



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  $\mu\text{mol/L}$ ) and holding for 6 h, and then cultured in Dulbecco Modified Eagle Medium (DMEM) within 0.45 mmol/L of palmitic 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 palmitic 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.

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## 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;

Walgren et al., 2000). The mechanism of RSV in antisense-silencing is extremely complicated and has not been clarified. The antioxidant properties and the ability of activating silencing information regulator (SIRT1) may mediate the antisense-silencing 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  $\mu\text{mol/L}$ . 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 concen-

trate are used for setting aside. Nutrient solution concentration is 0.45 mmol/L.

### 2.2. Analytical methodology

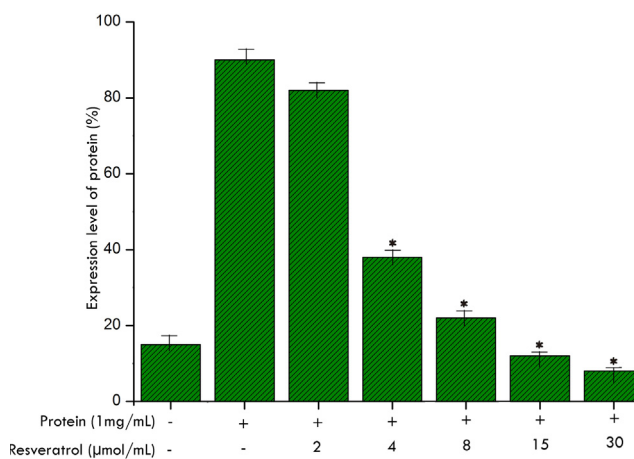
Isolation, culture and identification of original generation of endothelial cells are controlled by the investigator. Aseptic operation was carried out in medical university and general hospital maternity healthy newborn umbilical cord of 10~15 cm soaked in phosphate buffered solution (PBS), at 5° is saved, for 4 h. Umbilical vein endothelial cells are separated by 0.25% trypsin perfusion method. Vaccination in 25 cm<sup>2</sup> 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

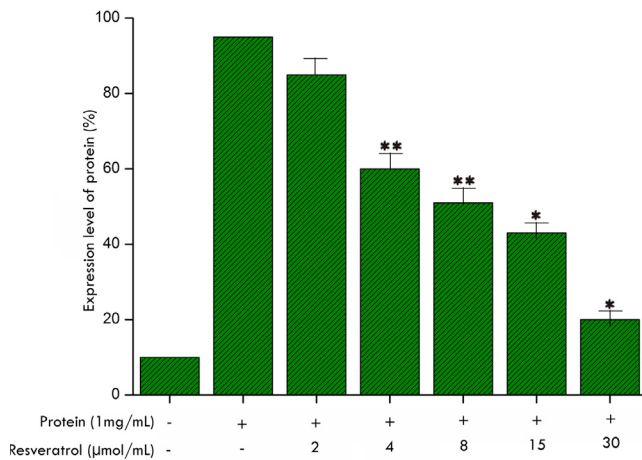
The second or third generation cells are grown well and used in the experiment. Start when the cells in 80% fusion experiments, serum-free synchronization for the night before the beginning of the experiment. Experiment groups are divided

**Table 1** E-selected element and  $\beta$ -actin gene amplification primer sequences.

| Gene name           | Primer sequences  | Gene product size (bp) |
|---------------------|---|------------------------|
| E-selected          | Up: 5'CTATTTGTTTCTCTGTATGTTAG3'<br>Down: 5'CTCTGCTGTTCTGATCCTTATC3' | 335                    |
| $\beta$ -actin gene | Up: 5'CGTGACATTAAGGAGAAGCTG3'<br>Down: 5'CTAGAAGCATTGCGGTGGAC3'     | 550                    |



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



**Figure 2** The expression level of protein in vascular endothelial cell (comparison with blank control group, \*\* $P < 0.05$ , \* $P < 0.01$ ).

into normal control group, high glucose and lipid group (32.8 mmol/L glucose and 0.45 mmol/L palmitic acid), pre-treatment of high sugar and 0.1 μmol/L resveratrol for 4 h group, which are cultured for 12 h containing 20% fetal bovine serum in DMEM medium (Batool et al., 2015; Khaskheli et al., 2015).

