Antidiabetic activity of Cissus sicyoides in KK-Ay mice

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The antidiabetic activity of Cissus sicyoides (Vitaceae) was investigated in KK-Ay mice, an animal model of genetically type 2 diabetes with hyperinsulinemia. The water extract of Cissus sicyoides (CS) (500 mg/kg body weight) improved hyperglycemia after an oral administration of maltose in KK-Ay mice. CS significantly reduced maltase activity in small intestine of KK-Ay mice. CS reduced the blood glucose of KK-Ay mice 2 weeks after repeated administration without changing plasma insulin level. These results suggest that the antidiabetic activity of CS is derived, at least in part, due to the inhibition of maltase activity.

Key words antidiabetic activity, KK-Ay mouse, Cissus sicyoides, maltase.

Introduction

Despite considerable progress in the management of diabetes mellitus by synthetic drugs, the search for indigenous natural antidiabetic agents is ongoing. The plant kingdom offers a wide field to look for effective oral hypoglycemics. More than 400 species have been reported to display hypoglycemic effects, but only a few of them have been investigated.

Cissus sicyoides (Vitaceae) (Inushina in Japanese name) is a tropical plant widely used in Brazilian folk medicine to treat diabetes mellitus, so much so that it is usually called "vegetable insulin". Pepato et al. reported on the hypoglycemic activity of Cissus sicyoides in streptozotocin-induced diabetic rats, an animal model of type 1 diabetes. Moreover, anthocyanins present in the fruit may have potential as a food colorant. However, adequate characterization of antidiabetic activity is yet to be done and few studies have been performed on type 2 diabetes models. In the present study, we examined the effect of Cissus sicyoides on blood glucose in type 2 diabetic mice, and also the effect of maltase activity in order to clarify the antidiabetic mechanism.

Materials and Methods

Cissus sicyoides which was provided by Space-three Co., LTD. (Tokyo, Japan) were used in the present experiment. Three hundred grams of Cissus sicyoides leaf was extracted with 2 l of water (50°C, 2 h). After filtration (ADVANTEC No.131), the water extracts were lyophilized (CS) and stored at room temperature until use. The yield was 16.0%.

Animals. KK-Ay mice (Clea, Tokyo, Japan), 6 weeks old, were used. KK-Ay mice with blood glucose level above 200 mg/100ml were considered to be diabetic and used in this study. The mice were housed in an air-conditioned room at 22±2°C with a 12 hour light-12 hour dark cycle (light: 9:00 a.m. to 9:00 p.m.). The animals were kept in an experimental animal room for 7 days with free access to food (CE-2, Clea, Tokyo) and water (tap water). Blood samples were withdrawn from the cavernous sinus with a capillary to determine blood glucose levels under non-anesthesia and non-fasting. CS was dissolved in distilled water. The distilled water was administered to the control mice. The studies were started at 10:00-11:00 a.m., and blood samples after repeated administration of CS were taken at 10:00-11:00 a.m. CS was administered orally on a compulsory basis (repeated administration, once a day). Animals were treated in accordance with the Guideline for the Care and Use of Laboratory Animals (the Prime Minister's Office no.6, 1980).

Oral maltose tolerance test. After overnight (18 hours) fasting, KK-Ay mice were given CS (500 mg/kg) orally and 0.5 hours later, the maltose (2 g/kg body weight) solution was administered orally. Blood samples were collected before the administration of the maltose and 30, 60 and 120 minutes later.

Maltase activity. After overnight fasting, KK-Ay mice were given CS (500 mg/kg) orally and 0.5 h later, mouse small intestine was excised, mucous membrane was removed on a slide and homogenized individually with Potter-Elvehjem homogenizer. Maltase activity was assayed by the method of Dahlqvist with some modifications. Maltose (50 mM) was incubated with diluted enzyme. The reaction was terminated by placing it in a boiling water bath for 3 min and glucose content was determined.

