Possible Mechanism of Hypoglycemic Effect of 4-Hydroxybenzoic Acid, a Constituent of *Pandanus odoratus* Root

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Received August 5, 1998 Accepted September 29, 1998

ABSTRACT—We studied the hypoglycemic effect of 4-hydroxybenzoic acid, a constituent of the root of *Pandanus odoratus* Ridl. (Pandanaceae, Thai name: *Toei-hom*), in streptozotocin-diabetic rats. Oral administration of 4-hydroxybenzoic acid caused a decrease in plasma glucose levels dose-dependently in the diabetic rat. The constituent did not affect serum insulin level and liver glycogen content in the diabetic model, but increased glucose consumption in normal and diabetic rat diaphragms. These results suggest that 4-hydroxybenzoic acid produces a hypoglycemic effect mediated by an increase in the peripheral glucose consumption.

Keywords: *Pandanus odoratus*, Hypoglycemic effect, 4-Hydroxybenzoic acid

It was reported that oral administration of an aqueous extract of the root and rhizome of *Pandanus odoratus* Ridl. (Thai name: *Toei-hom*, Pandanaceae) significantly decreased plasma glucose level in mild and severe alloxan-diabetic female rats (1). Additionally, the root extract showed hypoglycemic and hypolipidemic effects in streptozotocin-diabetic rats (2). One of the hypoglycemic constituents isolated from *Pandanus odoratus* Ridl. root was 4-hydroxybenzoic acid, which caused a decrease in plasma glucose level and an increase in serum insulin level and liver glycogen content in normal rats (3). The aim of the present study was to evaluate the hypoglycemic effect of 4-hydroxybenzoic acid in streptozotocin-diabetic rats. The serum insulin, liver glycogen levels and peripheral glucose consumption were also measured to clarify the possible mechanism of the hypoglycemic action.

Prior to the experiment, six-week-old male Wistar rats, obtained from SLC (Shizuoka), weighing 120–140 g, were housed under a 12-hr light/dark cycle in an air-conditioned room ($23 \pm 1^\circ\text{C}$ with $55 \pm 5\%$ humidity) for at least one week with free access to food and water. Streptozotocin (STZ; Sigma Aldrich Japan, Tokyo) in citrate buffer, pH 7.5 (75 mg/kg, i.p.) was injected after overnight fasting to produce experimentally diabetic rats. The diabetic condition was daily checked by a urine glucose strip throughout the experiment. Five days after the in-

jection, the fasting plasma glucose level was determined, and the rats with a level of 180 mg/dl or more were used as diabetic rats. A modified oral glucose tolerance test (OGT) was used to examine the hypoglycemic effect. No food was given to the rat during the 15 hr prior to the experiment. Groups of six to nine rats then received oral administration of distilled water (control) or 4-hydroxybenzoic acid. Thirty minutes after 4-hydroxybenzoic acid, glucose (1.25 g/kg) was orally administered to each rat. In the insulin-treated group, insulin (Bovine insulin crystal, dissolved in normal saline; Sigma Aldrich Japan) was subcutaneously injected immediately after glucose administration. Ninety minutes later, blood samples were taken from vessels of the rat tail or abdominal aorta in order to assay plasma glucose or insulin content. All experiments and treatments follow the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society.

Plasma or serum glucose concentration was determined by the glucose oxidase and glucose peroxidase method (4), measuring the optical density with a spectrophotometer at the wavelength of 505 nm (model DU 68; Beckman, Palo Alto, CA, USA). To measure liver glycogen level, 30% KOH solution (2.0 ml) was added to one gram of the liver and the mixture was heated at 100°C for 20 min. An ice-cold 95% ethanol solution (4.0 ml) was then added to the mixture and kept for 30 min at 4°C . After two additional treatments with ethanol, the combined

