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Tyrosol, an olive oil polyphenol, inhibits ER stress-induced apoptosis in pancreatic β-cell through JNK signaling

Hyunjung Lee [a], Sung Won Im [a], Chang Hwa Jung [a, b], Young Jin Jang [a], Tae Youl Ha [a, b], Jiyun Ahn [a, b, *]

[a] Research Group of Metabolic Mechanism, Korea Food Research Institute, Seongnam 13539, South Korea
[b] Division of Food Biotechnology, University of Science & Technology, Daejeon 34113, South Korea

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ABSTRACT

Dysfunction of pancreatic β-cell is a major determinant for the development of type 2 diabetes. Because of the stimulated insulin secretion in metabolic syndrome, endoplasmic reticulum (ER) stress plays a central mediator for β-cell failure. In this study, we investigated whether an antioxidant phenolic compound, tyrosol protects against β-cell dysfunction associated with ER stress. To address this issue, we exposed pancreatic β cells, NIT-1 to tunicamycin with tyrosol. We found tyrosol diminished tunicamycin-induced cell death in a dose-dependent manner. We also detected tyrosol decreased the expressions of apoptosis-related markers. Exposure to tunicamycin evoked UPR response and co-treatment of tyrosol led to reduction of ER stress. These effects of tyrosol were mediated by the phosphorylation of JNK. Moreover, we confirmed supplement of tyrosol ameliorated β-cell loss induced by high fat feeding. Taken together, our study provides a molecular basis for signaling transduction of protective effect of tyrosol against ER stress-induced β-cell death. Therefore, we suggest tyrosol could be a potential therapeutic candidate for amelioration of type 2 diabetes.

1. Introduction

The accumulation of misfolded proteins in the endoplasmic reticulum (ER) generates a stress condition that engages the unfolded protein response (UPR) and this condition is referred to as 'ER stress'. The UPR is initiated by the activation of three distinct types of stress sensors located at the ER membrane, including protein kinase-like endoplasmic reticulum kinase (PERK), activating transcription factor 6 (ATF6) α and β, and inositol-requiring kinase 1α (IRE1α).

Pancreatic β cell, which stores and secrets insulin, is one of the specifically vulnerable cells to ER stress. In pancreatic β cells, ER is an important cellular compartment for insulin biosynthesis, which accounts for half of total protein production in these cells [1]. Metabolic dysregulation associated with obesity, such as excess nutrients and insulin resistance, has also been implicated in the secretory burden of the β-cell leading to ER stress and severely compromising cell function [2]. If ER homeostasis is disturbed as a result of increased demand for insulin secretion, ER stress proceeds and leads to β-cell apoptosis. A reduction of β-cell mass, due to increased β-cell apoptosis and defective β-cell regeneration, is a key component of diabetes [3].

Tyrosol, 2-(4-hydroxyphenyl)-ethanol, is one of the major phenolic compound contained in olive oil and has been reported to possess various physiological activities by its potent antioxidant activity [4]. Tyrosol shows antigenotoxic activity and prevents apoptosis in keratinocyte [5]. It also prevents the endothelial dysfunction by decreasing the expression of cell adhesion molecule [6] and inhibits platelet-induced aggregation [7]. Recently, tyrosol was reported to ameliorate hyperglycemia in diabetic rats [8]. However, little data exists on the molecular mechanisms involved in the beneficial effects of tyrosol on diabetes.

Therefore, the aim of the present study is to investigate the effects of tyrosol on ER stress-induced apoptosis of β-cell. For this, we

Abbreviations: ER, endoplasmic reticulum; UPR, unfolded protein response; PERK, protein kinase-like endoplasmic reticulum kinase; ATF6, activating transcription factor 6; IRE1α, inositol-requiring kinase 1α; HFD, high-fat diet; ND, normal-fat diet; Bip, binding immunoglobulin protein; CHOP, C/EBP-homologous protein; XBP-1, X box-binding protein 1; T2D, type 2 diabetes.

* Corresponding author. Research Group of Metabolic Mechanism, Korea Food Research Institute, 1201-62 Anyangpangyo-ro, Bundang, Seongnam, Gyeonggi 13539, South Korea.
E-mail address: jyan@kfri.re.kr (J. Ahn).

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examined the protective effect of tyrosol on ER stress-evoked apoptosis of NIT-1, pancreatic β-cell lines. We also measured whether tyrosol reduced ER stress-mediated UPR. Finally, we confirmed the protective effect of tyrosol against pancreatic β-cell dysfunction induced by high fat feeding.

2. Materials and methods

2.1. Cell culture and reagents

NIT-1 cells (ATCC, Manassas, VA), mouse insulinoma cell lines, were maintained in DMEM (Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C in 5% CO_2. Tunicamycin, a chemical inducer of UPR, and tyrosol were obtained from Sigma Aldrich (San Diego, CA).

2.2. Cell viability assay

Cell viability was analyzed using MTT assay. Cells were plated at 1×10^4 cells/well in 96-well plate and the next day exposed to indicated compounds for 24 h. MTT solution was added to the cells at a final concentration of 500 μg/ml, followed by incubation at 37 °C for 4 h. The medium was then removed, and the formazan product was solubilized using dimethyl sulfoxide. Cell viability was determined by measuring the OD at 570 nm.

