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Cinnamic acid exerts anti-diabetic activity by improving glucose tolerance *in vivo* and by stimulating insulin secretion *in vitro*

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ABSTRACT

Although the anti-diabetic activity of cinnamic acid, a pure compound from cinnamon, has been reported but its mechanism(s) is not yet clear. The present study was designed to explore the possible mechanism(s) of anti-diabetic activity of cinnamic acid in *in vitro* and *in vivo* non-obese type 2 diabetic rats. Non-obese type 2 diabetes was developed by injecting 90 mg/kg streptozotocin in 2-day-old Wistar pups. Cinnamic acid and cinnamaldehyde were administered orally to diabetic rats for assessing acute blood glucose lowering effect and improvement of glucose tolerance. Additionally, insulin secretory activity of cinnamic acid and cinnamaldehyde was evaluated in isolated mice islets. Cinnamic acid, but not cinnamaldehyde, decreased blood glucose levels in diabetic rats in a time- and dose-dependent manner. Oral administration of cinnamic acid with 5 and 10 mg/kg doses to diabetic rats improved glucose tolerance in a dose-dependent manner. The improvement by 10 mg/kg cinnamic acid was comparable to that of standard drug glibenclamide (5 mg/kg). Further *in vitro* studies showed that cinnamaldehyde has little or no effect on glucose-stimulated insulin secretion; however, cinnamic acid significantly enhanced glucose-stimulated insulin secretion in isolated islets. In conclusion, it can be said that cinnamic acid exerts anti-diabetic activity by improving glucose tolerance *in vivo* and stimulating insulin secretion *in vitro*.

Abbreviations

STZ	streptozotocin
OGTT	oral glucose tolerance test
CA	cinnamic acid
CD	cinnamaldehyde
GB	glibenclamide
TB	tolbutamide
BSA	bovine serum albumin
Db	diabetic

Introduction

Cinnamon (*Cinnamomum cassia*) has been used in several cultures for centuries as a spice and traditional medicine and is known as an anti-diabetic agent all over the world. Cinnamon contains volatile oils such as cinnamaldehyde, eugenol, and cinnamic acid; phenolic compounds such as tannin, catechins, and proanthocyandins; monoterpenes, sesquiterpenes; and trace coumarin (Anderson et al. 2004; Barceloux 2009). Among these compounds, proanthocyandins, cinnamic acid and cinnamaldehyde are found to be major components of cinnamon aqueous extract (Jiao et al. 2013). We have recently reported that cinnamaldehyde and cinnamic acid are major compounds of cinnamon aqueous extract (Shaukat 2013). Proanthocyandins has been reported to inhibit aggregation of human islet amyloid polypeptide and works as an anti-diabetic agent (Jiao et al. 2013). Cinnamaldehyde has been shown to exert several pharmacological effects such as anti-inflammatory, anti-oxidant, anti-microbial, and anti-diabetic activities (Gowder 2006; Chao et al. 2007; Zhang et al. 2008; Pei et al. 2009). Cinnamic acid modulates glycogenesis and gluconeogenesis (Huang 2012) and improves insulin sensitivity (Arlt et al. 2004) in diabetic rats. Though the anti-diabetic activity of cinnamic acid is reported, however, the anti-diabetic mechanism is not yet clear. It has been reported that cinnamaldehyde is partially metabolized into cinnamic acid in the stomach and small intestine, and is almost completely metabolized into cinnamic acid in the liver before it is absorbed into the blood in rats (Chen 2009). Though cinnamaldehyde is metabolized to cinnamic acid, we hypothesize that cinnamic acid

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may be the active principle of cinnamon that is responsible for the anti-diabetic activity of cinnamon. To test this hypothesis, insulin secretory activity of cinnamic acid and cinnamaldehyde in isolated islets was performed. Additionally, acute blood glucose lowering effect and glucose tolerance test in the presence of cinnamic acid and cinnamaldehyde were performed in neonatally streptozotocin-induced non-obese type 2 diabetic rats. Cinnamic acid lowered blood glucose and improved glucose tolerance in diabetic rats. Additionally, cinnamic acid showed insulin secretory activity in isolated islets.

Materials and methods

Materials

Streptozotocin (STZ), glibenclamide (GB), tolbutamide (TB), collagenase V and bovine serum albumin (BSA) were obtained from Sigma (St. Louis, MO, USA). Trans-cinnamic acid (>99% pure) and trans-cinnamaldehyde (>98% pure) were purchased from Alfa Aesar (Karlsruhe, Germany). Mouse ultrasensitive insulin ELISA kit was purchased from Crystal Chem Inc. (IL, USA).

Animals and development of non-obese type 2 diabetic rats

Wistar rats of either sex from the animal house of the International Center for Chemical and Biological Sciences (ICCBS), University of Karachi, Pakistan, were used throughout the study. The animals were kept under temperature and humidity control (25 ± 2 °C; 50–55% humidity) with a 12-h light:12-h dark cycle. The rats were maintained in clean cages with free access to water and food *ad libitum*. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care with stating the guidelines (NIH publication) with the approval of the Institutional Ethics Committee of ICCBS, University of Karachi, Pakistan.

A freshly prepared STZ solution of 90 mg/kg in citrate buffer (pH 4.5) was injected intraperitoneally (i.p.) to 2-day-old Wistar rat pups to obtain the model of non-obese type 2 diabetic rats. After three months, an oral glucose tolerance test (OGTT) was performed with 3 g/kg glucose. The rats having fasting blood glucose levels of 7.6–11.1 mmol/l at 0 min and showed the maximum rise at 45 min (12.6–22.0 mmol/l) were included in this study.

