Lowering Effect of Phenolic Glycosides on the Rise in Postprandial Glucose in Mice

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Glycosides were screened for their lowering effect on the postprandial blood glucose rise in vivo. The effect of phlorizin and other phenolic glycosides on the postprandial blood glucose response to glucose ingestion was evaluated in Std ddY mice. When phlorizin was simultaneously added, the peak blood glucose level was significantly decreased by 51% (p < 0.01) compared to vehicles following glucose ingestion by mice, while the blood insulin responses were generally similar. Screening experiments were conducted with different classes of phenolic glycosides added to a glucose solution. Reductions of 40–52% (p < 0.05) were observed in vehicles containing arbutin, 4-hydroxyphenyl-α-d-glucopyranoside (hydroquinone-α-glucoside) or glycyrrhizin, and of only 15–31% (not significant) in vehicles containing neohesperidin dihydrochalcone, glycyrrhetinic acid monogluconoridine, or 3,4-dimethoxyphenyl-β-d-glucopyranoside. No lowering effect was observed in vehicles containing salicin. Since glycyrrhizin, arbutin, and hydroquinone-α-glucoside blunted to varying degrees the postprandial blood glucose rise following glucose ingestion, they may be useful adjuvants for the treatment of diabetic subjects.

Key words: phlorizin; glycosides; mice; hypoglycemic effect; postprandial blood glucose

The prevention of hyperglycemia and hyperinsulinemia by retarding glucose absorption from the small intestine is one successful approach to improving insulin resistance in subjects with diabetes mellitus. It has been demonstrated that the intestinal α-glucosidase inhibitor, acarbose, which delays the time-course effect of sugar and starch digestion in male Streptozotocin-induced diabetic rats1 and male Zucker rats,2 prevented hyperglycemia and enhanced the expression of glucose transporter type 4, by which the majority of the glucose transport response in insulin-sensitive tissues is mediated, in the muscle.3,4 Gymnema sylvestre leaves have been demonstrated to selectively suppress the sweet taste sensation in humans, and the active principle, named gymnemic acid, was determined to be a glucuronide of a triterpene and was found to inhibit Na+−dependent active glucose transport in the small intestine.3,4 On the other hand, the non-sugar fraction of crude black sugar caused reductions in the blood triglyceride, lipid peroxide, and insulin levels of rats fed on a high-sugar diet.5 Materials that are able to manipulate glucose absorption from the small intestine are widely found in plants. The phenolics are widespread in nature and are primarily found in foods of plant origin9 such as fruits, vegetables and legumes, and in coffee, tea and wine.6,7 High levels of tannins have been found in cereal grains such as sorghum and sunflower seeds which contain considerable amounts of chlorogenic acid.8 However, satisfactory information is not available because of a lack of systematic screening in view of the interaction with carbohydrate absorption in vivo.

Phenolic glycosides, which have been reported to exist in a wide variety of plants and related artificial materials and are synthesized by glycosidases, have been reported to have an affinity for the intestinal glucose transport system9 and shown to slow the rate of glucose absorption from the gut. Each glycoside has its own affinity against intestinal glucose transport, so that glycosides which have an appropriate inhibitory action on glucose absorption would be effective for preventing postprandial hyperglycemia.

In this study, we prepared convenient methods for screening glycosides which delayed the time-course effect of sugar absorption in mice. Some of the glycosides investigated reduced the rise in postprandial blood glucose and consequently halted the effect in the pancreas by triggering insulin secretion when ingested with a glucose solution.

Materials and Methods

Animals. Male 6-week-old Std ddY mice, which are frequently used in pharmacological and toxicological experiments in Japan10 and were obtained from Japan SLC (Hamamatsu, Japan), were housed in standard cages (33 × 23 × 12 cm, six mice per cage). All the mice were kept in an air conditioned room at 22 ± 2°C with a lighting schedule of 12 h light (0600–1800) and 12 h darkness until the experiments were started. The animals were fed on a standard pelleted diet (MF; Oriental Co., Tokyo, Japan) containing (g/100 g diet) water, 8; protein, 24.6; fat, 5.6; fiber, 3.1; and carbohydrates, 52.3. Food and water were available freely. After 3 days of familiarization with the new environment, food was withheld for 17 h (2000–1300), but with free access to water allowed, before the experiments. The care and treatment of the experimental animals conformed with Kyoto University guidelines for the ethical treatment of laboratory animals.

