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Research Article

Clinical Evaluation of Vitamin A Supplementation on Disease Development, Progression and Treatment of Multiple Sclerosis: Current Evidence and Future Perspectives

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Abstract

Vitamin A constitutes an essential nutrient with important actions in immunological responses and the Central Nervous System (CNS). Neuro-immunological functions of vitamin A are mediated through its active metabolite, Retinoic Acid (RA). RA contributes to the regeneration and plasticity of the CNS, exerting also a key role in enhancing tolerance and reducing inflammatory responses by regulating T- and B- cells, as well dendritic cells' populations. Several important studies have documented low plasma vitamin A levels in patients suffering from Multiple Sclerosis (MS). Vitamin A deficiency also leads to dysregulation of immune tolerance and pathogenic immune cell production in MS.

In view of the above, the present review aims to critically summarize and discuss the currently available clinical studies, focusing on the potential beneficial effects of vitamin A on controlling MS pathophysiology.

Cochrane Register of Controlled Trials (CENTRAL), BioMed Central, and MEDLINE databases database was thoroughly searched, using relative keywords, in order to identify clinical trials published in English. According to the existing clinical studies, the role of vitamin A in MS could be dual: it may decrease inflammation, while, at the same time, it may increase autoimmunity tolerance, also contributing to brain protection of MS patients. However, it must be stated that, at the present time, there is no clear clinical indication for using vitamin A as a complimentary treatment for MS.

Further clinical trials with vitamin A supplementation as a potential co-treatment agent or as an add-on option are strongly recommended.

Keywords: Vitamin A; Multiple sclerosis; Inflammation; Immunological response; Central nervous system

Introduction

Vitamin A is a lipid soluble vitamin that cannot be synthesized by mammals and must be obtained from the diet, as either pre-formed vitamin A (e.g. liver) or carotenoid-containing fruits and vegetables [1]. Retinoid homeostasis is a tightly regulated process with a number of specific carrier proteins and enzymes, being involved in transportation, storage, metabolism and clearance. Vitamin A (and pro-vitamin A in the form of carotenoids) is absorbed via the mucosal cells of the small intestine [2,3] and can either be transported to target tissues or stored in the liver [4]. Through binding to Retinoic Acid Receptors (RARs) and Retinoid X Receptors (RXRs), this ligand regulates target gene transcription [5,6]. Retinoic acid can enhance or decrease expression of more than 500 genes depending on the target cell type and the physiological state of the organism [6,7].

Multiple Sclerosis (MS) is a demyelinating disease, in which the insulating covers of nerve cells in the brain and the spinal cord are damaged [8]. MS constitutes a chronic inflammatory disease that leads to degeneration of the brain and spinal tissue. Imbalance of CD4+ T-cells, their secreted cytokines and their relative gene expression are all-important aspects of MS immune-pathogenesis [9]. MS usually appears in adults in their late twenties or early thirties, whereas it rarely starts in childhood or after 50 years of age [10]. Similar to many other autoimmune disorders, MS is more common in women, and this trend prevalence is expected to in-crease in the future [8]. As of 2008, it is about two times more common in women than in men globally [10].

The formation of lesions in the CNS (also called plaques), the inflammation and the destruction of myelin sheaths of neurons are considered to be the three basic characteristics of MS. The interaction of these features to produce the breakdown of the nerve tissue and, as a result, the following disease symptoms, are very complicated and not entirely well understood, so far [8]. It is believed that the interaction of the individuals' genetics and several currently unidentified environmental factors may lead to increased risk for MS development [11,12]. Also, MS involves the loss of oligodendrocytes, which are the

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responsible cells for creating and maintaining myelin, which supports the neurons to carry electrical signals [8]. When myelin is destroyed, a neuron can no longer effectively conduct electrical signals. Then, a repair process, called remyelination, takes place in early phases of the disease [13], but repeated attacks from the immune system lead to successively less effective remyelination, until a scar-like plaque is built up around the damaged axons [14]. Regardless of the underlying conditions for MS, some damage is triggered by a Cerebrospinal Fluid (CSF) unknown soluble factor, which is produced in meningeal areas and diffuses into the cortical parenchyma. This factor can destroy myelin either in a direct or indirect way through microglia activation [15].

Several disease phenotypes have been the focus of many studies. The course of MS is used by phenotypes in order to predict future disease progression. They are important not only for disease prognosis, but also for treatment decisions. In 1996, the United States National Multiple Sclerosis Society described four clinical courses [16]. In 2013, these courses were reviewed by an international panel, adding clinically isolated syndrome and radiologically isolated syndrome as phenotypes, without changing the main structure [17]. These phenotypes include Relapsing-Remitting MS (RRMS), characterised by periods of neurological relapses, followed by remissions; Secondary Progressive MS (SPMS), in which there is a gradual progression of neurological dysfunction with fewer or no relapses; Primary Progressive MS (PPMS), in which there is neurological deterioration from disease onset. It should be noted that the Progressive Relapsing MS (PRMS) entity was removed in the 2013 review [17].

