Note

Inhibition of Alpha-glucosidase and Amylase by Luteolin, a Flavonoid

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Twenty-one naturally occurring flavonoids were tested for inhibitory activities against alpha-glucosidase (EC 3.2.1.20) and alpha-amylase (EC 3.2.1.1). Luteolin, amentoflavone, luteolin 7-O-glucoside, and daidzein were the strongest inhibitors among the compounds tested. Luteolin inhibited alpha-glucosidase by 36% at the concentration of 0.5 mg/ml and was stronger than acarbose, the most widely prescribed drug, in inhibitory potency, suggesting that it has the possibility to effectively suppress postprandial hyperglycemia in patients with non-insulin dependent diabetes mellitus. Luteolin also inhibited alpha-amylase effectively although it was less potent than acarbose. The clinical value of luteolin needs to be further evaluated.

Key words: alpha-glucosidase inhibitor; alpha-amylase; luteolin; flavonoids

It is widely accepted that the most challenging goal in the management of patients with diabetes mellitus is to achieve blood glucose levels as close to normal as possible. In addition, postprandial hyperglycemia (PPHG) or hyperinsulinemia are independent risk factors for the development of macrovascular complications of diabetes mellitus. Alpha-glucosidase is an enzyme that catalyzes the final step in the digestive process of carbohydrates, and hence alpha-glucosidase inhibitors could retard the use of dietary carbohydrates to suppress PPHG. Alpha-glucosidase inhibitors such as acarbose, miglitol, and voglibose are known to reduce PPHG primarily by interfering with the carbohydrate-digesting enzymes and delaying glucose absorption. Alpha-amylases, endoglucanases that catalyze the hydrolysis of internal alpha-1,4-glucosidic linkages in starch and other related polysaccharides, have also been target for suppression of PPHG. Inhibitors of alpha-amylases are expected to be better suppressor of PPHG, since the inhibitor would not result in an abnormal accumulation of maltose which causes side effects such as abdominal pain, flatulence, diarrhea, and soft feces in the colon. Although several drugs targeted for carbohydrate-hydrolyzing enzymes are in clinical use, it is necessary to have a large inhibitor pool as diabetic patients can develop resistance to current regimens. Flavonoids, which are widely distributed in the plant kingdom and present in considerable quantities in common food products, spices, and beverages, have been used since ancient times by physicians and laymen to treat a great variety of human diseases such as diabetes, coronary heart disease, and cancers. In this study we investigated alpha-glucosidase and alpha-amylase inhibitory activities of some naturally occurring flavonoids. Genistein, daidzein, para-nitrophenyl-alpha-d-glucopyranoside, para-nitrophenyl-alpha-d-maltopentoglycoside, porcine pancreatic alpha-amylase, and yeast alpha-glucosidase were purchased from Sigma (St Louis, MO, USA). Acarbose (purity: 96.1%) were kindly provided by Bayer Korea Ltd. (Seoul, Korea).

Astragalin and kaempferol-3-O-[6'-O-(3-hydroxy-3-methylglutaroyl)glucoside] were isolated from the leaves of Polygala japonica and identified according to the previously published method of Do et al. Baicalin was obtained from the roots of Scutellaria baicalensis and its spectral data were consistent with those of an authentic standard. Pectolinarin was isolated from the aerial parts of Cirsium nipponicum. Hesperidin was purified from Citrus unshiu and its structure was identified according to data from the literature. Rutin and isorhamnetin-3-O-rutinoside were isolated from Sophora unshiu and identified with the spectral data of authentic standards. Quercitin and hyperin were isolated from the leaves of Kalopanax pictum, and linarin was obtained from Lycopus lucidus. Luteolin, lonicerin, and rhoifolin were isolated from Lonicera japonica and structurally identified according to the previous procedures of Son et al. Ginkgetin, isoginkgetin, bilobetin, and amentoflavone were purified from the leaves of Ginkgo biloba, and luteolin 7-O-glucoside was isolated from Salix gracilistyla (unpublished data). Twenty-two compounds were dissolved in dimethylsulfoxide at the concentration of 5 mg/ml and used as test solutions. The alpha-glucosidase inhibitory assay was done by

