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Differential anti-diabetic effects and mechanism of action of charantin-rich extract of Taiwanese *Momordica charantia* between type 1 and type 2 diabetic mice

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ABSTRACT

Momordica charantia Linn. (Cucurbitaceae), also called bitter melon, has traditionally been used as a natural anti-diabetic agent for anti-hyperglycemic activity in several animal models and clinical trials. We investigated the differences in the anti-diabetic properties and mechanism of action of Taiwanese *M. charantia* (MC) between type 1 diabetic (T1D) and type 2 diabetic (T2D) mice. To clarify the beneficial effects of MC, we measured non-fasting glucose, oral glucose tolerance, and plasma insulin levels in KK/HIJ mice with high-fat diet-induced diabetes (200 mg/kg/day of charantin-rich extract of MC [CEMC]) and in ICR mice with STZ-induced diabetes. After 8 weeks, all the mice were exsanguinated, and the expression of the insulin-signaling-associated proteins in their tissue was evaluated, in coordination with the protective effects of CEMC against pancreatic β-cell toxicity (*in vitro*). Eight weeks of data indicated that CEMC caused a significant decline in non-fasting blood glucose, plasma glucose intolerance, and insulin resistance in the KK/HIJ mice, but not in the ICR mice. Furthermore, CEMC decreased plasma insulin and promoted the sensitivity of insulin by increasing the expression of GLUT4 in the skeletal muscle and of IRS-1 in the liver of KK/HIJ mice; however, CEMC extract had no effect on the insulin sensitivity of ICR mice. *In vitro* study showed that CEMC prevented pancreatic β cells from high-glucose-induced cytotoxicity after 24 h of incubation, but the protective effect was not detectable after 72 h. Collectively, the hypoglycemic effects of CEMC suggest that it has potential for increasing insulin sensitivity in patients with T2D rather than for protecting patients with T1D against β-cell dysfunction.

1. Introduction

According to the projections of the World Health Organization, the diabetic population is likely to increase to 300 million or more by the year 2025 (King et al., 1998). Restoring normoglycemia reverses much of the beta-cell dysfunction, which has led to the belief that hyperglycemia or hyperlipidemia may play a causative role in the development of diabetes. Hence, these states are referred to as glucose toxicity or lipotoxicity (Robertson, 2004; Robertson et al., 2004). Clinically, 90% of diabetic patients are T2D (Modak et al., 2007). Insulin resistance has been defined as a

Abbreviations: MC, *Momordica charantia*; T1D, type 1 diabetic; T2D, type 2 diabetic; CEMC, charantin-rich extract of *Momordica charantia*; MCE, *Momordica charantia* extract; STZ, streptozocin; GHb, glycated hemoglobins; HFD, high-fat diet; TAG, triacylglycerol; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvate transaminase.

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condition of low insulin sensitivity, which is the ability of insulin to reduce blood glucose concentration (Kahn, 2003). Reduction of blood glucose concentration is achieved by stimulating glucose uptake in muscle and adipose tissues (Shiota et al., 2012). Insulin resistance contributes to multiple diseases, including T2D, dyslipidemia, hypertension, and central (abdominal) obesity (Carr and Brunzell, 2004).

The clinical manifestations and biochemical abnormalities of insulin resistance syndrome (also called dysmetabolic syndrome) are well defined (Schrauwen et al., 1998). Biochemical abnormalities in patients with insulin resistance syndrome include hyperinsulinemia, high triglyceride levels, high cholesterol levels, and low high-density lipoprotein (HDL). Currently available therapies for diabetes include insulin and various oral antidiabetic agents, such as sulfonylureas, biguanides, β-glucosidase inhibitors, and glinides, which are used as monotherapies or in combination to achieve better glycemic regulation (Silva et al., 2012). However, many of these oral antidiabetic agents have a number of serious adverse effects. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of investigation.

Momordica charantia Linn. var. abbreviata Ser (*Cucurbitaceae*), locally known as Ampalaya, which has been used as a traditional anti-diabetic agent in China, India, Africa, and the southeastern US, is a wild variety of bitter melon (BM) in Taiwan. Studies involving both animals and humans suggest that the fruits, seeds, and leaf extracts of this plant possess hypoglycemic activity in anti-hyperglycemic activity in alloxan (Rathi et al., 2002b; Kar et al., 2003) or streptozotocin-induced (Sitasawad et al., 2000; Grover et al., 2002; Rathi et al., 2002a) as well as genetic models of diabetes (Miura et al., 2001). Theoretical mechanisms of action include increased insulin-like effects and stimulation of pancreatic secretion, leading to decreased hepatic gluconeogenesis, increased hepatic glycogen synthesis, and increased peripheral glucose oxidation (Shibib et al., 1993; Yeh et al., 2003; Dans et al., 2007). No serious adverse effects were reported in clinical trial data (Ooi et al., 2012) and several studies of its toxicity reported that an acute (~4800 mg/kg) and a sub-chronic (1000 mg/kg) dose of *M. charantia* extract (MCE) were safe and caused no abnormal behaviors or biochemical alterations in rats (Fernandes et al., 2007).

