

Oxidized graphene-aggravated allergic asthma is antagonized by antioxidant vitamin E in Balb/c mice

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Abstract Nanomaterials have been widely used in a number of applications; however, these nanomaterials may potentially be risky for human health, particularly for the respiratory system. In this study, we used a mouse asthma model to study whether graphene oxide (GO), a promising carbonaceous nanomaterial with unique physicochemical properties, aggravates allergic asthma via the oxidative stress pathway. Mice were sensitized with ovalbumin (OVA) to trigger immune reactions, while vitamin E (Ve) was administered as an antioxidant. Our results showed that GO aggravated OVA-induced allergic asthma in mice, as suggested by increased reactive oxygen species (ROS), elevated total immunoglobulin E (IgE), upregulated Th2 response, and the aggravation of allergic asthma symptoms, such as airway remodeling, collagen deposition with mucus hypersecretion, and airway

hyperresponsiveness (AHR). The administration of Ve dramatically attenuated all of the above effects. In conclusion, Ve showed anti-allergic properties in antagonizing the exacerbation of allergic asthma induced by GO, providing a new possibility for the treatment of allergic asthma.

Keywords Graphene oxide · Allergic asthma · Exacerbation effect · Oxidative stress · Vitamin E

Introduction

Rapid development in nanotechnology has resulted in large amounts of advanced nanomaterials being fabricated. Since the start of this century, various nanomaterials have been extensively applied in many fields including food, health care products, sensors, electronics, catalysts, photonics, biomedicine, cosmetics, and garments. Increased use of nanomaterials is accompanied by heightened risk to human health due to nanomaterials' intrinsic properties such as surface, quantum size, small size, and macroscopic quantum tunneling effects.

Graphene is one of the most application prospects of nanomaterials. As an important derivative of grapheme, graphene oxide (GO) is characterized by its many surface oxygenic functional groups (epoxy, hydroxyl, and carboxyl). GO exhibits remarkable chemical, electrical, and mechanical properties and is therefore used in a variety of fields ranging from electronics to medical application (Jang et al. 2015; Singh et al. 2012). As a result of the widespread usage, elevated emission of GO into the environment poses potential risks to human health, especially to the respiratory system. Duch found that GO is hypertoxic when directly injected in the lungs of mice, resulting in severe and persistent lung damage (Duch et al. 2011). Sangiliyandi demonstrated that when in vitro cells were exposed to GO, they produced superoxide radical anions and

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lost their viability (Gurunathan et al. 2012). Moreover, GO was able to cause plasma membrane damage in HepG2 cells and high levels of oxidative stress were found in the process (Lammel et al. 2013). However, there are few studies on the effects of GO in patients with respiratory disease, the mechanism of toxicity, and the means or strategies in antagonizing or reducing the adverse effects of GO.

Allergic asthma, a common chronic inflammatory disease of the distal airways, has been a critical public health problem in terms of prevalence, morbidity, and mortality (Edwards et al. 2009). It is characterized by immunoglobulin E (IgE) production, airway inflammation, mucus hypersecretion, and airway hyperresponsiveness (AHR), a measure of bronchial constriction ordinarily observed in asthma patients (Kannan et al. 2013). Th cells are believed to fall into two major categories: Th1 and Th2, while the imbalance of Th1/Th2 may be the basis of asthma (Romagnani 2004). Because Th2 produces interleukin-4 (IL-4) and leads to increased production of IgE, the rise in Th2 is strongly associated with asthma.

Environmental pollutants are perceived as important inductive agents of allergic asthma. Numerous studies have now shown that a variety of nanomaterials can aggravate allergic asthma symptoms in experimental animal models (Inoue et al. 2010; Jonasson et al. 2013). Oxidative stress is widely acknowledged as the toxicity mechanism resulting in the exacerbation effect (Kang et al. 2011; Madl et al. 2014). Vitamin E (Ve), one of the most commonly used antioxidants, is used extensively in experiments to antagonize the adverse effects induced by environmental pollution. Li concluded that Ve was effective as a protective reagent for weakening single-walled carbon nanotube (SWCNT) exacerbation on allergen-induced allergic asthma (Li et al. 2014).

In this study, an experimental allergic asthma mice model was established to assess the aggravating effects of GO by evaluating serum immunoglobulin E concentrations, cytokines, histopathological analyses of lung tissues, and AHR tests. Ve was applied to demonstrate the mechanism of oxidative stress in GO-exacerbated asthma, and we expect that our results will be useful in developing an effective method for protecting allergic asthma patients from further asthma exacerbations induced by nanomaterials.

Materials and methods

Experimental animals and ethical statement

Fifty-six male SPF Balb/c mice, 6–8 weeks old, 20–25 g body weight, were purchased from the Experimental Animal Center of Hubei province. All animal experiments were conducted in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Office of Scientific Research Management of Central China Normal University (No. CCNU-SKY-201-008).

Physicochemical characterization of GO

Pristine GO was obtained from XFNANO Co. Ltd. (Nanjing, China). Morphologies of the GO were imaged using scanning electron microscope (SEM, JSM-6510LV, JEOL, Tokyo, Japan) and transmission electron microscope (TEM, JEM-2100, JEOL, Tokyo, Japan). Raman spectra of GO were collected using a Raman microscope with a $\times 50$ air objective lens and a 633-nm (1.96 eV) laser (Renishaw inVia Plus, Renishaw Inc., Hoffman Estates, IL, USA). UV-Vis spectrum of GO suspensions was recorded with a TU1901UV-Vis spectrophotometer (Beijing Purkinje General) at wavelengths ranging from 200 to 800 nm.

Experimental design and animal exposure

Different concentrations of GO solutions were diluted in Milli-Q water. These were then placed into an ultrasound processor for 2 h and subjected to ultrasound pulses 30 min before each injection to ensure that the particles were evenly distributed in solution.

