

In vitro and in vivo Antiallergic Effects of Taurine on Allergic Rhinitis

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Keywords

Taurine · Oxidative stress · Extracellular superoxide dismutase · Peroxisome proliferator-activated receptor-γ · Allergic rhinitis

Abstract

Background: The current treatment for allergic rhinitis (AR) is inadequate. **Objective:** The present study aimed to investigate the therapeutic effect of taurine on AR and to identify the underlying molecular mechanisms. **Methods:** The serum level of the antioxidant enzyme extracellular superoxide dismutase (SOD3) was determined in AR patients and in healthy controls. The antiallergic inflammatory effects of taurine were evaluated in a dinitrophenyl-human serum albumin (DNP-HSA)-stimulated human mast cell line (HMC-1) and in an ovalbumin (OVA)-induced AR mouse model. **Results:** Clinically, a reduction in serum level of SOD3 was observed in AR patients. Taurine treatment led to dose-dependent increases in SOD3 at both protein and mRNA levels in HMC-1 cells. SOD3 production was regulated by peroxisome proliferator-activated receptor-γ (PPAR-γ) in response to taurine. SOD3 overexpression inhibited the release of proinflammatory cytokines including tumor necrosis factor-α, interleukin (IL)-4, and IL-6. Its overexpression also ameliorated the loss of interferon-γ. SOD3 and PPAR-γ influenced inflamma-

tory cytokine production via regulation of the phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2). An OVA-induced AR animal model study showed that taurine was efficacious in alleviating allergic inflammatory reactions by relieving behavior symptoms of AR mice and reducing eosinophilic and mast cell infiltration into the nasal cavity. In addition, taurine treatment increased the production of SOD3 and PPAR-γ, which, in turn, suppressed expression of proinflammatory cytokines through phosphorylation of ERK1/2. **Conclusion:** Taurine could potentially serve as a therapeutic treatment for allergic disorders.

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Introduction

Allergic rhinitis (AR) is an immunoglobulin E (IgE)-mediated, nasal mucosal hypersensitivity reaction to allergens such as pollen, molds, animal dander, and dust mites. Symptoms include sneezing, rhinorrhea, and nasal congestion and irritation [1]. AR has become a serious public health problem with increasing prevalence that currently affects 1 in 6 individuals worldwide [2].

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Upon introduction of an allergen, inflammatory cells including eosinophils, basophils, mononuclear cells, and mast cells enter the nasal mucosa and release a number of allergic mediators such as histamine, leukotrienes, interleukin (IL)-4, IL-6, interferon- γ (IFN- γ), and tumor necrosis factor (TNF)- α , thus activating inflammatory reactions [3]. AR negatively impacts the quality of life of sufferers. Despite extensive options to treat the symptoms of AR, there is no known cure, and about 20% AR patients do not respond well to current pharmacotherapy [4].

The role of oxidative stress in the pathogenesis of airway allergic inflammation such as asthma and AR has been studied extensively [5]. During oxidative stress, eosinophils release cytotoxic mediators such as eosinophilic peroxidase, resulting in severe damage to the epithelia [6]. Extracellular superoxide dismutase (SOD3) is an antioxidant enzyme that may protect tissues from oxidative stress by removing superoxide radicals [7]. Administration of SOD3 to mice with allergic asthma tempered allergic responses [8]. Peroxisome proliferator-activated receptor- γ (PPAR- γ) is a member of the nuclear hormone receptor superfamily [9]. PPAR- γ plays a pivotal role in the regulation of lipid and glucose metabolism, cellular proliferation, and control of inflammation [10, 11]. Studies have demonstrated that activation of PPAR- γ inhibits the release of inflammatory mediators from various cell types [12, 13]. Activation of PPAR- γ reduces human airway hyperresponsiveness and asthma-induced eosinophil activation [14, 15]. Interactions between SOD and PPAR- γ on the survival of diabetes mellitus patients have been detected [16]. However, the relationship between SOD3 and PPAR- γ in the pathogenesis of allergic disorders such as AR has not been investigated.

Taurine (2-aminothanesulfonic acid) is the most abundant free amino acid in humans. It has been implicated in essential physiological functions such as bile acid conjugation, maintenance of calcium homeostasis, osmoregulation, antioxidation, modulation of neurotransmitters, and membrane stabilization [17]. The concentrations of taurine in tissues upon the exposure to elevated levels of oxidants are particularly high, suggesting its association with oxidative stress [18]. As an effective antioxidant, taurine has been demonstrated to control proinflammatory cytokine production [19]. Both in vitro and clinical studies revealed potential therapeutic effects of taurine and its derivatives on various topical infections and chronic inflammatory diseases [20–22]. However, little is known about the pharmacological effects and un-

derlying mechanisms of taurine on allergic inflammatory disorders such as AR. The aim of the present study was to test the potential therapeutic effects of taurine on AR and to elucidate molecular mechanisms for those effects. We hypothesize that the antiallergic effect of taurine on AR is through regulation of the antioxidant enzyme SOD3 which is mediated by PPAR- γ activation.

