CONCISE COMMUNICATION

Vitamin A Supplementation and Human Immunodeficiency Virus Type 1 Shedding in Women: Results of a Randomized Clinical Trial

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Observational studies have associated vitamin A deficiency with vaginal shedding of human immunodeficiency virus (HIV) type 1–infected cells and mother-to-child HIV-1 transmission. To assess the effect of vitamin A supplementation on vaginal shedding of HIV-1, a randomized, double-blind, placebo-controlled trial of 6 weeks of daily oral vitamin A (10,000 IU of retinyl palmitate) was conducted among 400 HIV-1–infected women in Mombasa, Kenya. At follow-up, there was no statistically significant difference in the prevalence of HIV-1 DNA (18% vs. 21%, P = .4) or the quantity of HIV-1 RNA (3.12 vs. 3.00 log10 copies/swab, P = 1.0) in vaginal secretions of women receiving vitamin A, compared with women receiving placebo. No significant effect of supplementation on plasma HIV-1 load or CD4 or CD8 cell counts was observed, and no effect was seen among women who were vitamin A deficient at baseline. Vitamin A supplementation is unlikely to decrease the infectivity of women infected with HIV-1.

Strategies to control the human immunodeficiency virus type 1 (HIV-1) pandemic should ideally combine interventions that reduce infectivity of HIV-1–infected persons with those that reduce susceptibility of exposed individuals. We previously reported the results of a large cross-sectional study of correlates of shedding of HIV-1–infected cells in female genital secretions [1]. Low serum vitamin A concentration was strongly related to vaginal shedding. These findings reinforced earlier studies that associated low vitamin A levels with shedding of HIV-1–infected cells during pregnancy [2], shedding of HIV-1–infected cells in breast milk [3], and mother-to-child HIV-1 transmission [4], as well as with lower CD4 cell counts and faster HIV-1 disease progression [5].

Vitamin A plays a key role in normal immune function [6]. Deficiency leads to depressed humoral and cellular immunity and heightened morbidity from infectious diseases. Large randomized trials showed that vitamin A supplementation can increase CD4 cells among deficient children, reduce childhood mortality, and decrease pregnancy-related mortality among adult women [6, 7].

The consistency of these observational data relating vitamin A deficiency and HIV-1 infectivity and disease progression and the positive results of supplementation trials in HIV-1–uninfected populations suggested that vitamin A supplementation might benefit HIV-1–infected persons. Thus, we initiated a randomized, double-blind, placebo-controlled trial of vitamin A supplementation among HIV-1–infected Kenyan women. The goal of this study was to assess the effect of supplementation on vaginal shedding of HIV-1 and HIV-1–infected cells, plasma HIV-1 load, and CD4 and CD8 cell counts.

Methods

Participants and procedures. Between September 1998 and June 2000, HIV-1–seropositive women attending Coast Provincial General Hospital outpatient clinics in Mombasa, Kenya, were enrolled. Exclusion criteria included age < 18 or > 45 years, pregnancy, or use of vitamin supplements or oral contraceptive pills [1]. A standard questionnaire was administered, and blood was obtained. Vaginal secretions were collected by rolling plastic-handled Dacron swabs 3 full turns against the lateral vaginal wall, as described elsewhere [1, 8]. Women were randomized by computer-generated block randomization to a 6-week daily supply of gelatin capsules containing...
either 10,000 IU of vitamin A as retinyl palmitate or placebo (Tishcon). This dosage is recommended by the World Health Organization for correction of symptomatic vitamin A deficiency in women of childbearing age [9]. The regimens were indistinguishable, and field researchers were blinded to treatment assignments. Prescription bottles containing an alarm were used to remind patients of their daily dosage (RemindRx; IBV Technologies). Vitamin A capsules tested after 12 months of field storage retained 100% activity. After 100 participants were enrolled, a multivitamin supplementation study arm was added, and women were thereafter randomized to vitamin A, multivitamin, or placebo. The current analysis is restricted to the vitamin A and placebo comparison. Women returned after 6 weeks for follow-up. Samples were collected as at enrollment, and a pill count was done. All women were then given 4 weeks of vitamin A supplements to treat deficiency in the placebo group.

