3,3',4',5'-Tetrahydroxyflavone Induces Formation of Large Aggregates of Amyloid β Protein

Hiroko Ushikubo, Yui Tanimoto, Kazuho Abe, Tomohiro Asakawa, Toshiyuki Kan, and Tatsuhiro Akaishi

Laboratory of Pharmacology, Faculty of Pharmacy and Research Institute of Pharmaceutical Sciences, Musashino University; Tokyo 202–8585, Japan; and Synthetic Organic & Medicinal Chemistry, School of Pharmaceutical Sciences, University of Shizuoka; Shizuoka 422–8526, Japan.

Received September 9, 2013; accepted February 26, 2014

Amyloid β protein (Aβ) self-assembles into insoluble fibrils, and forms the senile plaques associated with Alzheimer’s disease. 3,3',4',5'-Tetrahydroxyflavone, a synthetic analogue of the natural flavonoid fisetin, has been found to potently inhibit Aβ fibril formation. In the present study, we investigated how inhibition of Aβ fibril formation by this flavonoid affects Aβ conformation and neurotoxicity. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of Aβ1-42 (20 µM) incubated with or without 3,3',4',5'-tetrahydroxyflavone demonstrated that 3,3',4',5'-tetrahydroxyflavone (100 µM) rapidly caused formation of atypical Aβ conformers, which appeared as a very broad, smear-like band in the high molecular weight region and were distinguishable from soluble Aβ oligomers or mature Aβ fibrils. Transmission electron microscopy (TEM) revealed that large spherical Aβ aggregates were preferentially formed in the presence of 3,3',4',5'-tetrahydroxyflavone. The SDS-resistant, smear-like band on SDS-PAGE and the large spherical aggregates in TEM both disappeared after heat treatment (100°C, 10 min). Furthermore, a neurotoxicity assay with cultured rat hippocampal neurons demonstrated that Aβ incubated with 3,3',4',5'-tetrahydroxyflavone was significantly less toxic than Aβ incubated without the flavonoid. These results suggest that the newly synthesized fisetin analogue 3,3',4',5'-tetrahydroxyflavone directly produces atypical, large Aβ aggregates and reduces Aβ toxicity.

Key words fisetin; amyloid β protein; 3,3',4',5'-tetrahydroxyflavone; neurotoxicity

Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by selective neuronal loss, senile plaques and neurofibrillary tangles in the brain. Amyloid β protein (Aβ), the major component of senile plaques, is a 40-42 amino acid peptide that self-assembles into insoluble fibrillar aggregates and promotes neurotoxicity. Based on the hypothesis that abnormal aggregation of Aβ into insoluble fibrillar deposits causes neurodegeneration in AD, extensive efforts have been made to find drugs that can inhibit formation of Aβ fibrils. Aβ vaccine therapy has also been developed as an attractive therapeutic strategy to suppress AD. However, the original amyloid cascade hypothesis for AD has been questioned in recent years and is currently quite controversial. Aβ aggregation is a complicated process and appears to involve more than a simple conversion of soluble monomers to insoluble fibrils. Assemblies ranging from dimers to 24-mers, or those of even higher molecular weight, have been reported as Aβ fibrils. Assemblies ranging from dimers to 24-mers, or those of even higher molecular weight, have been reported as Aβ fibrils. Assemblies ranging from dimers to 24-mers, or those of even higher molecular weight, have been reported as Aβ fibrils. Assemblies ranging from dimers to 24-mers, or those of even higher molecular weight, have been reported as Aβ fibrils. Assemblies ranging from dimers to 24-mers, or those of even higher molecular weight, have been reported as Aβ fibrils.

Fisetin (3,3',4',5'-tetrahydroxyflavone, Fig. 1) is a flavonoid present in a number of commonly eaten foods, such as strawberries, and has a variety of biological effects that may be beneficial for the treatment of AD. For example, fisetin protects nerve cells from oxidative stress-induced death and promotes the differentiation of nerve cells, indicating that it is neurotrophic. Furthermore, fisetin has been reported to inhibit Aβ fibril formation in vitro. To find more effective drugs than fisetin, we have studied the structure–activity relationship by comparing the anti-amyloidogenic effects of several naturally occurring flavonoids and synthetic fisetin analogues, and found that the 7-hydroxy group is not necessary for the anti-amyloidogenic activity and increasing the number of hydroxyl groups on the B ring potentiates the activity. The compound 3,3’,4’,5’-tetrahydroxyflavone (Fig. 1) was selected as a synthetic fisetin analogue that inhibits Aβ fibril formation more potently than fisetin.

