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RESEARCH NOTE

Ameliorating Effects of a Nopal (*Opuntia ficus-indica*) Complex on Blood Glucose in *db/db* Mice

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Abstract This study investigated the effects of a nopal (*Opuntia ficus-indica*) complex (OF) on blood glucose metabolism in *db/db* mice for 4 weeks. Food and water intake significantly decreased in OF-treated mice compared to controls ($p<0.05$). In addition, blood glucose and plasma insulin levels were significantly reduced in the OF-treated group compared to the controls ($p<0.05$). Histopathological analysis showed that the morphology of pancreatic islets was improved in the OF-treated *db/db* mice. Immunohistochemistry of the pancreatic islets showed that insulin production and the number of β-cells apparently increased.

Keywords: *Opuntia ficus-indica*, hypoglycemic, insulin, pancreatic islet, *db/db* mice

Introduction

Diabetes mellitus (DM) is a major public health concern affecting approximately 2% of the world population (1). The major metabolic characteristic of DM is hyperglycemia, commonly caused by defects in insulin-related functions (2). Blood glucose levels are controlled by the balance between glucose production and utilization, which is often regulated by insulin. Therefore, improving plasma insulin levels via insulin injection or sulfonylurea treatment is one of the major therapeutic approaches for DM treatment.

In northeastern Asia, including Korea, China, and Japan, medicinal plants have been used to treat patients for thousands of years, and Oriental medicine practices still flourish. In Oriental medicine, 85-90% of the treatment materials are composed of phytochemicals derived from medicinal herbs (3), and usually, several herbs are formulated together to optimize health benefits. The compositions of formulated medicines, called Oriental herbal medicinal complexes, have been empirically determined for hundreds of years without scientific examination. Thus, evaluating the biological functions and effectiveness of currently prescribed herbal medicinal complexes is important.

Several herbal medicines have been reported to show preventive and treatment effects on DM. Of those, nopal (*Opuntia ficus-indica*) has been used for the management of diabetes in Korea as well as in the non-Asian country of Mexico (4-6). Nopal belongs to the family Cactaceae, and its fruits are called cactus pears (7,8). It appears that nopal may have multiple effects on metabolism, thus regulating glucose, lipid, total cholesterol, high density lipoprotein (HDL) cholesterol, and low density lipoprotein (LDL) cholesterol levels (7-10). Moreover, nopal has been reported to contain multiple potentially bioactive compounds. Two types of alkaloids (indicaxanthin and neonetanin) (11,12) and 5 types of flavonoids (isorhamnetin, quercetin, kaempferol, dihydrokaempferol, and dihydroquercetin) (13) have been isolated from nopal and analyzed.

We previously developed a formula that can optimize the hypoglycemic effects of nopal, which includes the addition of 6 other plants according to Oriental medicine

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theory. In Oriental medicine, *jiwhang* (*Rehmannia glutinosa* Liboschitz) is known to orchestrate bodily functions among different organs and to have antidiabetic and hepatoprotective effects (14). *Hanultari* (*Trichosanthes kirilowii* Maxim.) has been effective in treating diabetes in streptozotocin-treated diabetic rats (15). *Horopa* (*Trigonella foenum-graecum*) was found to control lipid metabolism (16,17) and improve liver function and hyperglycemia in diabetic rats (18). *Sanyak* (*Dioscorea radix*) has shown hypoglycemic and hypolipidemic effects in alloxan-treated diabetic animal models (19). *Mokdanpi* (*Paeonia suffruticosa*) has antioxidant activities by scavenging superoxide anions (20). Finally, *samchilgun* (*Panax notoginseng* (Burk) F.H. Chen), similar to ginseng, is known to synergize the overall pharmacological effects of antidiabetic drugs and normalize plasma glucose levels in *ob/ob* and *db/db* mice (21).

Here, we investigated the effects of a nopal complex (OF) on glucose metabolism in mice. The *db/db* mice were fed OF for 4 weeks.

Materials and Methods

Animals and samples Male *db/db* mice (type 2 diabetes induced mice) aged 12 weeks (44-46 g) were purchased from Samtako (Seoul, Korea). Primary antibodies (Insulin AB-5, Glucagon AB-1, Ki67) were purchased from Lab Vision (Fremont, CA, USA). 3,3'-Diaminobenzidine (DAB) solution was obtained from DakoCytomation (Glostrup, Denmark). The OF powders were provided by Dr. Paul Y. of Emperor's Acupuncture and Natural Herbs. The formula for the OF powders contained 7 types of medicinal plants. The composition was 65% nopal, 7% *hanultari*, 7% *mokdanpi*, 6% *jiwhang*, 6% *horopi*, 6% *sanyak*, and 3% *samchilgun*. Each plant was dried and ground with a cyclonic plant grinder and formulated precisely.

