Antiobesity and Antidiabetic Actions of a New Potent Disaccharidase Inhibitor in Genetically Obese-Diabetic Mice, KKA^y

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Summary AO-128 is a potent and structurally novel inhibitor of the intestinal disaccharidases, such as maltase and sucrase. Genetically obese-diabetic mice, KKA^y, were used to examine the acute or long-term effectiveness of this compound. AO-128 decreased a postprandial rise in blood glucose after sucrose solution loading dose-dependently; the ED₅₀ to reduce a delta increment of blood glucose by 50% was 0.22 mg/kg. The intestinal sucrase and maltase activities were suppressed to 7 and 48% of the control levels, respectively, at a dose of 0.21 mg/kg. Four-week-old female KKA^y mice were kept on a laboratory diet containing 10 or 50 ppm of AO-128 for 12 weeks. The high dose of AO-128 reduced food intake and body weight gain throughout the experimental period. On the other hand, the low dose reduced body weight gain for the first 4 weeks without any effect on food intake. Development of the hyperglycemia and hyperinsulinemia characteristic of KKA^y mice was moderately prevented by the low dose, and completely by the high dose. Hypertriglyceridemia tended to be suppressed by the AO-128 treatment. The high dose decreased the hemoglobin A1 level and parametrial adipose tissue weight. Hepatomegaly and fatty liver were ameliorated by AO-128 dose-dependently. Nephropathy was ameliorated by the high dose. These findings indicate that AO-128 may be useful for treating human obesity and diabetes.

Key Words disaccharidase inhibitor, obesity, diabetes, KKA^y mice, hyperglycemia, hyperinsulinemia, sucrase, maltase

A steep rising in the postprandial blood glucose level is a therapeutic problem in carbohydrate-related disorders, such as obesity, diabetes mellitus, and hypertriglyceridemia (1, 2), because the high blood glucose level accelerates lipogenesis and fat accumulation through hypersecretion of insulin, and increases insulin requirement. Recent research indicates that alpha-glucosidase inhibitors, which delay

Fig. 1. Chemical formula of AO-128.

absorption of carbohydrates by inhibiting conversion of carbohydrates to monosaccharides, are useful to treat the above-mentioned metabolic diseases (3–7).

On the basis of its inhibitory actions on the porcine alpha-glucosidase activities, 1 L-[1 (OH), 2,4,5 / 3]-5-[2 -hydroxy- 1-(hydroxymethyl)-ethyl] amino-1-C-(hydroxymethyl)-1,2,3,4-cyclohexanetetrol (AO-128, Fig. 1) has been selected as one of the promising candidates from a series of valioline derivatives (8). This compound strongly suppresses maltase and sucrase activities of the rat intestinal sucrase-isomaltase complex, but does not suppress the alpha-amylase activity of porcine pancreas (9). The present article describes the antiobesity and antidiabetic actions of this compound in genetically obese-diabetic mice, KKA^y (10).

MATERIALS AND METHODS

Animal. The genetically obese-diabetic mice, KKA^y, were bred in the Laboratory Animal Unit of our Division and weaned at 4 weeks of age. These mice were maintained on a laboratory chow diet consisting of 52.7% carbohydrate, 23.6% protein, 4.4% fat, 4.9% fiber, and 6.6% minerals and vitamins (CE-2, Clea Japan Inc., Tokyo) and water *ad libitum*. In order to measure food intake, the laboratory chow diet was changed to a CE-2 powdered diet 3 days before the start of the long-term experiment, and a powdered diet in each group was weighed in the lump every week after drying at room temperature for 24 h and sifting off the feces. Food efficiency (%) was calculated as body weight gain (g) per food intake (g) $\times$ 100 every 2 or 4 weeks. Throughout the experiment, the mice were housed individually in metal cages in a room with controlled temperature ($23 \pm 1^\circ\text{C}$), humidity ($55 \pm 5\%$), and light (08:00–20:00 h).

