Antidiabetic Mechanism of Bakumondo-inshi

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To determine the antidiabetic mechanism of Bakumondo-inshi (BI), we examined its effects on glucose absorption, α-glucosidase activity, sodium-dependent glucose transporter and facilitative glucose transporter isoform 5 (SGLUT) in small intestine. The oral administration of BI into KK-Ay mice caused a significant decrease in the glucose absorption in small intestine. The small intestine content of active glucose transporter isoform (SGLUT) protein content from KK-Ay mouse significantly decreased in the BI-treated KK-Ay mouse compared to that in the controls. However, the small intestine content of facilitative glucose transporter isoform, GLUT5 protein content did not change. The α-glucosidase activity in small intestine significantly decreased in the BI-treated KK-Ay mice. These results suggest that the antidiabetic effect of BI is derived at least in part, from a decrease of glucose absorption in small intestine, due to the reduction of SGLUT protein content in total membrane of the small intestine and the reduction of α-glucosidase activity. Because of its therapeutic mechanism, BI could be a new category of therapeutic agent for non-insulin dependent diabetic mellitus.

Key words Bakumondo-inshi; glucose absorption; SGLUT; GLUT5; α-glucosidase; Western blotting

Increased sugar absorption is known as one of the major pathogenic factors of non-insulin-dependent diabetes mellitus (NIDDM), together with the insulin resistance in peripheral tissues and the impairment of glucose-induced insulin secretion from pancreatic beta cells. Although the therapeutic agents to inhibit α-glucosidase (for example, acarbose or voglibose) have been used for NIDDM patients, drugs to decrease glucose absorption are not yet directly available clinically.

Bakumondo-inshi (BI) was used for the treatment of diabetes mellitus in oriental traditional medicine. 11 We recently reported that following its oral administration to diabetic mice, there was a significant improvement in hyperglycemia. 22 However, the precise mechanism by which it improves the hyperglycemic effect has not been elucidated.

Glucose transport across the plasma membrane is mediated by carrier proteins termed glucose transporters. 33 Recent cDNA cloning has demonstrated that the glucose transporters comprise a family of structurally related proteins with differing tissue distribution. 44 The protein content of glucose transporters has been found to be altered under pathological conditions such as diabetes mellitus. 55–77 In the present study, we examined the effect of Bakumondo-inshi (BI) on glucose absorption and α-glucosidase activity. Investigating the protein content of the small intestine glucose transporters (sodium-dependent glucose transporter; SGLUT and facilitative glucose transporter isoform 5; GLUT5) to identify the mechanism by which BI improves the hyperglycemic effect, we found that the substance decreases the glucose absorption, reduces α-glucosidase activity and SGLUT protein content in the total membrane fraction from mouse small intestine.

MATERIALS AND METHODS

Materials BI was obtained from Tsumura Co, Ltd, Tokyo, Japan (Lot No. 243193010). The constituents were 10 raw ingredients, Ophiopogonis Tuber (ratio 7.0), Ginseng Radix (ratio 2.0), Trichosanthis Radix (ratio 2.0), Anemarhenae Rhizoma (ratio 3.0), Puerariae Radix (ratio 3.0), Rehmanniae Radix (ratio 4.0), Poria (ratio 6.0), Schizandrae Fructus (ratio 1.0), Glycyrrhizae Radix (ratio 1.0), Lophatheri Herba (ratio 1.0). BI contains spray-dried water extracts of 10 crude drugs as a mixture. The yield was 29.27%. This agent (1400 mg) was dissolved in distilled water (20 ml) for oral administration.

Animals Male KK-Ay mice (12 weeks old, Clea, Japan) were kept in an experimental animal room for 7 d with free access to food and water. They were housed individually in an air-conditioned room at an ambient temperature of 24±2°C with a 12 h light–dark cycle. Mice with blood glucose above 300 mg/dl were considered to be diabetic and were used in this study. BI was force orally.

Absorption of Glucose in Small Intestine After overnight (18 h) fasting, the mice were given BI (1400 mg/kg body weight) orally, or distilled water as a control. After 0.5 h, glucose (100 mg, 0.5 ml) solution was injected into a loop of the small intestine under anesthesia (an adjoining part was used (to calculate) prevalue and was washed out immediately). After 0.5 h, glucose content in small intestine was determined for the evaluation of glucose absorption, as described below. Glucose inhibition rate (%)=glucose content after 0.5 h/glucose content in the adjoining part.

α-Glucosidase Activity After overnight (18 h) fasting, mice were given BI (1400 mg/kg body weight) orally, or distilled water as a controls. After 0.5 h, mouse small intestine was excised, mucous membrane was removed on a slide and individually homogenized using a Potter-Elvehjem homogenizer. α-Glucosidase activity was assayed by the method of Dahlqvist 53 with some modifications. Sucrose and maltose (50 mm) were incubated with diluted enzyme. The reaction was terminated by placing in a boiling water bath for 3 min and glucose content was determined.

