Bioorganic & Medicinal Chemistry 26 (2018) 1538–1546

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Mechanistic analyses of the suppression of amyloid β42 aggregation by apomorphine

Mizuho Hanaki [a], Kazuma Murakami [a], Sumie Katayama [b], Ken-ichi Akagi [b], Kazuhiro Irie [a,*]

[a] Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
[b] National Institute of Biomedical Innovation, Health and Nutrition, Osaka 567-0085, Japan

ARTICLE INFO

Article history:
Received 7 November 2017
Revised 29 January 2018
Accepted 31 January 2018
Available online 2 February 2018

Keywords:
Alzheimer's disease
Amyloid β-protein
Aggregation
(*R*)-Apomorphine
NMR
LC–MS

ABSTRACT

(*R*)-Apomorphine (**1**) has the potential to reduce the accumulation of amyloid β-protein (Aβ42), a causative agent of Alzheimer's disease (AD). Although the inhibition of Aβ42 aggregation by **1** is ascribable to the antioxidative effect of its phenol moiety, its inhibitory mechanism at the molecular level remains to be fully elucidated. LC–MS and UV analyses revealed that **1** is autoxidized during incubation to produce an unstable *o*-quinone form (**2**), which formed a Michael adduct with Lys 16 and 28 of Aβ42. A further autoxidized form of **1** (**3**) with *o*-quinone and phenanthrene moieties suppressed Aβ42 aggregation comparable to **1**, whereas treating **1** with a reductant, tris(2-carboxyethyl)phosphine diminished its inhibitory activity. ^{1}H-^{15}N SOFAST-HMQC NMR studies suggested that **1** interacts with Arg5, His13,14, Gln15, and Lys16 of the Aβ42 monomer. These regions form intermolecular β-sheets in Aβ42 aggregates. Since **3** did not perturb the chemical shift of monomeric Aβ42, we performed aggregation experiments using 1,1,1,3,3,3-hexafluoro-2-propanol-treated Aβ42 to investigate whether **3** associates with Aβ42 oligomers. Compounds **1** and **3** delayed the onset of the oligomer-driven nucleation phase. Despite their cytotoxicity, they did not exacerbate Aβ42-mediated neurotoxicity in SH-SY5Y neuroblastoma cells. These results demonstrate that extension of the conjugated system in **1** by autoxidation can promote its planarity, which is required for intercalation into the β-sheet of Aβ42 nuclei, thereby suppressing further aggregation.

1. Introduction

Amyloid fibrils of senile plaques in patients with Alzheimer's disease (AD) consist mainly of 40- and 42-residue amyloid β-proteins (Aβ40 and Aβ42).[1,2] Aβ42 is regarded as a leading cause of AD because its aggregative ability, and it exhibits greater neurotoxicity than Aβ40.[3] In contrast, Aβ40 could be involved in the progression of AD due to its greater abundance. The aggregation mechanism of Aβ42 is generally explained by a nucleation-dependent polymerization model that includes nucleation and elongation phases.[4] During the nucleation phase, Aβ42 monomer gradually forms low-molecular-weight intermediates called "nuclei". In the subsequent elongation phase, each nucleus acts as a template that associates most frequently with Aβ42 monomers, and occasionally with Aβ40 monomers, to polymerize, resulting in the formation of amyloid fibrils with abundant β-sheet structure.

Several lines of evidence in terms of Aβ aggregation have shown that neuronal death and cognitive dysfunction are caused mainly by transient oligomers or protofibrils of Aβ rather than end-stage mature fibrils.[5,6] The nucleus in the nucleation-dependent polymerization model can be related to toxic Aβ42 oligomers.[7,8] "Aggregation" is defined in this work as the change from Aβ monomers into fibrils *via* either oligomers or protofibrils. It is widely accepted that compounds that either stop the formation of toxic oligomers or disassemble them are beneficial in the treatment of AD. Alternatively, small molecules that convert the toxic oligomers of Aβ into non-toxic fibrils would also be useful for treating AD.[9]

Drug repositioning has attracted increasing attention due to the high failure rates and production costs involved in new drug development. (*R*)-apomorphine (**1**, Fig. 1E) is currently in clinical use as a dopaminergic agonist in order to supply dopamine to the motor

