediate postpartum period is effective in improving the vitamin A status of mothers and their breastfed infants (15) and has enormous programmatic feasibility throughout the developing world, where vitamin A deficiency and HIV infection are prevalent. However, high-dose supplementation results in transient but elevated concentrations of circulating retinyl esters; plasma concentrations rise from ~50 nM to ~15,000 nM during the 6 hours after dosing, and then rapidly fall near baseline within 6 to 24 hours (16). The effects of this brief but unique metabolic state on HIV expression may be different than that of physiologic doses of vitamin A.

METHODS

Design and Subjects

The study was a randomized, double-masked, placebo-controlled trial. Study subjects were recruited from the Moore Clinic in the Johns Hopkins Hospital, Baltimore, MD, U.S.A. Eligible patients were HIV-seropositive women between 18 and 45
years of age who were not pregnant or breastfeeding an infant, were not receiving any antiretroviral drugs or were receiving a stable regimen of antiretroviral drugs, and had an absolute CD4 count of >200 cells/mm³. Although vitamin A deficiency has been reported in HIV-infected people in the United States (1), by excluding women with CD4 counts <200 cells/mm³, our population was less likely to be deficient. This was done because our primary purpose was to identify potential adverse immunologic responses to a large dose of vitamin A, which may be more likely in individuals with sufficient levels of vitamin A, in whom clearance of retinyl esters may be slower. Because large doses of vitamin A may be teratogenic (17), we required that women be using a highly effective method of contraception (i.e., levonorgestrel [Norplant], medroxyprogesterone acetate [Depo-Provera], sterilization, or regular use of birth control pills or sexual abstinence with regular menstrual periods) in addition to having a negative result on a serum pregnancy test on the day before dosing. Women were requested not to take supplements containing vitamin A from 1 week before and throughout the entire study period. Following written informed consent, eligible women were scheduled for a baseline appointment. History of any AIDS-defining illnesses (18) and of an AIDS-associated illness (e.g., swollen glands, anorexia, diarrhea, weight loss, fever or night sweats, and fatigue) during the previous month were documented. Because this was a safety study, the trial was conducted among two cohorts of women to limit the number of women exposed should the intervention prove to be harmful. The first 18 women participated from January 20 to March 20, 1996. Following a review of these data by an outside Data Safety and Monitoring Committee (DSMC), a second cohort of 22 women was studied from May 5 to July 5, 1996. One of these women failed to return for all follow-up visits so data are reported for 39 women. The investigators remained masked to treatment allocation until after the DSMC conducted a second review of the data generated by all 39 women. The purpose of this second review was to detect any trends in the data that might reach statistical significance if additional women were studied. The DSMC concluded that the sample size was sufficient.

Treatment

Although the current World Health Organization (WHO)-recommended dose for supplementing postpartum women is 200,000 IU (19), a larger dose of 300,000 IU was chosen for this study because that level has been shown to elevate breast milk retinol concentrations for 6 to 9 months among Indonesian lactating women and improve vitamin A status in their infants (15). Furthermore, a more recent report demonstrated that the 200,000 IU dose was not enough to correct vitamin A deficiency in Bangladeshi women or to build adequate vitamin A stores in their breastfed infants and concluded that the recommended dose should be increased (20).

Treatment capsules were kindly donated by Tishcon Corporation (Westbury, NY, U.S.A.) and contained either 3300 µmol (100,000 IU) vitamin A as retinyl acetate in soybean oil (plus 2 IU vitamin E as an antioxidant to protect vitamin A activity), or soybean oil alone. Three capsules, yielding a 9900-µmol (300,000-IU) dose of vitamin A or placebo, were wrapped together in foil and placed in individual airtight plastic bags to protect them from light and humidity. Another scientist, not involved with the study, randomly allocated sequential two-digit ID numbers to vitamin A or placebo, and appropriately labeled each bag. Stability of the supplement was confirmed in our vitamin A reference laboratory midway through and at the end of the study.
Consenting women came to the General Clinical Research Clinic (GCRC) 1 day prior to being randomized to treatment (day -1) for baseline tests. These included a serum pregnancy test, viral load, serum retinol, total lymphocytes, lymphocyte subsets (CD3, CD4, CD8, CD45), activation markers (CD4-DR, CD4-38, CD8-DR, and CD8-38), and lymphocyte proliferation to phytohemagglutinin A (PHA) and Candida. The next day (day 0), following confirmation of a negative result on a serum pregnancy test and continued contraception, patients were assigned the next available ID number and given the capsules labeled with that ID number. Patients swallowed the three capsules under direct observation of an investigator. After dosing, the ID label was removed from the bag and placed on the data collection form for data entry. On the day after dosing (day 1), measurements of viral load and lymphocyte subsets were repeated. Viral load studies were repeated 1, 2, 3, and 4 weeks after dosing. Lymphocyte subset and activation marker enumeration was repeated at 1, 2, 4, and 8 weeks after dosing. Lymphocyte proliferation and cytokine studies were repeated at weeks 2, 4, and 8 and serum retinol was measured again at week 4. At the day 1 and week 1 visits, women were queried for possible side effects to vitamin A including headache, nausea, vomiting, fever, and diarrhea. At each visit, women were asked which, if any, antiretroviral drugs or vitamin supplements they were currently taking and at what dosage to facilitate interpretation of study outcomes. A 1-month history for AIDS-associated illness was elicited at baseline, and a 1-week history for AIDS-associated illness was then elicited at each follow-up visit.

