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Regulation of signaling molecules associated with insulin action, insulin secretion and pancreatic β-cell mass in the hypoglycemic effects of Korean red ginseng in Goto-Kakizaki rats

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ABSTRACT

Ethnopharmacological relevance: Korean red ginseng (KRG) has long history as herbal remedy for antidiabetic effect.
Aim of the study: To study molecular mechanisms by which KRG ameliorates diabetes mellitus, we investigated whether the supplementation with the aqueous extract of KRG as a dietary admixture (1%, w/w) regulates the expressions of signaling molecules that are associated with insulin action, insulin secretion and pancreatic β-cell mass in spontaneously diabetic Goto-Kakizaki (GK) rats.
Methods: An aqueous extract of KRG was supplemented for the estimated dosage to be 0.2 g/kg rat/day beginning at 5 weeks of age for 12 weeks in male GK rats. Plasma glucose levels were measured every 4 weeks. The expressions of signaling molecules that are associated with insulin action, insulin secretion and β-cell mass in tissues were determined by Western blotting.
Results: The 12-week supplementation with KRG significantly ($P < 0.05$) decreased blood glucose compared to control. It up-regulated the expression of glucose transporter (GLUT) 4 in adipose tissue, and down-regulated the expression of protein tyrosine phosphatases (PTP)-1B in adipose tissue and skeletal muscle. It also up-regulated the expression of insulin and down-regulated the expression of uncoupling protein (UCP) 2, Bax and poly (ADP-ribose) polymerase (PARP) in pancreas.
Conclusions: These results suggest that GLUT4, PTP-1B, insulin, UCP2, Bax and PARP may be the primary targets of KRG that result in increase in insulin action and in insulin secretion, and decrease in β-cell mass, and that cause the normalization in glucose homeostasis.

1. Introduction

Korean red ginseng (KRG) (*Panax ginseng* C.A. Meyer Radix rubra Araliaceae), which is produced by steaming raw ginseng (*Panax ginseng* C.A. Meyer) at 98–100 °C for 2–3 h, has long been used as a traditional medicine in Korea to treat diabetes mellitus (The editing committee for the History of Korean Ginseng, 2001). KRG has been reported to have a hypoglycemic effect in type 2 diabetic animals (Franz et al., 2002; Kim et al., 2005; Liu et al., 2005; Lee et al., 2009) and humans (Sievenpiper et al., 2003; Vuksan et al., 2003, 2006; De Souza et al., 2011). KRG might mediate its hypoglycemic effect through a variety of mechanisms including actions on the insulin-secreting pancreatic β-cells and the target tissues that take up glucose. *In vitro* studies have demonstrated that KRG treatment increases insulin secretion from rat islets (Kim and Kim, 2008) and inhibits β-cell apoptosis (Kim and Kim, 2007). It has been reported that KRG administration improved insulin sensitivity in Otsuka Long-Evans Tokushima fatty rats (Lee et al., 2009). However, its molecular mechanisms of antidiabetic action have not been evaluated in animal models which have defects in insulin action, glucose-stimulated insulin secretion, and pancreatic β-cell mass. Non-obese Goto-Kakizaki (GK) rats are a highly inbred strain of Wistar rats that spontaneously develop a mild diabetic state (Goto et al., 1976). The genetic roden model develops hyperglycemia with defects in insulin action, glucose-stimulated insulin secretion and pancreatic β-cell mass (Movassat et al., 2008).

The pathways that regulate insulin action, insulin secretion and pancreatic β-cell mass are crucial in the development of type 2 diabetes. Among the pathways that regulate the metabolic action of insulin, glucose transporter 4 (GLUT4) transports glucose (Berger et al., 1989; Kahn et al., 1989), and protein tyrosine phosphatases (PTP)-1B terminates insulin signal in adipose tissue and skeletal muscle (Kennedy and Ramachandran, 2000). In β-cells, uncoupling protein (UCP) 2 negatively regulates glucose-stimulated insulin secretion (Chan et al., 2001; Zhang et al., 2001;

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Krauss et al., 2003). The loss of β-cell mass is mainly caused by apoptosis (Lee and Pervaiz, 2007). Among the pathways contributing to β-cell apoptosis, Bax expression (Mathis et al., 2001) and poly (ADP-ribose) polymerase (PARP) cleavage plays a crucial role (Mabley et al., 2001). To elucidate the mechanism on the antidiabetic effect of KRG, we examined the effect of KRG on the expression of signaling molecules associated with insulin action, insulin secretion and β-cell mass in Goto-Kakizaki (GK) rats.

2. Materials and methods

2.1. Materials

Specific antibodies for GLUT4, PTP-1B, insulin, UCP-2, Bax, cleaved PARP and β-actin were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Aqueous extract of KRG (Hong Sam Jung™; Korea Ginseng Cooperation, Daejeon, Korea) was purchased. The extract was made using KRG, which is a *Panax ginseng* strain, six-years-old, and cultivated in Korea. The contents of moisture, crude protein, crude fat, and crude ash were analyzed by the Food Analysis Center at Korea Food Research Institute according to the method outlined in the Korea Food Code (Korea Food and Drug Administration, 2007). The HPLC chromatogram of the KRG extract has been shown in our previous study (Kim and Kim, 2008).