Materials and Methods

Clinical Study

A nested case-control study was conducted to compare the serum level of SOD3 in normal controls ($n = 60$) and AR patients ($n = 60$). These AR patients, aged between 20 and 50 years, do not have any other chronic medical conditions or allergic disorders except AR. Age-matched healthy people without chronic medical conditions or allergic disorders were selected as controls. This study has been approved by the Ethics Committee of Dahua Hospital (Shanghai, China). All experiments were performed in accordance with relevant guidelines and regulations. All participants provided written informed consent.

Cell Culture and Stimulation

Human mast cells (HMC-1) were purchased from Millipore and grown at 37 °C/5% CO₂ in HMC expansion medium supplemented with Iscove's medium, 1.2 mM α -thioglycerol, 10% fetal bovine serum (GIBCO catalog #16000-044) and 1 $\times$ penicillin/streptomycin (GIBCO). HMC-1 cells were treated with 0, 5, 10, 20, 40, and 80 mmol/L taurine. SOD3 expression was measured by real-time (RT) PCR and Western blot.

To sensitize the HMC-1 cells, cells were incubated with anti-dinitrophenyl (DNP) IgE (final concentration of 200 μ g/L) in culture medium overnight. Then antigen DNP-human serum albumin (HSA) (final concentration of 20 μ g/L) was added to stimulate immunization. In one treatment group, 20 mmol/L taurine was added with DNP-HSA. In another treatment group, 20 mmol/L taurine and 1 μ mol/L PPAR- γ antagonist GW9662 were both added together with DNP-HSA. The treated and untreated control groups were incubated for 30 min at 37 °C. After centrifugation, the levels of SOD3 and β -hexosaminidase A in the supernatant in each experimental group of HMC-1 cells were quantified by enzyme-linked immunosorbent assay (ELISA). The SOD3 and PPAR- γ expression levels were measured by RT-PCR and Western blot.

Effect of SOD3 Overexpression in DNP-HSA-Induced HMC-1 Cells

The HMC-1 cells were transfected with SOD3 overexpression vector in order to overexpress SOD3. Transfected HMC-1 cells were sensitized with anti-DNP IgE and immunized by antigen DNP-HSA as previously described. As a positive control, 5 μ mol/L PPAR- γ agonist rosiglitazone was added. ELISA was used to quantify the expression level of SOD3, β -hexosaminidase A, IFN- γ , IL-4, IL-6, and TNF- α in the cell supernatant. Expression levels of SOD3, PPAR- γ , extracellular signal-regulated kinase 1/2 (ERK1/2) and its phosphorylated form p-ERK1/2 were measured by RT-PCR and Western blot.

Mast Cell Degranulation Assay

Normal and transfected HMC-1 cells, after sensitization with anti-DNP IgE and immunization with DNP-HSA, were washed and resuspended in HEPES buffer. Taurine and/or the PPAR- γ antagonist GW9662 were added. All samples were run in duplicate. After 30-min incubation, the supernatant was combined with *p*-nitrophenyl N-acetyl- β -D-glucosamide substrate solution and assayed for the presence of β -hexosaminidase A using ELISA (Cusabio, catalog #CSB-E09462h, USA).

Flow Cytometric Analysis

Sensitized normal and transfected HMC-1 cells, immunized with DNP-HSA, treated with taurine and/or PPAR- γ antagonist GW9662, were collected and treated with trypsin/EDTA solution. The cells were stained with 10 μ mol/L Fluo-3, Am, calcium indicator (Thermo Fisher Scientific, catalog #F1241, USA) and analyzed using a FACS-Accuri C6 cytometer (BD Bioscience, USA). Intracellular calcium concentration is calculated by the following formula:

$$[Ca^{2+}]_{free} = K_d \frac{F - F_{min}}{F_{max} - F}$$

F represents the value of each cell's fluorescence intensity. Maximum fluorescence intensity (F_{max}) was determined by permeabilizing cells with PBS plus 1% Triton X-100 for 30 min followed by administering a saturating concentration (1 mmol/L) of calcium chloride for 10 min. Minimum fluorescence intensity (F_{min}) was determined by chelating free calcium by incubating cells in 10 mM EDTA for 10 min. Fluorescence measured at 488 nm is F_{min} . K_d = 390 nm.