To explore the potentiality of the fisetin analogue 3,3’,4’,5’-tetrahydroxyflavone as a therapeutic drug candidate for AD, the present study addressed the following two important issues. First, what kind of Aβ conformer(s) is (are) present in Aβ solutions when fibril formation is inhibited by 3,3’,4’,5’-tetrahydroxyflavone? Second, does inhibition of fibril formation by 3,3’,4’,5’-tetrahydroxyflavone result in a decrease or increase in Aβ neurotoxicity? To answer these questions, Aβ samples incubated in the absence and presence of 3,3’,4’,5’-tetrahydroxyflavone were analyzed by SDS-PAGE. The results showed that 3,3’,4’,5’-tetrahydroxyflavone caused the formation of atypical Aβ conformers, which were different from the soluble Aβ oligomers or mature Aβ fibrils. These results suggest that 3,3’,4’,5’-tetrahydroxyflavone directly produces atypical, large Aβ aggregates and reduces Aβ toxicity.

Key words fisetin; amyloid β protein; 3,3’,4’,5’-tetrahydroxyflavone; neurotoxicity

Fig. 1. Chemical Structures of Fisetin and 3,3’,4’,5’-Tetrahydroxyflavone
of 3,3′,4′,5′-tetrahydroxyflavone were analyzed by sodium do- decyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) or transmission electron microscopy (TEM), and neurotoxicity was investigated using primary cultured rat hippocampal neurons.

MATERIALS AND METHODS

Chemicals Aβ1-42 was purchased from Peptide Institute (Lot No. 600911; Osaka, Japan). An Aβ1-42 stock solution (250 µM) was prepared by dissolving the peptide in 0.1% am- monia solution, and stored in small aliquots at −85°C until use. Fisetin was purchased from Sigma (St. Louis, MO, U.S.A.). 3,3′,4′,5′-Tetrahydroxyflavone was synthesized using our recently developed synthetic method for flavones.18,19) Fise- tin and 3,3′,4′,5′-tetrahydroxyflavone were dissolved at 50 mM in 100% dimethyl sulfoxide and stored at −30°C until use. Immediately before use, flavonoids were diluted with 50 mM phosphate buffer (pH 7.4) to give the desired concentrations.

Thioflavin T Fluorescence Assay Fibril formation of Aβ1-42 was quantitatively measured by the Thioflavin T (ThT) fluorescence assay, in which ThT exhibits enhanced fluorescence upon binding to Aβ fibrils.20) Aβ1-42 was diluted to 20 µM in 50 mM phosphate buffer (pH 7.4) and incubated for 0, 2, 6 or 24 h at 37°C. Ten microliters of the Aβ solution was mixed well with 990 µL of 5 µM ThT in 50 mM glycine–NaOH buffer (pH 8.4), and the fluorescence intensity was measured at 450 nm excitation and 480 nm emission with a fluorescence spectrometer (RF-5300PC; Shimadzu Corp., Kyoto, Japan).

SDS-PAGE and Immunoblotting SDS-PAGE was performed as described by Okajima et al.21) Briefly, Aβ samples were mixed in a 1:2 ratio (vol/vol) with 1.5% Triton X-100 stock solution buffer (Bio-Rad Laboratories, Hercules, CA, U.S.A.) supplemented with 0.15M dithiothreitol. In experiments testing the influence of heat on Aβ conformers, the samples were boiled (100°C) for 10 min. The samples were loaded onto SDS-polyacrylamide gels containing 3 M Tris–HCl (pH 8.85), and resolved in a running buffer containing 0.05 M Tris (pH 8.3), 0.38 M glycine and 0.1% SDS. After transfer to poly- vinylidene fluoride (PVDF) membrane, the membrane was blocked with 5% nonfat milk in Tris-buffered saline containing 0.01% Tween 20 (TBS-T) for 1 h at room temperature, and incubated with monoclonal anti-Aβ antibody 6E10 (1:1000 in TBS-T; Convance, Emeryville, CA, U.S.A.) overnight at 4°C. The PVDF membrane was then incubated with horseradish peroxidase-conjugated rabbit anti-mouse antibody (Cell Signaling Technology, Beverly, MA, U.S.A.) diluted 1:5000 in TBS-T for 1 h at room temperature. Antibody labeling was detected using an enhanced chemiluminescence detection kit (GE Healthcare, U.K.). The membrane was scanned using an LAS-3000 system (FUJIFILM, Japan), and densitometric analysis was performed using Multi Gauge V3.0 software (FUJIFILM).

