Ginsenoside Rg3 Alleviates Rotenone-induced Lung Injury in Mice by Its Anti-oxidative Properties

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ABSTRACT

Objectives: Acute lung injury is commonly found in patients with insecticide poisoning and the pathogenesis is related to oxidative stress. Ginsenoside Rg3, one of the main constituents of Panax ginseng C.A. Meyer, shows an anti-oxidative activity. The aim of this study was to evaluate whether ginsenoside Rg3 can alleviate lung injury induced by rotenone in mice.

Methods: C57BL/6J male mice were divided into five groups (n=11). The mice in ginsenoside Rg3 groups were treated with ginsenoside Rg3 at a dose of 5, 10 or 20 mg/kg. Except for the control group, mice were challenged intragastrically with rotenone at a dose of 30 mg/kg, once a day for 6 weeks. Subsequently, the lung tissues of mice were collected. The effect of ginsenoside Rg3 on rotenone-induced lung injury was observed by hematoxylin and eosin staining. The oxidative stress in lung tissues were also examined.

Results: Rotenone induced substantial hemorrhage, alveolar wall thickness and neutrophils infiltration. These structural damages were attenuated significantly by ginsenoside Rg3 treatment. The lung injury induced by rotenone was associated with oxidative stress in lung tissues of mice. Compared with the control group, rotenone exposure resulted in the increase of malondialdehyde (MDA), the decreases of the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and the glutathione (GSH) content. Nevertheless, ginsenoside Rg3 treatment not only reduced MDA production but also increased the activities of SOD, GSH-Px, and the content of GSH in lung tissue of mice.

Conclusion: Taken together, this study demonstrated that ginsenoside Rg3 has potential to ameliorate rotenone-induced lung injury and the mechanism of action of ginsenoside Rg3 is mediated by its anti-oxidative properties.

Key words: Ginsenoside Rg3, rotenone, acute lung injury, oxidative stress

INTRODUCTION

Insecticide abuse causes a serious pollution to the environment during agricultural activities. Moreover, the data of World Health Organization (WHO) show that the insecticide poisoning is the leading cause of suicide worldwide (1). And insecticide poisoning induces more than 200,000 people’s death every year (2). This severe insecticide poisoning causes a damage to organs of human body. The symptoms of insecticide poisoning are coma, renal injury, acute lung injury (ALI), and respiratory failure (3). ALI, initial state of acute respiratory distress syndrome (ARDS), presents mainly with acute respiratory insufficiency, which is implicated in alveolar epithelial injury and capillary endothelial cell injury. Patients with ALI/ARDS occupy for 10% in intensive care unit (ICU) and 4% in total inpatients. It is the main reason of mortality in critically ill patients. Generally, the mortality of patients with ALI is up to 60%, which is a major medical issue to be resolved so far (4).
Rotenone is used commonly as an insecticide in agriculture. It has the effect of inhibiting mitochondrial complex I which is an important metabolic enzyme involved in redox reactions (3). The inhibition of mitochondrial complex I leads to mitochondrial dysfunction and trigger oxidative stress occurrence (6–7). In animal experiments, many reports demonstrated that lung injury of rat were induced by an exposure of rotenone (8–9). Previous study shows that oxidative stress is a key contributing factor for the lung injury and the impairment of lung function (10). Meanwhile, reactive oxygen species-induced oxidative stress also plays an important role in the pathogenesis of respiratory diseases (11). Recently, researches demonstrate that using antioxidants against oxidative stress reduces the airway inflammation in animal models of asthma (12). Epigallocatechin-3-gallate (EGCG), a catechin in green tea, shows an antioxidant activity and therefore attenuates lung injury (13). Thus, reducing oxidative stress may be an effective strategy to against insecticide poisoning-induced lung injury.