Laboratory methods. HIV-1 serology was done with 2 ELISAs (Detect HIV-1/2, BioChem ImmunoSystems; Recombigen, Cambridge Biotech). CD4 and CD8 cells were counted by use of a semi-automated system (Zymnune; Bartels) with a lower limit of quantification of 25 cells/µL. Light-protected serum samples were tested for vitamin A by high-pressure liquid chromatography [10]. Deficiency was defined as < 30 µg/dL, and severe deficiency was defined as < 20 µg/dL [6].

Swabs were tested for HIV-1 DNA by nested polymerase chain reaction amplification of the gag gene [8]. An HIV-1 load assay (Gen-Probe) was used for quantitative detection of HIV-1 RNA in plasma and vaginal samples [8]. The lower limits of quantification were 50 HIV-1 RNA copies/mL for plasma and 125 HIV-1 RNA copies/swab for vaginal swabs.

Data analysis. The study was designed with 200 participants per arm to detect a 3-fold difference in vaginal HIV-1 DNA prevalence with 80% power, a 2-sided test, α = 0.05, 15% loss to follow-up, and 14% baseline shedding [1]. Analyses were on an intent-to-treat basis. Vaginal and plasma virus loads were log transformed. Categorical variables were compared by χ² tests and continuous variables by Mann-Whitney U test and Spearman’s correlation coefficient. We used multivariate linear and logistic regression models. Analyses were done with SPSS 10.0 software.

Results

Population. Of 2021 women tested, 1026 (51%) were HIV-1 seropositive, of whom 857 (84%) received their results. In all, 650 (76%) were enrolled: 200 received vitamin A, 200 received placebo, and 250 were in the multivitamin arm. Of the remaining 207 HIV-positive women, 1026 (84%) received their results. In all, 200 received vitamin A, 200 received placebo, and 250 were in the multivitamin arm. Of the remaining 207 HIV-positive women, 1026 (84%) received their results. In all, 650 (76%) were enrolled: 200 received vitamin A, 200 received placebo, and 250 were in the multivitamin arm. Of the remaining 207 HIV-positive women, 1026 (84%) received their results. In all, 650 (76%) were enrolled: 200 received vitamin A, 200 received placebo, and 250 were in the multivitamin arm. Of the remaining 207 HIV-positive women, 1026 (84%) received their results.
concentration and vaginal HIV-1 RNA quantity (Spearman’s \( r = -0.10; P = .04 \)).

Follow-up. In total, 354 women (89%) returned for follow-up, including 176 (88%) of those randomized to vitamin A and 178 (89%) randomized to placebo (\( P = .8 \)). Median time to follow-up was 42 days (range, 32–445 days); 326 women (92%) returned within 8 weeks.

Participants lost to follow-up had more advanced HIV-1 disease than those who completed the study (median CD4 cells, 98 vs. 239, \( P < .001 \); median plasma virus load, 5.99 vs. 5.36 \( \log_{10} \) copies/mL, \( P < .001 \)). They were more likely to be vitamin A deficient (78% vs. 56%; \( P = .004 \)) or severely vitamin A deficient (43% vs. 24%; \( P = .004 \)). At follow-up, 318 women returned medication bottles. The median number of pills remaining was 0 (range, 0–15), and 307 (97%) had \( \leq 2 \) pills remaining. Among the 36 women not returning their bottle, 34 (94%) reported \( \leq 2 \) pills remained. Thus, 341 women (96% of those with follow-up) took \( \geq 95 \)% of their prescribed pills.

Effect of vitamin A. At follow-up, there was no statistically significant difference in the prevalence of HIV-1 DNA detected in vaginal swabs from women randomized to vitamin A when compared with women randomized to placebo (18% vs. 21%; \( P = .4 \); table 2). Similarly, the median vaginal HIV-1 RNA concentration at follow-up did not differ significantly between the randomization groups (3.12 vs. 3.00 \( \log_{10} \) copies/swab; \( P = 1.0 \)). Adjustment for baseline factors did not substantially alter these results. Plasma HIV-1 load was also similar for women receiving vitamin A or placebo, and multivariate analysis revealed no significant effect of supplementation. There were slight differences in follow-up CD4 and CD8 cell counts between the 2 groups, but, in multivariate models, no statistically significant effect of supplementation was retained. In addition, for all outcome measures, no effect of vitamin A was observed for women who were vitamin A deficient or severely vitamin A deficient at baseline (data not shown).

