Marginal Vitamin A Deficiency Exacerbates Memory Deficits Following Aβ1-42 Injection in Rats

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Abstract: Background: Although clinical vitamin A deficiency (VAD), which is a public health problem developing throughout the world, has been well controlled, marginal vitamin A deficiency (MVAD) is far more prevalent, especially among pregnant women and preschool children in China. Increasing evidence suggests that VAD is involved in the pathogenesis of Alzheimer’s disease (AD). However, whether MVAD, beginning early in life, increases the risk of developing AD has yet to be determined.

Objective: The goal of this study was to investigate the long-term effects of MVAD on the pathogenesis of AD in rats.

Method: An MVAD model was generated from maternal MVAD rats and maintained with an MVAD diet after weaning. The males were bilaterally injected with aggregated amyloid β (Aβ) 1–42 into the CA3 area of the hippocampus, and the AD-associated cognitive and neuropathological phenotypes were examined.

Results: We found that MVAD feeding significantly aggravated Aβ1-42-induced learning and memory deficits in the Morris water maze test. MVAD did not induce the mRNA expression of retinoic acid receptors (RARs), a disintegrin and metalloprotease 10 (ADAM10) or insulin-degrading enzyme (IDE) in Aβ1-42-injected rats. Moreover, RARα and RARγ mRNA were positively correlated with ADAM10 mRNA, whereas RARβ mRNA was positively correlated with IDE mRNA.

Conclusion: Our study suggests that MVAD beginning from the embryonic period perturbs the AD-associated genes, resulting in an enhanced risk of developing AD.

Keywords: Alzheimer’s disease, amyloid β, vitamin A, marginal vitamin A deficiency, memory deficits, retinoic acid receptors, ADAM10, IDE.

INTRODUCTION

Alzheimer’s disease (AD) is the most common neurodegenerative disorder that leads to dementia. Patients with AD typically display progressive memory loss and cognitive impairment. The neuropathology of AD is characterized by extracellular neuritic plaques, intracellular neurofibrillary tangles, synaptic loss, glial cell activation and neuronal death. The neuritic plaques are primarily composed of amyloid β (Aβ) protein, which is derived from the sequential cleavage of amyloid β precursor protein (APP) via β-secretase and then γ-secretase. The abnormal accumulation of Aβ initiates neuronal dysfunction and plays an important role in AD pathogenesis. Aβ40 and Aβ42 are the major species of Aβ in the brain. Of these two species, Aβ42 is the more toxic form; furthermore, it is hydrophobic, prone to aggregation and predominant in neuritic plaques [1]. Under physiological conditions, the majority of APP is cleaved by α-secretase within its Aβ region to preclude Aβ generation [2-4]. The reduction of a disintegrin and metalloprotease 10 (ADAM10), the central component of α-secretase, promotes AD pathogenesis by shifting α-cleavage to β-cleavage of APP [5]. In addition, the dysfunction of the Aβ clearance mediated by Aβ-degrading enzymes (e.g., insulin degrading enzyme; IDE) is crucial for the accumulation of Aβ in the AD brain [6].

Vitamin A (VA) is one of the most essential micronutrients in humans. In the central nervous system (CNS), VA and its active metabolite retinoic acid (RA) are involved in several essential biological processes including early development of brain structure and function, neuronal patterning,
proliferation and differentiation, neurite outgrowth and synaptogenesis [7]. RA primarily exerts its biological effects by activating retinoic acid receptors (RARs) and/or retinoid X receptors (RXRs), which act on the retinoic acid responsive elements (RAREs) in the promoter regions of target genes [7]. Both RARs and RXRs have three subtypes: α, β and γ. Over 500 genes might be regulated by RA.

Vitamin A deficiency (VAD) is a serious public health problem throughout the developing world and is primarily caused by chronically low VA intake. VAD affects an estimated 190 million preschool children and 19.1 million pregnant women worldwide [8]. Evidence regarding the involvement of VAD in the pathogenesis of AD has increased. The first evidence was provided by studies that reported decreased serum VA and β-carotene in patients with AD [9-12]. A nutritional analysis of the risk factors for AD also revealed that low dietary VA or β-carotene is associated with an increased risk of AD [13]. Furthermore, the depletion of VA or the dysfunction of RARs results in Aβ deposition, impairment of hippocampal long-term synaptic plasticity and memory deficits in rodents; these effects can be restored via VA or RAR agonist replenishment [14-22]. However, the potential mechanisms between VAD and AD remain elusive. Currently, severe VAD has been largely brought under control; however, more widespread marginal states of deficiency have been revealed, designated as mild or marginal vitamin A deficiency (MVAD). MVAD primarily affects pregnant/lactating women and preschool children [23-25]. Our previous study found that MVAD is also prevalent among the elderly. Furthermore, Lahiri first proposed the Latent Early-life Associated Regulation (LEARn) model, suggesting that environmental risk factors (e.g., dietary factors) perturb gene regulation in a long-term fashion, beginning at early developmental stages, which plays a vital role in the etiology of AD [26, 27]. Thus, we hypothesized that long-term MVAD, beginning early in life, might regulate the AD-associated gene and protein expression, thereby increasing the risk for developing AD.

The present study established an MVAD rat model from the embryonic period to the sacrifice. Aggregated Aβ1–42 was injected into the hippocampus of these rats to investigate the effects of MVAD on AD pathogenesis. This study demonstrates aggravated hippocampus-dependent memory deficits in these MVAD rats following Aβ1–42 injection. We subsequently examined the AD-associated gene expression and its correlation with RARs. Our work suggests that MVAD, beginning during the embryonic period, enhances the risk of developing AD.

