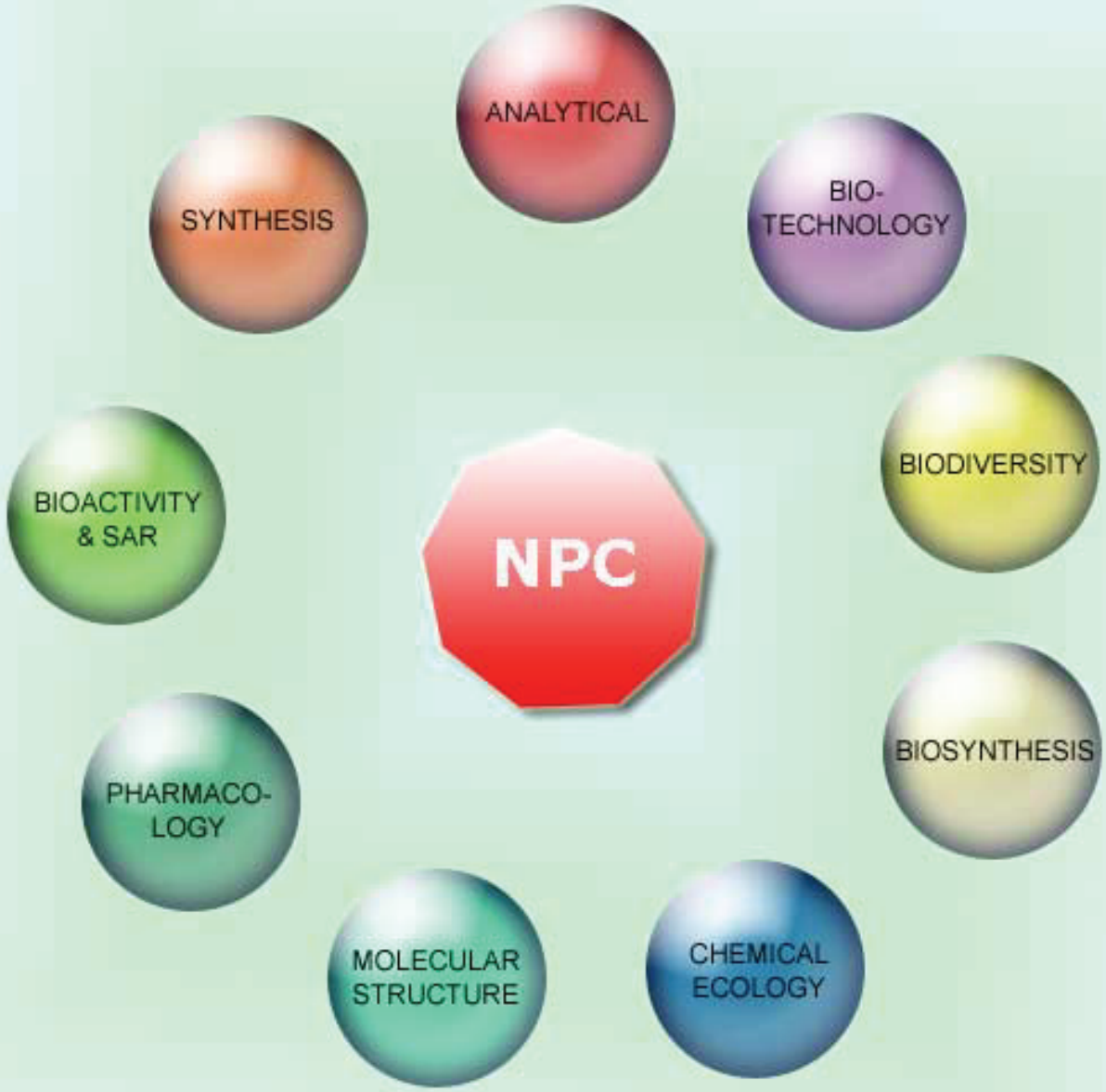
NATURAL PRODUCT COMMUNICATIONS

An International Journal for Communications and Reviews Covering all Aspects of Natural Products Research



Volume 13. Issue 8. Pages 919-1096. 2018
ISSN 1934-578X (printed); ISSN 1555-9475 (online)
www.naturalproduct.us

NPC Natural Product Communications

INFORMATION FOR AUTHORS

Full details of how to submit a manuscript for publication in Natural Product Communications are given in Information for Authors on our Web site http://www.naturalproduct.us.

