Fermented Soybean–Derived Water-Soluble Touchi Extract Inhibits α-Glucosidase and Is Antiglycemic in Rats and Humans after Single Oral Treatments

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ABSTRACT A water-soluble extract of Touchi, a traditional Chinese food, was found to exert a strong inhibitory activity against rat intestinal α-glucosidase. We orally administered sucrose (2 g/kg) with or without Touchi extract (TE) to normal rats at 100 and 500 mg/kg. Postprandial increases in blood glucose levels at 30 and 60 min after the administration of TE were significantly depressed compared with controls. In humans, eight borderline diabetic subjects were administered 0.1–10.0 g TE before sucrose loading (75 g). TE decreased the glycemic response dose dependently after sucrose loading. Compared with the area under the curve of the postprandial rise in blood glucose with various doses, TE elicited a significant antiglycemic effect at a minimum effective dose of 0.3 g. In addition, when four diabetics were administered 0.3 g TE before eating 200 g of cooked rice, the postprandial increase in blood glucose and mean insulin levels were significantly depressed at 60 and 120 min, respectively, after ingestion compared with levels when no TE was administered. TE, which exhibits α-glucosidase inhibitory activity, demonstrated an antihyperglycemic effect and may have potential use in the management of patients with non–insulin-dependent diabetic mellitus. J. Nutr. 131: 1211–1213, 2001.

KEY WORDS: • Touchi • α-glucosidase inhibitory action • antihyperglycemic effect • non–insulin-dependent diabetic mellitus • rats • humans

Agents with α-glucosidase inhibitory action delay carbohydrate digestion in the small intestinal tract and thereby reduce meal-induced rises in blood glucose and plasma insulin levels (1,2). Acarbose and voglibose are typical therapeutic agents that display such a mechanism of action (1–4) on postprandial blood glucose levels and are useful oral antidiabetic additions to the drugs currently available for the treatment of patients with type 2 non–insulin-dependent diabetic mellitus (3,4).

The importance of biologically active substances in foods has recently received much attention, and many physiological effects of foods (5–14) have been reported. The antihyperglycemic effects of a variety of foods with indigestible dextrin (8), resistant starch (9), α-glucosidase (10–13) or α-amylase inhibitory action (14) isolated from various sources have been investigated in animals or humans. In the present study, we screened for α-glucosidase inhibitory action in many foodstuffs and found a water-soluble Touchi extract (TE)2 that exhibited potent inhibitory action on rat intestinal α-glucosidase. Touchi, a traditional Chinese food, is used mainly for seasoning. Touchi is obtained through first steaming and then fermenting soybeans with koji (Aspergillus sp.). In addition to the effects of TE on the postprandial rise in blood glucose after sucrose loading in normal rats and borderline diabetic subjects, serum insulin levels in diabetic humans were examined in this study.

MATERIALS AND METHODS

TE preparation

Touchi (100 g), obtained from commercial sources, was milled and suspended in 900 mL of water before being boiled for 60 min. This was followed by centrifugation at 2050 × g for 30 min at room temperature, and the supernatant was filtered with paper (No. 5C; Toyo Roshi, Tokyo, Japan). The filtrate was electro dialysed with microacalyzer-G3 (Asahikasei Industry Ltd., Kawasaki, Japan), and the dialysate was concentrated before being dried under a stream of air. The powder thus obtained was the TE used in this study. The IC50 value of TE in rat intestinal α-glucosidase inhibition using sucrose as a substrate measured 0.34 g/L according to the method described by Miwa et al. (15).

In vivo studies with animals

Experiments were performed on 250- to 300-g normal male rats (Shimizu Laboratory Supplies, Kyoto, Japan). After a 12-h food deprivation, the rats were administered a sucrose solution (2 g/kg) orally with or without TE (100 and 500 mg/kg) using a stomach tube. Blood samples were collected from the tail vein and placed into heparinized (0.1 mg) tubes. Glucose levels were determined according to the oxidase method with Glucose Test Wako (Wako Pure Chemical Industries, Osaka, Japan) before (0 min) and 15, 30, 60 and 120 min after sucrose administration. Animals were treated according to National Research Council 1985 guidelines for the care and use of laboratory animals.

