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The Effect of Supplemental Beta-Carotene on Immunologic Indices in Patients with AIDS: A Pilot Study

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Patients with the acquired immunodeficiency syndrome (AIDS) are characterized by a decrease in the number of T helper cells, a defect that is linked to the impaired immunologic competence. Vitamin A and its dietary precursor, beta-carotene, increase absolute T helper cell counts as well as indices of T cell function in both human and animal models. To determine if short-term beta-carotene treatment affects T lymphocyte subsets in patients with AIDS, a single-blind, non-randomized clinical trial of beta-carotene was performed in seven patients with AIDS. Enrollment criteria included no evidence of: a) active opportunistic infection; b) greater than 1 kilogram change in weight in the month preceding enrollment; c) chronic diarrhea or malabsorption; and d) hepatic disease or significant anemia. Beta-carotene was given with meals in two divided doses of 60 mg/day for four weeks; this was followed by no therapy for six weeks. Samples for total white blood cell, lymphocyte and T lymphocyte subset counts were measured at baseline, at the end of four weeks of treatment and another six weeks after treatment had stopped. P24 antigen, beta-2 microglobulin and liver function tests were also measured.

All subjects tolerated the treatment well without evidence of toxicity. In response to beta-carotene, total lymphocyte counts rose by 66 percent ($.05 < p < .10$), and CD4+ cells rose slightly, but insignificantly, in the entire group. In all three of the patients who had baseline CD4+ cells greater than 10/μl, however, the mean absolute increase in CD4+ cells in response to beta-carotene was 53 ± 10 cells/μl ($p < .01$). Six weeks off beta-carotene treatment, the absolute CD4+ cell count returned to pretreatment levels ($p < .01$). No change was observed in CD8+ cells. P24 antigen and beta-2 microglobulin did not change during treatment. These preliminary observations suggest that short-term treatment with beta-carotene may increase CD4+ cell counts in patients with AIDS who have greater than 10 cells/μl.

INTRODUCTION

Interest in vitamin A and related compounds to modulate human diseases has expanded in recent years. In children, dietary supplementation with vitamin A reduces mortality due to measles by 60 percent [1], even without clinical signs of deficiency [1]. In animals, vitamin A deficiency augments mortality due to a variety of infective organisms [2], and conversely, dietary supplementation enhances survival [2] and lessens morbidity [3]. Although all of the mechanism(s) of vitamin A action are not known on an immunologic

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[f]*Abbreviations:* AIDS, acquired immunodeficiency syndrome; ALT, alanine aminotransferase; ARC, AIDS-related complex; AST, aspartate aminotransferase; PCP, pneumocystis carinii pneumonia; KS, Kaposi's sarcoma; SEM, standard error of the mean.

basis, vitamin A increases absolute T helper cell counts [4] as well as indices of T cell function, such as skin graft rejection [5, 6] or mixed lymphocyte response [7].

Beta-carotene, the major dietary precursor of vitamin A, has similar effects on the immune system. In normal humans, beta-carotene increased OKT4 positive T cells by 40 percent [8, 9] without affecting OKT8 cells. Given these properties of vitamin A and beta-carotene, we conducted a preliminary trial of beta-carotene in patients with the acquired immunodeficiency syndrome (AIDS)[f], which is characterized by impaired immunologic defenses in the setting of a dearth of T helper cells. We chose beta-carotene over vitamin A because of a lower incidence of toxicity [10].

METHODS

Seven patients (six male/one female; mean age 34 ± 3 yr) with Centers for Disease Control and Prevention clinical criteria for AIDS were enrolled in this single-blind study (Table 1). The AIDS defining diagnoses were: pneumocystis pneumonia (four patients), Kaposi's sarcoma (one), candida esophagitis (two). The mean time after diagnosis of AIDS was 67 ± 20 weeks (range 26-164). Eligibility criteria included no evidence of: opportunistic infection, > 1 kg change in weight in the month preceding enrollment, chronic diarrhea or malabsorption, hepatic disease (defined by AST or ALT > 2x normal) or significant anemia (hemoglobin < 10g/dl).

