Luteolin, a Flavone, Does Not Suppress Postprandial Glucose Absorption Through an Inhibition of α-Glucosidase Action

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In order to clarify the postprandial glucose suppression via α-glucosidase (AGH) inhibitory action by natural compounds, flavonoids were examined in this study. Among the flavonoids (luteolin, kaempferol, chrysin, and galangin), luteolin showed the potent maltase inhibitory activity with the IC₅₀ of 2.3 mM, while less inhibitions were observed against sucrose. In addition, the effects of maltase inhibition by flavonoids were observed in the descending order of potency of luteolin > kaempferol > chrysin > galangin. Apparently, the AGH inhibition power greatly increased with the replacement of hydroxyl groups at 3' and 4'-position of the B-ring. However, the inhibitory power of luteolin was poorer than a therapeutic drug (acarbose; ICS₅₀: 430 nM). As a result of a single oral administration of maltose or sucrose (2 g/kg) in SD rats, no significant change in blood glucose level with the doses of 100 and 200 mg/kg of luteolin was observed. These findings strongly suggested that luteolin given at less than 200 mg/kg did not possess the ability to suppress the glucose production from carbohydrates through the inhibition of AGH action in the gut.

Key words: α-glucosidase; flavonoids; phenolic acids; noninsulin-dependent diabetes mellitus; luteolin

To assess the prophylaxis of noninsulin-dependent diabetes mellitus (NIDDM) disease by dietary food intake, many natural resources have been examined with respect to the exertion of an α-glucosidase (AGH, EC 3.2.1.20) or α-amylase inhibitory activity. The retardation of membrane-bound AGH reaction and/or inhibition of passive gastric transport would successfully flatten the postprandial blood glucose excursions or reduce hyperglycemia. In our studies on AGH inhibition by food components, acylated anthocyanins were found to cause the benefit of suppression of glucose production from dietary carbohydrates. To date, many studies on the antioxidant, antimitagenic, and antihyperglycemic effects of flavonoids have been done. In addition, their alternative physiological function of suppression of glucose absorption at the small intestine has been also reported. Among the flavonoids, tea polyphenols such as catechins have been found to inhibit AGH activity and glucose transport. These findings led us to make a further investigation of flavonoids commonly present in plant and food products for any anti-hyperglycemic effect. In this paper, we have examined the in vitro and in vivo AGH inhibition abilities of naturally occurring flavonoids, i.e., luteolin and chrysin as flavones, kaempherol and galangin as flavonols.

α-Glucosidase (AGH, EC 3.2.1.20, 2.2 U/mg) from rat intestinal acetone powder was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All of the flavonoids used in this study were purchased from Wako Pure Chemical Institute, Co. (Osaka, Japan). The AGH inhibitory assay was done according to our proposed immobilized AGH (iAGH) assay system. The immobilization of AGH partially purified from rat acetone powder on CNBr-activated Sepharose 4B (Pharmacia Biotech AB, Upsala, Sweden) were described in detail in our previous paper. In the iAGH assay, the iAGH support (10 mg wet gel, 4.1 mg/mg wet gel) was taken in an end-capped ASSIST Mini-column with 45-90 μm of polystyrene filter (CC-07, 5 ml, ASSIST, Tokyo, Japan), and the assay was started after adding 100 μl of inhibitor solution and 900 μl of the model intestinal fluid containing maltose (10 mM) or sucrose (45 mM) to it. After incubation with a rotating cultivator (4 rpm, RT-5, TAITEC, Saitama, Japan) at 37°C for 30 min (maltase assay) or 60 min (sucrase assay), the reaction was stopped by filtration of the solution in the column. Maltase activity was measured by the liberated glucose from maltose in the filtrate by Glucose-Test Wako (Wako Pure Chemical Institute, Co., Osaka, Japan). When sucrose was
used as a substrate, F-kit Glucose (Roche Diagnostics, Co., Tokyo, Japan) was used for measuring sucrase activity, since sucrose itself interfered with the glucose measurement by the Glucose-Test Wako. The flavonoids assayed in this system were dissolved in dimethylsulfoxide (DMSO). One unit of maltase or sucrase activity was defined as the amount of enzyme that hydrolyzed $1 \mu$mol of substrate per min under the above assay conditions. The concentration of AGH inhibitor required for inhibiting 50% of the AGH activity under these assay conditions was defined as the IC$_{50}$ value. The animal experiments in SD rat were done as follows. Male 6-week-old Sprague-Dawley rats (SPF/VAF Crj:SD, Charles River Japan, Kanagawa) were fed a laboratory diet (CE-2, Clea Japan, Tokyo) and given water ad libitum. All rats were housed for 1 week at 21 ± 1°C and 55 ± 5% humidity under controlled lighting from 8:30 to 20:30. Before the experiment, food was withheld for 16 h. A single oral administration of a flavonoid sample via a stomach sonde was done in SD rats ($n = 4$, 238.7 ± 4.3 g) with either a dosage of 100 or 200 mg/kg sample. The sample dissolved in 1 ml of DMSO was orally administered. After 5 min, 2 g/kg of substrate (maltose or sucrose) dissolved in 1 ml of deionized water was administered to each rat. Control rats were administered with the same volume of substrate solution without flavonoid. At each sample time to 120 min, about 20 μl of blood sample was collected from the tail vein, then immediately the blood glucose level (BGL) was measured by a disposable glucose sensor (Glutest Pro, Sanwa Chemical Research, Co., Tokyo, Japan). Each result for the administration study is expressed as the mean of BGL (mg/dl) ± SEM (%). Statistical differences of BGL in control (without flavonoid) and flavonoid groups at each administration time were evaluated by the unpaired Student’s $t$-test. $P$ values < 0.05 were considered to be significant. The care and treatment of the experimental rats conformed to Kyushu University guidelines for the ethical treatment of laboratory animals.

