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Octaphlorethol A, a marine algae product, exhibits antidiabetic effects in type 2 diabetic mice by activating AMP-activated protein kinase and upregulating the expression of glucose transporter 4

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ABSTRACT

Octaphlorethol A (OPA), a type of phlorotannin isolated from Ishige foliacea has been shown to have antidiabetic activities. However, the mechanism of action of OPA in type 2 diabetes has not been investigated extensively. Here, we investigated the antidiabetic effects and mechanism of OPA in C57BL/KsJ-db/db mice, a model of type 2 diabetes. Levels of postprandial blood glucose were significantly lower in OPAtreated db/db mice than in control db/db mice. In addition, the OPA supplements significantly improved fasting blood glucose level and impaired glucose tolerance compared to control db/db mice. OPA also significantly decreased the level of serum insulin, augmented the activation of AMP-activated protein kinase (AMPK), and increased the expression of glucose transporter 4 (GLUT4) protein in skeletal muscle. In addition, it significantly suppressed the increases in hepatic mRNA expression level of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), gluconeogenesis-related enzymes. Therefore, the mechanisms of OPA may involve suppression of gluconeogenesis by inhibiting PEPCK and G6Pase activity in the liver and affecting GLUT4-mediated glucose uptake in skeletal muscle through activation of AMPK. These findings provide a new insight into the antidiabetic clinical applications of OPA and demonstrate the potential of OPA as a new drug candidate for type 2 diabetes.

1. Introduction

Diabetes mellitus is the most serious chronic disease that is associated with increasing obesity and aging in the general world population. It is largely classified into insulin-dependent diabetes mellitus (type 1 diabetes) and non-insulin-dependent diabetes mellitus (type 2 diabetes). Particularly, type 2 diabetes is a disorder of glucose metabolism characterized by induced hyperglycemia due to insulin resistance and is the most prevalent form of diabetes (Zimmet et al., 2001). Over the last few decades, the prevalence of type 2 diabetes has considerably increased worldwide and in Korea and is expected to increase further in the future because of increased consumption of a high-fat and high-glycemic diet resulting from economic development and changes toward a more sedentary lifestyle (Chan et al., 2009; Kim, 2011; Wild et al., 2004).

Currently available oral antidiabetic drugs such as sulfonylureas, metformin, rosiglitazone, α-glucosidase inhibitors, and thiazolidinediones, have a number of limitations, such as adverse effects and high rates of secondary failure. Therefore, recently, there has been a growing interest in alternative therapies and in the therapeutic use of natural products for type 2 diabetes, especially those derived from herbs (Chang et al., 2006; Hu et al., 2013; Jung et al., 2008; Lee et al., 2012a, Lee and Jeon, 2013). This is because plant sources are usually considered less toxic with fewer side effects than synthetic ones.

Marine algae and food are popular food and medicinal ingredients mainly in Asian countries such as Korea, Japan, and China

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and are known to have an abundance of bioactive compounds with great potential for use in the pharmaceutical, functional food, and biomedical industries (Lee et al., 2015; Ko and Jeon, 2015; Samarakoon et al., 2014). Several studies have focused on the isolation of phlorotannin compounds from marine algae, and those compounds have been found to show promising antidiabetic effects (Kang et al., 2013; Lee et al., 2012a, Lee and Jeon, 2013). *Ishige foliacea* is abundant along the coast of Korea's Jeju Island and is considered an edible brown alga. Octaphlorethol A (OPA) a type of phlorotannin, is one of the major and most active compounds in *I. foliacea*.

Our previous studies have proposed that OPA can be explored as a potential antidiabetic agent. For example, OPA influences glucose uptake in skeletal muscle cells (Lee et al., 2012b). Another study indicated that OPA alleviates postprandial hyperglycemia in streptozotocin-induced diabetic mice (Lee et al., 2014). Despite such results indicating the potential use of OPA as an antidiabetic agent, there is no study reporting its effects in type 2 diabetes thus far. Therefore, the purpose of this study was first to evaluate the antidiabetic effects of OPA in C57BL/KsJ-*db*/*db* (*db*/*db*) type 2 diabetic mice, and then investigate the mechanism underlying this effect.

2. Materials and methods

2.1. Materials

The brown alga *I. foliacea* (Phylum: Phaeophyta, Class: Phaeophyceae, Order: Ishigeales, Family: Ishigeaceae) was collected from the coast of Jeju Island, Korea. The samples were washed three times with tap water to remove salt, sand, and epiphytes attached to the surface, followed by careful rinsing with fresh water and maintenance in a medical refrigerator at −20 °C. Next, the frozen samples were lyophilized and homogenized with a grinder prior to extraction. All chemicals and reagents used were of analytical grade and were obtained from commercial sources.