To Subscribe: Natural Product Communications is a journal published monthly. 2018 subscription price: US$2,595 (Print, ISSN# 1934-578X); US$2,595 (Web edition, ISSN# 1555-9475); US$2,995 (Print + single site online); US$595 (Personal online). Orders should be addressed to Subscription Department, Natural Product Communications, Natural Product Inc., 7963 Anderson Park Lane, Westerville, Ohio 43081, USA. Subscriptions are renewed on an annual basis. Claims for nonreceipt of issues will be honored if made within three months of publication of the issue. All issues are dispatched by airmail throughout the world, excluding the USA and Canada.

NPC Natural Product Communications

2018
Vol. 13
No. 8
1007 – 1010

Evaluation of Anti-glycation Activities of Phlorotannins in Human and Bovine Serum Albumin-glyceraldehyde Models

Shingo Sugiura[a,f], Ryosuke Taniguchi[a,f], Yoshihiko Nishioka[a], Ryota Iwase[b], Reiji Tanaka[a,d,f], Hideo Miyake[a,d,f], Tetsushi Mori[c,d,f], Mitsuyoshi Ueda[e,f] and Toshiyuki Shibata[a,d,f*]

[a]*Graduate School of Bioresources*, *Mie University*, *1577 Kurimamachiya-cho*, *Tsu*, *Mie 514-8507*, *Japan*

[b]*Faculty of Bioresources*, *Mie University*, *1577 Kurimamachiya-cho*, *Tsu*, *Mie 514-8507*, *Japan*

[c]*Department of Biotechnology and Life Science*, *Tokyo University of Agriculture and Technology*, *2-24-16 Naka-cho*, *Koganei*, *Tokyo 184-8588*, *Japan*

[d]*Seaweed Biorefinery Research Center*, *Mie University*, *1577 Kurimamachiya-cho*, *Tsu, Mie 514-8507*, *Japan*

[e]*Graduate School of Agriculture*, *Kyoto University*, *Kitashirakawa Oiwake*, *Sakyo-ku*, *Kyoto 606-8502*, *Japan*

[f]*Japan Science and Technology Agency*, *CREST*, *4-1-8 Hon-cho*, *Kawaguchi*, *Saitama 332-0012*, *Japan*

shibata@bio.mie-u.ac.jp

Received: June 1st, 2018; Accepted: June 22nd, 2018

The anti-glycation activities of phlorotannins contained in the Japanese Lessoniaceae (*Ecklonia cava*, *Eck. kurome*, *Eck. stolonifera*, *Eisenia arborea*, and *Eis. bicyclis*) were tested using serum albumin-glyceraldehyde (GA) models. In the human serum albumin (HSA)-GA model and the bovine serum albumin (BSA)-GA model, the concentrations of crude phlorotannins at 50% inhibition (IC_{50}) of fluorescent advanced glycation end products (AGEs) formation was in the range of 0.48 to 0.70 mg/mL and 0.52 to 0.75 mg/mL, respectively. In tests using phloroglucinol and purified phlorotannins (eckol, fucofuroeckol A, phlorofucofuroeckol A, dieckol, and 8,8′-bieckol), dieckol had the highest inhibitory activity (IC_{50}: 5.5 x 10^2 μM) against fluorescent AGEs formation in HSA-GA model and showed about 18 times inhibition compared with aminoguanidine hydrochloride of positive control. In the BSA albumin model, 8,8′-bieckol had about 27 times AGEs formation inhibitory activity (IC_{50}: 6.2 x 10^2 μM) against aminoguanidine hydrochloride. In tests on GA scavenging activity, it was shown that compounds with phloroglucinol tetramer or higher had a scavenging rate of 70%, or more, with a reaction time of 120 minutes. These results suggest that among the phlorotannins, in particular the dimers of eckol (dieckol and 8,8′-bieckol), there are effective compounds for inhibiting the formation of AGEs derived from GA.

Keywords: Advanced glycation end products, Anti-glycation, 8,8′-Bieckol, Glyceraldehyde, Dieckol, Phlorotannins, Lessoniaceae.