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Laboratory Methods

Blood samples were held at room temperature and processed within 4 hours. Measurement of HIV-1 RNA in plasma samples was performed using AIDS Clinical Trials Group (ACTG) known standards. Quantitative HIV-1 RNA polymerase chain reaction (PCR) was performed on batched samples using an HIV-1 monitor assay according to the manufacturer’s instructions (AMPLICOR HIV-1 Monitor Test, Roche Molecular Systems, Inc. Somerville, N.J., U.S.A.). In each case, 50 µl of each prepared RNA sample was used for PCR. Following amplification and detection of the PCR product, the starting HIV-1 RNA load in each sample was calculated by comparison with the internal quantitation standard, and results were expressed as HIV-1 RNA copies/ml plasma. Lymphocyte subsets and activation markers were measured using the Coulter Q-prep procedure (Miami, FL, U.S.A.). Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation over a Ficoll-Hypaque gradient centrifugation and washed twice in phosphate-buffered saline solution. Cells were suspended to 1 × 10^6 PMBC/ml in RPMI-1640 culture medium with 10% pooled human AB+ serum. Aliquots of 100 µl were cultured in microtiter plate wells with PHA for 3 days or Candida antigen (Greer Labs, Lenoir, NC, U.S.A.) for 6 days. Lymphocyte proliferation was assessed by measuring ³H-thymidine uptake (21). Serum retinol was measured by high performance liquid chromatography following the method of Bieri et al. (22), with quality control monitored using reference standards from the National Institute for Standards and Technology (Gaithersburg, MD, U.S.A.).

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Statistical Analysis

Proportions were compared with [chi]² or Fisher’s exact tests and means were compared using t-tests. Medians and geometric means were calculated as measures of central tendency for skewed data (i.e., viral load, lymphocyte subsets) and groups were compared using nonparametric tests. All statistical tests were two-tailed, except the side effect data in which one-tailed tests were used. Level of significance used was p < .05.
Ethical Approval

The study was approved by the Joint Clinical Committee for Investigation of The Johns Hopkins School of Medicine.

RESULTS

Comparability of Treatment Groups

At baseline, no significant differences were found between the vitamin A-treated and placebo-treated groups in age, the proportion of women who had had at least one documented AIDS-defining illness, or the proportion of women who reported at least one episode of AIDS-associated illness in the previous month (Table 1). In the vitamin A group, 10 (45.4%) and in the placebo group, 11 (61.1%) women were not receiving antiretroviral drugs ($p = .50$), and the remaining women in both groups were taking zidovudine (ZDV), didanosine (ddI), dideoxycytidine (ddC), stavudine (d4T), lamivudine (3TC), or a combination of two of these drugs. One woman in the vitamin A group was concurrently enrolled in ACTG-276 and taking her prescribed drug. None of the women reported taking any of the new protease inhibitors.

Tolerance

Administration of a single 9900-µmol dose of vitamin A was well tolerated: rates of headache, nausea, vomiting, diarrhea, and fever reported by vitamin A recipients were no different compared with those reported by placebo recipients at either 24 hours or 1 week after dosing (Table 2).

Impact of Supplementation on Vitamin A Status

Serum retinol concentration was normal (>1.05 µmol/L) in the majority of women at baseline, though concentrations were below this cutoff for four women (3 vitamin A, 1 placebo). No change was found in the mean or median serum retinol concentrations in either treatment group 4 weeks after supplementation (Table 3).

