Bioorganic & Medicinal Chemistry 20 (2012) 5844–5849

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

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

Protective effects of caffeoylquinic acids on the aggregation and neurotoxicity of the 42-residue amyloid β-protein

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ARTICLE INFO

Article history:
Received 12 July 2012
Revised 1 August 2012
Accepted 3 August 2012
Available online 12 August 2012

Keywords:
Alzheimer's disease
Amyloid β aggregation
Neuroprotective effect
Caffeoylquinic acid
Structure–activity relationship

ABSTRACT

Alzheimer's disease (AD), a neurodegenerative disorder, is characterized by aggregation of 42-mer amyloid β-protein (Aβ42). Aβ42 aggregates through β-sheet formation and induces cytotoxicity against neuronal cells. Aβ42 oligomer, an intermediate of the aggregates, causes memory loss and synaptotoxicity in AD. Inhibition of Aβ42 aggregation by small molecules is thus a promising strategy for the treatment of AD. Caffeoylquinic acid (CQA), a phenylpropanoid found widely in natural sources including foods, shows various biological activities such as anti-oxidative ability. Previously, our group reported that 3,5-di-*O*-caffeoylquinic acid (3,5-di-CQA) rescued the cognitive impairment in senescence-accelerated-prone mice 8. However, structure–activity relationship of CQA derivatives on the aggregation and neurotoxicity of Aβ42 remains elusive. To evaluate the anti-amyloidogenic property of CQA-related compounds for AD therapy, we examined the effect of CQA and its derivatives on the aggregation and neurotoxicity of Aβ42. In particular, 4,5-di-*O*-caffeoylquinic acid (4,5-di-CQA) and 3,4,5-tri-*O*-caffeoylquinic acid (3,4,5-tri-CQA) strongly inhibited the aggregation of Aβ42 in a dose-dependent manner. Structure–activity relationship studies suggested that the caffeoyl group in CQA is essential for the inhibitory activity. These CQAs also suppressed the transformation into β-sheet and cytotoxicity against human neuroblastoma cells of Aβ42. Furthermore, 3,4,5-tri-CQA blocked the formation of Aβ42 oligomer. These results indicate that 3,4,5-tri-CQA could be a potential agent for the prevention of AD.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized with the deposits of amyloid fibrils.[1] The deposits mainly consist of 40- and 42-mer amyloid β-protein (Aβ40 and Aβ42), which are produced from amyloid β-protein precursor (APP) by two proteases, β- and γ-secretases.[2,3] Aβ42 plays a more important role in the pathogenesis of AD than Aβ40 because of its stronger aggregative ability and neurotoxicity.[1] Accumulated evidence shows that Aβ oligomers (intermediates of Aβ aggregates), but not the monomer nor fibril, induce cognitive dysfunction and synaptic impairment during AD progression.[4,5] Recently, several small molecules were reported to inhibit Aβ-related pathologies in vitro and in vivo, especially polyphenols, some of which (resveratrol and epigallocatechin gallate) are currently in pre-clinical or clinical trial.[6]

Caffeoylquinic acids (CQAs) are bioactive phenylpropanoids found in natural sources such as coffee beans, sweetpotatoes, propolis, and other plants.[7–10] Our previous investigation using several derivatives of CQAs (Table 1) proposed that 3,5-di-*O*-caffeoylquinic acid (3,5-di-CQA) and 3,4,5-tri-*O*-caffeoylquinic acid (3,4,5-tri-CQA) inhibited Aβ42-induced cytotoxicity on human neuroblastoma SH-SY5Y cells by enhancing the expression of mRNA of glycolytic enzymes and intracellular ATP.[11–13] We also reported that 3,5-di-CQA treatment improved spatial learning memory on senescence accelerated-prone mice 8 through increasing the mRNA expression of phosphoglycerate kinase 1.[11] However, there are no comprehensive reports on the effects of CQA derivatives on the aggregation and neurotoxicity of Aβ42.

To evaluate the potential role of CQAs in the treatment of AD, we examined the effects of CQAs on the aggregation of Aβ42 and its neurotoxicity on SH-SY5Y cells using thioflavin T (Th-T) assay, transmission electron microscopy (TEM), circular dichloism (CD) spectroscopy, MTT test, dot blotting, and Western blotting. This paper describes the potent inhibition of Aβ42's aggregation (oligomerization) and cytotoxicity by CQA derivatives. In particular, 3,4,5-tri-CQA had the most potent inhibitory activity, suggesting that the caffeoyl group plays an important role in the inhibition.

