Heliyon 7 (2021) e08101

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Glavonoid, a possible supplement for prevention of ATTR amyloidosis

Hiroaki Matsushita [a,*], Aito Isoguchi [b], Masamitsu Okada [b], Teruaki Masuda [b], Yohei Misumi [b], Chiharu Tsutsui [a], Narumi Yamaguchi [a], Yuko Ichiki [a], Jinko Sawashita [c], Mitsuharu Ueda [b], Mineyuki Mizuguchi [d], Yukio Ando [a,b,**]

[a] Department of Amyloidosis Research, Faculty of Pharmaceutical Sciences, Nagasaki International University, 2825-7 Huis Ten Bosch Sasebo, Nagasaki 859-3298, Japan
[b] Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-0811, Japan
[c] Pharma & Supplemental Nutrition Solutions Vehicle, Kaneka Corporation, Osaka, Japan
[d] Faculty of Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama 930-0914, Japan

ARTICLE INFO

Keywords:
TTR
Glavonoid
Glabridin
Amyloidosis
Tetramer

ABSTRACT

Transthyretin (TTR) is an amyloidogenic protein associated with hereditary and nonhereditary transthyretin amyloidoses (ATTR). Dissociation of the tetramer of TTR to the monomer induces TTR misfolding, which leads to amyloid fibril formation and triggers the onset of ATTR amyloidosis. Stabilizers of tetrameric TTR have been accepted as an effective ATTR amyloidosis treatment while effect is limited and they are too expensive. The aim of our study was to find more effective and cheep natural compound to suppress TTR amyloid formation. Glabridin, a prenylated isoflavan isolated from *Glycyrrhiza glabra* L., stabilized the TTR tetramer in vitro. The effects of licorice-derived flavonoid oil—Glavonoid, a natural substance that includes glabridin and several polyphenols—on stabilizing the TTR tetramer must still be elucidated. To examine plasma TTR stabilization by Glavonoid in vitro, we investigated the feasibility of utilizing glabridin plus Glavonoid to prevent TTR amyloid fibril formation. Glavonoid mixed with human plasma samples at 24 h incubation in vitro increased the tetramer level ($P < 0.05$) and reduced the monomer level ($P < 0.01$) and the monomer/tetramer ratio ($P < 0.05$) of TTR compared to those without Glavonoid by immunoblot analysis, such effect could not observe in the presence of glabridin. Oral Glavonoid (300 mg for 12 weeks) in 7 healthy volunteers effectively increased the plasma glabridin concentration. Glavonoid increased the TTR tetramer level and reduced the monomer/tetramer ratio of TTR ($P < 0.05$) in plasma at 12 weeks in healthy volunteers compared to those of age matched control subjects without the supplement. Thus, oral Glavonoid may effectively prevent TTR amyloid fibril formation via TTR tetramer stabilization. Glavonoid may become a promising supplement before onset of ATTR amyloidosis.

1. Introduction

Amyloidogenic proteins have been identified as important molecules in amyloid-related disorders such as Alzheimer's disease, AA amyloidosis, prion disease, cerebral amyloid angiopathy, and transthyretin (TTR) amyloidosis (Sperry et al., 2018; Ueda et al., 2019). TTR, the precursor protein in transthyretin amyloidosis (ATTR), binds to thyroxin and retinol-binding protein, which transport vitamin A. Moreover, the protein TTR has been well known as an amyloidogenic protein associated with ATTR amyloidosis, hereditary (ATTRv) amyloidosis, and nonhereditary (ATTRwt, wild-type ATTR) amyloidosis (Ueda et al., 2019). It has been well documented that ATTR amyloidosis is an ageing related disorder, and the number of the patients is increasing year by year, because aged people is increasing year by year (Ueda et al., 2011). In fact, most of the autopsy of supercentenarians had ATTRwt amyloidosis (Leslie, 2008). Cardiac dysfunction and locomotive syndrome found in ATTRwt amyloidosis should be treated before they become life-threatening complications.

TTR is 14 kD protein which binds to T4 and retinol binding protein and forms tetramer in plasma. TTR tetramer dissociation and monomer misfolding cause misassembly into numerous aggregate morphologies including amyloid fibrils. Misfolding of the TTR monomer form is the trigger for the amyloid fibril formation of TTR, and stabilization of the TTR tetramer has been effective for prevention and treatment of TTR amyloidosis (Sekijima, 2015).

* Corresponding author.
** Corresponding author.
E-mail addresses: hiroakim@niu.ac.jp (H. Matsushita), andoy430@niu.ac.jp (Y. Ando).

https://doi.org/10.1016/j.heliyon.2021.e08101
Received 7 July 2021; Received in revised form 17 September 2021; Accepted 28 September 2021

Diflunisal and tafamidis have been well documented as effective drugs for halting ATTR amyloidosis progression by stabilizing the tetramer form of TTR. Use of these drugs led to improvement in polyneuropathy and cardiac failure (Coelho et al., 2012; Lamb and Deeks. 2019). However, diflunisal is nephrotoxic, tafamidis is costly ($30,000 per year per patient in Japan), and effects were limited to the early stage of disease in patients with ATTR. Moreover, these agents cannot be used as prophylactic drugs. Because organ dysfunction induced by amyloid deposition is irreversible, especially in the advanced stage of disease, prophylactic drugs for amyloidosis or a supplement administered to asymptomatic patients should be developed.