Animal care and experimental design The mice were fed a standard chow diet and water *ad libitum,* and were kept in a specific-pathogen-free room at 23±2°C with an alternating 12-hr light/dark cycle. After an 1-week acclimatization period, the *db/db* mice were randomly divided into 2 groups: *db/db* mice fed a control chow diet and *db/db* mice fed an OF diet (n=6/group). The OF diet group was fed chow containing 5%(w/w) OF extract for 4 weeks. Blood was collected retro-orbitally after anesthetization with 1% avertin. Blood glucose levels were measured every week for 4 weeks. After the experimental period, the mice were fasted for 12 hr and then blood samples were collected in purple-topped ethylenediamine tetraacetic acid (EDTA) tubes for further analysis. The pancreas was collected from each mouse and fixed in formaldehyde for histopathological analysis.

Blood assay Blood glucose concentrations were determined using a glucose analyzer (MyCare GAM 2200; Green Cross Co., Seoul, Korea). Plasma insulin levels were measured using a radioimmunoassay kit (Samkwang Medical Laboratory, Seoul, Korea).

Histology and immunohistochemistry of pancreatic islets from each mouse After 4 weeks of feeding, the pancreas was isolated, fixed in formalin, and embedded in paraffin. The specimens were thin-sectioned (4 μm) for slide preparation and deparaffinized in xylene 3 times for 5 min. The slides were rehydrated with graded ethanol. The sections were heated in 0.01 M citrate buffer for 10 min, treated with 3% H_2O_2 solution for 6 min, and then washed with Tris-buffered saline (TBS) for 3 min. The slides were stained with hematoxylin and eosin. Additionally, immunohistochemical staining was conducted with anti-insulin, anti-glucagon, and anti-Ki67 antibodies from Lab Vision. The sections were incubated with goat blocking serum for 20 min and then incubated with diluted primary antibody at 25°C for 30 min. After another wash in TBS, the sections were incubated with biotin-conjugated secondary antibody at 25°C for 30 min. After a third wash in TBS, DAB solution was added to stain the nuclei, and the color reactions were allowed to proceed at 25°C for 2 min. Hematoxylin was used for counterstaining.

Cell counts of pancreatic islets The number of immuno-stained pancreatic islets and cells was manually counted and confirmed by 3 independent trained technicians. The number of pancreatic islets and each cell type from the 3 technicians was averaged and used for data analysis.

Statistical analysis The results are expressed as means± standard error (SE). Two-group comparisons were performed using Student's t-test. Analysis of variance (ANOVA) was performed for multiple group comparisons, if necessary, and differences among samples were examined by Duncan's multiple range tests using SAS (version 9.13; SAS Institute, Cary, NC, USA). A $p<0.05$ was considered to indicate significance.

Results and Discussion

Food intake and blood assay The OF-fed group showed significantly lower food intake (Table 1). The OF-treated group also showed a slight increase in total water intake, whereas the untreated group showed a marked increase.

After 4 weeks of OF feeding, fasting blood glucose levels of the mice significantly decreased compared to mice fed the chow diet. This reduction in fasting glucose was observed after 2 weeks of OF feeding. At 4 weeks,

Table 1. Body weight change, total feed intake, total water intake, blood glucose, and plasma insulin levels in OF-treated and control *db/db* mice during 4 weeks of treatment

Group[1)]	Body weight change (g)	Total food intake (g)	Total water intake (mL)	First blood glucose (mg/dL)	Last blood glucose (mg/dL)	Plasma insulin (μU/dL)
db-C	7.4±0.7[2)]	172.7±3.2	336.7±8.2	409±70	492±77	2.2±0.4
db-OF	7.0±1.7	164.3±3.1*	345.0±9.4*	447±67	312±39*	3.5±0.6*

[1)]C, control, fed normal diet; OF, normal diet containing 5% OF powder
[2)]Data are mean±SE, n=6; *p<0.05 compared to control

fasting blood glucose was 492±77 in the control group and 312±39 mg/dL in the OF-fed mice (p<0.05; Table 1). Fasting insulin levels increased significantly after 4 weeks of OF feeding (3.5±0.6 μU/mL, p<0.05; Table 1). These data suggest that OF treatment resulted in hypoglycemic effects potentially due to improved insulin secretion from pancreatic β-cells.