Sucrose tolerance test. After being fasted for 18 h, 3 groups of 9- or 10-week-old female KKA^y mice received an oral sucrose load (2.5 g/10 ml/kg) containing AO-128 (0.21, 0.63, and 1.89 mg/10 ml/kg, respectively). Blood samples (20 μl) were taken from the orbital sinus 0, 30, 60, and 120 min after loading to measure blood glucose. Delta increments from basal levels of plasma glucose were calculated as: $\Delta \text{area} = (a + 2b + c) \times 1/4 - a$ (mg/dl $\cdot$ h), where a represents the level of plasma glucose before an oral glucose load, and b and c are the levels at 30 and 60 min after glucose, respectively. At the end of the test, the mice were killed to examine the sucrase and maltase activities in the small intestine as described below.

Antiobesity and antidiabetic actions. Four-week-old female KKA^y mice,

weighing 13.4–21.7 g, were kept on a CE-2 powdered diet containing 10 (low dose) or 50 (high dose) ppm (w/w) of AO-128 for 12 weeks. Blood samples (100 μ l) were taken from the orbital sinus every 2 or 4 weeks to measure plasma glucose, triglyceride, and insulin. Hemoglobin A1, and sucrase and maltase activities in the small intestine were determined at the end of the experiment. The adipose tissue and liver were weighed and the liver lipid composition was examined as we described previously (11). The right kidney was fixed in 10% neutral formalin, embedded in paraffin, and stained with hematoxylin/eosin to examine the progress of the nephropathy present in KKA^y mice (10).

Sucrase and maltase activities. The small intestine between the part just below the duodenum and the part just above the cecum was cut longitudinally, rinsed with ice-cold saline, and homogenized with 10 ml of saline using a polytron homogenizer. The homogenate (5–20 μ l) was incubated with 40 mM sucrose or 40 mM maltose in 0.5 ml of reaction mixture at 37°C for 1 h. The protein content was determined by the method of Lowry *et al.* (12). Sucrase and maltase activities were calculated by glucose concentration converted from sucrose or maltose, and were indicated as μ mol/mg protein/h.

Analytical procedures. The glucose concentration in the blood, plasma and the reaction mixture, and the triglyceride concentration in the plasma were determined enzymatically using EncoreTM (Baker Instruments Co., Allentown, PA). Plasma insulin was measured by the double-antibody method based on Hales and Randle (13) using a commercial kit (Amersham, England) with human insulin as a standard. Hemoglobin A1 was determined using a commercial kit (NC-ROPET, Nihon-Chemical Co.). The statistical significance of differences between sample means was assessed using Duncan's multiple range test.

RESULTS

Effect on postprandial hyperglycemia

AO-128 dose-dependently decreased the postprandial rise in blood glucose after an oral sucrose load (Fig. 2). The ED₅₀ value required to reduce a delta increment of the blood glucose area by 50% was 0.22 mg/kg. The intestinal sucrase and maltase activities measured 120 min after AO-128 load were suppressed to 7 and 48% of the control levels even at the lowest dose; further suppression of these activities by increased dosages of AO-128 was small (Table 1).

Antiobesity and antidiabetic actions

Four-week-old female KKA^y mice were kept on a CE-2 powdered diet containing 10 (low dose) or 50 (high dose) ppm (w/w) of AO-128 for 12 weeks. The daily intake of AO-128 calculated from food intake every week changed from 2.5 to 1.4 mg/kg/day in the low dose group and from 9.6 to 7.4 mg/kg/day in the high dose group with advancing age. The high dose of AO-128 induced diarrhea and soft feces only within the first 5 days; the low dose did not. The high dose reduced food

Fig. 2. Effect of AO-128 on sucrose tolerance in KKA^y mice. After being fasted for 18 h, 9- or 10-week-old female KKA^y mice received oral administration of sucrose (2.5 g/kg/10 ml) containing AO-128 at a dose of 0 (○), 0.21 (●), 0.63 (□) or 1.89 (■) mg/kg. The data points represent means $\pm$ SD for $n = 6$. Differences between four groups at each time were analyzed by Duncan's multiple range test: points without a common letter differ significantly ($p < 0.05$).

