The effect of vitamin A supplementation on stimulated T-cell proliferation with myelin oligodendrocyte glycoprotein in patients with multiple sclerosis

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ABSTRACT

Background: Multiple sclerosis (MS) is an autoimmune disease whereby myelin sheath of the central nervous system is destroyed. Vitamin A is known to play a role in the immune system. It has been recognized that some metabolites of vitamin A can be used effectively to treat experimental autoimmune encephalomyelitis (EAE). **Aims:** The effect of vitamin A as retinyl palmitate on T-cell proliferation in MS patients. **Setting and Design:** This study is a double blind clinical trial of two test groups over a period of 6 months. **Materials and Methods:** Thirty five multiple sclerosis (MS) patients were divided into two groups. One group received 25,000 IU/day vitamin A (as retinyl palmitate) and the other group were administered a placebo. The peripheral blood mononuclear cells (PBMCs) were separated and stimulated with myelin oligodendrocyte glycoprotein (MOG) and phytohemagglutinin (PHA) before and after the trial period. BrdU calorimetric assay was performed to measure cell proliferation. **Statistical Analysis:** Analysis of covariance (ANCOVA) and paired t-test were used to analyze the data. **Results:** Observations showed statistical significant differences in the reduction of cell proliferation in the presence of MOG and fetal calf serum (FCS) in the culture medium, between patients receiving vitamin A and the placebo ($P = 0.046$). Although, this difference was not significant between the two vitamin A and placebo groups in MOG treatment with human serum, a decrease was observed in the group of patients taking vitamin A supplements ($P = 0.019$). Phytohemagglutinin did not cause any change in cell proliferation between the two groups. **Conclusion:** The results suggest supplementation with retinyl palmitate in patients with MS reduce MOG stimulatory effects on T-cells.

Key words: Cell proliferation, multiple sclerosis, myelin oligodendrocyte glycoprotein, vitamin A

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease and major disorder of the central nervous system (CNS), which usually begins between the ages of 20 to 40 years.[1] The exact etiology of MS remains unknown but there is strong evidence to support the theory that autoimmune mechanisms such as Th1 like response from myelin specific CD4^{+} T-cells are considered a part of the pathophysiology of this disease.[2,3] The main event in the pathogenesis of MS is myelin sheath destruction. Therefore, proteins in the myelin of the CNS are likely candidates to be involved in the pathogenic self- response.[4]

Myelin oligodendrocyte glycoprotein (MOG) is one of a few autoantigens on the surface of the myelin sheath that is readily available to autoreactive T-cells and because MOG is the only protein that is expressed in CNS, the response of T-cells likely plays an important role in the pathogenesis of MS.[4]

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Additionally, vitamin A plays an important role in the immune system and retinoids have been identified as having many functions that regulate immune responses.[5] Retinoids, metabolites of vitamin A and ligands of its steroid receptor superfamily are useful in the treatment of experimental autoimmune encephalomyelitis (EAE).[6] Experimental autoimmune encephalomyelitis is an animal model of MS and occurs by immunization with myelin antigens such as myelin basic protein (MBP), proteolipid protein (PLP) or MOG.[7-12] The studies that were conducted on retinoic acid (RA) make the assumption that vitamin A may perform a direct immune function through its effect on mitogenic adjustment signals in T-cells.[13]

Retinyl palmitate is a retinol ester that can be converted to RA in the body.[14] According to the positive impact of vitamin A on the immune system as well as on EAE, the animal model, and as a high level of RA is potentially toxic, this study aimed to examine the potential of vitamin A (as retinyl palmitate) supplement to reduce a mitogenic response of MOG as an autoantigen in patients with MS.

Materials and Methods

Participants

Thirty six relapsing-remitting MS (RRMS) patients consented to participate in this survey (written consent was obtained from all of them); they were under the guidance of two neurologists, (one subject withdrew for personal reasons in mid-project, so 35 people remained until the end of the study). The Ethics Committee of Tehran University of Medical Sciences approved the study. All patients used Interferon beta-1a (Avonex) as treatment. This study was a randomized double blind clinical trial with vitamin A (as retinyl palmitate) and placebo. A general questionnaire was provided for each patient that set out an explanation of the essential information about vitamin A supplementation. The supplements were taken for a period of 6 months as 25,000 IU retinyl palmitate (Zahravi, Tabriz, Iran) per day, to this way that they had been given either vitamin A or placebo for 3 months and after this period, their liver function and clinical signs were checked and either vitamin A or placebo was given to them for a further 3 months if no problems were diagnosed.

