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GINSENOSIDE RG1 COUNTERACTS PM2.5-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 $PM_{2.5}$-induced endothelial cell injury and the cytoprotective effects of the Rg1.

Methods After stimulation with $PM_{2.5}$ and ginsenoside Rg1 for the indicated concentration ($PM_{2.5}$: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 $PM_{2.5}$ 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 $PM_{2.5}$ (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 $PM_{2.5}$, 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_{50} value for $PM_{2.5}$ 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 $PM_{2.5}$. Coincubation with Rg1 2.5, 10, 40 μg/ml, the IC_{50} of $PM_{2.5}$ increased to 592.3, 825.6 and 1232.1 μg/ml respectively. $PM_{2.5}$ 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 $PM_{2.5}$ 800 μg/ml were 148.0±4.0 and 241.0±7.0 ($p<0.05$), respectively. $PM_{2.5}$ (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 $PM_{2.5}$ 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. $PM_{2.5}$ (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 $PM_{2.5}$-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 $PM_{2.5}$ 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_{2.5}$400 μg/ml), 36.0% ($PM_{2.5}$400 μg/ml with Rg1), and ROS was increased by 40.2% ($PM_{2.5}$400 μg/ml), 38.0% ($PM_{2.5}$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 $PM_{2.5}$ and Rg1 can attenuate $PM_{2.5}$-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 $PM_{2.5}$ in Nrf2 pathway in HUVECs.