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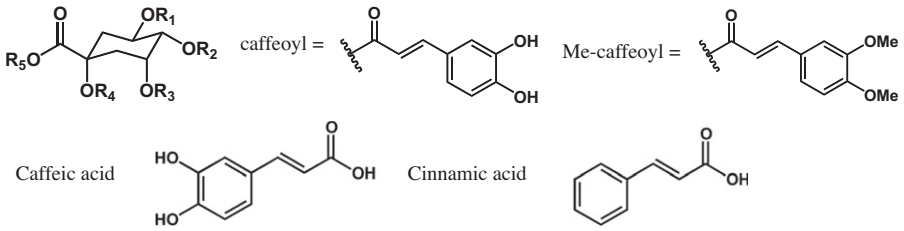
http://dx.doi.org/10.1016/j.bmc.2012.08.001

Table 1
Structures of test compounds



Compound	R_1	R_2	R_3	R_4	R_5
Quinic acid	H	H	H	H	H
Caffeic acid	—	—	—	—	—
Cinnamic acid	—	—	—	—	—
Chlorogenic acid	Caffeoyl	H	H	H	H
Methyl chlorogenic acid	Me-caffeoyl	H	H	H	H
3,5-Di-*O*-caffeoylquinic acid	Caffeoyl	H	Caffeoyl	H	H
3,4-Di-*O*-caffeoylquinic acid	Caffeoyl	Caffeoyl	H	H	H
4,5-Di-*O*-caffeoylquinic acid	H	Caffeoyl	Caffeoyl	H	H
1,4,5-Tri-*O*-caffeoylquinic acid	H	Caffeoyl	Caffeoyl	Caffeoyl	H
3,4,5-Tri-*O*-caffeoylquinic acid	Caffeoyl	Caffeoyl	Caffeoyl	H	H
3,4,5-Tri-*O*-caffeoylquinic acid methyl ester	Caffeoyl	Caffeoyl	Caffeoyl	H	Me

To the best of our knowledge, this is the first report to show that CQAs possess the anti-amyloidogenic effects.

2. Results

2.1. Structure–activity relationship of CQA derivatives on Aβ42 aggregation

To investigate the inhibitory effects on Aβ42 aggregation by CQA derivatives (Table 1), Th-T fluorescence assays were performed (Fig. 1). Caffeic acid and chlorogenic acid prevented the aggregation of Aβ42 in a dose-dependent manner (Fig. 1B, D), whereas quinic acid, cinnamic acid, and methyl chlorogenic acid did not (Fig. 1A, C, E). In addition, 3,5-di-CQA, 3,4-di-CQA, 4,5-di-CQA, 1,4,5-tri-CQA, 3,4,5-tri-CQA, and 3,4,5-tri-CQA methylester with more than two caffeoyl groups inhibited the aggregation more potently compared with caffeic acid and chlorogenic acid (Fig. 1F–K). These results suggest the significance of a caffeoyl group and vicinal phenolic hydroxyl groups in the inhibitory activity against Aβ42 aggregation.

The IC_{50} values of these compounds are shown in Table 2, in which the ability to prevent Aβ42 aggregation is in the following order: 4,5-di-CQA = 3,4,5-tri-CQA > 3,4-di-CQA > 1,4,5-tri-CQA = 3,4,5-tri-CQA methylester > 3,5-di-CQA > caffeic acid > chlorogenic acid > quinic acid = cinnamic acid = methyl chlorogenic acid. We also tested their inhibitory potency against Aβ fibrillogenesis by transmission electron microscopy (TEM) (Fig. 2). Typical fibril formation was observed in Aβ42 alone, while the fibril formation was potently reduced in the presence of 4,5-di-CQA or 3,4,5-tri-CQA. In previous study, we demonstrated that 3,5-di-CQA treatment improved spatial learning memory on senescence accelerated-prone mice 8 through increasing the mRNA expression of phosphoglycerate kinase 1.[11] However, among the tested compounds in Table 1, 4,5-di-CQA and 3,4,5-tri-CQA had most potent inhibitory activities against Aβ42 aggregation. In the following experiment, we focused mainly on 4,5-di-CQA and 3,4,5-tri-CQA.

2.2. Effects of CQA derivatives on the secondary structure of Aβ42

Aβ42 aggregates through the formation of β-sheet to induce neurotoxicity.[1] To investigate the secondary structure of Aβ42 in the presence of CQA derivatives, CD spectra were measured. Shown in Figure 3A are the data of Aβ42; both the positive peak at 207 nm and the negative peak at 220 nm increased during 24 h incubation, meaning that a random organization transformed into a β-sheet structure. In contrast, both 4,5-di-CQA and 3,4,5-tri-CQA blocked these structural changes of Aβ42 (Fig. 3B, C).

2.3. Effects of CQA derivatives on the Aβ42-induced toxicity in SH-SY5Y cells

To estimate the effects of 4,5-di-CQA and 3,4,5-tri-CQA on Aβ42-induced neurotoxicity, MTT assay was carried out using SH-SY5Y cells. Incubation with Aβ42 at 2 μM for 48 h significantly decreased the cell viability to 66% ± 2.1 compared to the vehicle-treated cells (P <0.01) (Fig. 4). The treatment of SH-SY5Y cells with 4,5-di-CQA and 3,4,5-tri-CQA resulted in a dose-dependent protection against Aβ42 toxicity at concentrations of 1, 10, and 20 μM. In particular, 20 μM 4,5-di-CQA and 3,4,5-tri-CQA restored cell viability to 78.9% ± 4.1 and 96.1% ± 6.2, respectively (P <0.01 versus Aβ42 alone). In previous studies[11,12], we have already demonstrated that CQA derivatives exhibit no cytotoxicity on SH-SY5Y cells at concentration of 20 μM. These results suggest that 4,5-di-CQA and 3,4,5-tri-CQA have a potent protective effect against Aβ42 toxicity in SH-SY5Y cells.