Sample blood collection

Blood samples were collected from the subjects in sterile tubes with Heparin as the anticoagulant. 1 ml of blood samples were saved in a sterile microtube without any anticoagulant for serum blood isolation. Peripheral blood mononuclear cells (PBMCs) were isolated on Ficoll-Histoprep gradients (BAG Health Care GmbH, Germany). Cells were counted and diluted to 1×10^6 cells per ml culture medium (RPMI 1640; PAA laboratories GmbH, Austria). 500 µl of this cell dilution was separated and 10% fetal calf serum (FCS; Gibco, Invitrogen, UK) was added to the remaining cells. 75,000 PBMC were cultured in 96-well flat-bottomed microtiter plates (Nunc, Thermo Fisher Scientific Inc, Denmark) in the presence of 5 µl/ml MOG [(35-55) human; Anaspec, USA]. 75,000 PBMCs from diluted cells without FCS were cultured in the presence of MOG as above and then 10 µl of serum from coagulated blood was added to these cells. 75,000 cells were left stimulated with 5 µl/ml phytohemagglutinin (PHA; Sigma, USA) and untreated. Cells were incubated at 37 ºC in a 5% CO_2 incubator for 96 h. After this period, a cell proliferation ELISA BrdU kit (Roche Diagnostics GmbH, Germany) was used for cell proliferation assessment. Briefly BrdU was added to each well and incubated for 4 h in the same incubator. After this period, the next stages were performed according to company protocol. The absorbance was measured at 450 nm using ELISA plate reader. The design of study has been summarized in a flow chart [Figure 1].

Statistical analysis

Data were analyzed using SPSS version 17.0 for windows. Analysis of covariance (ANCOVA) was used to test the main and interaction effects of categorical variables on a continuous dependent variable. Data taken from each
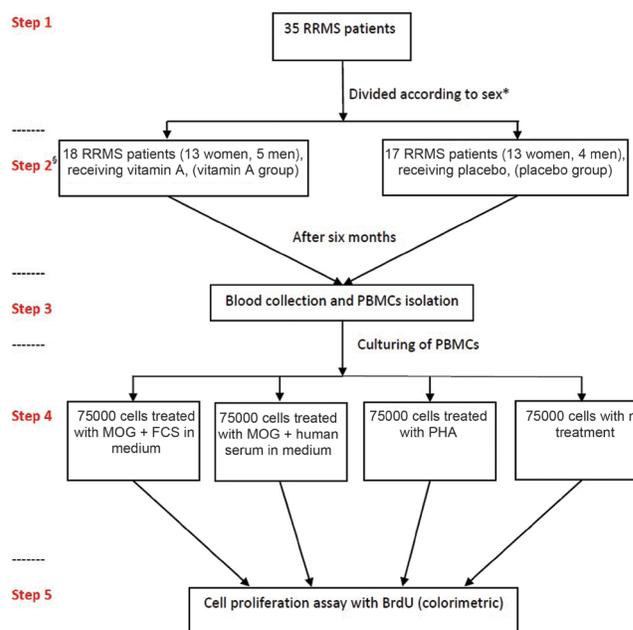
*This was a randomized double blind clinical trial.

§Steps 3,4 and 5 were also performed for all participants before supplementation.

Figure 1: Study design to determine the effects of vitamin A on cell proliferation in RRMS patients

group was compared by paired-sample T-test. *P*-value less than 0.05 was considered statistically significant.

Results

The thirty five subjects in this study were divided into two groups: the vitamin A group (18 patients) and the placebo group (17 patients). 74.3% of participants were female and 25.7% were male. The ratio of women to men was approximately 2.9. The mean age was 32 years. Table 1 shows the profile of patients participating in the study.