Acute effect of cinnamic acid and cinnamaldehyde on blood glucose

Diabetic rats were fasted overnight and divided into 6 groups (5 rats/group). Group 1, diabetic control rats received only 1 ml of distilled water (Db); Group 2, diabetic rats received 5 mg/kg cinnamic acid (Db+CA-5); Group 3, diabetic rats received 10 mg/kg cinnamic acid (Db+CA-10); Group 4, diabetic rats received 5 mg/kg cinnamaldehyde (Db+CD-5); Group 5, diabetic rats received 10 mg/kg cinnamaldehyde (Db+CD-10); Group 6, diabetic rats received 5 mg/kg glibenclamide (Db+GB). Cinnamic acid or cinnamaldehyde was given orally in 1 ml of water suspension by a gavage to the experimental rats. Blood glucose levels were measured at 0, 1, 2 and 3 h after oral administration of cinnamic acid or cinnamaldehyde.

OGTT with cinnamic acid and cinnamaldehyde in diabetic rats

Thirty type 2 diabetic rats were divided into five groups (6 rats/group). Group I, received only distilled water (Db); Group II, received 5 mg/kg glibenclamide (Db+GB); Group III, diabetic rats received cinnamaldehyde at a dose of 10 mg/kg (Db+CD-10); Group IV, diabetic rats received cinnamic acid at a dose of 5 mg/kg (Db+CA-5); Group V, diabetic rats received cinnamic acid at a dose of 10 mg/kg (Db+CA-10). Cinnamaldehyde or cinnamic acid was given orally in 1 ml of water suspension to the experimental rats.

All the diabetic rats were fasted overnight (14 h) prior to the OGTT test. Sixty minutes following the cinnamic acid or cinnamaldehyde administration, 3 g/kg oral glucose load was given to each rat. All rats were tested for blood glucose levels at −60 min (just before the administration of cinnamic acid or cinnamaldehyde) and at 0, 45, 60 and 120 min after glucose load. OGTT was also performed in non-diabetic control rats for comparison.

Determination of cinnamic acid in serum samples

Cinnamic acid (10 mg/kg body weight) was fed orally to diabetic rats and blood samples were collected after 1, 2, and 4 h. Samples preparation and HPLC analysis of cinnamic acid in blood were done according to the procedure described by Song et al. (2002). Blank sample was prepared from the blood collected prior to feed cinnamic acid and standard was prepared by spiking 100 μl (100 ppm) aqueous cinnamic acid into blank serum. Prepared samples were analyzed on HPLC (Agilent Chemstation, USA) at 254 nm by using 250 mm × 4.6 mm i.d. ODS C18, 5-μm particle size (Merck, Germany).

Islets isolation and insulin secretion assay

Isolation of islets and insulin secretion assay were done as described previously (Siddiqui et al. 2014). In brief, batches of five size-matched islets were incubated for 60 min in KRB buffer solution with 3 mM (basal) or 16.7 mM (stimulatory) glucose, supplemented with cinnamic acid or cinnamaldehyde in different concentrations. At the end of incubation, 100 μl aliquots were removed from each tubes and secreted insulin was measured using an ultra sensitive mouse insulin ELISA kit. Insulin concentrations were normalized for the number of islets.

Statistical analysis

All statistical analyses were performed by SPSS 12.0 statistical package for Windows (SPSS, Inc., Chicago, IL, USA). All values were expressed as mean ± SEM. Statistical difference among groups was assessed by one-way ANOVA with Dunnett's *post hoc* test. To compare data within the group, paired *t*-test was performed. Values were considered to be statistically significant at $p < 0.05$.

Results and discussion

Cinnamic acid lowers blood glucose and improves glucose tolerance in non-obese type 2 diabetic rats

Non-obese type 2 diabetic rats (body weight 160–180 g) were treated with cinnamic acid or cinnamaldehyde and their blood glucose lowering effect was observed with an acute experiment. No significant changes of blood glucose levels were observed in control diabetic rats or cinnamaldehyde-treated diabetic rats during the 3 h of experimental period (Fig. 1A). Interestingly, after oral administration to diabetic rats, cinnamic acid significantly decreased blood glucose levels in a time- and dose-dependent manner (Fig. 1A). Blood glucose lowering effect reached significant level at 2 h (7.28 ± 0.17 mmol/l; $p < 0.05$) and 3 h (6.95 ± 0.22 mmol/l; $p < 0.01$) compared to their 0 h value (8.08 ± 0.28 mmol/l) with the dose of 5 mg/kg. The dose 10 mg/kg could lower blood glucose level significantly ($p < 0.05$) at 1 h (7.43 ± 0.23 mmol/l *vs.* 8.18 ± 0.27 mmol/l). A further decrease ($p < 0.01$) of blood glucose lowering effects was found at 2 h (6.97 ± 0.18 mmol/l) and 3 h (6.62 ± 0.19 mmol/l) with 10 mg/kg cinnamic acid. GB (5 mg/kg) lowered blood glucose level more efficiently than cinnamic acid. The anti-diabetic effect of cinnamic acid lasts for 4 h, whereas the activity lasts for 8–12 h for GB. The concentrations of cinnamic acid in serum were 30.5 ± 4.3, 23.7 ± 3.2 and 17.2 ± 2.8 μg/ml after 1, 2 and 4 h of oral administration of cinnamic