Advanced glycation end products (AGEs) are a general term for structures generated by nonenzymatic reactions between proteins and reducing sugars such as glucose and fructose [1,2]. In previous studies [3-6], it has been clarified that AGEs are produced not only from the reducing sugars but also from sugar metabolic intermediates and intermediates of Maillard reactions. It is known that dicarbonyl compounds (methylglyoxal, glyoxal, and 3-deoxyglucosone) generated from autoxidation, and degradation products of glucose, have higher blood concentrations in diabetic patients than in healthy subjects [7,8]. In addition, it has been considered that the dicarbonyl compounds have high reactivity with proteins because there are two carbonyl groups in the molecule. From this scientific background, the relationship between AGEs derived from the dicarbonyl compounds and lifestyle-related diseases has been drawing attention. In recent years, according to Takeuchi *et al*.'s report [9], it was revealed that α-hydroxy aldehydes such as glyceraldehyde (GA) and glycolaldehyde are more reactive with proteins than dicarbonyl compounds. Among AGEs generated *in vivo*, it has been reported that AGEs derived from GA (GA-AGEs) accelerate intracellular oxidative stress through its binding to its receptor, and can cause strong cytotoxicity [10-12]. It is also pointed out that the GA-AGEs are involved in the onset and progression of diabetic vascular complications [9,13,14], Alzheimer's disease [9,15,16], nonalcoholic steatohepatitis [9,17,18], hypertension [9,14], and cancer [9,19]. Therefore, suppression of GA-AGEs formation and scavenging of GA can be regarded as effective for prevention and treatment of these diseases.

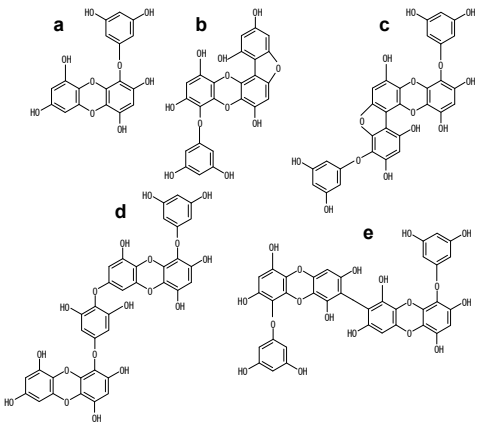


Figure 1: Chemical structures of phlorotannins isolated from the Japanese Lessoniaceae. a: eckol, b: fucofuroeckol A, c: phlorofucofuroeckol A, d: dieckol, e: 8,8′-bieckol.

In a preceding report [20], we isolated phlorotannins (eckol, fucofuroeckol A, phlorofucofuroeckol A, dieckol, and 8,8′-bieckol) (Figure 1) from Japanese Lessoniaceae (*Ecklonia cava*, *Eck. kurome*, *Eck. stolonifera*, *Eisenia arborea*, and *Eis. bicyclis*) and evaluated their anti-glycation properties in the serum albumin-methylglyoxal models. In the report [20], we clarified the following two facts: (1) phlorofucofuroeckol A and fucofuroeckol A with a benzobisbenzofuran skeleton have inhibitory activities far superior to aminoguanidine hydrochloride against the formation of fluorescent AGEs, and (2) eckols (eckol, dieckol, and 8,8′-bieckol) have higher methylglyoxal scavenging activity than aminoguanidine

hydrochloride. Phlorotannins are polyphenols that are specifically contained in brown algae and have a structure in which phloroglucinol (1,3,5-trihydroxybenzen) is polymerized [21]. In this study, to further evaluate the effectiveness of phlorotannins as a novel anti-glycation substance derived from natural plants, their inhibitory activity against the formation of fluorescent AGEs in the serum albumin-GA models and the GA scavenging activity of phlorotannins were examined.

In both human serum albumin (HSA)-GA and bovine serum albumin (BSA)-GA models, all the crude phlorotannins tested in this study inhibited the formation of fluorescent AGEs in a dose-dependent manner. Table 1 shows the concentration (IC_{50}) of the crude phlorotannins at 50% inhibition of fluorescent AGEs formation. The IC_{50} values of crude phlorotannins were in the range of 0.48 to 0.70 mg/mL in the HSA-GA model and 0.52 to 0.75 mg/mL in the BSA-GA model, respectively (Table 1). In both models, crude phlorotannins prepared from *Eis*. *bicyclis* showed the highest inhibitory activity against fluorescent AGEs formation among the tested samples (Table 1). Aminoguanidine is a synthetic glycation inhibitor that inhibits the formation of AGEs and suppresses crosslinking and polymerization of proteins *in vitro* [22]. Aminoguanidine is not clinically applied because it has several adverse side effects on humans, but it is frequently used as a positive control in studies in the search for compounds having anti-glycation activity. Since the IC_{50} values of aminoguanidine hydrochloride obtained in this study were 1.10 mg/mL in HSA-GA model and 1.93 mg/mL in BSA-GA model, it was found that the crude phlorotannins of *Eis*. *bicyclis* has anti-glycation activity of about 2.3 times and 3.7 times with respect to aminoguanidine hydrochloride. Currently, *Eck*. *kurome* is cultivated as a supply source of phlorotannins in Kumamoto prefecture, Japan [23]. The crude phlorotannins were prepared from both naturally occurring and cultured versions of *Eck*. *kurome*, and their inhibitory activities on the formation of fluorescent AGEs were evaluated. As shown in Table 1, IC_{50} values of crude phlorotannins from cultured *Eck*. *kurome* in each model were almost the same as those of the natural plants of *Eck*. *kurome*. Therefore, as with the natural plants of Lessoniaceae, it was confirmed that crude phlorotannins of cultured *Eck*. *kurome* can be utilized as a natural product having an inhibitory effect against AGEs formation.