Patients received beta-carotene (Solatene, Hoffmann-LaRoche, Inc.) 60 mg/day in two divided doses with meals for four weeks as well as a daily multi-vitamin to supply 100 percent of the recommended daily allowance of zinc and vitamin E. No new medications were given to any of the patients during the study. Each patient continued, however, with his/her ongoing medications, including zidovudine. The duration of zidovudine therapy was 52 ± 11 weeks (range 16-94). Prior to treatment, all subjects had single baseline measurements of: complete blood count and differential; serum electrolytes and liver function tests; and total lymphocytes and CD4+ and CD8+ subsets. Repeat samples were drawn at approximately the same time of day at the end of four weeks of beta-carotene and then at week 10 (six weeks later off therapy). Conventional and acid dissociated p24 antigen and beta-2 microglobulin were also sampled in all subjects at the baseline and

Table 1. Baseline characteristics of study patients.

Patient	Age (yrs)	Gender	AIDS diagnosis	Duration of AIDS (wks)	AZT Therapy (wks)	CD4+ cells/μl	Weight (kg)
1	45	Male	Candida esophagitis	56	64	144	83
2	38	Male	AIDS dementia	88	92	99	60
3	37	Male	PCP	20	20	7	80
4	24	Male	PCP	91	94	5	71
5	27	Male	PCP	164	40	100	76
6	34	Male	KS	26	28	5	57
7	33	Female	Candida esophagitis	26	16	9	61
Mean ± SEM	33 ± 3	—	—	67 ± 20	52 ± 11	60 ± 21	70 ± 3

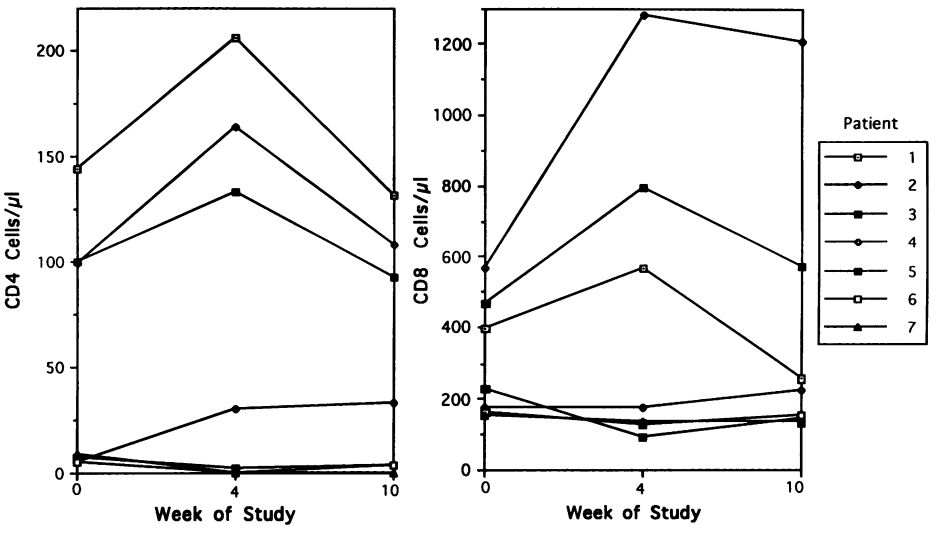

Figure 1. Effect of beta-carotene on T cell subsets. Total CD4+ and CD8+ cell counts before (0), at the end of four weeks (4) and six weeks off treatment (10) in seven patients with AIDS.

week-four time points and at week 10 for subjects 1 to 3. The T cell measurements were done using flow cytometry. All subjects gave informed consent prior to participation, which was approved by the Human Investigation Committee of the Yale University School of Medicine. An ANOVA for repeated measures with post-hoc testing (Duncan's) was used for statistical comparisons of data (True Epistat, Richardson, Texas). Data are reported as mean ± SEM.

RESULTS

All patients tolerated the treatment well; the only side-effect was carotenodermia, which faded after discontinuation of the beta-carotene. No changes in weight or hepatic function were observed. For the entire group, total white blood cell counts did not change but total lymphocyte counts increased from 0.56 ± 0.09 to 0.93 ± 0.24 (x 10^3/µl; .05 < p < .10) between baseline and at the end of beta-carotene treatment. Figure 1 summarizes T cell subset changes during the study. For the entire group of patients, CD4+ cells tended to increase in response to beta-carotene, but this change was statistically insignificant (p < .14). Three of these patients had baseline CD4+ cells greater than 10/µl. Their CD4+ counts demonstrated an average increase of 53 ± 10 cells/µl (or 43 ± 9 percent over baseline; p < .01 by ANOVA, p < .01 baseline vs. four weeks). Total white blood cell counts increased 23 percent in these three patients, from 3.0 ± 0.3 to 3.7 ± 0.03 (x 10^3/µl) due to an increase in total lymphocytes (0.82 ± 0.01 to 1.47 ± 0.29, x 10^3/µl). Six weeks after discontinuing beta-carotene, CD4+ cells returned to baseline values in these three patients (p < .01, 4 vs. 10 weeks). It is noteworthy that patient two also had T cell subsets measured after two weeks of beta-carotene. His CD4+ cells had shown an increase to 134/µl from 94/µl at baseline. At four weeks of treatment, his CD4+ cell count was 164/µl.