#### 2.4. Detection of RT-PCR

Cultured cells are using Trizol chloroform method for the extraction of total RNA. Application of RT-PCR method to detect a select element E gene expression, only chooses house-keeping genes β-actin.

Detection index primers are shown in Table 1. The reverse transcription step was carried out in accordance with the specifications. The target genes of PCR reaction system are as follows: the DDW 16 μL, 10 × buffer 2.5 μL, upstream primer 2 μL, downstream primer 2 μL, 10 × dNTP 1 μL, template 1 μL, 0.5 μL Taq polymerase, a total of 25 μL. Reaction steps are as follows: 90° for 10 min, 90° for 5 min, 60° for 5 min, 75° for 5 min, 75° for 10 min. A total of 20 cycles are tracked.

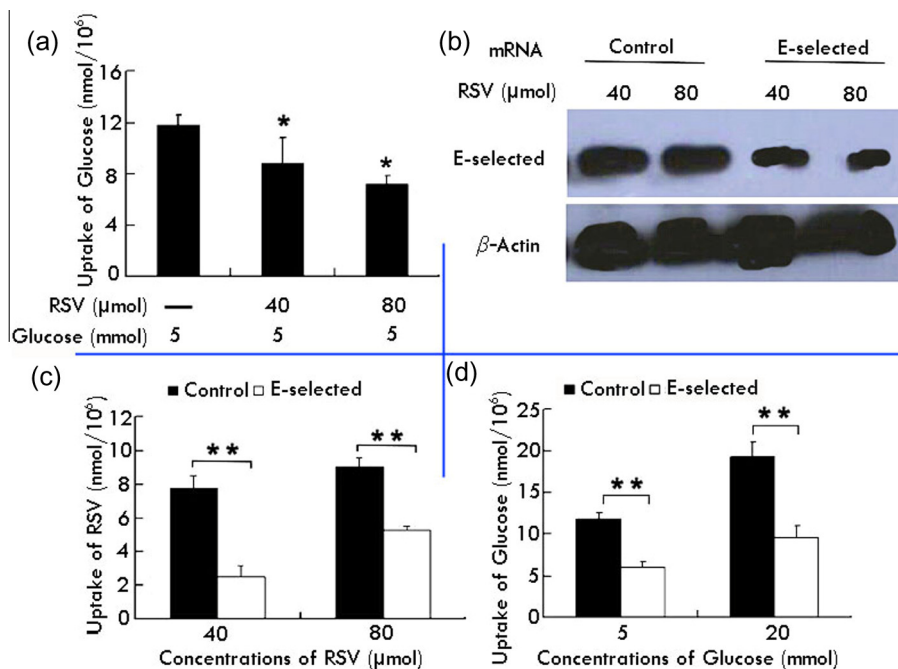
#### 2.5. Statistical analysis

It deals with all data ( $\bar{x} \pm s$ ) via the statistical software SPSS 17.0, the testing of  $\chi^2$  methods is used to compare with enumeration data, and its difference is considered statistically significant while  $P < 0.05$ .

### 3. Results

#### 3.1. Expression of protein

The expression level of protein in vascular endothelial cell is shown in Fig. 1. Two aspects of influence factors are resveratrol and expression of vascular endothelial cell by high glucose treatment (Towbin et al., 1979; Sanchez-Lozada et al., 2010). In comparison with blank control group, \* $P < 0.01$ , as seen in Fig. 1 expression level of the protein (1 mg/mL) and resveratrol (4 μmol/mL) is obviously lower than that of protein (1 mg/mL) and resveratrol (2 μmol/mL). Therefore, the expression level of protein (1 mg/mL) and resveratrol (15 μmol/mL) is obviously lower than that of protein (1 mg/mL) and resveratrol (8 μmol/mL), these trends are changing with the increase in concentration of resveratrol.