Table 1: IC_{50} values of crude phlorotannins from Lessoniaceae against fluorescent AGEs formation.

Algae	Specific area of origin	HSA-GA (mg/mL)	BSA-GA (mg/mL)
Eck. *cava*	Mie	0.70	0.75
Eck. *kurome*	Fukuoka	0.58	0.55
Eck. *kurome*	Kumamoto	0.61	0.59
Cultured *Eck*. *kurome*	Kumamoto	0.52	0.58
Eck. *stolonifera*	Yamaguchi	0.54	0.56
Eis. *arborea*	Mie	0.51	0.61
Eis. *bicyclis*	Fukuoka	0.48	0.52

All the data are expressed as the mean of three independent measurements. The IC_{50} values of aminoguanidine hydrochloride were 1.10 mg/mL in the HSA-GA model and 1.93 mg/mL in the BSA-GA model.

Table 2: IC_{50} values of phloroglucinol and isolated phlorotannins against fluorescent AGEs formation

Compounds	HSA-GA (μM)	BSA-GA (μM)
Phloroglucinol	3.8×10^3	3.8×10^3
Eckol	1.1×10^3	1.3×10^3
Fucofuroeckol A	7.4×10^2	1.4×10^3
Phlorofucofuroeckol A	7.3×10^2	1.4×10^3
Dieckol	5.5×10^2	8.7×10^2
8,8′-Bieckol	5.7×10^2	6.2×10^2

All the data are expressed as the mean of three independent measurements. The IC_{50} values of aminoguanidine hydrochloride were 1.0×10^4 μM in the HSA-GA model and 1.7×10^4 μM in the BSA-GA model.

In order to further analyze the inhibitory activity of phlorotannins against fluorescent AGEs formation in the albumin-GA models, tests were carried out using phloroglucinol and five kinds of isolated compounds (eckol, fucofuroeckol A, phlorofucofuroeckol A, dieckol, and 8,8′-bieckol). Similar to the results in the albumin-methylglyoxal models obtained in the preceding report [20], phloroglucinol and the isolated eckols inhibited the formation of fluorescent AGEs in a concentration-dependent manner in both serum albumin-GA models (data not shown). As a result of calculating the IC_{50} value, dieckol and 8,8′-bieckol showed the effective activities in both models (Table 2). In the HSA-GA model, the IC_{50} value of dieckol was 5.5×10^2 μM (Table 2), which was found to have about 18 times activity compared with aminoguanidine hydrochloride. The IC_{50} value of 8,8′-bieckol obtained in the BSA-GA model was 6.2×10^2 μM (Table 2), and it had about 27 times inhibitory activity with respect to aminoguanidine hydrochloride. Even compounds with the lowest inhibition of AGEs also had activity about 9.1 times (eckol) in the HSA-GA model and about 12 times (fucofuroeckol A and phlorofucofuroeckol A) in the BSA-GA model as compared with aminoguanidine hydrochloride. Therefore, it was strongly suggested that the phlorotannins contained in Lessoniaceae, in particular dimers of eckol (dieckol and 8,8′-bieckol) have very excellent inhibitory activity on the formation of AGEs derived from GA.