Animal experiments. Groups of 4 to 9 mice were used per experiment. Following fasting for 17 h (2000–1300), the mice were orally administered, via a stomach sonde, a glucose solution with or without phlorizin or various glycosides. The mice were killed by decapitation 0 to 30 min after the administration of the glucose solution. Blood samples were collected from the severed neck veins, and serum was obtained by centrifugation.

Abbreviations: Nhp-DIC, neohesperidin dihydrochalcone; HQ, hydroquinone; HQ-αG, 4-hydroxyphenyl-α-d-glucopyranoside (hydroquinone-α-glucoside); Grt-1G glycyrrhetinic acid monoglucuronide; 3,4-DMPG, 3,4-dimethoxyphenyl-β-d-glucopyranoside.
and maintained at −20°C until assayed. After the stomach had been extirpated, the gastric juice was weighed to investigate gastric emptying.

**Analytical techniques.** Blood glucose concentration was determined by the glucose oxidase method with a commercial kit (Wako Pure Chemical Ind., Osaka, Japan). Blood insulin was measured by a double-antibody radio-immunoassay (SRL, Tokyo, Japan). Blood glucose and insulin were calculated as the change after correction for the baseline (zero time) following the glucose administration.

**Glycosides.** Phlorizin, arbutin, neohesperidin dihydrochalcone (NHDHC), salicin, and glycyrhrizine were purchased from Sigma (St. Louis, U.S.A.). Glycyrrhetinic acid monoglucuronide (Grt-1G) was donated by Maruzen Seiyaku (Tokyo, Japan). Hydroquinone-a-glucoside (HQ-aG) was enzymatically synthesized from hydroquinone (HQ) by using the HQ glucosylating enzyme, and 3,4-dimethoxyphenyl-β-D-glucopyranoside (3,4-dimethoxyphenol-β-glucoside; 3,4-DMP) was synthesized from 3,4-dimethoxyphenol (3,4-DMP, Sigma-Aldrich Japan, Tokyo) by using a plant tissue culture in our laboratory.

**Statistical analysis.** Each result is expressed as the mean ± SEM. Comparisons between the means of 2 groups were performed by an unpaired Student’s t test, and statistical calculations were done with INSTAT software (Macintosh Version 2.00, GraphPad Software, San Diego, CA). A level of p < 0.05 is used as the criterion for statistical significance.

**Results**

**Effects of phlorizin on postprandial glycemic responses**

To confirm a screening method for substances that would reduce the rise in postprandial blood glucose level, we used phlorizin as a positive control. In this experiment, the mean glycemic response in mice is expressed as the change in blood glucose level. Figure 1 indicates that the glycemic response showed a dose-dependent increase 5 or 10 min after the administration of a 2–10% glucose solution. The blood glucose response curve to a 2% glucose load was lower than that from any other glucose treatment and produced no peak. On the other hand, the peak glycemic response was reached 10 min after a 5 or 10% glucose load in mice. It is well known that a high-osmotic-pressure solution is transported to the small intestine more slowly than a low-pressure one. Therefore, we decided to use a 5% glucose solution to take account of the physiological conditions in this experiment.

