Red Ginseng Administration Before Islet Isolation Attenuates Apoptosis and Improves Islet Function and Transplant Outcome in a Syngeneic Mouse Marginal Islet Mass Model

J.S. Kim[a], H.J. Jang[b,*], S.S. Kim[a], M.Y. Oh[b], H.J. Kim[b], S.Y. Lee[b], D.W. Eom[c], J.Y. Ham[d], and D.J. Han[e]

[a]Departments of Anesthesia and Pain Medicine, [b]Surgery, and [c]Pathology, Ulsan University College of Medicine, Gangneung Asan Hospital, Gangneung, South Korea; [d]Natural Medicine Center, Korea Institute of Science and Technology (KIST), Gangneung, Seoul, South Korea; and [e]Asan Medical Center, Seoul, South Korea

ABSTRACT

Background. Transplantation of isolated islets is a promising treatment for diabetes. Red ginseng (RG) is steamed ginseng and has been reported to enhance insulin secretion–stimulating and anti-apoptotic activities in pancreatic β-cells. In this study, we examined the hypothesis that pre-operative RG treatment enhances islet cell function and anti-apoptosis and investigated whether RG improves islet engraftment by transplant of a marginal mass of syngeneic islets pretreated with RG in diabetic mice.

Methods. Balb/c mice were randomly divided into 2 groups, and 1 group was administered RG (400 mg/kg/day orally) for 7 days before islet isolation. In vitro islet viability and function were assessed. After cytokine treatment, cell viability, function, and apoptosis of islet cells were analyzed. Furthermore, we studied the effects of RG in a syngeneic islet graft model. A marginal mass of syngeneic mouse islets was transplanted into diabetic hosts.

Results. Islet pretreatment with RG showed 1.4-fold higher glucose-induced insulin secretion than did control islets. RG pretreatment upregulated B-cell lymphoma 2 (Bcl-2) expression and downregulated Bcl-associated X protein (BAX), caspase-3, and inducible nitric oxide synthase (iNOS) expression. Glucose-induced insulin release, NO, and apoptosis were significantly improved in RG-pretreated islets compared with cytokine-treated islets. RG-pretreated mice exhibited improved marginal mass islet graft survival compared with controls.

Conclusions. These results suggest that pre-operative RG administration enhanced islet function before transplantation and attenuated cytokine-induced damage associated with apoptosis. These studies indicate that inhibition of apoptosis by RG significantly improved islet cell and graft function after isolation and transplantation, respectively.

THE TRANSPLANTATION of isolated islets of Langerhans has emerged as a promising treatment for diabetes mellitus [1]. However, the success of islet transplantation still faces numerous challenges mainly related to cell damage during islet isolation and early after transplantation [2–4]. This therapeutic approach has a major limitation that islets from 2 to 4 donors are required to achieve insulin independence in one recipient with diabetes [5]. Furthermore, the viability of islets is lost in the course of isolation to transplantation [6]. Therefore, developing new strategies to prevent islet quality loss during the isolation procedure is of great interest and necessary for improving engraftment and reducing the number of islets required to achieve normoglycemia in diabetes.

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*Address correspondence to Hyuk Jai Jang, Department of Surgery, Gangneung Asan Hospital, Gangwon-do, Gangneung, Sachean Myeon, Bangdong gil 38, South Korea. E-mail: jhj@gnah.co.kr

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Islets are already susceptible to various harmful stresses during organ preservation, digestion, isolation, and culture procedures. Several factors may contribute to the decrease of islet viability during isolation including ischemia, changes in temperature, exposure to collagenase and pancreatic enzymes, mechanical disruption, and osmotic stress [7]. These procedures are therefore expected to lead islets to apoptosis, which is the major cause of islet loss [6,8].

Panax ginseng has been widely used traditionally in oriental countries for its pharmacological effects such as anti-diabetic, anti-inflammatory, anti-oxidant, and anti-apoptotic properties. The anti-inflammatory and anti-apoptotic properties of ginseng, acting directly on β-cells, have been supported by numerous studies. Ginseng and its active compounds have been shown to prevent β-cell apoptosis triggered by different stimuli such as lipotoxicity, pro-inflammatory cytokines, and streptozotocin [9–11]. Red ginseng (RG, Ginseng Radix Rubra) is produced by steaming raw ginseng. RG had been reported to enhance insulin secretion–stimulating and anti-apoptotic activities in pancreatic β-cells [10,12].

