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Original article

Cleaving effect of melatonin on crosslinks in advanced glycation end products

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Abstract

***Objective*:** The cleavage of AGE crosslinks represents a mechanism for the degradation of advanced glycation end products (AGEs) of proteins. This study was conducted to evaluate the effects of melatonin (a sleep-related hormone) on AGE degradation, in comparison with representative plant-containing components.

***Methods*:** Melatonin (0.4 nmol/L) was chosen as the test material, with *N*-phenacylthiazolium bromide (PTB) as a positive control, and 28 plant-containing flavonoids (0.4 nmol/L) and 6 tea plant-containing catechins (10 mg/mL) as reference controls. The evaluation of AGE crosslink cleaving activity was based on the phenomenon in which an equimolar amount of benzoic acid is produced upon degradation of 1-phenyl-1,2-propanegione (PPD). After the reaction of PPD and each sample solution or PTB (0.4 and 10 nmol/L), the benzoic acid released was quantified using high performance liquid chromatography. [All measurements are expressed herein as mean ± standard deviation (n=3).]

***Results*:** AGE crosslink degradation activity was determined to be 15.76 ± 0.38% for melatonin, 6.37 ± 2.71% for PTB (0.4 nmol/L), and 23.00 ± 7.50% for PTB (10 nmol/L); melatonin was found to be more potent than the same amount of PTB. Of the plant-containing flavonoids examined, urolithin B (14.89 ± 0.01%) and carnosic acid (9.01 ± 0.93%) were more active than the same amount of PTB, but less active than melatonin. Of the catechins (10 mg/mL) examined, (-)-epigallocatechin gallate (8.78 ± 0.05%) and (-)-epigallocatechin (8.45 ± 0.64%) were highly active, but less active than melatonin (0.4 nmol/L).

***Conclusion*:** Since melatonin proved to be markedly effective in AGE crosslink degradation, it may be biologically active in reducing AGE accumulation in living organisms.

***KEY WORDS*:** advanced glycation endproducts (AGEs) , melatonin, *N*-phenacylthiazolium bromide (PTB), catechin, AGE breaker,

Introduction

Accelerated degenerative changes in senescence due to the production and accumulation of advanced glycation end products (AGEs) of protein in living organisms have recently been recognized to be problematic in view of the concept of glycation stress [1,2]. The AGE production process is mediated by a wide variety of reactions, including oxidation and crosslinking, and it is thought to be influenced by lifestyle habits such as diet, exercise, sleep, and stress [3-6].

Our laboratory previously quantified skin fluorescent AGEs in healthy humans in a non-invasive manner, and reported that the AGE content increased with aging, and that individual differences widened accordingly [7]. The increased individual differences were shown to be related to lifestyle factors such as smoking, drinking, and lack of sleep [8]. Melatonin, a representative sleep-related hormone, has been investigated from a broad range of perspectives [9-14]; however, only a few studies have been focused on glycative stress.

One study showed that melatonin is weakly active in suppressing AGE production [15]. On the other hand, to facilitate AGE degradation in living organisms, and thus contribute to the prevention of senescence and disease, studies were undertaken to determine substances that promote AGE degradation. Based on these studies, pomegranate [16], water chestnut [17], and rosemary [18] extracts were found to be such substances. The present study was conducted, with a focus on crosslink-cleaving action as a mechanism for AGE degradation [19], to evaluate the AGE crosslink cleaving effects of melatonin.

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Methods

Test materials

The main compound tested was melatonin and the comparison included 28 kinds of flavonoids as follows; apigenin, 7,4'-dihydroxyflavone, liquiritigenin, chrysin, pinocembrin, 5-hydroxyflavone, 7-hydroxyflavanone, naringenin, flavone, 4'-hydroxflavanone, 7-hydroxyflavone, luteolin, scutellarein, quercetagetin, apigenin-7-O-glucoside, daidzein, genistein, acacetin, carnosic acid, kaempferol, corosoric acid, diosmetin, pinitol, maslinic acid, rutin, rubusoside, rosmarinic acid, urolithin B. remove it These compounds were dissolved and with distilled water, 100% ethanol or 100% dimethyl sulfoxide (DMSO) and were adjusted to a concentration of 0.4 mmol/L. Furthermore, 6 kinds of catechins, which are known compounds in tea leaf (*Camelia sinensis*) [20] were used for comparison; (+)-catechin, (-)-epicatechin, (-)-epicatechin gallate, (-)-epigallocatechin, (-)-epigallocatechin gallate, (-)-gallocatehin. Catechins were adjusted to a concentration of 10 mg/mLwith distilled water. As a positive control for AGE-derived crosslink cleaving activity, 10 mmol/L *N*-phenacylthiazolium bromide (PTB) were used.