Lymphocyte Response to Vitamin A Supplementation

The two most informative lymphocyte parameters for predicting survival (i.e., disease progression) among HIV-infected people are a decline in CD4 percentage (slightly more predictive than absolute levels [23]) and an increase in the proportion of CD8 cells that are CD38+ (CD8+CD38+) (24,25). Median CD4 percentage was no different between the treatment groups at baseline (26% versus 25.5% in the vitamin A and placebo groups, respectively; $p = .84$) and thereafter tended to be higher in the vitamin A group, although this difference only reached statistical significance at 2 weeks (29% versus 23.5%; $p < .05$; Fig. 1). No difference was shown between treatment groups in mean or median concentration of CD8+CD38+ cells before or at any time point following treatment (Fig. 2). Available power to detect a difference of >=10 percentage points in mean CD8+CD38+ cells between treatment groups varied from 36% at baseline (day -1) to 91% at week 8. These data were also evaluated using two arbitrary cutoffs: 20%, the approximate percentage of CD8 cells that are CD38+ in seronegative people and 50%, a level suggested to be a very poor prognostic indicator for HIV-infected people (24). The proportions of women in the two treatment groups with CD8+CD38+ cells exceeding each of these cutoffs were not different at any time point ($p >= .38$). No significant differences were found in the means or medians of any other lymphocyte...
subset measured, in the CD4/CD8 ratio, or in total lymphocyte count at any time point between treatment groups (p values for tests comparing means ranged from 0.08-0.68; p values for tests comparing medians ranged from 0.19-0.89).

Responsiveness to mitogens (PHA and Candida) was assessed as an in vitro marker of immune function. No consistent trend up or down existed in the vitamin A-treated group in terms of mean counts. Data were also analyzed in terms of the number of responders to each mitogen in which response was defined in three different ways: for PHA >5000 CPM, >10,000 CPM, or stimulation index <10; for Candida >2500 CPM, >5000 CPM, and stimulation index <5. Using any definition, no significant difference was obtained in the proportion of nonresponders in the vitamin A group compared with the placebo group for either mitogen.

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Plasma HIV-1 RNA (Viral Load) Response to Vitamin A Supplementation

Serial measures of log10 plasma HIV RNA (copies/ml) are plotted for each woman in the vitamin A group (Fig. 3, top) and placebo group (Fig. 3, bottom). Dashed lines in Figure 3 represent ±0.5 log10 of the woman's baseline viral load, the conventional cutoff used for disease progression or improvement; in this paper, we define a change in viral load of this magnitude or greater as being clinically “significant.” We carefully compared all reported morbidity and changes in type or dosage of anti-retroviral drug therapy and were unable to explain fluctuations in viral load based on these factors for any woman except ID 43 who began 3TC at week 2 and thereafter experienced a significant decline in viral load. Geometric mean viral load concentration was very similar for the two groups at all time points (p >= .44; Fig. 4). We had 80% power to detect a difference of 0.72 log in geometric means. Similarly, no differences were observable between groups in median viral load levels at any time point (p >= .24; data not shown).

Figure 5 illustrates the median change in log viral load from baseline to each follow-up time point by treatment group. Most delta viral load values for both the vitamin A and placebo recipients were <0.5 log10 of baseline value and the median change was very near zero for both treatment groups at each of the 5 follow-up time points. Similarly, mean changes were <0.2 log at all time points for both treatment groups. However, greater variability existed in both directions in the vitamin A group at week 1 and week 2: the standard deviation (SD) of the mean change was significantly greater for the vitamin A group compared with the placebo group at these two time points.

We examined baseline characteristics of the vitamin A recipients that might distinguish women who experienced at least one significant increase (>=0.5 log10) in viral load compared with women who did not. There was no difference in baseline serum retinol concentrations (1.50 ± 0.44 µmol/L versus 1.53 ± 0.42 µmol/L for those who had a significant increase compared to those who did not, respectively), suggesting that vitamin A status did not influence viral load response to vitamin A supplementation. In addition, no significant differences existed in any lymphocyte subset or activation marker between vitamin A recipients who experienced a significant viral load increase compared with those who did not (data not shown). The only baseline characteristic that did significantly distinguish these women was baseline viral load concentration: the mean plasma HIV-1 RNA concentration at baseline was significantly lower among vitamin A recipients who experienced at least one significant increase in viral load compared with vitamin A.
recipients who did not have any significant rises (8094 ± 9534 versus 42,632 ± 56,373; p < .04). No distinct pattern was found in viral load changes over time among the seven vitamin A recipients who experienced a significant rise in viral load; for two study subjects, this increase appeared at weeks 2 and 3 only, for two others, this increase appeared at week 2 or 3 and then again at week 4, one woman experienced rises at every time point (ID 42) and two women experienced both an increase and a decrease of >=0.5 log10: one woman had a decrease at week 2 and an increase at week 4, the other had an increase at week 2 and a decrease at week 4.