Fifty-six male Balb/c mice were randomly divided into eight exposure groups with seven mice each: saline control group (saline); OVA-sensitized group (OVA); 0.04 mg GO/kg body weight (bw) combined with OVA group (GO 0.04 + OVA); 0.40 mg GO/kg bw combined with OVA group (GO 0.40 + OVA); 4.00 mg GO/kg bw combined with OVA group (GO 4.00 + OVA); OVA-sensitized combined with Ve-protected group (OVA + Ve); 0.40 mg GO/kg bw combined with OVA and Ve-protected group (GO 0.40 + OVA + Ve); and 4.00 mg GO/kg bw combined with OVA and Ve-protected group (GO 4.00 + OVA + Ve).

In order to establish an animal allergic asthma model, the mice sensitized with OVA + Al(OH)₃ (200 mg OVA and 6.5 mg Al(OH)₃ in 1 ml saline each time) on days 6, 20, and 27 by subcutaneous injection. Then, the mice were exposed to an aerosol challenge of 1 % OVA or saline (30 min/day) using an ultrasonic nebulizer (Yuyue 402A type I, China) from days 33 to 39. The mice were injected intraperitoneally in doses of 0.01 ml/g body weight of three different GO suspensions or saline solution for 32 consecutive days to explore the aggravating effects of GO. Vitamin E (100 mg/kg), an antioxidant, was given intraperitoneally 3 h after administration of GO (0.40 or 4.00 mg/kg bw) in this study.

To avoid the effect of methacholine (MHC) used in AHR test, two sets of experiments were conducted. In the first round, after administration, the 56 mice were used directly sacrificed for testing Th cytokine concentrations and oxidative stress-related biomarkers in serum Ig production and lung homogenates. In the second experiment, a separate set of 56 mice were treated under the same conditions and then used for lung histological evaluation and AHR tests.

Preparation of serum and lung homogenate

The mice were anesthetized intraperitoneally with pentobarbital sodium (0.01 ml/g bw) 24 h after the final challenge in the first round of the experiment. Blood was obtained by heart puncture, and the serum samples were collected by centrifugation (3000 rpm at 25 °C for 10 min) and stored at −70 °C.

After serum collection, the mice were sacrificed by cervical dislocation and the whole lung was weighed and then homogenized using 10 ml/g of ice-cold PBS at pH 7.5 to get a tissue homogenate with a concentration of 10 %. The homogenate was then centrifuged at 10,000 rpm and 4 °C for 10 min and the supernatant collected and stored at −70 °C for later assessment.

Measurement of the levels of serum Ig and Th cytokines

According to the manufacturer protocols, the serum concentrations of T-IgE and lung protein levels of IL-4 and IFN- γ were measured using ELISA kits. Sensitivities of ELISA kits were 4 ng/ml for total IgE, 4 pg/ml for IL-4, and 15 pg/ml for IFN- γ .

Estimation of oxidative stress

Reactive oxygen species (ROS) Concentrations were determined based on the reactions between ROS and 2', 7'-dichlorofluorescein diacetate (DCFH-DA). Supernatant of lung tissue homogenate was diluted 10-fold in PBS (12 μ l tissue homogenate and 108 μ l PBS), and then 100 diluted supernatant was mixed with 100 μ l DCFH-DA (diluted 1000-fold with 10 μ M dimethylsulfoxide, DMSO) and placed in a 96-well microplate. The reaction mixture was kept in complete darkness at 37 °C for 5 min and the ROS contents then measured using a fluorescence reader with 485 and 520 nm for excitation and emission, respectively.

GSH assay In accordance with the manufacturer's instructions, GSH content was measured by assay kit (Nanjing Jiancheng Bioengineering Institute, A006-2, China).

MDA assay Malondialdehyde (MDA) concentration was assayed by the thiobarbituric acid (TBA) method (Ma et al. 2013). A sample of 100 μ l of lung tissue homogenate supernatant was mixed with 2 ml of 0.6 % TBA solution (0.6 g solid TBA dissolved in 100 ml 10 % TCA) and incubated at 100 °C for 15 min, and then washed by flowing tap water until cooling to room temperature and centrifuged at 10,000 r/min for 10 min. The absorbance of the sample was measured at 450, 532, and 600 nm.

Lung function measurement

According to the manufacturer's instructions, AHR was measured 24 h after the final aerosol challenge using the AniRes2005 lung function system (Bestlab, version 2.0, China) in the second round of the experiment. Mouse airway function was measured by detecting changes in lung resistance in response to increasing concentrations (0.025, 0.05, 0.1, and 0.2 mg/kg) of MCH injected into the jugular vein of the anesthetized mouse. The inspiratory and expiratory resistance R-area (Ri and Re), the graphic area between the peak value and baseline during the 250-s period, and the valley value of Cldyn were recorded for further analysis (Guo et al. 2012).

Lung histological assay

The left lung of each mouse was removed to make lung sections. All samples were fixed in 4 % paraformaldehyde solution for 24 h at room temperature, embedded in paraffin, and cut into pieces, each of which was stained with hematoxylin and eosin (H&E), Masson's trichrome (MT), and periodic acid-Schiff (PAS), respectively.