2.3. Detection of apoptosis

Apoptotic cells were measured using the TUNEL assay (TUNEL Apoptosis Detection Kit, Millipore, Billerica, MA) which detects DNA strand breaks formed during apoptosis in accordance with the manufacturer's instructions. NIT-1 cells were plated at 4-well chamber slide and treated in a similar way for cell proliferation assays. At the end of the treatment, cells were washed with PBS, fixed with 4% paraformaldehyde for 15 min and permeabilized with 0.5% Triton X-100 for 15 min. The nuclei were stained using 4′, 6-diamidino-2-phenylindole (DAPI). Results were expressed as percentage of TUNEL-positive cells among the total number of cells.

2.4. Western blot

Cells were lysed in ice-cold RIPA buffer (Thermo Scientific, Waltham, MA) with complete protease inhibitor cocktail (Roche diagnostics). Western blot analysis was performed as previously described [9]. Total protein extracts were subjected to immunoblotting with antibides to Bip, p-PERK, PERK, p-eIF2α, eIF2α, CHOP, and β-actin (Cell Signaling, Danvers, MA).

2.5. Histological examination of pancreatic tissues

Six-week-old male C57BL/6J mice were fed a high-fat diet (HFD: 45% of total calories from fat) for 10 weeks. Then, mice were divided into 2 groups (n = 10 per group) and fed either HFD or HFD containing 0.05% tyrosol (HFD + Ty) for additional 8 weeks. Control mice were fed a normal-fat diet (ND: 10% of total calories from fat) throughout the experimental period. After 18 weeks on the diet, mice were sacrificed and pancreatic tissues were isolated. For histological examination, pancreatic tissues were fixed and stained with hematoxylin and eosin (H&E) or anti-insulin (BioGenex, Fremont, CA). The stained areas were observed using a light microscope (Olympus, Tokyo, Japan) with a magnification of 100×. All animal studies were conducted in accordance with a protocol approved by the Institutional Animal Care and Use Committee of the Korea Food Research Institute.

2.6. Statistical analysis

Results were expressed as the mean ± standard deviation. Statistical analyses were performed using Prism 6 software (GraphPad software, San Diego, CA). One-way analysis of variance (ANOVA) was used to compare quantitative data among groups. The Bonferroni post-hoc test was used if ANOVA indicated significance ($P < 0.05$).

3. Results

3.1. Tyrosol inhibits tunicamycin-induced cell death in NIT-1 cells

We first examined the effect of ER stress on NIT-1 cells viability. To induced ER stress, we treated NIT-1 with 2 μg/mL of tunicamycin for 6, 24, 48 h. As shown in Fig. 1A, tunicamycin significantly decreased cell viability and the reduction was increased according to exposure time. Then, we determined the effect of tyrosol on cell viability and found tyrosol (10, 25, and 50 μM) has no effect on NIT-1 cell viability after 24 and 48 h of treatments (Fig. 1B). To access whether tyrosol protects against tunicamycin-induced cell death, we exposed NIT-1 cells to tunicamycin and various concentrations of tyrosol simultaneously for 24 h. Interestingly, co-treatment of tyrosol effectively reduced tunicamycin-induced cell death in a dose-dependent manner (Fig. 1C).

3.2. Tyrosol protects against tunicamycin-induced apoptosis in NIT-1 cells

Normally, cells regulate the capacity of the ER to fold and process proteins and therefore control the balance between protein demand and folding capacity. However, when the imbalance occurs, the accumulation of unfolding proteins can lead to apoptosis [10]. To explore whether tyrosol suppresses apoptotic cell death induced by tunicamycin, we performed TUNEL assay. Treatment of NIT-1 cells with tunicamycin increased the number of apoptotic cells from 9.14% to 64.46%. However, co-treatment with 25 and 50 μM of tyrosol decreased the percentage to 29.24% and 16.53%, respectively (Fig. 2A and B). To determine if the protection was associated with an inhibition of the mitochondrial apoptotic pathway, we measured the expressions of apoptosis-related markers in protein levels. We observed tunicamycin induced activation of Bax and decrease of Bcl-1. This means tunicamycin evoked apoptosis through mitochondrial apoptotic pathway (Fig. 2C). And tyrosol effectively inhibited the changes of Bax and Bcl-2. Next, the extent of cleavage for caspase 3 and PARP was assessed. Tyrosol distinctly decreased the cleavages of full length caspase 3 and PARP which are the result of tunicamycin treatment. These results indicate that tyrosol effectively prevents tunicamycin-induced cell death by suppressing mitochondrial apoptotic death pathway.

3.3. Tyrosol decreased tunicamycin-evoked UPR in NIT-1 cells

To determine whether tyrosol inhibits tunicamycin-induced UPR, we measured protein expression levels of several ER stress markers. Induction of ER chaperone is an essential events of the UPR and a well-known ER stress marker. As shown in Fig. 3A, exposure to tunicamycin increased the expressions of binding immunoglobulin protein (Bip). Bip, also referred to GRP78, is an ER chaperone and associates with ER stress receptors. On accumulation unfolded proteins, Bip dissociates and triggers the UPR [11]. The addition of tyrosol reduced the tunicamycin-induced increase of Bip. PERK is responsible for the attenuation of general protein translation through phosphorylation of eIF2α. Phosphorylated eIF2α is known to stimulate expression of C/EBP-homologous