Ginsenosides are the compounds extracted from Panax ginseng C.A. Meyer. Up to date, more than 30 ginsenosides including Rg3, Rg2, Re, and Rh2 were separated (14). Ginsenoside Rg3 is a tetracyclic triterpenoid saponin. Previous studies show that ginsenoside Rg3 possesses a good many of pharmacological activities, such as antioxidant (15), anticancer (16), cardiovascular protection (17), immune improvement (18), anti-inflammation (19), and anti-aging effects (20). Ginsenoside Rg3 alleviates the dopaminergic nerve injury in Parkinson’s disease mice through its antioxidant effects (21–22). Ginsenoside Rg3 exerts an antioxidant activity and then ameliorates myocardial ischemia-reperfusion injury in rats (23). This study aims to investigate whether ginsenoside Rg3 can ameliorate lung injury induced by rotenone in mice.

MATERIALS AND METHODS

Chemicals and Reagents

Rotenone was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ginsenoside Rg3 was provided by Dalian Fusheng Natural Medicine Development Co., Ltd. (Dalian, China). Carboxymethylcellulose sodium was purchased from Guangfu Fine Chemical Company (Tianjin, China). Malondialdehyde (MDA) kit (Lot. 20200508), superoxide dismutase (SOD) kit (Lot. 20200508), glutathione (GSH) kit (Lot. 20200416), and glutathione peroxidase (GHS-Px) kit (Lot. 20200416) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Animals

C57BL/6j male mice (18 - 22 g) were from Jinan Pengyue Experimental Animal Breeding Company (Jinan, China). The animals were housed in a specific pathogen-free conditions and temperature-controlled facility with a 12-hr light/dark cycle, and had ad libitum access to a standard animal diet. All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Publication 86–23, revised in 1986), and the protocols were approved by the Ethics Committee of Yantai University (No. 58-ECYU-19).

Experimental Design

Animals were randomly divided into five groups (n=11) including the control, model, and ginsenoside Rg3 (5, 10, and 20 mg/kg) groups. Rotenone and ginsenoside Rg3 were dissolved in 0.5% carboxymethylcellulose sodium. Mice in control and model groups were administered intragastrically with 0.5% carboxymethylcellulose sodium. While mice in ginsenoside Rg3 groups were treated intragastrically with ginsenoside Rg3 at doses of 5, 10, or 20 mg/kg. Two hours later, mice in the control group were intragastrically with 0.5% carboxymethylcellulose sodium again. Mice in the other groups were intragastrically challenged with rotenone (30 mg/kg) to induce the lung injury model. The treatment with ginsenoside Rg3 and the challenge with rotenone were performed once a day for 6 weeks.

Histomorphology

Mice were anesthetized with ketamine (90 mg/kg) and xylazine (5 mg/kg). The lung tissues were washed with 0.9% saline and then they were fixed with 4% paraformaldehyde. After dehydrated and embedded, the lung tissues were consecutively cut into 4-μm thick sections for hematoxylin and eosin (H&E) staining. The pathological changes were evaluated by three pathologists who were blinded to the design with an inverted microscope (IX71, Olympus, Japan). The lung injury was detected by Smith scoring criteria: pulmonary hyaline membrane formation, alveolar cavity enlargement, alveolar wall thickening, hemorrhage, necrosis, and inflammatory cell infiltration. The severity of lung injury was graded from 0 to 4.

Oxidative Stress Measurement

Oxidative stress indicators in the lung tissues of mice, such as MDA, SOD, GSH, and GHS-Px, were assayed according to the manufacturer’s instructions. Briefly, the lung tissues from eight mice in each group were weighed and homogenized in phosphate-buffered saline at a ratio of 1:10 (weight to volume). The homogenates were centrifuged at 16, 000 g at 4 °C for 10 min. The supernatant was used to measure oxidative stress indicators. Data were expressed as the absorbance after normalization by the protein content.

Statistical Analysis

Data are presented as the Mean ± SD. Experimental data were analyzed by SPSS 19.0 statistical analysis (IBM Corporation, Armonk, NY, USA). Differences among multiple groups were analyzed by One-way ANOVA followed by Fisher’s LSD test. A p-value < 0.05 was considered to indicate a significant difference.