GA is thought to be caused by three pathways: the glycolytic pathway, the polyol pathway, and the fructose metabolic pathway *in vivo* [24]. It has also been shown that GA-AGEs are produced more rapidly *in vivo* than other AGEs such as AGEs derived from glucose and AGEs derived from methylglyoxal [4,25,26]. Therefore, it is considered that the scavenging of GA may contribute to the reduction of glycation stress. As a result of experiments at a concentration of 5 mg/mL, each type of crude phlorotannins prepared from the five kinds of Lessoniaceae scavenged GA over time (data not shown). The GA scavenging rate at the reaction time of 120 minutes was in the range of 62.3% (*Eck*. *stolonifera*) to 78.2% (*Eck*. *cava*). Furthermore, GA scavenging activity was measured for phloroglucinol and five kinds of eckols, and the data obtained are shown in Figure 2. Except for eckol, the tested compounds scavenged GA is a roughly linear manner over time (Figure 2). The GA scavenging rate at a reaction time of 120 minutes was 63.8% for phloroglucinol, 58.1% for eckol, 77.3% for fucofuroeckol A, 70.0% for phlorofucofuroeckol A, 73.9% for dieckol, 75.0% for 8,8′-bieckol, and that of aminoguanidine hydrochloride was 86.2% (Figure 2). Although the GA scavenging activity of tested compounds was lower than aminoguanidine hydrochloride, it was revealed that compounds having phloroglucinol tetramer or higher had a scavenging activity of approximately 70%, or more.

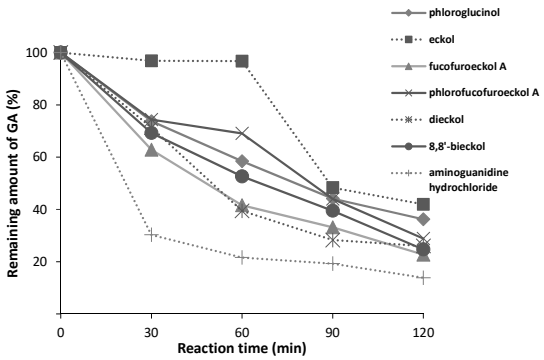


Figure 2: GA scavenging activity of phloroglucinol, isolated phlorotannins, and aminoguanidine hydrochloride. All the data are expressed as the mean of three independent measurements.

In comparison with polyphenols of terrestrial plants such as catechins, there are few reports on the toxicity evaluation of phlorotannins to animal cultured cell lines, the influence on cell

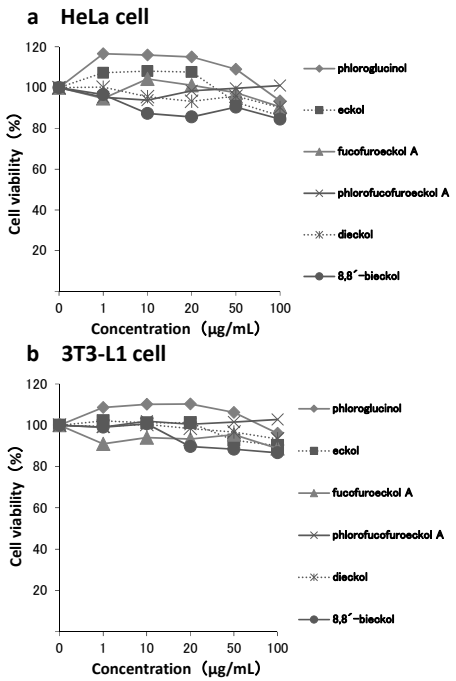


Figure 3: Cytotoxicity assay of phloroglucinol and isolated phlorotannins using MTT assay. Each value is the average of triplicate cultures.

proliferation, and bioavailability. Using phloroglucinol and the isolated compounds, their effect on the growth of HeLa (cancer cell line) and 3T3-L1 (normal cell line) cells was evaluated by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay (Figure 3). As a result of adding each compound to the medium at a concentration of 1 to 100 μg/mL, the survival rate of both cell lines was approximately 85%, or more (Figure 3). As a result of testing at concentrations of 50 and 100 μg/mL, each compound showed inhibitory activity in the range of about 10 to 20% against formation of fluorescent AGEs. Therefore, it was suggested that phloroglucinol and phlorotannins may not exhibit cytotoxicity at concentrations that exert anti-glycation properties. To our knowledge, there is no report on the evaluation of the anti-glycation properties of plant extracts or natural products in the serum albumin-GA models. The Lessoniaceae used in this study are the same algae used as a foodstuff in Japan and Korea. The results obtained in the preceding [20] and present studies strongly suggest that phlorotannins are superior anti-glycation substances derived from natural plants and that they may contribute to both alleviation of symptoms and prevention of onset of diseases caused by AGEs generated *in vivo*.

