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In silico and *in vitro* characterization of anti-amyloidogenic activity of vitamin K3 analogues for Alzheimer's disease

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ABSTRACT

Background: Aggregation of amyloid-beta (Aβ) has been proposed as the main cause of Alzheimer's disease (AD). Vitamin K deficiency has been linked to the pathogenesis of AD. Therefore, 15 synthesized vitamin K3 (VK3) analogues were studied for their anti-amyloidogenic activity.
Methods: Biological and spectroscopic assays were used to characterize the effect of VK3 analogues on amyloidogenic properties of Aβ, such as aggregation, free radical formation, and cell viability. Molecular dynamics simulation was used to calculate the binding affinity and mode of VK3 analogue binding to Aβ.
Results: Both numerical and experimental results showed that several VK3 analogues, including VK3-6, VK3-8, VK3-9, VK3-10, and VK3-224 could effectively inhibit Aβ aggregation and conformational conversion. The calculated inhibition constants were in the μM range for VK3-10, VK3-6, and VK3-9 which was similar to the IC_{50} of curcumin. Cell viability assays indicated that VK3-9 could effectively reduce free radicals and had a protective effect on cytotoxicity induced by Aβ.
Conclusions: The results clearly demonstrated that VK3 analogues could effectively inhibit Aβ aggregation and protect cells against Aβ induced toxicity. Modified VK3 analogues can possibly be developed as effective anti-amyloidogenic drugs for the treatment of AD.
General significance: VK3 analogues effectively inhibit Aβ aggregation and are highly potent as anti-amyloidogenic drugs for therapeutic treatment of AD.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia within the senior population, and is characterized pathologically by the progressive intracerebral accumulation of amyloid-beta (Aβ) peptides [1,2]. These peptides are proteolytic byproducts of the Aβ protein precursor, and are most commonly composed of 40 (Aβ1−40) and 42 (Aβ1−42) amino acids. Aβ peptides appear to be unstructured in their monomeric state but aggregate to form fibrils with an ordered cross β-sheet pattern [3–6]. Increasing evidence from recent studies indicates that soluble oligomers as well as mature fibrils are the toxic agents [7–9].

Presently there is no cure or treatment for AD, and significant effort has been made to find drugs to cope with this disease. Based on the amyloid cascade hypothesis, small molecules which enable to stabilize the conformation of monomeric Aβ or to inhibit and reverse misfolding and aggregation could be potent drug candidates for the therapeutic treatment of AD [4,5,7,10–13]. In general, two classes of inhibitors are known, bioactive molecules and drugs unrelated to AD [10]. Many of the known compounds such as curcumin [14], polyphenols from wine [15], apomorphine [16], omega-3 fatty acids [17], vitamin A, and β-carotene [18] belong to the first class. The drugs included in the second class are anti-inflammatory [19] and anti-Parkinson agents such as dopamine, selegiline, and L-dopa [20,21].

Moreover, based on their anti-amyloidogenic activities, the compounds which inhibit Aβ aggregation can be further divided into four groups. The strongest anti-amyloidogenic group, including dopamine

Abbreviations: Aβ, amyloid-beta; VK, vitamin K; AD, Alzheimer's disease; IC_{50}, half maximal inhibitory concentration; ApoE, apolipoprotein E; MD, molecular dynamics; MM-PBSA, molecular mechanics-Poisson−Boltzmann surface area; SI, supplemental information; DCFH-DA, dichlorofluorescein diacetate; DCF, dichlorofluorescein; ThT, thioflavin-T; FT-IR, Fourier-transform infrared; ROS, reactive oxygen species

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and tannic acid, has a half maximal inhibitory concentration (IC_{50}) value of 0.01 μM [15,20,22]. The second group, including nordihydroguaiaretic acid, curcumin, and myricetin, has an IC_{50} value of 0.1 μM [14,20,23]. The third group, including L-dopa and β-carotene, has an IC_{50} value of 1 μM [18,20]. The fourth group, including tetracycline and rifampicin, has an IC_{50} value of 10 μM [24,25]. The effects of the antioxidant vitamins A, B2, B6, C, and E on the inhibition of Aβ aggregation has been studied [18]. Among these vitamins, vitamin A shows the most potent inhibition of Aβ aggregation *in vitro*. The IC_{50} of vitamin A is approximately 0.1 μM [18], whereas the IC_{50} for vitamins C and E are much higher, at around 200–500 μM for vitamin C [26] and 10 μM for vitamin E [21].