Most of the currently available evidence, as far as MS aetiology is concerned, suggests that disease prevalence depends on the interaction between diet and visible sunlight exposure. The recommended diet program includes supplementation with fish oils, avoidance of saturated fats, and the associated intake of antioxidants with unsaturated fatty acids [18]. The antioxidant properties of vitamin A may lead to inhibition of leukotriene synthesis [19], increasing tolerance and decreasing inflammation [20]. Moreover, vitamin D is considered to exert an important immune function. Notably, low serum levels of 25-Hydroxyvitamin D (25OH-vitamin D) were associated with enhanced disease progression over 5 years in a large population of individuals presenting a first demyelinating episode [21]. Visible solar radiation could be of benefit due to vitamin A releasing from visual pigment rhodopsin [19]. It should be noted that 1,25 (OH)-vitamin D and RA may exert synergistic effects on the regulation of T-cells, in particular T helper 17 (Th17) cells [22]. Thus, the epidemiological observations on the prevalence of MS may be attributed to the inter-action of both factors [19].

Fragoso et al. have documented that several environmental modifiable factors may be involved in MS, such us low adherence to treatment, smoking, obesity, low levels of liposoluble vitamins A and D, high salt consumption, and a sedentary life-style [12]. A recent meta-analysis has also shown that smoking is strongly associated with increased MS prevalence [23]. Notably, smokers are in two times greater risk of developing MS compared to non-smokers [24]. MS patients who smoke also tend to have a more severe disease course and a faster disability progression rate [25]. Moreover, individuals, who were overweight or obese during childhood or adolescence, are more likely to develop MS in adulthood. It has also been reported that physical exercise may induce favorable changes in T-cells, by reducing plasma levels of both interferon gamma (INF-γ) and interleukin-17 (IL-17) [18]. The somatic-affective improvement in mood also occurs due to regular physical activities [26]. Moreover, vitamin A adequacy may ameliorate several pro-inflammatory states that enforce MS disease onset [20]. Considerable improvement in MS in-flammatory conditions could be achieved by smoking cessation, reducing over-weight or obesity, enhancing physical activities or increasing vitamin levels. The possibility of modification of these environmental risk factors could be an important approach in MS management [12]. Notably, there are more than 136 ongoing clinical trials on National MS Society website, testing different FDA approved therapeutic agents for MS relapsing forms [27]. This indicates that a new approach for the treatment of MS disease is being investigated.

Materials and Methods

Cochrane Register of Controlled Trials (CENTRAL), BioMed Central, and MED-LINE databases were thoroughly searched until December 2017. The selection criteria were clinical trials, *in vivo* trials and English language. The keywords that were used were "vitamin A", "multiple sclerosis", "supplementation". Another se-lection criterion for the trials was the correlation of vitamin A supplementation with the effect of vitamin A or its derivatives on the progression or against disease development, progression and treatment of multiple sclerosis.

Results

Utility of Vitamin A and its metabolites against MS pathogenesis: Potential beneficial actions

The role of Th17 cells and T regulatory (Treg) cells in MS pathogenesis, the effect of vitamin A and its active metabolite RA, as well as the management of inflammation have been analyzed by many, mainly *in vitro*, studies. Also, it is known that in MS, the balance between Th17 cells and Treg cells is impaired [28]. It was shown by magnetic resonance imaging that serum-retinol may predict new T1Gd (+) and T2 lesions six months ahead. Notably, an increase of retinol by 1μmol is likely to decrease the risk of developing Gadolinium (Gd) enhancing lesions (Gd-enhancement is a marker for blood brain barrier breakdown, and histologically is correlated with the inflammatory phase of lesion development), new T2 lesions and active lesions by 49%, 42% and 46%, respectively [29].

Vitamin A and its metabolites also appear to be effective in preventing progression of several autoimmune diseases, including MS. More to the point, vitamin A has been considered to be an essential nutrient that exerts important actions in immuno-logical responses and CNS [30]. Specifically, neuro-immunological functions of vita-min A are mediated through its active metabolite, RA. In the CNS, RA contributes to regeneration and plasticity, playing also a key role in enhancing tolerance and reducing inflammatory responses by regulating T- and B- cell and dendritic cell populations. Substantial clinical evidence has indicated that MS patients are characterised by significantly low plasma vitamin A levels and that vitamin A deficiency may lead to dysregulation of immune tolerance, inducing pathogenic immune cell production [30].