2.4. Effects of CQA derivatives on thermodynamic stability and oligomerization of Aβ42

To test the potential of 3,4,5-tri-CQA to inhibit the formation of Aβ42 oligomers, we performed a dot blotting using the oligomer-specific antibody (A11)[14] (Fig. 5A). A11-immunoreactive oligomers were readily detected in Aβ42 alone, but not in the presence of 3,4,5-tri-CQA. These data suggest that 3,4,5-tri-CQA blocks the formation of Aβ42 oligomer as well as the fibrilization, the formation of Aβ42 fibril. To analyze the molecular size of blocked oligomers in Fig. 5A, we carried out Western blotting using the antibody (82E1)[15] that recognizes the N-terminus of Aβ (Fig. 5B). Trimer was formed after 2 h-incubation of Aβ42 alone. The treatment of 3,4,5-tri-CQA suppressed slightly the formation of the trimer, and the amount of the non-toxic monomer increased apparently. On the other hand, quinic acid hardly changed the amount of Aβ42 monomer. Band quantification also showed that the Aβ42 trimer/

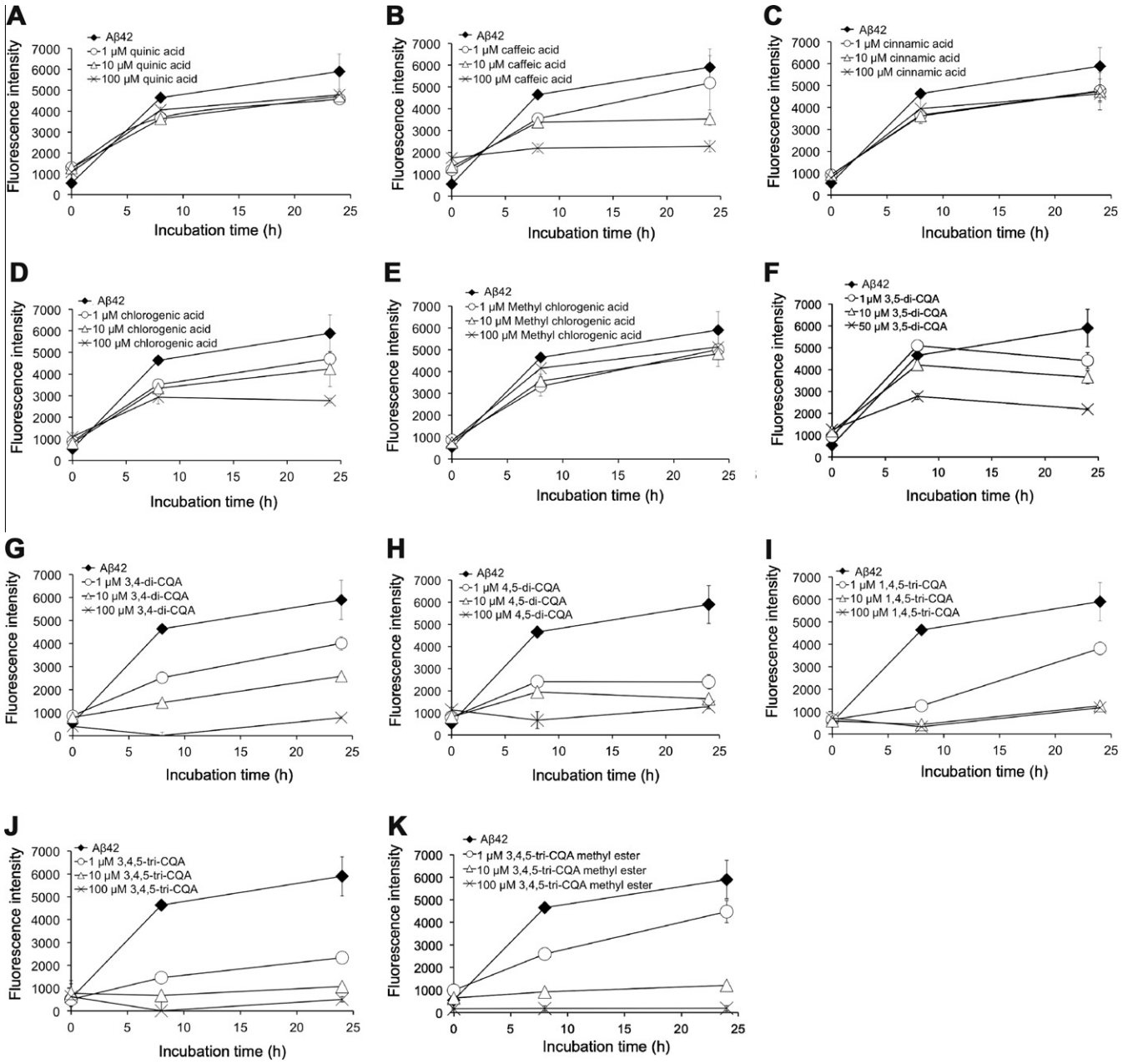


Figure 1. Effect of test compounds on the aggregation of Aβ42 in Th-T test. Aβ42 (25 μM) was monitored by Th-T fluorescence in the absence or presence of 1, 10, and 100 μM (A) quinic acid, (B) caffeic acid, (C) cinnamic acid, (D) chlorogenic acid, (E) methyl chlorogenic acid, (G) 3,4-di-CQA, (H) 4,5-di-CQA, (I) 1,4,5-tri-CQA, (J) 3,4,5-tri-CQA, and (K) 3,4,5-tri-CQA methyl ester, 1, 10, and 50 μM (F) 3,5-di-CQA. Fluorescence intensity was measured at an excitation wavelength of 420 nm and emission wavelength of 480 nm. Values represent the mean ± SD ($n = 6$).

monomer ratio was decreased by the treatment of 3,4,5-tri-CQA. In fact, compared with Aβ42 alone, the incubation with 50 μM quinic acid, 25 μM 3,4,5-tri-CQA, and 50 μM 3,4,5-tri-CQA decreased the trimer/monomer ratio to 100.3%, 87.6%, and 63.1%, respectively.

