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ER stress attenuation by *Aloe*-derived polysaccharides in the protection of pancreatic β-cells from free fatty acid-induced lipotoxicity

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ABSTRACT

Insulin resistance, a pathophysiology of type 2 diabetes, is associated with obesity. Lipotoxicity in obesity leads to the dysfunction and death of pancreatic β-cells and inadequate insulin production, thereby aggravating type 2 diabetes. The present study was conducted to determine the effect of *Aloe vera* polysaccharides (APs) as an anti-hyperglycemic agent and their mechanisms of action. Gel polysaccharides from *Aloe* extracts were separated using ultrafiltration devices with molecular weight-cutoff membranes, and the protective effect of APs on pancreatic β-cells in response to free fatty acids (FFAs) was determined. Hamster pancreatic β-cell line HIT-T15 was treated with palmitate and APs to analyze cellular responses. We observed a large number of apoptotic β-cell death after treatment with high levels of palmitate, but this was efficiently prevented by the addition of APs in a dose-dependent manner. It was found that the anti-apoptotic properties of APs were largely due to the relief of endoplasmic reticulum (ER) stress signaling. APs were effective in interfering with the FFA-induced activation of the PERK and IRE1 pathways as well as ROS generation, thereby protecting pancreatic β-cells from lipotoxicity. Although variation in the chain length of APs can influence the activity of FFA-mediated ER stress signaling in different ways, polysaccharide mixtures with molecular weights higher than 50 kDa showed greater antiapoptotic and antioxidant activity in β-cells. After oral administration of APs, markedly lowering fasting blood glucose levels were observed in *db/db* mice, providing evidence of the potential of APs as an alternative insulin sensitizer. Therefore, it was concluded that APs have a protective effect against type 2 diabetes by modulating obesity-induced ER stress in pancreatic β-cells.

1. Introduction

Type 2 diabetes mellitus is associated with lipotoxicity, which is one of the major causes of insulin resistance [1]. Obese and diabetic humans have elevated plasma levels of free fatty acids (FFAs) in their circulatory system [2]. Accumulating evidence suggest that long-chain FFAs, such as palmitate, mediate pancreatic β-cell failure and death *in vitro* and *in vivo*, and inhibit proinsulin synthesis and eventually glucose-stimulated insulin secretion [3]. Although the exact mechanisms underlying FFA-induced insulin resistance are not clear, many studies have suggested that FFA-induced ER stress is involved in the induction of lipotoxicity [4,5]. Accordingly, the role of FFAs in the pathogenesis of insulin resistance has received increasing attention in basic study and clinical practice. However, improvements in glucose control with fewer adverse effects are still required.

Aloe barbadensis Miller, commonly called *Aloe vera*, is the most widely used for the general promotion of health. For example, the administration of *Aloe vera* extracts has been shown to improve hyperlipidemia and hyperglycemia in diabetic rats [6,7]. Because *Aloe* plants contain multiple compounds with a variety of potential biological activities, many studies have sought to identify the primary components of *Aloe* extracts [7]. Analysis of the extracts has

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found that the amount of carbohydrates, including non-starch polysaccharides, was substantially higher than that of other materials. It is generally agreed that functional *Aloe vera* polysaccharides (APs) consist of linear chains of glucose and mannose molecules, with a molecular weight (MW) ranging from a few hundred Da to several thousand kDa. Different AP chain lengths may differ in their biological activity, but their physiological roles and exact modes of action remain to be clarified.

Prolonged exposure to high concentrations of FFAs has been shown to cause insulin resistance and β-cell dysfunction [5,8]. Obesity-induced apoptosis leads to a decrease in the number of pancreatic β-cells, where insulin is produced. Thus, pancreatic β-cell apoptosis may contribute to the increasing prevalence of type 2 diabetes mellitus. Of many ways to alter β-cell function and viability, Endoplasmic reticulum (ER) calcium depletion and ER stress may play a crucial role in FFA-induced β-cell death because insulin resistance significantly enhances the secretary demand in β-cells. ER is responsible for synthesis, maturation, ER-Golgi trafficking, and quality control for a variety of proteins. Under sustained or irresolvable ER impairment, ER stress switches from an adaptive to an apoptotic role. In pathological settings, stimuli that elicit ER stress can also cause not only disruption of ER homeostasis but also depolarization of the mitochondrial membrane, thereby activating JNK, IKK and downstream molecules, leading to inflammatory responses and cell death.