The natural small molecule glabridin, a prenylated isoflavan isolated from *G. glabra* L. (Favaceae), was shown to stabilize the TTR tetramer in vitro. Glabridin forms a complex with TTR, and the inclusion of this molecule leads to stabilization of the dimer-dimer interface (Yokoyama et al., 2014). Glabridin is believed to be a phytoestrogen and is associated with numerous biological properties, including anti-obesity, antioxidant, anti-inflammatory, and neuroprotective activities. Glabridin inhibited TTR aggregation in a thioflavin assay. Glavonoid, whose major component is glabridin, is a natural supplement that is commonly known as licorice-derived flavonoid oil and is used in cosmetics, food, and tobacco and in both traditional medicine and herbal medicine (Tominaga et al., 2006; Kuroda et al., 2010; Lee et al., 2012). Glavonoid is sold commercially as an effective supplement for those purposes; it is also inexpensive and without obvious side effects. It may become a medicine that will be useful for prevention of ATTR amyloidosis by stabilizing the TTR tetramer and reducing the progression of the disease.

In this study, we used plasma of healthy volunteers to analyze the TTR-stabilizing effect of glabridin and Glavonoid in vitro. We also analyzed the plasma glabridin concentration in healthy volunteers. Next, to examine the stabilization effect of Glavonoid in vivo, we administered 300 or 600 mg of the supplement to healthy volunteers for 12 or 4 weeks, and we evaluated changes in the forms of TTR in plasma.

2. Materials and methods

2.1. Materials

Glabridin was purchased from Sigma-Aldrich, St. Louis, MO, USA (Code G9548). Glavonoid was provided by Kaneka Corporation (Osaka, Japan). We prepared Kaneka Glavonoid Rich Oil™ (Kaneka Corporation) for use in this study. Glavonoid is an extract rich in polyphenol-type substances, which derives from licorice (*G. glabra*) using previously described methods (Aoki et al., 2005; Tominaga et al., 2006). In brief, we obtained crude extract from root or rootstock of licorice by using ethanol and then performed an extraction of this ethanol extract with medium-chain triglycerides (fatty acid composition C8: C10 = 99:1). We standardized Glavonoid by using medium-chain triglycerides to a final concentration of 3.0% of the prenylated flavonoid glabridin, which is the most abundant constituent of the polyphenol fraction contained in the extract. Glavonoid contains glabrene, glabrol, and 4′-*O*-methylglabridin as minor prenylated flavonoids, as well as 45 other identified polyphenol-type substances, and less than 0.005% (w/w) glycyrrhizin, as described previously (Aoki et al., 2005; Kuroda et al., 2010). In human tests, we used Kaneka Glavonoid, which is a commercially available soft gel capsule (Kaneka Your Health Care Co., Ltd., Osaka, Japan). One capsule contains 300 mg of Glavonoid including 3.0% glabridin as a guaranteed value.

2.2. Subjects and Glavonoid administration to healthy volunteers

Subjects were healthy Japanese volunteers (3 males, 4 females; 49.9 ± 6.4 years old) who recruited by Kaneka Corporation. Volunteers who had diseases were excluded from this study at Kaneka Corporation. To investigate TTR stability in humans after use of Glavonoid supplementation, we obtained plasma samples from the healthy subjects (Table 1).

Table 1. Results of laboratory examinations of plasma from healthy volunteers.

	0 week	12 weeks
Weight (kg)	69.89 ± 6.7	-
Age (y)	49.9 ± 6.4	-
Total Protein (g/dL)	7.40 ± 0.33	7.24 ± 0.26
Albumin (g/dL)	4.40 ± 0.18	4.27 ± 0.27
AST (IU/L)	21.1 ± 6.5	19.4 ± 6.1
ɣ-GTP (IU/L)	24.0 ± 10.7	22.0 ± 11.5
ALP (IU/L)	220.1 ± 27.2	224.0 ± 36.3
LDH (IU/L)	184.7 ± 29.4	180.1 ± 18.0
CK (IU/L)	103.9 ± 63.6	91.9 ± 25.0
Total-Cho (mg/dL)	225.6 ± 53.0	213.1 ± 46.7
LDL-Cho (mg/dL)	137.6 ± 36.6	129.4 ± 28.2
HDL-Cho (mg/dL)	66.3 ± 18.7	63.0 ± 15.9
TG (mg/dL)	101.4 ± 69.4	120.0 ± 91.0
fGlc (mg/dL)	94.0 ± 7.2	92.3 ± 6.6
HbA1c (%)	5.53 ± 0.38	5.57 ± 0.35
Insulin (μIU/mL)	7.59 ± 3.19	6.63 ± 2.01
Uric acid (mg/dL)	5.16 ± 1.45	5.00 ± 1.05
BUN (mg/dL)	15.37 ± 2.65	12.47 ± 1.42
Creatinine (mg/dL)	0.719 ± 0.148	0.677 ± 0.120

± SD, n = 7.