Evaluation for AGE-derived crosslink cleaving activity

The AGE-derived crosslink cleaving activity was evaluated by the modified method [16] of Vasan *et al.* [19]. Briefly, 1-phenyl-1,2-propane dione (PPD), dissolved in 50% acetonitrile, was used as a reactive substrate in the AGE crosslink model. For the measurement of AGE-derived crosslink cleaving activity, the samples (500 µL) were mixed with 10 mmol/L PPD 100 µL and 0.2 mol/L phosphate buffered saline (PBS) 400 µL, and then incubated at 37°C for 8 hours. The reaction was stopped by adding 2 mol/L hydrochloric acid (HCl) 200 µL, followed by centrifugation at 10,000 rpm (9,170g) for 2 minutes. The benzoic acid amount in the supernatant was measured by high performance liquid chromatography (HPLC).

An HPLC (LC-10A; Shimadzu, Nakagyo-ku, Kyoto, Japan) equipped with Cadenza CD-C18 75 x 4.6 mmID (Imtakt, Shimogyo-ku, Kyoto, Japan) was used. Analytical conditions were as follows: eluate, 0.2% acetic acid/acetonitrile (70/30) containing 2 mmol/L ethylenediamine-N,N,N',N'-tetraacetic acid-disodium salt (EDTA-2Na)-dehydrate; flow rate, 1.0 mL/min; column temperature, 40°C; detection wave length, UV 270 nm; injection volume, 50 µL.

When 1 mol of PPD breaks down, 1 mol of benzoic acid is formed [3]. Calculation of AGE crosslink cleaving activity ratio was conducted after measuring bezoic acid by HPLC as previously reported [16]. The measurement was conducted in triplets (n = 3) and the results were expressed as mean ± standard deviation.

Results

The AGE-derived crosslink cleaving activity is presented in ***Table 1***. Among the 29 kinds of compounds examined, the cleaving activity was the highest in melatonin (15.76 ± 0.38%). The cleaving activity was higher in melatonin, urolithin B and carnosic acid of 0.4 mmol/L than that in a positive control PTB of the same concentration. The activity of (-)-epigallocatechin gallate and (-)-epigallocatechin (10 mg/mL) was higher than that in 0.4 mmol/L PTB.

Discussion

Etiological associations between sleep time and lifestyle-related diseases with severe glycation stress, such as obesity, dyslipidemia, and type 2 diabetes mellitus have recently been attracting attention.

In these days many people have a wide variety of sleep-related problems, including short sleeping hours due to lengthy commutes and long working hours and time-shift work, sleep time reductions, and decreased quality of sleep; the number of people with such problems has recently been increasing year by year [21,22]. Several lines of evidence [23-27] indicate that such sleep problems represent a risk factor for diabetes mellitus and obesity; a recent study even insist upon advocating sound sleep as a third-choice treatment for diabetes mellitus [28].

A U-shaped relationship exists between sleep time and lifestyle-related disease, with a negative correlation found between too long or short of a sleep time and lifestyle-related disease [29]. The percentage complication of diabetes mellitus in people with a sleep time of ≤ 5 hr is approximately 2.5 times higher than in those with a sleep time of 7 to 8 hr [30]. In fact, many diabetic patients have sleep disorders as a diabetic complication [31]. Sleep environment deterioration can further worsen blood glucose control in diabetic patients [31].

In a cohort study of lifestyle-related disease in 8,218 children, known as the Toyama Study, sleep disorders such as a late bedtime and short sleeping hours were shown to be associated with conditions such as overweight and obesity [32,33]. Since sleep disorders represent a causal factor for childhood obesity, and can progress to type 2 diabetes mellitus with increased insulin resistance, diabetes mellitus is often preceded by sleep problems. Our past study revealed sleep time shortening to be a factor for increasing the skin accumulation of fluorescent AGEs [8]; sleep disorders are thought to intensify glycation stress.

Sleep disorders cause alteration of the hormone balance in the body. Abnormalities of the balance among eating-related hormones such as leptin and ghrelin due to sleep time shortening, secondary increased insulin resistance due to intensified obesity, and increased anti-insulin hormones such as cortisol secretion in nighttime are involved in the onset of type 2 diabetes mellitus [30]. Increased secretion of the adrenal medullary hormones adrenalin and noradrenalin is involved in hypertension [34]. Growth hormones secreted during sleep, classified in a class of anti-insulin hormones, have blood glucose elevating effects; however, their secretion can be rather decreased with sleep time shortening, and seem to be little involved in the onset of diabetes mellitus.