Determination of blood glucose and insulin. Blood glucose levels in the mice were determined by the glucose oxidase method, and plasma insulin was measured by the double antibody method. All the data were expressed as mean ± S.E.M, and a Student’s t test and an ANOVA followed by a Student-Neuman-Kuels post hoc test was used for statistical analysis. The values were considered to be significant when the p value was less than 0.05.

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Results

Effect of CS on maltose tolerance test in KK-Ay mice. The effects of CS on maltose tolerance are shown in Fig. 1. CS improved hyperglycemia after maltose loading (30 and 60 min, \( p<0.05 \)).

Effect of CS on maltase activity in small intestine of KK-Ay mice. The effects of CS on maltase activity in small intestine are shown in Table 1. CS significantly reduced maltase activity in small intestine of KK-Ay mice (\( p<0.01 \)).

Effects of CS on blood glucose in KK-Ay mice (repeated administrations). The effect of the repeated administration of CS on blood glucose is shown in Fig. 2. CS-treated animals (100 mg/kg) showed lower blood glucose levels from 2 weeks after the administration when compared to the controls (\( p<0.05 \)). The plasma insulin level in CS-treated KK-Ay mice was not affected 2 weeks after the administration (Control 136±34 \( \mu \)U/ml, CS 113±20 \( \mu \)U/ml). CS did not change the body weight of KK-Ay mice (Fig. 3).

Discussion

This study clearly showed that the water extract of Cissus sicyoides produces a consistent hypoglycemic effect. It was reported that CS showed antidiabetic activity at 500 mg/kg after oral administration. Therefore, we studied the effect of CS at the dosage of 500 mg/kg. We examined the therapeutic effects of CS on hyperglycemia in KK-Ay mice, an animal model of type 2 diabetes mellitus. These mice, which are known for genetically induced diabetes and which

![Fig. 2 Effects of CS on Blood Glucose in KK-Ay Mice (repeated administrations)](image)

CS 500 mg/kg was administered orally to KK-Ay diabetic mice. After 2 weeks, blood samples were taken to determine blood glucose level. Each value represents the mean±S.E. of 5-7 mice. Significantly different from control, \( *p<0.05 \).

![Fig. 3 Effect of CS on Body Weight in KK-Ay Mice (repeated administrations)](image)

CS 500 mg/kg was administered orally to KK-Ay diabetic mice. Each value represents the mean±S.E. of 5-7 mice.
include ob/ob mice\(^{10}\) and KK mice\(^{11}\) had hyperinsulinemia as a result of insulin resistance.\(^{12}\) Their treatment with CS resulted in hypoglycemia without changing plasma insulin. These results indicate that CS may be useful for type 2 diabetes.

We have found further that CS reduced maltase activity in small intestine. From this finding, it seems likely that CS exhibits its inhibition of sugar absorption by decreasing maltase activity. The maltase inhibition of CS is weaker than that of acarbose, a synthetic drug, however.\(^{13}\) Because folk medicines contain many compounds, further studies are needed to clarify the details.

The toxicity of CS seems to be very low (LD\(_{50}>>2000\) mg/kg body weight) (data not shown). CS-treated (2000 mg/kg) mice did not show any obvious stimulus action. CS did not change the body weight of KK-Ay mice in repeated administration. These findings suggest that CS is a supplement with low toxicity.

Diabetics also often have elevated blood cholesterol and triglyceride. Further study would show how CS could become a useful drug in the treatment of diabetes through this unique therapeutic mechanism. The above experimental results suggest that *Cissus sicyoides* supports the traditional medical use of type 2 diabetes.

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**References**


**Japanese abstract**

*Cissus sicyoides*（インスリーナ）の抗糖尿作用を2型糖尿模型の1つ KK-Ayマウスで検討した。*Cissus sicyoides*の水抽出物（CS）500 mg/kgはマルトース負荷後の血糖値の上昇を抑制し、小腸のマルターゼ活性を抑制した。CSは2週間連続投与によりインスリン値に影響することなく血糖値を低下させた。これらの結果より、CSの血糖低下機序は小腸のマルターゼ活性を抑制することにより血糖値を低下させると考えられた。

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