Biochemical data of plasma samples from 7 healthy volunteers who were given Glavonoid for 12 weeks are provided. Data represent means ± SD.

AST aspartate aminotransferase, *γ-GTP* γ-glutamyl transpeptidase, *ALP* alkaline phosphatase, *LDH* lactate dehydrogenase, *CK* creatine kinase, *Cho* cholesterol, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *TG* triglyceride, *fGlc* fasting glucose, *HbA1c* glycated hemoglobin, BUN blood urea nitrogen.

These subjects had participated in a randomized placebo-controlled double-blind parallel group comparison study in 2019 (VF1-1801; UMIN ID, UMIN000034285). This clinical study was performed by a contract research organization named Apo Plus Station Co., Ltd., (Tokyo, Japan) together with Kaneka Corporation. The objective of this study to investigate health promotion in healthy Japanese people by using the supplement Glavonoid. People were excluded from the study if they used licorice-containing medicinal products or health supplements; had a history of serious disease such as diabetes, hepatic disease, renal disease, or cardiac disease; had a history of food allergy (especially to licorice components), idiosyncrasy, or excessive alcohol use; underwent exercise programs for weight loss or were taking health supplements for weight loss; or were pregnant or wanted to become pregnant during the study period (women). Fasting subjects visited the clinic in the morning before (0 week) and after (4, 8, and 12 weeks) the intervention. We determined their physical condition and obtained their fasting blood samples. At every evaluation visit, we separated plasma from blood by using a standard method and stocked spare plasma in a deep freezer. After the intervention with the Glavonoid supplement (300 mg/capsule/day) or placebo capsule (0 mg/capsule/day) for 12 weeks, we obtained glabridin concentrations in plasma samples from all participants according to an improved HPLC method (Sumika Chemical Analysis Service, Ltd., Osaka, Japan) based on a procedure described previously (Aoki et al., 2007). We then selected 7 subjects from the Glavonoid group and obtained samples of the spare plasma, which we stocked in a deep freezer, to analyze plasma TTR stability (VF7-1904; UMIN ID, UMIN000038870). We obtained 4 samples (0, 4, 8, and 12 weeks with the supplementation) of plasma from 7 subjects to analysis for TTR stability. For all subjects in both studies, approval from the ethical committee of the medical institution (Kanazawabunko Hospital, Kanagawa, Japan) was provided before the study commenced. All subjects signed informed consent forms before enrolling in the study, which was performed in accordance with the Declaration of Helsinki. We tested TTR tetrameric stability after administration of 300 mg/day Glavonoid.