As with CD4+ cells, CD8+ cells did not change in the group as a whole. In all three subjects with CD4+ cells greater than 10/µl, CD8+ cells increased from 479 ± 49 to 881 ± 211/µl. Due to a large change in single subject, this was not statistically significant. No change was observed in individual CD4+/CD8+ ratios. Acid dissociated p24 antigen was

detectable in only three of seven patients and did not change in response to beta-carotene. Similarly, beta-2 microglobulin levels were unaffected by beta-carotene (data not shown).

DISCUSSION

The data from this pilot study suggest that beta-carotene increases the CD4+ cell population in AIDS patients who have greater than 10 cells/μl. The response of the small number of patients in the present study is consistent with that observed in normal adults and related effects of vitamin A in animals, i.e., biologically significant increases in CD4+ cells [4, 8, 9]. Although this study focused on patients with AIDS [11], three other trials of beta-carotene in HIV-infected patients have recently been reported. Coodley et al. [12] treated HIV-seropositive patients with beta-carotene (180 mg/day in three divided doses) for four weeks. In comparison to placebo, significant fractional increases were observed in their patients, whose helper cell counts ranged from less than 200 to greater than 500/μl. Those patients with helper cell counts less than 200/μl demonstrated the greatest change in helper cells (+30 percent with beta-carotene compared with -36 percent on placebo). Garewal et al. [13] treated 11 HIV-positive patients with a single, daily dose of beta-carotene (60 mg) for four months. These investigators observed an approximate 50 percent increase in NK cells but without changes in CD4+ cells in the entire group. No data were provided on responses to beta-carotene when analyzed as a function of baseline measurements. Although both Coodley et al. [12] and Garewal et al. [13] studied HIV-seropositive patients, it is not known if any of the patients had clinical manifestations that characterize AIDS. Bianchi-Santamaria et al. [14] recently treated nine AIDS-related complex (ARC) patients with beta-carotene for six to 21 months. In response to treatment, CD4+ cell counts rose by 11.5 percent, a change that was statistically insignificant. Clinically, however, symptomatic asthenia, feverishness, sweating and diarrhea resolved. None of these patients progressed to AIDS during the six to 21-month observation period.

In spite of the positive response observed in the present study, the CD4+ counts at four weeks were still subnormal. It has been demonstrated, however, that the risks for acquiring different opportunistic infections are related to the absolute helper cell count within the subnormal range [15]. Increases in helper T cell number, therefore, may alter these risks. The lack of response in the more significantly CD4+ depleted group may be due to end-stage immunologic dysfunction or a time-dependent factor, since the short treatment period may not have been long enough to elicit an increase.

Recent observations have suggested that vitamin A deficiency is strongly associated with an increased risk of death in patients with AIDS [16]. In this study of a cohort of drug abusers in Baltimore, along with depressed CD4 counts, serum retinol levels below 1.05 μmol/L predicted a four-fold increased risk of death. Moreover, serum retinol [16] or carotene [17] levels correlate with CD4 counts. In HIV positive mothers and their offspring, maternal vitamin A deficiency was associated with an increased risk of HIV transmission to the neonate [18]. Thus, available indicators suggest that vitamin A deficiency is associated with an increased risk of death. It is recognized that although these studies provide strong associations between vitamin A deficiency and adverse outcome in patients with AIDS, many other variables are also affected in these patients, such that vitamin A deficiency may be a harbinger but not a cause of death. For the present study, it is emphasized that firm, statistically sound conclusions about the efficacy of beta-carotene cannot be based upon the responses of the three subjects with CD4 counts greater than 10/μL. Rather, these results need to be considered preliminary. However, when considered in combination with other cited work, the present study forms the spark to encourage larger and longer trials of beta-carotene in this patient population, specifically a double-blind,

placebo and diet-controlled study with an adequate number of patients treated for at least three months time.