Although the pineal hormone melatonin is expected to be largely influenced by sleep disorders, much remains unknown. In most regions in the Northern Hemisphere, diabetic patients have seasonal changes in their blood glucose levels irrespective of the type of diabetes. HbA1c values change seasonally in 12-month cycles, tending to minimize in spring and summer and maximize in winter, with a variation of about 0.2 to 0.6% [35]. Melatonin secretion increases in the

Table 1. AGE-derived crosslink cleaving activity.

Sample	Cleaving activity (%)	Relative ratio [1)]	Relative ratio [2)]
Melatonin	15.76 ± 0.38	69	248
Urolithin B	14.89 ± 0.01	65	234
Carnosic acid	9.01 ± 0.93	39	142
Rosmarinic acid	1.93 ± 0.11	8	30
Quercetagetin	1.29 ± 0.13	6	20
Scutellarein	1.20 ± 0.06	5	19
7-Hydroxyflavone	0.82 ± 0.89	4	13
7,4'-Dihydroxyflavone	0.65 ± 0.55	3	10
Apigenin	0.06 ± 0.42	0	1
Flavone	0.00 ± 0.28	0	0
Apigenin-7-O-glucoside	-0.07 ± 0.07	NE	NE
4'-Hydroxflavanone	-0.18 ± 0.03	NE	NE
Naringenin	-0.23 ± 0.05	NE	NE
Luteolin	-0.24 ± 0.06	NE	NE
5-Hydroxyflavone	-0.28 ± 0.13	NE	NE
7-Hydroxyflavanone	-0.28 ± 0.20	NE	NE
Pinocembrin	-0.29 ± 0.11	NE	NE
Chrysin	-0.38 ± 0.08	NE	NE
Corosoric acid	-0.68 ± 0.56	NE	NE
Rubusoside	-0.70 ± 0.17	NE	NE
Kaempferol	-0.70 ± 0.19	NE	NE
Rutin	-0.93 ± 0.17	NE	NE
Maslinic acid	-0.93 ± 0.07	NE	NE
Liquiritigenin	-1.09 ± 0.96	NE	NE
Diosmetin	-1.31 ± 0.03	NE	NE
Pinitol	-1.32 ± 0.07	NE	NE
Daidzein	-1.34 ± 0.37	NE	NE
Acacetin	-1.46 ± 0.08	NE	NE
Genistein	-2.23 ± 0.34	NE	NE
(-)-epigallocatechin gallate	8.78 ± 0.05	38	138
(-)-epigallocatechin	8.45 ± 0.64	37	133
(-)-gallocatechin	5.77 ± 0.04	25	91
(+)-catechin	5.12 ± 0.59	22	80
(-)-epicatechin	3.57 ± 0.06	15	56
(-)-epicatechin gallate	1.95 ± 0.01	8	31
PTB (10 mmol/L)	23.00 ± 7.50	100	–
PTB (0.4 mmol/L)	6.37 ± 2.71	–	100

1) : Relative ratio of AGE crosslink cleaving activity when the ratio of 10 mmol/L PTB is assumed to be 100.
2) : Relative ratio of AGE crosslink cleaving activity when the ratio of 0.4 mmol/L PTB is assumed to be 100.
Concentrations of components are 0.4 mmol/L except catechins of which concentration are 10 mg/mL. Results are expressed as mean ± standard deviation, n = 3. AGE, advanced glycation end product; NE, not effective; PTB, N-phenacylthiazolium bromide.

long-night season of winter, and may someway respond to increased blood glucose levels in the season.

As stated above, patients with a lack of sleep as a diabetic complication are severely affected by glycative stress [8], and can therefore be at increased risk of disease caused by glycative stress. We hypothesized that melatonin, a sleep-related hormone, lessened glycative stress. Methods of reducing glucose-associated glycative stress include the following: i) glucose consumption reduction and glucose absorption retardation, ii) AGE production inhibition, iii) promotion of AGE degradation, and iv) inhibition of AGEs/RAGE binding/activation [1,2]. In addition, since AGE production is in part related to oxidizing reactions, antioxidants act to reduce AGE production. Melatonin has antioxidant action, and in this regard, it may have some contribution to the suppression of AGE production; however, melatonin did not suppress AGE production in an *in vitro* glucose/human serum albumin reaction model [15]. In the present study, melatonin was found to have AGE degradation promoting effects. The mechanism is based on the cleavage of AGE crosslinks [19]. This may account, in part, for the fact that skin deposition of AGEs is relatively common among persons with lack of sleep [8].

