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Note

The Effects of an Arabinogalactan-Protein from the White-Skinned Sweet Potato (*Ipomoea batatas* L.) on Blood Glucose in Spontaneous Diabetic Mice

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We examined the effects of an arabinogalactanprotein (WSSP-AGP) from *Ipomoea batatas* L. on hyperglycemia in db/db mice. An oral glucose tolerance test indicated significantly decreased plasma glucose levels by WSSP-AGP. Additionally, an insulin tolerance test found improvement in insulin sensitivity due to treatment with WSSP-AGP. This suggests that amelioration of insulin resistance by WSSP-AGP causes to lead its hypoglycemic effects.

Key words: arabinogalactanprotein; oral glucose tolerance test; insulin tolerance test; hypoglycemic effects; high-sensitive C-reactive protein

The white-skinned sweet potato (WSSP) is a kind of sweet potato (*Ipomoea batatas* L.) belonging to the Convolvulaceae family, used as a food and in traditional medicine in Brazil[1] and Japan. The tuberous root is beneficial in the treatment of diabetes mellitus.[2–4] Recently, we isolated an arabinogalactanprotein (WSSP-AGP) as an anti-diabetic compound from the tuberous cortex of WSSP.[5] WSSP-AGP is a glycoprotein with a weight-average molecular weight of about 130,000 *g*/mol that consists of 5% w/w protein and 95% w/w carbohydrate. As shown in Fig. 1, the structure is composed of a partial structure 1, (1 → 3)-*β*-D-galactan highly branched at O-6 with 2, (1 → 6)-*β*-D-galactan, in which the branched chains are replaced at the O-3 position with 3, *α*-L-Ara-(1 → and 4, *α*-L-Ara-(1 → 5)-*α*-L-Ara-(1 →, and at the O-6 position typically with 5, *α*-L-Rha-(1 → 4)-*β*-D-GlcA-(1 → as a terminal group.[5] However, there are few studies related to the effects of AGPs on hyperglycemia, and the details remain unclear. Hence, in the present study, we performed continuous oral administration of WSSP-AGP to db/db mice to examine the effects of WSSP-AGP on sugar metabolism.

Female BKS.Cg-*Lepr*^{db}/*Lepr*^{db}/J mice (db/db, 4 weeks old) were purchased from Charles River Laboratories Japan (Yokohama, Japan). All the animals were housed under controlled conditions (temperature, 24 ± 2 °C; humidity, 50 ± 10%; light, 12-h light-dark cycle). They were maintained on a basal diet (CE-2; Clea Japan, Tokyo) and water *ad libitum* throughout the experiment. After an acclimatization period of 1 week, all the mice were randomly divided into groups. The present study was conducted following the Guidelines for Animal Experimentation, No. 88, established by the Prime Minister's Office of Japan in 2006 with the approval of the Animal Care and Use Committee of Fuji-Sangyo Co., Ltd.

