



Note

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 IC_{50} of 2.3 mM, while less inhibitions were observed against sucrase. 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; IC_{50} ; 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.^{1,2)} The retardation of membrane-bound AGH reaction³⁾ and/or inhibition of passive glucose transport⁴⁾ would successfully flatten the postprandial blood glucose excursions or reduce hyperglycemia. In our studies on AGH inhibition by food components,^{5,6)} acylated anthocyanins were found to cause the benefit of suppression of glucose production from dietary carbohydrates. To date, many studies

on the antioxidant,⁷⁾ antimutagenic,⁸⁾ and antihypertensive effects⁹⁾ of flavonoids have been done. In addition, their alternative physiological function of suppression of glucose absorption at the small intestine has been also reported.^{1,10)} Among the flavonoids, tea polyphenols such as catechins have been found to inhibit AGH activity²⁾ and glucose transport.⁴⁾ 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, kaempferol 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 (*i*AGH) assay system.¹¹⁾ 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.¹¹⁾ In the *i*AGH assay, the *i*AGH support (10 mg wet gel, 4.1 mU/mg wet gel) was taken in an end-capped ASSIST Mini-column with 45–90 μ m of polyethylene filter (CC-07, 5 ml, ASSIST, Tokyo, Japan), and the assay was started after adding 100 μ l of inhibitor solution and 900 μ l 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

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Abbreviations: AGH, α -glucosidase; noninsulin-dependent diabetes mellitus, NIDDM; BGL, blood glucose level

used as a substrate, F-kit Glucose (Roche Diagnostics, Co., Tokyo, Japan) was used for measuring sucrose 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 sucrose activity was defined as the amount of enzyme that hydrolyzed 1 μ 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 $21 \pm 1^\circ C$ and $55 \pm 5\%$ 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 μ l 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) \pm 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 *i*AGH 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 *i*AGH 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. Sucrose 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 sucrose. Among them, luteolin was the strongest maltase inhibitor with the inhibitory ratio

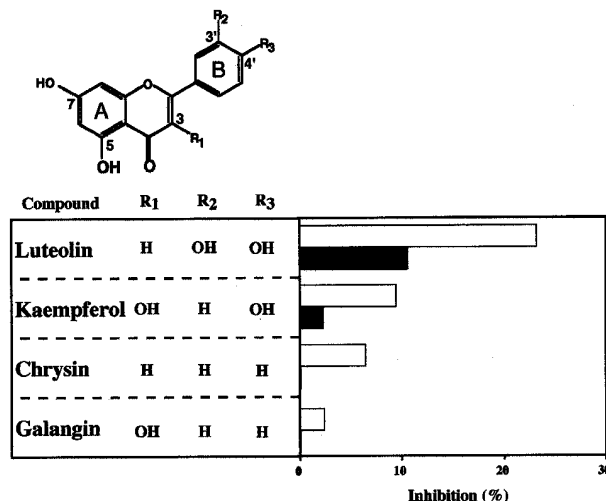


Fig. 1. AGH Inhibitory Activity of Flavonoids Estimated by the Immobilized AGH Assay System.

One mg of flavonoid per 1 ml of DMSO solution was put through the immobilized AGH assay. Maltase (open bars) and sucrose (closed bars) inhibitory activities were evaluated by using maltose (10 mM) and sucrose (45 mM) as substrates at $37^\circ C$.

of 23.2%: the IC_{50} value for luteolin, 2.3 mM; kaempferol, 17.3 mM. However, the *i*AGH (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.¹¹⁾

On the basis of the result that luteolin had the strongest *i*AGH (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 *i*AGH 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_{30min, control}; 152.7 ± 1.5 mg/dl, BGL_{30min, luteolin}; 154.0 ± 2.0).

It has already been proved that the catechins typical in tea polyphenols elicited potent sucrose inhibitory activity, in particular esterified catechins such as epigallocatechin gallate.²⁾ Matsumoto *et al.*¹²⁾ demonstrated the favorable BGL reduction at > 10 mg dose of catechin/rat, following a significant suppression

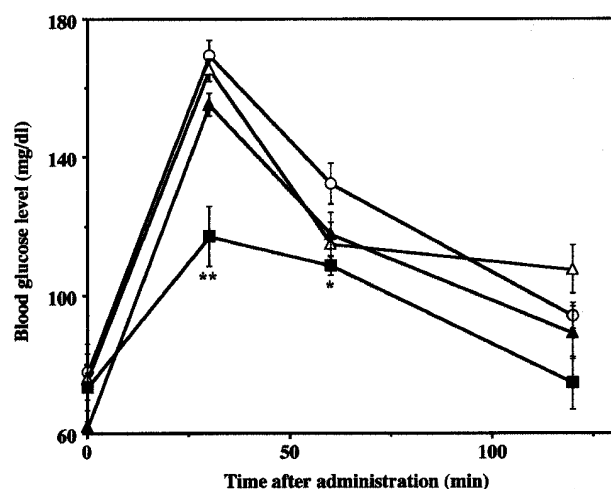


Fig. 2. Effects of Luteolin on Blood Glucose Levels after Single Oral Administration of 2 g/kg Maltose in SD Rats.

One ml of 100 (Δ) and 200 mg/kg (\blacktriangle) luteolin was given to male 7-week-old SD rats. Acarbose (\blacksquare) with the dose of 3 mg/kg was used as a positive control. After 5 min, 1 ml of 2 g/kg of maltose solution was administered to each rat. Control rats (\circ) were administered the same volume of substrate solution without flavonoid. At each time to 120 min, about 20 μ l of blood samples were collected from the tail vein, the blood glucose level was immediately measured by a disposable glucose sensor. Data are the mean (mg/dl) \pm SEM. The significant difference versus control was examined with an unpaired Student's *t*-test ($n=4$, * $P<0.05$, ** $P<0.01$).

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.⁴⁾ 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 *i*AGH 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 *i*AGH (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.*¹³⁾ 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 origins¹⁴⁾ and the extent to which AGH was inhibited was largely influenced by its membrane-bound state or not,¹¹⁾ as we have already reported in our previous papers. As a result of *i*AGH 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 *i*AGH 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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