AGEs are degraded mainly in two ways: cleavage of the α-diketone structure in crosslinked AGEs [19] and the action of oxidized protein hydrolase (OPH), an enzyme that preferentially degrades oxidized and glycated proteins [36]. AGE crosslink degradation activity is attracting attention in attempt to determine a therapeutic approach to degrade and eliminate AGEs by cleaving AGE crosslinks, and it is suggested to suppress AGE accumulation in blood vessels, and contribute to the treatment of diabetic vascular complications [37]. PTB, when ingested, can pose safety issues, including adverse reactions [38]. Accordingly, research is ongoing to identify natural substances that can suppress AGE accumulation with little influence on the body. Substances reported to cleave AGE crosslinks include mugwort and rooibos extracts [39], as well as pomegranate [16], water chestnut [17], and rosemary [18] extracts.

The positive control *N*-phenacylthiazolium bromide (PTB) is a compound that cleaves the C-C bonds of the α-diketone structure in AGEs to degrade the AGEs [19]. Here, melatonin had potent AGE crosslink cleaving effects which were equal to or higher than those of PTB at the same concentrations. A comparison with flavonoid polyphenol controls revealed that melatonin possessed higher activity than 28 representative flavonoids contained in vegetables and herbs.

Measurements of the cleaving activity of catechins contained in tea revealed (-)-epigallocatechin gallate [40] to be the most potent single component, suggesting that (-)-epigallocatechin gallate in tea is associated with AGE crosslink cleaving effects. Tea also contains (-)-epigallocatechin and (-)-gallocatechin [41], which were ranked second to (-)-epigallocatechin gallate in cleaving activity. Hence, catechins with a benzenetriol structure are expected to have AGE crosslink cleaving effects. Although it was difficult to accurately compare prepared concentrations, 0.4 mmol/L melatonin was found to be more potent than tea plant-derived catechins (10 mg/mL) in terms of AGE crosslink degradation activity.

Among various fruit extracts, pomegranate extracts possess relatively high activity to suppress AGE production [42]; a pilot study in human subjects reported an improvement of serum HbA1c with pomegranate extracts [43]. A study to evaluate the AGE crosslink cleaving effects of pomegranate components revealed that gallic acid (0.4 nmol/L) was had an extremely high activity of 26.02 ± 0.07%, followed by pinicalagin (0.4 nmol/L) at 15.32 ± 2.57%, eucalbanin B at 13.93 ± 0.31%, and pomegraniin A at 13.51 ± 0.08%, with other components accounting for less than 13% each (0.4 nmol/L concentration each) [16]. Although its activity was weaker than that of gallic acid, melatonin was found to be as active as other components or higher.

Among various extracts from vegetables, root crops, and nuts/berries, water chestnut extracts possess a relatively high activity to suppress AGE production [44]. With regard to AGE crosslink cleaving effects, water chestnut extracts had activities of 7.2% (100μg/mL) and 32.49% (1 mg/mL), which were equal to or higher than the activity of the positive control PTB [17]. When comparing the activity at a dose of 0.4 nmol/L, melatonin is approximately 2.5 times more potent than PTB, and it is comparable to water chestnut extracts in terms of activity potency.

Taken toether, the above results suggest that melatonin is biologically active to prevent AGE accumulation in the body via its more potent AGE crosslink degradation effects compared to those of the positive control PTB and representative plant components. Melatonin secretion decreases with aging and qualitative deteriorations of sleep, including sleep under bright conditions and sleep time shortening. This situation is likely to retard AGE degradation, causing AGE accumulation, and increasing the risk of onset of glycation stress-induced disease.

Conclusion

Melatonin proved to be potently effective in AGE crosslink degradation in vitro, and was suggested to be biologically active in reducing AGE accumulation in living organisms. Decreased melatonin secretion with aging and sleep disorders may intensify glycative stress.

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Conflict of Interest Statement

The authors state that the performance of this study entailed no issues representing a conflict of interest.