**Figure 3** (a) Effect of resveratrol (RSV) on glucose uptake in HUVECs was also detected. (b) E-selected was knocked down by E-selected transfection; (c) Thereafter, resveratrol; (d) Glucose uptake by endothelial cells was analyzed. Values are presented as  $\bar{x} \pm s$ . ( $n = 5$ ); \*\* $P < 0.01$ , \* $P < 0.05$ , compared with the blank control group.

While the statistical significance difference  $P < 0.05$ , the protein (1 mg/mL) and resveratrol (8  $\mu\text{mol/mL}$ ) are obviously lower than that of protein (1 mg/mL) and resveratrol (4  $\mu\text{mol/mL}$ ), and in another aspect of that the protein (1 mg/mL) and resveratrol (30  $\mu\text{mol/mL}$ ) are obviously lower than that of protein (1 mg/mL) and resveratrol (15  $\mu\text{mol/mL}$ ), the statistical significance difference  $P < 0.01$  (see Fig. 2).

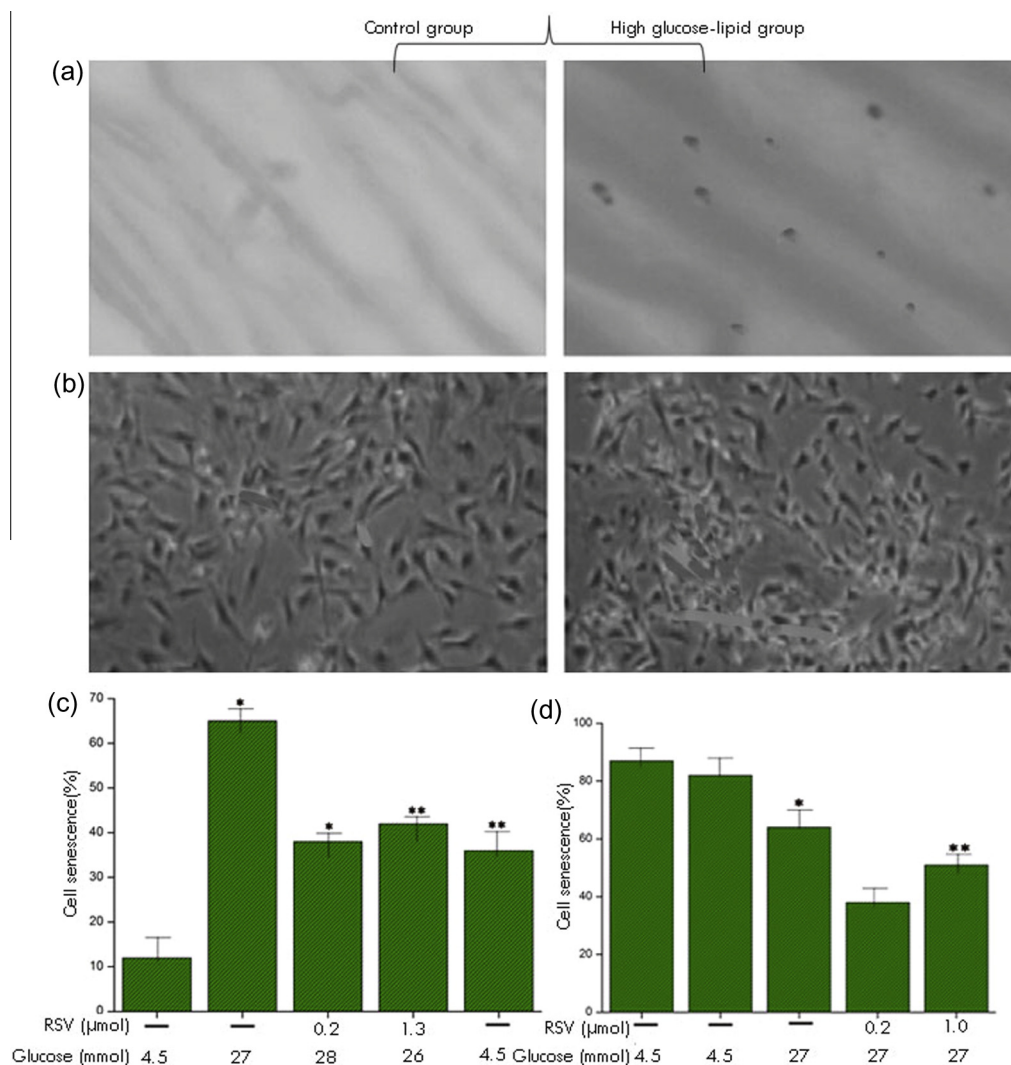
### 3.2. Effect of resveratrol on glucose uptake

