Antidiabetic and Beta Cell-Protection Activities of Purple Corn Anthocyanins

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Abstract
Antidiabetic and beta cell-protection activities of purple corn anthocyanins (PCA) were examined in pancreatic beta cell culture and db/db mice. Only PCA among several plant anthocyanins and polyphenols showed insulin secretion activity in culture of HIT-T15 cells. PCA had excellent antihyperglycemic activity (in terms of blood glucose level and OGTT) and HbA1c-decreasing activity when compared with glimepiride, a sulfonylurea in db/db mice. In addition, PCA showed efficient protection activity of pancreatic beta cell from cell death in HIT-T15 cell culture and db/db mice. The result showed that PCA had antidiabetic and beta cell-protection activities in pancreatic beta cell culture and db/db mice.

Key Words: Antidiabetes, Purple corn anthocyanins, Beta cell-protection, Antihyperglycemic activity, Pancreatic beta cell, db/db Mice

INTRODUCTION
Type 2 diabetes mellitus is characterized by insufficient insulin secretion and insulin resistance (Goldstein, 2002). The insulin resistance results in hyperinsulinemia, amyloid deposits, and inflammation in pancreatic beta cells (Lebovitz and Banerji, 2004). Consequently, pancreatic beta cells mass and insulin secretion are gradually decreased. Sulfonylurea-based hypoglycemic agents that have been most prescribed for type-2 diabetic patients, directly stimulate insulin secretion from pancreatic beta cells sufficiently to lower blood glucose level through blocking potassium channel (Ashcroft, 1988; Ashcroft and Ashcroft, 1992). The sulfonylurea has well been prescribed with the medicine for insulin resistance like metformin. Disadvantages of using sulfonylurea-based medicines are insulin oversecretion and induction of pancreatic beta cells death (Efanova et al., 1998; Iwakura et al., 2000; Del Guerra et al., 2005; Maedler et al., 2005). Therefore, better medicine with sufficient insulin secretion and maintenance of pancreatic beta cell mass has been searched.

Anthocyanins are a class of antioxidant polyphenols and are present in various colored fruits, crops, beans and vegetables. The anthocyanins found most commonly are the glycosides of anthocyanidins. More than 400 naturally occurring anthocyanins are present in various colored fruits, crops, beans and vegetables. The anthocyanins found most commonly are the glycosides of anthocyanidins. More than 400 naturally occurring anthocyanins have been identified (Kong et al., 2003). Dietary intake of anthocyanins is associated with various therapeutic benefits including anti-inflammatory, cardioprotective, neuroprotective, and anticarcinogenic properties (Bickford et al., 1999; Juranic and Zizak, 2005; Bobe et al., 2006; Cirico and Omaye, 2006). Purple corn anthocyanins also reported to contain insulin secretion activity (Jayaprakasam et al., 2005). Cyanidine 3-glucoside-rich purple corn anthocyanins improved the high fat diet-induced obesity and hyperglycemia in mice (Tsuda et al., 2003). Cyanidine 3-glucoside reported to ameliorate hyperglycemia and insulin sensitivity due to down-regulation of retinol binding protein 4 expression in diabetic mouse (Sasaki et al., 2007). Dietary anthocyanin-rich bilberry extract reported to ameliorate hyperglycemia and insulin sen-
Materials and Methods

Materials

Powder of purple corn (Zea mays var. kculi) anthocyanin extract (extracted with ethanol, total anthocyanin content: 10%) was purchased from Zana Export Co. (Peru). Glimepiride (a sulfonfonylurea), dieoxyadenosine (DDA, an inhibitor of adenylate cyclase activity) and H89 (a PKA inhibitor) were purchased from Sigma-Aldrich Co. Various anthocyanins and polyphenols from plants were prepared for measuring insulin secretion activity as follows. 200 g of dried plants were crushed in thermomix then 400 ml of water were added and the mixture was heated to 80°C for 5 min. After 5 min the heating was turned off, and 600 ml of methanol (with 10 ml of acetic acid) was added. The anthocyanins were extracted for 2.5 h. The extract was then centrifuged (4°C, 30 min) and the supernatants were removed. The sample was then extracted again in 600 ml of 60% methanol (1% acetic acid) using the same procedure, and the supernatants were then combined. The supernatants were then evaporated at 40°C under vacuum to remove solvents. The residue was dissolved in water (0.1% acetic acid) and applied to a column of nonionic polymeric absorbent (Amberlite XAD-16). After washing with water (0.1% acetic acid) the anthocyanins were collected by elution with methanol (0.1% acetic acid) and then evaporated to dryness using a rotary evaporator. The residue was dissolved with HPLC grade water (0.1% acetic acid). Polyphenols were extracted with ethanol. Polyphenols were applied to Amberlite XAD-16 column and collected by elution with ethanol. Polyphenols were then evaporated to dryness using a rotary evaporator.