Vitamin A may ameliorate MS pathogenesis through several mechanisms, including reduction of inflammatory processes by

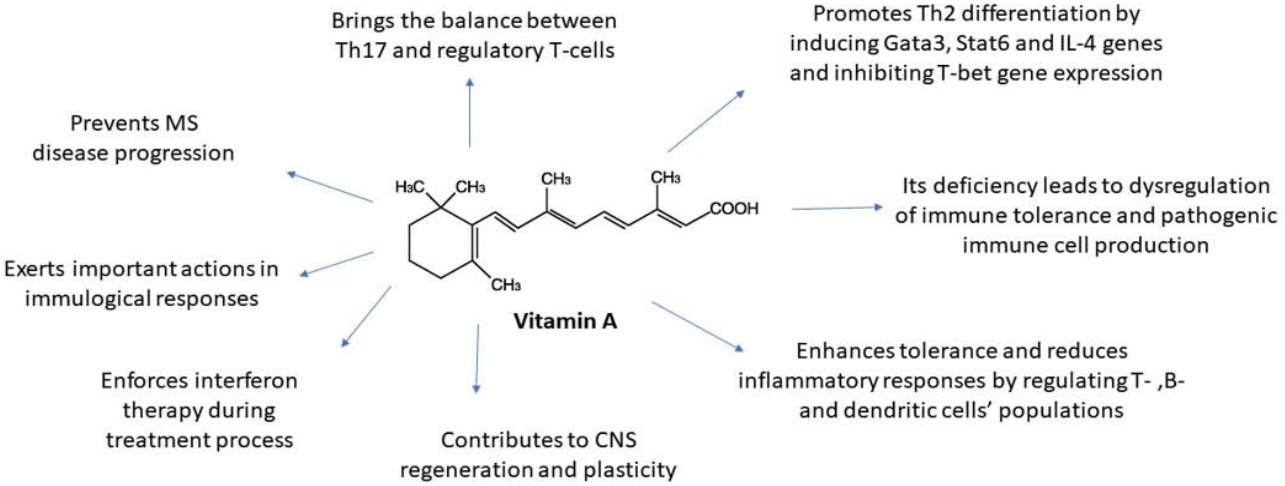

Figure 1: The potential beneficial actions of vitamin A against Multiple Sclerosis.

re-establishing the balance between pathogenic (Th1, Th17, Th9) and immune-protective (Th2, Treg) cells, modulation of B-cells and dendritic cells functions, as well as increase of autoimmunity and regeneration tolerance in the CNS [30,31]. Thus, vitamin A could be considered as a potential co-treatment agent in MS disease management [30,31]. Vitamin A supplementation also inhibits Th1 cells' and promotes Th2 cells' differentiation, both *in vitro* and *in vivo* [20]. Moreover, RA promotes Th2 cells' differentiation by inducing Gata3, Stat6, and IL-4 genes and inhibiting T-bet gene expression [20]. Vitamin A could also decrease peripheral blood mononuclear cells proliferation in the presence of myelin oligodendrocyte glycoprotein [25]. In addition, it has been reported that RA can modulate gene expression of specific nuclear receptors, including Fork box P3 (FoxP3) [3]. Several studies have also shown that RA may elicit pro-inflammatory Th1 and Th17 cells' responses to infection [20]. Notably, RA receptor alpha (RARα) seems to be a critical mediator of the above beneficial effects. A functional role for RA-RARα axis in the development of both regulatory and inflammatory reactions governing adaptive immune system has also been suggested [13]. In Figure 1, the potential beneficial actions of vitamin A by its metabolites, against MS are depicted.

Clinical evidence for the potential effects of vitamin A on MS development and progression

The deficiency of vitamin A in the human body and its association with the development of free oxidative radicals, as well as the development of MS, have been investigated by several studies [32]. Moreover, the low concentration of lipophilic antioxidants in the blood of MS patients has been reported to negatively affect the bioenergetics reparative remyelinating processes (Bioenergetics are the part of biochemistry concerning the energy relationships and energy transformations and transductions in living organisms). This leads to neurodegeneration, while peroxidation, which is correlated with MS, seems to be affected by lipophilic antioxidants, since beta-carotene, the most well-known provitamin A carotenoid, has been associated with IgF intrathecal synthesis and free radicals elimination [27]. From a clinical point of view, there are currently X clinical studies evaluating the potential beneficial effects of vitamin A on MS disease development, progression and co-treatment (Table 1).

In addition, Runia et al. compared vitamin A levels between MS patients and healthy controls in order to investigate whether vitamin A levels may be associated with disease relapse risk [33]. In this prospective longitudinal study, 73 RRMS patients participated and their serum samples for all-trans-retinol measurements were taken every 8 weeks. Potential associations between all-trans-retinol concentrations and relapse rates were evaluated, as well as potential associations between vitamin A and vitamin D [33]. Mean vitamin A levels were found to be lower in MS patients compared to healthy individuals at a borderline significant level, though. During 1.7 years of follow-up, 58 patients experienced 139 relapses in total. It should be noted that serum all-trans-retinol and vitamin D were positively correlated, however this correlation was weak. Moreover, the researchers did not find convincing evidence that vitamin A can exert any positive impact on the disease course of RRMS patients. However, they found a week association between vitamin A and vitamin D levels in the RRMS patients, possibly due to the intake of dietary products that contain the two fat-soluble vitamins [33]. In another study, Røsjø et al. explored the relationships between vitamins A, D and E and inflammation in RRMS patients [34]. For this purpose, the associations between vitamin A, D and E, and 11 inflammation markers of 85 RRMS patients, before and during IFN-β1α treatment were evaluated. A negative association between vitamin A and pentraxin 3, independently of IFN-β1α use, was recorded. Moreover, positive associations between vitamin D and IL-1 receptor antagonist and secreted frizzled-related protein 3 were documented. These findings were observed both before and during IFN-β1α treatment. The above findings may suggest associations with diverse inflammatory pathways, which could differentially be influenced by IFN-β1α treatment in RRMS [34].

