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Antioxidant Activities and Hyaluronidase Inhibitory Activities of Flavonoids

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フラボノイド類の抗酸化活性とヒアルロニダーゼ阻害活性

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Abstract

Eight flavonoids belonging to defferent structural groups (flavone, flavonol and flavanone) and possessing from three to five free hydroxy groups were investigated by measurement of their antioxidant activities and hyaluronidase inhibitory activities. Luteolin showed the strongest those activities among eight tested flavonoids. The 2,3-double bond and the hydroxy substitution at the A and B rings enhanced the activities, while 3-OH, the introduction of one methoxy group and the presence of a glycoside substituent reduced the activities. Hyaluronidase inhibitory activity of eriodictyol has been reported for the first time.

Introduction

Flavonoids are widespread in the plant kingdom and comprise a large group of naturally occurring compounds found in fruits, vegetables, nuts, seeds, grains, tea, and wine (Miyake *et al*., 1998). Flavonoids have been used since ancient times by physicians to treat a great variety of diseases such as cancers, cerebral apoplexy, coronary heart disease, and diabetes (Kim *et al*., 2000). The primary structure of flavonoids consists of two moieties: benzopyran (A and C rings) and phenyl (B ring) groups (Tadera *et al*., 2006). Natural antioxidants contained in dietary plants may play an important role in the prevention of carcinogenesis and in extending the life span of animals (Miyake *et al*., 1998). Superoxide dismutase (SOD), free radical scavenging enzyme, is one of the first line of cellular defence against oxidative injury (Karthick *et al*., 2006). Hyaluronidase inhibitors are expected to be useful for treating allergic diseases like pollenosis and gastrointestinal allergy, since the inhibitors are assumed to suppress the degranulation of mast cells and possess anti-allergic activity (Kuppusamy *et al*., 1990).

In this study, we investigated the antioxidant activities and hyaluronidase inhibitory activities of eight commercially available flavonoids for the prevention of lifestyle related diseases. And we attempted to determine the fundamental structure of flavonoids related to the activity, since comparative studies of flavonoids under the same conditions could be considered an effective

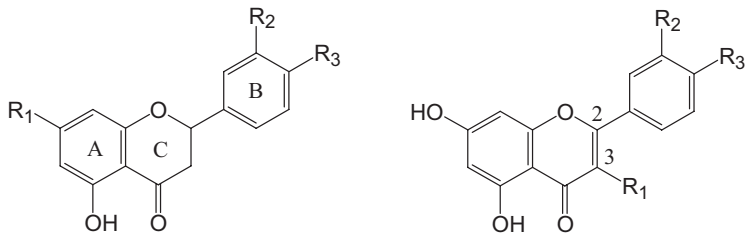
approach to provide important comparable information on nutraceuticals. For the structure-activity relationship, we selected naringenin, eriodictyol, hesperetin and hesperidin (flavanone group and its glycoside), apigenin and luteolin (flavones group), and quercetin and rutin (flavonol group and its glycoside) on the basis of the structure of the C ring, the number and position of free hydroxy group, the aglycone-glycoside pairs.

Material and Methods

Chemicals Apigenin (APG), *p*-Dimethylaminobenzaldehyde, hesperetin (HPT), hesperidin (HPD), luteolin (LTL), maltose, naringin (NRG), quercetin (QCT), rutin (RTN), and superoxide dismutase kit SOD Test Wako were purchased from Wako Pure Chemical Industries, Ltd, Osaka, Japan. Compound 48/80, eriodictyol (EDO), hyaluronic acid sodium salt, and hyaluronidase were purchased from Sigma-Aldrich Co., St. Louis, USA. Eight tested flavonoids were dissolved in 5% dimethylsulfoxide at a concentration of 0.3 mM and used as sample solutions.

Antioxidant assay The antioxidant activity was determined by using a superoxide dismutase kit SOD Test Wako (Yamaguchi *et al*., 2006). Briefly, superoxide radicals were generated by the xanthine/xanthine oxidase system, and reduced nitro blue tetrazolium to water-soluble formazane which exhibited an absorption maximum at 560 nm. Decreased absorption of the reaction mixture indicated increased super oxide scavenging activity. The reaction mixture was incubated at 37°C for 20 min, and the absorbance was measured at 560 nm with a Hitachi U-1800 spectrophotometer. The antioxidant activity was calculated according to the following equation: antioxidant activity (%) = [(Control Abs − Control $_{\text{blank}}$ Abs) − (Sample Abs − Sample $_{\text{blank}}$ Abs)]/ (Control Abs − Control $_{\text{blank}}$ Abs) × 100.

Hyaluronidase inhibitory assay The assay of hyaluronidase inhibitory activity was done according to the method of Murata *et al*. (2010). Each sample solution (0.1 mL) was mixed with hyaluronidase (Type IV-S: From bovine testes, 2140 units/mg solid) in 0.1 M acetate buffer (pH 4.0; final concentration: 0.1 mg/mL, 0.1 mL) and incubated at 37°C for 20 min. Then, compound 48/80 in the same buffer (final concentration: 0.1 mg/mL, 0.1 mL) was added and incubated 37°C for 20 min. After hyaluronic acid sodium salt in the same buffer (final concentration: 0.4 mg/mL, 0.25 mL) had been added, the mixture was incubated at 37°C for 40 min. Then, the reaction was stopped by adding 0.4 M NaOH (0.1 mL) and 0.8 M bolate solution (pH 9.1; 0.1 mL) and then boiling the mixture in a water bath for 5 min. *p*-Dimethylaminobenzaldehyde (100 mg/mL in glacial acetic acid, diluted 1:10 with 10 M HCl, 3 mL) was then added and incubated at 37°C for 20 min. The absorbance was measured at 585 nm with a Hitachi U-1800 spectrophotometer. The percentage inhibition of the hyaluronidase activity was calculated according to the following equation: the inhibitory activity (%) = [(Control Abs − Control $_{\text{blank}}$ Abs) − (Sample Abs − Sample $_{\text{blank}}$ Abs)]/ (Control Abs − Control $_{\text{blank}}$ Abs) × 100.