The possible role of vitamin K in the pathogenesis of AD was first reported by Allison [27]. When compared to other apolipoprotein E (ApoE 2 and ApoE3) genotypes, the concentration of vitamin K was lower in the circulating blood of ApoE4 carriers, which is a genetic risk factor for late-onset AD. Therefore, it was suggested that vitamin K deficiency may contribute to the pathogenesis of AD and that vitamin K supplementation may have a beneficial effect in preventing or treating AD. Although vitamin K has been shown to regulate functions in the brain, such as sulfotransferase activity, and the activity of a growth factor/tyrosine kinase receptor, the molecular mechanism of action of vitamin K on AD remains unclear. This could be due to a lack of interest because of its neurotoxicity.

In the present study, we experimentally studied the effects of 15 vitamin K3 (VK3) analogues on Aβ1–40 aggregation and cellular toxicity. Although many VK3 analogues such as VK3–9, VK3–10, and VK3–6 inhibited the aggregation of Aβ1–40, only VK3–9 was able to protect cells against Aβ1–40 induced toxicity. The effective dose of VK3–9 was approximately 0.1 μM, which is as effective as amyloidogenic compounds such as curcumin [14,15,21]. Further simulation analyses revealed that the electrostatic and van der Waals forces, rather than hydrogen bonding networks, are the key factors governing binding affinities of VK3 analogues to Aβ1–40. The binding energies of Aβ1–40–VK3 analogue complexes displayed a high correlation with the experimental aggregation rates. In conclusion, although most VK3 analogues did not protect cells against Aβ induced toxicity, both simulation and experimental results suggest that VK3–9 is a potent compound for preventing aggregation of amyloid peptides. Other VK3 analogues such as VK3–10 and VK3–6 could be further modified for potential use as therapeutic drugs to treat AD.

2. Material and methods

2.1. Docking of Vitamin K3 analogues to Aβ1–40

Because Aβ peptides are highly aggregation prone in water, their monomeric structures have not yet been experimentally resolved. Therefore, to obtain suitable Aβ1–40 structures in an aqueous environment for use in simulation of binding of VK3 analogues, we modeled the Aβ structure in water using the PDB code 1BA4 [28] as the initial structural model. This model is Aβ1–40 determined in the water-micelle environment. The structure taken from 1BA4 was first heated to $T=$ 500 K. The 5 ns MD simulations [see Supplemental Information (SI) for details on MD simulations] were carried out at this temperature until random coil structures were obtained in explicit water using the GROMOS96 43a1 force field [29]. A random coil structure was used as the starting configuration for subsequent 300 ns MD simulations at $T=310$ K. Snapshots collected at equilibrium during the last 200 ns were grouped by the Cα-RMSD conformational clustering method implemented in the Gromacs software. With the clustering tolerance of 1 Å, 5 representative structures with the lowest energy (Fig. S3 in SI) were used for further docking of VK3 analogues to Aβ1–40.

To dock VK3 analogues to full-length Aβ1–40, both Aβ1–40 and VK3 analogues were prepared as PDBQT files using AutodockTools 1.5.4 [30]. The Autodock Vina version 1.1 was employed [31], as it is much more efficient than Autodock 4.0. To describe atomic interactions, a modified version of the CHARMM force field was implemented [32]. In the Autodock Vina software the Broyden–Fletcher–Goldfarb–Shanno method was employed for local optimization [33]. To obtain reliable results, the exhaustiveness of global search was set to 400, and the maximum energy difference between the best and worse binding mode was chosen as 7. Twenty binding modes (20 modes of docking) were generated with random starting positions of each VK3 analogue, which had fully flexible torsional degrees of freedom. The center grids were placed at the center of the mass of Aβ1–40, and grid dimensions were 60×40× 40, which are large enough to cover the entire Aβ1–40. In this approach, the binding energy was the average of five obtained Aβ1–40–VK3 analogue complex models (Fig. S5 in SI).

