Ginsenoside Rg1 reduces toxicity of fine particulate matter on human alveolar epithelial cells: A preliminary observation

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Abstract. Fine particulate matter ($PM_{2.5}$) is a significant environmental pollutant responsible for a number of human diseases. Ginsenoside Rg1 (Rg1) is likely to have the potential to relieve $PM_{2.5}$-induced cell injury. The present study is designed to preliminarily observe the harmful effect of $PM_{2.5}$ and the protective effect of Rg1 against $PM_{2.5}$ on human A549 lung epithelial cells *in vitro*. The cytotoxic effects of the $PM_{2.5}$ or Rg1 on A549 cells were measured by means of cell viability, and then exposure concentration of $PM_{2.5}$ and pretreatment concentration of Rg1 used in the following assays were established. The A549 cells were pretreated with Rg1 for 1 h and then exposed to $PM_{2.5}$ for 24 h. The levels of lactate dehydrogenase (LDH) in the cell culture supernatant and malondialdehyde (MDA) within the cells were assayed. The present results revealed that 200-1,200 μg/ml of $PM_{2.5}$ decreased the viability of A549 cells significantly in a concentration-dependent manner; however, 50-400 μg/ml of Rg1 had no significant effect. Pretreatment with 100, 200 or 400 μg/ml Rg1 significantly diminished the 200 μg/ml $PM_{2.5}$-induced A549 cell viability and decreased LDH leakage and MDA generation in a concentration-dependent manner. These results indicated that $PM_{2.5}$ induced cell injury and Rg1, antagonized $PM_{2.5}$-induced cell injury to a certain extent.

Introduction

A well-known hazard to human health is air pollution. One significant environmental pollutant is ambient airborne particular matter (PM), which has been linked to several types of cancer and cardiopulmonary diseases (1). Over the last few decades, several studies have shown that a significant parameter in determining the potential to cause inflammatory injury, oxidative damage and other biological effects is the size and surface area of PM (2). Fine particles (diameter <2.5 μm; termed as $PM_{2.5}$) had stronger effects due to their ability to enter deeper into the airways of the respiratory tract. As a consequence, $PM_{2.5}$ can reach the alveoli whereby 50% are retained in the lung parenchyma (3). In recent years, the hazardous effects of $PM_{2.5}$ have captured increasingly more public attention. However, whether there are alternative methods to protect us from the effects of $PM_{2.5}$, whether its discharge into the atmosphere can be reduced and if certain herbal additive intake can actively defend the body against the damaging effects of $PM_{2.5}$ remain to be elucidated.

Panax ginseng has been safely used in China for >2000 years. In Asia, *Panax ginseng* is a general tonic and an adaptogen to maintain the body's resistance to adverse factors and homeostasis, including enhanced physical functions, general vitality, anti-stress and anti-aging (4,5). Ginsenoside Rg1, a steroidal saponin abundantly contained in *Panax ginseng*, is one of its most active components and contributes to a number of its effects (6). A previous study reveals that Rg1 has protective effects on glutamate-induced lung injury (7). Based upon this literature, we hypothesize that Rg1 may offset the ill effects of $PM_{2.5}$ on human A549 lung epithelial cells. To date, there are limited studies on the protective effects of Rg1 on an organism exposed to $PM_{2.5}$ *in vivo* or *in vitro*. Only a recent study shows that Rg1 reduces the toxicity of $PM_{2.5}$ on human umbilical vein endothelial cells by upregulating the intracellular antioxidative state (8). However, whether Rg1 has similar protective effects on lung cells remains unclear considering the lung is a major target organ attacked by $PM_{2.5}$ (9). This preliminary study was designed to examine the toxic effects of $PM_{2.5}$ and the protective effects of Rg1 on the A549 cells.

Materials and methods

Reagents. The following reagents were used: Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS; Gibco-BRL, New York, NY, USA), Ginsenoside Rg1 with purity >98% was purchased from Shanghai Winwerb Medical Science Co., Ltd) and dissolved in double distilled water and dimethyl sulfoxide (DMSO) and penicillin-streptomycin (both from Invitrogen, Carlsbad, CA, USA). An assay kit of MDA

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was purchased from Nanjing Jiancheng Biological Engineering Co., Ltd. (Nanjing, China).

Cell culture. The A549 human alveolar type II epithelial cells were obtained from the Cell Bank of Peking Union Medical College (Beijing, China) and were maintained in low-glucose DMEM supplemented with 10% heated-inactivated FBS (Hyclone, Atlanta, GA, USA), 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere of 5% CO_2 and 95% air at 37˚C. The cells were serum-starved for 24 h prior to being treated with the $PM_{2.5}$. The Rg1 were added to the cells 1 h prior to the $PM_{2.5}$ treatment.