Abbreviations: Aβ, amyloid β-protein; AD, Alzheimer's disease; HFIP, 1,1,1,3,3,3,-hexafluoroisopropyl alcohol; HMQC, heteronuclear multiple quantum coherence; HPLC, high performance liquid chromatography; LC–MS, liquid chromatography-mass spectrometry; LC/Q Tof-MS, liquid chromatography/quadrupole time-of-flight mass spectrometry; NMR, nuclear magnetic resonance; PBS, phosphate-buffered saline; SOFAST, band-selective optimized flip angle short transient; TEM, transmission electron microscopy; Th-T, thioflavin-T.

* Corresponding author.
E-mail address: irie@kais.kyoto-u.ac.jp (K. Irie).

https://doi.org/10.1016/j.bmc.2018.01.028
0968-0896/

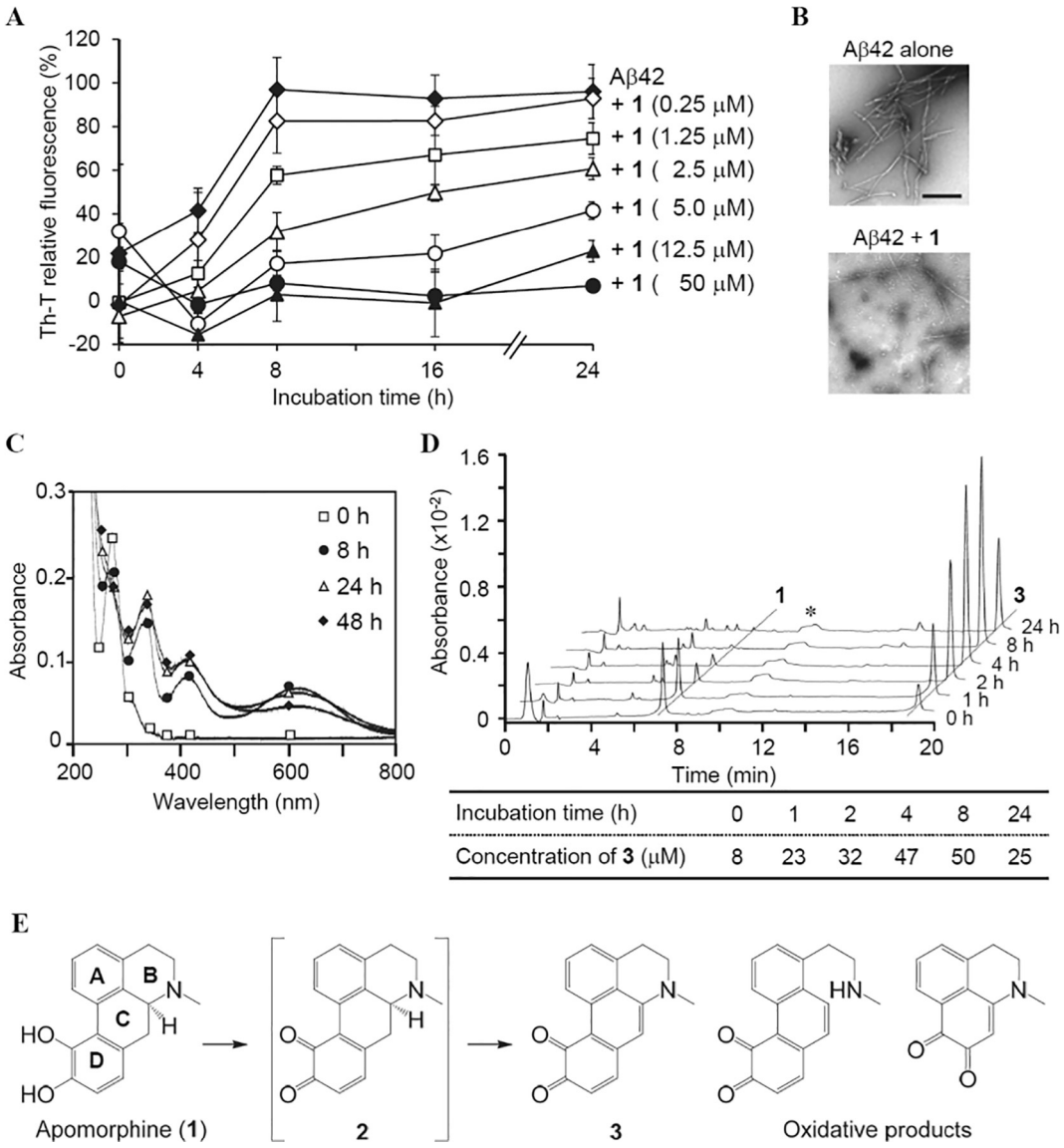

Fig. 1. Autoxidation of apomorphine (**1**) and its ability to inhibit Aβ42 aggregation. (A) The ability of **1** to prevent the aggregation of Aβ42 was determined by the thioflavin-T (Th-T) test. Aβ42 (◆; 25 μM) and **1** (◇; 0.25, □; 1.25, △; 2.5, ○; 5, ▲; 12.5, ●; 50 μM) were incubated in PBS (pH 7.4) at 37 °C. Data are presented as the mean ± SD (n = 8). (B) TEM analysis of Aβ42 aggregates after incubation for 48 h in the Th-T test. Scale bar = 200 nm. *Left*, Aβ42; *right*, Aβ42 treated with **1**. (C) UV–visible spectra of **1** (50 μM) treated with Aβ42 (25 μM) in PBS (pH 7.4) at 37 °C after incubation for 0 (□), 8 (●), 24 (△), and 48 (◆) h. (A) Autoxidation procedure of **1**. (D) HPLC analysis of **1** (50 μM) incubated in PBS (pH 7.4) at 37 °C for the indicated duration. Chromatograms were detected at 350 nm. The concentration of **3** was calculated by the corresponding calibration curve. It was impossible to prepare the calibration curve of **1** because of its instability in the eluent condition of HPLC analysis. Asterisk (*) means artifact. (E) Autoxidation procedure of **1**.