2.2. Experimental design

Four-week-old male GK rats and age-matched, non-diabetic Wistar rats were purchased from Japan SLC, Inc. (Shizuoka Prefecture, Japan). All rats were individually housed in a temperature- and humidity-controlled room with a 12 h light/dark cycle. All animals were acclimated for 1 week prior to the experiment. At 5 weeks of age, the Wistar rats (Wistar-control) were assigned to a modified AIN-93G diet (Reeves et al., 1993) without the KRG extract. The GK rats were assigned by a stratified random design based on blood glucose and body weight to 1 of 2 modified AIN-93G diets for 12 weeks: (1) a control diet without KRG extract (GK-control); (2) the same diet but containing 1% KRG extract (GK+1% KRG extract). The concentration of KRG extract in the rat diet as 1% was calculated based on the daily dosage prescribed by the producers, which is 3 g per human. The pharmacological effect of a drug is assumed to be dependent on metabolic rate (MR). It is established that MR scales allometrically with relative body size (W) according to the power law $Y=aW^b$, where, for mammals, W means relative body size (W) in no unit, Y is the MR of one species of which the MR is unknown, a is the MR of another species of which the MR is known, the exponent b is generally taken to be 3/4 (Weibel et al., 2004). Thus MR per unit body mass of a 250 g rat is about four times higher than that of a 60 kg human. Therefore, 0.2 g/kg rat/day was calculated from 0.05 g/kg human/day, and 1% (w/w) diet was calculated assuming the diet consumption of a rat as 20 g/day. Each group consisted of eight animals. Rats had free access to the diets and water throughout the study. The composition of the semi-synthetic diet is shown in Table 1. The protein, fat, carbohydrate, and energy contents in the diets were formulated to be the same based on the composition analysis of the KRG extract (Table 2). Body weights were recorded weekly and food intake was measured twice a week for each rat by determining the pre- and post-weights of the food jars. The experiment was performed under the US guidelines for the care and use of animals in research (NIH publication #85-23, revised in 1985), and approved by the Institutional Animal Care and Use Committee of the Korea Food Research Institute.

Table 1
Composition of the experimental diets (%, w/w).[a]

Ingredients	Control (g/1000 g)	1% KRG diet (g/1000 g)
Casein	20.0	19.838
Cornstarch	39.7486	39.7486
Sucrose	23.2	22.4698
Cellulose	50.00	50.00
Soybean oil	7.0	6.9681
t-Butylhydroquinone	0.0014	0.0014
Mineral mixture[b]	3.500	3.4241
Vitamin mixture[c]	1.000	1.000
L-Cystine	0.3	0.3
Choline bitartrate	0.25	0.25
KRG extract	0	1
Energy (kJ/1000 g)	16857	16857

[a] Modified AIN-93G purified rodent diet.
[b] AIN-93VX Vitamin Mixture (Dyets, USA).
[c] AIN-93G mineral mixture (Dyets, USA).

Table 2
Contents of moisture, crude protein, crude fat, crude ash, carbohydrate and energy in extract of Korean red ginseng.[a]

Component	Wet content (g/100 g wet weight of extract)	Dry content[b] (g/100 g dry weight of extract)
Moisture	36.1 ± 0.113	–
Crude protein	10.4 ± 0.107	16.2 ± 0.167
Crude fat	2.04 ± 0.134	3.19 ± 0.210
Crude ash	4.85 ± 0.099	7.59 ± 0.155
Crude carbohydrate[c]	46.6	72.9
Energy[d]	1029 kJ/100 g	1611 kJ/100 g

[a] Values are means ± SD, $n=3$.
[b] Calculated from the wet contents.
[c] By difference.
[d] By calculation.

2.3. Blood glucose concentrations and parameters to tissue collection

Blood was collected under fasting conditions at 10:00 every 4 weeks from the tail vein into heparinized tubes and centrifuged at 2500*g* for 10 min at 4 °C. The supernatant fraction (plasma) was collected and stored at −70 °C until analyzed. Plasma glucose concentrations were measured by the glucose oxidase method using a commercially available kit from Young Dong Diagnostics (Yong-in, Kyonggi-do, Korea). At week 12 of the supplementation (age 17 weeks), after a 16 h starvation period, the rats were anaesthetized with diethyl ether and killed. Blood was collected from the vena cava, plasma was obtained, and plasma glucose concentration was measured by the glucose assay kit. The perirenal fat pad (adipose tissue), hind-limb muscle (Musculus gastrocnemius) and pancreata and were excised, frozen in liquid N_2, and stored at −70 °C until analysis.

2.4. Preparation of tissue homogenates and Western blotting

Adipose tissue, muscle and pancreas were washed twice with ice-cold phosphate-buffered saline (PBS, pH 7.4) and centrifuged at 800*g* for 3 min. Tissues were homogenized in lysis buffer (cellLytic MT Mammalian Tissue lysis/extraction reagent, Sigma Chemical Co., St. Louis, MO, USA) for 30 min at 4 °C. The lysates were centrifuged at 13,000*g* at 4 °C for 20 min. Protein concentrations were determined using the Bradford protein assay reagent (Quick Start Bradford Dye Reagent) according to the manufacturer's instructions (Bio-Rad Laboratories, Hercules, CA, USA) and 0.1 mg protein was loaded onto a 10% SDS-polyacrylamide gels,