Is Vitamin D Beneficial to Alzheimer Disease? A Surprising Dilemma

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Alzheimer disease (AD) is a degenerative disease of the central nervous system, characterized clinically by progressive cognitive decline and, histologically, by senile plaques and neurofibrillary tangles. The major component of senile plaques is the β -amyloid protein ($A\beta$), which is produced by the sequential proteolysis of a ubiquitous transmembrane protein, amyloid precursor protein (APP). In the amyloidogenic pathway, $A\beta$ is produced by the initial action of β -secretase cleavage, which creates an $A\beta$ -containing C-terminal fragment, known as β -CTF. The latter is further cleaved by γ -secretase complex, producing $A\beta$ and a smaller γ -CTF. Conversely, in the nonamyloidogenic pathway, APP is first cleaved at the α -secretase site, which results in the release of N-terminal sAPP- α and the generation of α -CTF [1]. The prevailing view through most of the twentieth century was that $A\beta$ is only a marker of the disease, but does not play a role in the neurodegenerative process. This view has changed since the 1990s with the elucidation of the amyloid hypothesis, which suggests that abnormal accumulation of $A\beta$ in the brain is a direct cause of neurodegeneration and cognitive decline in AD. The hypothesis is supported by the identification of mutations in APP and presenilins (PS) 1 and 2, which increase $A\beta$ generation, with an increased propensity for $A\beta$ oligomer formation and aggregation [2].

Current drugs for AD mainly include four categories: acetylcholinesterase inhibitors (such as huperzine A, rivastigmine, donepezil, etc.), N-methyl-D-aspartate (NMDA) receptors inhibitors (memantine), selective serotonin re-uptake inhibitors, and recently the use of anti-histamine agent, dimebolin [3]. Generally speaking, these drugs at best slightly delay cognitive decline and

treat symptoms of AD, however, are not known to be disease modifying, as these agents do not modify the underlying pathogenic mechanisms mentioned above. Recently, $A\beta$ aggregation or clearance, genes that are associated with AD, oxidative stress, trophic factors, and TGF- β 1 have been proposed to be the therapeutic targets [4,5]; however, to date γ -secretase inhibitors have been largely ineffective and come with deleterious side effects [6].

In the last decade, inflammaging, a low-grade, chronic up-regulation of certain inflammatory cytokines, such as TNF- α and IL-6 has been proposed as a "prodrome" to AD [7]. Indeed, it is known that microglial activation and its release of proinflammatory cytokines plays critical role in the inflammatory process of AD. In light of this, researchers have focused on vitamin D for its anti-inflammatory and immunomodulatory effects. We preliminarily evaluated the effect of vitamin D₃ (cholecalciferol) administration on microglial cells in terms of TNF- α release as well as in neuron like N2a cells *via* analysis of APP processing, respectively.

In order to determine the effect of vitamin D₃ administration on TNF- α release from microglia, N9 cells were plated in 24-well tissue culture plates (Costar, Cambridge, MA, USA) at 5×10^4 cells/well ($n = 12$ for each condition) for 24 h. They were then stimulated with LPS (10 ng/mL) for 2 h in the presence of pretreatment (1 h) with different dosage of vitamin D₃, or PBS (control). Cell-free supernatants were collected and assayed by TNF- α ELISA kit (Minneapolis, MN, USA) in strict accordance with the manufacturer's instruction. The BCA Protein Assay (Pierce, Rockford, IL, USA) was performed to measure total cellular protein from each of the cell groups under consideration just before quantification of TNF- α release [8].

Figure 1 Vitamin D₃ (VD) significantly increases the Aβ production (upper, Mean ± SEM, pg/mL) and decreases sAPP-α release (lower, mean ± SEM, μg/mL) from Swe APP N2a cells, (**P* < 0.01 compared with controls or positive controls of EGCG [epigallocatechin-3-gallate, extracted from green tea, an α-secretase promoter], by *t*-test, the experiment was conducted twice, with six replicates for each condition).

In order to determine the effect of vitamin D₃ on Aβ production, we cultured Swe APP N2a cells according to previously described methods. Briefly, N2a cells were seeded at 16×10^5 cells/well (*n* = 12 for each condition) in 24-well tissue culture plates containing 0.5 mL of complete DMEM medium, and incubated at 37 °C overnight. These cells were treated with 20 μM EGCG (epigallocatechin-3-gallate, an α-secretase promoter extracted from green tea, as a positive control) and different dosage of vitamin D₃ for 3 h. Conditioned medium was collected and total Aβ was determined according to published methods. Briefly, 6E10 was coated at 2 mg/mL in PBS in 96-well immunoassay plates overnight at 4 °C. The plates were then washed with 0.05% Tween-20 in PBS five times and blocked with blocking buffer (PBS with 1% bovine serum albumin, 5% horse serum) for 2 h at room temperature. Conditioned media or Aβ standards

Figure 2 Vitamin D₃ (VD) also significantly inhibits the release of TNF-α from N9 cells after LPS stimulation in a dosage dependent manner (mean ± SEM, pg/mL, **P* < 0.01 compared with controls and LPS only, by *t*-test, the experiment was conducted four times, with three replicates for each condition), with the most potent concentration at 10 μg/mL.

were added to the plates and incubated overnight at 4 °C. Following three washes, biotinylated antibody 4G8 (0.5 mg/mL in PBS with 1% bovine serum albumin) was added to the plates and incubated for 2 h at room temperature. After five washes, streptavidin-horseradish peroxidase (1:200 dilutions in PBS with 1% bovine serum albumin) was added to the 96-well plates for 30 min at room temperature. TMB substrate was then added to the plates and incubated for 15 min at room temperature. Then 50 μL of stop solution (2N H₂SO₄) was added to each well of the plate. The optical density of each well was immediately determined by a microplate reader at 450 nm. For sAPP-α assay, we replaced the primary antibody with 22C11 antibody, and the second antibody with biotinylated 6E10 antibody [8].

From Figure 1, we can see that vitamin D₃ can significantly increase the Aβ production (Figure 1, left), and accordingly decrease the production of sAPP-α (Figure 1, right) from Swe APP N2a cells; while also inhibiting the release of proinflammatory cytokine, TNF-α, from microglia cells (Figure 2). The latter effect may be beneficial to Alzheimer disease, while the former may counteract this advantageous aspect. sAPP-α has neurotrophic properties, promoting neuronal survival in aging and neuropathological conditions. When neurotrophin synthesis is decreased, spatial memory and navigation will be compromised.

Previous community studies showed that residents with AD and other dementias had lower serum concentrations of vitamin D, and its concentrations were significantly elevated after year-round sun exposure. However, whether sun exposure had improved cognitive function was not mentioned [9].

On the other hand, some authors found that vitamin D could upregulate γ-glutamyl transpeptidase and subsequently increase glutathione. Glutathione is an innate antioxidant which protects oligodendrocytes and the integrity of the nerve conduction pathway critical to mental processing [10]. Future *in vivo*

studies will be needed to quantify the degree to which vitamin D's apparent detrimental effect on sAPP- α production may be neutralized or even outweighed by its promotion of glutathione production.

In conclusion, vitamin D deficiency may be associated with Alzheimer disease, but, up to date, there is still lack of *in vivo* or clinical evidence that directly supports its preventive or therapeutic effects on AD animal models or patients.

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Conflict of Interest

The authors declare no conflict of interest.