MATERIAL AND METHODS

Animals and Diets

All Wistar rats used in this study were obtained from the Experimental Animals Center of Beijing, China. The rats were housed in a room with a constant airflow system, controlled temperature (22-24°C) and a 12-hour light/dark cycle. The rats were provided with food and water ad libitum. The female rats were randomly divided into a control group and an MVAD group and fed a VA-normal (VAN) diet (6,500 IU VA per kilogram of the basic diet) and an MVAD diet (400 IU VA per kilogram of the basic diet), respectively, for 4 weeks to generate maternal control and MVAD rats [28]. After confirming that the serum retinol levels matched the recommended standards for MVAD (0.70-1.05 μmol/L) and the VAN control (1.05-2.07 μmol/L) [29], the female rats were mated with the VAN males. After giving birth, the dams continued to be maintained on their respective diets. The male weanlings were subjected to experiment and were maintained throughout the study on a VAN or MVAD diet. This study was conducted in strict accordance with the recommendations of the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals. The Animal Experimentation Ethical Committee of Chongqing Medical University in Chongqing, China, approved the protocol.

Aggregated Aβ1–42 Preparation and Surgery

The preparation of the aggregated Aβ1–42 and the brain stereotaxic surgery were performed as previously described [30]. Aβ1–42 (Sigma-Aldrich, USA) was dissolved in 0.01 M phosphate-buffered saline (PBS), pH 7.4, and then vigorously agitated (200 rpm) at room temperature for 36 hours. The Aβ1–42 peptide was precipitated via centrifugation at 15,000×g for 10 minutes. Sediments were dissolved with PBS to a 10−4 M stock, aliquoted, and stored at -80°C.

After the baseline Morris water maze tests were conducted with 18-week-old pups, the pups in both the control and MVAD groups were randomly divided into the Aβ group (full dose of aggregated Aβ1–42), the 1/2Aβ group (half dose of aggregated Aβ1–42) and the vehicle group. All subjects were anaesthetized, and the injections were made bilaterally into the CA3 area of the dorsal hippocampus with the injectates deposited slowly over 10 minutes. The stereotaxic coordinates were 2.6 mm lateral and 3.5 mm posterior to the bregma, and 3.7 mm ventral. The injections were 5.0 μl of 1×10−4 M aggregated Aβ1–42 suspension, 5.0 μl of 0.5×10−4 M aggregated Aβ1–42 suspension and 5.0 μl of PBS for the Aβ group, the 1/2Aβ group and the vehicle group, respectively.

Serum Retinol Assay

The maternal rats at preconception and five to six pups from each group at postnatal 24 hours, 4 weeks, 8 weeks, 12 weeks, 18 weeks, 22 weeks and 26 weeks were randomly selected to detect serum retinol levels. The pups younger than 8 weeks old were decapitated to collect the blood; and the blood from the pups at the age of 8 weeks and older were collected via the caudal vein. All pups were decapitated after the last behavioral tests to detect serum retinol levels and confirm the success of the animal models. Serum retinol was measured using high performance liquid chromatography (HPLC) as previously described [31]. Briefly, 200 μl of serum was deproteinized with an equal volume of dehydrated alcohol. The retinol was extracted from the serum by adding 1,000 μl of hexane and evaporated with nitrogen gas. The retinol residue was dissolved in 100 μl of the mobile phase mixture (methanol:water =97:3). Then, 20 μl of the dissolved liquid was tested in the HPLC apparatus (DGU-20As, Shimadzu Corporation, Kyoto, Japan) on a silica column with a 315-nm ultraviolet photodiode array detector. All procedures were performed in a dark room to protect the samples from light.
light at the Children’s Nutrition Research Center in the Children’s Hospital of Chongqing Medical University.

**Real-time Polymerase Chain Reaction (RT-PCR)**

All pups were sacrificed after the last behavioral tests. Total RNA was extracted from the hippocampus of rats using TRI Reagent (Sigma) and reverse-transcribed into cDNA using the PrimeScript RT Reagent Kit (TaKaRa) according to the manufacturer’s instructions. Real-time PCR was conducted using a SYBR Premix Ex Tag (Perfect Real Time; TaKaRa) and a Bio-Rad Real-Time PCR system. The primer sequences for RARα, RARβ, RARγ, ADAM10, IDE and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) are listed in Table 1. The gene expression levels of all genes were normalized to GAPDH.

**Table 1. Primer sequences for the genes of rats.**

| Gene name | Sequences | PCR products |
|-----------|-----------|--------------|
| RARα      | F: CAGAGGAGGAGAAGACGTCAC-3' | 200 bp |
|           | R: ATGGCTTATGTTGACGAC-3' |
| RARβ      | F: CAATGCTGCTGTCCCTGCC-3' | 137 bp |
|           | R: CCTAGGCTGTGCGGCTCTC-3' |
| RARγ      | F: CTGACCTGAAACCGACCC-3' | 144 bp |
|           | R: TCCACAAGATAGCAGGATGCA-3' |
| ADAM10    | F: GCAGTCAATGTCGACTACGC-3' | 179 bp |
|           | R: GAGCCACACTCCTCGACATG-3' |
| IDE       | F: CTCCTCGCCGCTACTGCACC-3' | 200 bp |
|           | R: GCTAGCCACGACCTGCTGAAAG-3' |
| GAPDH     | F: CAGGTCACAAGGACGACAC-3' | 149 bp |
|           | R: TGCTGGAAGAGGCCAGGACTC-3' |

RARα, retinoic acid receptor α; RARβ, retinoic acid receptor β; RARγ, retinoic acid receptor γ; ADAM10, a disintegrin and metalloprotease 10; IDE, insulin degrading enzyme; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