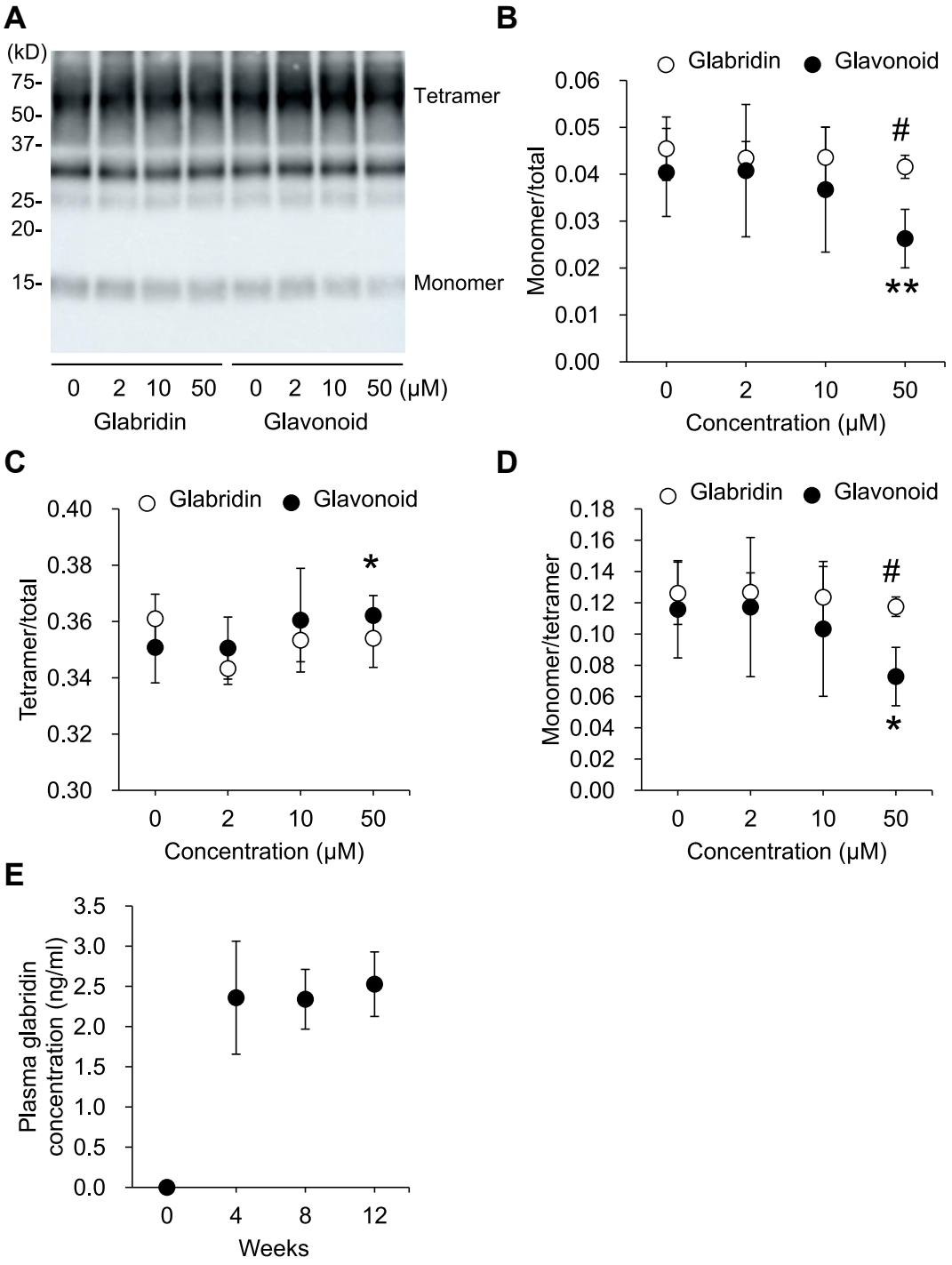

Figure 1. TTR stabilization in plasma after in vitro Glavonoid. (A) Immunoblot of tetramer stability with glabridin or Glavonoid in vitro ($n = 3$). (B) Quantitative analysis of TTR monomers. (C) Quantitative analysis of TTR tetramers. (D) Analysis of the monomer/tetramer ratio of TTR. Data represent means ± SD. $*P < 0.05$, $**P < 0.01$ vs. 0 μM, for samples with and without glabridin or Glavonoid. $\#P < 0.05$ vs. glabridin group. (E) Plasma concentration of glabridin after 300 mg/day Glavonoid administration. Plasma concentration of glabridin after administration of Glavonoid at 300 mg/day for 12 weeks ($n = 7$). Data represent means ± SD.

2.3. Evaluation of Glavonoid in vitro via chemical cross-linking

To evaluate the effects of drugs on the tetramers stability of TTR to urea denaturation, samples of human plasma in healthy volunteers were incubated at 25 °C for 30 min with or without 0, 2, 10 or 50 μM glabridin or Glavonoid in test tubes followed by denaturation with 4.5 M urea for 24 h at 25 °C, as described previously (Ueda et al., 2019). We added 5 μl of 25% glutaraldehyde for 4 min at room temperature followed by quenching via addition of 5 μl of 7% $NaBH_4$ in 0.1 M NaOH. Cross-linked samples were analyzed by western blotting.

2.4. Immunoblot analysis

Western blotting was performed as described previously (Tojo et al., 2006). Samples of human plasma were separated by using sodium dodecyl sulfate–polyacrylamide gel electrophoresis and then were