It is well-established that vitamin A and its derivatives may modulate the immune system via RAR. In this aspect, Bitarafan et al. explored the impact of retinyl palmitate supplementation on RAR subtype gene expression in peripheral blood mononuclear cells derived from MS patients [35]. In this aspect, a double-blind randomized clinical trial was performed on 19RRMS patients. In

Table 1: Clinical studies evaluating vitamin A supplementation against Multiple sclerosis disease.

Study population	Studyperiod /and dose	Mainresults	Ref.
SPMS patients, males (n=11) and females (n=28), aged 21-44 years	Vitamin A (25,000 IU retinyl palmitate) for 6 months	Anti-inflammatory response by upregulation of TGF-β and FoxP3 expression gene Promising complementary approach and disease slowdown.	[31]
SPMS patients (n=17) and healthy controls (n=25)	Venous blood sample before starting INF-β1b treatment and 1 week later	INF-β1b partially restored defective T suppressor cell function. INF-β1b beneficial action was synergistically potentiated by RA.	[37]
SPMS patients(n=19), aged 20 to 40 years, BMI<30	Daily administration of 25,000 IU retinyl palmitate for 6 months	Vitamin A significantly downregulated RAR-α gene expression in PBMCs by *in vivo* regulatory mechanisms for its action on the immune system.	[34]
SPMS patients, males (n=11) and females (n=28), aged 21-44 years	Daily administration of 25,000 IU retinyl palmitate, for 6 months	Vitamin A down regulates IL-17 and RORγt gene expression. No changes in gene expression occurred in the placebo group.	[36]
RRMS patients (n=85)	Omega-3 or corn-oil (placebo) administration for 24 months	Negative association between vitamin A and pentraxin 3, independently of IFN-β1α use.	[33]
RRMS patients (n=101), aged 20-45 years, BMI: 18.5-30	25000 IU/dretinyl palmitate for 6 months followed by 10000 IU/d retinylpalitate for another 6 months	Reduction of progression of disability, upper limb and cognitive functions.	[28]
RRMS patients, (n=101), aged 20-45 years	25000 IU/dretinyl palmitate for 6 months followed by 10000 IU/d retinylpalitate for another 6 months	Vitamin A improved the depression through the modulation of inflammatory conditions.	[29]

PBMCs: Peripheral blood mononuclear cells.

fact, both intervention and placebo groups received one capsule of 50,000IU vitamin D per 2 weeks and one intramuscular injection IFN-β1α per week. Moreover, the intervention group received one 25,000IU retinyl palmitate capsule daily for 6 months, whereas the placebo group received one placebo capsule daily. Peripheral blood mononuclear cells were isolated from participants and RAR-α and RAR-γ genes expression levels were assessed. After supplementation, RAR-α gene expression levels were significantly decreased in the intervention group compared to the placebo one. On the other hand, RAR-γ gene expression levels were not significantly altered. In view of the above findings, it was speculated that vitamin A supplementation may significantly reduce RAR-α gene expression levels in peripheral blood mononuclear cells of MS patients, supporting evidence for potential *in vivo* regulatory mechanisms of vitamin A action on the immune system [35]. In another study, Mohammadzadeh Honarvar et al. [34] investigated the role of vitamin A on RAR-related orphan receptor gamma (RORγt) and IL-17 gene expression in 39RRMS patients [36]. In particular, the patients in the vitamin A intervention group received 25,000IU retinyl palmitate per day for 6 months, while the patients in the placebo group received one capsule of placebo per day for 6months. In this clinical study, it was clearly found that vitamin A downregulated both IL-17 and RORγt gene expression, whereas no such changes in the placebo group were found [36].

An important issue that deserves special consideration is the fact that vitamin A is a fat-soluble compound and its long-term consumption in high doses may exert some adverse effects. In this aspect, Jafarirad S et al. investigated its possible complications, searching for potential solutions to minimize its adverse effects [37]. In this double blind, randomized clinical trial, vitamin A (as retinyl palmitate) was administrated to 35 RRMS patients in order to investigate whether it could regulate their immune system with a dose of 25000IU/day for a period of 6 months. Lipid profiles, fasting blood sugar and liver enzymes were assessed to explore the possible biochemical complications [37]. It was found that vitamin A did not affect lipid pro-files, fasting blood sugar and liver enzymes in both the intervention and placebo groups. However, the authors suggested that frequent clinical and biochemical evaluations were strongly required along with vitamin A supplementation [37].