We considered whether immunosuppression limited response to vitamin A supplementation by including an interaction term to allow assessment of whether the intervention operated differently among women with \( \geq 200 \) CD4 cells. No evidence was found of statistical interaction for any of these outcome measures, suggesting the effect of vitamin A did not differ by CD4 cell count.

At follow-up, women receiving vitamin A had higher serum vitamin A concentrations than those receiving placebo (median, 29.4 vs. 26.8 \( \mu g/\text{dL}; P = .03 \)). This was true among those who were deficient at enrollment (25.1 vs. 21.2 \( \mu g/\text{dL}; P = .03 \)), but not among those who were severely deficient (19.0 vs. 17.9 \( \mu g/\text{dL}; P = .5 \)).

### Table 2. Effect of vitamin A supplementation.

<table>
<thead>
<tr>
<th>Outcome measure</th>
<th>Vitamin A arm ((n = 176)^a)</th>
<th>Placebo arm ((n = 178)^b)</th>
<th>( P )</th>
<th>Adjusted OR (95% CI)</th>
<th>Adjusted regression coefficient (95% CI)</th>
<th>Adjusted ( P )^c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal HIV-1 DNA</td>
<td>31 (18)</td>
<td>38 (21)</td>
<td>.4</td>
<td>0.7 (0.4–1.4)</td>
<td></td>
<td>.3</td>
</tr>
<tr>
<td>Vaginal HIV-1 RNA, ( \log_{10} ) copies/swab</td>
<td>3.12 (&lt;2.10–5.63)</td>
<td>3.00 (&lt;2.10–5.85)</td>
<td>1.0</td>
<td>0.15 (–0.05–0.35)</td>
<td></td>
<td>.2</td>
</tr>
<tr>
<td>Vaginal HIV-1 RNA, ( \geq 125 ) copies/swab</td>
<td>121 (69)</td>
<td>117 (66)</td>
<td>.6</td>
<td>1.5 (0.9–2.5)</td>
<td></td>
<td>.1</td>
</tr>
<tr>
<td>Plasma HIV-1 RNA, ( \log_{10} ) copies/mL</td>
<td>5.34 (2.63–7.20)</td>
<td>5.49 (&lt;1.70–7.17)</td>
<td>.1</td>
<td>0.06 (–0.02–0.15)</td>
<td></td>
<td>.2</td>
</tr>
<tr>
<td>CD4 cells/( \mu L ), ( \geq 25 )–997</td>
<td>272 (&lt;25–997)</td>
<td>225 (&lt;25–840)</td>
<td>.04</td>
<td>0.34 (–0.22–0.90)^d</td>
<td></td>
<td>.2</td>
</tr>
<tr>
<td>CD8 cells/( \mu L ), 719 (97–2001)</td>
<td>581 (166–3242)</td>
<td>.08</td>
<td>0.06 (–0.91–1.04)^d</td>
<td></td>
<td>.9</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** CI, confidence interval; HIV, human immunodeficiency virus; OR, odds ratio.

^a Data are median (range) or no. (%) of subjects.

^b One woman in placebo arm did not have vaginal specimens collected at follow-up.

^c For categorical variables (vaginal HIV-1 DNA and vaginal HIV-1 RNA \( \geq 125 \) copies/swab), adjusted odds ratio (OR) and \( P \) are from multivariate logistic regression model. For continuous variables (vaginal HIV-1 RNA, plasma HIV-1 RNA, CD4 cell count, and CD8 cell count), regression coefficient and \( P \) are from multivariate linear regression model. HIV-1 RNA concentrations and CD4 and CD8 cell counts below limits of quantification were set at half those limits.

^d Adjusted regression coefficients for CD4 and CD8 cell counts are from multivariate models and used the square root of CD4 or CD8 cell count at follow-up as the dependent variable in order to approximate normality.