Transmission Electron Microscopy (TEM) TEM was used to morphologically confirm Aβ fibril formation. After incubation, 10 µL of Aβ1-42 solution was placed on 400-mesh copper grids coated with collodion (Nissin EM, Tokyo, Japan). The grids were negatively stained with 2% phosphotungstic acid for 15 s, and observed under an electron microscope (H-7650; Hitachi High-Technologies Corp., Tokyo, Japan) operated at an accelerating voltage of 100 kV.

Neurotoxicity Assay All animal experiments in this study were performed in accordance with the “Guiding Principles for the Care and Use of Laboratory Animals” approved by the Japanese Pharmacological Society. Primary neuronal cultures were prepared from the hippocampi of 18-d-old embryos of Wistar rats as described in our previous paper22) with some modifications. Briefly, dissociated hippocampal cells were suspended in Neurobasal medium (Life Technologies, Gaithersburg, MD, U.S.A.) supplemented with 2% B27 (Life Technologies), 25 µM l-glutamate and 10% fetal bovine serum, and seeded on polylysine-coated 96-well culture plates at a density of 1×10⁵ cells/cm². The culture medium was changed to serum-free, B27-supplemented Neurobasal medium 1 d after plating and again to B27-supplemented Neurobasal medium containing cytosine arabinoside (5 µM) 3 d after plating. The cells were then cultured for a further 4 d. The culture medium was switched to unsupplemented Neurobasal medium 24 h prior to the toxicity assay. Aβ1-42 (20 µM) was incubated for 24 h in the presence of vehicle or 3,3′,4′,5′-tetrahydroxyfla- vone, and the solution was added to the culture at a dilution of 1:40. Since Aβ fibrils rapidly induced neuronal cell death, the cells were fixed with 4% paraformaldehyde 6 h after addition of Aβ. Since colorimetric methods are sometimes interfered by factors unrelated to cell viability,23) we chose the visual cell counting for quantitating Aβ toxicity as described previ- ously.24) The cells were stained with 0.1% cresyl violet, and the number of neurons bearing processes at least twice as long as the cell body diameter, as an index of neuronal survival, was counted under a microscope.

RESULTS

To examine the time-course of changes in Aβ conformation, Aβ1-42 (20 µM) was incubated for 0, 2, 6 or 24 h at 37°C in 50 mM phosphate buffer (pH 7.4) and subjected to ThT fluorescence assay. As shown in Fig. 2A, ThT fluorescence intensities rapidly increased between 2–6 h of incubation and remained unchanged during 6–24 h after incubation, which is consistent with our previous study.16) Next, changes in soluble Aβ monomers and oligomers were analyzed by SDS-PAGE and immunoblotting with the anti-Aβ antibody 6E10. Imme- diately after preparation of Aβ1-42 solution (0 h), abundant Aβ monomers and substantial Aβ dimers and tetramers were observed on SDS-PAGE (Fig. 2B). Aβ dimers were rarely found at 0–2 h, but increased considerably 6 h after incubation (Fig. 2B). Six to 24 h after incubation, Aβ monomer, trimer and tetramer bands disappeared, and only a very low level of Aβ dimers remained (Fig. 2B). It is likely that most Aβ monomers and oligomers are converted into Aβ fibrils between 2–6 h, and that mature Aβ fibrils do not appear on SDS-PAGE gels in our experimental conditions, as they are highly insoluble and of very high molecular weight.