Figure 1 shows the iAGH inhibition behavior of four flavonoids, i.e., luteolin, kaempferol, chrysin, and galangin at the final concentration of 0.1 mg/ml-DMSO. For maltase inhibition, the effects were observed in the descending order of potency of luteolin > kaempferol > chrysin > galangin. Apparently, this suggested that the iAGH inhibition power was greatly affected by the replacement of hydroxyl groups at 3'- and 4'-position of the B-ring, not by the difference in the aglycone structure of flavone and flavonol. Sucrase inhibitions showed the same descending order as maltase inhibition, but their power was lower than maltase inhibition. Thus, the flavonoids used in this study inhibited maltase in preference to sucrase. Among them, luteolin was the strongest maltase inhibitor with the inhibitory ratio of 23.2%: the IC$_{50}$ value for luteolin, 2.3 mM; kaempferol, 17.3 mM. However, the iAGH (maltase) inhibition power of four flavonoids was much less than those of acarbose (IC$_{50}$; 430 nM) and voglibose (IC$_{50}$; 5.5 nM) as a therapeutic AGH inhibitor.³

On the basis of the result that luteolin had the strongest iAGH (maltase) inhibitory activity among the four flavonoids (Fig. 1), changes in the BGL after the administration of luteolin with maltose were examined in SD rats. Acarbose with the dose of 3 mg/kg was used in this study as a positive control. As seen in Fig. 2, no dose-dependent and no significant change in the BGL with the doses of 100 and 200 mg/kg of luteolin was observed against control SD rats administered maltose during the experimental period of 120 min. The BGL of 200 mg-dose of luteolin at 0 h seems to be lower than other groups, but there was no significant difference among the groups. On the other hand, acarbose showed a marked BGL reduction of 52.3 mg/dl 30 min after administration ($P < 0.01$ vs. control). Thus, to elicit the postprandial BGL reduction by luteolin, a dosage of more than 200 mg/kg ($> 0.17$ mol/rat body) would be needed. This strongly supported the finding that luteolin was a poor iAGH inhibitor with the IC$_{50}$ of 2.3 mM against maltase (Fig. 1). Though data are not shown, the in vivo experiment of sucrose administration in SD rats also showed no effect (BGL$_{30\text{min, control}}$ 152.7 ± 1.5 mg/dl, BGL$_{50\text{min, luteolin}}$ 154.0 ± 2.0).

It has already been proved that the catechins typical in tea polyphenols elicited potent sucrase inhibitory activity, in particular esterified catechins such as epigallocatechin gallate.³ Matsumoto et al. demonstrated the favorable BGL reduction at >10 mg dose of catechin/rat, following a significant suppression
of insulin secretion after administering 4 g of sucrose/rat in Wistar rats. Catechins were also involved in an alternative function with respect to the inhibition of transport activity of glucose transporter at the mucosal brush border membrane. Thus, both functions of catechins would be presumable for preventing the hyperglycemia effect. In the case of flavone and flavonol, however, no potent anti-hyperglycemia effects through the inhibition of AGH were observed in the iAGH inhibitory assay (Fig. 1) and in vivo SD rat study (Fig. 2). Even an excessive dose (200 mg/kg) of luteolin that showed a weak iAGH (maltase) inhibitory activity (IC_{50}; 2.3 mM) did not affect the in vivo BGL. These findings strongly demonstrated that the flavonoids used in this study had poor ability to delay or inhibit the production of glucose from carbohydrates in the gut.

Contrary to our negative results against AGH inhibition by flavonoids, Kim et al. reported the potent in vitro effectiveness of luteolin on the inhibition of AGH and α-amylase rather than acarbose as a therapeutic drug. However, we thought that they obtained erroneous results, because AGH inhibitory action varied with its origins and the extent to which AGH was inhibited was largely influenced by its membrane-bound state or not, as we have already reported in our previous papers. As a result of iAGH assay of luteolin, the magnitude of maltase inhibitory activity of it was estimated to be about 1/5400 lower than that of acarbose (Fig. 1). Although their experimental results might be correct within the conventional baker’s yeast AGH inhibitory assay system, the fact that the BGL in SD rat administered luteolin was not suppressed (Fig. 2) supported the validity of the in vitro results from our proposed iAGH assay system (Fig. 1).

In conclusion, it was found that luteolin did not possess an in vivo suppression effect on glucose production from carbohydrates through AGH inhibition in the gut.

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