Histology and immunohistochemistry of pancreatic islets The effects of OF on pancreatic islet morphology were also examined. Histological data show that the *db/db* mice had reduced numbers of pancreatic islets and smaller islet sizes as compared to non-diabetic mice. Pancreatic islet morphology was heterogeneous in the *db/db* mice compared to a regular homogeneous islet shape in the non-diabetic mice. β-Cells were the major cell type in the islets, and were found in the center of the islet; non-β-cells existed in the peripheral region of the islet. However, the islet structure appeared damaged and β-cells and non-β-cells tended to coexist in the islets of the *db/db* mice. OF feeding for 4 weeks improved pancreatic islet integrity in *db/db* mice. The number of pancreatic islets significantly increased in the mice after OF feeding (182±16.2 for OF-treated vs. 72±7.5 in control chow groups, p<0.05; Fig. 1, 2).

Immunohistochemical data showed that insulin synthesis was greatly reduced and occurred in β-cells scattered throughout the islet. In mice fed OF, the insulin synthesis pattern assessed by immunostaining was similar to that seen for non-diabetic mice. The percentages of insulin-positive β-cells from OF- and chow-fed *db/db* mice were 81±16.4 and 18±1.2%, respectively (p<0.05; Fig. 1, 2).

In addition, glucagon synthesis appeared to be altered by OF feeding. Immunohistochemical data showed a dramatic increase in glucagon-producing cells in the control *db/db* mice, and the staining pattern was scattered. However, glucagon production in the islets of OF-fed *db/db* mice markedly normalized and approached levels comparable to those of non-diabetic mice. The percentages of glucagon-positive cells were 51.3±2.2 and 24.9±2.7% for the control and OF groups, respectively (p<0.05; Fig. 1, 2).

Finally, pancreatic cell proliferation was assessed by Ki67-immunostaining. The results showed that cell proliferation

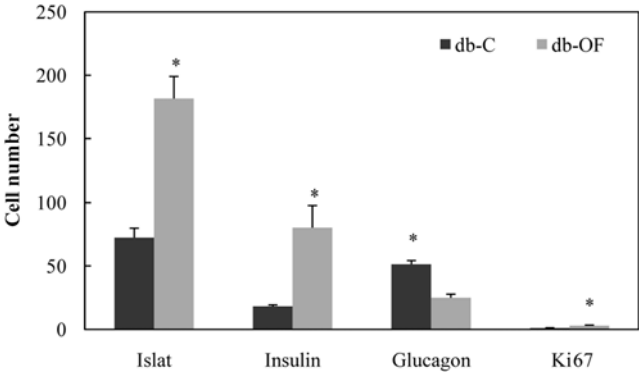

Fig. 1. Comparison of Langerhan's islet cell numbers (islet), insulin-positive cell numbers (insulin), glucagon-positive cell ratios (glucagon), and Ki67-immunostaining ratios (Ki67) in 15-week-old *db/db* mice after 4-week treatment with C, control (= fed normal diet) and OF, normal diet containing 5% OF powder. Data are mean±SE; *Indicates a p<0.05 vs. db-C

increased significantly in OF-fed mice. The percentage of actively proliferating islet cells was 3.0±0.3 in the OF-fed mice while it was 0.7±0.1% in the control mice (p<0.05; Fig. 1, 2).

The immunohistochemistry results demonstrated that cell proliferation after OF feeding predominantly occurred in β-cells in the pancreatic islets, and Ki67-immunostaining suggested that OF feeding may induce pancreatic β-cell proliferation followed by the induction of insulin secretion. Diabetes hyperglycemia is the result of multiple metabolic consequences, including increased hepatic glucose production and decreased hepatic glycogen synthesis and glycolysis. These are often caused by insulin resistance, hallmarked by reduced glucokinase activity and elevated levels of glucose-6-phosphate and PEPCK activities (22-24). All these contribute to the postprandial hyperglycemia and insulin resistance of individuals with diabetes (25). Since insulin is a key regulator of plasma glucose homeostasis, the function of the pancreatic β-cells is critical to normal glucose metabolism and chronic insulin deficiency and insulin insensitivity are the major causes of diabetes (26). Thus, induction of insulin release from the pancreatic cells by sulfonylureas has been used as one of the major therapeutic strategies to treat patients with diabetes.