Experiment 1 was conducted to evaluate the effect of WSSP-AGP on hyperglycemia. In experiment 1, all the animals were randomly divided into three groups (n = 6), and were orally administered distilled water (control) or 20 mg/kg of WSSP-AGP or Pioglitazone (PIO; Takeda Pharmaceuticals, Osaka, Japan) solutions *via* a stomach tube, daily for 8 weeks. The doses of test materials were decided after it was confirmed that they were sufficient to cause the physiological effect. WSSP-AGP was isolated by a method described previously.[5] PIO, a typical anti-diabetic drug, was used as positive control. After treatment for 6 weeks, all the mice were given an oral glucose tolerance test (OGTT), as follows: they were fasted for 15 h, followed by oral administration of a 10% glucose solution (1 g/kg). Blood samples were collected from the tail veins at 0, 30, 60, 120, and 180 min after oral administration. Plasma samples were obtained by centrifugation to measure glucose and insulin levels. After treatment for 8 weeks, the mice were anesthetized with a pentobarbital solution (40 mg/kg; Kyoritsu Pharmaceuticals, Tokyo), and were sacrificed by exsanguination from the inferior vena cava. Then the plasma levels of the biochemical markers were measured using commercial kits, as follows: glucose C-II test, triglyceride (TG) E-test, non-esterified fatty acid (NEFA) C-test (Wako Pure Chemical Industries, Osaka, Japan), an insulin ELISA kit, an ELISA mouse leptin kit (Morinaga Institute of Biological Science, Yokohama, Japan), a mouse/rat adiponectin ELISA kit (Otsuka Pharmaceuticals, Tokyo), and a mouse high-sensitive C-reactive protein (hs-CRP) ELISA (Kamiya Biomedical Company, Seattle, WA). Experiment 2 aimed to assess the mechanism of the effect of WSSP-AGP on plasma glucose gain. After treatment for 6 weeks, an insulin tolerance test (ITT) was done as follows: After a 15-h fast, all the mice were intraperitoneally injected an insulin solution (2 units/kg; Wako). Blood samples were collected from the tail veins at 0, 30, 60, and 120 min after intraperitoneal injection

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Abbreviations: WSSP, white-skinned sweet potato; AGP, arabinogalactan-protein; SEM, standard error of the mean; OGTT, oral glucose tolerance test; ITT, insulin tolerance test; AUC, area under the curve; hs-CRP, high-sensitive C-reactive protein

1: →3)-β-D-Gal-(1→3)-β-D-Gal-(1→3)-β-D-Gal-(1→3)-β-D-Gal-(1→
6 6 6 6
↑ ↑ ↑ ↑

2: →6)-β-D-Gal-(1→6)-β-D-Gal-(1→6)-β-D-Gal-(1→ **3:** α-L-Ara-(1→
3 3 3
↑ ↑ ↑

4: α-L-Ara-(1→5)-α-L-Ara-(1→ **5:** α-L-Rha-(1→4)-β-D-GlcA-(1→

Fig. 1. The Partial Structures of WSSP-AGP.

and glucose levels were measured using a commercial kit. Experiment 2 was conducted under conditions similar to those for experiment 1 until an ITT was examined, except that PIO was not administered as positive control. Data were expressed as mean ± standard error of the mean (SEM). Significant differences in experiment 1 were determined by Tukey-Kramer's test using Excel add-in software Statcel2, and those in experiment 2 were determined by Student's *t*-test. p values of less than 0.05 were considered to be statistically significant.

In an OGTT (experiment 1), as shown in Fig. 2A, the WSSP-AGP group showed significantly reduced plasma glucose levels as compared to the control group (30, and 60 min, $p < 0.01$; 180 min, $p < 0.05$). Similarly, the PIO group showed significantly reduced plasma glucose levels as compared to those for the other groups (for all points, $p < 0.01$ *vs.* the control group; for 30, and 60 min, $p < 0.01$; for 120, and 180 min, $p < 0.05$ *vs.* the WSSP-AGP group). In addition, the area under the curve for plasma glucose concentration during the OGTT (AUC_G) was also calculated to assess the total absorbed amount. AUC_G in the WSSP-AGP (1,415 ± 173 mg·h/dL, $p < 0.01$) and PIO groups (808 ± 58 mg·h/dL, $p < 0.01$) was significantly lower than in the control group (2,034 ± 105 mg·h/dL). As for insulin levels (Fig. 2B), the PIO group was significantly higher at 30 min ($p < 0.01$) than the control group. On the other hand, the area under the curve for plasma insulin concentration during the OGTT (AUC_I) in the WSSP-AGP group (26.0 ± 2.0 ng·h/mL) was similar to that in the PIO group (26.5 ± 2.0 ng·h/mL), and those in both groups were not significant as compared to that in the control group (19.8 ± 2.2 ng·h/mL). These results suggest that treatment with WSSP-AGP leads to improvements in insulin sensitivity.