$PM_{2.5}$ sampling and preparation. Urban atmospheric $PM_{2.5}$ was kindly provided by Professor Xiaohong Zhao from the College of Arts and Sciences of Beijing Union University (Beijing, China). $PM_{2.5}$ was collected on 150-mm diameter nitrocellulose filters (HAWP, Sartorius, La Ferté-sous-Jouarre, France) with a high volume sampler machine (DA-80 Digitel, Cugy, Switzerland; flowrate, 30 m^3/h) on the roof of a five story building on Xueyuan Road (Beijing, China) which was taller than surrounding buildings. The particles were processed as described previously (10). Briefly, the $PM_{2.5}$ samples were extracted from sampled filter strips by immersing them in deionized water and then sonicating them for 30 min in a water bath sonicator (KQ-700 V and 700 W; Asha Analytical Instruments Pvt. Ltd., Secunderabad, India). The $PM_{2.5}$ samples were then stored at -80˚C until further use.

Cell viability. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay was used to measure cell viability. Following treatment with $PM_{2.5}$ or Rg1, medium was discharged and the cells were rinsed with PBS and the MTT (final concentration, 0.5 mg/ml) was added for 4 h (11). The medium was removed and the MTT reduction product dissolved in 1 ml DMSO. The absorbance of each sample was assessed by Multiskan Ascent (Thermo Scientific Inc., Waltham, MA, USA) at 565 nm. The test was replicated three times and the cell viability was calculated as follows: % cell viability = $[(OD_{experiment} - OD_{blank}) / (OD_{control} - OD_{blank})] \times 100$.

Cell toxicity. The cytotoxicity of the $PM_{2.5}$ was measured by the lactate dehydrogenase (LDH) activity in the cell medium using ELISA. The absorbance was read using an ELISA reader (Bio-Tek, Colmar, France) at 450 nm. An increase in the number of dead or cell membrane-damaged cells increases the LDH activity in the cell culture supernatant. In these experiments, the A549 cells were exposed to $PM_{2.5}$ for 24 h after pretreatment of Rg1 for 1 h. This incubation period is required to obtain reliable measurements of the cytotoxicity.

Measurement of MDA. MDA was quantitated by spectrophotometry. Following removal of the media, the membranes were solubilized in 400 μl of 8% SDS, 25 μl of 4% butylated hydroxy toluene in ethanol was added and 500 μl of 10% phosphotungstic acid in 0.5 M sulfuric acid in a serial manner. Following addition of 250 μl of 0.7% thiobarbituric acid, the tubes were placed in a boiling bath for 50 min. Next, 1 ml of 1-butanol was added, the tubes were centrifuged and the supernatant containing thiobarbituric acid reactants was collected to measure the absorbance at 532 nm. Thiobarbituric acid reactants were quantitated using a standard curve prepared with a 1 mM solution of tetrahydroxypropane hydrolyzed in 1% sulfuric acid.

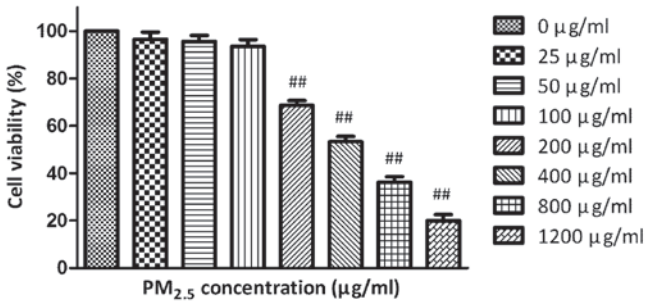

Figure 1. Viability of the cultured human A549 lung epithelial cells exposed to 0-1,200 μg/ml $PM_{2.5}$ was determined by an MTT assay. The MTT assay revealed that incubation with 200-1,200 μg/ml $PM_{2.5}$ for 24 h decreased the A549 cells viability in a concentration-dependent manner ($^{##}P<0.01$ compared with A549 cells not exposed to $PM_{2.5}$).