Name	R_1	R_2	R_3	Name	R_1	R_2	R_3
Naringenin	OH	H	OH	Apigenin	H	H	OH
Eriodictyol	OH	OH	OH	Luteolin	H	OH	OH
Hesperetin	OH	OH	OMe	Quercetin	OH	OH	OH
Hesperidin	ORu	OH	OMe	Rutin	ORu	OH	OH
Ru: rutinose				Ru: rutinose			

Fig. 1. Structures of eight flavonoids.

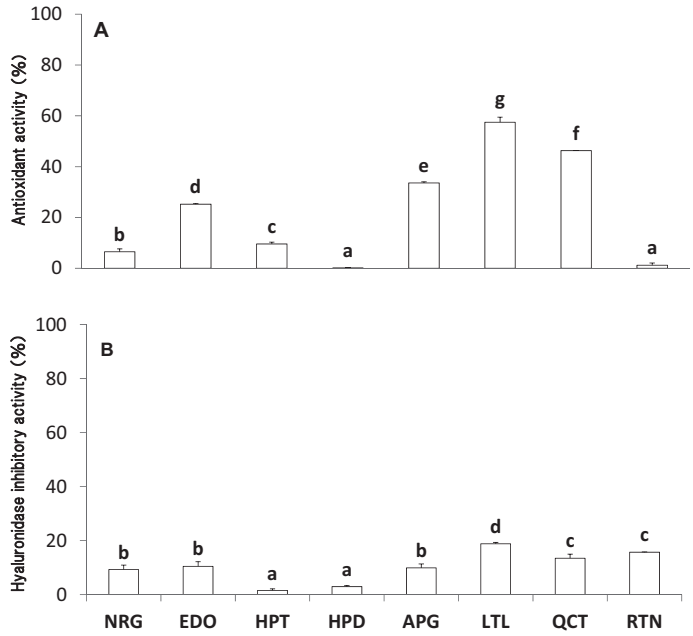


Fig. 2. Effects of eight flavonoids on antioxidant activity (A) and hyaluronidase inhibitory activity (B). Bars indicate standard error of the mean. The significance of the difference was evaluated by Duncan's multiple-range test, $P<0.05$. NRG: naringenin, EDO: eriodictyol, HPT: hesperetin, HPD: hesperidin, APG: apigenin, LTL: luteolin, QCT: quercetin, RTN: rutin.

Results

Eight tested flavonoids in Fig. 1 were examined for their effects on the antioxidant activities and hyaluronidase inhibitory activities. The percentage inhibition of each flavonoid was determined with respect to control assay run simultaneously.

Antioxidant activities of flavonoids Eight flavonoids in Fig. 1 were evaluated for the antioxidant activities (Fig. 2). Luteolin showed the strongest antioxidant activity among eight tested flavonoids. Quercetin, apigenin and eriodictyol also showed the antioxidant activities, but the others had only a weak activity.

Hyaluronidase inhibitory activities of flavonoids Eight flavonoids in Fig. 1 were evaluated for the hyaluronidase inhibitory activities (Fig. 2). Luteolin showed the strongest hyaluronidase inhibitory activity among the tested flavonoids, and quercetin, rutin, eriodictyol apigenin, and naringenin showed weak hyaluronidase inhibitory activities.

Discussion

We have evaluated for the antioxidant activities and the hyaluronidase inhibitory activities of eight flavonoids belonging to defferent structural groups and possessing from three to five free hydroxy groups. This study is the first report on hyaluronidase inhibitory activity of eriodictyol. Luteolin showed the strongest antioxidant activity and hyaluronidase inhibitory activity among eight tested flavonoids.

The results confirmed the importance of the unsaturated C ring and the hydroxy substitution observed by other authors (Ito *et al*., 1984; Huguet *et al.*, 1990; Kuppusamy *et al.*, 1990 & 1991; Tadera *et al*., 2006; Li *et al*., 2009). Flavone and flavonol groups, which possess the 2,3-double bond in the C ring, exhibited higher activity than flavanone group lacking the double bond. These results suggested that flavones and flavonol exhibited a similar behavior with increase in the potency resulting from the resonance between B and C rings but the saturation of the 2,3-double bond (flavanone) might disrupt the planarity and the conjugation of the C ring and therefore contribute to decrease the activity (Kuppusamy *et al*., 1991; Huguet *et al*., 1990). The differences in the activity were accounted for by the number and position of free hydroxy groups, since the activity increased with the number of free hydroxy groups in the molecule but the 3-hydroxy substitution in C ring decreased the activity. The introduction of one methoxy group decreased the activity, as seen for hesperetin, which is monomethoxy derivative of eriodictyol. Blockade of hydroxy group by glycosylation decreased the activity, as seen for the aglycone-glycoside pair of quercetin-rutin. These results suggested that the activity might result through the ability to act as electron donors to the peroxy radical and the conformational change of the enzyme caused by hydrogen bond and hydrophobic interaction between the enzyme and flavonoids (Kuppusamy *et al*., 1990; Kim *et al*., 2005).

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