Sulfonylureas, which are often used to treat type II

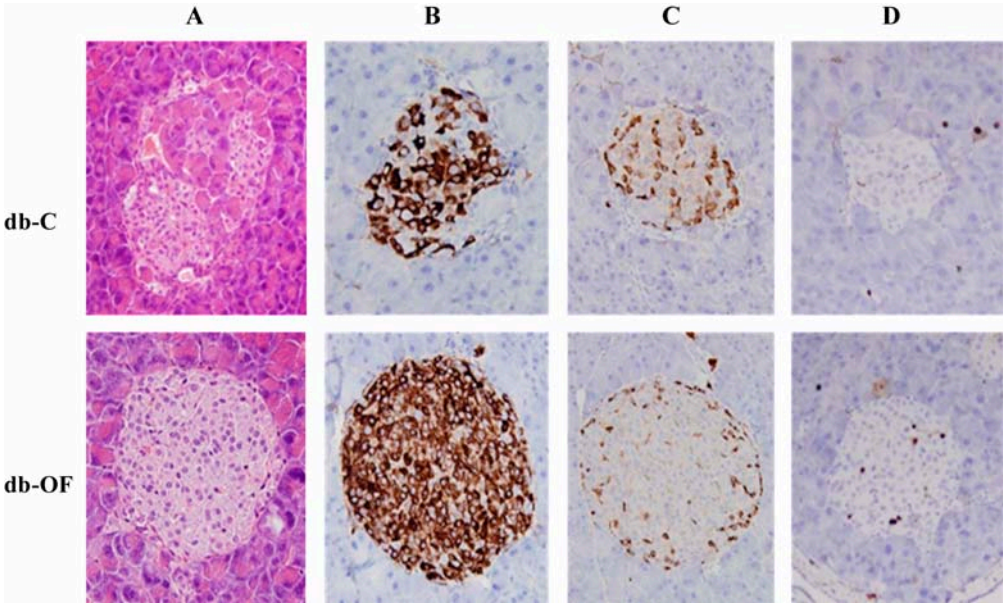

Fig. 2. Histopathological examination of the pancreas from untreated *db/db* mice. db, *db/db* mice; C, control (normal chow diet); OF, normal diet containing 5% OF powder. A, hematoxylin and eosin staining; B, anti-insulin antibody staining; C, anti-glucagon antibody staining; D, anti-Ki67 antibody staining (original magnification ×200)

diabetes, bind to an ATP-dependent K^+ channel called the sulfonylurea receptor (SUR1) on the cell membrane of pancreatic β-cells and inhibit a tonic, hyperpolarizing efflux of potassium, thus causing the electric potential over the cell membrane to become more positive. This depolarization opens voltage-gated Ca^{2+} channels and the resulting rise in intracellular calcium leads to increased fusion of insulin granules with the cell membrane, and therefore, increased insulin secretion (27). Other diabetic drugs, such as PPAR-γ agonists and AMPK activators, primarily affect insulin sensitivity in adipose tissue and only marginally influence pancreatic β-cell functions (28).

The results indicated that OF feeding enhanced plasma insulin levels and glucose tolerance. We cannot rule out the possibility that OF, especially a compound from nopal, may have sulfonylurea-like activity in pancreatic β-cells. However, based on our results, it appears that OF increased plasma insulin levels not by improving pre-synthesized insulin secretion, but by recovering overall β-cell function accompanied by islet cell proliferation. Therefore, it was assumed that the hypoglycemic effects of OF may operate differently from conventional diabetic drugs such as sulfonylureas. This approach could be especially beneficial for patients with chronic diabetes who have damaged islet cells. Further studies are needed to elucidate the active insulin-secreting compound produced as a result of OF treatment.

Since OF was formulated with 7 different herbs, OF feeding showed additional physiological effects. Note that OF reduced food intake and weight gain in *db/db* mice during the feeding period. The anorexigenic effects may also have contributed to the hypoglycemic effects of OF in the *db/db* mice. Several studies, including one by Kim *et al*. (29), reported that insulin secretion decreased in *db/db* mice due to damaged β-cells in the pancreatic islets. Our results indicate the same phenotype in *db/db* mice, but OF feeding clearly improved islet morphology. In *db/db* mice fed OF, islet cells showed round or ellipsoid shapes and were arranged in an orderly manner, while in untreated *db/db* mice, islet cells showed irregular shapes and β-cells and non-β-cells coexisted. Accordingly, the number of β-cells was 2.5 times higher in *db/db* mice fed OF compared to control *db/db* mice. When cell proliferation was assessed by Ki67 staining, OF feeding significantly ($p<0.05$) increased cell proliferation, probably in the β-cells, in *db/db* mice fed OF.

Our data show that 4 weeks of OF feeding significantly reduced fasting glucose levels, increased serum insulin concentrations, markedly alleviated glucose tolerance, and improved insulin production from pancreatic β-cells in *db/db* mice. These results support the idea that OF may have potent hypoglycemic effects mediated through improved pancreatic β-cell function, resulting in the induction of insulin secretion. Further studies are needed to confirm these effects in humans.

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