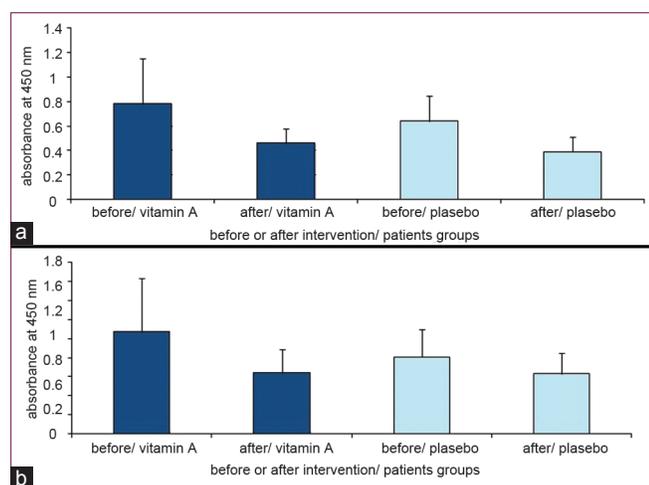In Figure 2, results of cell stimulation with MOG are shown. From two different treatments of MOG, the treatment that contained the fetal calf serum (FCS) showed significant difference between the group treated with vitamin A and the placebo group (*P* = 0.046), as determined with ANCOVA statistical analysis [Figure 2a].

In another assay MOG with human serum was used to stimulate cells, but no statistically significant differences were observed in stimulated cells between the vitamin A treated group and the placebo group (*P* = 0.21, ANCOVA). This indicated that the proliferation of cells treated with MOG and human serum has been decreased in both vitamin A and placebo groups. Although, the reduction in cell proliferation occurred in both groups after the supplementation period, this reduction in the group receiving vitamin A had a statistically significant difference compared with before of supplementation (*P* = 0.019 and *P* = 0.054 in vitamin A and placebo groups respectively; analyzed by paired sample T-test) [Figure 2b]

Results of stimulated cell proliferation with PHA are shown in Figure 3 and reveals no differences between the two groups before and after 6 months supplementation period (*P* = 0.22; analyzed by ANCOVA; *P* = 0.84 and *P* = 0.9 in vitamin A and placebo groups respectively; analyzed by paired sample T-test). Cells cultured without mitogen treatment showed no difference in proliferation between the two groups (*P* = 0.47 analyzed by ANCOVA) [Figure 4].

Discussion

Myelin oligodendrocyte glycoprotein is a membrane glycoprotein with 218 amino acids from a superfamily of immunoglobulin that has a lower amount than other

Figure 2: (a) Cell proliferation with MOG in the presence of FCS in culture medium in the two groups; the group receiving vitamin A supplement and that which received the placebo. (b) Cell proliferation with MOG in the presence of his/her blood serum (Data are presented as mean ± SD)

Table 1: Profile of patients participating in the study

|		Vitamin A	Placebo	Total
Age (years) †		33.22 ± 7.26	31.05 ± 5.90	32.17 ± 6.60
BMI †	Before	24.10 ± 4.2	25.1 ± 4.4	65.8 ± 13.6
	After	24.8 ± 4.2	25.6 ± 4.6	67.4 ± 14.0
Weight (kg) †	Before	64.6 ± 14.7	67.2 ± 12.74	24.6 ± 4.2
	After	66.4 ± 15.1	68.5 ± 13.3	25.2 ± 4.30
Sex#	Male	5 (14.3%)	4 (11.4%)	9 (25.7%)
	Female	13 (37.15%)	13 (37.15%)	26 (74.3%)
Education#	Under diploma	0 (0.0%)	2 (5.7%)	2 (5.7%)
	High School diploma	7 (20.0%)	2 (5.7%)	9 (25.7%)
	Batcher of Science	10 (28.6%)	11 (31.4%)	21 (60.0%)
	Master of Science	1 (2.9%)	2 (5.7%)	3 (8.6%)
Date of disease detection#	6-12 months prior the study	3 (8.6%)	3 (8.6%)	6 (17.2%)
	1-3 years prior the study	8 (22.9%)	5 (14.2%)	13 (37.1%)
	More than 3 years	7 (20.0%)	9 (25.7%)	16 (45.7%)
Marriage#	Single	8 (22.8%)	6 (17.2%)	14 (40.0%)
	Couple	10 (28.6%)	11 (31.4%)	21 (60.0%)