To investigate the effects of fisetin and 3,3′,4′,5′-tetra- hydroxyflavone on conversion of soluble Aβ into insoluble fibrils, Aβ1-42 (20 µM) was incubated for 0, 2, 6 or 24 h in the presence of vehicle (0.2% dimethyl sulfoxide) or each fla- vonoid, and analyzed by SDS-PAGE and immunoblotting with 6E10. Time-dependent changes in the amounts of Aβ monomers and oligomers were virtually the same in the absence (Fig. 2B) and presence of vehicle (Figs. 3A, B), indicating that the vehicle has no effect on Aβ aggregation. Aβ monomers,
trimers and tetramers disappeared 6 h after incubation in the presence of vehicle or 10 μM fisetin, while they remained 6 h after incubation in the presence of 100 μM fisetin (Figs. 3A, C), indicating that fisetin delayed the conversion of Aβ monomers and oligomers into fibrils. Aβ monomers, trimers and tetramers disappeared 24 h after incubation in the presence of 100 μM fisetin (Figs. 3A, C), indicating that fisetin delayed the conversion of Aβ monomers and oligomers into fibrils. Aβ monomers, trimers and tetramers disappeared 24 h after incubation in the presence of 10 μM fisetin, while they remained 6 h after incubation in the presence of 100 μM fisetin (Figs. 3A, C), indicating that fisetin delayed the conversion of Aβ monomers and oligomers into fibrils. Aβ monomers, trimers and tetramers disappeared 24 h after incubation in the presence of 100 μM fisetin, indicating that the inhibitory effect of fisetin is not persistent. 3,3',4',5'-Tetrahydroxyflavone at 10 μM had the same effect as 100 μM fisetin, but at 100 μM showed very different effects (Figs. 3B, D). Two hours after incubation in the presence of 100 μM 3,3',4',5'-tetrahydroxyflavone, a prominent smear-like band appeared in the high molecular weight region, and it remained unchanged 6–24 h later (denoted by an asterisk in Fig. 3B). The smear-like band was also observed 1 h after incubation in the presence of 100 μM 3,3',4',5'-tetrahydroxyflavone (data not shown). The smear-like band is likely to represent SDS-resistant, high molecular weight Aβ aggregates that are distinguishable from soluble Aβ oligomers and mature Aβ fibrils. Aβ monomers, trimers and tetramers remained 24 h after incubation in the presence of 100 μM 3,3',4',5'-tetrahydroxyflavone (Figs. 3B, D).

In general, heat can break the bonds that maintain the three-dimensional structure of proteins. To characterize the smear-like Aβ band produced in the presence of 3,3',4',5'-tri-...
hydroxyflavone, the effect of heat treatment was investigated. Heat treatment (100°C for 10 min) resulted in a decrease of the high molecular weight, smear-like band and an increase of Aβ monomers on SDS-PAGE (Fig. 4Aa), indicating that the SDS-resistant Aβ aggregates are formed by weak bonding and disassemble into monomers upon heating. When Aβ fibrils were subjected to the same heat treatment, Aβ monomers did not appear on SDS-PAGE (Fig. 4Ab). In addition, the same heat treatment did not affect the ThT fluorescence intensity of Aβ fibrils (Fig. 4B) nor morphology of mature Aβ fibrils confirmed by TEM (Fig. 4Ca,b), indicating that mature Aβ fibrils are formed by stronger bonding and are relatively resistant to heat. The ThT fluorescence intensity of Aβ in the presence of 3,3',4,5'-tetrahydroxyflavone (100µM) was very low, which was unchanged after heating (n=3, data not shown). In addition, TEM examination revealed that 3,3',4,5'-tetrahydroxyflavone produced very large (500–1000 nm in diameter), spherical Aβ aggregates, which disappeared after heating (Fig. 4Cc,d).

To determine whether inhibition of fibril formation by 3,3',4,5'-tetrahydroxyflavone results in a decrease or increase of Aβ neurotoxicity, cytotoxicity was investigated using primary cultures of hippocampal neurons. Rat hippocampal neurons extended long processes and survived stably in our culture conditions (Fig. 5A, none). When Aβ1-42 (20µM) was incubated for 24 h in the presence of vehicle and added at a dilution of 1:40 to hippocampal neuron cultures, almost all cells lost their processes and died within 6 h (Fig. 5A, Aβ+/vehicle). When Aβ1-42 (20µM) was incubated for 24 h in the presence of 100µM 3,3',4,5'-tetrahydroxyflavone and added at a dilution of 1:40, the neurons retained normal morphology and were alive at 6 h after addition (Fig. 5A, Aβ+/3,3',4,5'-tetrahydroxyflavone). Quantitative analysis by counting of viable neurons bearing processes demonstrated that Aβ incubated in the presence of 100µM 3,3',4,5'-tetrahydroxyflavone is significantly less toxic than Aβ incubated in the presence of vehicle (Fig. 5B). For comparison, 3,3',4,5'-tetrahydroxyflavone (100µM) alone was added at a dilution of 1:40 to hippocampal neuron cultures, but it had no effect on cell viability in this experimental condition (data not shown, n=5).