The effect of phlorizin on the glycemic response in mice following glucose ingestion is shown in Fig. 2A. The incremental blood glucose curve following ingestion of the glucose solution with phlorizin was lower at all points than that of the vehicle (without phlorizin), and the difference was statistically significant (p < 0.05) at 10 min, indicating that phlorizin suppressed the postprandial blood glucose rise. Figure 2B shows that the mean blood glucose concentration following glucose ingestion with phlorizin decreased significantly and dose-dependently. The phlorizin doses used were 2 to 20 mM, with 5% glucose in a 5% EtOH solution being given as a control. The blood glucose level in mice given more than 10 mM of phlorizin was significantly lower than that in the controls. In those animals given

![Fig. 1. Glycemic Response (mean ± SEM; n = 9) Expressed as Blood Glucose above the Fasting Level Following the Ingestion of a 2%, 5%, or 10% Glucose Solution. Mice were administered with 16–80 mg of glucose/100 g of body weight, and blood samples were collected 0, 5, 10, and 20 min after glucose loading.](image1)

![Fig. 2. (A) Glycemic Response (mean ± SEM; n = 7–9) Following 5% Glucose Ingestion (40 mg of glucose/100 g of body wt) with or without 15 mM Phlorizin (5.7 mg of phlorizin/100 g of body wt) in Mice. (B) Effect of Phlorizin Dose (0.8–7.6 mg of phlorizin/100 g of body wt) on the Glycemic Response (mean ± SEM; n = 9) Following 5% Glucose Ingestion (40 mg of glucose/100 g of body wt) in Mice. *Significantly different from the vehicle (p < 0.05).](image2)
Table I. Inhibitory Effects of Glycosides on the Postprandial Blood Glucose Response in Mice Given a 5% or 10% Glucose Solution

<table>
<thead>
<tr>
<th>Addition (mm)</th>
<th>Vehicle glucose concentration 5%</th>
<th>Vehicle glucose concentration 10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phlorizin (15)</td>
<td>50.7±6.8**</td>
<td>63.2±6.9**</td>
</tr>
<tr>
<td>Nhp-DHC (15)</td>
<td>25.6±9.3</td>
<td>0±8.7</td>
</tr>
<tr>
<td>Arbutin (30)</td>
<td>51.5±12.0**</td>
<td>7.6±7.7</td>
</tr>
<tr>
<td>HQ-aG (30)</td>
<td>40.0±9.0*</td>
<td>0±14.9</td>
</tr>
<tr>
<td>Glycyrrhizin (30)</td>
<td>44.3±13.7*</td>
<td>43.9±29.9*</td>
</tr>
<tr>
<td>Grt-IG (15)</td>
<td>27.3±16.0</td>
<td>31.3±14.7</td>
</tr>
<tr>
<td>3,4-DMPG (15)</td>
<td>15.4±12.4</td>
<td>23.2±12.9</td>
</tr>
<tr>
<td>Salicin (15)</td>
<td>8.6±13.7</td>
<td>0±23.4</td>
</tr>
</tbody>
</table>

* Each value represents the mean±SEM for 5-10 mice.
* Blood glucose change rate (BGCR) was calculated by dividing the incremental blood glucose (IBG) of the glycoside-added group by the incremental glucose of the vehicle group at 10 min. The percentage inhibition is presented as follows:

\[
\text{BGCR} = \frac{\text{IBG (glycoside)} - \text{IBG (vehicle)}}{\text{IBG (vehicle)}} \times 100
\]

* Significant difference from vehicles (p<0.05).
** Significant difference from vehicles (p<0.01).

Table II. Influence of Glycoside Dose on the Postprandial Blood Glucose Response in Mice Given a 5% Glucose Solution

<table>
<thead>
<tr>
<th>Addition</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 mm</td>
</tr>
<tr>
<td>Phlorizin</td>
<td>50.7±6.8**</td>
</tr>
<tr>
<td>Nhp-DHC</td>
<td>25.6±9.3</td>
</tr>
<tr>
<td>Arbutin</td>
<td>18.1±6.9</td>
</tr>
<tr>
<td>HQ-aG</td>
<td>NT*</td>
</tr>
<tr>
<td>Glycyrrhizin</td>
<td>34.8±10.6*</td>
</tr>
<tr>
<td>Grt-IG</td>
<td>38.4±18.2</td>
</tr>
<tr>
<td>3,4-DMPG</td>
<td>15.4±12.4</td>
</tr>
<tr>
<td>Salicin</td>
<td>8.6±13.7</td>
</tr>
</tbody>
</table>

* Each value represents the mean±SEM for 5-10 mice.
* Indicated in Table I.
* Significant difference from vehicles (p<0.05).
** Significant difference from vehicles (p<0.01).
* NT, not tested.

less than 10 mm of phlorizin in the glucose solution, no statistically significant decrease was observed.