RESULTS

The effect of ginsenoside Rg3 on rotenone-induced lung injury in mice

As shown in Fig. 1, the pathological section of lung tissues in the control group demonstrated that the bronchial epithelial structure was complete, the structure of epithelial cells was normal and arranged tightly, and the alveolar structure was clear. Furthermore, there was no obvious thickness of the alveolar wall and inflammation in the control group. However, substantial hemorrhage, alveolar wall thickness, and neutrophils infiltration were observed in
the model group. Treatment with ginsenoside Rg3 alleviated the rotenone-induced lung injury. Especially, there were only slight alveolar epithelial cell hyperplasia and alveolar septal thickness in the lungs of the mice of ginsenoside Rg3 at dose of 20 mg/kg group. The score of lung injury was shown in Fig. 2. Compared with the control group, the score of lung injury in the model group increased significantly \((p<0.01)\). However, treatment with ginsenoside Rg3 at dose of 5, 10, and 20 mg/kg decreased the score of lung injury when compared with that of the model group \((p<0.01)\). These results demonstrated that ginsenoside Rg3 had a property to alleviate the lung injury induced by rotenone in mice.

**Fig. 1. The effect of ginsenoside Rg3 on rotenone-induced lung injury in mice.** Lung histopathology of mice stained with H&E \((n=3)\). A, Control; B, Model; C, Ginsenoside Rg3 5 mg/kg; D, Ginsenoside Rg3 10 mg/kg; E, Ginsenoside Rg3 20 mg/kg. \((\times 100)\). F, Control; G, Model; H, Ginsenoside Rg3 5 mg/kg; I, Ginsenoside Rg3 10 mg/kg; J, Ginsenoside Rg3 20 mg/kg. \((\times 400)\). Red arrowheads indicate hemorrhage, yellow arrowheads indicate alveolar wall thickening and alveolar epithelial cell hyperplasia, black arrowheads indicate inflammatory cell infiltration.

**Fig. 2. The effect of ginsenoside Rg3 on rotenone-induced lung injury in mice.** The score of lung injury. Data were expressed as the Mean ± SD \((n = 3)\). Statistical analyses were performed using One-way ANOVA followed by Fisher’s LSD test. \#\#p<0.01 compared with the control group; \**p<0.01\) compared with the model group.

**The effect of ginsenoside Rg3 on SOD activity in the lung tissues**

SOD is an important antioxidant enzyme and presents a property to against oxidative stress. As shown in Fig. 3, the SOD activity of model group was lower than that of the control group \((p<0.01)\). However, treatment with
ginsenoside Rg3 at dose of 10 and 20 mg/kg markedly increased the SOD activity in the lung tissues of mice (p<0.05 or 0.01).

![Fig. 3. The effect of ginsenoside Rg3 on SOD activity in the lung tissues.](image)

The effect of ginsenoside Rg3 on GSH-Px activity and GSH content in the lung tissues

In Fig. 4, GSH-Px activity of the model group decreased significantly when compared with the control group (p<0.01). Ginsenoside Rg3 augmented the GSH-Px activity (p< 0.05 or p< 0.01). Consistently, GSH in the lung tissues of model group was also decreased when compared with the control group (p<0.01, Fig.5). Treatment with ginsenoside Rg3 at dose of 5, 10 and 20 mg/kg increased the GSH content in the lung tissues (p<0.05 or p< 0.01).

![Fig. 4. The effect of ginsenoside Rg3 on GSH-Px activity in the lung tissues.](image)
The effect of ginsenoside Rg3 on MDA content in the lung tissues

MDA is a parameter reflecting the degree of lipid peroxidation. As shown in Fig. 6, there was an increase of MDA in the lung tissues of the mice of the model group (p<0.01). Compared with the model group, the MDA content in ginsenoside Rg3 groups was decreased significantly (p<0.01). The results indicated that ginsenoside Rg3 alleviates rotenone-induced lung injury in mice by its anti-oxidative properties.