**Morris Water Maze Test**

Learning and memory at baseline was examined *via* the Morris water maze tests at 18 weeks of age, and the rats were re-tested at 30 days and 80 days after the injection of aggregated Aβ1-42 to evaluate their spatial learning and memory abilities as previously described [32]. The test was performed in a 1.5-m (in diameter) pool with a 10-cm (in diameter) platform. The procedure consisted of 1 day of visible platform tests and 4 days of hidden platform tests, plus a probe trial 24 hours after the last hidden platform test. In the visible platform test performed on the first day, the rats were trained for 5 continuous trials, with an inter-trial interval of 60 minutes. For the hidden platform tests, the platform was placed in the southwest quadrant of the pool. Rats were trained for 5 trials, with an inter-trial interval of 60 minutes. Each rat was allowed 60 seconds to search for the platform. If the rat was unable to find the platform, then it was placed on the platform and allowed to stay for 20 seconds. On the last day of the test, each rat was subjected to the probe trial in which the platform was removed. Each rat was given 60 seconds to locate where the platform was originally placed. Animal movement was tracked and recorded using ANY-maze tracking software (Stoelting).

**Statistical Analysis**

All results are presented as the means ± SEMs. Serum retinol data between the MVAD and control groups prior to Aβ1-42 injection were analyzed by Student’s *t* test. After Aβ1-42 injection, either the serum retinol data or mRNA data were analyzed with the general linear model univariate ANOVA for the main effects of diet (control or MVAD), Aβ1-42 injection (vehicle, 1/2Aβ or Aβ), and their interactions (two-way ANOVA); all significant main effects were further analyzed using Bonferroni post hoc tests. Behavioral data were analyzed by a two-way ANOVA with repeated measures, with diet as the between-subjects factor and learning day as the within-subjects factor. Correlation analyses between the ADAM10/IDE and RARs mRNA levels were conducted by calculating the Pearson’s correlation coefficient (r). All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS 17.0) software. Differences were considered significant at *P*<0.05. Graphs were built in GraphPad Prism 5.0.

**RESULTS**

**MVAD Exacerbates Memory Deficits in Aβ1-42-injected Rats**

To investigate the effects of embryonic-onset MVAD following an Aβ1-42 injection on spatial learning and memory, we first generated a maternal MVAD model by feeding female adult rats an MVAD diet for 4 weeks. Compared with control rats, the serum retinol levels were significantly reduced in the MVAD maternal rat model (0.79±0.07 μmol/L vs. 1.33±0.20 μmol/L, *P*<0.05; Fig. 1A). After confirming that the maternal model was established, the females were mated with normal males, and the male offspring were used for the subsequent experiments. The serum retinol levels of the pups at 4, 8 and 12 weeks of age were significantly lower in the MVAD group than in the control animals (*P*<0.05; Fig. 1B). After a half- or full-dose injection of aggregated Aβ1-42 (1/2Aβ or Aβ), only significant main effects of diet (*P*<0.05; Fig. 1C) without a main effect of Aβ or a significant diet×Aβ interaction were observed at all ages of 18, 22 and 26 weeks (*P*<0.05; Fig. 1C), indicating that the Aβ1-42 injection did not significantly affect the serum retinol content while the MVAD diet markedly lowered the serum retinol levels. The rats were sacrificed immediately after the last behavioral tests at 80 days post-aggregated Aβ1-42 injection, and the serum retinol levels were measured. A two-way ANOVA revealed a significant main effect of diet in the absence of a main effect of Aβ or a significant diet×Aβ interaction (diet: *F*(1,59)=95.47, *P*<0.001; Aβ: *F*(1,59)=2.59, *P*>0.05; diet×Aβ: *F*(1,59)=0.72, *P*>0.05). Post hoc Bonferroni tests indicated that serum retinol levels were significantly reduced in PBS-, 1/2Aβ- and Aβ-injected MVAD rats compared with those of their respective controls (*P*<0.001; Fig. 1D). Since there was no significant difference in serum retinol levels among the PBS, 1/2Aβ and Aβ groups together for statistical analysis. The results further confirmed that MVAD treatment decreased the serum retinol levels compared with those of the control group (0.94±0.05 μmol/L vs. 1.20±0.053 μmol/L,
These data demonstrate that the MVAD model, which began during the embryonic period, was successfully established. We also detected the Aβ_{1-42} levels in the hippocampus of both MVAD and control groups after the last behavioral tests at 80 days post-Aβ_{1-42} injection. Western blot analysis showed that Aβ_{1-42} expression was significantly elevated after 1/2Aβ or Aβ injection compared with the PBS vehicle injection in either the MVAD or control rats (P<0.01; Supplemental Fig. S1), confirming that Aβ_{1-42} was successfully injected.

To determine whether MVAD affects spatial learning and memory, Morris water maze tests were used for both the MVAD and control pups after an aggregated Aβ_{1-42} injection. First, we examined learning and memory at baseline (prior to the Aβ_{1-42} injection at 18 weeks old). On the visible platform tests, all rats exhibited similar escape latencies and path lengths (data not shown), indicating that MVAD did not affect the rats’ mobility or vision. On the hidden platform tests, the MVAD pups exhibited similar escape latencies relative to the control group (P>0.05; Fig. 2A). At 30 days post-injection of vehicle PBS or 1/2Aβ, no significant differences were found between the MVAD and control groups with regard to escape latency (P>0.05; Fig. 2B, left and middle), whereas Aβ-injected MVAD rats showed a significantly prolonged escape latency compared with that of their respective controls (P<0.01; Fig. 2B, right). However, 80 days after the injection of vehicle PBS and 1/2Aβ, the MVAD pups showed markedly longer escape latencies than those of controls (P<0.01; Fig. 2C, left and middle), whereas the escape latency of MVAD rats was similar to that of controls after the Aβ injection (P>0.05; Fig. 2C, right). These results suggest that MVAD significantly exacerbated the cognitive deficits induced by a half dose of Aβ_{1-42} during late stages of rat development and that a full dose of Aβ_{1-42} could accelerate this aggravation.