Determination of Blood Glucose and Protein Blood samples were taken from the eye with a capillary to determine blood glucose level. Five animals were used for each

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Fig. 1. Effect of BI on Glucose Absorption in Small Intestine
Data shown are the mean±S.E.M. and results are from 3 animals/group. Significantly different from the control (distilled water), **p<0.01.

Western Blot Analysis  After overnight (18 h) fasting, KK-Ay mice were given BI (1400 mg/kg) orally and, 0.5 h later, glucose solution (2g/kg) was administered orally. After 0.5 h, the small intestine was resected for the experiment. The antibody used in Western blotting (East Acres, U.S.A.) was raised against a synthetic peptide corresponding to the extracellular loop of rabbit SGLUT (residues 402—419), as reported by Hirayama et al.11 and mouse GLUT5 as reported by Rand et al.12 To prepare the total membrane particulate fractions, the mouse small intestine was excised, mucous membrane was removed on a slide and individually homogenized in 5 ml of 5 mM EDTA. The homogenates were then centrifuged at 450g for 10 min at 4°C and the resulting pellet was centrifuged at 450g for 10 min at 4°C to yield a pellet designated as the membrane fraction of the small intestine in this study. The membrane fractions (0.1 mg) prepared were suspended in 1% SDS and 50 mM dithiothreitol and subjected to SDS-polyacrylamide (9%) gel electrophoresis. Electrophoretic transfer to nitrocellulose paper and detection of the immunocomplex with enhanced chemiluminescence (Amersham, Buckinghamshire, UK) were carried out as previously described.13 The sheet was exposed on RX X-ray film and intensifying screen (Fuji, Tokyo, Japan). Prestained molecular weight standard (Bio-Rad, Richmond, VA, U.S.A.) was used for estimation of the molecular weight. The experiments were performed at least twice for each tissue with similar results.

Statistical Analysis  All data are expressed as means±S.E.M. Student’s t-test was used for the statistical analysis. Values were considered to be significantly different when p value was less than 0.05.

RESULTS

Absorption of Glucose in Small Intestine  The suppressive effect of BI on glucose absorption in small intestine is shown in Fig. 1. BI (1400 mg/kg) significantly reduced glucose absorption in the mice after oral administration compared with control.

α-Glucosidase Activity  Effect of BI on α-glucosidase activity is shown in Fig. 2. BI significantly reduced maltase and sucrase activity (maltase: p<0.05, sucrase: p<0.05).

Fig. 2. Effect of BI on α-Glucosidase Activity (A: Maltase Activity, B: Sucrase Activity)
Each value represents the mean±S.E.M. (n=3). Significantly different from control, *p<0.05.

Fig. 3. Effect of BI on SGLUT Protein Content in Small Intestine
Each value represents the mean±S.E.M. (n=3). Significantly different from control, **p<0.01.
Western Blot Analysis  The quantitation of SGLUT and GLUT5 protein in membrane in mouse small intestine was assessed by Western blotting in the mice. Quantitation of the 85 kDa SGLUT and 50 kDa GLUT5 glucose transporter band isolated from nitrocellulose paper demonstrated that relative amount of SGLUT protein in the small intestine from BI treated mice was 216% of that observed in the control mice (p<0.01) (Fig. 3). However, there was no significant difference between BI-treated and control groups on GLUT5 protein content in small intestine (Fig. 4).

DISCUSSION

The present study clearly shows that Bakumondo-inshi (BI) has a significant suppressive effect on glucose absorption in small intestine on KK-Ay mice. In the previous study, we examined dose-dependence (140—1400 mg/kg) after the treatment of BI in an oral glucose tolerance test, and found that the most effective dose was 1400 mg/kg.21 We have new found further that BI reduced α-glucosidase activity in small intestine. From these findings, it seems likely that BI exhibits its inhibition of sugar absorption by decreasing the small intestine glucose uptake and α-glucosidase activity. An α-glucosidase inhibitor (for example, acarbose and voglibose) general inhibits only α-glucosidase and does not inhibit glucose absorption at all. Therefore, it is interesting that BI inhibited not only α-glucosidase but also glucose absorption. The α-glucosidase inhibition of BI is much weaker than that of acarbose, a synthetic drug, however.14 Because chinese medicines contain many compounds, further studies are needed to clarify the details.

To elucidate the mechanism of the reduction of small intestine glucose uptake, we examined the effects of BI on SGLUT and GLUT5 glucose transporters in mouse small intestine, since it has been reported that SGLUT and GLUT5 play a crucial role in the process of sugar intake of the small intestine.15 The SGLUT protein content was decreased in the small intestine of BI-treated KK-Ay mice, while the substance did not change the GLUT5 protein content in this organ. These findings suggested decreased SGLUT protein in the total membrane fraction is due to the decreased glucose absorption, since glucose absorption was observed to be suppressed.

Since we have shown previously that BI treatment inhibits hyperglycemia after oral administration of glucose, sucrose or maltose in KK-Ay mice, further study could show how BI could become a useful drug in the treatment of NIDDM through this therapeutic mechanism.

REFERENCES