We also estimated the thermodynamic stability of Aβ42 aggregates by calculating the monomer concentration (critical concentration: C_r) after 48-hr incubation, when Aβ42 solution came to the equilibrium between monomer and aggregates (Fig. 5C). Because a high concentration of test compounds (50 μM) used in other assays induced over 25 μM of C_r on the bias by reducing Cu^{2+} *itself* in the preliminary test, this assay was performed in the concentration of 10 μM. The C_r of Aβ42 alone was 1.2 ± 0.10 μM, meaning the thermodynamic stability of the fibril to be stable. In contrast, 4,5-di-CQA and 3,4,5-tri-CQA decreased the stability of Aβ42 aggregates ($C_r = 10 \pm 0.73$ and 27 ± 1.9 μM, respectively), and the destabilizing potency of 3,4,5-tri-CQA was higher than that of 4,5-di-CQA. These results support the increase of Aβ42 monomer in the presence of 3,4,5-tri-CQA (Fig. 5B, lane 4). Quinic acid did not significantly affect the stability ($C_r = 1.2 \pm 0.030$ μM). Taken together, 3,4,5-tri-CQA would block the oligomerization by shifting the equilibrium into the non-toxic monomer.

3. Discussion

In this study, we investigated the modulation of CQA derivatives on the fibril formation (Figs. 1 and 2), the β-sheet transformation (Fig. 3), the neurotoxicity on SH-SY5Y cells (Fig. 4), the thermodynamic stability, and oligomer formation of Aβ42

Table 2
Effect of each compound on Aβ42 aggregation

Compound	IC_{50}[a] (μM)
Quinic acid	>100
Caffeic acid	32.8
Cinnamic acid	>100
Chlorogenic acid	92.9
Methyl chlorogenic acid	>100
3,5-Di-CQA	16.4
3,4-Di-CQA	4.7
4,5-Di-CQA	0.1
1,4,5-Tri-CQA	2.2
3,4,5-Tri-CQA	0.3
3,4,5-Tri-CQA methyl ester	3.0

[a] IC_{50} value was calculated from the inhibitory rate (%) of each compound on Aβ42 aggregation after 24 h incubation using Th-T assay.

(Fig. 5). There have been many reports on the preventive effects on Aβ aggregation and neurotoxicity of various flavonoids and polyphenols originated from common foods, such as red wine, green tea, and turmeric.[16,17] Ladiwala et al. reported that resveratrol, a constituent of red wine, remodels soluble oligomers and fibrils of Aβ42 into non-toxic insoluble disordered aggregates.[18] Wanker and colleagues reported that epigallocatechin gallate redirects toxic oligomers of Aβ42 into non-toxic unstructured, off-pathway oligomers.[19] The prevention of neurotoxicity on SH-SY5Y cells by 3,4,5-tri-CQA could be associated with shifting the equilibrium from the trimer into the non-toxic monomer and/or with blocking the function of A11-immunoreactive toxic oligomers (Fig. 5).

Recent failure of a number of clinical trials[20] in AD therapy motivates us to understand the molecular mechanisms of Aβ-related biological phenomena more precisely. Several inhibitors of Aβ42 aggregation, such as iAβ5,[21] empirically designed as a β-sheet breaker, might cause unexpected adverse effects by the non-specific interactions. Based on the structure-activity studies of CQA derivatives (Table 2), a caffeoyl group proved to be indispensable to the inhibitory effects on Aβ42 aggregation. Since catechol unit is susceptible to auto-oxidation, the resulting *o*-quinone might form a covalent bond with some residues of Aβ42, as is the case of α-synuclein, a culprit of Parkinson's disease.[22] Such covalent modification may destabilize the β-sheet structure in amyloidogenic polypeptides. Indeed, 4,5-di-CQA and 3,4,5-tri-CQA, with two or three catechol units, inhibited β-sheet formation of Aβ42 (Fig. 3). These implications did not contradict with the results that 3,4,5-tri-CQA methylester was active, while caffeic acid and chlorogenic acid, with one catechol unit, were slightly active (Fig. 1, Table 2).

Gazit proposed that blocking of the β-sheet formation by polyphenols could be originated from π–π stacking interaction between Aβ42 and polyphenols.[23] π-Orbital of the aromatic ring of the caffeoyl moiety in CQAs would also contribute to the π–π stacking effects, resulting in the inhibition of Aβ42 aggregation. Further analysis will be required to clarify the inhibitory mechanism of CQA derivatives against Aβ42 aggregation.