Animals

Our study was reviewed and approved by the Animal Care and Use Committee of Hallym University (Hallym 2009-87). Six-week-old male C57BL/KsJ db/db mice were purchased from Orient Bio (Sungnam, Korea), and they were acclimated for 1 week before being randomly assigned into the experimental groups. The animals were housed in a room with a 12-12 h light-dark cycle (8:00 AM to 8:00 PM), a temperature of 23 ± 1°C, and a humidity of 55 ± 5%. During the acclimatization period, animals were fed standard rodent chow (LabDiet, Richmond, VA, USA) and water ad libitum.

Measurement of insulin secretion from pancreatic beta cells

HIT-T15 cells (ATCC CRL-1777, hamster pancreatic beta cell line) were cultured in RPMI 1640 media containing 11.1 mM glucose with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin. The medium was changed every 2 days, and cells were subcultured every 5-6 days. HIT-T15 cells were seeded into a 24-well plate at a density of 2×10⁴ cells per well and grown for 24 h. The cells were washed twice and preincubated for 30 min in Krebs-Ringers bicarbonate (KRB) buffer (115 mM NaCl, 4.7 mM KCl, 2.56 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 20 mM NaHCO₃, 16 mM HEPES, and 0.3% bovine serum albumin, pH 7.4). Various anthocyanins and polyphenols were added to see insulin secretion activity. Cells were then incubated with KRB buffer containing 11.1 mM glucose for 1 h at 37°C. After incubation, cells were centrifuged and aliquots of supernatants were stored at -20°C until insulin measurement. Insulin levels were determined by mouse insulin ELISA kit (Shibayagi Co.).

Oral glucose tolerance test (OGTT) in db/db mice

The db/db mice were allowed to fast for 12 h prior to experiment and PCA (purple corn anthocyanins, 10 mg/kg of body weight) was administered orally 30 min prior to the glucose challenge. Glucose (2 mg/kg) was orally administered at 0 min, and the blood was withdrawn from the orbital venous plexus at 0, 30, 60, and 120 min after glucose administration. Blood glucose and insulin levels were determined by blood glucose meter (Accu-check Active, Roche) and mouse insulin ELISA kit (Shibayagi Co.), respectively.

Administration of purple corn extract to animals

Seven week old, C57BL/KsJ db/db mice were randomly divided into four groups (8 mice per group, the diabetic control group and three treatment groups. Purple corn (Zea mays var. kculi) anthocyanin extracts (Zana Export Co., Peru) were fed to the animal by dissolving in drinking water in the concentration of 10 mg/kg/day for 7 weeks. As a positive control, glimepiride, a sulfonfonylurea was added to drinking water and administered at a dose of 10 mg/kg/day. No addition to drinking water was a negative control. During the experiment, body weight and blood glucose levels were measured every week. OGTT, HbA1C, blood insulin level was measured at the first day and the last day of experiment.

Immunostaining

The pancreas was removed at the last day of experiment and fixed in 10% neutral buffered formalin. The tissues were subsequently embedded in paraffin and sectioned with thickness of 5 μm using a microtome (Leica, Wetzlar, Germany). The sections prepared onto aminosilane-treated slides were deparaffinized and rehydrated through graded alcohols to distilled water, and incubated with 0.1% trypsin and normal rabbit serum blocking solution for 30 min to block nonspecific binding of immunoglobulin. The sections were incubated with goat anti-insulin A (C-12) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h at room temperature and treated with 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity. The tissues were then incubated with biotinylated rabbit anti-goat IgG for 30 min at room temperature. The tissues were labeled using a modification of the avidin-biotin complex immunoperoxidase staining procedure (Vectorstain Elite ABC kit, Vector Laboratories, Burlingame, USA). Positive staining was visualized using DAB peroxidase substrate solution for 5-10 min, and tissues were counterstained with hematoxylin.

Cell viability and apoptosis analysis in HIT-T15 cells

The viability of HIT-T15 cells was estimated by measuring the rate of mitochondrial reduction of a methylthiazoletetrazolium (MTT, Sigma) after treatment of PCA and glimepiride (100 μg/ml) for 2 days. 1×10⁴ HIT-T15 cells were seeded in