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total precipitate was dissolved in 1 ml of water. The glycogen content was then measured by the anthrone- H_2SO_4 method (5). Serum insulin level was determined by means of the EIA method, and optical density was measured with a spectrophotometer at the wavelength of 492 nm (DU68, Beckman). Peripheral glucose consumption was studied in rat diaphragm preparation from animals fasted for 36 hr previous to the experiment. The diaphragm was divided into five pieces and incubated in the nutrient solution with constant oxygenation and shaking (90 cycles/min) at 37°C for 90 min in accordance with the Vallance-Owen technique (6). The nutrient solution was prepared with the following formula: 125 ml of 1.3% NaHCO_3 was aerated with carbon dioxide for 3 min and then added to 750 ml of buffer solution. The buffer solution consisted of: 162.56 mM NaCl, 5.37 mM KCl, 2.70 mM CaCl_2 , 4.17 mM NaHCO_3 , 1.42 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 1.46 mM KH_2PO_4 . The resultant mixture was aerated for 10 min and used immediately. Glucose was added to a final concentration of 300 mg%. Each piece of diaphragm was incubated in 2 ml of glucose nutrient mixture. The results were expressed as glucose consumption per 10 mg dry diaphragm (by subtracting glucose concentration after incubation from glucose before incubation). The dry weight was determined after oven drying the diaphragm at 105°C for 120 min. A glucose oxidase kit and Glazyme insulin-EIA test (Wako Pure Chemicals, Osaka) were used for the measurement of plasma or serum glucose and serum insulin level, respectively. Statistical analyses were performed by one way analysis of variance followed by Dunnett's test, two way analysis of variance followed by the Student-Newman-Keuls method, Student's *t*-test or paired *t*-test to evaluate the statistical differences between the control and the experimental samples, and *P*

Fig. 1. Effect of 4-hydroxybenzoic acid on plasma glucose level in streptozotocin-diabetic rats. After 15 hr of fasting, water (control) or 4-hydroxybenzoic acid was orally administered to rats. Thirty minutes later, 1.25 g/kg of glucose was orally administered to each rat. Ninety minutes after glucose administration, blood was collected from the tail vein to measure plasma glucose levels. Vertical bar shows the S.E.M. ($n=6$). * $P<0.05$, ** $P<0.01$ vs control (Dunnett's test).

values of 0.05 or less were considered significant.

Oral administration of 4-hydroxybenzoic acid decreased plasma glucose level dose-dependently in STZ-diabetic rats (Fig. 1). 4-Hydroxybenzoic acid at a dose of 5 mg/kg, which significantly decreased serum glucose level in the diabetic rat, did not affect the liver glycogen and serum insulin levels significantly (Table 1A), while insulin (5 U/kg, s.c.) significantly decreased the serum glucose level in diabetic rats (Table 1B). 4-Hydroxybenzoic acid at the concentration of 10^{-4} to 10^{-2} mg/ml increased the

Table 1. Effects of 4-hydroxybenzoic acid on serum glucose, liver glycogen content and serum insulin in streptozotocin-diabetic rats

	Serum glucose (mg%)	Liver glycogen (mg/g wet weight)	Serum insulin ($\mu\text{U/ml}$)
A			
Control (6)	381.6 ± 24.1	42.9 ± 11.9	41.5 ± 9.9
4-Hydroxybenzoic acid 5 mg/kg, p.o. (6)	$274.0 \pm 35.6^*$	39.3 ± 9.6	38.3 ± 9.4
B			
Control (7)	418.9 ± 28.8	42.9 ± 11.6	47.0 ± 10.9
Insulin, 5 U/kg, s.c. (6)	$57.1 \pm 37.2^{**}$	—	—

The same procedure as used in Fig. 1 was employed. Ninety minutes after glucose administration, all blood was collected from the abdominal aorta to assay serum glucose and insulin levels and the liver was also removed to assay liver glycogen. The data represent the mean $\pm$ S.E.M. The number of animals is shown in parentheses. * $P<0.05$, ** $P<0.01$ vs control (A: Student-*t* test, B: Dunnett's test).