In vivo studies with humans

After being briefed on the experiments, volunteers gave consent for participation in the study. The protocol study was compiled according to guidelines stipulated in the revised Helsinki Declaration of 1989.

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2 Abbreviations used: BMI, body mass index; TE, Touchi extract.

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Borderline diabetic subjects. The age range of eight male borderline diabetic subjects was 29–56 y (mean, 40.1 ± 3.0 y), and their weight was 55–80 kg (mean, 66.1 ± 2.8 kg) with a body mass index (BMI) of 19.7–28.7 kg/m² (mean, 23.1 ± 1.2 kg/m²). Their fasting blood glucose was <6.1 mmol/L (mean, 6.0 ± 0.2 mmol/L), and their blood glucose level 1 h after loading with 75 g glucose in the oral glucose tolerance test was >8.3 mmol/L (mean, 11.6 ± 0.5 mmol/L). After a 12-h fast, subjects were orally loaded with sucrose (75 g) with and without 0.1, 0.3, 1.0, 3.0 or 10 g TE. Blood samples were taken by fingerprick at 0, 30, 60, 90 and 120 min after TE administration on d 0, 7, 14, 21, 28 and 35 after the weekly administration of sucrose with or without TE. Blood glucose levels were determined after oral sucrose loading in a dose-dependent manner.

Diabetic patients. The age range of the four diabetic subjects (one man and three women) was 50–64 y (mean, 59.3 ± 3.9 y), and their weight was 52–74 kg (mean, 62.5 ± 4.5 kg) with a BMI of 23.4–26.2 kg/m² (mean, 25.1 ± 0.6 kg/m²). Their fasting blood glucose levels were >6.0 mmol/L (mean, 11.6 ± 1.6 mmol/L), and glycated hemoglobin (HbA1c) levels were >6.5% (mean 8.8 ± 1.3%). After a 12-h fast, patients consumed 200 g of rice (~1260 kJ) with or without TE (0.3 g). Blood glucose and insulin levels were determined before (0 min) and 30, 60, 90 and 120 min after rice consumption with or without TE administration. Rice was used in our study because it has been used extensively in human studies of diabetes (14,15) and is served as a daily staple food in the Japanese population.

**Statistical analysis**

Results are expressed as means ± SEM. All data were initially analyzed by ANOVA for each group. When a significant F-value (P < 0.05) was obtained, this test was followed by the Duncan’s multiple range test (SAS Institute, Tokyo, Japan). The area under the curve was calculated using the trapezoidal method (16).

**RESULTS AND DISCUSSION**

In vivo studies with animals. At a dose of 500 mg/kg, TE significantly (P < 0.01) decreased the postprandial rise in blood glucose compared with the control group (n = 10) at 15–60 min after sucrose loading (Fig. 1). In addition, TE at a lower dose of 100 mg/kg significantly (P < 0.05) depressed the postprandial rise in blood glucose compared with the control group at 30 min after sucrose loading. Furthermore, the TE-treated groups (100 and 500 mg/kg) had significantly (P < 0.01) lower postprandial rises in blood glucose levels (17.96 ± 0.42 and 16.76 ± 0.39 h−1 mmol/L for 100 and 500 mg/kg TE, respectively) compared with the control group (19.91 ± 0.58 h−1 mmol/L) when area-under-the-curve values were compared. TE significantly depressed the postprandial rise in blood glucose after oral sucrose loading in a dose-dependent manner.

In vivo studies with humans. We previously used biochemical and blood analyses (data not shown) to confirm the safety of the TE (10 g) with single oral bolus administration in four healthy volunteers. In borderline diabetic subjects who were administered TE, postprandial increases after oral sucrose loading were markedly reduced (Table 1). At doses of ≥0.3 g, TE significantly suppressed the postprandial blood glucose levels compared with the controls at 60 and 90 min after sucrose loading. Furthermore, TE manifested dose-dependent antiglycemic effects after sucrose administration based on evaluation of the area-under-the-curve data (Table 1). The minimum effective dose was 0.3 g.