**MVAD Suppresses Aβ_{1-42}-induced ADAM10 Expression Via RARα/γ and IDE Expression Via RARβ in the Hippocampus of Rats**

To further investigate the effect of MVAD on APP processing enzymes, we examined the hippocampal gene expression of RARs, including RARα, RARβ and RARγ, as well as the AD-associated gene expression of ADAM10 and IDE 80 days after the injection of Aβ_{1-42}. A two-way ANOVA of RARα, RARβ, RARγ and ADAM10 revealed significant main effects of diet, Aβ and diet×Aβ interaction (P<0.05; Fig. 3A-D). Furthermore, significant main effects of Aβ and diet×Aβ interaction (P<0.05) in the absence of a significant main effects of diet were observed for IDE (P>0.05; Fig. 3E). In addition, post hoc Bonferroni tests showed that a full dose of Aβ_{1-42} injection appeared to dramatically upregulate the mRNA levels of RARα, RARγ and ADAM10 in the control group (P<0.001); however, upregulation occurred to a much lesser extent in the MVAD group with no significant
Fig. (2). MVAD with Aβ1-42 injection exacerbates memory deficits in rats. Both the MVAD and control rats were subjected to the Morris water maze test at 18 weeks of age and at 30 and 80 days after vehicle PBS, 1/2Aβ or Aβ injection. (A) At 18 weeks of age, prior to Aβ1-42 injection at baseline, the MVAD pups showed similar escape latencies relative to those of the control group. *P<0.001; **P<0.0001 by two-way ANOVA with repeated measures. (B) At 30 days post-injection of vehicle PBS (left) or 1/2Aβ (middle), no significant differences in escape latencies were observed between the MVAD and control rats; however, the MVAD rats showed a prolonged escape latency compared with the control rats at 30 days post-injection of Aβ (right). (C) At 80 days post-injection of vehicle PBS (left) or 1/2Aβ (middle), the MVAD rats showed a longer escape latency than the control rats, whereas similar escape latencies were found between the MVAD and control rats; however, the MVAD rats showed a prolonged escape latency compared with the control rats after the Aβ injection (right). Control group: n vehicle=5, n1/2Aβ=6, and nAβ=10; MVAD group, n vehicle=6, n1/2Aβ=9, and nAβ=6; *P<0.001 and **P<0.01 by two-way ANOVA with repeated measures. All values are expressed as the means ± SEMs. 1/2Aβ, half dose of aggregated Aβ1-42; Aβ, full dose of aggregated Aβ1-42; MVAD, marginal vitamin A deficiency.

differences (P>0.05; Fig. 3A-C). In addition, the mRNA levels of both RARβ and IDE were increased by the half dose of Aβ1-42 but reduced by the full dose of Aβ1-42 in control rats (P<0.01); however, no significant differences were observed in the MVAD rats following Aβ1-42 injection (P>0.05; Fig. 3D and E).

Pearson’s correlation analysis showed that ADAM10 mRNA was positively correlated with both RARα and RARγ mRNA (r_RARα*ADAM10=0.9946, P<0.0001; r_RARγ*ADAM10 = 0.9964, P<0.0001; Fig. 4A and B). A positive correlation was also found between RARα and RARγ mRNA (r_RARα*RARγ=0.9951, P<0.0001; Fig. 4C), whereas no significant correlation was found between ADAM10 and RARβ mRNA levels (r_RARβ*ADAM10=-0.2444, P=0.13). Furthermore, IDE mRNA was positively correlated with RARβ mRNA (r_RARβ*IDE=0.6780, P<0.0001; Fig. 4D) but negatively correlated with both RARα and RARγ mRNA (r_RARα*IDE=-0.5118, P<0.001; r_RARγ*IDE=-0.5152, P<0.001; Fig. 4E and F). These findings suggest that MVAD suppressed Aβ-induced ADAM10 expression through RARα and RARγ and IDE expression through RARβ.

DISCUSSION

VA is an essential micronutrient throughout the life span of mammals. VA and its derivatives, the retinoids, play vital roles in the development, regeneration and maintenance of the CNS in both developing and mature brains. Despite increasing evidence suggests that VA nutrition is related to neurodegenerative diseases including AD, the mechanisms of VAD and MVAD in the pathogenesis of AD remain unclear.