Using these results (Figs. 1 and 2), we established a screening method for substances that could reduce the rise in postprandial blood glucose level with the following parameters: concentration of the glucose solution given to mice, 5%; glycoside concentration added to the glucose solution, more than 15 mm; blood sampling time, 10 min after glucose administration.

Screening tests

By using the screening method just described, we investigated the inhibitory effect of various glycosides on the postprandial blood glucose level following glucose loading. Tables I and II show the effects of glycosides on the postprandial blood glucose response, while the percentage inhibition of glycemic response to various glycosides is summarized in Table I. Phlorizin, glycyrrhizin, and Grt-IG similarly inhibited the peak blood glucose increment, and 3,4-DMPG slightly inhibited it when 5% or 10% glucose was ingested. The ingestion of 5% glucose with Nhp-DHC, arbutin, or HQ-aG reduced the rise in postprandial blood glucose level. However, using 10% glucose with Nhp-DHC, arbutin or HQ-aG did not reduce the elevation in glucose level. The addition of salicin to either 5% or 10% glucose had no effect on the rise in glucose level at all. Table II shows the relationship between the percentage inhibition of the glycemic response and the glycoside dose.

None of the glycosides affected gastric emptying in mice (Table III). The blood insulin response to a 5% glucose solution loaded with or without 15 mm of phlorizin 5 min and 10 min after oral administration was at the same level (Table IV).

Discussion

With type II (non-insulin-dependent) diabetes in humans, a considerable body of evidence has been accumulated indicating that a chronic physiological increment in the blood glucose concentration leads to the progressive impairment of insulin secretion and may contribute to insulin resistance as well. The precise biochemical mechanism responsible for the hyperglycemia-induced defect in insulin secretion remains to be defined, but may be related to a defect in phosphoinositide metabolism. The importance of controlling the blood glucose level in a diabetic subject has prompted active research to find ways of stabilizing the postprandial glucose level. One such attempt has been
through the use of specific enzyme inhibitors of intestinal \(\alpha\)-glucoside hydrolases.\(^{15}\) We thought that substances which inhibit or delay glucose absorption from the small intestine following carbohydrate ingestion would be useful for improving hyperglycemia. We therefore attempted to construct an easy system to detect changes in postprandial blood glucose level after glucose loading by using mice in vivo. Mice have several advantages over other animals such as their little requirement of specimens and ease of handling and treatment. Phlorizin competes with glucose for binding sites in the transport system on the outer surface of the proximal tubular brush-border membrane. High-affinity phlorizin binding appears to be \(Na^+\) dependent and is competitively inhibited by \(\alpha\)-glucose and those sugar analogs which share the glucose transport system,\(^{14}\) suggesting that it is a high-affinity binding site that is associated with the \(Na^+\)/glucose co-transporter.\(^{15}\) The glycermic response following the ingestion of a glucose solution was dose-dependent on the amount of glucose (Fig. 1). The peak glycermic response was reached 10 min after a 5% or 10% glucose load in the mice and indicated a sharp peak.