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Abbreviation: PPHG, postprandial hyperglycemia
the chromogenic method described by Watanabe\textsuperscript{2)} using a readily available yeast enzyme. Briefly, yeast alpha-glucosidase (0.7 U, Sigma) was dissolved in 100 mM phosphate buffer (pH 7.0) containing 2 g/liter bovine serum albumin and 0.2 g/liter NaN\textsubscript{3}, and used as an enzyme solution. 5 mM para-Nitrophenyl-alpha-D-glucopyranoside in the same buffer (pH 7.0) was used as a substrate solution. The 50 \( \mu \)l of enzyme solution and 10 \( \mu \)l of test compounds dissolved in dimethylsulfoxide at the 5 mg/ml concentration were mixed in a well of a microtiter plate and measured for titer (Abs 405 nm) at zero time with microplate reader (model 550, Biorad, Hercules, California, USA). After incubation for 5 min, substrate solution (50 \( \mu \)l) was added and incubated for another 5 min at room temperature. The increase in absorbance from zero time was measured. Inhibitory activity was expressed as 100 minus relative absorbance difference (%) of test compounds to absorbance change of the control where test solution was replaced by carrier solvent. alpha-Amylase inhibitory activity was assayed in the same way as described for

alpha-glucosidase inhibitory assay except that porcine pancreatic amylase (100 U, Sigma) and blocked para-nitrophenyl-alpha-D-maltopentaglycoside (Sigma, St Louis, MO, USA) were used as enzyme and substrate, respectively.

In this study twenty-two flavonoids were evaluated for the inhibitory activity of alpha-glucosidase and alpha-amylase as shown in Fig 1 and 2. Luteolin (K), amentoflavone (O), luteolin 7-O-glucoside (R), and daidzein (T) showed strong inhibition against yeast alpha-glucosidase while luteolin among the compounds tested had the strongest inhibitory activity. Yeast alpha-glucosidase inhibitory activity of luteolin was stronger than acarbose with IC\textsubscript{50} of about 5 mg/ml, a drug used clinically, and its IC\textsubscript{50} was 0.5~1 mg/ml. Compounds A, E, F, G, H, and I, which are flavonol glycosides, showed little alpha-glucosidase inhibitory activity. In fact some compounds including B, F, and I caused an increase in alpha-glucosidase activity maybe due to a conformational change derived from binding of compounds to the enzyme. However flavonol glycosides tended to inhibit pig pancreatic alpha-amylase activity. Some flavone glycosides (P, Q, R) inhibited alpha-glucosidase significantly while the other flavone glycosides (B, C, J) showed no enzyme inhibition. Compound D, a flavan glycoside, had little inhibitory action.

against both enzymes. Most biflavones (L, M, N) did not have inhibitory activity for alpha-glucosidase and alpha-amylase except that compound O with free OH groups at C7, C4', and C4'' positions had relatively strong inhibitory activity for alpha-glucosidase but not for alpha-amylase. Genistein and daidzein, isoflavones present abundantly in sojbeans, showed strong inhibitory activities against both enzymes while genistin, a genistein glucoside, showed little inhibitory activity. From the chemical structure and inhibitory activity relationship polyhydroxyl groups in the flavonoids seem play an important role in the inhibition of alpha-glucosidase. Luteolin and foods containing the compound might improve the symptoms caused by hyperglycemia in type II diabetic patients. However there is a possibility that its inhibitory activity against human alpha-glucosidase may be different from that against the yeast enzyme. Luteolin also inhibited porcine pancreatic alpha-amylase activity strongly and its IC50 was in the range of 50 to 500 μg/ml while IC50 of acarbose against alpha-amylase ranged 5 to 50 μg/ml. Kaempferol 3-O-[6'-O-(3-hydroxy-3-methylglutaryl)glucoside] and luteolin 7-O-glucoside also showed strong inhibitory activity against alpha-amylase at the concentration of 5 mg/ml.

Both dietary and pharmacological tools are now available for the management of PPHG. The pharmacological agents with the greatest effect on PPHG include insulin lispro, amylin analogues, and alpha-glucosidase inhibitors. Insulin lispro, which differs from human insulin only in the order of 2 amino acids in the B-chain, mimics the physiologic insulin response to meals with a more stable action than insulin. Amylin, a pancreatic beta-cell hormone co-secreted with insulin in response to various insulin secretagogues is involved in the regulation of gastric emptying, suppression of postprandial glucagon secretion, and replenishment of hepatic glycogen stores. Meanwhile alpha-glucosidase inhibitors are currently the most commonly used oral agents for ameliorating PPHG because of the lack of a hypoglycemic threat, and more importantly the prospect of blood glucose control without hyperinsulinemia and body weight gain. At the present three glucosidase inhibitors, including acarbose, miglitol, and voglibose, are available for the treatment of patients with type II diabetes mellitus. Inhibition of glucosidases and amylases should result in delayed carbohydrate digestion and glucose absorption with attenuation of postprandial hyperglycemic excursions. It has been reported that alpha-glucosidase inhibitors usually do not alter the total amount of carbohydrate absorbed and therefore do not cause any net nutritional caloric loss although they slow down carbohydrate digestion. In addition to flavonoids, N-para-coumaroyl tyramine and kotalanol isolated from plants have been reported to be strong inhibitors of alpha-glucosidase. In vivo efficacy and the clinical usefulness of luteolin remains to be evaluated. Also it might be worthwhile to evaluate its inhibitory activity against other carbohydrate-degrading enzymes such as sucrase and isomaltase.

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