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ORIGINAL ARTICLE

The effect and action mechanism of resveratrol on the vascular endothelial cell by high glucose treatment

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Abstract To investigate the effect and action mechanism of resveratrol on the vascular endothelial cell by high glucose treatment. Primarily cultured human umbilical vein endothelial cells (HUVECs) were pretreated by resveratrol (0.2 μmol/L) and holding for 6 h, and then cultured in Dulbecco Modified Eagle Medium (DMEM) within 0.45 mmol/L of palmimte acid and 32.8 mmol/L of glucose, which is holding for 12 h. The cells were collected to analyze the expression of E-selected element. Supernatant of cultured cells, induced by 100 nmol/L insulin for 30 min, was used to analyze the nitric oxide content. Compared with normal control cells, the secretion of nitric oxide is stimulated by insulin decrease, however, the expression of E-selected element increased in HUVEC. Resveratrol treatment increased the secretion of nitric oxide stimulated by insulin and decreased the expression of E-selected element and partly counteracts the impairment of high glucose and palmitate acid on the function of endothelial cells. Resveratrol can improve and protect the function of high glucose and fatty acid cultured endothelial cell, and therefore may be a promising medicine in the prevention or therapy of diabetic macrovascular diseases.

1. Introduction

Resveratrol (trans-3,5,4′-trihydroxystilbene, RSV), is a polyphenol phytoalexin, which has a variety of diverse biochemical and physiological functions, and antiaging effects, and has attracted extensive attention (Bertelli et al., 1998; Lancon et al., 2004; Banerjee et al., 2009; Kelly, 2010;

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Walgren et al., 2000). The mechanism of RSV in antisenescence is extremely complicated and has not been clarified. The antioxidant properties and the ability of activating silencing information regulator (SIRT1) may mediate the antisenescence effect of RSV (Zorzano et al., 1997; Biasutto et al., 2010; Juan et al., 1998; Lagouge et al., 2006; Nagareddy et al., 2005; Wang et al., 2011). For example, RSV has direct free-radicals-scavenging capacity due to its structure of hydroxyl groups. Besides, RSV can decrease lipid peroxidation by chelating metals (Surhio et al., 2014; Ashraf et al., 2013). Nevertheless, the free-radical-scavenging ability is very limited relative to the strong free-radical-producing ability of the human body itself.

As well known, resveratrol belongs to phenolic phytoalexin, which is a natural antioxidant and free radical clearance agent (Towbin et al., 1979; Naureen et al., 2014; Kiyani et al., 2014). Recent studies have found that resveratrol, in addition to having resistance to atherosclerosis effect also has a significantly lower blood sugar which improves diabetes. Whether it has improved the effect of type 2 diabetes vascular lesions has not yet been reported. Vascular endothelial cell injury is early complications related to diabetes vascular lesions, its specificity protein E a select element is reflect endothelial cell damage, the reliability index of the activation (Sanchez-Lozada et al., 2010; Zeng et al., 2000; Leonard et al., 2003; Spanier et al., 2009). Nitric oxide is secreted by vascular endothelial cells vasodilatation factors, which have blood vessel protection. This study with high sugar and high fat cultivate people the original generation of umbilical vein endothelial cells (HUVECs) as the research object, research on resveratrol on endothelial cells E-selected element expression and secretion of nitric oxide effect (Sugimoto et al., 2005; Wang et al., 2008).

The aim of study in this paper is to investigate the effect and action mechanism of resveratrol on the vascular endothelial cell by high glucose treatment. Using in vitro culture human umbilical vein endothelial cells (HUVECs) induced by the hydrogen peroxide HUVECs damage model, the study of RSV on oxidative damage protection of endothelial cells and its relationship with apoptosis, RSV prevention mechanism of the heart, cerebrovascular disease, for the development of RSV and their analogs treatment traumatic disease of heart head blood-vessel providing experimental data and theoretical basis was performed.

2. Methods

2.1. Reagent

Pancreatic enzymes are produced by Invitrogen company; resveratrol, dimethyl sulfoxide (DMSO), palmitic acid, insulin were purchased from Sigma company; bovine serum albumin, glucose, DMEM medium and fetal bovine serum (FBS) are the products of Gibco company; Primers and reverse to record a polymerase chain reaction (RT-PCR) kit were purchased from Shanghai Sangon Biological Engineering Company; A fight of von Willebrand factor, second fight are the products of Wuhan Boster Biological; The nitric oxide test kit is the product of Nanjing Institute of Biological. Resveratrol was dissolved in DMSO, and mixed 1000 times that of the mother liquor by 1.5 μmol/L. DMSO culture cell concentration is less than 0.035%. Palmitic acid with bovine serum albumin (BSA) and NaOH as 10 mmol/L tendency for palmitic acid/BSA concentrate are used for setting aside. Nutrient solution concentration is 0.45 mmol/L.

2.2. Analytical methodology

Isolation, culture and identification of original generation of endothelial cells are controlled by the investigator. Aseptic operation was carried out in medical university and general hospital maternity healthy newborn umbilical cord of 10∼15 cm soaked in phosphate buffered solution (PBS), at 5° is saved, for 4 h. Umbilical vein endothelial cells are separated by 0.25% trypsin perfusion method. Vaccination in 25 cm^2 containing 20% fetal bovine serum DMEM medium cultivation in the bottle, day in liquid to pour out non adherent cells, after 2∼3 d in liquid. The cells after about 5∼7 d which totally integrate into 0.25% trypsin and 0.02% EDTA digestion are represented. The immunohistochemical method is used to identify the von Willebrand factor in endothelial cells (Safi et al., 2015; Butt et al., 2015).

2.3. Experimental groups

The second or third generation cells are grown well and used in the experiment. Start when the cells in 80% fusion experiments, serum-free synchronization for the night before the beginning of the experiment. Experiment groups are divided

Table 1 E-selected element and β-actin gene amplification primer sequences.

Gene name	Primer sequences	Gene product size (bp)
E-selected	Up: 5′CTATTTGTTTTCTTCTGTATGTTAG3′ Down: 5′CTCTGCTGTTCTGATCCTTATC3′	335
β-actin gene	Up: 5′CGTGACATTAAGGAGAAGCTG3′ Down: 5′CTAGAAGCATTTGCGGTGGAC3′	550

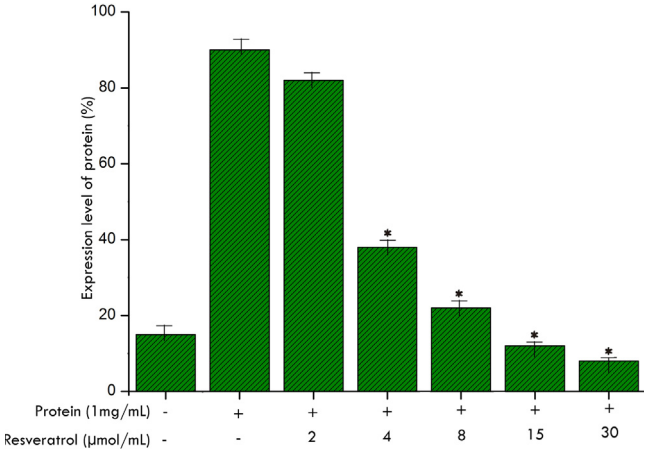

Figure 1 The expression level of protein in vascular endothelial cell (comparison with blank control group, $^{*}P < 0.01$).