Protective Effect of Paeoniflorin on PM2.5-Induced BEAS-2B Cell Damage: Post-Print

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Date: 2018-06-15T00:00:00+00:00

Abstract

Objective: To investigate the protective effect of paeoniflorin against PM2.5-induced injury in human bronchial epithelial cells (BEAS-2B cells) and its underlying mechanism. Methods: A factorial design was adopted. MTT assay, TBA method, and chemifluorescence method were employed to measure the effects of different doses of paeoniflorin on PM2.5 exposure-induced inhibition of BEAS-2B cell growth, malondialdehyde (MDA) content in cell culture supernatant, and intracellular reactive oxygen species (ROS) levels. Results: With increasing PM2.5 intervention concentrations, cell viability decreased significantly ($P<0.05$), exhibiting a dose-response relationship ($r=-0.759$, $P<0.05$). With increasing co-treatment concentrations of paeoniflorin, the magnitude of PM2.5-induced decrease in BEAS-2B cell viability was significantly attenuated ($P<0.05$), although no dose-response relationship was observed ($P>0.05$). Additionally, PM2.5 exposure significantly increased both MDA content in cell culture supernatant and intracellular ROS levels ($P<0.05$). High-concentration paeoniflorin intervention significantly reduced both MDA content in cell culture supernatant and intracellular ROS levels compared with the exposure control group ($P<0.05$). Conclusion: Paeoniflorin demonstrates a significant protective effect against PM2.5-induced growth inhibition in BEAS-2B cells, and its mechanism may be associated with the antioxidant activity of paeoniflorin.

Full Text

Preamble

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Abstract

Objective: To investigate the protective effects of paeoniflorin against PM2.5-induced damage in BEAS-2B cells and explore the underlying mechanisms. **Methods:** Using a factorial design, we examined the effects of different doses of paeoniflorin on PM2.5-induced BEAS-2B cell growth inhibition, as well as its influence on malondialdehyde (MDA) content in cell culture supernatant and intracellular reactive oxygen species (ROS) levels, measured by MTT assay, TBA method, and chemiluminescence assay, respectively. **Results:** Exposure to increasing PM2.5 concentrations caused a significant decrease in cell survival rate ($P<0.05$) with a clear dose-response relationship ($r=-0.759$, $P<0.05$). Co-treatment with increasing paeoniflorin concentrations significantly attenuated the PM2.5-induced decline in BEAS-2B cell survival ($P<0.05$), though no dose-response relationship was observed for paeoniflorin alone ($P>0.05$). PM2.5 exposure significantly increased both MDA content in cell culture supernatant and intracellular ROS levels ($P<0.05$). High-concentration paeoniflorin intervention significantly reduced both MDA and ROS levels compared to the PM2.5-exposed control group ($P<0.05$). **Conclusion:** Paeoniflorin exerts significant protective effects against PM2.5-induced growth inhibition in BEAS-2B cells, and the mechanism may be related to its antioxidant properties.

Keywords: paeoniflorin; PM2.5; BEAS-2B cells; antioxidant effects

Introduction

Inhalable particulate matter (PM) has become an increasingly concerning air pollutant, with PM2.5 posing the most severe health risks to humans. The respiratory system serves as the primary target organ for PM2.5 exposure and action, and epidemiological studies have confirmed that PM2.5 exposure contributes to the onset and progression of numerous respiratory diseases [1-2]. Toxicological experiments have further revealed that the mechanisms underlying PM2.5-induced respiratory injury are primarily associated with oxidative stress and inflammatory responses [3].

Paeoniflorin, the active component of the traditional Chinese herb *Paeonia lactiflora*, is a natural antioxidant with diverse pharmacological effects including anti-inflammatory and analgesic properties [4], anti-allergic effects [5], anti-fibrotic activity [6], immunomodulatory functions [7], anti-hyperlipidemic effects [8], and anti-platelet aggregation [9]. This compound also demonstrates protective effects on various cardiopulmonary cells, alleviating damage induced by lipopolysaccharide, hydrogen peroxide, and hypoxia, with mechanisms likely related to its antioxidant and anti-inflammatory actions [10-12]. Guided by the traditional Chinese medicine principle of "preventive treatment," this study investigates the potential therapeutic value of paeoniflorin for PM2.5-induced

respiratory system injury, leveraging its antioxidant function to prevent and control environmental PM2.5 pollution-related health hazards [13].