Furthermore, Salzer et al. examined whether vitamin A levels were associated with MS risk in samples collected prospectively and during gestation [38]. More to the point, this clinical study evaluated Retinol Binding Proteins (RBPs), a family of proteins with diverse functions, and high-sensitivity C-Reactive Protein (hs-CRP) levels in prospectively collected bio-bank blood samples derived from MS patients and healthy individuals, as well as in gestational samples where the offspring had later developed MS, and gestational healthy individuals. Interestingly, this clinical study supported evidence that sub-optimal vitamin A levels may be associated with increased MS risk. The association between hs-CRP levels and MS risk that was recorded in young subjects may further support the role of the hygiene hypothesis in MS aetiology [38]. Another cohort study also investigated the association between retinol and disease activity in MS [29]. In particular, 88 RRMS patients, receiving omega-3 fatty acids, were included in this randomised placebo-controlled clinical trial. Study population was followed prospectively for 24 months with repeated assessments of serum-retinol levels and magnetic resonance imaging scans. Moreover, RRMS patients were initiated on IFN-β1a treatment after 6months [29]. In this clinical study, it was found that a 1μmol/L increase in serum-retinol levels reduced the odds for new T1 gadolinium (Gd (+)) enhanced lesions by 49%, new T2 lesions by 42%, and combined unique activity by 46% in simultaneous magnetic resonance imaging scans, 63% for new T1Gd (+) lesions, 49% for new T2 lesions and 43% for combined unique activity at the subsequent month. Importantly, serum retinol levels also predicted new T1Gd (+) and T2 lesions, 6 months ahead. Thus, it was speculated that serum retinol levels may inversely be associated with simultaneous and subsequent magnetic resonance imaging outcomes in RRMS [29].

In another substantial clinical study, Royal et al. assessed retinol levels and retinoid receptor gene expression in RRMS patients [39]. In this study, blood and CSF retinol levels and naïve and memory Th cells' subset percentages in samples from MS patients were evaluated [39]. Retinol receptor expression in peripheral blood cells derived from MS patients with or without a history of IFN-β1a pre-treatment was also assessed. Notably, plasma retinol levels for untreated relapsing-remitting RRMS patients were lower compared to patients with non-inflammatory neurological disease. Among IFN-β1a-treated patients, plasma retinol levels were slightly higher than for untreated patients. Lower plasma retinol levels among MS patients were associated with higher CSF retinol index measurements - a measure that was calculated to correct for nonspecific leakage of retinol from blood into CSF. In addition, a borderline direct

correlation between CSF retinol index measurements and CSF memory T-helper cell percentages was recorded. The above findings supported evidence for a potential association between plasma retinol levels and clinical dis-ease activity in MS patients, suggesting also that IFN-β1a treatment may be associated with specific retinoid receptor subtypes activation [39]. Another clinical study determined the serum levels of antioxidant vitamins, ascorbic acid, beta-carotene, retinol and alpha-tocopherol, as well as lipid peroxidation (as estimated by thiobarbituric acid reacting substances -TBARS- generation) in 24 MS patients and 24 sex- and age-matched healthy individuals [40]. It was found that antioxidant vitamins levels were significantly lower in MS patients compared to healthy individuals. Moreover, TBARS levels were significantly higher in MS patients compared to healthy individuals. Regarding MS patients, the levels of beta-carotene, alpha-tocopherol and ascorbic acid were significantly associated with each other. Importantly, it appeared that there was an inverse correlation between the serum levels of ascorbic acid and beta-carotene, but not amongst alpha-tocopherol, retinol and TBARS levels. It was also documented that serum antioxidant vitamins levels were decreased in MS patients during an attack, and that this decrease may be dependent on the increased oxidative burden, as reflected by lipid peroxidation products [40].

Clinical evidence for the potential effects of vitamin A on MS treatment

Several clinical studies have shown that active vitamin A derivatives may sup-press the formation of pathogenic T-cells in MS patients. Notably, a recent study by Bitarafan et al. determined the effect of vitamin A on disease progression in MS patients [41]. In fact, a total of 101 RRMS patients were enrolled in a 1-year placebo-controlled randomized clinical trial. The treated group firstly received 25000IU/d retinyl palmitate for 6 months followed by 10000 IU/d retinyl palmitate for another 6 months. The results of the Expanded Disability Status Scale (EDSS) and MS Functional Composite (MSFC) were recorded both at the beginning and at the end of this study. Moreover, the relapse rate was recorded during the intervention period. Notably, it was shown that MSFC was significantly improved in the treatment group, whereas there were no significant differences between EDSS changes in the treated and placebo groups. Enhanced brain active lesions were recorded in both groups. Moreover, no significant difference in the volume of T2 hyper-intense lesions between the two groups after intervention was recorded. Thus, this clinical study sup-ported evidence that vitamin A may improve total MSFC score in RRMS patients, however it cannot affect EDSS, relapse rate and brain active lesions [41]. A year later, Bitarafan et al. applied a modified scale of effects based on fatigue and Beck Depression Inventory-II scales at both at the beginning and the end of a one year study [42]. Notably, a significant improvement in the treated group for fatigue and depression was found. In view of the above, this study supported evidence that vitamin A supplement may contribute to interferon therapy; improving also mental health out-comes [42].