CQA was reported to show an anti-oxidative potency.[24] Oxidative stress is one of the contributing factors to AD progression.[25,26] Aβ-induced cytotoxicity has been related to the oxidative damage through the radicalization of proteins and lipids in vitro[27,28] and in vivo.[29,30] Recently, we demonstrated the restoration of AD-like phenotypes (behavioral abnormalities and Aβ oligomerization) by vitamin C (an anti-oxidant) using a transgenic AD mouse.[31] The anti-oxidative ability of CQA derivatives might be effective to inhibit the formation of Aβ42 fibril by suppressing the radical-mediated aggregation of Aβ42.[28] It is worth noting that 3,5-di-CQA mitigated the disturbance of spatial learning and memory function of senescence accelerated-prone mice 8 through the up-regulation

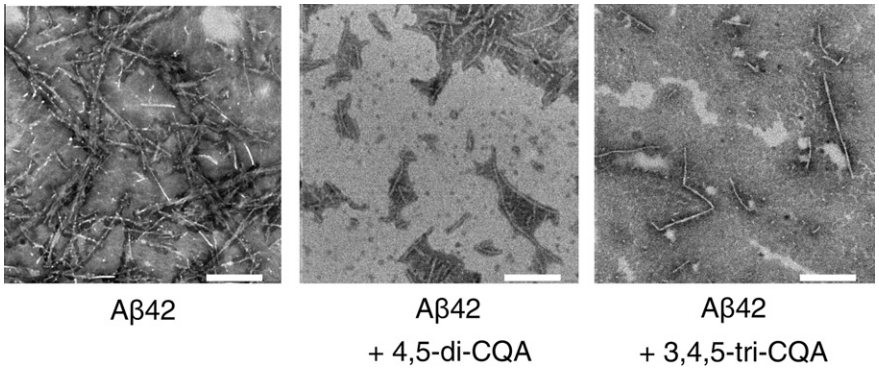


Figure 2. Effects of CQA derivatives on the fibril formation of Aβ42 analyzed by TEM. The fibril formation was observed after 24 h incubation in 50 mM PBS: *left*, 25 μM Aβ42; *middle*, 25 μM Aβ42 + 50 μM 4,5-di-CQA; *right*, 25 μM Aβ42 + 50 μM 3,4,5-tri-CQA. Scale bar represents 200 nm.

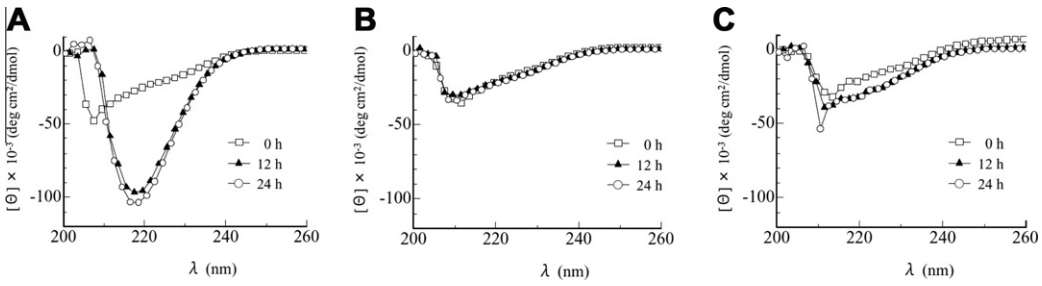


Figure 3. Effects of CQA derivatives on the secondary structure of Aβ42 analyzed by CD spectra. (A) 25 μM Aβ42, (B) 25 μM Aβ42 + 50 μM 4,5-di-CQA, (C) 25 μM Aβ42 + 50 μM 3,4,5-tri-CQA with the indicated time point (0, 12, and 24 h).

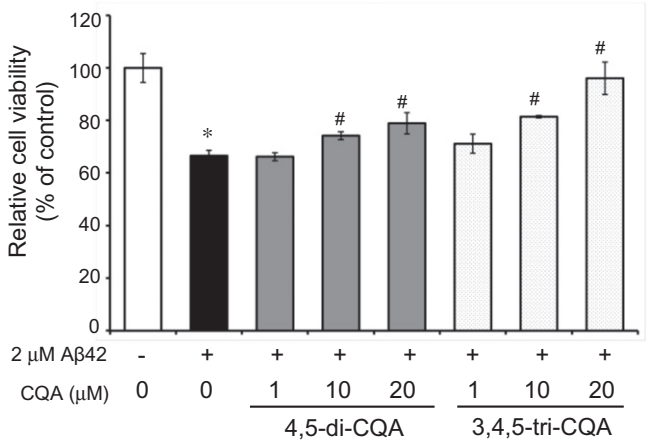


Figure 4. Effects of CQA derivatives on Aβ42-induced neurotoxicity in MTT assay. SH-SY5Y cells were incubated with Aβ42 (2 μM) and each compound (1, 10, and 20 μM) at 37 °C for 48 h. Each bar represents the mean ± SD (n = 6). *P <0.01 (vs vehicle), #P <0.01 (vs. Aβ42).