After treatment for 8 weeks, the plasma biochemical parameters were as shown in Table 1. The glucose levels in the WSSP-AGP and PIO groups were significantly lower ($p < 0.05$ and $p < 0.01$ respectively) than in the control group. The insulin, TG, NEFA, leptin, and adiponectin levels in the WSSP-AGP group showed no changes as compared to those in the control group. The PIO group showed several changes, as follows: The levels of TG and NEFA were significantly lower (TG, $p < 0.05$ *vs.* the WSSP-AGP group; NEFA, $p < 0.01$ *vs.* the other groups), and the levels of leptin and adiponectin were markedly higher (leptin, $p < 0.01$ *vs.* the control group and $p < 0.05$ *vs.* the WSSP-AGP group; adiponectin, $p < 0.01$ *vs.* the other groups). Hs-CRP in the WSSP-AGP group was significantly lower ($p < 0.05$) than in the control group. CRP release from the liver and adipose tissue is promoted by inflammatory cytokines such as interleukin-6 and tumor necrosis

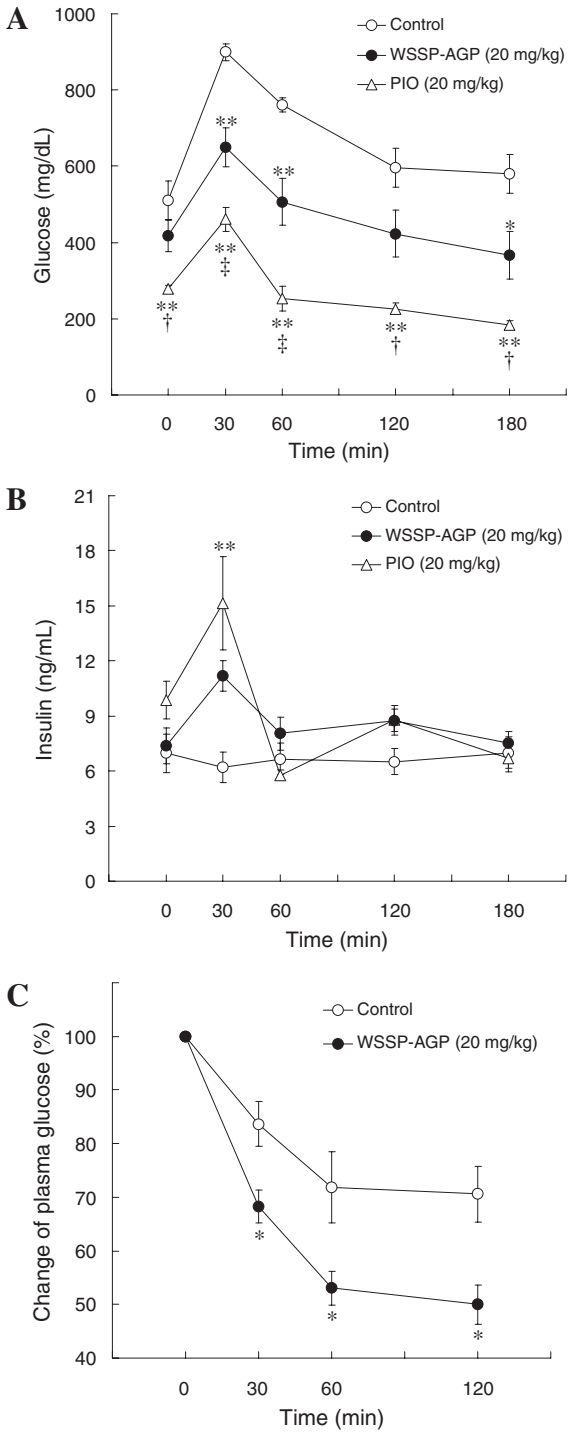

Fig. 2. Effects of WSSP-AGP on the Oral Glucose Tolerance Test (OGTT) and the Insulin Tolerance Test (ITT).