Discussion

In this randomized trial, we found no statistically significant effect of vitamin A on vaginal shedding of HIV-1 or HIV-1–infected cells, HIV-1 plasma virus load, or CD4 or CD8 cell counts, even among women who had serum vitamin A concentrations indicative of deficiency. This trial was motivated by an earlier study from Mombasa that found low serum vitamin A concentrations strongly predicted vaginal shedding of HIV-1–infected cells [1]. Prospective studies of sexually transmitted disease (STD) treatment [8] and antiretroviral therapy [11] have demonstrated that genital tract shedding is amenable to intervention.
We were disappointed that vitamin A supplementation did not reduce shedding in a similar fashion, and we considered possible explanations for our results. The first explanation could be a lack of statistical power. However, we had > 95% power to detect a 3-fold difference in vaginal HIV-1 DNA between randomization groups at follow-up and 74% power to detect a 2-fold difference. We also had high power to detect even small effects of supplementation on our other outcome measures.

Second, the vitamin A dosage may have been inadequate. However, this dosage was nearly 4-fold higher than the US recommended dietary allowance [12] and is the recommended treatment for women with corneal lesions as a result of very severe vitamin A deficiency [9]. No women in this study had such lesions.

Third, our intervention may have been of insufficient length. However, treatment of symptomatic vitamin A deficiency requires only 4 weeks of supplementation [9], and reductions in vaginal HIV-1 shedding have been demonstrated within 3 weeks of STD treatment [8]. This suggests that our 6 weeks of follow-up was sufficient to demonstrate a change in vaginal shedding.

A fourth reason for our results may be that the participants were too advanced in HIV-1 disease stage. However, we found no evidence for a difference in effect between women with >200 and ≤200 CD4 cells. Overall, it seems unlikely that insufficient sample size, vitamin A dose, time to follow-up, or prevalence of immunosuppression in the study population led to our results.

A fifth explanation for our findings is that serum vitamin A levels are not indicative of true vitamin A deficiency in this population. The observational studies relating vitamin A deficiency to HIV-1 progression and transmission used serum vitamin A levels to classify deficiency, and, although they adjusted for CD4 cell count, incomplete control for disease severity may have biased those findings. Some have suggested that the acute-phase response, which lowers serum vitamin A levels during infections even when vitamin A stores are adequate, operates in persons infected with HIV-1 [13]. We found that vitamin A supplementation raised vitamin A levels in our population, except among severely deficient women, although this subgroup would seem most likely to respond to therapy. If low vitamin A levels in part reflect more active HIV-1 infection rather than true deficiency, this may explain the failure to respond to supplementation. Detailed studies of serum vitamin A levels, HIV-1 disease stage, and the acute-phase response may help explain the disappointing findings of trials of vitamin A for HIV-1 infection.

Finally, a sixth possible explanation for our trial results may be that the relationship between vitamin A and HIV-1 vaginal shedding was different among our trial population than among previously studied populations. We found no strong relationship between serum vitamin A and vaginal HIV-1 shedding at baseline. This suggests either that low vitamin A concentrations truly do not predict shedding or that this relationship is not present in all populations. In either case, supplementation seems unlikely to have widespread efficacy in reducing HIV-1 transmission.

Our results are consistent with those of other clinical trials of vitamin A. β-carotene did not increase CD4 cell counts [14], and vitamin A had no effect on HIV-1 plasma load [5] in US trials. In 3 randomized trials in Africa, vitamin A failed to decrease mother-to-child HIV-1 transmission [13, 15]. The disparity between these results and those of earlier observational studies associating low serum vitamin A levels with faster HIV-1 disease progression and increased infectivity emphasizes the importance of randomized clinical trials in evaluating intervention strategies to prevent and treat HIV-1 infection. On the basis of our results and those of other randomized trials, it appears unlikely that there is a role for vitamin A supplementation in reducing infectivity or preventing transmission of HIV-1.

Acknowledgments

We thank the clinic, laboratory, and administrative staff (Virginia Njuki, Mary Wamugunda, Florence Murigi, Khamis Mwinyikai, Amina Abdalla, Gladwell Maina, Bhavna Chohan, Jonathan Saha, Jeanne Gow, Sifuna Cherambai, Collins Mpoya, Hassan Saleh, Del Landicho, Teresa Whisler, Olubayo Johnson, Dana Panteleeff, Abby Petty, Chris Kealy, Eileen Seese, Susan Mello, and Christine Clase-man), for their dedication; the administration of Coast Provincial General Hospital, for providing clinic and laboratory space; and Gen-Probe, for providing reagents for RNA assays. We are grateful to the women whose participation made this study possible.

References