Experimental

Materials: For samples of brown algae (*Ecklonia cava* Kjellman, *Eck. kurome* Okamura, *Eck. stolonifera* Okamura, *Eisenia arborea* Areschoug, and *Eis. bicyclis* Kjellman), the algal plants used in the preceding report [20] were used. The cultured *Eck. kurome* was purchased from the Fisheries Cooperative Association of Amakusa in Kumamoto Prefecture, Japan, in 2015. The algae used for the extraction of phlorotannins were washed with filtered seawater, air-dried, and pulverized *via* pulverizing mill (ABS-W, Osaka Chemical). The algal powders were stored at -30°C until use. Aminoguanidine hydrochloride, HSA, and GA were purchased from Wako Pure Chemical Industries. BSA was obtained from Sigma-Aldrich. 1,3-Cyclohexanedione was purchased from Tokyo Chemical Industry. All other reagents used in this study were of analytical grade.

Extraction and purification of phlorotannins: Extraction of phlorotannins from algal powder was prepared according to the method described in the previous report [23]. Phlorotannins were purified by column chromatography and preparative HPLC using the same conditions described in the previous reports [27,28]. Each of the obtained phlorotannins (eckol, fucofuroeckol A, phlorofucofuroeckol A, dieckol, and 8,8′-bieckol) was confirmed to have a purity of 98% or more by three-dimensional HPLC (SPD-M10AV, Shimadzu) with an Inertsil ODS-3 column (4.6 mm i.d. x 250 mm, GL Science) [28]. The identification of the purified phlorotannins was carried out using LC/ESI/MS with the analysis condition reported in the preceding study [23]. The purified phlorotannins were stored at -30°C until used as samples.

Serum albumin-GA assay: Sample, GA (400 mM), and albumin (HSA or BSA, 20 mg/mL) solutions were prepared separately by dissolving in a 100 mM phosphate buffer (pH 7.4). The measurement was carried out using a 96-well black plate (FLUOTRAC600, Greiner) and a microplate reader (Infinite 200, Tecan). The sample solution (40 μL), GA solution (10 μL), and albumin solution (50 μL) were added to the well in the plate. Measurement was carried out with fluorescence intensity at an excitation of 370 nm and an emission of 440 nm, and the obtained value was taken as a control value. After incubation at 37°C for 24 hours, the fluorescence intensities of each well were measured under the same measurement conditions. For blank wells, a 100 mM phosphate buffer was used instead of a sample solution. Aminoguanidine hydrochloride was used as a positive control. The inhibition rate (%) of fluorescent AGEs formation was calculated using the following formula:

Inhibition rate (%) = {1 – [(fluorescence intensity of sample after incubation for 24 hours – fluorescence intensity of control of sample) / (fluorescence intensity of blank after incubation for 24 hours – fluorescence intensity of control of blank)]} x 100.

The IC_{50} value was calculated from the logarithmic function obtained by plotting the inhibitory rate of fluorescent AGEs formation against the sample concentration.

GA-scavenging assay: The GA-scavenging activity of phlorotannins was measured using a modification of the derivatization method of GA by Usui *et al* [29]. Twenty-five mM GA was prepared using a 200 mM phosphate buffer (pH 7.4). The crude phlorotannins extracted from each brown algae were dissolved in the phosphate buffer to a concentration of 5 mg/mL. Purified phlorotannins, phloroglucinol, and aminoguanidine hydrochloride were dissolved in the phosphate buffer to a concentration of 25 mM each. 1,3-Cyclohexanedione (0.25 g) was dissolved in a mixture consisting of ammonium acetate (10 g), acetic acid (5 mL), and ultrapure water (50 mL), and it was used as a derivatization reagent. Each sample solution (50 μL) and a GA solution (50 μL) were mixed in well of a 96-well microplate (BioLite, Thermo Scientific) and incubated at 37°C for 30, 60, 90, and 120 minutes, respectively. After incubation, the derivatization reagent (100 μL) was added to the mixture and maintained at 60°C for 30 minutes. The amount of GA remaining in the reaction solution was measured at 370 nm using a microplate reader (Infinite 200, Tecan). For the blank test, the phosphate buffer was used instead of a GA solution. Aminoguanidine hydrochloride was used as a positive control. The scavenging rate of GA was calculated using the following formula:

Scavenging rate of GA (%) = {1 – [concentration of GA remaining in the reaction solution (mM) / 25]} x 100.