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DISCUSSION

This study revealed no evidence of adverse effects following a 9900 µmol dose of vitamin A among HIV-seropositive women. No differences were found in the rates of any sign or symptom commonly reported during acute vitamin A toxicity (26) between vitamin A-treated and placebo-treated women. Although no side effects were reported from previous studies giving women this dose or higher (15,27,28), women were not explicitly queried about side effects in those studies. Comparing women treated with vitamin A and those with placebo, no differences were found in any lymphocyte subset or activation marker measured at any time, including the two most prognostic lymphocyte subset indicators of disease progression (CD4 percentage and CD8CD38). Indeed, if anything, vitamin A supplementation may have resulted in slightly higher CD4 percentage levels, an effect previously observed (29). In addition, no differences were measured between treatment groups in in vitro lymphocyte responsiveness to either PHA or Candida. Finally, mean and median viral load concentrations were not different between groups at any time point.

Vitamin A supplementation did not result in an increase in mean or median serum retinol concentration, suggesting the women were near their homeostatic concentration before supplementation (30). Two women in the vitamin A group, however, had serum retinol concentration in the deficient range (<0.70 µmol/L) at baseline and still did not respond to vitamin A supplementation. Serum retinol is often a poor indicator of vitamin A status (30), especially during the acute phase of an infection (31). It is possible the low serum retinol concentration in these women reflected illness rather than poor vitamin A status.

Although mean and median changes in plasma HIV RNA concentrations between baseline and each follow-up point were not different between treatment groups, the variance around this mean change was significantly higher for vitamin A compared with placebo recipients at two time points (week 1 and week 2). Of potential concern were women who experienced a significant increase in viral load at one or more time points following vitamin A supplementation. In our study, these women could be distinguished from vitamin A recipients whose viral load did not increase, based on lower viral load concentrations before supplementation. Two factors argue that this greater variability has little or no clinical significance. First, at lower concentrations of viral load, smaller absolute changes in viral load would be classified as relative changes of >=0.5 log10 compared with changes at higher viral load concentrations. Second, no distinct pattern of viral load changes over time was manifest among the vitamin A recipients who experienced at least one increase in viral load. Further research among a larger number of subjects on the impact of vitamin A supplementation not only on short-term changes in viral load but also on clinical outcomes (i.e., progression to AIDS, vertical transmission) will be needed to
understand whether this increased variability is a real effect and whether it has clinical significance.

Our findings confirm and extend those of two previous reports. Coutsoudis et al. reported no increase in viral load at one week post partum among 12 HIV-seropositive women following daily doses of 5000 IU vitamin A during the last trimester of pregnancy and a single 200,000 IU dose at delivery (32). Semba et al. found no impact on viral load or CD4 count among 120 (mostly male) injection drug users at 2 and 4 weeks following a single dose of 200,000 IU (33). Our study reports for the first time an absence of impact on viral load at 24 hours following a large dose of vitamin A when retinyl ester concentrations transiently but dramatically increase. Even a transient increase in viral load immediately after dosing with vitamin A could have serious implications for the breastfed neonate of the supplemented mother even when no effect on the mother's health was found. Thus, our findings are particularly reassuring for use of large-dose vitamin A for newly delivered postpartum women. In addition, we included additional assays including lymphocyte proliferation to PHA and Candida and CD8+CD83+ cells, which may be more sensitive of adverse response to the large dose vitamin A. Taken together, these three studies provide no evidence that high-dose vitamin A supplementation of HIV-infected women results in significant clinical or immunologic adverse effects and render support for pursuing vitamin A supplementation as a therapy during HIV infection.

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**Bibliographic Links**

Key Words: Vitamin A; Viral load; Women; HIV/AIDS

**IMAGE GALLERY**

*Table 1*

*Table 2*

*Table 3*

*Fig. 1*

*Fig. 2*

*Fig. 3*

*Fig. 4*

*Fig. 5*