Statistical analyses

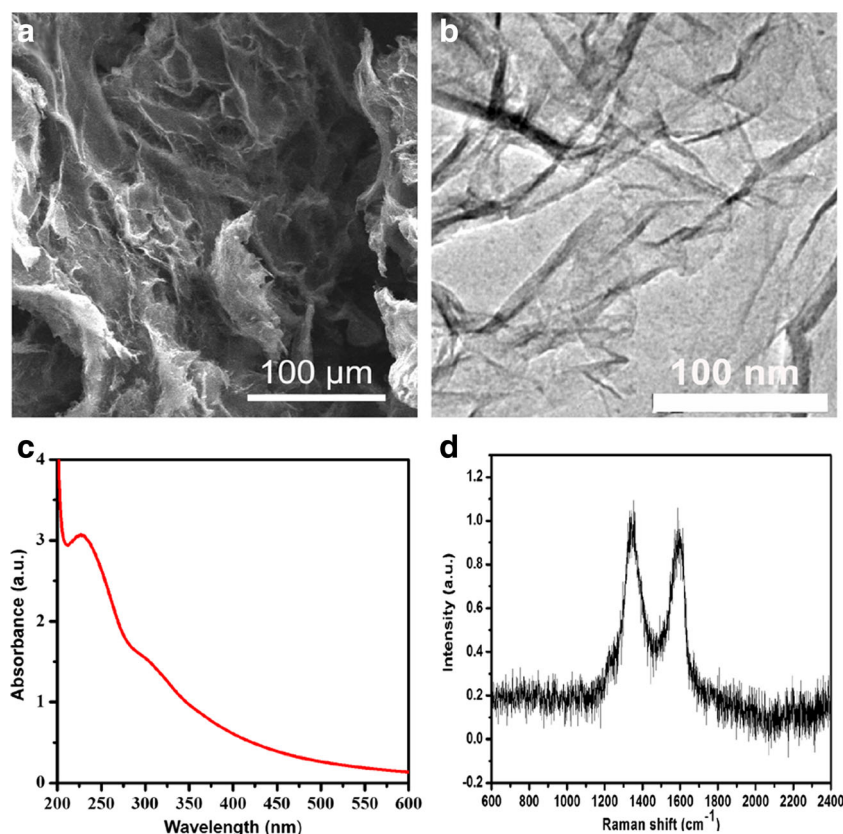
The data is presented as the mean $\pm$ standard error of the mean and the statistical graphs were made by GraphPad Prism 5.0 (San Diego, CA, USA). Results were evaluated statistically using a one-way analysis of variance (ANOVA). $p < 0.05$ and $p < 0.01$ were considered to be significant and extremely significant differences, respectively. Data analyses were carried out using SPSS ver13 (SPSS, Chicago, IL, USA).

Results

Characterization of GO

It is generally known that the physical and chemical properties of nanomaterials have a major influence on their induced biological effects. In an effort to further understand the biological implications, SEM, TEM, Raman spectroscopy, and UV-Vis spectrometry were carried out to characterize GO. SEM and TEM images (Fig. 1a, b) show that the GO materials are smooth nanosheets with small wrinkles. The UV-Vis spectrum (Fig. 1c) demonstrates a typical absorption peak at 232 nm on the graphitic structure (Chen et al. 2014), corresponding to π - π transitions of aromatic C=C bonds in GO nanosheets. In the Raman spectrum (Fig. 1d), GO sheets exhibit two prominent peaks at 1596 and 1350 cm^{-1} , which can be indexed to the G and D bands, respectively.

Fig. 1 Characterization of graphene oxide. **a** Scanning electron microscope (SEM) image of GO. **b** Transmission electron microscope (TEM) image of GO. **c** UV-Vis spectra of GO. **d** Raman spectrum of GO



Effects of GO on serum total IgE levels and cytokine concentrations in lung

All OVA-sensitized groups displayed heightened (Fig. 2a, $p < 0.01$) IgE concentrations compared with the saline group. Low dose of GO (0.04 mg/kg) exposure significantly elevated the content of IgE (Fig. 2a, $p < 0.05$), while higher concentrations (0.40 and 4.00 mg/kg) were detected to induce even more increases (Fig. 2a, $p < 0.01$) in serum compared with the OVA-sensitization group. IL-4 concentrations (Fig. 2b, $p < 0.05$) increased (Fig. 2c, $p < 0.05$) and IFN- γ concentrations (Fig. 2c, $p < 0.01$) decreased in OVA-sensitized groups compared with the saline group. Compared with the OVA-sensitization group, the OVA combined with 0.40 mg/kg GO and OVA combined with 4.00 mg/kg GO groups showed significant (Fig. 2b, c, $p < 0.05$) and extremely significant (Fig. 2b, c, $p < 0.01$) differences in both IL-4 and IFN- γ levels, respectively.

GO treatment effects visualized as histopathological lung changes in the presence of OVA

As shown in Fig. 3, H&E, MT, and PAS staining methods were directly used to observe the histopathological changes in the lung. Thickened subepithelial cell layers, bent airway lumen, and the infiltration of inflammatory cells into peribronchial areas were observed in the GO + OVA groups by H&E staining. MT staining showed subepithelial collagen deposition, while PAS staining

displayed mucus hypersecretion, a symptom of airway remodeling. In conclusion, all the results showed that compared with the saline and the OVA groups, the GO exposure groups appeared to have seriously worsened the histological changes in the lung. Furthermore, as data suggests, increasing GO concentration is characterized by more severe histopathological changes (Fig. 3).

Effects of GO treatment on AHR in mice

In this study, AHR, a hallmark of asthma, has been measured according to expiratory and inspiratory resistances as well as dynamic lung compliance (Cldyn) by using MCH challenge tests. As expected in our investigation of the effect of GO on airway hyperresponsiveness in response to increasing concentrations of MCH, we found a significant increase in the R-areas of inspiratory and expiratory resistances and a significant decrease in Cldyn in all OVA exposure groups. Meanwhile, as the GO concentrations increased from 0.04 to 4.00 mg/kg, continuous upward shifts of the Ri and Re curves were detected (Fig. 4a, b, respectively, $p < 0.01$), suggesting that GO adversely affects the airways.