One study (Rathi et al., 2002a) reported that chronic treatment with aqueous MC fruit extract (200 mg/kg, orally) caused a significant fall in plasma glucose levels in alloxanized rats and streptozocin (STZ)-induced diabetic mice, respectively. A subchronic study (Fernandes et al., 2007) of MCE in rats with alloxan-induced diabetes showed significant antihyperglycemic activity: blood glucose and the percentage of glycated hemoglobins (GHb%) were lowered, and the pattern of the glucose tolerance curve of the rats was also significantly altered. Oral aqueous MC extract (400 mg/day for 15 days) given to rats fed a fructose rich diet substantially prevented hyperglycemia and hyperinsulinemia (Vikrant et al., 2001). Additionally, insulin resistance has been implicated as a major contributor to the development of hyperglycemia in patients with T2D, and is linked to obesity, hypertension, dyslipidemia, and atherosclerosis (Reaven, 1998). MC extract greatly reduced body weight, improved glucose metabolism, and increased skeletal muscle insulin signaling in high-fat diet (HFD)-C57BL/6J mice after 12 weeks of treatment (Wang et al., 2011). It also reduced the fasting insulin, triacylglycerol (TAG), cholesterol and epididymal fat, which were increased by a high-fat diet in Wistar rats for 10 weeks. These data suggested that MC extract attenuates insulin sensitivity, improves glucose tolerance, and increases insulin signaling in high-fat-diet-induced insulin resistance and elevated serum lipids: it appears to be useful for managing T2D (Chen et al., 2003). Although an alcoholic extract of the whole fruit of MC has also been reported to improve oral glucose tolerance by significantly reducing plasma glucose, pancreatic β-cells (Sarkar et al., 1996; Hafizur et al., 2011), serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) activity (Sundaram et al., 2009), and hepatic glucose-6-phosphatase and fructose-1,6-bisphosphatase activity (Shibib et al., 1993) in rats with STZ-induced diabetes, and improving the islets of Langerhans in albino rats with alloxan-induced diabetes (Singh et al., 2008).

The MC fruit has been claimed to contain anti-diabetic phytomedicines. Charantin, one of its major active compounds, is believed to have an insulin-like chemical structure and properties (Patel et al., 2010; Hazarika et al., 2012). However, charantin did not show any hypoglycemic effects in male ddY mice (400 mg/kg) with alloxan-induced diabetes (Harinantenaina et al., 2006). The efficacy of low-dose oral charantin from MC on diabetes have not been well characterized (Raman and Lau, 1996); therefore, further studies on its mechanism of action are needed, as are further studies on its mechanism of action in T2D mice. In this study, we fed a high-fat diet to a strain of KK/HIJ mice in which the Ay mutation is introduced onto a KK-strain background, which is susceptible to T2D; metabolic abnormalities similar to those in humans are thereby induced in an obese mouse model of T2D (Lane, 2011). The aim of this study was to provide a guide for estimating how much charantin-rich extract of MC (CEMC) is needed to control diabetes, and to determine the efficacy and mechanisms of CEMC in HFD-controlled type 2 diabetes (T2D) mice compared with those in mice with STZ-induced type 1 diabetes (T1D).

In this study, in order to clarify the hypoglycemic effect of CEMC, the assessment of body weight, non-fasting blood glucose, oral glucose tolerance, and plasma insulin levels after oral CEMC (200 mg/kg/day for 8 weeks) were tested on high-fat diet-induced KK/HIJ mice (T2D) and STZ-induced ICR mice (T1D).

2. Methods

2.1. Chemicals and reagents

Culture medium RPMI-1640, fetal bovine serum, sodium bicarbonate, L-glutamine, and 0.05% trypsin–EDTA were from Gibco Ltd. Streptozotocin and sulforhodamine B sodium salt (SRB) were from Sigma (Saint Louis, Missouri, USA). Rabbit polyclonal antibodies against GLUT-4, IRS-1; mouse monoclonal antibodies against PPAR-γ; HRP (Horseradish peroxidase); anti-rabbit IgG; and HRP anti-mouse IgG were from Santa Cruz Biotechnology, Inc. (Delaware, California, USA). Nitrocellulose membranes were purchased from NEN Life Science Products (Boston, Massachusetts, USA). The Apoptosis Detection Kit was from Strong Biotech Co. (Washington, DC, USA). The mouse insulin ELISA kit was from Mercodia (Sylveniusgatan, Uppsala, Sweden).

2.2. Preparations of crude extracts of M. charantia

About 1 kg of pulverized crude extract of Taiwanese *M. charantia* (Hualien No. 1) fruit (the level of charantin is often less than 0.2% (w/w)) was suspended in 10 L of double-distilled water (ddH2O) and extracted by the 100% ddH2O with an extraction temperature of 20–22 °C, extraction frequency of 40 kHz, and extraction time of 0.5 h, which was provided by the MesoPhase Technologies, Inc. (Tainan, Taiwan). After the extraction, the size of residual powder particles was determined to be 70–300 nm using a laser particle-size analyser (Zetasizer Nano ZS; Malvern Instruments, UK), and so as to determine its steroidal saponin contents (charantin) by spectrophotometry. The charantin-rich extract of MC (CEMC) was then concentrated and dehydrated through the process of spray drying (Kingmech, FD 20L-6S, Taiwan) at class 10,000 cleanroom. All dried MC aqueous extracts (35% (w/w) charantin) were combined, and subsequently used for experiments in this study.

2.3. Animals and experimental design

The 6-week-old male KK/HIJ mice, weighing 19–22 g, were purchased from the Jackson Laboratory (Biolasco, Taiwan), and the 6-week-old male ICR mice, weighing 27–31 g, were from the National Laboratory Animal Center in Taipei, Taiwan. All animals were maintained in laminar flow cabinets under specific pathogen-free (SPF) conditions in facilities approved for Accreditation of Laboratory Animal Care and in accordance with Institutional Animal Care and Use Committee (IACUC) of the Animal Research Committee in Chi-Mei Medical Center, Tainan, Taiwan. The cages, bedding, food, and water were autoclaved. The two groups of mice were

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