FFAs are believed to be an important factor in the onset and progression of insulin resistance and type 2 diabetes. The pancreas may be associated with ER stress because of its heavy duty of insulin synthesis and secretion in response to insulin resistance. In addition, *Aloe* has been known to have an anti-diabetic effect. These facts raise the possibility that APs may provide protective activity against FFA-induced ER stress and β-cell death, thereby improving insulin resistance. Thus, in this study, we attempted to determine whether APs, as a major component of *Aloe*, play a role in the attenuation of FFA-induced detrimental ER stress in β-cells and the amelioration of hyperglycemia in *db/db* mice.

2. Materials and methods

2.1. Cell culture and reagents

Hamster pancreatic β-cell line HIT-T15 was purchased from the American Type Culture Collection (Rockville, MD, USA). HIT-T15 cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum, 25 mM HEPES, 100 IU/ml penicillin-G and 0.1 mg/ml streptomycin, and 5% glutamax (Gibco-BRL). ER stress inducer, thapsigargin (Sigma) was dissolved in DMSO as recommended by the manufacturers.

2.2. Aloe preparation

APs were provided by KJM Aloe, Inc. (Seoul, Korea). *Aloe vera* gel was processed by patented concentration protocol in combination of ultra-filtration (UF) devices (Tami Industries, France) as described previously [9]. UF filtration using 50 and 150 kDa MW cutoff membranes allowed *Aloe* gel polysaccharides to be fractionated in some ranges of MW. The fresh AP fractions were immediately lyophilized. In order to prepare an aqueous form, each AP material was rehydrated in distilled water at 60 °C overnight with gentle shaking. After centrifugation at 3000 × g for 10 min, the supernatant was collected and stored at 4 °C. The polysaccharide content of each AP was determined by the quantitative colorimetric assay [10].

2.3. Western blotting

All procedures for western blotting were followed as previously described [11]. Antibodies against phosphorylated eIF2α at Ser51, phosphorylated JNK and phosphorylated p65 were purchased from Cell Signaling. Antibodies against GADD153 and actin were purchased from GeneTex.

2.4. RNA preparation and RT-PCR

Total RNAs were extracted using TRIzol reagent (Invitrogen). First strand cDNA conversion was done using SuperScript III reverse transcriptase according to manufacturer's instructions (Invitrogen). The cDNA products were PCR-amplified by primers specific for the indicated genes. The primer set used are as follows: XBP1 (forward), 5′-AAACAGAGTAGCAGCGCAGACTGC-3'; XBP1(reverse), 5′-TCCTTCTGGGTAGACCTCTGGGAG-3'. PCR products were separated on an agarose gel and stained with ethidium bromide. Quantitative intensity of the bands was determined using densitometry.

2.5. Cytotoxicity assay

Cytotoxicity was determined with the trypan blue exclusion assay. Briefly, cells were treated with variable concentrations of palmitate and APs and then incubated for 24 h. After harvest, the cell suspension in PBS was mixed with an equal volume of 0.4% trypan blue, and transferred to a hemocytometer for counting the number of both live and dead cells.

2.6. Annexin V apoptosis detection assay

2×10^5 HIT-T15 cells were seeded in 6-well plates and treated with palmitate and APs. After incubation, analysis was carried out using flow cytometer (FACS Caliber, Becton and Dickinson) with FITC-labeled annexin V antibody.

2.7. Measurement of intracellular ROS

The level of superoxide anion was analyzed using the superoxide detection system (Enzo Life Sci.) as described previously [11]. Briefly, cells were seeded in 6 well plates. After treatment, the cells were washed, incubated with superoxide staining solution for 30 min at 37 °C, and immediately analyzed using a flow cytometer (FACS Caliber, Becton and Dickinson).

2.8. Animals and experimental treatments

Five-week-old C57BL/KsJ-db/db male mice were used as spontaneous type 2 diabetic animal model. Mice were fed high fat diet (45% fat, HFD, Harlan Teklad) until stable hyperglycemia was achieved. Fasting blood glucose levels and body weight (BW) were measured regularly. Once the stable hyperglycemia (>250 mg/dl) was achieved, mice were fed HFD alone, HFD containing APs (100 μg/g) or metformin (100 μg/g) for 3 weeks. Blood samples were obtained from tail veins and blood glucose levels were measured using an electrochemical meter (OneTouch Ultra2, Lifescan). All experimental procedures were performed in strict compliance with the Guidelines for the Care and Use of Laboratory Animals issued by Korea University (IACUC number: KUIACUC-2016-18).

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