Table. 1. Effect of a single administration of AO-128 on intestinal sucrase and maltase activities of KKA^y mice.

Dose of AO-128 (mg/kg)	<i>n</i>	Sucrase (μ mol/mg protein/h)	Maltase
0	6	7.52 ± 1.27^a	30.5 ± 4.3^a
0.21	6	0.49 ± 0.15^b	14.5 ± 1.2^b
0.63	6	0.35 ± 0.10^b	12.7 ± 1.2^b
1.89	6	0.19 ± 0.07^b	11.4 ± 2.5^b

After being fasted for 18 h, 9- or 10-week-old female KKA^y mice received an oral administration of sucrose (2.5 g/kg/10 ml) containing AO-128. Two hours later, the small intestine was removed and enzyme activities were measured. Values represent means $\pm$ SD. Differences between four groups were analyzed by Duncan's multiple range test: values without a common letter differ significantly ($p < 0.05$).

intake and body weight gain throughout the experiment, and food efficiency for the first 4 weeks of treatment (Fig. 3). On the other hand, the low dose reduced body weight gain and food efficiency for the first 4 weeks without affecting any effect on food intake. The control KKA^y mice developed hyperglycemia, hypertriglyceridemia, and hyperinsulinemia (Fig. 4). The high dose completely prevented the development of hyperglycemia and hyperinsulinemia; the low dose did partially. Hypertriglyceridemia tended to be suppressed by the AO-128 treatment. Hemoglobin A1 measured at the end of the experiment was decreased significantly only in the high dose group (Fig. 5). The parametrial adipose tissue weight was significantly lighter in the high dose group than in the control group (1.7 ± 0.8 vs. 3.1 ± 0.4 g, $p < 0.01$); the weight in the low dose group (3.0 ± 0.7 g) did not differ from the control group. The control KKA^y mice showed hepatomegaly and an

Fig. 3

Fig. 3. Effect of AO-128 on body weight, food intake and food efficiency of KKA^y mice. Four-week-old female KKA^y mice were kept on CE-2 diet containing AO-128 at a dose of 0 (○), 10 (●) or 50 (□) ppm for 12 weeks. Food intake was calculated by dividing food consumption of each group by number of mice in each group. The data points represent means $\pm$ SD or means for $n=5$ or 6. Differences of body weight between three groups at each age were analyzed by Duncan's multiple range test: points without a common letter differ significantly ($p < 0.05$).

Fig. 4

Fig. 4. Effect of AO-128 on plasma glucose, triglyceride and insulin levels of KKA^y mice. Four-week-old female KKA^y mice were kept on CE-2 diet containing AO-128 at a dose of 0 (○), 10 (●) or 50 (□) ppm for 12 weeks. The data points represent means $\pm$ SD for $n=5$ or 6. Differences between three groups at each age were analyzed by Duncan's multiple range test: points without a common letter differ significantly ($p < 0.05$).

increase in hepatic triglyceride content (Table 2). The treatment with AO-128 dose-dependently decreased liver weight and the lipid control.

Effects on spontaneous renal lesions

The control KKA^y mice showed various changes in the glomeruli and renal tubuli (Table 3, Fig. 6). The high dose of AO-128 suppressed the development of the exudative type glomerulosclerosis and of hyaline casts and atrophy in the renal tubuli (Table 3, Fig. 6).

Fig. 5. Effect of AO-128 on hemoglobin A1 of KKA^y mice. Four-week-old female KKA^y mice were kept on CE-2 diet containing AO-128 at a dose of 0, 10 or 50 ppm for 12 weeks. Values represent means $\pm$ SD for $n=5$ or 6. Differences between three groups were analyzed by Duncan's multiple range test: bars without a common letter differ significantly ($p < 0.05$).