GO effects on oxidative stress in the lung and the elimination effects of Ve

By assessing the effects of GO on lung oxidative damage in the presence of OVA, we found that OVA treatment could lead to

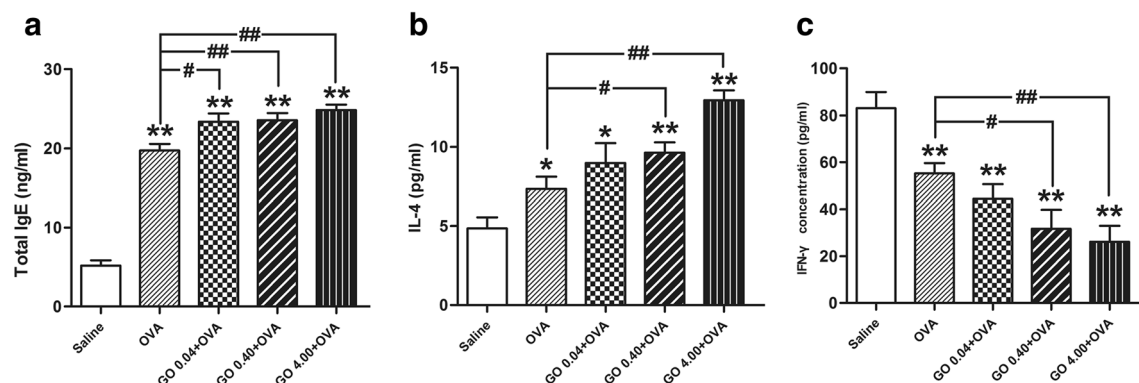


Fig. 2 Effects of GO on total serum IgE levels and cytokine concentrations in the lung. **a** Serum Ig concentrations in the presence of OVA. **b**, **c** Concentrations of IL-4 and IFN-γ in lung with OVA.

* $p < 0.05$, ** $p < 0.01$, compared with the saline group; # $p < 0.05$, ## $p < 0.01$, compared with the OVA group

increases in ROS, indicating the production of oxidative stress. The ROS level in the 4.00 mg/kg GO exposure group displayed an extremely significant difference ($p < 0.01$) compared with the control group and a significant difference ($p < 0.05$) compared with the OVA group (Fig. 5a, $p < 0.01$). After the injection of Ve, increase in ROS content was inhibited significantly (Fig. 5d, $p < 0.05$ or $p < 0.01$). Meanwhile, the results (Fig. 5b) showed that the GSH levels in the OVA and GO exposure with OVA groups were lower than that of the saline group, and that Ve administration restored the GSH activities and boosted the antioxidant system in the lung. Figure 5c showed the difference of MDA levels in response to various treatments. As shown in the results, co-exposure with OVA (0.40 mg/kg GO exposure) caused a significant accumulation of MDA in the lung

($p < 0.05$). Furthermore, the amount of MDA content in the lung for the 4.00 mg/kg GO exposure group was significantly higher ($p < 0.01$) than that of the saline and the OVA groups. However, compared with the 0.40 and 4.00 mg/kg exposure groups, administration of Ve significantly decreased the MDA content ($p < 0.05$ or $p < 0.01$), suggesting that Ve could be used as an antioxidant to inhibit the effect of the oxidative damage.

The elimination effects of Ve on total serum IgE levels and cytokine concentrations in the lung

The amount of IgE reduction due to Ve injection was significantly different between the GO exposure group (0.40 and 4.00 mg/kg) and the protection group (Fig. 6, $p < 0.05$). In

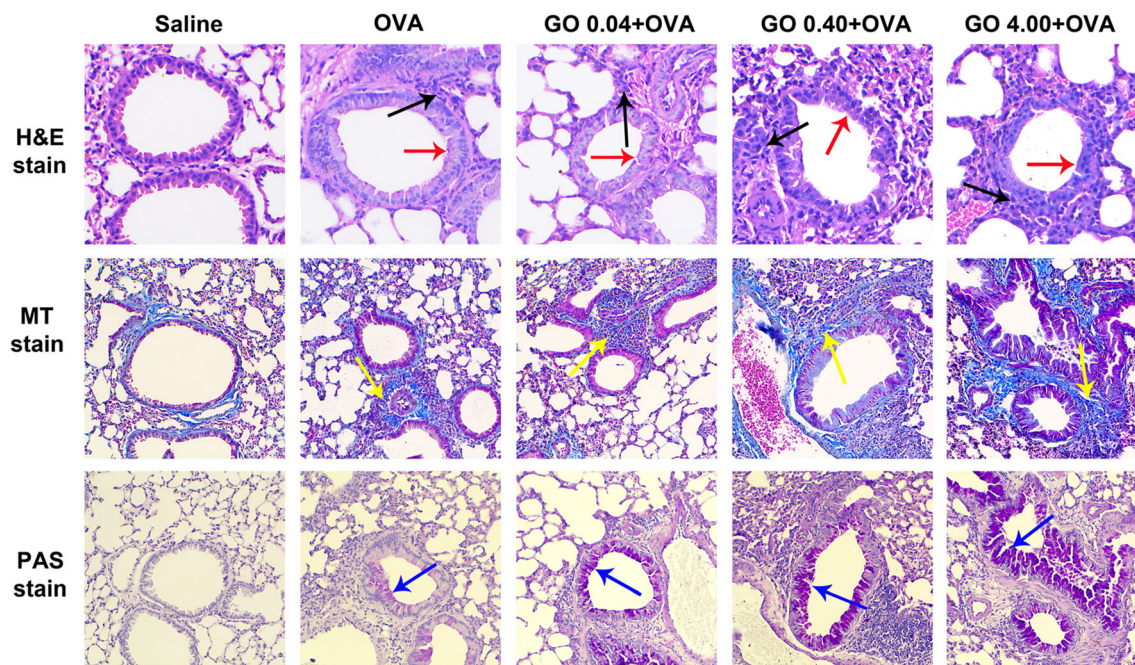


Fig. 3 GO treatment effects visualized as histopathological lung changes in the presence of OVA. Black arrow, lung tissue cell infiltration; red arrow, bronchial remodeling; yellow arrow, subepithelial collagen

deposition (blue-colored stain); blue arrow, mucus hypersecretion (pale pink-colored stain)