RG treatment has not been used in islet transplantation and therefore we performed this study to examine the hypothesis that pre-operative RG administration to a donor might improve islet cell function and attenuate apoptosis, which are important to improve the islet transplantation success rate. Furthermore, we investigated whether RG improved islet engraftment after the transplantation of a marginal mass of syngeneic islets in diabetic mice.

METHODS

Animals

Male Balb/c mice (8 weeks old) were purchased from Dae Han Bio-Link Co, Ltd, (Eumseong, Korea). Our study protocol was reviewed and approved by the Animal Care and Use Committee of the Korea Institute of Science and Technology, Gangneung, Korea. Male 8-week-old inbred BALB/c mice were used in this experiment as donors and recipients. Diabetes was induced by intraperitoneal injections of streptozotocin (Sigma-Aldrich, St Louis, Mo, United States) at a dose of 180 mg/kg. Mice that exhibited nonfasting blood glucose concentrations greater than 350 mg/dL for 3 days consecutively were randomly assigned to 2 groups for transplantation.

Red Ginseng

The Korean RG extract was purchased from the Korea Ginseng Corporation (Seoul, Korea) and diluted with sterile water. According to the manufacturer's data, the main components of the Korean RG extract were ginsenoside Rg1 (2.01%), Rb1 (8.27%), Rg3 (s) (1.04%), Re (2.58%), Rc (3.90%), Rb2 (3.22%), Rd (1.09%), Rf (1.61%), Rh1 (0.95%), and Rg2 (s) (1.35%).

Mouse Islet Isolation

Islets were isolated from the Balb/c mice through the use of the collagenase digestion technique. In brief, 3.0 mL of collagenase P (0.8 mg/mL, Roche, Mannheim, Germany) was injected into the bile duct of the mouse, the swollen pancreas were gently excised, and other attached tissues were removed. The pancreas was then incubated with collagenase at 37.5°C for 15.5 minutes with gentle shaking. After incubation, cold Hank's balanced salt solution (HBSS) was added to stop the digestion. The tissue was then passed through a 400-mm screen and centrifuged on densities 1.108, 1.096, 1.069, and 1.037 Ficoll gradients, islets were collected from the interface, washed with cold HBSS, and individual islets were then hand-picked. The isolated islets were counted under a scaled microscope at ×40 to calculate islet equivalents (IEQ). One IEQ was the islet tissue mass equivalent to a spherical islet of 150-μm diameter. Islets were hand-picked individually with the use of the microscope to ensure pure islet preparations. After isolation, the islets were cultured overnight in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco Life Technologies, Grand Island, NY, United States) supplemented with 10% fetal bovine serum in an atmosphere of 95% air and 5% CO_2 at 37°C.

Treatment With RG

Pancreatic islets were isolated from Balb/c mice after they were randomly divided into 2 groups, which were the untreated control group and the treatment group, which was administered RG. Before islet isolation, the donor mice received RG by oral gavage at 400 mg/kg/day for 7 days; the islet cell yield, viability, function, and apoptosis were then assessed in vitro.

Isle Cell Viability

Islet viability was assessed through the use of fluorescent staining with diacetate-propidium iodide immediately and 24, 48, and 72 hours after the islets were isolated.

Islet Cell Function

Islet cell function was evaluated immediately after they were isolated, using static glucose incubation. The result was expressed as the stimulation index (SI), calculated by dividing the insulin secretion of islets challenged with a high glucose concentration (16.7 mmol/L) by the insulin secretion under low glucose conditions (1.6 mmol/L). The secreted insulin level was measured with the use of a mouse insulin enzyme-linked immunosorbent assay (ELISA) kit (ALPCO, Salem, NH, United States) according to the manufacturer's instructions.

Islet Insulin and DNA Content

The islets were washed with phosphate-buffered saline and extracted with hydrochloric acid (0.18 mol/L) in 75% ethanol for 18 hours at 4°C. The acid-ethanol extracts were collected for the determination of insulin content with the use of mouse insulin ELISA kits (ALPCO, Salem, NH, United States). The islet DNA content was quantified through the use of a PicoGreen kit (Molecular Probes, Eugene, Ore, United States) according to the manufacturer's instructions.

Islet Cell Apoptosis Assay

After islet cell isolation, cell death was evaluated through the use of a programmed cell death detection ELISA^{PLUS} kit (Roche, Burgess Hill, United Kingdom), according to the manufacturer's instructions. The absorbance of the reaction mixture was measured at 405 nm against a 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) solution, and ABTS stop solution was used as the blank with the use of a Novostar plate reader (BMG Labtech, Aylesbury, United Kingdom). The results are expressed in arbitrary units of oligonucleosome-associated histone.