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# GINSENOSIDE RG1 COUNTERACTS PM<sub>2.5</sub>-INDUCED CELL INJURY BY MODULATING INTRACELLULAR REDOX VIA NRF2 PATHWAY IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

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**Objectives** The purpose of this study was to explore the possible mechanism of the organic component of fine particle PM<sub>2.5</sub>-induced endothelial cell injury and the cytoprotective effects of the Rg1.

**Methods** After stimulation with PM<sub>2.5</sub> and ginsenoside Rg1 for the indicated concentration (PM<sub>2.5</sub>: 0–800 µg/ml, Rg1: 2.5–40 µg/ml), the cell viability of HUVECs were assessed by CCK-8 assay, the oxidative stress of HUVECs were assessed by ROS and MDA assay. Furthermore, the influence of PM<sub>2.5</sub> and ginsenoside Rg1 on the expression of Nrf2, Keap1 and HO-1 was investigated by RT-PCR and Western blotting assays. The confocal microscopy was applied to observe the Nrf2 expression and nuclei translocation. Design and construct the short hairpin carrier direct against Nrf2, transfect cells with Lipofectamine2000, select the ShRNA, study the toxicity of PM<sub>2.5</sub> (400 µg/ml) with or without Rg1 (40 µg/ml) after Nrf2 is interfered, assay the cell viability by CCK-8, measure the reactive oxygen species, the expression of HO-1 protein.

**Results** PM<sub>2.5</sub>, at concentrations of 200, 400, and 800 µg/ml, reduced HUVECs viability by 30.7±2.8%, 54.8±3.9% and 70.2±2.8%, respectively, and the IC<sub>50</sub> value for PM<sub>2.5</sub> to reduce HUVEC viability by 50% was 526.9 µg/ml. Incubation for 48 h with Rg1 2.5, 10 and 40 µg/ml concentration-dependently antagonised the HUVECs viability decrease induced by PM<sub>2.5</sub>. Coincubation with Rg1 2.5, 10, 40 µg/ml, the IC<sub>50</sub> of PM<sub>2.5</sub> increased to 592.3, 825.6 and 1232.1 µg/ml respectively. PM<sub>2.5</sub> dose-dependently stimulated oxidative stress generation in HUVECs. In addition to cell death, pretreatment with Rg1 could also significantly increase HO-1 mRNA and protein expression and decrease ROS level and MDA production. The green fluorescence intensities in control and HUVECs incubated with PM<sub>2.5</sub> 800 µg/ml were 148.0±4.0 and 241.0±7.0 (p<0.05), respectively. PM<sub>2.5</sub>

(100–800 µg/ml) increases intracellular fluorescence in a concentration dependent manner. In presence of Rg1 10 µg/ml and 40 µg/ml, the maximal green fluorescence intensity increases induced by PM<sub>2.5</sub> 800 µg/ml were reduced to 216.0±2.0 and 206.0±3.0 (p<0.05) respectively. MDA concentration within control HUVECs was 0.87±0.07 nmol/mg protein. PM<sub>2.5</sub> (100–800 µg/ml) concentration dependently increased MDA with the maximum being 1.96±0.09 nmol/mg protein. Coculture with Rg1 (10 and 40 µg/ml) decreased PM<sub>2.5</sub>-induced MDA production by 72.96% (p<0.05) and 58.66% (p<0.05), respectively. Analysis using laser confocal microscopy revealed that Rg1 treatment led to a dramatic increase in the level of Nrf2. The ratio of cytoplasmic Nrf2 protein to nuclear Nrf2 protein was markedly reduced and reached maximum at 40 µg/ml (0.09) compared with that of the PM<sub>2.5</sub> only (0.3) and control (0.4). However the Keap1 has no changed. When the RNA of Nrf2 was interfered, the HO-1 protein expression was decreased, the cell viability was descend by 39.1% (PM<sub>2.5</sub>400 µg/ml), 36.0% (PM<sub>2.5</sub>400 µg/ml with Rg1), and ROS was increased by 40.2% (PM<sub>2.5</sub>400 µg/ml), 38.0% (PM<sub>2.5</sub>400 µg/ml with Rg1), compared with negative control group, and they both are of significant difference in statistics. The protective effect of Rg1 on cells was decreased.

**Conclusions** our results suggest that Nrf2 was activated and protect the cells from cytotoxicity caused by the organic component of fine particle PM<sub>2.5</sub> and Rg1 can attenuate PM<sub>2.5</sub>-induced cell damage by augmenting the cellular antioxidant defense including higher HO-1 expression. In addition, Rg1 also promoted nuclear translocation of Nrf2, which put new evidence on the cardiovascular-protective mechanism of Rg1 against the oxidative stress of PM<sub>2.5</sub> in Nrf2 pathway in HUVECs.