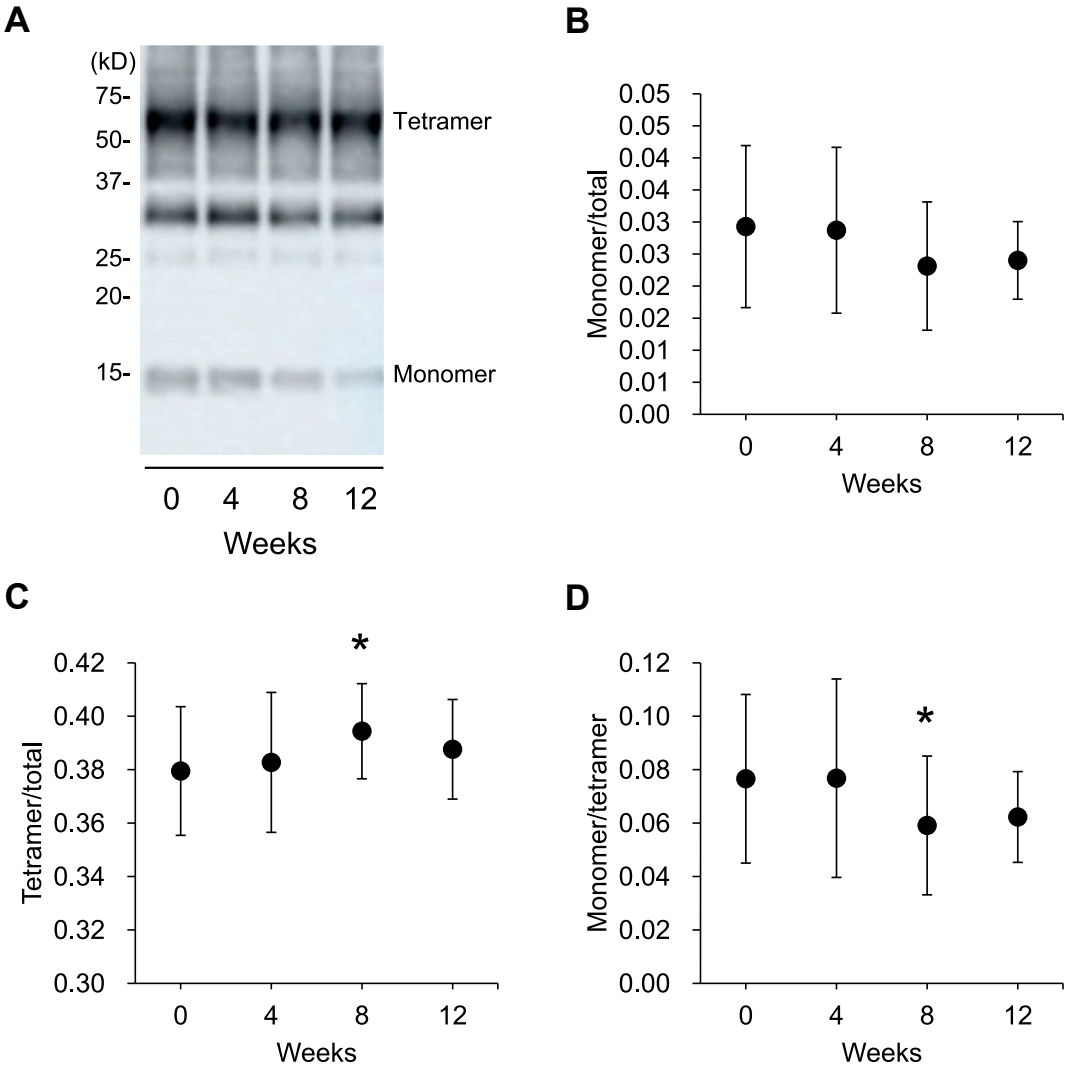

Figure 2. TTR stabilization in plasma after 300 mg/day Glavonoid administered for 12 weeks. (A) Immunoblot of TTR tetramer stability after 12 weeks of 300 mg/day Glavonoid ($n = 7$). (B) Quantitative analysis of TTR monomers. (C) Quantitative analysis of TTR tetramers. (D) Analysis of the monomer/tetramer ratio of TTR. Data represent means ± SD. *$P < 0.05$ vs. 0 week.

transferred onto polyvinylidene difluoride membranes. The membranes were blocked with 2.5% skim milk in 1×Tris-buffered saline with Tween 20 (TBST) for 1 h at room temperature. Then membranes were washed 3 times with 0.2% skim milk in 1×TBST for 5 min and were incubated overnight at 4 °C with rabbit polyclonal antibody against TTR (anti-TTR; Dako, CA, USA) (Tojo et al., 2006) at 1:1500 dilution. After the membranes were washed, they were incubated with secondary antibodies conjugated with horseradish peroxidase (anti-rabbit IgG for anti-TTR at 1:4000 dilution) (Sigma-Aldrich) for 1 h at room temperature. Western blot bands were detected by using the enhanced chemiluminescence technique with the ECL prime detection kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK).

2.5. Analyses of laboratory data

During 4 weeks, we assessed the plasma TTR concentration and TTR stability, hsCRP after oral administration of Glavonoid at 600 mg/day to subjects via routine studies at the Department of Laboratory Medicine, Kumamoto University Hospital, registered ISO 15189. Five healthy volunteers were included in that study.

2.6. Statistics

Data shown are means ± standard deviation (SD). Data were analyzed for statistical significance by using Student's *t*-test. *P* values less than 0.05 were considered to be significant.

3. Results

3.1. Plasma TTR stabilization by Glavonoid in vitro

To investigate TTR stabilization in human plasma, we performed immunoblot analysis. Figure 1A shows the bands for plasma TTR with or without 0, 2, 10, or 50 μM glabridin or Glavonoid. Glavonoid added to human plasma significantly reduced the monomer level ($P < 0.01$; Figure 1B) and increased the tetramer level ($P < 0.05$; Figure 1C), so that the monomer/tetramer ratio of TTR decreased in a dose-dependent manner ($P < 0.05$; Figure 1D) compared with samples without Glavonoid. Glabridin, however, did not have such effects. Thus, Glavonoid alone showed stabilizing effect for tetrameric form of TTR.