Eriksen et al. explored the potential beneficial effects of RA on B-cells of 25 female RRMS patients [43]. This clinical study showed that B-cells derived from MS patients, which were co-stimulated via the Toll-Like Receptors (TLRs), TLR9 and RP105, secreted lower levels of the anti-inflammatory cytokine IL-10 compared to B-cells derived from 15 healthy controls. Importantly, it was found that RA increased IL-10 secretion by MS-derived B-cells without influencing the levels of the pro-inflammatory cytokine tumor necrosis factor-α (TNF-α). Moreover, RA exerted the same ability to induce IL-10, as well as interferon-β-1b (IFN-β-1b). The B-cells of MS patients treated with glatiramer acetate or IFN-β-1b still displayed the beneficial effects of RA on the IL-10/TNF-α ratio. In view of the above, it was suggested that RA may enhance IL-10 levels in TLR-stimulated B-cells of RRMS patients [43]. Given that there were few elements as far as the effect of vitamin A supplementation on the balance of T-cells is concerned, Saboor-Yaraghi et al. focused on the function of RA, as an active metabolite of vitamin A [36]. In fact, they examined whether RA could modulate gene expression of specific nuclear receptors, including FoxP3. For this purpose, 36RRMS patients received vitamin A (25,000IU retinyl palmitate) or placebo (one capsule of placebo per day) for 6months. Peripheral blood mononuclear cells were isolated from RRMS patients and the expression of FoxP3 and Transforming Growth Factor (TGF)-β gene expression were evaluated. Notably, it was found that vitamin A upregulated both TGF-β and FoxP3 gene expression. Therefore, this clinical study supported substantial evidence that vitamin A supplementation may contribute to MS prevention and treatment by upregulating both TGF-β and FoxP3 gene expression levels [36].

Another study assessed whether three novel interventions, which were formulated based on a systems medicine therapeutic concept, could reduce disease activity in 80RRMS patients, who were either treated with disease-modifying treatment or received placebo [44]. In fact, this randomized, double-blind, placebo-controlled, phase II clinical study was conducted at the Cyprus Institute of Neurology and Genetics for 30 months [44]. The intervention (B, PLP10) was a combination of A and γ-tocopherol, being administered once daily, 30min before dinner [44]. It should be noted that the primary end point was the annualized relapse rate of the 3 interventions versus the placebo at 2 years, while the secondary end point was the time to confirm disability progression at 2 years. The majority of patients (51%) completed the 30-month trial. Overall, for the per-protocol analysis of the 2-year primary end point, 8 relapses were recorded in the PLP10 group versus 25 relapses in the placebo group, representing a 64% adjusted relative rate reduction for the PLP10 group. The per-protocol analysis for the secondary outcome at 2 years, the time to disability progression, was significantly longer only for PLP10 group. The cumulative probability of disability progression at 2 years was 10% in PLP10 group and 58% in placebo group. In a subgroup analysis that excluded patients receiving natalizumab, the cu-mulative progression probability was 10% for the 10 patients in PLP10 group and 70% for the 12 patients in placebo group, representing a relative 86% decrease in the risk of the sustained progression of disability in PLP10 group. No adverse events were reported. Interventions A (10 patients) and C (9 patients) showed no significant efficacy [44]. In spite of the above promising findings, larger clinical studies are strongly recommended to further assess the safety and efficacy of PLP10 treatment [44].

Furthermore, Qu ZX et al. [45] explored the potential effects of a combination of all-trans RA and INF-β1b therapy on immune system functions, which are considered potentially relevant to MS [45]. More to the point, INF-γ-secreting cells, T- sup-pressor cell function, and lymphocyte proliferative responses were assessed using peripheral blood mononuclear cells derived from both SPMS patients and healthy

individuals, under control conditions and in the presence of INF-β1b, RA, and their combination. This clinical study was performed in a university hospital MS clinic, in which 17 SPMS patients and 25 healthy individuals participated. This clinical study showed that INF-β1b treatment partially restored defective T- suppressor cell function in MS patients. Interestingly, this potentially beneficial action was synergistically potentiated by RA co-treatment [45]. In another study, Wong et al. investigated whether there were differences in fat absorption, beta-carotene and vitamin A ab-sorption in MS disease [46]. In this double-blind and randomized clinical study, 24 patients with clinically definite MS and 36 healthy individuals were evaluated. This study did not find substantial evidence for fat malabsorption in MS. Thus, the authors concluded that there were no differences between these two populations with regard to fat absorption, as well as beta-carotene and vitamin A absorption [46].