Table 2. Effect of AO-128 on liver weight, and hepatic triglyceride (TG), cholesterol (CHOL) and phospholipid (PL) contents of KKA^y mice.

Dose of AO-128 (ppm)	<i>n</i>	Liver weight (g)	Hepatic lipid contents (mg/g liver)		
			TG	CHOL	PL
0	5	2.17 $\pm$ 0.29 ^a	26.5 $\pm$ 3.5 ^a	3.60 $\pm$ 0.37 ^a	32.3 $\pm$ 1.4 ^b
10	6	1.95 $\pm$ 0.30 ^a	17.2 $\pm$ 6.2 ^b	3.34 $\pm$ 0.25 ^{ab}	33.6 $\pm$ 1.1 ^{ab}
50	6	1.21 $\pm$ 0.16 ^b	4.1 $\pm$ 1.4 ^c	3.10 $\pm$ 0.35 ^b	37.4 $\pm$ 5.0 ^a

Four-week-old female KKA^y mice were kept on CE-2 diet containing AO-128 at a dose of 0, 10 or 50 ppm for 12 weeks. Values represent means $\pm$ SD. Differences between three groups were analyzed by Duncan's multiple range test: values without a common letter differ significantly ($p < 0.05$).

Effects on intestinal sucrase and maltase activities

Sucrase activities were markedly suppressed by both doses of AO-128 (Fig. 7), whereas maltase activities tended to be lower in the high dose group than in other two groups (Fig. 7).

DISCUSSION

AO-128 decreased the increment of blood glucose after an oral sucrose load in a dose-dependent manner. The ED₅₀ value to reduce a delta increment of the blood glucose area by 50% was 0.22 mg/kg. The almost same dose, 0.21 mg/kg, suppressed the intestinal sucrase and maltase activities to 7 and 48% of control levels, respectively. Thus, it is expected that AO-128 can decrease a postprandial rise in blood glucose, one of the problems in treating diabetes and obesity, by inhibiting alpha-glucosidases.

Table 3. Effect of AO-128 on the spontaneous renal lesions in KKA^y mice.

Dose of AO-128 (ppm)		0						10						50					
		1	2	3	4	5		1	2	3	4	5	6	1	2	3	4	5	6
Mouse number																			
Glomerulosclerosis																			
Diffuse type		++	++	++	+	++		++	++	++	++	++	+	+	++	++	+	++	+
Exudative type		±	±	++	++	++		+	+	++	++	++	+	-	±	+	±	±	+
Nodular type		+	+	+	++	+		+	±	±	++	+	+	+	+	++	-	+	+
Tubular lesions																			
Hyaline cast		±	+	+	++	+		+	+	++	+	+	-	-	+	++	-	-	±
Atrophy		-	±	±	+	±		+	±	+	+	+	±	-	-	++	±	-	-
Dilatation of pelvis		-	-	-	+	±		-	-	-	-	±	-	-	-	±	-	-	-

Four-week-old female KKA^y mice were kept on CE-2 diet containing AO-128 at a dose of 0, 10 or 50 ppm for 12 weeks. Grade of lesions: -, no; ±, slight; +, mild; ++, moderate; +++, severe.

Fig. 6. Light micrographs of glomeruli and renal tubuli from KKA^y mice in the control and AO-128-treated groups. Exudative type of glomerulosclerosis is observed in the control mouse (A), but disappears in mouse treated with a high dose of AO-128 (B). PAS stain. $\times 260$.

Fig. 7. Effect of AO-128 on sucrase and maltase activities in the small intestine of KKA^y mice. Four-week-old female KKA^y mice were kept on CE-2 diet containing AO-128 at a dose of 0, 10 or 50 ppm for 12 weeks, and were then sacrificed under the fed condition. Differences between three groups were analyzed by Duncan's multiple test: bars without a common letter differ significantly ($p < 0.05$).