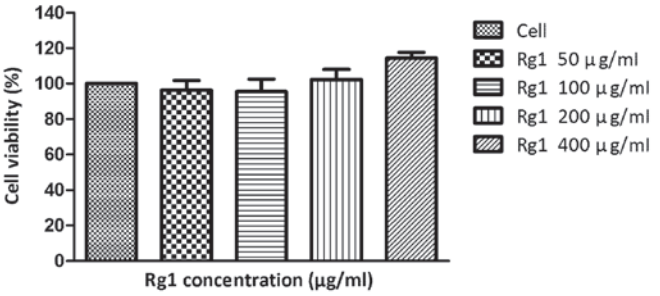

Figure 2. Viability of the cultured human A549 lung epithelial cells incubated with Rg1 was determined by the MTT assay. MTT assay demonstrated that incubation with 50-400 μg/ml Rg1 for 24 h did not significantly change the A549 cells viability.

Statistical analysis. Statistical analysis of the data was performed using one-way analysis of variance with the Newman-Keuls multiple comparison post-hoc tests by using SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA). All the data are expressed as the mean ± standard deviation. $P<0.05$ was used to indicate a statistically significant difference.

Results

Effect of $PM_{2.5}$ on viability of A549 cells. The MTT assay demonstrated that incubation with 0-1,200 μg/ml $PM_{2.5}$ for 24 h decreased the A549 cells viability in a concentration-dependent manner (Fig. 1).

Effect of Rg1 on viability of A549 cells. The MTT assay revealed that treatments with 50-800 μg/ml Rg1 for 24 h did not change the A549 cells viability significantly (Fig. 2).

Effect of Rg1 on viability of A549 cells exposed to 200 μg/ml $PM_{2.5}$. The MTT assay revealed that pretreatments with 50-400 μg/ml Rg1 for 1 h increased the viability of A549 cells exposed to 200 μg/ml $PM_{2.5}$ in a dose-dependent manner (Fig. 3).

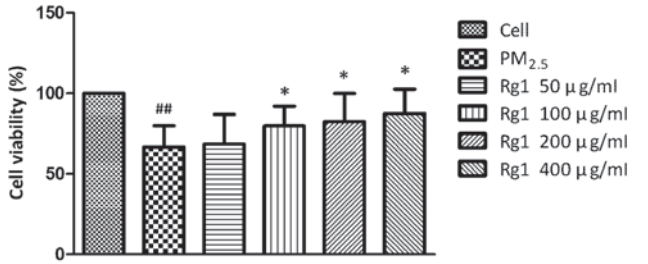

Figure 3. Effect of Rg1 on viability of cultured human A549 lung epithelial cells exposed to $PM_{2.5}$. The MTT assay revealed that 200 μg/ml $PM_{2.5}$ decreased the viability of the untreated cells compared with the untreated control cells ($^{\#\#}$P<0.01 compared with untreated control A549 cells), but pretreatments with 50-400 μg/ml Rg1 for 1 h increased the viability of the A549 cells exposed to $PM_{2.5}$ in a dose-dependent manner (*P<0.05 compared with untreated A549 cells exposed to 200 μg/ml $PM_{2.5}$).

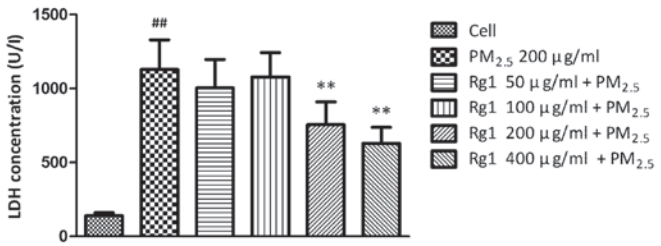

Figure 4. Effect of Rg1 on the lactate dehydrogenase (LDH) concentration in culture supernatants of A549 cells exposed to 200 μg/ml $PM_{2.5}$. $PM_{2.5}$ increased the LDH release from the untreated cells compared with the untreated control cells ($^{\#\#}$P<0.01 compared with untreated control A549 cells), but co-culture with 200 and 400 μg/ml Rg1 decreased $PM_{2.5}$-stimulated LDH leakage from cells significantly (**P<0.01 compared with untreated A549 cells exposed to 200 μg/ml $PM_{2.5}$).