Discussion

Several well-designed and well-organized clinical studies have showed that vitamin A supplementation may exert beneficial effects on MS disease development and progression. The potential beneficial role of vitamin A in MS, at a molecular level, seems to be exerted by decreasing inflammatory-related disease progression effects, as well as by increasing autoimmunity tolerance, which may contribute to overall brain protection. However, the currently available clinical studies, although well-designed and well-organized, they suffer from several limitations, which render their basic findings as scientifically supportive and informative but not convincing and conclusive.

Most of the currently available studies have been conducted on small MS patients' populations, while there is also a significant heterogeneity concerning the dose and the duration of vitamin A supplementation amongst them. Moreover, some clinical studies evaluated the potential beneficial effects of vitamin A as a supplement, whereas other studies investigated its active metabolites, e.g. RA. In addition to this, it should be noted that several studies reported either dropouts or low compliance, or subjects that did not come back for follow up. Furthermore, it should be noted that, up to date, most of the potential molecular mechanisms, as far as the beneficial effects of vitamin A against MS development and progression are concerned, have been derived by *in vitro* and *in vivo* animal studies. Such data, although significant and absolutely essential, should not evidently be extrapolated to human population suffering from MS.

Conclusions

Collectively, although the currently available clinical studies have supported prelimi-nary evidence for the potential beneficial effects of vitamin A supplementation as part of MS treatment, there is at present no clear clinical indication for vitamin A supplementation as a co-treatment for MS disease management. Consequently, fur-ther clinical trials focusing on vitamin A, as a potential supplementation or as an add-on option are strongly recommended, also focusing on the distinct MS pheno-types as distinct disease subpopulations.

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References

1. Harrison EH. Mechanisms involved in the intestinal absorption of dietary vitamin A and provitamin carotenoids. Biochim Biophys Acta. 2012; 1821: 70-77.
2. Erdman JW Jr, TL Bierer and ET Gugger. Absorption and transport of carotenoids. Ann N Y Acad Sci. 1993; 691: 76-85.
3. Parker RS. Absorption, metabolism, and transport of carotenoids. Faseb j. 1996; 10: 542-551.
4. Harrison EH. Mechanisms of digestion and absorption of dietary vitamin A. Annu Rev Nutr. 2005; 25: 87-103.
5. Chawla A, et al. Nuclear receptors and lipid physiology: opening the X-files. Science. 2001; 294: 1866-1870.
6. Balmer JE and R Blomhoff. Gene expression regulation by retinoic acid. J Lipid Res. 2002; 43: 1773-1808.
7. Tang XH and LJ Gudas. Retinoids, retinoic acid receptors, and cancer. Annu Rev Pathol. 2011; 6: 345-364.
8. Compston A and A Coles. Multiple sclerosis. Lancet. 2008; 372: 1502-1517.
9. Harrirchian MH, et al. The effect of vitamin A supplementation on disease progression, cytokine levels and gene expression in multiple sclerotic patients: study protocol for a randomized controlled trial. Acta Med Iran. 2014; 52: 94-100.
10. World Health Organization, Atlas Multiple Sclerosis Resources in the World. 2008; 2008: 51.
11. Coles AJ, et al. Monoclonal antibody treatment exposes three mechanisms underlying the clinical course of multiple sclerosis. Ann Neurol. 1999; 46: 296-304.
12. Fragoso YD. Modifiable environmental factors in multiple sclerosis. Arq Neuropsiquiatr. 2014; 72: 889-894.
13. Comabella M and SJ Khoury. Immunopathogenesis of multiple sclerosis. Clin Immunol. 2012; 142: 2-8.
14. Chari DM. Remyelination in multiple sclerosis. Int Rev Neurobiol. 2007; 79: 589-620.
15. Lassmann H. Multiple sclerosis: Lessons from molecular neuropathology. Exp Neurol. 2014; 262: 2-7.
16. Lublin FD and SC Reingold. Defining the clinical course of multiple sclerosis: Results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. Neurology. 1996; 46: 907-911.
17. Lublin FD, et al. Defining the clinical course of multiple sclerosis: the 2013 revisions. Neurology. 2014; 83: 278-286.
18. Weinstock-Guttman B, et al. Low fat dietary intervention with omega-3 fatty acid supplementation in multiple sclerosis patients. Prostaglandins Leukot Essent Fatty Acids. 2005; 73: 397-404.
19. Hutter C. On the causes of multiple sclerosis. Med Hypotheses. 1993; 41: 93-96.
20. Hall JA, et al. Essential role for retinoic acid in the promotion of CD4 (+) T cell effector responses via retinoic acid receptor alpha. Immunity. 2011; 34: 435-447.
21. Ascherio A, et al. Vitamin D as an early predictor of multiple sclerosis activity and progression. JAMA Neurol. 2014; 71: 306-314.
22. Ikeda U, et al. 1alpha, 25-Dihydroxyvitamin D3 and all-trans retinoic acid synergistically inhibit the differentiation and expansion of Th17 cells. Immunol Lett. 2010; 134: 7-16.
23. O'Gorman C and SA Broadley. Smoking and multiple sclerosis: evidence for latitudinal and temporal variation. J Neurol. 2014; 261: 1677-1683.
24. Alonso A, et al. A case-control study of risk factors for multiple sclerosis in Iran. Mult Scler. 2011; 17: 550-555.