In the developing countries, the dietary pattern is primarily an insufficient amount of dominated by grain and vegetables, with meat and VA-rich foods. VAD or MVAD is primarily caused by chronically low VA intake. Furthermore, MVAD is far more prevalent than VAD; however, it has been grossly neglected because its clinical symptoms are obscure. In many countries, including China, postnatal rather than prenatal supplementation is recommended for cases of VAD to avoid the teratogenic effect of excess VA during pregnancy. This convention has resulted in widespread MVAD among pregnant women and preschool children. Our previous work also revealed that twice as many MVAD cases than VAD cases exist in the elderly (25.8% vs. 13.3%). Though highly prevalent of MVAD among these people, human studies on MVAD during pregnancy and the child’s development are difficult to be conducted. Thus, the present study established the MVAD rat model by partially depriving VA in diets from preconception to the sacrifice of offspring, which was considered to be a long-term status of MVAD.
Fig. (3). MVAD suppresses the Aβ-induced mRNA levels of RARα/β/γ, ADAM10 and IDE in the hippocampus of rats. The rats were sacrificed immediately after behavioral tests at 80 days post-injection of vehicle PBS, 1/2 Aβ or Aβ, and the mRNA levels of RARα (A), RARβ (D), RARγ (B), ADAM10 (C) and IDE (E) were detected in the hippocampus of both the MVAD and control groups. (A-C) Aβ injection dramatically induced RARα (A), RARγ (B) and ADAM10 (C) mRNA levels in the control group, whereas the increased levels of these mRNAs induced by Aβ were not significant in the MVAD group. (D and E) RARβ (D) and IDE (E) mRNA levels were increased by 1/2Aβ and decreased by Aβ in control rats; however, no significant difference was observed in these mRNA levels among the vehicle PBS, 1/2Aβ and Aβ groups with regard to the MVAD rats. Control group: n vehicle=8, n1/2Aβ=8, and nAβ=10; MVAD group: n vehicle=8, n1/2Aβ=9, and nAβ=8; *significant effects (P<0.05 in all cases), **P<0.01 and ***P<0.001 by two-way ANOVA with Bonferroni post hoc tests. Values indicate means ± SEMs. 1/2Aβ, half dose of aggregated Aβ1-42; Aβ, full dose of aggregatedAβ1-42; ADAM10, a disintegrin and metalloprotease 10; IDE, insulin degrading enzyme; RARα, retinoic acid receptor α; RARβ, retinoic acid receptor β; RARγ, retinoic acid receptor γ.

The serum retinol content was found to be significantly reduced in this MVAD model in both mother and offspring, confirming that the model was successfully established.

Some previous studies have found that the deprivation of VA or disruption of RARs induces memory impairment in rodents [16, 17, 33]; however, these relationships have not been investigated in an AD mode. In addition, few studies
have investigated the involvement of MVAD in the development of AD. To investigate the long-term effects of MVAD on AD pathogenesis, the current study established the AD model in MVAD rats through the bilateral injection of aggregated Aβ1-42 into the CA3 area of the hippocampus in male rats using a single intrahippocampal injection. Aβ1-42 levels were significantly increased after this injection (Supplementary Fig. S1), confirming that Aβ1-42 was successfully injected. This rodent model represents a valid tool for investigating AD because the injection can induce synaptic and memory dysfunction and can facilitate an AD-like pathology [34, 35]. Nevertheless, there have been few studies establishing the AD model on this long-term MVAD model. Behavioral deficits were not apparent during the first 30 days following aggregated Aβ injection; however, significant deficits became apparent approximately 60 to 80 days after injection [30, 36]. Moreover, the current study employed Morris water maze tests at 30 and 80 days post-injection with aggregated Aβ1-42. Given that both MVAD and Aβ administration can impair behavioral performance, we injected rats with not only a full dose of Aβ1-42 but also a half dose [30]. We found that the half dose of Aβ1-42 induced learning and memory deficits compared with the vehicle PBS injection in both the MVAD and control rats at 80 days post-injection but not at 30 days post-injection (Supplemental Fig. S2), confirming that the half dose of Aβ1-42 induced cognitive impairments during the later stages of development. We also found no significant differences in spatial learning and memory between the MVAD and control rats after 30 days of either vehicle PBS injection or a half dose of Aβ2-42 injection. However, at 80 days post-injection of vehicle PBS or a half dose of Aβ1-42, the MVAD rats showed significant memory deficits compared with the control animals. The delayed behavioral deficits induced by MVAD might be because of the time required for the neurons to become damaged following a half dose of Aβ1-42 injection and the age-related decline in the rats’ ability to protect their neurons from Aβ-induced neurotoxicity. However, the memory deficits induced by a full dose of Aβ1-42 in the MVAD rats were apparent at 30 days but not 80 days post-injection. Previous studies have demonstrated that intracerebral fibrillar Aβ1-42 induces proliferation and activation of microglia [37, 38] and RA suppresses microglial activation in an AD mouse model [39], so it is likely that MVAD accelerates the activation of microglia following Aβ1-42 injection. A full dose of Aβ1-42 might as well facilitate the damage of neurons. All these results demonstrate that MVAD aggravated Aβ1-42-induced cognitive deficits. Furthermore, the age-related decline of anti-neurotoxicity properties might play an important role in the pathogenesis of AD [37].

Numerous in vitro and in vivo studies have associated RA and its signaling to APP processing genes or protein expression [40]. Furthermore, RAR/RXR has been long reported to be involved in regulation of ADAM10 or IDE following Aβ1-42 injection, suggesting that MVAD might impair the regulatory feedback of ADAM10 and IDE induced by Aβ1-42 injection, resulting in an incompetent protective response. We subsequently investigated the correlations between RARs and each of ADAM10 and IDE. We found that both RARα and RARγ were positively correlated with ADAM10, which somewhat corroborates previous studies reporting that RARα and RARβ increased ADAM10 expression in vivo and in vitro [43, 44, 47]. In addition, a positive correlation was observed between RARβ and IDE in the current study. This result supports a recent study reporting that RARα and RARγ were negatively correlated with IDE, which is inconsistent with a previous in vitro study showing that RA-elicited RARα-induced IDE expression [46]. Our study of an elderly population also revealed a positive correlation between ADAM10 and RARα and between IDE and RARγ. Together, these data indicated that different AD-associated enzymes were regulated by different RARs. Our findings are of great significance for interpreting the MVAD mechanism concerning AD pathogenesis. Additional studies are needed to gain insight regarding RA signaling and AD-associated gene expression.