Cytotoxicity assay: MTT (Dojindo) was used as an indicator of cell viability. Briefly, cell lines (HeLa or 3T3-L1) were cultured in 96-

well microplates (BioLite, Thermo Scientific) at a density of 5 x 10^3 cells per well. After 24 hours cultivation in a CO_2 incubator (CPE-2601, Hirasawa) with 5% CO_2 at 37°C, the cell lines were washed with fresh medium (Dulbecco's modified Eagle's medium with glucose and pyruvate, containing 10% fetal bovine serum and 1% antibiotic-antimycotic, Gibco) and then treated with each sample solution (10 μL) for 24 hours in the incubator. Sample solutions were prepared by dissolving in Dulbecco's phosphate buffered saline (DPBS) without calcium and magnesium (Gibco). For blank and control wells, DPBS was used instead of a sample solution. The cell lines were then rewashed with the medium, treated with 10 μL of MTT solution, and cultured for 4 hours in the incubator at 37°C. MTT solution was prepared by dissolving MTT (25 mg) in the DPBS (5 mL). After removing the medium containing the MTT solution, 200 μL of the DPBS was added to each well and allowed to maintain for 1 minute at 37°C. Finally, in order to dissolve the formed formazan salt, dimethyl sulfoxide (200 μL) was added to each well from which DPBS had been removed. The absorbance of each well was measured at 535 nm using a microplate reader (Infinite 200, Tecan). The cell viability (%) was calculated using the following formula:

Cell viability (%) = [(absorbance of sample well – absorbance of blank well) / (absorbance of control well – absorbance of blank well)] x 100.

Acknowledgments - This work was financially supported by the Japan Science and Technology Agency, CREST.

References

[1] McPherson JD, Shilton BH, Walton DJ. (**1988**) Role of fructose in glycation and cross-linking of proteins. *Biochemistry*, ***27***, 1901-1907.

[2] Singh R, Barden A, Mori T, Beilin L. (**2001**) Advanced glycation end-products: a review. *Diabetologia*, ***44***, 129-146.

[3] Thornalley PJ, Langborg A, Minhas HS. (**1999**) Formation of glyoxal, methylglyoxal and 3-deoxyglucosone in the glycation of proteins by glucose. *Biochemical Journal*, ***344***, 109-116.

[4] Takeuchi M, Makita Z, Bucala R, Suzuki T, Koike T, Kameda Y. (**2000**) Immunological evidence that non-carboxymethyllysine advanced glycation end-products are produced from short chain sugars and dicarbonyl compounds *in vivo*. *Molecular Medicine*, ***6***, 114-125.

[5] Takeuchi M, Makita Z. (**2001**) Alternative routes for the formation of immunochemically distinct advanced glycation end-products *in vivo*. *Current Molecular Medicine*, ***1***, 305-315.

[6] Sato T, Iwaki M, Shimogaito N, Wu X, Yamagishi S, Takeuchi M. (**2006**) TAGE (toxic AGEs) theory in diabetic complications. *Current Molecular Medicine*, ***6***, 351-358.

[7] McLellan AC, Thornalley PJ, Benn J, Sonksen PH. (**1994**) Glyoxalase system in clinical diabetes mellitus and correlation with diabetic complications. *Clinical Science*, ***87***, 21-29.

[8] Odani H, Shinzato T, Matsumoto Y, Usami J, Maeda K. (**1999**) Increase in three α,β-dicarbonyl compound levels in human uremic plasma: specific *in vivo* determination of intermediates in advanced maillard reaction. *Biochemical and Biophysical Research Communications*, ***256***, 89-93.

[9] Takeuchi M. (**2012**) Participation of toxic AGEs (TAGE) in a variety of diseases. *Folia Pharmacologica Japonica*, ***139***, 193-197.

[10] Takeuchi M, Bucala R, Suzuki T, Ohkubo T, Yamazaki M, Koike T, Kameda Y, Makita Z. (**2000**) Neurotoxicity of advanced glycation end-products for cultured cortical neurons. *Journal of Neuropathology and Experimental Neurology*, ***59***, 1094-1095.

[11] Yonekura H, Yamamoto Y, Sakurai S, Petrova RG, Abedin MJ, Li H, Yasui K, Takeuchi M, Makita Z, Takasawa S, Okamoto H, Watanabe T, Yamamoto H. (**2003**) Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. *Biochemical Journal*, ***370***, 1097-1109.

[12] Iwamoto K, Kanno K, Hyogo H, Yamagishi S, Takeuchi M, Tazuma S, Chayama K. (**2008**) Advanced glycation end products enhance the proliferation and activation of hepatic stellate cells. *Journal of Gastroenterology*, ***43***, 298-304.