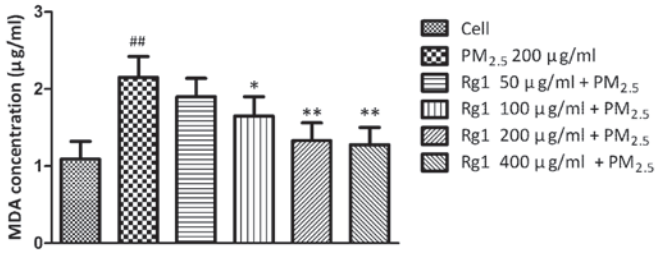

Figure 5. Effect of Rg1 on malondialdehyde (MDA) concentration in the A549 cells exposed to 200 μg/ml $PM_{2.5}$. $PM_{2.5}$ increased the MDA production of the untreated cells compared with the untreated control cells ($^{\#\#}$P<0.01 compared with untreated control A549 cells), but co-culture with 100, 200 and 400 μg/ml Rg1 decreased $PM_{2.5}$-stimulated MDA production of the cells significantly (*P<0.05 and **P<0.01 compared with untreated A549 cells exposed to 200 μg/ml $PM_{2.5}$).

Effect of Rg1 on LDH concentration in culture supernatants of A549 cells exposed to 200 μg/ml $PM_{2.5}$. The level of LDH in cell-free culture supernatants significantly increased in untreated A549 cells exposed to $PM_{2.5}$ (1129.944±199.428 U/l; P<0.01) compared with untreated control cells (140.248±20.771 U/l). Co-culture with 200 and 400 μg/ml Rg1 decreased $PM_{2.5}$-stimulated LDH leakage to 755.858±153.423 U/l (P<0.01) and 629.738±108.065 U/l (P<0.01), respectively (Fig. 4).

Effect of Rg1 on MDA concentration in A549 cells exposed to 200 μg/ml $PM_{2.5}$. The level of the oxidative stress based on the MDA assay significantly increased in untreated A549 cells exposed to $PM_{2.5}$ (2.15±0.27 μg/ml; P<0.01) compared with untreated control cells (1.09±0.23 μg/ml). Co-culture with 100, 200 and 400 μg/ml Rg1 decreased $PM_{2.5}$-induced MDA production to 1.65±0.25 μg/ml (P<0.05), 1.33±0.23 μg/ml (P<0.01) and 1.28±0.22 μg/ml (P<0.01), respectively (Fig. 5).

Discussion

In the present study, the 25-1,200 μg/ml of $PM_{2.5}$ decreased the A549 cells viability in a dose-dependent manner, which was in accordance with previous studies (12,13). Since 200 μg/ml was the minimum effective dose of $PM_{2.5}$, this dose was selected as the exposure concentration in the following assays. Ginsenoside Rg1 was a compound extracted from *Panax ginseng* and had been studied extensively. The MTT assay demonstrated that 50-400 μg/ml Rg1 did not alter the cell viability in A549 cells significantly. Although there were no previous studies on the viability of A549 cells co-incubated with Rg1, numerous studies indicated that Rg1 had no marked cytotoxic effect on fibroblasts (14) or neuroblasts (15). Doses of 50-400 μg/ml were selected as pretreatment concentrations of Rg1 in the following assays. While the A549 cells were pretreated with Rg1 for 1 h at concentrations of 50-400 μg/ml, followed by exposure to 200 μg/ml $PM_{2.5}$, Rg1 at three concentrations of 100, 200 and 400 μg/ml was observed to be capable of increasing the cells viability significantly, which implied that Rg1 possessed a cytoprotective effect in order to antagonize the lesion from $PM_{2.5}$.

The measurement of LDH in the culture supernatant as a result of leakage is an indicator of cell membrane integrity (16). In the present study, 200 μg/ml $PM_{2.5}$ increased the level of LDH in the A549 cell culture supernatant compared with the control cells. However, 200 and 400 μg/ml Rg1 could decrease the LDH generation significantly in a concentration-dependent manner. These results indicated that Rg1 had inhibitory effects on the cytotoxicity induced by $PM_{2.5}$.

The cellular level of MDA is a sensitive marker for oxidative damage, particularly lipid peroxidation and has been widely used (17). In the present study, the A549 cells exposed to $PM_{2.5}$ produced more MDA than the control cells indicating that $PM_{2.5}$ could induce more oxidative stress. This result was in accordance with a previous study (18). Rg1 had been proven to have an effect on antioxidative stress by inhibiting MDA production (19,20). To the best of our knowledge, the present study, for the first time, observed that 100, 200 and 400 μg/ml Rg1 could reduce the MDA level of the A549 cells exposed to $PM_{2.5}$ in a concentration-dependent manner. These results indicated that Rg1 had the potential to protect the alveolar epithelium from oxidative damage induced by $PM_{2.5}$.

The present study preliminarily implies that ginsenoside Rg1 is a promising candidate drug to antagonize the harmful effects of $PM_{2.5}$ on alveolar epithelium cells, but the usefulness of Rg1 for this purpose requires further investigation, in particular, clinical studies.

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