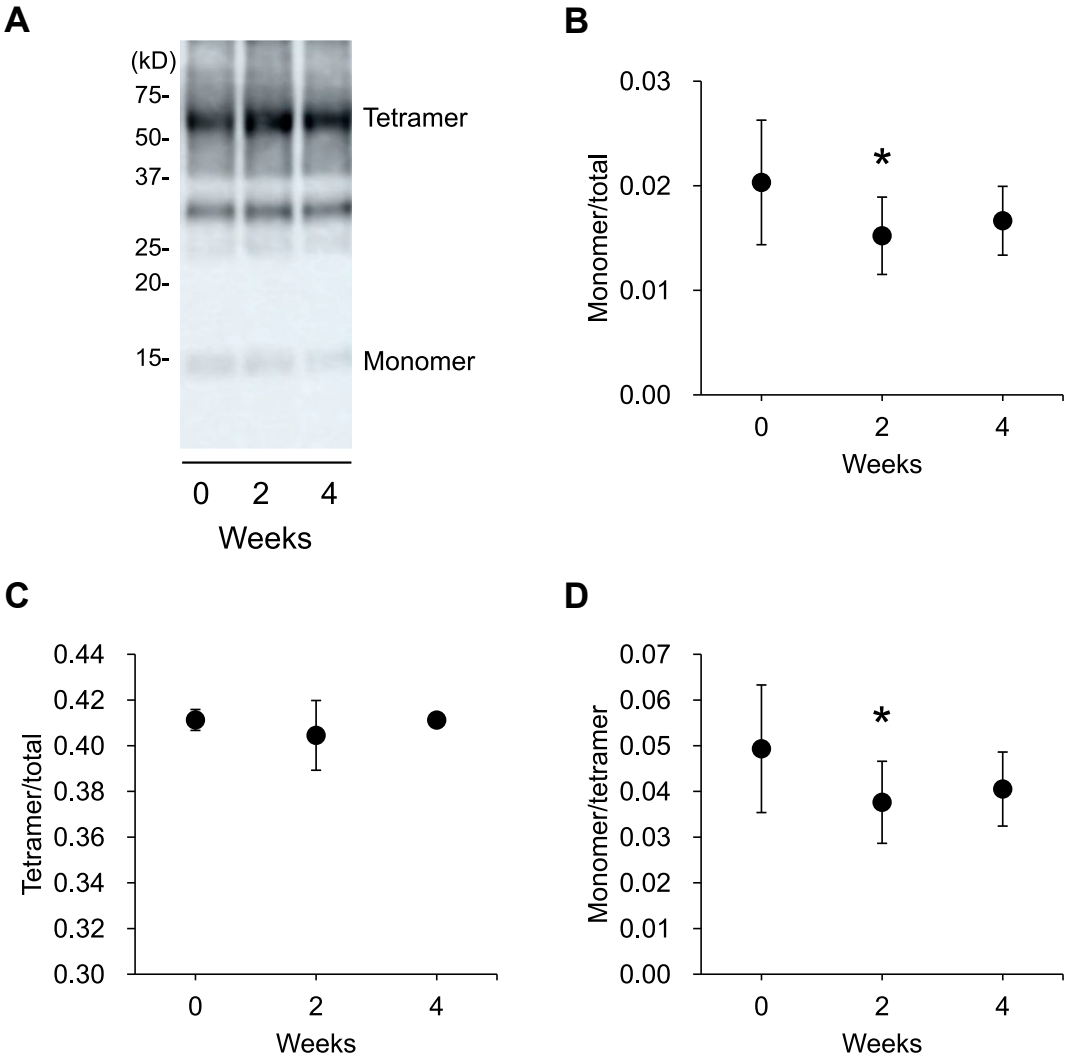

Figure 3. TTR stabilization in plasma after 4 weeks of 600 mg/day Glavonoid. (A) Immunoblot of TTR tetramer stability after 600 mg/day Glavonoid ($n = 3$). (B) Quantitative analysis of TTR monomers. (C) Quantitative analysis of TTR tetramers. (D) Analysis of the monomer/tetramer ratio of TTR. Data represent means ± SD. *$P < 0.05$ vs. 0 week.

3.2. Plasma glabridin concentration

To analyze the plasma pharmacokinetics of glabridin, the main component of Glavonoid, after administration of Glavonoid to healthy volunteers, we determined the plasma concentrations by using high-performance liquid chromatography (HPLC), as described previously (Aoki et al., 2005, Aoki et al., 2007). Orally administered Glavonoid at 300 mg/day for 12 weeks increased the plasma glabridin concentrations in healthy volunteers (Figure 1E).

3.3. TTR stabilization by 300 mg/day Glavonoid administered for 12 weeks

Figure 2A provides an immunoblot of plasma TTR after oral Glavonoid at 300 mg/day for 12 weeks. Orally administered Glavonoid gradually increased the TTR tetramer level ($P < 0.05$; Figure 2C), so that the result was a reduced TTR monomer/tetramer ratio ($P < 0.05$; Figure 2D) at 8 weeks that reached a plateau at 12 weeks after the initial Glavonoid administration, but the TTR monomer level (Figure 2B) did not change.