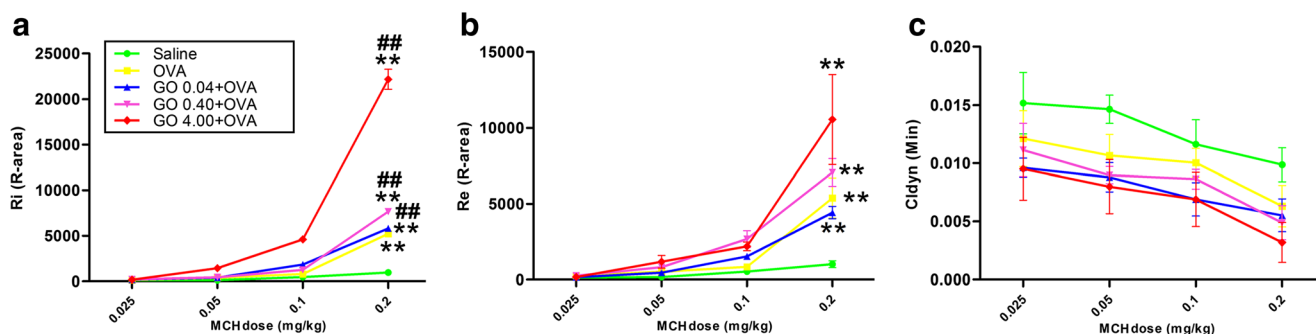


Fig. 4 Effects of GO treatment on AHR in rats. **a–c** Ri, Re, and Cldyn values, respectively, of saline, OVA, GO 0.04 + OVA, GO 0.40 + OVA, and GO 4.00 + OVA groups. $^{**}p < 0.01$, compared with the saline group; $^{##}p < 0.01$, compared with the OVA group

addition, IFN- γ concentrations in GO 0.40 + OVA and GO 4.00 + OVA groups were extremely lower after the injection of Ve. On the other hand, IL-4 concentrations expressed an inverse trend, indicating that the balance of Th1/Th2 was restored (Fig. 6b, c, $p < 0.05$, $p < 0.01$).

Protective effects of Ve on histopathological changes in lung tissue and AHR

H&E, MT, and PAS staining methods showed that treatment with Ve markedly reduced the magnitude of airway remodeling, the level of collagen deposition, and the overproduction of mucus (Fig. 7), indicating that Ve could abate the degree of the airway remodeling significantly. AHR assays were conducted to decide whether Ve could attenuate asthmatic effects exacerbated by GO.

Based on the AHR results, downward shifts of the Ri and Re curves (Fig. 8, $p < 0.01$) and upward shifts of the Cldyn curves were detected in Ve protection groups, suggesting that Ve has an inhibitory effect on the development of allergic AHR.

Discussion

The balance of Th1 and Th2 immune responses and total IgE production are significant in the clinical diagnosis of allergic asthma (Busse and Lemanske 2001; Park et al. 2009). Therefore, total IgE together with IL-4 (a Th2 cytokine) and IFN- γ (a Th1 cytokine) were detected to confirm the establishment of mice asthma model at the molecular level. All antigen-sensitized groups showed a decrease in IFN- γ

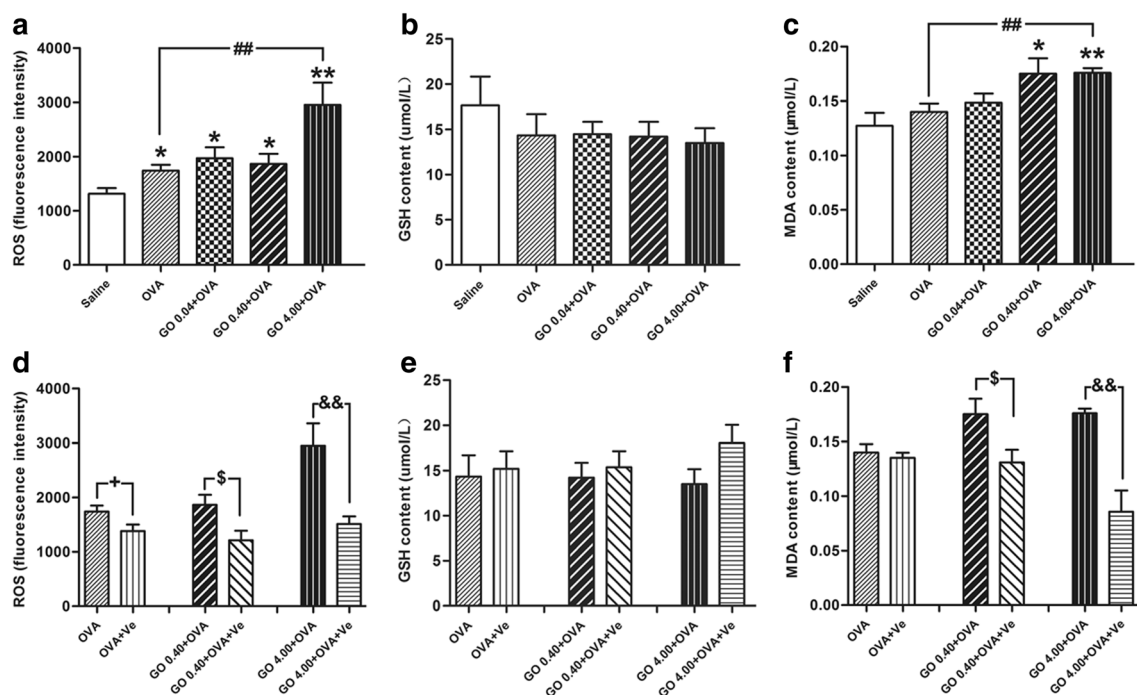


Fig. 5 GO effects on oxidative stress in the lung. **a–c** Effects of GO on ROS, GSH, and MDA in the presence of OVA; $^{*}p < 0.05$, $^{**}p < 0.01$, compared with the saline group; $^{##}p < 0.01$, compared with the OVA group. **d–f** The elimination effects of Ve on ROS, GSH, and MDA;