Fig. 4. Large Aβ Aggregates Produced in the Presence of 3,3',4,5'-Tetrahydroxyflavone Are Distinguishable from Aβ Fibrils

Aβ1-42 (20µM) was incubated for 24 h in the presence of vehicle or 100µM 3,3',4,5'-tetrahydroxyflavone, and subjected to heat treatment (100°C for 10 min). (A) SDS-PAGE of Aβ in the presence (a) or absence (b) of 3,3',4,5'-tetrahydroxyflavone before or after heat treatment. (B) ThT fluorescence of Aβ incubated in the presence (a) or absence (b) of 3,3',4,5'-tetrahydroxyflavone before or after heat treatment. (C) TEM images of Aβ incubated in the presence of vehicle (a,b) or 3,3',4,5'-tetrahydroxyflavone (c,d) before (a,c) or after (b,d) heat treatment. Scale bar, 200 nm.
In our previous study, incubation of Aβ with fisetin at 100µM, but not 10µM, resulted in a significant decrease in the ThT fluorescence intensity of Aβ. SDS-PAGE analysis in the present study demonstrated that fisetin at 100µM, but not 10µM, delayed disappearance of Aβ monomers, trimers and tetramers on gels. Furthermore, 3,3',4',5'-tetrahydroxyflavone at 10µM decreased the ThT fluorescence intensity of Aβ in our previous study, and delayed disappearance of Aβ monomers, trimers and tetramers in the present study. These results support the notion that our previous observation is not an artifact of the ThT fluorescence analysis, but represents inhibition of Aβ fibril formation by these flavonoids. In addition, 100µM fisetin and 10µM 3,3',4',5'-tetrahydroxyflavone delayed conformational changes in Aβ, but their effects were not persistent and did not result in a significant increase in Aβ oligomers.

In addition to the anti-amyloidogenic effect, 3,3',4',5'-tetrahydroxyflavone had a unique effect that could not be seen in the ThT fluorescence assay. SDS-PAGE analysis in the present study revealed that 3,3',4',5'-tetrahydroxyflavone at 100µM caused formation of atypical Aβ conformers, which appeared as a very broad, smear-like band in the high molecular weight region. The smear-like band is likely to represent SDS-resistant Aβ aggregates whose molecular weight is higher than tetramers and lower than mature fibrils. Furthermore, TEM examination revealed that 3,3',4',5'-tetrahydroxyflavone at 100µM caused formation of very large, spherical aggregates. Since the SDS-resistant, smear-like band in SDS-PAGE and the large spherical aggregates in TEM both disappeared after heat treatment (100°C for 10min), they are likely to be identical.

DISCUSSION

In our previous study, incubation of Aβ with fisetin at 100µM, but not 10µM, resulted in a significant decrease in the ThT fluorescence intensity of Aβ. SDS-PAGE analysis in the present study demonstrated that fisetin at 100µM, but not 10µM, delayed disappearance of Aβ monomers, trimers and tetramers on gels. Furthermore, 3,3',4',5'-tetrahydroxyflavone at 10µM decreased the ThT fluorescence intensity of Aβ in our previous study, and delayed disappearance of Aβ monomers, trimers and tetramers in the present study. These results support the notion that our previous observation is not an artifact of the ThT fluorescence analysis, but represents inhibition of Aβ fibril formation by these flavonoids. In addition, 100µM fisetin and 10µM 3,3',4',5'-tetrahydroxyflavone delayed conformational changes in Aβ, but their effects were not persistent and did not result in a significant increase in Aβ oligomers.