**DISCUSSION**

In this study, rotenone exposure caused a severe lung injury and an oxidative stress of lung in mice. However, ginsenoside Rg3 alleviated the lung injury induced by rotenone. Further experiments demonstrated that the mechanism of action of ginsenoside Rg3 is mediated by its anti-oxidative properties.

Insecticides, such as rotenone, omethoate, methyl parathion and dichlorvos, can cause oxidative stress and severe lung damage (24-26). Researches have demonstrated that exposure of insecticides for long time caused pulmonary impairments in mice and rabbits (27). Besides, oxidative stress and inflammation in lung were major features in the insecticides-challenged rats (28). Rotenone is mainly extracted from the roots and stems of Lonchocarpus and Derris species (29). It is a commonly used insecticide in agriculture. Previous studies indicated that rotenone could cause a mitochondrial dysfunction, which is implicated in the inhibition of mitochondrial electron transport chain complex I (30). Therefore, the leak of electrons from mitochondria will react with oxygen and produce reactive oxygen species (ROS). ROS leads to the mitochondrial disorders, abnormal nucleic acids, protein misfolding, and disorders of lipid metabolism. Previous study showed that rotenone caused the injury of lung in mouse models (31). In this study, histopathology evaluation was performed to determine the lung injury in the rotenone-challenged mice. The results showed that rotenone exposure induced a severe damage in lung tissue of mice, including hemorrhage and alveolar wall thickness. However, ginsenoside Rg3 treatment alleviated rotenone-induced lung injury in mice. These findings demonstrated that ginsenoside Rg3 can alleviates rotenone-induced lung injury in mice.

The molecular mechanisms of insecticides-induced lung injury are complex and diverse. But the main pathological mechanisms include inflammation, oxidative stress, and apoptosis (28,32). Accordingly, rotenone-induced lung injury was implicated in the increase of ROS under the condition of oxidative stress (4-5). Although lung has its antioxidant system, excessive ROS will result in the damage of epithelial cells of lung (33-34). Moreover, clinical studies also demonstrated that ROS plays a vital role in airway tissue damage of patients (4). Emerging evidence suggests that oxidative stress was one of the main factors which causes lung injury (35-37). Thus, oxidative stress plays an indispensable role in the occurrence and development of lung injury induced by rotenone. Therefore, reducing oxidative stress and/or increasing antioxidant capacity are primary treatment strategies for alleviating rotenone-induced lung injury. Cells can also prevent oxidative stress-induced damage by increasing the activities of the antioxidant enzymes (38). The antioxidant enzymes, including GSH-Px and SOD, can scavenge ROS and therefore prevent the damage of oxidative stress (39). GSH is a cardinal antioxidant in cells and it protects cells against exogenous and endogenous toxins, including ROS and nitrogen species. Previous study showed that ovalbumin inhalation increased the ROS generation in bronchoalveolar lavage fluids. But the augment of GSH content significantly reduced the lung injury induced by the ovalbumin inhalation (40). MDA is a stable product of lipid peroxidation of cells and therefore is the most frequently used biomarker of oxidative stress. In this study, rotenone exposure resulted in the increase of MDA, the decreases of the activities of SOD, GSH-Px, and the GSH content. Nevertheless, ginsenoside Rg3 treatment not only reduced MDA production but also increased the activities of SOD, GSH-Px, and the content of GSH in lung tissue of mice. The findings of these experiments demonstrated that the mechanism of action of ginsenoside Rg3 is mediated by its anti-oxidative properties.
There are several limitations in this study. ROS is an important indicator of insecticides-caused lung injury. However, the levels of ROS were not assayed directly in the present study. Additionally, lactate acid level and lactate clearance show a direct relationship with mortality in patients with lung injury in clinic (41). Therefore, it will be better to detect the lactate acid content in the blood of mice with rotenone exposure.

CONCLUSION

In summary, this study demonstrates that ginsenoside Rg3 exerts lung protective effects in rotenone-induced lung injury mice. And the mechanism of action of ginsenoside Rg3 is mediated by its anti-oxidative properties.

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