$^{+}p < 0.05$, compared with the OVA group; $^{§}p < 0.05$, compared with the GO 0.40 + OVA group; $^{&&}p < 0.01$, compared with the GO 4.00 + OVA group

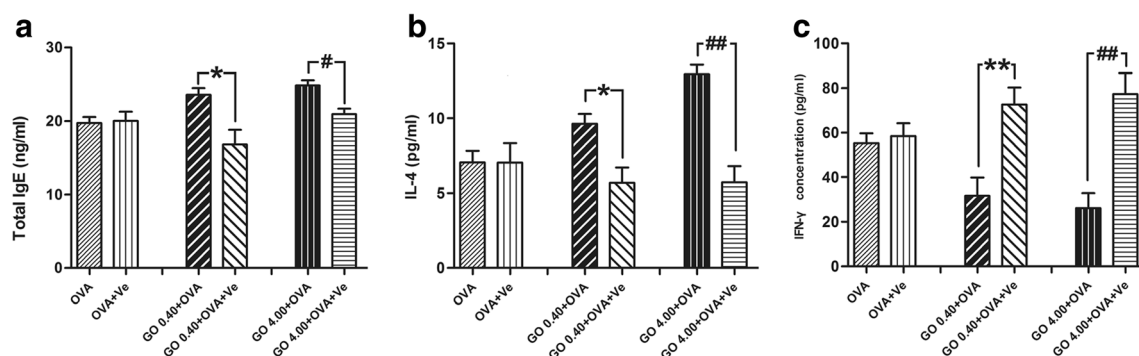


Fig. 6 The elimination effects of Ve on total serum IgE levels and cytokine concentrations in the lung. **a** Serum Ig concentrations with protective effects of Ve in the presence of combined GO + OVA treatment. **b, c** Concentrations of IL-4 and IFN- γ with protective effects

of Ve in the presence of combined GO + OVA treatment. * $p < 0.05$, ** $p < 0.01$, compared with the GO 0.40 + OVA group; # $p < 0.05$, ## $p < 0.01$, compared with the GO 4.00 + OVA group

contents and an increase in IL-4 levels when compared with the saline group, indicating that the balance of Th1/Th2 was disturbed and that the Th2 immune response played a dominant role in antigen-treated mice. Consistent with the results of the cytokine detection, after antigen sensitization, total IgE concentrations increased significantly, which corresponded well with the Ig indicators in the mice allergic asthma model. In addition, 0.40 and 4.00 mg/kg GO administrations accelerated Th2 immune responses in a dose-dependent manner. The concentration of total IgE was also triggered after 0.40 and

4.00 mg/kg GO administration in the presence of antigen. Serum total IgE and lung cytokine content demonstrated that airway inflammation developed after antigen treatment and that 0.40 and 4.00 mg/kg GO treatments exacerbated the antigen-induced allergic inflammation.

Airway remodeling, a consequence of airway inflammation, is a well-established feature of asthma (Gorowiec et al. 2012; Lezmi et al. 2015). In this study, we not only used H&E staining to indicate the thickened subepithelial cell layers and the infiltration of inflammatory cells in pulmonary tissue after