In addition to the anti-amyloidogenic effect, 3,3',4',5'-tetrahydroxyflavone had a unique effect that could not be seen in the ThT fluorescence assay. SDS-PAGE analysis in the present study revealed that 3,3',4',5'-tetrahydroxyflavone at 100µM caused formation of atypical Aβ conformers, which appeared as a very broad, smear-like band in the high molecular weight region. The smear-like band is likely to represent SDS-resistant Aβ aggregates whose molecular weight is higher than tetramers and lower than mature fibrils. Furthermore, TEM examination revealed that 3,3',4',5'-tetrahydroxyflavone at 100µM caused formation of very large, spherical aggregates. Since the SDS-resistant, smear-like band in SDS-PAGE and the large spherical aggregates in TEM both disappeared after heat treatment (100°C for 10min), they are likely to be identical.
The Aβ aggregates produced in the presence of 100 µM 3,3',4',5'-tetrahydroxyflavone are clearly distinguishable from mature Aβ fibrils. The structure of mature Aβ fibrils was not changed by heat treatment (100°C for 10 min), as indicated by the ThT fluorescence analysis and TEM examination. SDS-PAGE analysis demonstrated that heat treatment of Aβ samples containing fibrils did not produce Aβ monomers or oligomers. These results suggest that mature Aβ fibrils are formed by stronger bonding and are relatively resistant to heat. On the other hand, the large spherical Aβ aggregates produced in the presence of 100 µM 3,3',4',5'-tetrahydroxyflavone disappeared with heat treatment, as demonstrated by TEM examination. SDS-PAGE analysis revealed that heat treatment caused a decrease of the high molecular weight smear-like band, which was accompanied by a prominent increase of Aβ monomers. These results suggest that the large spherical Aβ aggregates are formed by weak bonding and are disassembled into monomers upon heating.

Another notable characteristic of the Aβ aggregates produced in the presence of 100 µM 3,3',4',5'-tetrahydroxyflavone is the rapidity with which they are formed. More than 2 h was required for the conversion of Aβ monomers or oligomers into Aβ fibrils in our experimental conditions, as indicated by the ThT fluorescence and SDS-PAGE analyses. On the other hand, the SDS-resistant, smear-like band appeared on SDS-PAGE within 1–2 h after incubation. This result suggests that, in the presence of 3,3',4',5'-tetrahydroxyflavone, atypical Aβ aggregates are formed faster than Aβ fibrils. It is likely that atypical Aβ aggregates are formed by a direct effect of 3,3',4',5'-tetrahydroxyflavone, but not as a result of inhibition of fibril formation.

It has recently been proposed that formation of amyloid fibers is a two-step process in which proteins first aggregate into colloidal spheres of approximately 20 nm diameter, and the spheres then join together to form linear chains. The spherical Aβ aggregates observed using TEM in the present study were very large, ranging from 500 nm to 1 µm in diameter, and are probably different from the colloidal spheres that can evolve into mature fibrils. Very recently, Ladwala et al. have reported using atomic force microscopy that resveratrol, a stilbenoid found largely in the skins of red grapes, remodels soluble oligomers and fibrils of Aβ into large spherical aggregates. The shape and size of Aβ aggregates observed in those studies are very similar to those found in the present study. However, it remains unclear how these small molecules cause the unusual conformational changes in Aβ. In our preliminary study, 100 µM 3,3',4',5'-trihydroxyflavone produced large spherical Aβ aggregates, very similar to 3,3',4',5'-tetrahydroxyflavone, suggesting that the 3,4',5'-trihydroxy group of the B ring is crucial for the effect of flavonoids. Considering that resveratrol (3,4',5'-trihydroxy stilbene) does not have the 3,4',5'-trihydroxy group, 3,3',4',5'-tetrahydroxyflavone and resveratrol may bind to Aβ in different manners. To clarify molecular mechanisms by which these flavonoids produce large spherical Aβ aggregates, computational modeling and molecular simulation studies are underway in our laboratory. Little is known about the physiological or pathological role of atypical Aβ aggregates produced by 3,3',4',5'-tetrahydroxyflavone. A neurotoxicity assay with cultured rat hippocampal neurons demonstrated that Aβ incubated in the presence of 100 µM 3,3',4',5'-tetrahydroxyflavone was significantly less toxic than Aβ incubated in the presence of vehicle, suggesting that the large spherical aggregates produced by 3,3',4',5'-tetrahydroxyflavone are less toxic Aβ conformers. Further investigations are underway in our laboratory to clarify how 3,3',4',5'-tetrahydroxyflavone affects Aβ conformation and neurotoxicity in the brains of animals.

In conclusion, we have found for the first time that 3,3',4',5'-tetrahydroxyflavone rapidly produces large, spherical Aβ aggregates whose bonding is resistant to SDS, but sensitive to heat. Furthermore, we have confirmed that inhibition of Aβ fibril formation by 3,3',4',5'-tetrahydroxyflavone results in a decrease of Aβ neurotoxicity. Therefore, this newly synthesized fisetin analogue is a promising lead compound for the development of therapeutic drugs for AD. For clinical application, further medicinal chemical studies are underway in our laboratory to find fisetin analogues with increased bioavailability.

REFERENCES


