Note

Luteolin, a Flavone, Does Not Suppress Postprandial Glucose Absorption Through an Inhibition of \( \alpha \)-Glucosidase Action

Toshiro MATSUI,† Mio KOBAYASHI, Sachiko HAYASHIDA, and Kiyoshi MATSUMOTO

Department of Bioscience and Biotechnology, Division of Bioresource and Bioenvironmental Sciences, Faculty of Agriculture, Graduate School, Kyushu University, 6-10-1 Hakozaaki, Higashi-ku, Fukuoka 812-8581, Japan

Received September 25, 2001; Accepted October 26, 2001

In order to clarify the postprandial glucose suppression via \( \alpha \)-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: \( \alpha \)-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 \( \alpha \)-glucosidase (AGH, EC 3.2.1.20) or \( \alpha \)-amylase inhibitory activity.\(^1,2\) The retardation of membrane-bound AGH reaction\(^3\) and/or inhibition of passive glucose transport\(^4\) 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,\(^7\) antimitagenic,\(^8\) and antihypertensive effects\(^9\) of flavonoids have been done. In addition, their alternative physiological function of suppression of glucose absorption at the small intestine has been also reported.\(^10\) Among the flavonoids, tea polyphenols such as catechins have been found to inhibit AGH activity\(^2\) 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, kaempherol and galangin as flavonols.

\( \alpha \)-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.\(^11\) 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 iAGH assay, the iAGH support (10 mg wet gel, 4.1 mU/mg wet gel) was taken in an end-capped ASSIST Mini-column with 45–90 \( \mu \)m of polyethylene filter (CC-07, 5 ml, ASSIST, Tokyo, Japan), and the assay was started after adding 100 \( \mu \)l of inhibitor solution and 900 \( \mu \)l of the model intestinal fluid containing maltose (10 mM) or sucrose (45 mM) to it. After incubation with a rotating culti

\(^{†}\) To whom correspondence should be addressed. Tel: 81-92-642-3012; Fax: 81-92-642-3012; E-mail: tmatsui@agr.kyushu-u.ac.jp

Abbreviations: AGH, \( \alpha \)-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 sucrase activity, since sucrase 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 μmol 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 IC50 value. The animal experiments in SD rats 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 sucrase) 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 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 AGH inhibitor with the IC50 of 2.3 mM against maltase (Fig. 1). Though data are not shown, the in vivo experiment of sucrase administration in SD rats also showed no effect (BGL10min,control; 152.7 ± 1.5 mg/dl, BGL30min,luteolin; 154.0 ± 2.0).

It has already been proven that the catechins typical in tea polyphenols elicited potent sucrase inhibitory activity, in particular esterified catechins such as epigallocatechin gallate. 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 of catechins would be presumable for which AGH was inhibited was largely influenced by flavonoid. At each time to 120 min, about 20 μl 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)±SEM. The significant difference versus control was examined with an unpaired Student’s t-test (n=4, *P<0.05, **P<0.01).

Acknowledgment

Part of this work was supported by a Grand-in Aid for Scientific Research on Priority Areas from the Ministry of Education, Science, and Culture of Japan (No. 11794007).

References

10) Suzuki, Y., Hayashi, K., Sakane, I., and Kakuda, T.