References

1) Nagai R, Mori T, Yamamoto Y, et al. Significance of advanced glycation end products in aging-related disease. Anti-Aging Medicine. 2010; 7: 112-119.
2) Ichihashi M, Yagi M, Nomoto K, et al. Glycation stress and photo-aging in skin. Anti-Aging Medicine. 2011; 8: 23-29.
3) Vlassopoulos A, Lean ME, Combet E. Oxidative stress, protein glycation and nutrition--interactions relevant to health and disease throughout the lifecycle. Proc Nutr Soc. 2014; 73:430-438.
4) Prasad C, Imrhan V, Marotta F, et al. Lifestyle and advanced glycation end products (AGEs) burden: Its relevance to healthy aging. Aging Dis. 2014; 5:212-217.
5) Couppé C, Svensson RB, Grosset JF, et al. Life-long endurance running is associated with reduced glycation and mechanical stress in connective tissue. Age (Dordr). 2014; 36(4):9665. doi: 10.1007/s11357-014-9665-9.
6) Turner DP. Advanced glycation end-products: a biological consequence of lifestyle contributing to cancer disparity. Cancer Res. 2015; 75:1925-1929.
7) Nomoto K, Yagi M, Arita S, et al. A survey of fluorescence derived from advanced glycation end products in the skin of Japanese: Differences with age and measurement location. Anti-Aging Medicine. 2012; 9: 119-124.
8) Nomoto K, Yagi M, Arita S, et al. Skin accumulation of advanced glycation end products and lifestyle behaviors in Japanese. Anti-Aging Medicine. 2012; 9: 165-173.
9) Oba S, Nakamura K, Sahashi Y, et al. Consumption of vegetables alters morning urinary 6-sulfatoxymelatonin concentration. J Pineal Res. 2008; 45:17-23.
10) Yonei Y, Hattori A, Tsutsui K, et al. Effects of melatonin: Basics studies and clinical applications. Anti-Aging Medicine. 2010; 7: 85-91.
11) Mihara T, Nakamura N, Ka K, et al. Effects of melatonin premedication to prevent emergence agitation after general anaesthesia in children: A systematic review and meta-analysis with trial sequential analysis. Eur J Anaesthesiol. 2015; 32: 862-871.
12) Kozaki T, Kubokawa A, Taketomi R, et al. Effects of day-time exposure to different light intensities on light-induced melatonin suppression at night. J Physiol Anthropol. 2015; 34(1):27. doi: 10.1186/s40101-015-0067-1.
13) Obayashi K, Saeki K, Iwamoto J, et al. Physiological levels of melatonin relate to cognitive function and depressive symptoms: The Heijo-Kyo Cohort. J Clin Endocrinol Metab. 2015; 100: 3090-3096.
14) Kawada T. Sleep parameters by actigraphy and relationship between plasma melatonin and intestinal permeability in alcoholics. Am J Physiol Gastrointest Liver Physiol. 2015 Aug 15; 309(4):G279. doi: 10.1152/ajpgi.00153.2015.
15) Moniruzzaman M, Takabe W, Yonei Y. Melatonin is not a carbonyl scavenger. Glycative Stress Research. 2016; 3: 00-00 (in press)
16) Yagi M, Mitsuhashi R, Watanabe A, et al. Cleaving effect of pomegranate (*Punica granatum*) extract on crosslink derived from advanced glycation endproducts. Glycative Stress Research. 2015; 2: 58-66.
17) Takeshita S, Yagi M, Uemura T, et al. Peel extract of water chestnut (*Trapa bispinosa* Roxb.) inhibits glycation, degrades α-dicarbonyl compound, and breaks advanced glycation end product crosslinks. Glycative Stress Research. 2015; 2: 72-79.
18) Jean D, Pouligon M, Dalle C. Evaluation in vitro of AGE-crosslinks breaking ability of rosmarinic acid. Glycative Stress Research. 2015; 2: 204-000.
19) Vasan S, Zhang X, Zhang X, et al. An agent cleaving glucose-derived protein crosslinks *in vitro* and *in vivo*. Nature. 1996; 382: 275-278.
20) Otake K, Yagi M, Takabe W, et al. Effect of tea (Camellia sinensis) and herbs on advanced glycation endproduct formation and the influence of post-fermentation. Glycative Stress Research. 2015; 2: 156-162.
21) Okawa M. Sleep disturbance and diabetes mellitus. Diabetes Frontier. 2011; 22: 119-128. (in Japanese)
22) Mishima K. Sleep medicine for the treatment and prevention of life style disease. Journal of Clinical and Experimental Medicine (Igaku No Ayumi). 2011; 236: 5-10. (in Japanese)
23) Kondo F, Nakajima T, Suzuki A, et al. Actigraph: Application for clinical situation Amount of activity increase during sleep in type-2 diabetes mellitus patients. Journal of International Society of Life Information Science. 2006; 24: 435-443.
24) Nakajima H, Kaneita Y, Yokoyama E, et al. Insomnia symptoms associated with hyperglycemia. Sleep and Biological Rhythms. 2010; 8: 203-211.
25) Hsieh SD, Muto T, Murase T, Tsuji H, Arase Y. Association of short sleep duration with obesity, diabetes, fatty liver and behavioral factors in Japanese men. Intern Med. 2011; 50: 2499-2502.
26) Fuse K, Yoshimine F, Kasai A, et al. Sleep disturbances in the diabetic patients. Japanese Journal of Psychosomatic Medicine. 2011; 51: 799-806. (in Japanese)
27) Tanaka M, Kaneko R, Kamata N, et al. The relation of the sleep indicator by Actigraph to fasting blood glucose and HbA1c: A study in the laborer. Occupational Mental Health. 2012; 20: 250-258. (in Japanese)
28) Kawamori R. Sleep and diabetes mellitus. The Journal of Adult Diseases. 2010; 40: 433-440. (in Japanese)
29) Nakagome S, Kaneita Y. Relationship of sleeping time to obesity and lifestyle-related diseases. Sleep and Clinical Practice. 2014; 7: 5-7. (in Japanese)
30) Yamamoto N, Otsuka K, Kim G, et al. Insomnia and diabetes mellitus. Journal of Chronobiology. 2010; 16: 42-47. (in Japanese)
31) Yamada S, Inaba M. Diabetes mellitus and sleep. Sleep and Clinical Practice. 2014; 7: 8-11. (in Japanese)
32) Numata N, Yamagami T, Soukejima S, et al. Physique changes from infancy to childfood: Lifestyle factors causing overweight. Journal of the Japanese Association for Cerebro-Cardiovascular Disease Control. 2000; 35: 35-43. (in Japanese)
33) Sekine M, Kanayama H, Kagamimori S. Formation and maintenance of exercise custom in infants and influence of the social family background: Epidemiological approach on the life course. Research-Aid Report. 2007; 22: 62-69. (in Japanese)