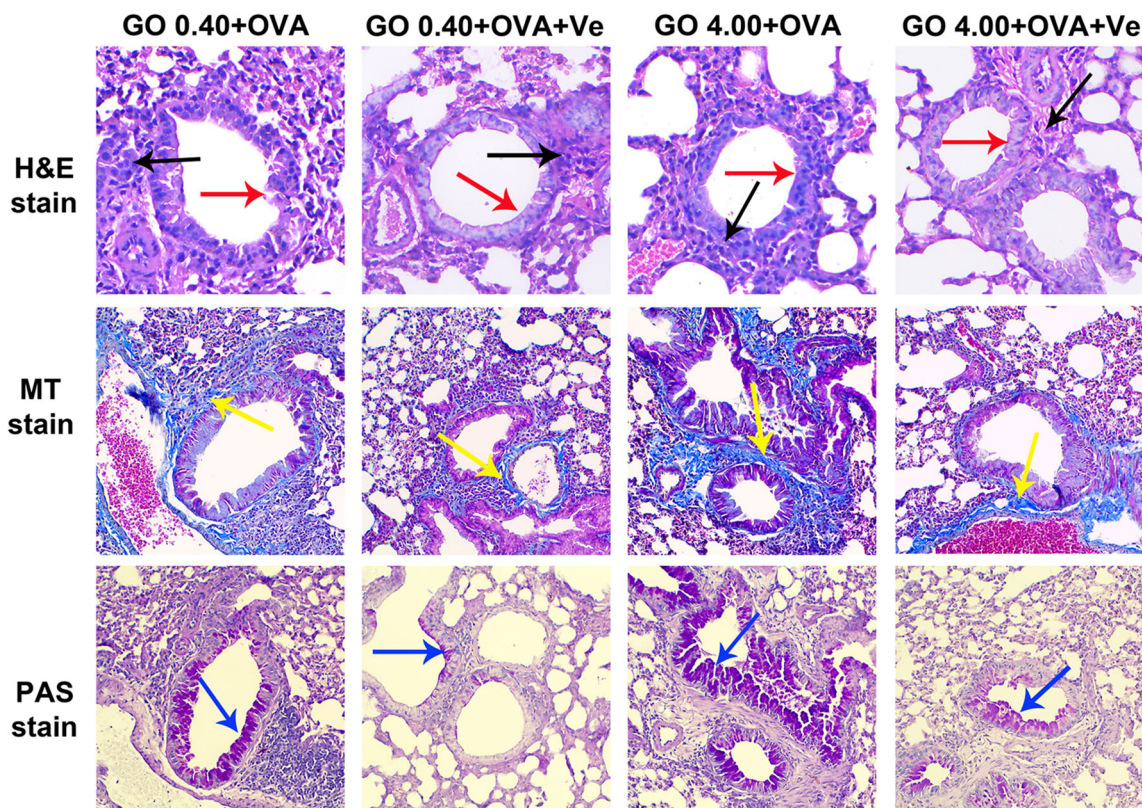


Fig. 7 Protective effects of Ve visualized as histopathological changes in lung tissue caused by combined GO + Ve treatments. *Black arrow*, lung tissue cell infiltration; *red arrow*, bronchial remodeling; *yellow arrow*,

subepithelial collagen deposition (*blue-colored stain*); *blue arrow*, mucus hypersecretion (*pale pink-colored stain*)

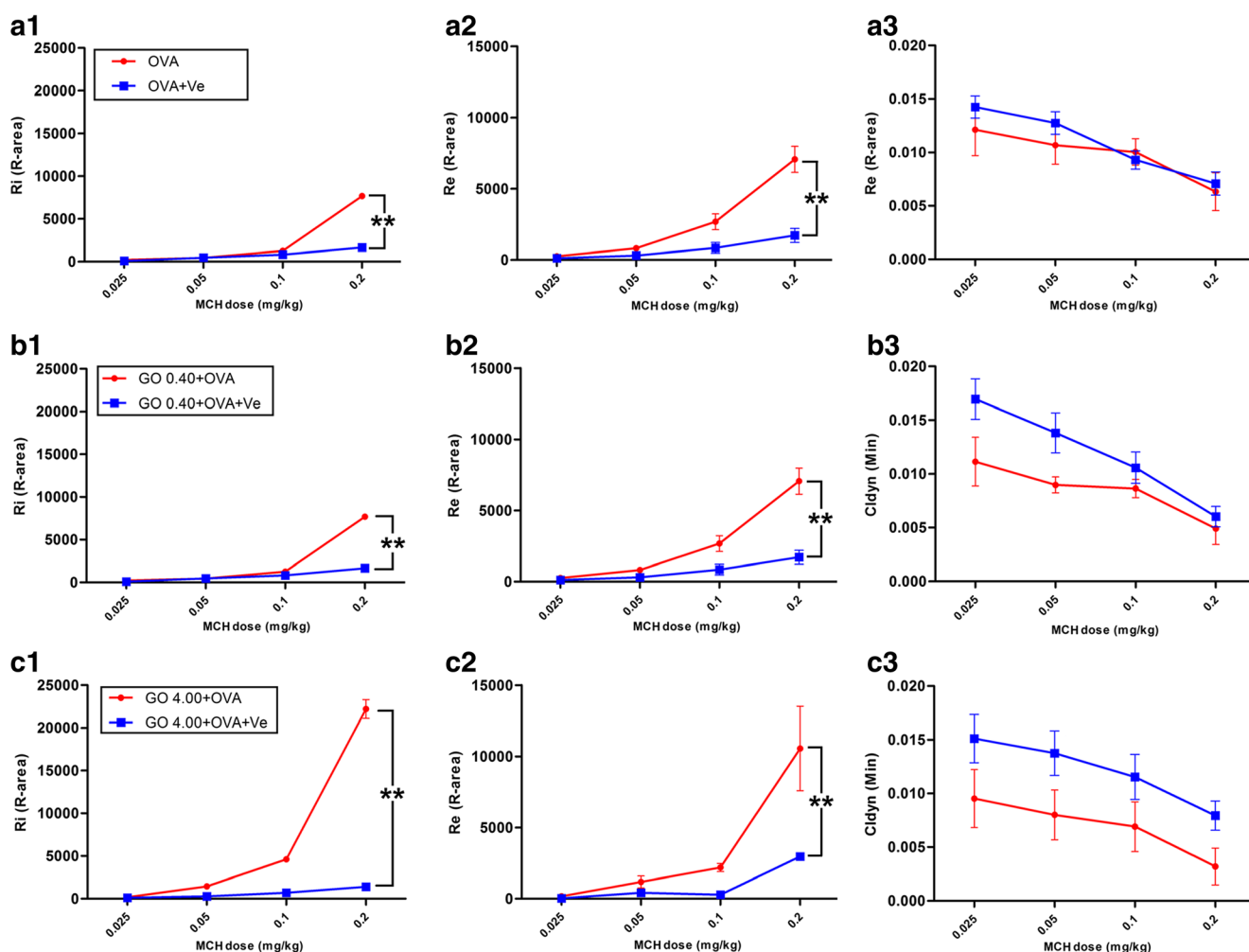


Fig. 8 Effects of Ve treatment on AHR in the presence of combined GO + OVA treatment. *A1–A3* represent Ri, Re, and Cldyn values, respectively, of the OVA and OVA + Ve groups. *B1–B3* represent Ri, Re, and Cldyn values, respectively, of the

GO 0.40 + OVA and GO 0.40 + OVA + Ve groups. *C1–C3* represent Ri, Re, and Cldyn values, respectively, of the GO 4.0 + OVA and GO 4.0 + OVA + Ve groups. **** $p < 0.01$, compared with the GO 0.40 + OVA group. **** $p < 0.01$, compared with the GO 4.0 + OVA group

GO treatment, but also applied PAS and MT staining to demonstrate mucus hypersecretion and subepithelial collagen deposition in the airway. These three staining techniques indicated that histopathological change in lung aggravated after antigen treatment and that 0.40 and 4.00 mg/kg GO exposure further promoted antigen-induced lesions in lung tissues. Another critical, clinical characteristic of allergic asthma is AHR (Hollingsworth 2015; Peters et al. 2001). The R-areas of the respiratory resistance and the variation in Cldyn represent changes in the large airways and the state of small airways, respectively (Han et al. 2011). Upward change of Ri and Re curves induced by the GO concentration increased from 0.40 and 4.00 mg/kg, indicating that GO negatively influence the large airways of the lung tissue.