3.4. TTR stabilization by 600 mg/day Glavonoid administered for 4 weeks

Figure 3A shows an immunoblot of plasma TTR after Glavonoid given orally at 600 mg/day for 4 weeks. No dropout volunteers were observed. Oral Glavonoid significantly reduced the TTR monomer level ($P < 0.05$; Figure 3B) and the monomer/tetramer ratio ($P < 0.05$; Figure 3D) at 2 weeks, compared with samples from control subjects, but the TTR monomer/tetramer ratio (Figure 3C) did not change. Compared with the 300 mg/day Glavonoid administration, 600 mg/day Glavonoid produced showed a more rapid effect.

3.5. Changes in serum TTR and CRP concentrations

Laboratory data did not differ between those before and 12 weeks administrating Glavonoid as demonstrated in Table 1. In serum TTR concentrations, which slightly increased at 4 weeks after the initial administration of Glavonoid (serum TTR concentration, 31.97 mg/dl at day 0, and 32.84 mg/dl at 4 weeks). The high-sensitivity C-reactive protein (hsCRP) level did not change during the study period (data not shown).

4. Discussion

The present study revealed the following important findings: 1. Orally administered Glavonoid increased the plasma concentration of glabridin, the major component of Glavonoid, in healthy volunteers. 2. Glavonoid added to plasma in vitro reduced the TTR monomer level and the monomer/tetramer ratio of TTR, compared with samples without Glavonoid, glabridin did not observed such effect. Although glabridin was know to an excellent stabilizer for tetrameric form of TTR, effect of Glavonoid was not known before this study (Yokoyama et al., 2014; Yokoyama and Mizuguchi, 2018). 3. Glavonoid given at 300 mg/day for 12 weeks to healthy volunteers reduced the TTR monomer level and increased the monomer/tetramer ratio; administration of Glavonoid at 600 mg/day caused more rapid effects. 4. Administration of Glavonoid to healthy volunteers did not affect plasma laboratory data, which suggested that Glavonoid produced no obvious side effects, especially in the liver and kidney (Table 1). Because TTR is an anti-acute phase protein, inflammation effects reduced the plasma TTR concentration (Ingenbleek and Bernstein, 2015). However, plasma TTR concentrations after Glavonoid administration increased slightly.

Our experiments suggest that oral administration of Glavonoid may effectively prevent ATTR amyloidosis via stabilization of the TTR tetramer. Several polyphenols have been well documented to produce stabilizing effects on amyloid formation in vitro in amyloid β and ATTR amyloidosis (Phan et al., 2019). Glavonoid may become a preventive supplement for Alzheimer's disease and ATTRwt amyloidosis, which are typical senescence disorders. Additional study is needed.

The TTR-glabridin binding of caused a rotation of the T119 side chain of the TTR molecule and the addition of a water molecule, which led to stabilization of the dimer-dimer interface (Yokoyama et al., 2014). However, glabridin did not demonstrate such effects in our assay systems, whereas Glavonoid increased the stability of tetrameric TTR compared with glabridin (Figure 1). Because Glavonoid contained several natural polyphenols in addition to glabridin (Kuroda et al., 2010), those polyphenols have an effect on the stabilization of tetrameric form of TTR (Porat et al., 2006; Yokoyama and Mizuguchi 2018).

Oral Glavonoid increased the plasma concentration of glabridin (Figure 1). The maximum peak glabridin concentration in plasma occurred 3.2 or 3.6 h after single oral administration of 300 or 600 mg Glavonoid (Aoki et al., 2007). Glavonoid given to healthy volunteers increased the stability of TTR (Figures 2 and 3). More frequent administration or higher doses of Glavonoid may be beneficial because Glavonoid is basically a safe supplement. When we administer Glavonoid to patients with ATTR amyloidosis, we must determine the proper dosage and the frequency of administration that will generate the maximum effect. Additional investigations are needed.

Tafamidis, a drug that stabilizes the tetramer form of TTR, has improved polyneuropathy and cardiac failure (Coelho et al., 2012; Lamb and Deeks, 2019). However, it is costly and its effect was limited to the early stage of disease in patients with ATTR amyloidosis. Diflunisal is another TTR stabilizer, and it stops the progression of polyneuropathy. However, it cannot be used for patients with ATTR amyloidosis because it is nephrotoxic, and these patients have kidney disorders during the course of illness because of amyloid deposition (Lobato and Rocha., 2012). In ATTRwt as well, amyloid deposition in the kidney is common. Moreover, this disorder is senescence related, so chronic kidney diseases are common.