The long-term administration of AO-128 produced antidiabetic and antiobesity actions in KKA^y mice. A high dose (7.4–9.6 mg/kg/day) completely prevented the development of hyperglycemia and hyperinsulinemia, tended to decrease hy-

pertriglyceridemia, and reduced body weight gain. These effects of a low dose (1.4–2.5 mg/kg/day) were observed clearly for the first 4 weeks and decreased gradually. Diarrhea and soft feces were induced by the high dose, but not by the low dose, for the first 5 days. Food intake in the high dose group was decreased markedly for the first 2 weeks and then returned to slightly smaller levels than those in the control group, suggesting that the sustained small food intake may be due to normoglycemia. It is generally known that one of causes for hyperphagia in the diabetic animals is a compensatory response to energy loss as urinary glucose excretion (14). Therefore, the above-mentioned effects of AO-128 observed for the long term are not explained by the undesirable effects in the gut observed for the first 5 days. These findings suggest that decreased and/or delayed carbohydrate absorption results in such beneficial effects.

Suppression of intestinal disaccharidase activities was observed even at the end of the experiment. Sucrase activities were significantly lower in both the low and high dose groups than in the control group. On the other hand, maltase activities were slightly lower only in the high dose group. The inhibitory action of AO-128 was slightly less on maltase than on sucrase (8). Generally, maltase activities are fivefold and more higher than sucrase activities in the small intestine of mammals (15). Disaccharidase activities in the distal part of the small intestine have been documented to be increased by long-term treatment with acarbose (16). Such increased activities are thought to be a compensatory response to the presence of undigested disaccharides and to be important for reducing the development of undesirable symptoms, such as diarrhea and flatulence. Therefore, it seems likely that the inhibitory action of AO-128 on maltase activities is underestimated by measuring maltase activities in the whole of the small intestine. Further studies will be required to examine the role of the distal part of the small intestine in animals treated with AO-128 for a long term.

AO-128 treatment at the high dose markedly decreased liver weight and hepatic lipid content. Hepatomegaly and an increase in hepatic lipid content are observed commonly in obese and diabetic animals with hyperinsulinemia (17,18). It was also reported that the liver of KKA^y mouse responds normally to plasma insulin levels, whereas the peripheral tissues, such as the muscle and adipose tissues, show decreased responsiveness, indicating the presence of insulin resistance in the latter organs (10,19–21). Therefore, the above-mentioned effects of AO-128 on the liver seem to be caused by decreased plasma insulin levels. On the other hand, the lowering action of AO-128 on plasma triglyceride was not prominent, suggesting that insulin resistance, for example on lipoprotein lipase, which plays a role in the uptake of triglyceride from plasma, still remains.

The KKA^y mouse strain was established by introduction of the yellow obese gene, A^y , into the KK mouse strain (10). As a result, the degrees of obesity, hyperglycemia, hyperlipidemia and hyperinsulinemia became much severer in KKA^y mice than in KK mice. KKA^y mice also showed the increases in liver weights and hepatic lipid content as compared with KK mice, suggesting that

hyperinsulinemia is closely related to these phenomena. AO-128 administration with the high dose in this study decreased the tissue weights to the levels less than those in age-matched KK mice (22), for example parametrial adipose tissue weight: 7.22, 4.96 and 6.22 g/100bw and liver weight: 5.03, 3.76 and 4.87 g/100bw for KKA^y, AO-128-treated KKA^y and KK mice, (means for 4–6 mice), respectively.

It was reported that KKA^y mice developed more advanced renal lesions as compared with non-obese, mild diabetic KK mice (10). AO-128 treatment at the high dose ameliorated some of these renal lesions partially. These results suggest that strict control of plasma glucose levels by AO-128 has a beneficial effect for treating diabetic nephropathy.

We conclude that AO-128 may be useful for treating human diabetes and obesity by suppressing intestinal alpha-glucosidase activities.

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