Our present study showed that MVAD, beginning from embryonic period, contributed to dysregulation of the Alzheimer-associate gene expression as well as exacerbated memory impairment late in life. Similar viewpoints have been demonstrated in the LEARn model, which was established to integrate environmental risk factors and the developmental basis of AD [26]. This model indicates that accumulated environmental hits, such as dietary factor and toxicological exposure, produce latent epigenetic changes (e.g., DNA methylation) in a long-term fashion, beginning at early developmental stages, until a pathological threshold is reached, resulting in the onset of AD [27, 48, 49]. In addition, deficiency of vitamin B12 or folate has been shown to result in gene promoter methylation with upregulation of AD-associated genes [26]. All these results suggested that environmental risk factors play a vital role in the etiology of late-life disorders, including AD. It was also reported that improving the environment could restore the impaired hippocampal neurogenesis and cognitive function in AD [50]. All these studies supported our hypothesis that VA malnutrition could bring about dysregulation of AD related genes which remain latent for many years until aging, poor mid-life diet or Aβ1-42 disturbance, resulting in sustained alterations in these genes to promote AD progression.

However, there are some limitations of our present study. 1) It was reported that the maternal nutritional and metabolic environment is critical in determining long-term health and viability with brain function being most sensitive to influences in the embryonic period [51]. Disturbed maternal nutrition may contribute to deficits in offspring’s development and health, resulting in offspring at increased risk of child and adult diseases [51-53]. However, whether maternal MVAD affects the cognitive function of the offspring and increases the risk of AD in the offspring was not investigated in the present study. Additional work is required to address...
this issue. 2) Additional research is required to explore the therapeutic potential of VA in MVAD-induced AD pathogenesis and the critical windows of therapy.

CONCLUSION

MVAD beginning during the embryonic period did not induce the AD-associated gene expression of ADAM10 and IDE via RARs following Aβ5-42 injection in rats. This nutritional gene effect might exacerbate hippocampus-dependent learning and memory deficits. Our results suggest that long-term MVAD results in an increased risk of AD. Long-term nutritional remediation, such as monitoring VA status and correcting low VA levels, during the early stage of life might reduce the risk for AD late in life. Evidently, more research on the therapeutic potential of VA in MVAD-induced AD pathogenesis is of crucial importance and may provide novel targets for the prevention or treatment of AD.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher’s web site along with the published article.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES

[1] Gravina SA, Ho L, Eckman CB, Long KE, Orvos LJ, Younkin LH, et al. Amyloid beta protein (A beta) in Alzheimer's disease brain. Biochemical and immunocytochemical analysis with antibodies specific for forms ending at A beta 40 or A beta 42(43). J Biol Chem 270(13): 7013-6 (1995).
[2] Sisodia SS, Koo EH, Beyreuther K, Unterbeck A, Price DL. Evidence that β-Amyloid protein in Alzheimer's disease is not derived by normal processing. Science 248(4954): 492-5 (1990).
[3] Li Y, Zhou W, Tong Y, He G, Song W. Control of APP processing and Abeta generation level by BACE1 enzymatic activity and transcription. FASEB J 20(2): 285-92 (2006).
[4] Kojro E, Fahrenholz F. The non-amyloidogenic pathway: structure and function of alpha-secretases. Subcell Biochem 38: 105-27 (2005).
[5] Kuhn PH, Wang H, Dislich B, Colombo A, Zeitschel U, Ellwalt JW, et al. ADAM10 is the physiologically relevant, constitutive alpha-secretase of the amyloid precursor protein in primary neurons. EMBO J 29(17): 3020-32 (2010).
[6] Edbauer D, Willem M, Lammich S, Steiner H, Haass C. Insulin-degrading enzyme rapidly removes the beta-amyloid precursor protein intracellular domain (AICD). J Biol Chem 277(16): 13389-93 (2002).
[7] Maden M. Retinoid acid in the development, regeneration and maintenance of the nervous system. Nat Rev Neurosci 8(10): 755-65 (2007).
[8] World Health Organization. Global prevalence of vitamin A deficiency in populations at risk 1995-2005: WHO Global Database on Vitamin A Deficiency. Geneva: WHO (2009).
[9] Zaman Z, Roche S, Fielden P, Frost PG, Niriella DC, Cayley AC. Plasma concentrations of vitamins A and E and carotenoids in Alzheimer’s disease. Age Ageing 21(2): 91-4 (1992).
[10] Jiménez-Jiménez FJ, Molina JA, de Bustos F, Orti-Pareja M, Benito-León J, Talíón-Barranco A, et al. Serum levels of beta-carotene, alpha-carotene and vitamin A in patients with Alzheimer’s disease. Eur J Neurol 6(4): 495-7 (1999).
[11] Rinaldi P, Polidori MC, Metastasio A, Mariani E, Mattioli P, Cherubini A, et al. Plasma antioxidants are similarly depleted in mild cognitive impairment and in Alzheimer’s disease. Neurobiol Aging 24(7): 915-19 (2003).
[12] Bourdel-Marchasson I, Delmas-Beauvieux MC, Peuchant E, Richard-Harston S, Decamps A, Reignier B, et al. Antioxidant defences and oxidative stress markers in erythrocytes and plasma from normally nourished elderly Alzheimer patients. Age Ageing 30(3): 235-41 (2001).
[13] Smith MA, Petot GJ, Perry G. Diet and oxidative stress a novel synthesis of epidemiological data on Alzheimer's disease. J Alzheimers Dis 1(4-5): 203-6 (1999).
[14] Corcoran JP, So PL, Maden M. Disruption of the retinoid signalling pathway causes a deposition of amyloid beta in the adult rat brain. Eur J Neurosci 20(4): 896-902 (2004).
[15] Misner DL, Jacobs S, Shimizu Y, de Uruquiza AM, Solomin L, Perlmann T, et al. Vitamin A deprivation results in reversible loss of hippocampal long-term synaptic plasticity. Proc Natl Acad Sci USA 98(20): 11714-9 (2001).
[16] Nomoto M, Takeda Y, Uchida S, Mitsuda K, Enomoto H, Saito K, et al. Dysfunction of the RAR/RXR signalling pathway in the forebrain impairs hippocampal memory and synaptic plasticity. Mol Brain 5: 8 (2012).
[17] Jiang W, Yu Q, Gong M, Chen L, Wen E, Bi Y, et al. Vitamin A deficiency impairs postsynaptic cognitive function via inhibition of neuronal calcium excitability in hippocampus. J Neurochem 121(6): 932-43 (2012).
[18] Jarvis CI, Goncalves MB, Clarke E, Hobbs C, Malmqvist T, Deacon R, Jack JW, et al. Evidence that β-Amyloid protein in Alzheimer's disease brain. Biochemical and immunocytochemical analysis with antibodies specific for forms ending at A beta 40 or A beta 42(43). J Biol Chem 270(13): 7013-6 (1995).
[19] Sisodia SS, Koo EH, Beyreuther K, Unterbeck A, Price DL. Evidence that β-Amyloid protein in Alzheimer's disease is not derived by normal processing. Science 248(4954): 492-5 (1990).
[20] Li Y, Zhou W, Tong Y, He G, Song W. Control of APP processing and Abeta generation level by BACE1 enzymatic activity and transcription. FASEB J 20(2): 285-92 (2006).
[21] Kojro E, Fahrenholz F. The non-amyloidogenic pathway: structure and function of alpha-secretases. Subcell Biochem 38: 105-27 (2005).
[22] Kuhn PH, Wang H, Dislich B, Colombo A, Zeitschel U, Ellwalt JW, et al. ADAM10 is the physiologically relevant, constitutive alpha-secretase of the amyloid precursor protein in primary neurons. EMBO J 29(17): 3020-32 (2010).
[23] Edbauer D, Willem M, Lammich S, Steiner H, Haass C. Insulin-degrading enzyme rapidly removes the beta-amyloid precursor protein intracellular domain (AICD). J Biol Chem 277(16): 13389-93 (2002).
[24] West KP. Extent of vitamin A deficiency among preschool children. J Nutr 132(9): 2587S-66S (2002).
[25] Li LM, Rao K, Kong LZ, Yao CH, Xiang HD, Zhai FY, et al. Retinoic acid receptor-alpha signalling antagonizes both intracellular and extracellular amyloid-beta production and prevents neuronal cell death caused by amyloid-beta. Eur J Neurosci 32(8): 1246-55 (2010).
[26] Ding Y, Qiao A, Wang Z, Goodwin JS, Lee ES, Block ML, et al. Retinoid acid attenuates beta-amyloid deposition and rescues memory deficits in an Alzheimer's disease transgenic mouse model. J Neurosci 28(45): 11622-34 (2008).
[27] Kawahara K, Suenobu M, Ohtsuka H, Kuniyasu A, Sugimoto Y, Nakagomi M, et al. Cooperative therapeutic action of retinoic acid receptor and retinoid x receptor agonists in a mouse model of Alzheimer's disease. J Alzheimers Dis 42(2): 587-605 (2014).
[28] Goncalves MB, Clarke E, Hobbs C, Malqvist T, Deacon R, Jack J, et al. Amyloid beta inhibits retinoid acid synthesis exacerbating Alzheimer disease pathology which can be attenuated by an retinoic acid receptor alpha agonist. Eur J Neurosci 37(7): 1182-92 (2013).
[29] Reinhardt S, Grimm MO, Stahlmann C, Hartmann T, Shudo K, Tomita T, et al. Rescue of hypovitaminosis A induces non-amyloidogenic amyloid precursor protein (APP) processing. Curr Alzheimer Res 13(11): 1277-89 (2016).
[30] Beer AJ, Orbea A, Ortega F, Ottesen EA, Global malnutrition. Parasitology 121: S5-22 (2000).
[31] West KP. Extent of vitamin A deficiency among preschool children and women of reproductive age. J Nutr 132(9): 2587S-66S (2002).
[32] Li LM, Rao K, Kong LZ, Yao CH, Xiang HD, Zhai FY, et al. A description on the Chinese national nutrition and health survey in 2002. Zhonghua Liu Xing Bing Xue Za Zhi 26(7): 478-84 (in Chinese) (2005).
[33] Lahiri DK, Maloney B. The "LEARN" (Latent Early-life Associated Regulation) model integrates environmental risk factors and the developmental basis of Alzheimer's disease, and proposes remedial steps. Exp Gerontol 45(4): 291-6 (2010).
[34] Lahiri DK, Maloney B, Basha MR, Ge YW, Zawia NH, How and when environmental agents and dietary factors affect the course of
Alzheimer's disease: the "LEARn" model (latent early-life associated regulation) may explain the triggering of AD. Curr Alzheimer Res 4(2): 219-28 (2007).

[28] Wei H, Huang H, Li T, Qu P, Liu Y, Chen J. Marginal vitamin A deficiency affects lung maturation in rats from prenatal to adult stage. J Nutr Sci Vitaminol 55(3): 208-14 (2009).

[29] Ross AC, Harrison EH. Vitamin A: Nutritional Aspects of Retinoids and Carotenoids. In: Zempleni J, Suttie JW, Gregory JF, III, Stover PJ, Eds. Handbook of Vitamins. Fifth ed. Boca Raton, FL: CRC Press; pp. 1-50 (2013).