Whether paeoniflorin exerts direct protective effects on bronchial epithelial cells exposed to PM2.5 suspension remains unreported in current literature. Therefore, this experiment employed human normal bronchial epithelial cells (BEAS-2B cells) exposed to concentrated atmospheric PM2.5 suspension, concurrently treating them with different paeoniflorin concentrations. We measured cell survival rates and toxicological indicators including intracellular MDA and ROS to investigate the interactive effects of various PM2.5 and paeoniflorin concentrations on bronchial epithelial cells. This study aims to evaluate the protective effects of paeoniflorin against PM2.5-induced bronchial epithelial cell injury and explore the underlying mechanisms at the cellular toxicology level.

Materials and Methods

1.1 Experimental Materials

Human bronchial epithelial cells (BEAS-2B cells) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Amresco) were used. Paeoniflorin (Solarbio Science & Technology, analytical grade, purity >98%) served as the intervention agent. Equipment included a KB-120F intelligent TSP-PM10 medium-volume sampler (Jinshida Electronic Technology), 2.0 m PTFE microporous membranes (Haichengshi Filtration Equipment), an Infinite M200 microplate reader (Tecan), an inverted fluorescence microscope (Qianxin Instruments), and a BD FACSCalibur flow cytometer.

1.2 Experimental Procedures

1.2.1 PM2.5 Sample Preparation Sampling was conducted from November 21, 2014, to March 15, 2015, on the seventh floor of the Health Law Building at Xi' an Jiaotong University Health Science Center. Samples were collected for 10 hours daily, with filter membranes replaced every two days. After collection, membranes were folded with the dust-containing side inward, stored in filter bags, and kept in a refrigerator protected from light. The collected PM2.5 filter membranes were cut into small pieces and completely immersed in 50 mL of triple-distilled water for ultrasonic separation to prepare a particle suspension. The suspension was aliquoted and stored overnight in a low-temperature refrigerator, then processed in a Scientz-10ND vacuum freeze dryer for dehydration. The resulting dry, gray, flocculent particles were stored in a low-temperature refrigerator for later use. PM2.5 particles were placed in cryovials and sterilized by freezing in liquid nitrogen for 24 hours. The particles were dissolved in 1640 culture medium to a final concentration of 2 mg/mL for subsequent experiments.

1.2.2 Measurement Methods Cell viability was measured by MTT assay using a microplate reader. Intracellular ROS levels were quantitatively detected by

flow cytometry and qualitatively observed by inverted fluorescence microscopy. MDA content in cell culture supernatant was determined by thiobarbituric acid (TBA) colorimetric method.

1.3 Statistical Analysis

This experiment employed a factorial design with two factors: paeoniflorin intervention concentration and PM2.5 intervention concentration. Factorial analysis of variance was used to compare the main effects and interaction effects of paeoniflorin and PM2.5 concentrations. One-way ANOVA was used to compare the individual effects of paeoniflorin intervention concentrations. The significance level was set at =0.05.

Results

2.1 Effects of Different PM2.5 Concentrations on BEAS-2B Cell Viability

BEAS-2B cells in good growth condition were treated with various PM2.5 concentrations for 24 hours, and MTT assay was used to evaluate the effects on cell growth and proliferation. A dose-response relationship was observed between PM2.5 concentration and cell survival rate (r=-0.759, P=0.018). PM2.5 exposure exhibited biphasic effects: low concentrations (0.1 and 1.0 g/mL) promoted cell proliferation, while high concentrations (10, 200, 400, and 800 g/mL) inhibited proliferation. Treatment with PM2.5 at 100, 200, 400, and 800 g/mL for 24 hours significantly reduced cell survival rates compared to the control group (0 g/mL) (P<0.05, [Figure 1: see original paper]).

2.2 Effects of Different Paeoniflorin Concentrations on BEAS-2B Cell Viability

BEAS-2B cells in good growth condition were treated with various paeoniflorin concentrations for 24 hours. MTT assay revealed that low paeoniflorin concentrations (0.001, 0.01, 0.1, and 1.0 mol/L) inhibited cell proliferation, with 0.01 and 0.1 mol/L significantly reducing cell survival rates. In contrast, high concentrations (10, 100, and 1000 mol/L) promoted cell proliferation, with 100 mol/L significantly increasing BEAS-2B cell survival (P<0.01, [Figure 2: see original paper]).