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REFERENCES

1. Fawzi, W.W., Chalmers, T.C., Herrera, M.G., and Mosteller, F. Vitamin A supplementation and child mortality: a meta-analysis. JAMA 269:898-903, 1993.
2. Cohen, B.E. and Elin, R.J. Vitamin A induced nonspecific resistance to infection. J. Infect. Dis. 129:597-600, 1974.
3. Seifter, E., Rettura, G., Padawer, J., Demetriou, A.A., and Levenson, S. Antipyretic and antiviral action of vitamin A in Moloney Sarcoma virus and poxvirus inoculated mice. JNCI 57:355-359, 1976.
4. Malkovsky, M., Edwards, A.J., Hunt, R., Palmer, L., and Medawar, P.B. T-cell mediated enhancement of host versus graft reactivity in mice fed a diet enriched in vitamin A acetate. Nature 302:338-340, 1983.
5. Floersheim, G.L. and Bollag, W. Accelerated rejection of skin homografts by vitamin A acid. Transplantation 15:564-567, 1974.
6. Malkovsky, M., Medawar, P.B., Thatcher, D.R., Toy, J., Hunt, R., Rayfield, L.S., and Dore, C. Acquired immunological tolerance of foreign cells is impaired by recombinant interleukin 2 or vitamin A acetate. Proc. Natl. Acad. Sci. 82:536-538, 1985.
7. Cohen, B.E., Gill, G., Cullen, P.R., and Morriss, P.J. Reversal of postoperative immunosuppression in man by vitamin A. Surg. Gynecol. Obstet. 149:658-662, 1979.
8. Alexander, M., Newmark, H., and Miller, R.G. Oral beta-carotene can increase the number of OKT4+ cells in human blood. Immunol. Lett. 9:221-224, 1985.
9. Watson, R.R., Prabhala, R.H., Plezia, P.M., and Alberts, D.S. Effect of beta-carotene on lymphocyte subpopulations in elderly humans: evidence for a dose-response relationship. Am. J. Clin. Nutr. 53:90-94, 1991.
10. Hathcock, J.N., Hattan, D.G., Jenkins, M.Y., Mcdonald, J.T., Sundaresan, P.R., and Wilkening, V.L. Evaluation of vitamin A toxicity. Am. J. Clin. Nutr. 52:183-202, 1990.
11. Fryburg, D.A., Mark, R.J., Griffith, B.P., Askenase, P.W., and Patterson, T.F. The immunostimulatory effects and safety of beta-carotene in patients with AIDS. VIII International Conference on AIDS/III STD World Congress. Netherlands: Amsterdam; 1992, pp. B163.
12. Coodley, G.O., Nelson, H.D., Loveless, M.O., and Folk, C. Beta-carotene in HIV infection. J. Acquir. Immune. Defice. Syndr. 6:272-276, 1993.
13. Garewal, H.S., Ampel, N.M., Watson, R.R., Prabhala, R.H., and Dols, C.L. A preliminary trial of beta-carotene in subjects infected with the human immunodeficiency virus. J. Nutr. 122:728-732, 1992
14. Bianchi-Santamaria, A., Fedeli, S., and Santamaria, L. Possible activity of beta-carotene in patients with the AIDS-related complex: a pilot study. Med. Oncol. 9:151-153, 1992.
15. Phair, J., Munoz, A., Detels, R., Kaslow, R., Rinaldo, C., and Saah, A. The Multicenter AIDS Cohort Study Group: the risk of pnemocystis carinii pneumonia among men infected with human immunodeficiency virus type I. N. Engl. J. Med. 322:161-165, 1990.
16. Semba, R.D., Graham, N.M.H., Caiaffa, W.T., Margolick, J.B., Clement, L., and Vlahov, D. Increased mortality associated with vitamin A deficiency during human immunodeficiency virus type I infection. Arch. Intern. Med. 153:2149-2154, 1993.
17. Ullarich, R., Schneider, T., Heise, W., Schmidt, W., Averdunk, R., Reicken, E.-O., and Zeitz, M., for the Berlin Diarrhoea/Wasting Syndrome Study Group: serum carotene deficiency in HIV-infected patients. AIDS 8:661-665, 1994.
18. Semba, R.D., Miotti, P.G., Chiphangwi, J.D., Saah, A.J., Canner, J.K., Dallabetta, G.A., and Hoover, D.R. Maternal vitamin A deficiency and mother to child transmission of HIV-1. Lancet ii:1539-1599, 1994.