†mean ± SD, #number (percent)
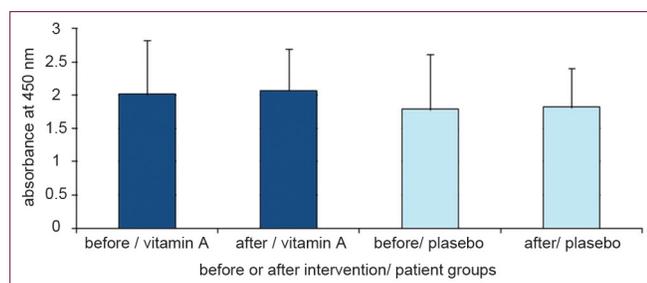
Figure 3: Cell proliferation in the presence of PHA in culture medium. Retinyl palmitate could not change T-cell proliferation in presence of PHA (Data are presented as mean ± SD)

Figure 4: Cell proliferation without any treatment. No differences have been obtained in two groups who receiving vitamin A supplement and placebo. (Data are presented as mean ± SD)

myelin proteins and is located in the outer surface of the oligodendrocyte membrane. This location makes it directly accessible to antibodies and is therefore a target for immune responses in MS.[1] Among other myelin antigens such as MBP or PLP, MS patient's lymphocytes are the most responsive to MOG.[15] In this study, it has been demonstrated that a vitamin A supplement taken by people with relapsing-remitting MS was able to reduce lymphocyte proliferation stimulated by MOG compared with those that received placebo.

The ability of vitamin A to control growth and differentiation is dependent on the metabolism of retinol to RA.[16,17] In the first step alcohol dehydrogenases (ADH) converts retinol to retinaldehyde; the next step is oxidation of retinaldehyde to RA by retinaldehyde dehydrogenases (RALDH). Retinoic acid acts as a ligand for RA receptor signaling.[16,17] Many studies have indicated that vitamin A regulates the secretion of IL-10 from Th2 cells. IL-10 can inhibit the synthesis of IL-2 inflammatory cytokine from Th1 cells.[18] According to reports, RA can stimulate the proliferation of normal peripheral lymphocytes mediated IL-2.[13] Two consecutive signals are required for resting lymphocyte proliferation. First, the antigenic stimulation of TCR/CD3 complex leads the cell cycle from a quiescent state to the G1 phase;[19,20] secondly IL-2 binding to its receptor drives the G1 phase to S, so that proliferation occurs.[21] It has been shown that RA can reduce the production of IL-2[18,22,23] through its direct effect on IL-2 gene expression,[24] suggesting that reduction in cell proliferation stimulated by MOG in this study could be due to reduced production of IL-2.

Further studies on cell proliferation under the stimulation of MBP *in vitro* showed reduced proliferation in a dose-dependent procedure of all *trans*-RA (ATRA). [6] In another study, different concentrations of ATRA on MBP-specific lymph node cell proliferation in SJL/J mice stimulated with MBP revealed that ATRA inhibited T-cell response to MBP in a dose-dependant manner. [22] The effect of 13-*cis*-RA on lymphocyte proliferation (without the
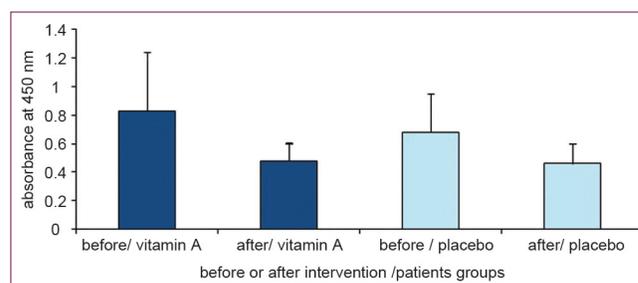
presence of a specific antigen) after oral administration to Lewis rats showed that when serum levels of 13-*cis*-RA reached the desired level, lymphocyte proliferation responses in these animals decreased.[25] In another survey on MS patients, lymphocyte proliferation was studied by adding RA dissolved in DMSO to a culture medium and no changes were observed in proliferation.[26] This study revealed that no significant difference in cell proliferation was stimulated with PHA and without any treatment between two groups, that which received vitamin A and that which received placebo, perhaps due to an only moderating effect of vitamin A on mitogenic reactions influenced by MOG antigens in these patients.

Conclusion

The results show vitamin A effects on the proliferation of PBMCs stimulated with MOG but no effect on the proliferation of PBMCs stimulated with PHA. The vitamin A effect on MOG can decrease the activity of this specific antigen on T-cell stimulation *in vitro*.

Acknowledgements

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