2.2. Extraction and isolation

Dried *I. foliacea* powder was extracted three times with 80% methanol and filtered. The filtrate was evaporated at 40 °C to obtain the methanol extract, which was suspended in distilled water and partitioned using ethyl acetate. The active compound was separated from the ethyl acetate fraction using silica gel and Sephadex-LH 20 column chromatography. The active compound was finally purified by high-performance liquid chromatography (HPLC), and the structure of the active compound (Fig. 1) was identified by comparison of its NMR spectral data with those reported in the literature (Lee et al., 2012b).

2.3. Experimental animals and treatment

Male C57BL/KsJ-*db*/*db* (*db*/*db*) mice (5 weeks of age) and their age-matched non-diabetic mice (normal mice) were purchased from Joong Ang Lab Animal Co (Seoul, Korea). All mice were maintained under a 12 h light/dark cycle, temperature of 22 ± 1 °C, and humidity of 50 ± 5%. The mice were allowed free access to commercial pellet chow and water. Nine-week-old *db*/*db* mice were used in the experiment. The *db*/*db* mice were randomly divided into two groups ($n = 8$), namely, control *db*/*db* mice group and OPA-treated group. Control *db*/*db* mice were intraperitoneally injected with saline, while OPA-treated mice were intraperitoneally injected with OPA (5 mg/kg body weight) daily for 5 weeks. The dose of OPA was determined based on results from a preliminary study. The non-diabetic mice ($n = 8$) as normal group were compared with diabetic groups. The postprandial blood glucose level and body weight were measured weekly during the experimental period. The blood glucose level was monitored using the venous blood from the tail vein using a glucometer (Roche Diagnostics Gmbh, Mannheim, Germany). At the end of the experimental period, the mice were anesthetized after withholding food for 12 h, and blood samples were taken from the inferior vena cava to determine biochemical parameters. The liver and skeletal muscle were then removed, rinsed, immediately frozen in liquid nitrogen, and stored at −70 °C. The experimental protocol was approved by the Laboratory Animal Administration Committee of Jeju National University and performed according to the University Guidelines for Animal Experimentation.

2.4. Fasting blood glucose, insulin level, and intraperitoneal glucose tolerance test (IPGTT)

To determine the fasting blood glucose level, the mice were fasted for 12 h before sacrifice. The blood glucose concentration was monitored in the venous blood from the tail vein using a glucometer (Roche Diagnostics Gmbh). Blood samples from the inferior vena cava were collected and serum was obtained by centrifugation at 1000 × *g* for 20 min at 4 °C. The serum was carefully removed from the sample. The levels of serum insulin were determined using radioimmunoassay with an enzyme-linked immunosorbent assay ELISA kit (Linco Research Inc., Billerica, MA, USA). An intraperitoneal glucose tolerance test (IPGTT) was performed during the last week of the experimental period. Following an overnight fast, the mice were injected intraperitoneally with glucose (0.5 g/kg body weight), and the blood glucose levels were determined in tail blood samples 0, 30, 60, and 120 min after glucose administration.

2.5. Homeostatic index of insulin resistance

Homeostatic index of insulin resistance (HOMA-IR) was determined as a surrogate for insulin sensitivity (Haffner et al., 1997). The HOMA-IR was calculated by using the homeostasis model assessment as follows:

$$\text{HOMA} - \text{IR} = \text{fasting glucose(mmol/l)} \times \text{fasting insulin(mU/l)}/22.51$$

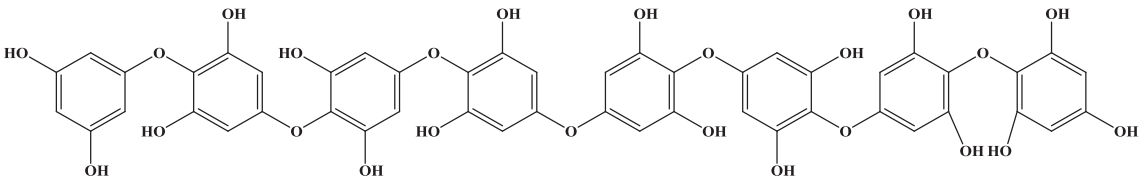

Fig. 1. The chemical structure of OPA isolated from *I. foliacea*.