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# Menadione sodium bisulfite inhibits the toxic aggregation of amyloid-β(1–42)

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ABSTRACT

Protein misfolding and aggregation are associated with amyloidosis. The toxic aggregation of amyloid-β 1–42 (Aβ42) may disrupt cell membranes and lead to cell death and is thus regarded as a contributing factor in Alzheimer's disease (AD). 1,4-naphthoquinone (NQ) has been shown to exhibit strong anti-aggregation effects on amyloidogenic proteins such as insulin and α-synuclein; however, its high toxicity and poor solubility limit its clinical application. Menadione sodium bisulfite (MSB, also known as vitamin K3), is used clinically in China to treat hemorrhagic diseases caused by vitamin K deficiency and globally as a vitamin K supplement. We hypothesized that MSB could inhibit amyloid formation since its backbone structure is similar to NQ. To test our hypothesis, we first investigated the effects of MSB on Aβ42 amyloid formation *in vitro*. We found that MSB inhibited Aβ42 amyloid formation in a dose dependent manner, delayed the secondary structural conversion of Aβ42 from random coil to ordered β-sheet, and attenuated the ability of Aβ42 aggregates to disrupt membranes; moreover, the quinone backbone rather than lipophilicity is esstial for the inhibitory effects of MSB. Next, in cells expressing a pathogenic APP mutation (Osaka mutation) that results in the formation of intraneuronal Aβ oligomers, MSB inhibited the intracellular aggregation of Aβ. Moreover, MSB treatment significantly extended the life span of *Caenorhabditis elegans* CL2120, a strain that expresses human Aβ42. Together, these results suggest that MSB and its derivatives may be further explored as potential therapeutic agents for the prevention or treatment of AD.

## 1. Introduction

Protein misfolding, aggregation and amyloid formation are associated with approximately 50 protein misfolding diseases (PMDs), including the neurodegenerative diseases Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD) [1–4]. During the course of the disease, protein monomers misfold and assemble into oligomers initially and subsequently fibrils characterized by rich β-sheet structures [5, 6]. Oligomers have been proven to be the most toxic species during protein aggregation [7–9], toxic oligomers are associated with the onset and development of amyloidogenic diseases [10–12], as a result of induced cell apoptosis *via* disruption of cell membranes, induction of oxidative stress and endoplasmic reticulum stress [8, 13, 14], as well as signaling through prion protein [15, 16]. Also, mature fibrils may accelerate the pathological process, for instance, α-synuclein fibrils can seed the aggregation of endogenous synuclein in mice [17].

AD is a common age-associated neurodegenerative disease [18]. Pathologically, one of the major features of AD brain tissue is deposition of amyloid plaques mostly comprised of insoluble fibrillar amyloid-β [18–20]. Amyloid-β peptides are derived from the cleavage of the amyloid precursor protein (APP), the majority of which are either amyloid-β (1–40) (Aβ40) or amyloid-β (1–42) (Aβ42; Fig. 1C) [21, 22], Aβ42 is more prone to aggregation than Aβ40 [23]. Aggregates of Aβ are reported to be toxic to nerve cells through disruption of the cell membrane, induction of oxidative stress, and mitochondrial dysfunction, which may eventually result in AD [14, 24, 25]. As the major toxic species, the structure and properties of Aβ oligomers have been

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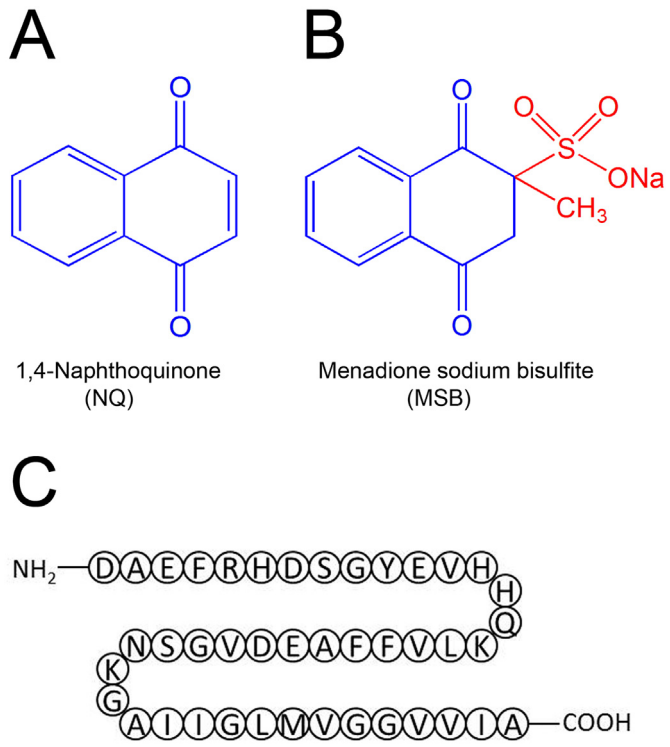


**Fig. 1.** Structure of Aβ42 and compounds. (A) 1,4-naphthoquinones (NQ); (B) Menadione sodium bisulfite (MSB); (C) Primary amino acid sequence of Aβ42.

extensively studied, which provide clues for development of effective therapy against AD [26–30].