[13] Takeuchi M, Takino J, Yamagishi S. (**2010**) Involvement of TAGE-RAGE system in the pathogenesis of diabetic retinopathy. *Journal of Opthalmology*, ***2010***, 170393.

[14] Takeuchi M, Takino J, Yamagishi S. (**2010**) Involvement of the toxic AGEs (TAGE)-RAGE system in the pathogenesis of diabetic vascular complications: a novel therapeutic strategy. *Current Drug Targets*, ***11***, 1468-1482.

[15] Choei H, Sasaki N, Takeuchi M, Yoshida T, Ukai W, Yamagishi S, Kikuchi S, Saito T. (**2004**) Glyceraldehyde-derived advanced glycation end products in Alzheimer's disease. *Acta Neuropathologica*, ***108***, 189-193.

[16] Koriyama Y, Furukawa A, Muramatsu M, Takino J, Takeuchi M. (**2015**) Glyceraldehyde caused Alzheimer's disease-like alterations in diagnostic marker levels in SH-SY5Y human neuroblastoma cells. *Scientific Reports*, ***5***, 13313.

[17] Takino J, Kobayashi Y, Takeuchi, M. (**2010**) The formation of intracellular glyceraldehyde-derived advanced glycation end-products and cytotoxicity. *Journal of Gastroenterology*, ***45***, 646-655.

[18] Sakasai-Sakai A, Takata T, Takino J, Takeuchi M. (**2017**) Impact of intracellular glyceraldehyde-derived advanced glycation end-products on human hepatocyte cell death. *Scientific Reports*, ***7***, 14282.

[19] Takata T, Ueda T, Sakasai-Sakai A, Takeuchi M. (**2017**) Generation of glyceraldehyde-derived advanced glycation end-products in pancreatic cancer cells and the potential of tumor promotion. *World Journal of Gastroenterology*, ***23***, 4910-4919.

[20] Sugiura S, Minami Y, Taniguchi R, Tanaka R, Miyake H, Mori T, Ueda M, Shibata T. (**2017**) Evaluation of anti-glycation activities of phlorotannins in human and bovine serum albumin-methylglyoxal models. *Natural Product Communications*, ***12***, 1793-1796.

[21] Ragan MA, Glombitza KW. (**1986**) Phlorotannins, brown algal polyphenols. *Progress in Phycological Research*, ***4***, 1-241.

[22] Brownlee M, Vlassara H, Kooney A, Ulrich P, Cerami A. (**1986**) Aminoguanidine prevents diabetes-induced arterial wall protein cross-linking. *Science*, ***232***, 1629-1632.

[23] Shibata T, Nagayama K, Sugiura S, Makino S, Ueda M, Tamaru Y. (**2015**) Analysis on composition and antioxidative properties of phlorotannins isolated from Japanese *Eisenia* and *Ecklonia* species. *American Journal of Plant Sciences*, ***6***, 2510-2521.

[24] Takeuchi M, Yamagishi S. (**2004**) Alternative routes for the formation of glyceraldehyde-derived AGEs (TAGE) *in vivo*. *Medical Hypotheses*, ***63***, 453-455.

[25] Takeuchi M, Makita Z, Yanagisawa K, Kameda Y, Koike T. (**1999**) Detection of noncarboxymethyllysine and carboxymethyllysine advanced glycation end products (AGE) in serum of diabetic patients. *Molecular Medicine*, ***5***, 393-405.

[26] Takeuchi M, Yanase Y, Matsuura N, Yamagishi S, Kameda Y, Bucala R, Makita Z. (**2001**) Immunological detection of a novel advanced glycation end-product. *Molecular Medicine*, ***7***, 783-791.

[27] Shibata T, Ishimaru K, Kawaguchi S, Yoshikawa H, Hama Y. (**2008**) Antioxidant activities of phlorotannins isolated from Japanese Laminariaceae. *Journal of Applied Phycology*, ***20***, 705-711.

[28] Fujii Y, Tanaka R, Miyake H, Tamaru Y, Ueda M, Shibata T. (**2013**) Evaluation for antioxidative properties of phlorotannins isolated from the brown alga *Eisenia bicyclis*, by the H-ORAC method. *Food and Nutrition Sciences*, ***4***, 8A, 78-82.

[29] Usui T, Yoshino M, Watanabe H, Hayase F. (**2007**) Determination of glyceraldehyde formed in glucose degradation and glycation. *Bioscience, Biotechnology, and Biochemistry*, ***71***, 2162-2168.

Accounts/Reviews

Natural Product Communications
2018
Volume 13, Number 8

Contents

Continued inside backcover