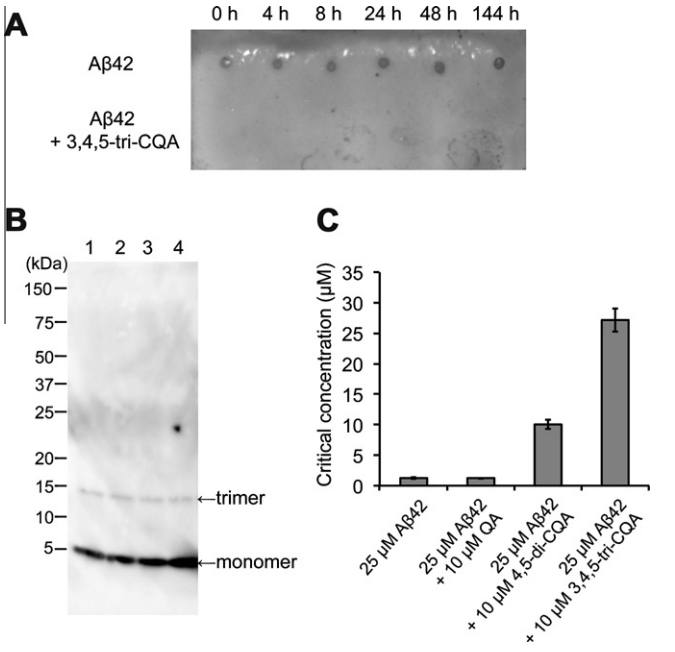


Figure 5. Effects of CQA derivatives on the thermodynamic stability and oligomer formation of Aβ42. (A) Dot blotting using an oligomer-specific antibody (A11). Aβ42 (45 μM) and 90 μM 3,4,5-tri-CQA were incubated at 37 °C for 0, 4, 8, 24, 48, and 144 h. (B) Western blotting of Aβ42 and each compound after 2 h incubation at 37 °C using 82E1 antibody. *Lane 1*, Aβ42; *lane 2*, Aβ42 + 50 μM quinic acid; *lane 3*, Aβ42 + 25 μM 3,4,5-tri-CQA; *lane 4*, Aβ42 + 50 μM 3,4,5-tri-CQA. (C) Thermodynamic stability of Aβ42 aggregates evaluated by critical concentration (C_r). C_r was calculated from the molar concentration of soluble monomer present in equilibrium after 48 h incubation of treated Aβ42 at 37 °C. Each bar represents the mean ± SD (n = 6).

of phosphoglycerate kinase-1.[11] In a similar manner, 4,5-di-CQA and 3,4,5-tri-CQA also might rescue the memory loss and synaptic dysfunction in a mouse model of AD.

In conclusion, 3,4,5-tri-CQA could be a promising therapeutics to inhibit Aβ42-mediated pathology in AD. Importantly, 3,4,5-tri-CQA blocked the formation of A11-reactive oligomers, one of which might be a trimer. Our group has proposed the toxic conformer of Aβ42 with the turn structure at positions 22 and 23 based on solid-state NMR studies, and the toxic conformer accelerated the formation of the trimer.[32] Further structural analysis of the Aβ42 oligomers could help to develop more potent inhibitors for AD therapy.

4. Materials and methods

4.1. Chemicals

Quinic acid, caffeic acid, cinnamic acid, and chlorogenic acid were purchased from Sigma (St. Louis, MO). 4,5-Di-CQA, 1,4,5-tri-CQA, 3,4,5-tri-CQA, 3,4,5-tri-CQA methyl ester, and methyl chlorogenic acid were synthesized as previously described.[13] 3,4-di-CQA was isolated from Brazilian propolis.[12] Aβ42 peptide was synthesized by the standard protocol.[28,33]

4.2. Th-T assay

The aggregative ability of Aβ42 was evaluated by the Th-T method developed by Naiki et al.[34] The procedure was described elsewhere.[35] Briefly, Aβ42 was dissolved with 0.1% NH_4OH at 250 μM. The resultant solution was diluted 10 times with 50 mM sodium phosphate buffer (PBS) containing 100 mM NaCl (pH 7.4), and 25 μM Aβ42 was incubated with or without test compounds at 37 °C. At each time point, 2.5 μL of Aβ42 solution was added to 250 μL of 1 mM Th-T in 50 mM Gly-NaOH (pH 8.5). Fluorescence intensity was measured at 420 nm excitation and 485 nm emission on Multidetection Microplate Reader powerscan HT (Dainippon Sumitomo Pharma).

4.3. Transmission electron microscopy

The procedure was previously reported[35] with slight modifications. Five microliters of Aβ42 sample after Th-T assay were spotted onto a glow-discharged, carbon-coated Formvar grid, and were incubated for 2 min before washing with 5 μL of distilled water twice. The resultant grid was negatively stained for 1 min with 5 μL of 0.4% silicotungstic acid twice. After air-dry for 5 min, samples were subjected to JOEL JEM-1400 electron microscope.

4.4. CD spectra

The procedure was described elsewhere[36] with slight modifications. In brief, Aβ42 solution (25 μM) treated with 4,5-di-CQA or 3,4,5-tri-CQA (50 μM) was loaded into 10 mm path length quartz cell, and CD spectra were recorded at 200–270 nm using J-820 spectropolarimeter (JASCO).