Table 2. Effect of 4-hydroxybenzoic acid on peripheral glucose consumption in diaphragm of normal and streptozotocin-diabetic rats

	Glucose consumption (mg/10 mg diaphragm dry weight)				
	Control	4-Hydroxybenzoic acid (mg/ml)			Insulin
		10^{-4}	10^{-3}	10^{-2}	5 U/ml
Normal rats (9)	0.55 ± 0.07	0.81 ± 0.18	$0.97 \pm 0.21^*$	$1.15 \pm 0.17^{**}$	$1.00 \pm 0.20^*$
Diabetic rats (8)	0.44 ± 0.09	$0.71 \pm 0.14^*$	$0.70 \pm 0.16^*$	$0.75 \pm 0.14^{*,\#}$	$0.86 \pm 0.25^*$

After 36 hr of fasting, the diaphragm was removed and then divided into five pieces and incubated at 37°C, with constant oxygenation for 90 min and shaking at 90 cycles/min. The results were expressed as glucose consumption per 10 mg dry diaphragm. The data represent the mean $\pm$ S.E.M. The number of animals is shown in parentheses. * $P < 0.05$, ** $P < 0.01$ vs control (paired *t*-test). # $P < 0.05$ vs normal rats at the same dose (Student-Newman-Keuls method).

peripheral glucose consumption of the rat diaphragm in vitro (Table 2). At the concentration of 10^{-2} mg/ml, the peripheral glucose consumption was markedly increased in both normal and diabetic rat diaphragm. However, the peripheral glucose consumption of diabetic rat diaphragm was less than that of normal rat diaphragm [$F_{\text{diabetic rats}}(1,75) = 4.41$, $P < 0.05$]. Insulin increased the peripheral glucose consumption in normal and diabetic rats.

The compound isolated from *Pandanus odoratus* Ridl. root, which was identified to be 4-hydroxybenzoic acid, reduced plasma glucose level and increased serum insulin and liver glycogen levels in normal rats (3). However, 4-hydroxybenzoic acid showed the hypoglycemic effect without the increase of serum insulin in the diabetic rat. It may due to the action of STZ to selectively destroy B cells of the pancreas, which only leaves less active B cells to secrete insulin. Thus, the mechanism of action of 4-hydroxybenzoic acid in IDDM rats is not the stimulation of insulin release. Theoretically, the blood glucose level after glucose load depends on factors like intestinal motility (7), glucose absorption (8), insulin secretion and metabolic factors (9) or glucose consumption. In the case of 4-hydroxybenzoic acid, the mechanism of action might not be due to the inhibition of glucose absorption, because *Pandanus* extract did not inhibit glucose absorption in mouse jejunum in vitro (W. Vonglieng et al., unpublished data) and *Pandanus* extract also produced a hypoglycemic effect in the intravenous glucose tolerance test (IVGT) in which glucose was administered intravenously and was not absorbed from the gastrointestinal tract (2). Insulin level and hepatic glucose uptake in terms of glycogen storage also did not increase. Thus, effect of 4-hydroxybenzoic acids on peripheral glucose consumption was further investigated. Total body glucose disposal is primarily related with insulin action in the muscle (10). It was found that 4-hydroxybenzoic acid increased peripheral glucose consumption in the rat dia-

phragm both of diabetic and normal rats in vitro, especially at the concentration of 10^{-2} mg/ml, which was calculated to be approximately equivalent to 5 mg/kg in total body water. However, at this dose, a significant difference in the peripheral glucose consumption between diabetic and normal rat diaphragm was observed. Although it was reported that at the 7th day of diabetes, an early defect in the pathway of glucose utility in the muscle was probably at the step of glucose transport (11), we must further investigate the effect of high concentration of 4-hydroxybenzoic acid.

In conclusion, the hypoglycemic effect of 4-hydroxybenzoic acid is due to an increase in peripheral glucose consumption, the action being independent of serum insulin level. Thus, 4-hydroxybenzoic acid might have an insulin-like activity, causing the hypoglycemic effect in the diabetic rat.

Acknowledgments

This work was supported by a JSPS Ronpaku Program (Ph.D. dissertation) grant, which was provided to P. Peungvicha by the Japan Society for the Promotion of Science (JSPS) through the National Council of Thailand and a Mahidol University Research Grant.

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