Amyloid deposition in targeted organs induces irreversible impairment, especially in the advanced stage of disease. Early administration of drugs or supplements is advantageous and produces a better outcome. Prophylactic therapeutic drugs or supplements for amyloidosis are needed and should be developed. Glavonoid is inexpensive as a supplement and has been widely used without obvious side effects.

5. Conclusions

Our experiments suggest that oral administration of Glavonoid should effectively prevent ATTR amyloidosis. Although existing stabilizers of tetrameric TTR, which are widely accepted as therapeutic drugs worldwide, cannot be administered as prophylactic treatment, ATTR amyloidosis is an ageing related disorder, and the number of the patients is increasing year by year, because aged people is increasing year by year. Glavonoid, an inexpensive and safe supplement, may be administered to asymptomatic subjects who are expected to start to manifest ATTRwt or ATTRv amyloidosis.

Declarations

Author contribution statement

Hiroaki Matsushita: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Aito Isoguchi; Masamitsu Okada; Chiharu Tsutsui; Narumi Yamaguchi; Yuko Ichiki: Performed the experiments; Analyzed and interpreted the data.

Jinko Sawashita: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Teruaki Masuda; Yohei Misumi: Conceived and designed the experiments.

Mitsuharu Ueda: Conceived and designed the experiments; wrote the paper.

Mineyuki Mizuguchi: Conceived and designed the experiments; Wrote the paper.

Yukio Ando: Conceived and designed the experiments; Wrote the paper.

Funding statement

This work was supported by Japan Society for the Promotion of Science Grants-in-Aid for Scientific Research (KAKENHI grant number 19H03565).

Data availability statement

The authors do not have permission to share data.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

Aoki, F., et al., 2005. Determination of glabridin in human plasma by solid-phase extraction and LC-MS/MS. J. Chromatogr. B 828, 70–74.
Aoki, F., et al., 2007. Clinical safety of licorice flavonoid oil (LFO) and pharmacokinetics of glabridin in healthy humans. J. Am. Coll. Nutr. 26, 209–218.
Coelho, T., et al., 2012. Tafamidis for transthyretin familial amyloid polyneuropathy: a randomized, controlled trial. Neurology 79, 785–792.
Ingenbleek, Y., Bernstein, L.H., 2015. Plasma transthyretin as a biomarker of lean body mass and catabolic states. Adv. Nutr. 6, 572–580.
Kuroda, M., et al., 2010. Phenolics from Glycyrrhiza glabra roots and their PPAR-γ ligand-binding activity. Bioorg. Med. Chem. 18, 962–970.
Lamb, Y.N., Deeks, E.D., 2019. Tafamidis: a review in transthyretin amyloidosis with polyneuropathy. Drugs 79, 863–874.
Lee, J.W., et al., 2012. AMPK activation with glabridin ameliorates adiposity and lipid dysregulation in obesity. J. Lipid Res. 53, 1277–1286.
Leslie, M., 2008. Aging. Searching for the secrets of the super old. Science 321, 1764–1765.

Lobato, L., Rocha, A., 2012. Transthyretin amyloidosis and the kidney. Clin. J. Am. Soc. Nephrol. 7, 1337–1346.
Phan, H.T.T., et al., 2019. Polyphenols modulate Alzheimer's amyloid beta aggregation in a structure-dependent manner. Nutrients 11, 756.
Porat, Y., et al., 2006. Inhibition of amyloid fibril formation by polyphenols: structural similarity and aromatic interactions as a common inhibition mechanism. Chem. Biol. Drug Des. 67, 27–37.
Sekijima, Y., 2015. Transthyretin (ATTR) amyloidosis: clinical spectrum, molecular pathogenesis and disease-modifying treatments. J. Neurol. Neurosurg. Psychiatry 86, 1036–1043.
Sperry, B.W., et al., 2018. Tenosynovial and cardiac amyloidosis in patients undergoing carpal tunnel release. J. Am. Coll. Cardiol. 72, 2040–2050.
Tojo, K., et al., 2006. Diflunisal stabilizes familial amyloid polyneuropathy-associated transthyretin variant tetramers in serum against dissociation required for amyloidogenesis. Neurosci. Res. 56, 441–449.
Tominaga, Y., et al., 2006. Licorice flavonoid oil effects body weight loss by reduction of body fat mass in overweight subjects. J. Health Sci. 52, 672–683.
Ueda, M., et al., 2011. Clinicopathological features of senile systemic amyloidosis: an ante- and post-mortem study. Mod. Pathol. 24, 1533–1544.
Ueda, M., et al., 2019. A cell-based high-throughput screening method to directly examine transthyretin amyloid fibril formation at neutral pH. J. Biol. Chem. 294, 11259–11275.
Yokoyama, T., et al., 2014. Crystal structures of human transthyretin complexed with glabridin. J. Med. Chem. 57, 1090–1096.
Yokoyama, T., Mizuguchi, M., 2018. Inhibition of the amyloidogenesis of transthyretin by natural products and synthetic compounds. Biol. Pharm. Bull. 41, 979–984.