34) Fujii M, Akamine Y, Kuroda K, et al. Effect of melatonin on the pathogenesis of hypertension in the Spontaneous Hypertensive Rats (SHR). Japanese Journal of Applied Physiology. 1993; 23: 525-532. (in Japanese)
35) Mineyama T, Noda M. Diabetes mellitus and seasonal changes. Japanese Journal of Sleep Medicine. 2014; 8: 219-224. (in Japanese)
36) Fujino T, Tada T, Beppu M, et al. Purification and characterization of a serine protease in erythrocyte cytosol that is adherent to oxidized membranes and preferentially degrades proteins modified by oxidation and glycation. J Biochem. 1998; 124: 1077-1085.
37) Cooper ME, Thallas V, Forbes J, et al. The cross-link breaker, *N*-phenacylthiazolium bromide prevents vascular advanced glycation end-product accumulation. Diabetologia. 2000; 43: 660-664.
38) Uniwersytet J, Olszanecki R, Bujak-Giżycka B, et al. The new application of 2-pyrrolidone derivatives. Int. Patent: WO 2011-049475: 2011-04-28.
39) Tada A. An evaluation and the material choice of the natural ingredient for anti-glycation cosmetics. Cosmetic Stage. 2011; 5: 33-38. (in Japanese)
40) Yoshino K, Hara Y. Determination of catechins and theaflavins in infusions by high-performance liquid chromatography. Numazu College of Technology. 1993; 27: 87-91. (in Japanese)
41) Huang S, Inoue K, Li Y, et al. Analysis of catechins in autoclaved tea leaves and drinks. Jpn J Food Chem. 2004; 11: 99-102. (in Japanese)
42) Parengkuan L, Yagi M, Matsushima M, et al. Anti-glycation activity of various fruits. Anti-Aging Medicine. 2013; 10: 70-76.
43) Yagi M, Parengkuan L, Sugimura K, et al. Anti-glycation effect of pomegranate (*Punica granatum* L) extract: An open clinical study. Glycative Stress Research. 2014; 1: 60-67.
44) Ishioka Y, Yagi M, Ogura M, et al. Antiglycation effect of various vegetables: Inhibition of advanced glycation end product formation in glucose and human serum albumin reaction system. Glycative Stress Research 2015; 2: 22-34.