Previous studies have shown that GO exposure in a model of OVA-induced asthma leads to potentiation of airway remodeling and hyperresponsiveness with the induction of mammalian chitinases (Shurin et al. 2014). However, the mice

were injected intraperitoneally with three different GO suspensions for 32 consecutive days instead of intratracheally with one concentration of GO three times. According to the above results, a mice allergic asthma model was successfully established, and exacerbation effects of GO on antigen-induced allergic asthma were also determined by detecting serum total IgE, cytokine concentrations, histopathological changes in lung tissues, and AHR tests. The detection for GO effect in this study was consistent with other nanoparticle studies such as those for TiO₂, silica, gold nanoparticles, and single-walled carbon nanotubes (SWCNTs) (Gustafsson et al. 2014; Han et al. 2011; Inoue et al. 2010; Qian et al. 2014; Zhang et al. 2012). Considering the pervasive application of nanotechnology, more and more nanomaterials can be expected to enter the environment and the risk of exposure to nanomaterials will greatly increase for people with various diseases, such as allergic asthma. Therefore, there is an urgent need for a strategy to ensure the safe use of nanomaterial.

GO-induced nanotoxicity via the ROS-dependent pathway has been illustrated in the existing literature (Ding et al. 2014; Gurunathan et al. 2013; Qu et al. 2013). Recent reports have suggested that ROS plays an important role in the deteriorating effects of nanomaterials on airway inflammation (Inoue et al. 2010; Li et al. 2014; Sisler et al. 2014). Therefore, the ROS pathway could be a mechanism explaining the aggravating effects of GO on antigen-induced allergic asthma. In present research, an antioxidative reagent Ve was shown to effectively reduce GO-induced oxidative stress in the presence of antigen. According to these findings and others from the literature (Dworski et al. 2011; Li et al. 2014; Wang et al. 2012), it can be speculated that Ve might possibly be applied to antagonize the exacerbation effect of GO in the mouse asthma model.

In order to confirm this hypothesis, we looked at what effect Ve had on serum total IgE and cytokine contents, histopathological changes, and AHR in the presence of a combined administration with OVA + GO. The application of Ve did greatly change the IL-4 and IFN- γ contents, indicating that the balance of Th1/Th2 was restored. Decreases in total IgE levels further confirmed the protective effects of Ve on GO + OVA-induced allergic inflammation. Since Ve exhibited no effect on OVA-related increases in total IgE and cytokine concentrations, this suggests that the antioxidant might specifically target GO-induced responses. Meanwhile, other biomarkers (i.e., OVA-IgE and OVA-IgG1) should be detected to further determine the exacerbation effects of GO on antigen-induced allergic asthma and protect effective of Ve. Therefore, more studies are needed for understanding the mechanism on GO-induced pulmonary injury in order to effectively prevent related health problems in the future.

Airway remodeling is an important indicator for Ve's therapeutic effect at the histopathological level. The degree of airway remodeling, cell infiltration, subepithelial collagen deposition, and mucus hypersecretion were less in the GO 0.40 + OVA + Ve and GO 4.00 + OVA + Ve groups compared with the GO + OVA groups. Since asthma symptoms, including coughing, wheezing, chest tightness, and shortness of breath, are based on the presence of AHR, measures must be conducted to confirm whether Ve functions by attenuating the GO-induced asthma deterioration effects. Based on the AHR results for all Ve administration groups, a downward shift in the Ri and Re curves were obtained. Data from tissue level detection and AHR tests supported that Ve is able to attenuate the deterioration effects of GO on allergic asthma. From the above, we concluded that Ve is an effective protective reagent for attenuating GO deterioration in antigen-induced asthma.

Conclusion

In this study, by measuring changes in asthma-related serum total IgE, cytokine content, and histopathological changes in the lung tissue and AHR test, an antigen-sensitized mouse

asthma model was established in order to detect the aggravation effect of allergic asthma by GO. The results indicated that GO could worsen allergic asthma. Moreover, an antioxidative reagent Ve was used to antagonize GO-induced deterioration effects by reducing oxidative stress. Based on the above results, an underlying mechanism behind Ve's protective effect was proposed, referring to oxidative stress elimination, reduced IgE concentration, downregulation of Th2 responses, and relief of allergic asthma symptoms. The present research suggested that Ve might provide support for an effective strategy for protecting individuals with allergic asthma from GO or other nanoparticle-caused aggravating effects. In order to detect more therapeutic targets and provide more solid data, nanomaterial exposure experiments should be performed in combination with animal disease models in the future study.

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Author's contributions Shuai Shang, Jinquan Li, Zhuge Xi, Xu Yang, and Rui Li conceived and designed the experiments. Shuai Shang, Jinquan Li, and Yun Zhao performed the experiments. Shuai Shang, Jinquan Li, Zhisong Lu, Xu Yang, and Rui Li analyzed the data. Zhuge Xi, Zhisong Lu, Xu Yang, and Baizhan Li contributed reagents, materials, and analysis tools. Shuai Shang, Jinquan Li, Zhisong Lu, Xu Yang, and Rui Li wrote the manuscript. All authors have given approval to the final version of the manuscript.

Compliance with ethical standards Compliance with National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Office of Scientific Research Management of Central China Normal University (No. CCNU-SKY-201-008).

Conflict of interest The authors declare that they have no conflicts of interest.

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