25. Manouchehrinia A, et al. Tobacco smoking and disability progression in multiple sclerosis: United Kingdom cohort study. Brain. 2013; 136: 2298-2304.

26. Swank C, M Thompson and A Medley. Aerobic exercise in people with multiple sclerosis: Its feasibility and secondary benefits. Int J MS Care. 2013; 15: 138-145.

27. Loma I and R Heyman. Multiple sclerosis: Pathogenesis and treatment. Curr Neuropharmacol. 2011; 9: 409-416.

28. Abdolahi M, et al. Molecular Mechanisms of the Action of Vitamin A in Th17/Treg Axis in Multiple Sclerosis. J Mol Neurosci. 2015; 57: 605-613.

29. Loken-Amsrud KI, et al. Retinol levels are associated with magnetic resonance imaging outcomes in multiple sclerosis. Mult Scler. 2013; 19: 451-457.

30. Reza Dorosty-Motlagh A, et al. The Molecular Mechanisms of Vitamin A Deficiency in Multiple Sclerosis. J Mol Neurosci. 2016; 60: 82-90.

31. Cassani B, et al. Vitamin A and immune regulation: Role of retinoic acid in gut-associated dendritic cell education, immune protection and tolerance. Mol Aspects Med. 2012; 33: 63-76.

32. Warren TR. Multiple sclerosis and infants fed on diets deficient in vitamin A or in selenium and vitamin E. Med Hypotheses. 1982; 8: 443-454.

33. Runia TF, et al. Vitamin A is not associated with exacerbations in multiple sclerosis. Mult Scler Relat Disord. 2014; 3: 34-39.

34. Mohammadzadeh Honarvar N, et al. The effect of vitamin A supplementation on retinoic Acid-Related Orphan Receptor gammat (RORgammat) and interleukin-17 (IL-17) gene expression in Avonex-treated multiple sclerotic patients. J Mol Neu-rosci. 2013; 51: 749-753.

35. Bitarafan S, et al. Impact of vitamin A supplementation on RAR gene expression in multiple sclerosis patients. J Mol Neurosci. 2013; 51: 478-484.

36. Saboor-Yaraghi AA, et al. The Effect of Vitamin A Supplementation on FoxP3 and TGF-beta Gene Expression in Avonex-Treated Multiple Sclerosis Patients. J Mol Neurosci. 2015; 56: 608-612.

37. Jafarirad S, et al. The effect of vitamin a supplementation on biochemical parameters in multiple sclerosis patients. Iran Red Crescent Med J. 2013; 15: 194-198.

38. Salzer J, et al. Vitamin A and systemic inflammation as protective factors in multiple sclerosis. Mult Scler. 2013; 19: 1046-1051.

39. Royal W, S Gartner and CD Gajewski. Retinol measurements and retinoid receptor gene expression in patients with multiple sclerosis. Mult Scler. 2002; 8: 452-458.

40. Besler HT, S Comoglu and Z Okcu. Serum levels of antioxidant vitamins and lipid peroxidation in multiple sclerosis. Nutr Neurosci. 2002; 5: 215-220.

41. Bitarafan S, et al. Impact of Vitamin A Supplementation on Disease Progression in Patients with Multiple Sclerosis. Arch Iran Med. 2015; 18: 435-440.

42. Bitarafan S, et al. Effect of Vitamin A Supplementation on fatigue and depression in Multiple Sclerosis patients: A Double-Blind Placebo-Controlled Clinical Trial. Iran J Allergy Asthma Immunol. 2016; 15: 13-19.

43. Eriksen AB, et al. Retinoic acid enhances the levels of IL-10 in TLR-stimulated B cells from patients with relapsing-remitting multiple sclerosis. J Neuro-immunol. 2015; 278: 11-18.

44. Pantzaris MC, et al. A novel oral nutraceutical formula of omega-3 and omega-6 fatty acids with vitamins (PLP10) in relapsing remitting multiple sclerosis: a randomised, double blind, placebo-controlled proof-of-concept clinical trial. BMJ Open. 2013; 3: e002170.

45. Qu ZX, et al. All-trans retinoic acid potentiates the ability of interferon be-ta-1b to augment suppressor cell function in multiple sclerosis. Arch Neurol. 1998; 55: 315-321.

46. Wong EK Jr, et al. Intestinal absorption of dietary fat in patients with multiple sclerosis. Metab Pediatr Syst Ophthalmol (1985). 1993; 16: 39-42.