4.5. MTT assay

Neurotoxicity on SH-SY5Y cells was assessed by MTT assay according to the previously reported protocol.[12] In brief, SH-SY5Y (2×10^4 cells/well) were treated with 4,5-di-CQA or 3,4,5-tri-CQA (1–20 μM) in OPTI-MEM (GIBCO), and subsequently were exposed to 2 μM Aβ42 for 48 h. After change of culture medium with OPTI-MEM (100 μL), MTT was added to the culture. The absorbance of MTT formazan at 570 nm was determined using Multidetection Microplate Reader powerscan HT (Dainippon Sumitomo Pharma). The absorbance obtained by the addition of vehicle was taken as 100%.

4.6. Dot blotting

Aβ42 solution (45 μM) was incubated with or without 3,4,5-tri-CQA (90 μM) at 37 °C. Two microliters of each sample were applied to nitrocellulose membrane (0.2 μm pore size, Biorad), and the

membrane was equilibrated with PBS including 0.01% Tween-20 for 10 min. After blocking with 2% ECL Advance™ blocking agent (GE Healthcare), the membrane was probed with A11[14] at 0.1 μg/mL (Invitrogen, Carlsbad, CA), followed by the treatment of the secondary antibody (anti-rabbit IgG). Development was performed with ECL chemiluminescence (GE Healthcare).

4.7. Western blotting

Gel electrophoresis and Western blotting were performed basically according to the previous report.[32] After 2 h incubation with 50 μM quinic acid, 4,5-di-CQA, and 3,4,5-tri-CQA, Aβ42 solution (25 μM) was centrifuged at 15,000 rpm for 10 min at 4 °C, and the supernatant was diluted 50 times with 50 mM PBS pH 7.4. Ten microliters of the obtained solution was mixed with 10 μL of lithium dodecyl sulfate sample buffer (Invitrogen), and was incubated for 2 min at 70 °C. Ten microliters of the heated solution were applied to 10–20% Tricine gel (Invitrogen). PVDF membrane (0.2 μm pore size, Bio-Rad) was used for blotting. The membrane after blocking with Block Ace (Dainippon Sumitomo Seiyaku, Osaka, Japan) was incubated with the primary antibody 82E1[15] at 1 μg/mL (Immuno-Biological Laboratories, Gunma, Japan), followed by the treatment with horseradish peroxidase-conjugated anti-mouse IgG (1:1000) (Dako, Glostrup, Denmark). Development was performed with ECL chemiluminescence (GE Healthcare).

4.8. Thermodynamic stability

Thermodynamic stability of Aβ42 fibrils present at equilibrium was evaluated by critical concentration (C_r), which was measured after 48 h incubation by Micro-BCA protein assay,[32,37] according to the manufacture's directions (Thermo scientific). Aβ42 solution (25 μM) was incubated with 10 μM quinic acid, 4,5-di-CQA, and 3,4,5-tri-CQA for 48 h, when the equilibrium can achieve

4.9. Statistical analysis

The statistical significance of differences was analyzed by Student's *t*-test. The value of $P < 0.05$ was considered significant.

Acknowledgements

We thank Professor Yasuyuki Yamamoto and Dr. Hulin Thai, Graduate School of Pure and Applied Sciences, University of Tsukuba for kind support of CD measurement. This work was supported in part by Sasakawa Scientific Research Grant from The Japan Science Society (to Y.M.) and by Grant-in-Aid for the Ministry of Education, Science, Sports, and Culture of Japan.