The time course of the plasma glucose level (A) and the insulin level (B) in OGTT, and the time course of the plasma glucose level in ITT (C) are shown. OGTT (A and B): After a 15 h fast, a 10% glucose solution (1 g/kg) was administered orally. Blood samples were collected from the tail veins of the mice at 0, 30, 60, 120, and 180 min after oral administration. ITT (C): After a 15 h fast, an insulin solution (2 units/kg) was injected intraperitoneally. Blood samples were collected from the tail veins of the mice at 0, 30, 60, and 120 min after intraperitoneal injection. In Fig. 2A–C, symbols show control (open circle), WSSP-AGP (solid circle), and PIO (open triangle). All values were expressed as mean ± SEM (n = 6). OGTT and ITT were analyzed by Tukey-Kramer's test and Student's *t*-test respectively. p values less than 0.05 were considered to be significant. Significant differences from the control and WSSP-AGP groups are represented as follows: $^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* the control group; $^{\dagger}p < 0.05$, $^{\ddagger}p < 0.01$ *vs.* the WSSP-AGP group.

Table 1. Effects of Continuous Ingestion of WSSP-AGP on Plasma Biochemical Parameters

		Control	WSSP-AGP	PIO
Glucose	(mg/dL)	804.4 ± 78.7	$504.9 \pm 36.2^{*}$	$408.4 \pm 78.8^{**}$
Insulin	(ng/mL)	4.80 ± 0.89	7.87 ± 1.13	6.27 ± 0.93
TG	(mg/dL)	170.2 ± 43.4	208.0 ± 20.6	$96.3 \pm 14.8^{\dagger}$
NEFA	(mEq/L)	1.58 ± 0.11	1.47 ± 0.07	$0.88 \pm 0.07^{**\ddagger}$
Leptin	(ng/mL)	71.0 ± 9.6	76.3 ± 13.4	$126.2 \pm 7.6^{**\dagger}$
Adiponectin	(μg/mL)	20.4 ± 1.6	20.3 ± 1.7	$50.6 \pm 3.7^{**\ddagger}$
hs-CRP	(ng/mL)	6.04 ± 0.21	$5.26 \pm 0.10^{*}$	5.29 ± 0.24

All values represent mean ± SEM (n = 6). p values were calculated by the Tukey-Kramer's test. Significant differences from the control and WSSP-AGP groups are represented as follows: $^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* the control group; $^{\dagger}p < 0.05$, $^{\ddagger}p < 0.01$ *vs.* the WSSP-AGP group.
TG, triglyceride; NEFA, non-esterified fatty acid; hs-CRP, high-sensitive C-reactive protein

factor-α, which are implicated in inhibition of insulin action.[6–8] Hence, the decreased hs-CRP levels in the WSSP-AGP group suggest that WSSP-AGP can suppress the secretion of aggravating factors in insulin resistance. However, in this study, it remains unclear how an anti-inflammatory effect due to WSSP-AGP leads to hypoglycemic effects, because there are several pathways leading to insulin resistance and diabetes mellitus.[7–9]

These results suggest that treatment with WSSP-AGP decreases the elevation of plasma glucose levels through improvement in insulin resistance. Hence, an ITT (experiment 2) was conducted to identify the mechanism of action of treatment with WSSP-AGP. As shown in Fig. 2C, an ITT revealed a significant decrease in plasma glucose levels in the WSSP-AGP group (30, 60, and 120 min, $p < 0.05$ *vs.* the control group). This finding strongly indicates improved insulin sensitivity due to long-term treatment with WSSP-AGP.

In conclusion, we performed OGTT and ITT in db/db mice, and observed that treatment with WSSP-AGP decreased plasma glucose levels and that ameliorated impaired insulin sensitivity. These observations suggest that the hypoglycemic effects of WSSP-AGP are mediated by amelioration of insulin resistance. In addition, the plasma hs-CRP levels in the WSSP-AGP group were significantly lower than in the control group. However, it remains unclear how in detail the decreased inflammatory state is directly linked with insulin signaling and hypoglycemic effects. Further studies are in progress.

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