Inhibiting the formation of toxic aggregates of amyloid proteins using small molecules has been proposed as a potential therapeutic approach for protein misfolding diseases [31–34]. We and others have previously reported that polyphenols such as (−)-epigallocatechin 3-gallate (EGCG), curcumin, myricetin, caffeic acid, and proanthocyanidins have inhibitory effects on amyloid formation [35–41]. As oxidative intermediates of polyphenols, quinones, such as anthraquinone and anthraquinone derivatives are widely available in medicinal plants, *e.g.* rhubarb [42]. Recently, we and others showed that multiple quinones inhibit protein aggregation, among which, 1,4-naphthoquinone (NQ) is highly effective in inhibiting amyloid formation by insulin [43], α-synuclein [44], and Aβ(25–35), which is a core domain for Aβ aggregation [45]. However, high toxicity and poor solubility limit the potential clinical application of quinones [46]. We previously suggested that the quinone ring of NQ may be a key moiety to the anti-aggregation property [43]. Through a search of a structural database, we found that the compound menadione sodium bisulfite (MSB) shares a similar backbone structure to NQ while having better solubility (Fig. 1A & B). MSB, a member of the synthetic vitamin K3 family, is used clinically in China to treat hemorrhagic diseases resulting from vitamin K deficiency, such as hypoprothrombinemia and neonatal hemorrhage [47, 48]. MSB is also used globally as a vitamin K supplement for animals [49, 50]. We hypothesized that MSB may affect the toxic aggregation of amyloidogenic proteins. Using Aβ42 (Fig. 1C) as a model, we found that MSB not only inhibited the toxic aggregation of Aβ42 *in vitro*, but also reduced aggregation in cells overexpressing a pathogenic APP mutation and extended the life span of transgenic *Caenorhabditis elegans* CL2120 that expresses human Aβ42 [51].

## 2. Materials and methods

### 2.1. Materials

Synthetic amyloid-β (1–42) (> 90%) was purchased from Lifetein Biotechnology (Beijing, China). Menadione sodium bisulfite (MSB) and 1,4-naphthoquinone (NQ) were obtained from Aladdin-Reagents (Shanghai, China). Thioflavin-T (ThT), 5(6)-Carboxyfluorescein were obtained from Sigma-Aldrich (St. Louis, USA). Uranyl acetate was from Ted Pella Inc. (Redding, CA, USA). Coagulation reagent I (DOPE:DOPS:DOPC 5:3:2 w/w) and 2-Oleoyl-palmitoyl-sn-glycerol-3-phospho-rac (1-glycerol) sodium salt (POPG) were obtained from Avanti Polar Lipids (Alabaster, AL, USA). Compounds including benzoquinone, anthraquinone, phenanthraquinone, glycyrrhizic acid, berberine, daidzeine, 2,2-Bis(4-hydroxyphenyl)butane, bisphenol A, 4,4'-Sulfonyldiphenol and paeoniflorin were obtained from Aladdin-Reagents (Shanghai, China). Anti-oligomer antibody (A11, #AB9234, 1:2000), anti-fibril antibody (OC, #AB2286, 1:2000), anti-rabbit and anti-mouse IgG were from Merck Millipore (Billerica, USA). Anti-Aβ antibody (82E1, #10323, 1:100), anti-Aβ oligomer antibody (11A1, #10379A, 1:100) were from Immuno-Biological Laboratories, anti-Aβ antibody (6E10, #SIG-39300, 1:2000) was purchased from BioLegend (San Diego, USA). Anti-GFP (#50430-2-AP, 1:10000) was obtained from proteintech (Wuhan, China), and anti-β-actin (#GB13001, 1:2000) antibody were from Google Biology (Wuhan, China). BCA Protein Assay Kit was from the Beyotime Biotechnology (Jiangsu, China). Fresh blood was collected from healthy volunteers with approval by the ethics committees of the Central Hospital of Wuhan. Wild type *Caenorhabditis elegans* strain N2 and the transgenic strain CL2120 [(pCL12) unc-54::beta 1–42 + (pCL26) mtl-2::GFP] were obtained from the *Caenorhabditis* Genetics Center (University of Minnesota, MN, USA). All other chemicals used were of the highest grade available.

### 2.2. Amyloid formation and thioflavin-T (ThT) fluorescence assays

Aβ42 fibril formation was monitored by ThT fluorescence detection. For amyloid formation, Aβ42 was dissolved in 60 mM NaOH to a stock concentration of 200 μM and then diluted in 50 mM PBS (100 mM NaCl, pH 7.4) to a final concentration of 20 μM as previously reported [52]. NQ and MSB were dissolved in ultrapure water. Compounds including benzoquinone, anthraquinone, phenanthraquinone, berberine, glycyrrhizic acid, daidzeine, bisphenol A, 2,2-Bis(4-hydroxyphenyl)butane, 4,4'-Sulfonyldiphenol and paeoniflorin were dissolved with DMSO to make 100 mM stock solutions, before experiments, they were mixed with Aβ at a molar ratio of 1:1 (20 μM) in 50 mM PBS (100 mM NaCl, pH 7.4). All samples were incubated at 37 °C for amyloid formation. At designated time points, 3 μl of each sample was collected and mixed with ThT (final concentration of 20 μM), ThT fluorescence was then measured using a Hitachi FL-2700 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) with excitation and emission wavelengths set at 450 nm and 482 nm, respectively. All experimental groups were assayed in triplicate and all experiments were repeated at least three times.

### 2.3. Thioflavin-T (ThT) fluorescence assays with lipids

Lipid vesicles containing DOPE:DOPS:DOPC (5:3:2 w/w, Avanti Polar Lipids) were prepared as described previously [53, 54]. Powdered lipid was dissolved in chloroform then evaporated under nitrogen gas and dried overnight under vacuum. The resulting lipid film was rehydrated to a final concentration of 20 mM by vortexing in a buffer of 50 mM PBS (100 mM NaCl, pH 7.4). After ten freeze-thaw cycles, the lipid solution was sonicated on ice until the solution became clear. Lipid vesicles were diluted to 200 μM and incubated with 20 μM Aβ42 at 37 °C.