neurons of Parkinson's disease patients.[10] Ohyagi and colleagues reported that **1** not only enhanced the degradation of intracellular Aβ[11] but also attenuated the accumulation of intracellular Aβ[12] using a 3xTg-AD mouse model. Given its relevance to the aggregation of Aβ42 induced by reactive oxygen species,[13] Lashuel et al. described that the inhibition of Aβ40 aggregation by **1** may be ascribed to its antioxidative activity, which is acquired through the autoxidation of 10,11-dihydroxy group of D-ring (Fig. 1E).[14]

The autoxidation mechanism of **1** has been studied well enough for an *o*-quinone intermediate of **1** (**2**) to be proposed, which includes a biphenyl structure, as well as a further autoxidized form of **1** (**3**) with *o*-quinone and phenanthrene moieties (Fig. 1E),[15,16] in addition to a few degraded or dimerized forms of **1**.[17,18] We previously determined the significance of "a catechol moiety" in (+)-taxifolin targeting Lys16 and 28 for Michael addition, and the importance of planarity derived from α,β-unsaturated carbonyl groups in morin, datiscetin, and curcumin, targeting His13 and 14 and Phe19 and 20 for intercalation into β-sheet regions to suppress Aβ42 aggregation.[19,20] The structural features of **3** such as both structural factors, an *o*-quinone moiety and planarity, prompted us to investigate which amino acid residues in Aβ42 they target and whether they inhibit the nucleation or elongation phase of Aβ42 aggregation. Here, we have reported comprehensive physicochemical and spectroscopic studies on the association of **1** and **3** with Aβ42 that leads to the suppression of Aβ42 aggregation, in addition to their effects on Aβ42-induced neurotoxicity towards SH-SY5Y human neuroblastoma cells.

2. Results

2.1. Effects of *1* and its oxidants (*3*) on *Aβ42 aggregation*

(*R*)-Apomorphine (**1**) inhibited the aggregation of Aβ42 in a dose-dependent manner (Fig. 1A). Moreover, the significant sup-