2.3 Protective Effects of Paeoniflorin Against PM2.5-Induced Growth Inhibition in BEAS-2B Cells

Cells in good growth condition were co-treated with various PM2.5 and paeoniflorin concentrations for 24 hours. MTT assay was then performed to evaluate the protective effects of paeoniflorin against PM2.5-induced growth inhibition. The results of factorial analysis examining the main effects, interaction effects,

and individual effects of paeoniflorin and PM2.5 concentrations are presented in .

2.3.1 Effects of Different PM2.5 Concentrations on Cell Survival Factorial analysis of variance revealed that cell survival rates decreased significantly with increasing PM2.5 concentrations ($P<0.0001$). Across different paeoniflorin intervention concentrations, cell survival rates increased with rising paeoniflorin levels ($P<0.0001$). However, no significant interaction effect was observed between paeoniflorin and PM2.5 concentrations ($P>0.05$).

2.3.2 Effects of Different Paeoniflorin Concentrations on Cell Survival Across PM2.5 Groups Individual effect analysis of paeoniflorin intervention was performed using one-way ANOVA to compare differences in cell survival rates among paeoniflorin dose groups within each PM2.5 concentration. The results showed no statistically significant differences in most PM2.5 concentration groups, except for the 800 g/mL PM2.5 group, where cell survival rates increased significantly with paeoniflorin concentration ($P<0.05$).

2.3.3 Effects of Different Paeoniflorin Concentrations on Cell Survival Dunnett' s t-test was used for multiple comparisons between each paeoniflorin concentration and the control group (0 mol/L). The 1 mol/L paeoniflorin group showed significantly higher cell survival rates compared to the control ($P<0.05$). When comparing the 10 and 100 mol/L paeoniflorin groups to the PM2.5-exposed control group, cell survival rates were significantly increased ($P<0.0001$).

2.4 Protective Effects of Paeoniflorin on PM2.5-Induced BEAS-2B Cell Injury

Cells in good growth condition were co-treated with various PM2.5 and paeoniflorin concentrations for 24 hours, after which intracellular ROS levels and MDA content in cell culture supernatant were measured.

2.4.1 Effects of Different Paeoniflorin Concentrations on PM2.5-Induced Intracellular ROS Content Factorial analysis of variance revealed that intracellular ROS content increased significantly with rising PM2.5 concentrations ($P<0.0001$). Conversely, ROS content decreased with increasing paeoniflorin concentrations ($P<0.0001$). A significant interaction effect was observed between paeoniflorin and PM2.5 concentrations on intracellular ROS content ($P<0.0001$).

Individual effect analysis showed that within each PM2.5 concentration group, intracellular ROS content decreased significantly with increasing paeoniflorin dose ($P<0.0001$). Dunnett' s t-test multiple comparisons revealed that all

three paeoniflorin concentration groups (1.0, 10, and 100 mol/L) showed significantly reduced intracellular ROS content compared to the PM2.5-exposed control group (P<0.0001,).

2.4.2 Effects of Different Paeoniflorin Concentrations on PM2.5-Induced MDA Content Factorial analysis demonstrated that MDA content in cell culture supernatant increased significantly with rising PM2.5 concentrations (P<0.0001) and decreased with increasing paeoniflorin concentrations (P<0.0001). However, no significant interaction effect was observed for MDA production (P>0.05).

Individual effect analysis using one-way ANOVA revealed that in the 400 and 800 g/mL PM2.5 groups, MDA content in cell culture supernatant decreased significantly with increasing paeoniflorin dose (P<0.0001). In the 0 g/mL PM2.5 group, MDA content also decreased with rising paeoniflorin concentration (P<0.05). Dunnett' s t-test multiple comparisons showed that the 1.0 mol/L paeoniflorin group had significantly lower MDA content compared to the PM2.5-exposed control group (P<0.0001), while the 10 and 100 mol/L groups also showed reduced MDA levels (P<0.05,).

2.4.3 Qualitative Observation of ROS Content in PM2.5-Exposed BEAS-2B Cells Treated with Paeoniflorin Fluorescence microscopy was used for qualitative observation of ROS levels in BEAS-2B cells. The results showed that intracellular fluorescence intensity increased with rising PM2.5 concentrations, while fluorescence intensity decreased markedly with increasing paeoniflorin concentrations ([Figure 3: see original paper]).

Discussion

Previous studies have demonstrated that atmospheric PM2.5 exerts significant cytotoxic effects on BEAS-2B cells, affecting cell growth and proliferation [14-16]. Our findings are consistent with these reports, showing a clear dose-response relationship between PM2.5 concentration and cell survival rate, confirming the pronounced cytotoxicity of PM2.5. Additionally, we observed that low PM2.5 concentrations stimulated BEAS-2B cell proliferation, possibly related to hormesis—a phenomenon where low-dose toxins cause micro-disturbances to homeostasis, triggering repair and maintenance mechanisms such as activation of transcription factors and kinases that increase expression of cytoprotective and repair proteins.