As shown in Fig. 3a, immunofluorescence analysis indicated that HUVECs notably expressed E-selectin in cell membranes and cytoplasm. Moreover, in order to determine whether resveratrol uptake by endothelial cells involved E-selectin activity, we examined sodium dependence and inhibition of both glucose and protein on resveratrol transport (Zeng et al., 2000). As expected, the resveratrol uptake by cells in sodium-supplemented medium was dramatically higher than that in sodium-free medium. Moreover, we found that resver-

atrol supplement also impacts the interaction between E-selectin and its substrate glucose, leading to the reduction of glucose transport (Fig. 3c). Furthermore, the uptake of resveratrol (Fig. 3c) and glucose (Fig. 4d) was significantly reduced by E-selectin mRNA transfection (Fig. 3b). These results supported the hypothesis that the carrier mediated process of resveratrol uptake was, at least partially, E-selectin dependent on vascular endothelial cells.

### 3.3. Effect of resveratrol on E-selectin

The effect of resveratrol on E-selectin expression of high glucose-lipid treated vascular endothelial cell is shown in Table. 2, the detailed studies are divided into three groups of blank control group, high glucose-lipid group, resveratrol treated group where all the repeat times are five. It is obvious that the value of E-selectin expression is 0.52 lower in blank control group than that of 0.61 (Compared with the normal control group  $P < 0.05$ ) in high glucose-lipid group, while the



**Figure 4** RSV prevents the cell senescence within the exposure to high glucose. (a) Absence or presence of different amounts of RSV for 7 weeks. (b–c) BAECs were incubated for 7 days with different concentrations of glucose (4.5 mmol/L for control and 27 mmol/L for other groups) in the presence of RSV (0.1 or 1.0  $\mu\text{mol/L}$ ) as noted. (d) Effect of RSV on the viability of BAECs. \*Compared with the normal control group  $P < 0.05$ ; \*\*Compared with the high glucose-lipid group  $P < 0.05$ .



**Table 2** Effect of resveratrol on E-selected expression of high glucose–lipid treated vascular endothelial cell.

| Groups                    | Repeat time | E-selected    |
|---------------------------|-------------|---------------|
| Blank control group       | 5           | 0.52 ± 0.03   |
| High glucose–lipid group  | 5           | 0.61 ± 0.03** |
| Resveratrol treated group | 5           | 0.55 ± 0.02*  |
| F                         | 28.52       |               |
| P                         | 0.0007      |               |

\*\* Compared with the normal control group  $P < 0.05$ .

\* Compared with the high glucose–lipid group  $P < 0.05$ .

**Table 3** Effect of resveratrol on nitric oxide content expression of high glucose–lipid treated vascular endothelial cell.

| Groups                    | Repeat time | Nitric oxide <sup>#</sup> (μmol/L) |
|---------------------------|-------------|------------------------------------|
| Blank control group       | 10          | 76.34 ± 1.03                       |
| High glucose–lipid group  | 10          | 68.25 ± 3.05**                     |
| Resveratrol treated group | 10          | 110.27 ± 10.15*                    |
| F                         | 79.37       |                                    |
| P                         | <0.001      |                                    |

\*\* Compared with the normal control group  $P < 0.05$ .

\* Compared with the high glucose–lipid group  $P < 0.05$ .