[30] Richardson RL, Kim E-M, Shephard RA, Gardiner T, Cleary J, O'Hare E. Behavioural and histopathological analyses of ibuprofen treatment on the effect of aggregated Aβ(1–42) injections in the rat. Brain Res 954(1): 1-10 (2002).

[31] Liu X, Cui T, Li Y, Wang Y, Wang Q, Li X, et al. Vitamin A supplementation in early life enhances the intestinal immune response of rats with gestational vitamin A deficiency by increasing the number of immune cells. PLoS One 9(12): e114934 (2014).

[32] Bromley-Brits K, Deng Y, Song W. Morris water maze test for learning and memory deficits in Alzheimer's disease mouse models. J Vis Exp 53: 2920 (2011).

[33] Hou N, Ren L, Gong M, Bi Y, Gu Y, Dong Z, et al. Vitamin A deficiency impairs spatial learning and memory: the mechanism of abnormal CBP-dependent histone acetylation regulated by retinoic acid receptor alpha. Mol Neurobiol 51(2): 633-47 (2015).

[34] Tamano H, Ide K, Adlard PA, Bush AI, Takeda A. Involvement of hippocampal excitability in amyloid β-induced behavioral and psychological symptoms of dementia. J Toxicol Sci 41(4): 449-57 (2016).

[35] Sharma S, Verma S, Kapoor M, Saini A, Nehru B. Alzheimer’s disease like pathology induced six weeks after aggregated amyloid-beta injection in rats: increased oxidative stress and impaired long-term memory with anxiety-like behavior. Neurol Res 38(9): 838-50 (2016).

[36] O'Hare E, Weldon DT, Mantyh PW, Ghilardi JR, Finke MP, Kuskowski MA, et al. Delayed behavioral effects following intrahippocampal injection of aggregated Aβ(1–42). Brain Res 815(1): 1-10 (1999).

[37] Geula C, Wu CK, Saroff D, Lorenzo A, Yuan M, Yankner BA. Aging renders the brain vulnerable to amyloid beta protein neurotoxicity. Nat Med 4(7): 827-31 (1998).

[38] El Khoury JB, Moore KJ, Means TK, Leung J, Terada K, Toft M, et al. CD36 mediates the innate host response to beta-amyloid. J Exp Med 197(12): 1657-66 (2003).

[39] Takamura R, Watamura N, Nikki M, Ohshima T. All-trans retinoic acid improved impaired proliferation of neural stem cells and suppressed microglial activation in the hippocampus in an Alzheimer's mouse model. J Neurosci Res 95(3): 897-906 (2017).

[40] Koryakina A, Aeberhard J, Kiefer S, Hamburger M, Kuenzi P. Regulation of secretases by all-trans-retinoic acid. FEBS J 276(9): 2645-55 (2009).

[41] Goodman AB. Retinoid receptors, transporters, and metabolizers as therapeutic targets in late onset Alzheimer disease. J Cell Physiol 209(3): 596-603 (2006).

[42] Holback S, Adlerz L, Gatsinzi T, Jacobsen KT, Iverfeldt K. P13-K and PKC-dependent up-regulation of APP processing enzymes by retinoic acid. Biochem Biophys Res Commun 365(2): 298-303 (2008).

[43] Tippmann F, Hundt J, Schneider A, Endres K, Fahrenholz F. Up-regulation of the alpha-secretase ADAM10 by retinoic acid receptors and acitretin. FASEB J 23(6): 1643-54 (2009).

[44] Kitaoa K, Shimizu N, Ono K, Chikahisa S, Nakagomi M, Shudo K, et al. The retinoic acid receptor agonist Am80 increases hippocampal ADAM10 in aged SAMP8 mice. Neuropsycharmacology 72: 58-65 (2013).

[45] Fahrenholz F. Alpha-secretase as a therapeutic target. Curr Alzheimer Res 4(4): 412-7 (2007).

[46] Melino G, Draouei M, Bernardini S, Bellincampi L, Reichert U, Cohen P. Regulation by retinoic acid of insulin-degrading enzyme and of a related endoprotease in human neuroblastoma cell lines. Cell Growth Differ 7(6): 787-96 (1996).

[47] Dommez G, Wang D, Cohen DE, Guarente L. SIRT1 suppresses beta-amyloid production by activating the alpha-secretase gene ADAM10. Cell 142(2): 320-32 (2010).

[48] Maloney B, Lahiri DK. Epigenetics of dementia: understanding the disease as a transformation rather than a state. Lancet Neurol 15(7): 760-74 (2016).

[49] Maloney B, Sambamurti K, Zawia N, Lahiri DK. Applying epigenetics to Alzheimer's disease via the latent early-life associated regulation (LEARn) model. Curr Alzheimer Res 9(5): 589-99 (2012).

[50] Rodriguez JJ, Noristani HN, Olabarria M, Fletcher J, Somerville TD, Yeh CY, et al. Voluntary running and environmental enrichment restores impaired hippocampal neurogenesis in a triple transgenic mouse model of Alzheimer's disease. Curr Alzheimer Res 9(7): 707-17 (2011).

[51] Symonds ME, Stephenson T, Gardner DS, Budge H. Long-term effects of nutritional programming of the embryo and fetus: mechanisms and critical windows. Reprod Fertil Dev 19(1): 53-63 (2007).

[52] Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet 382(9900): 427-51 (2013).

[53] Victora CG, Adair L, Fall C, Hallal PC, Martorell R, Richter L, et al. Maternal and child undernutrition: consequences for adult health and human capital. Lancet 371(9609): 340-57 (2008).