2.2. Molecular mechanics–Poisson–Boltzmann surface area

The details of MM-PBSA are given in SI. Overall, in this method the binding free energy (ΔG_{bind}) of ligand to receptor is given as:

$$\Delta G_{bind} = \Delta E_{elec} + \Delta E_{vdw} + \Delta G_{sur} + \Delta G_{PB} - T\Delta S \quad (1)$$

where ΔE_{elec} and ΔE_{vdw} are contributions from electrostatic and van der Waals interactions, respectively. ΔG_{sur} and ΔG_{PB} are nonpolar and polar solvation energies. The entropic contribution of $T\Delta S$ was estimated using the normal mode approximation (see SI for more details). To calculate ΔG_{bind}, the MD simulations were carried out using the GROMOS force field 43a1 as described in SI. The structures of Aβ1–40–VK3 analogue complexes obtained in the best docking mode (see Fig. 6 and snapshot 5 in Fig. S4) were used as starting configurations for MD simulations. For each system, 4—6 MD trajectories of approximately 100 ns were generated. Snapshots collected in equilibrium were used to compute the binding free energy given by Eq. (1).

2.3. Synthesis of vitamin K3 analogues

The synthesis procedures of vitamin K3 analogues shown in Fig. 1 are described elsewhere [34]. Analogues were kindly provided by Professor C. P. Chen of National Dong Hwa University.

2.4. Synthesis and purification of Aβ1–40

Aβ1–40 was synthesized in a solid-phase peptide synthesizer (ABI 433A) using standard FMOC protocols with HMP resin. After cleavage from the resin with a mixture of trifluoroacetic acid/H_2O/ethanedithiol thiol anisole/phenol, the peptides were extracted with 1:1 (v:v) ether: H_2O containing 0.1% 2-mercaptothanol. The synthesized Aβ1–40 peptides were purified using a C_{18} reverse-phase column with a linear gradient from 0% to 78% acetonitrile. Peptide purity was over 95% as

VK3-1 : R1=-SC_2H_4OH, R2=-CH_3
VK3-2 : R1=-SC_3H_6OH, R2=-CH_3
VK3-3 : R1=-SC_4H_8OH, R2=-CH_3
VK3-4 : R1=-$SC_6H_{12}OH$, R2=-CH_3
VK3-5 : R1=-$SC_{11}H_{22}OH$, R2=-CH_3
VK3-6 : R1=-SC_2H_4COOH, R2=-CH_3
VK3-8 : R1=-$SCH_2CHOHCH_3$, R2=-CH_3
VK3-9 : R1=- $SCH_2CHOHCH_2OH$, R2=-CH_3
VK3-10 : R1=-S (C_6H_4)OH, R2=-CH_3
VK3-199 : R1=-SC_2H_4OH, R2=-H
VK3-221 : R1=-OH, R2=-CH_3
VK3-231 : R1=-SC_2H_4OH, R2=-SC_2H_4OH
VK3-232 : R1=- $SCH_2CHOHCH_2OH$, R2=- $SCH_2CHOHCH_2OH$
VK3-233-2d : R1=-$SC_6H_{12}OH$, R2=-$SC_6H_{12}OH$
VK3-224 : R1=-NHC_2H_4 ($NC_2H_4OC_2H_4$), R2=-CH_3

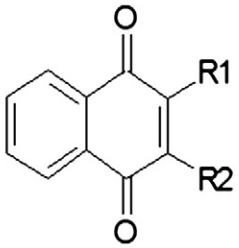

Fig. 1. Structures of the synthesized VK3 analogues.

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