<sup>#</sup> 100 nmol/L and 30 min nitric oxide content.

value of 0.55 in resveratrol treated group is lower than that of 0.61 in high glucose–lipid group.

### 3.4. Effect of resveratrol on nitric oxide content

The effect of resveratrol on nitric oxide content expression of high glucose–lipid treated vascular endothelial cell is shown in Table 3. The value of nitric oxide content is 76.34 in blank control group which is obviously higher than that of 68.25 in high glucose–lipid group (Compared with the normal control group  $P < 0.05$ ), at the same time, the value of nitric oxide content is 110.27 in resveratrol treated group which is obviously higher than that of 68.25 in high glucose–lipid group. Repeated time of the two is ten, and it has statistically significant difference.

## 4. Discussions

The histological appearances of representative vessels in different groups (Hung et al., 2000; Leonard et al., 2003) are shown in Fig. 1a. In contrast to vessels of controls, the high-glucose group showed an accumulation of positive cells in arteries. However, RSV can apparently reduce the blue cells in vessels. To investigate the concentration range of Res on protection of BAECs against senescence, we detected the effect of RSV (0.01, 0.1, 1.0, 3.0 μmol) on the viability of BAECs incubated in high glucose for 12 h with CCK-8 assay [29]. To determine whether or not the high glucose – induced change in viability of BAECs was due to the change in osmolarity, some BAECs were treated with a high concentration of mannitol. The high level of mannitol did not decrease viability, suggesting that the

high glucose-reduced viability of BAECs was caused by increased osmolarity (Spanier et al., 2009; Sugimoto et al., 2005).

RSV (0.01, 0.1, 2.0 μmol) protected against high-glucose induced decreases the viability of cells in a dose-dependent manner (Fig. 1d). To further study the effect of RSV on senescence of vascular cells, senescence was induced in BAECs by high glucose in the presence of 0–1 μmol of RSV. As shown in Fig. 1b–c, the number of positive cells was significantly increased by the exposure to high glucose. However, the increase was prevented by various amounts of RSV. The results of high-glucose-induced endothelial cells senescence were consistent with studies of other groups (Wang et al., 2008; Ota et al., 2010).

The strength of the vascular endothelial cell vitality reflected the state of cell proliferation, metabolism and vascular function, and LDH reflects the degree of cell membrane integrity and cell damage and death. The results show that the RSV reduces cell mitochondria injury and is induced by increasing H<sub>2</sub>O<sub>2</sub> cell vitality; H<sub>2</sub>O<sub>2</sub> damage and LDH leakage rate in cell cultures of model group is obviously higher than that of blank control group, and after giving RSV, cell culture that significantly reduces the amount of LDH in the supernatant fluid, prompt RSV can reduce the membrane damage caused by H<sub>2</sub>O<sub>2</sub>, which has protective effects on endothelial cells.

## 5. Conclusions

This study is found that high glucose and high fat culture endothelial cells can lead to insulin resistance, reducing endothelial protective factor nitric oxide secretion and increase atherosclerosis sex factor of E-selected element expression. Resveratrol has insulin resistance which improves endothelial cells, and promotes the secretion of nitric oxide and lowers E-selected element to express. Its effect on the improvement of the endothelial cell function may explain its improvement on glucolipid metabolism, insulin sensitivity, and the cause of the multiple roles of atherosclerosis. which is due to the structure of polyphenol of resveratrol and which provides a strong antioxidation role in atherosclerosis and protects the heart in a significant manner. Making it in the prevention and treatment of diabetic atherosclerosis has a unique advantage. In addition, resveratrol is a natural plant ingredient, has the safety of long-term use and has been proven in the prevention and treatment of Alzheimer's disease, malignant tumor, diabetes related diseases such as atherosclerosis, cardiovascular disease has obvious effects in the resistance, so the deep study of its effect on the prevention and treatment of diabetes and its chronic complications will be of great significance.

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.

### Conflict of interest

The authors have disclosed no potential conflicts of interest, financial or otherwise.

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