At this dose, we studied the effects of TE on the postprandial rise in blood glucose after the ingestion of rice with or without TE by patients with fully developed non–insulin-dependent diabetic mellitus. TE (0.3 g) significantly decreased both postprandial blood glucose and insulin levels at 60 and 120 min after ingestion, respectively (Table 2). Both borderline diabetics and diabetic patients had no complaints of any side effects such as abdominal pain.
TOUCHI EXTRACT IS ANTIGLYCEMIC IN VIVO

TABLE 2

Effects of oral Touchi extract (TE) on the glycemic response in diabetic patients

<table>
<thead>
<tr>
<th>Dose TE</th>
<th>Time after rice consumption with or without TE, min</th>
<th>g mmol/L</th>
<th>units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.0 ± 0.0</td>
<td>5.3 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>5.0 ± 0.3</td>
<td>3.9 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>5.0 ± 0.8</td>
<td>3.8 ± 0.6*</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>5.0 ± 0.9</td>
<td>3.1 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>5.0 ± 1.4</td>
<td>2.4 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>AUC (0–120 min)</td>
<td>9.7 ± 1.3</td>
<td>6.0 ± 1.0</td>
</tr>
</tbody>
</table>

1 Changes in blood glucose and serum insulin vs. before loading (zero time) are expressed as mean ± se, n = 4, * P < 0.05, ** P < 0.01 vs. 0 g TE, AUC was calculated with the trapezoidal method. Patients were orally loaded with rice (200 g) with (0.3 g) or without TE after a 12-h fast.

LITERATURE CITED


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diarrhea, retching or flatulence after TE ingestion. Moreover, abnormalities in hematological and biochemical blood data were not observed.

TE elicits inhibitory activity against maltase, although its potency was lower than that against sucrase, in our preliminary studies. The IC50 value of TE against rat intestinal α-glucosidase using maltose as a substrate was 1.1 g/L (data not shown). In addition, TE (100 mg/kg orally) exhibited significant antiglycemic effects after maltose (2 g/kg) administration (in our preliminary studies). However, TE does not show any inhibitory activity against α-amylases (data not shown) and therefore has specific action against the enzyme α-glucosidase. Because of this enzyme-specific action, TE may have elicited such an excellent outcome without inducing any side effects such as diarrhea, retching and flatulence, which are commonly encountered with the use of currently available α-glucosidase inhibitory therapeutic agents. Moreover, such favorable outcomes may be due to the lower potency of TE in inhibition of α-glucosidase in the small intestinal tract compared with currently used α-glucosidase inhibitory therapeutics (IC50 value of voglibose, an α-glucosidase inhibitory drug, was 11 pmol/L in our assay system). However, acarbose, an α-glucosidase inhibitor, was used in long-term (1 y) studies in diabetic patients and resulted in the passage of some dietary carbohydrate into the colon, although a significant loss of energy into the feces was not induced (17). Utilization of carbohydrates by colonic bacteria induces the production of hydrogen and short-chain fatty acids, which are inversely correlated with proliferation in the rectal upper crypt, a biomarker of risk for colonic neoplasia (17). TE ingestion may be used on a long-term basis without producing abdominal distention. Through timely regimen and proper dosing, TE may be useful for the management of borderline diabetics and diabetic patients. Before its therapeutic use in humans is actualized, studies on drug tolerance and the antiglycemic effects of long-term treatment with TE are warranted.

1 Changes in blood glucose and serum insulin vs. before loading (zero time) are expressed as mean ± se, n = 4, * P < 0.05, ** P < 0.01 vs. 0 g TE, AUC was calculated with the trapezoidal method. Patients were orally loaded with rice (200 g) with (0.3 g) or without TE after a 12-h fast.