The effect of phlorizin on the glycermic response in mice after glucose ingestion is shown in Fig. 2A. Phlorizin consistently suppressed the blood glucose level and the difference was statistically significant \((p<0.05)\) at 10 min. Although phlorizin did not dissolve in the glucose solution at above 20 mM (7.6 mg of phlorizin/100 g of body wt), a dose-dependent inhibitory effect of phlorizin on the postprandial blood glucose rise was observed (Fig. 2B). The results of phlorizin in this experiment agree with those of a previous study.\(^{16}\)

We therefore determined the effect of several glycosides on the postprandial blood glucose level (Tables I and II). Nhp-DHC has the dihydrochalcone structure which phlorizin contains. Nevertheless, the effect of Nhp-DHC on glucose absorption as means of reducing the blood glucose level when feeding glucose is different from that of phlorizin. Alvarado has reported that phlorizin competes with glucose and its analogs for a common membrane binding site, and that phloretin, an aglycone, inhibited glucose transport allosterically by binding to a different site closely associated with the binding site.\(^{14}\) The differences among tissues in their relative response to phlorizin and phloretin were attributed to a differing spatial relationship between the sugar site and the phenolic site. Therefore, the inhibitory effect of glycosides on the postprandial blood glucose rise may not be dependent on the dihydrochalcone structure, because the spatial relationship between the sugar site and the phenolic site may affect the glycermic response.

Arbutin is a \(\beta\)-glucoside, and HQ-zG is the \(\alpha\)-glucoside of HQ. HQ-zG was a specimen with limited availability, and was used only at a dose of 30 mM (6.6 mg of HQ-zG/100 g of body wt). Both HQ-glycosides at a dose of 30 mM (6.6 mg of HQ-zG or arbutin/100 g of body wt) had a significant inhibitory effect on the postprandial blood glucose level. A recent study of the \(\alpha\)-anomer and \(\beta\)-anomer of a glucoside of \(p\)-nitrophenol has shown that the glucose transport carrier preferred the \(\beta\)-anomer to the \(\alpha\)-anomer of this glucoside, and that the \(\alpha\)-glucoside was more hydrolyzed than the \(\beta\)-glucoside, although the aglycone tested was exclusively limited to \(p\)-nitrophenol.\(^{17}\) Arbutin was transported by the intestinal \(Na^+\)/glucose co-transporter (SGLT1) in hamster intestinal tissue\(^{16}\) and \(Xenopus\) oocytes.\(^{18}\) We think that arbutin was transported by intestinal SGLT1 and that HQ-zG was hydrolyzed to the aglycone, HQ, and glucose, and then that they were respectively transported by SGLT1. Therefore, arbutin and HQ-zG reduced the postprandial blood glucose rise by competitively inhibiting glucose absorption from the small intestine. Although we did not test HQ, it might have a similar ability to that of arbutin and HQ-zG.

Glycyrrhizin, glycyrrhetinic acid diglucuronide, the main component of kanzo (\(Glycyrrhiza\) radix), had a greater inhibitory effect on the postprandial blood glucose level than its derivative, glycyrrhetinic acid monoglucuronide, suggesting that the number of sugar residue might have changed its affinity for SGLT1.

Salicin did not have any inhibitory effect on the postprandial blood glucose rise, and our results with phlorizin, arbutin, and salicin correspond with those from an \textit{in vitro} study with \(Xenopus\) oocytes.\(^{19}\)

No glycosides affected gastric emptying in mice (Table III), suggesting that the reduced postprandial blood glucose rise in mice was not caused by retarding the transport of the glucose solution from the stomach to the small intestine, but was caused by glycosides inhibiting glucose absorption from the small intestine.

The blood insulin response was at the same level between the 5% straight glucose ingestion group and the 5% glucose with 15 mM phlorizin ingestion group (Table IV). The quantity of glucose ingested by the mice was 10 mg, which is equivalent to 20 g ingested/60 kg human. Since Std ddY mice at this age were non-diabetic and non-obese, no extra insulin was secreted.

In conclusion, we designed a convenient method to screen glycosides which inhibit glucose absorption by using mice in vivo. Some of the glycosides such as glycyrrhizin, arbutin, and HQ-zG led to a significant reduction in blood glucose rise following glucose ingestion. These glycosides might inhibit glucose transport or delay glucose uptake at the small intestine. Further studies are needed to examine this possibility and to establish a basis for reducing the postprandial blood glucose level.

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\textbf{References}

Inhibition of Glucose Absorption by Glycosides