References and notes

1. Haass, C.; Selkoe, D. J. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 101.
2. Masters, C. L.; Simms, G.; Weinman, N. A.; Multhaup, G.; McDonald, B. L.; Beyreuther, K. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4245.
3. Glenner, G. G.; Wong, C. W. *Biochem. Biophys. Res. Commun.* **1984**, *120*, 885.
4. Walsh, D. M.; Klyubin, I.; Fadeeva, J. V.; Cullen, W. K.; Anwyl, R.; Wolfe, M. S.; Rowan, M. J.; Selkoe, D. J. *Nature* **2002**, *416*, 535.
5. Roychaudhuri, R.; Yang, M.; Hoshi, M. M.; Teplow, D. B. *J. Biol. Chem.* **2009**, *284*, 4749.
6. Gravitz, L. *Nature* **2011**, *475*, S9.
7. Clifford, M. N.; Wu, W.; Kirkpatrick, J.; Kuhnert, N. *J. Agric. Food Chem.* **2007**, *55*, 929.
8. Farah, A.; de Paulis, T.; Trugo, L. C.; Martin, P. R. *J. Agric Food Chem.* **2005**, *53*, 1505.
9. Kurata, R.; Adachi, M.; Yamakawa, O.; Yoshimoto, M. *J. Agric Food Chem.* **2007**, *55*, 185.
10. Mishima, S.; Inoh, Y.; Narita, Y.; Ohta, S.; Sakamoto, T.; Araki, Y.; Suzuki, K. M.; Akao, Y.; Nozawa, Y. *Bioorg. Med. Chem.* **2005**, *13*, 5814.
11. Han, J.; Miyamae, Y.; Shigemori, H.; Isoda, H. *Neuroscience* **2010**, *169*, 1039.
12. Miyamae, Y.; Han, J.; Sasaki, K.; Terakawa, M.; Isoda, H.; Shigemori, H. *Cytotechnology* **2011**, *63*, 191.
13. Miyamae, Y.; Kurisu, M.; Han, J.; Isoda, H.; Shigemori, H. *Chem. Pharm. Bull.* **2011**, *59*, 502.
14. Kayed, R.; Head, E.; Thompson, J. L.; McIntire, T. M.; Milton, S. C.; Cotman, C. W.; Glabe, C. G. *Science* **2003**, *300*, 486.
15. Horikoshi, Y.; Sakaguchi, G.; Becker, A. G.; Gray, A. J.; Duff, K.; Aisen, P. S.; Yamaguchi, H.; Maeda, M.; Kinoshita, N.; Matsuoka, Y. *Biochem. Biophys. Res. Commun.* **2004**, *319*, 733.
16. Ono, K.; Hamaguchi, T.; Naiki, H.; Yamada, M. *Biochim. Biophys. Acta* **2006**, *1762*, 575.
17. Porat, Y.; Abramowitz, A.; Gazit, E. *Chem. Biol. Drug Des.* **2006**, *67*, 27.
18. Ladiwala, A. R.; Lin, J. C.; Bale, S. S.; Marcelino-Cruz, A. M.; Bhattacharya, M.; Dordick, J. S.; Tessier, P. M. *J. Biol. Chem.* **2010**, *285*, 24228.
19. Ehrnhoefer, D. E.; Bieschke, J.; Boeddrich, A.; Herbst, M.; Masino, L.; Lurz, R.; Engemann, S.; Pastore, A.; Wanker, E. E. *Nat. Struct. Mol. Biol.* **2008**, *15*, 558.
20. Mangialasche, F.; Solomon, A.; Winblad, B.; Mecocci, P.; Kivipelto, M. *Lancet Neurol.* **2010**, *9*, 702.
21. Soto, C.; Sigurdsson, E. M.; Morelli, L.; Kumar, R. A.; Castano, E. M.; Frangione, B. *Nat. Med.* **1998**, *4*, 822.
22. Zhu, M.; Rajamani, S.; Kaylor, J.; Han, S.; Zhou, F.; Fink, A. L. *J. Biol. Chem.* **2004**, *279*, 26846.
23. Gazit, E. *FASEB J.* **2002**, *16*, 77.
24. Hung, T. M.; Na, M.; Thuong, P. T.; Su, N. D.; Sok, D.; Song, K. S.; Seong, Y. H.; Bae, K. *J. Ethnopharmacol.* **2006**, *108*, 188.
25. Sayre, L. M.; Perry, G.; Smith, M. A. *Chem. Res. Toxicol.* **2008**, *21*, 172.
26. Barnham, K. J.; Masters, C. L.; Bush, A. I. *Nat. Rev. Drug Discov.* **2004**, *3*, 205.
27. Varadarajan, S.; Yatin, S.; Aksenova, M.; Butterfield, D. A. *J. Struct. Biol.* **2000**, *130*, 184.
28. Murakami, K.; Irie, K.; Ohigashi, H.; Hara, H.; Nagao, M.; Shimizu, T.; Shirasawa, T. *J. Am. Chem. Soc.* **2005**, *127*, 15168.
29. Li, F.; Calingasan, N. Y.; Yu, F.; Mauck, W. M.; Toidze, M.; Almeida, C. G.; Takahashi, R. H.; Carlson, G. A.; Flint Beal, M.; Lin, M. T.; Gouras, G. K. *J. Neurochem.* **2004**, *89*, 1308.
30. Murakami, K.; Murata, N.; Noda, Y.; Tahara, S.; Kaneko, T.; Kinoshita, N.; Hatsuta, H.; Murayama, S.; Barnham, K. J.; Irie, K.; Shirasawa, T.; Shimizu, T. *J. Biol. Chem.* **2011**, *286*, 44557.
31. Murakami, K.; Murata, N.; Ozawa, Y.; Kinoshita, N.; Irie, K.; Shirasawa, T.; Shimizu, T. J. *Alzheimers Dis.* **2011**, *26*, 7.
32. Masuda, Y.; Uemura, S.; Ohashi, R.; Nakanishi, A.; Takegoshi, K.; Shimizu, T.; Shirasawa, T.; Irie, K. *ChemBioChem* **2009**, *10*, 287.
33. Murakami, K.; Irie, K.; Morimoto, A.; Ohigashi, H.; Shindo, M.; Nagao, M.; Shimizu, T.; Shirasawa, T. *Biochem. Biophys. Res. Commun.* **2002**, *294*, 5.
34. Naiki, H.; Gejyo, F. *Methods Enzymol.* **1999**, *309*, 305.
35. Murakami, K.; Irie, K.; Morimoto, A.; Ohigashi, H.; Shindo, M.; Nagao, M.; Shimizu, T.; Shirasawa, T. *J. Biol. Chem.* **2003**, *278*, 46179.
36. Suzuki, T.; Murakami, K.; Izuo, N.; Kume, T.; Akaike, A.; Nagata, T.; Nishizaki, T.; Tomiyama, T.; Takuma, H.; Mori, H.; Irie, K. *Int. J. Alzheimers Dis.* **2010**, *25*, 431320.
37. Morimoto, A.; Irie, K.; Murakami, K.; Masuda, Y.; Ohigashi, H.; Nagao, M.; Fukuda, H.; Shimizu, T.; Shirasawa, T. *J. Biol. Chem.* **2004**, *279*, 52781.