As no previous studies have investigated paeoniflorin-induced cytotoxicity in BEAS-2B cells, its threshold dose remained unclear. Therefore, we treated BEAS-2B cells with various paeoniflorin concentrations and evaluated its toxic effects using MTT assay after 24 hours. The results revealed an S-shaped growth curve: low paeoniflorin concentrations inhibited BEAS-2B cell growth and proliferation, while high concentrations promoted cell proliferation. Factorial analysis and multiple comparisons further demonstrated that in PM2.5-exposed cells,

cell survival rates increased significantly with rising paeoniflorin concentrations, indicating a protective effect. Moreover, different paeoniflorin concentrations showed protective effects against PM2.5-induced BEAS-2B cell injury at various PM2.5 concentrations. Notably, in the 800 g/mL PM2.5 exposure group, cell survival rates in the 10 mol/L and 100 mol/L paeoniflorin intervention groups were significantly higher compared to the PM2.5-exposed control group, suggesting that paeoniflorin can mitigate PM2.5-induced cytotoxicity and protect against growth inhibition caused by high PM2.5 concentrations.

Yan et al. [13] previously demonstrated that PM2.5 exposure significantly increased intracellular ROS generation in BEAS-2B cells. Consistently, our quantitative and qualitative measurements of ROS levels showed that BEAS-2B intracellular ROS levels increased significantly with rising PM2.5 concentrations. Following paeoniflorin intervention, intracellular ROS content decreased markedly with increasing paeoniflorin concentration. In each PM2.5 exposure group, all paeoniflorin-treated groups showed significantly reduced intracellular ROS compared to their respective PM2.5-exposed controls.

Furthermore, our study found that MDA content increased significantly with rising PM2.5 concentrations, consistent with Deng et al.' s findings in PM2.5-exposed A549 cells [17]. This indicates that PM2.5 induced oxidative stress injury in BEAS-2B cells. Paeoniflorin intervention also significantly reduced MDA content in cell culture supernatant in a concentration-dependent manner, with significant decreases observed across different PM2.5 exposure groups compared to their respective controls.

Oxidative stress occurs when the body needs to eliminate senescent cells or encounters harmful stimuli, leading to excessive production of highly reactive molecules such as ROS. When oxidative capacity exceeds the scavenging ability, the balance between oxidative and antioxidant systems is disrupted, resulting in neutrophil inflammatory infiltration, increased protease secretion, and generation of numerous oxidative intermediates that cause tissue damage [19-20]. Previous studies have shown that paeoniflorin possesses potent antioxidant properties. Under conditions of cellular oxidative stress, paeoniflorin can reduce intracellular ROS generation, increase production of antioxidants such as glutathione and superoxide dismutase, and activate the Nrf2/HO-1 antioxidant pathway to induce heme oxygenase-1 gene expression, thereby protecting endothelial cells from oxidative damage and inhibiting apoptosis [21-23]. Moderate to high levels of ROS can induce apoptosis and even necrosis through oxidative stress responses. Increased intracellular ROS levels indicate activation of oxidative stress responses. In biological systems, free radicals act on lipids to produce MDA as the final product of lipid peroxidation [24]. MDA can indirectly measure the degree of membrane system damage, cause cross-linking and polymerization of vital macromolecules such as proteins and nucleic acids, and exhibit cytotoxicity by affecting mitochondrial respiratory chain complexes and key enzyme activities in vitro [25]. Studies have shown that paeoniflorin can significantly reduce MDA production in hypoxia-exposed neonatal rat cardiomy-

ocytes [10], thereby inhibiting oxidative hyperactivity. Increased MDA levels in cell culture supernatant indicate membrane damage and growth inhibition. In our experiment, paeoniflorin demonstrated significant intervention effects on the oxidative stress process in BEAS-2B cells, providing notable protection against oxidative stress-induced damage.

In summary, our main findings are: first, paeoniflorin inhibits BEAS-2B cell growth and proliferation at low concentrations but promotes cell proliferation at high concentrations. Second, paeoniflorin provides protective effects against PM2.5-induced growth inhibition in BEAS-2B cells, likely through its ability to significantly alleviate PM2.5-induced oxidative stress injury.

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