Lentiviral Overexpression and siRNA

For overexpression, PPAR- γ was inserted into the pLVX-Puro lentiviral vector. 293T cells (ATCC) were transfected using LipofectamineTM 2000 (Invitrogen) with pLVX-Puro PPAR- γ (1,000 ng) (Clontech), psPAX2 (100 ng) (Addgene), and pMD2G (900 ng) (Addgene). For siRNA, shPPAR- γ was inserted into pLKO.1-Puro lentiviral vector (Addgene). 293T cells were transfected using LipofectamineTM 2000 with pLKO.1-shPPAR- γ (1,000 ng), psPAX2 (900 ng), and pMD2G (100 ng) (Addgene). Four to six hours after transfection, cell medium was changed to growth medium. Viral particles were collected 48 and 72 h later.

Dual Luciferase Reporter Assay

MKN45 cells were transfected with pGL3-Enhancer-p SOD3 or internal control with PPAR- γ siRNA or PPAR- γ using LipofectamineTM 2000 according to the manufacturer's instructions. After 48 h, cells were harvested and lysed. Luciferase reporter activity was measured using the Dual-Luciferase Assay kit (Promega, catalog #E1910) and a Multilabel Plate Reader.

Chromatin Immunoprecipitation

Chromatin immunoprecipitation (ChIP) assays were conducted using the ChIP assay kit according to the manufacturer's protocol. Briefly, the HMC-1 cells were transfected with either a PPAR- γ overexpression vector or siRNA targeting PPAR- γ . Histones were cross-linked to DNA by adding formaldehyde directly to the culture media to a final concentration of 1%. Cells were washed twice using ice-cold PBS and then pelleted by centrifug-

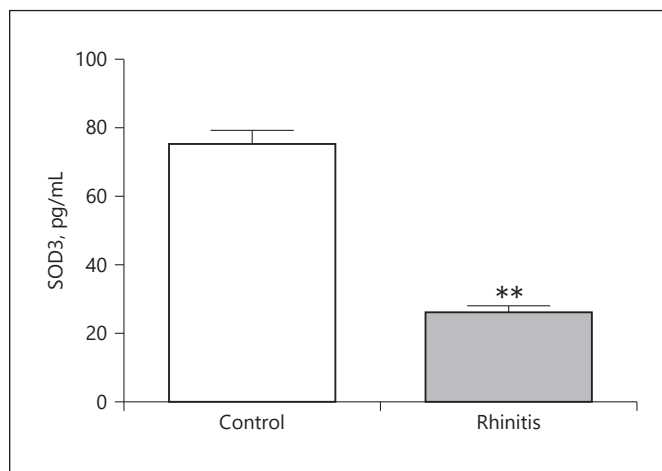


Fig. 1. The SOD3 concentration in serum was significantly decreased in AR patients ($n = 60$) compared to healthy controls ($n = 60$) as measured by ELISA. ** $p < 0.01$.

ing. Resuspended cells were lysed in SDS lysis buffer and subsequently sonicated on ice to shear DNA to lengths between 150 and 900 bp. Sheared chromatin was diluted in ChIP dilution buffer and precleared with protein agarose-A beads. Precleared chromatin was immunoprecipitated with an antibody against PPAR- γ or control normal IgG. Antibody and chromatin mixtures were rotated overnight at 4°C and precipitated with protein agarose-A. Beads were washed by rotation at 4°C with low salt followed by high salt immune complex wash buffer. Protein-DNA complexes were eluted with elution buffer. Formaldehyde cross-links were reversed by exposure to NaCl, proteinase K and heating at 65°C for 2 h. DNA was purified and analyzed by PCR. For amplification of enhancer elements (856 bp) of the SOD3 gene, the following primers were used: SOD3 forward, 5'-GGAC-GGTAGGGTGAAGGG-3' and reverse, 5'-AGCGGGTGGG-TCGTGATG-3'. PCR products were separated and examined using electrophoresis.

RNA Isolation and RT-PCR Analysis

Total RNA was extracted by TRIzol reagent (Invitrogen) according to the manufacturer's protocol. Briefly, the cells were resuspended in 1 mL of TRIzol reagent and incubated for 5 min. Chloroform (200 μ L) was added and vortexed, followed by another 2 min of incubation. The RNA-containing aqueous phase was extracted after centrifugation at 12,000 g for 10 min at 4°C. RNA was precipitated with isopropyl alcohol, and the pellet was washed with 75% ethanol and air-dried. Isolated RNA was reverse transcribed into cDNA. RT-PCR was performed with a Light Cycle System (Roche Applied Science) according to the manufacturer's protocol (Thermo, catalog #K0223, Fermentas, catalog #K1622). Two microliters of cDNA were amplified using the primer sets for SOD3 (primer F: 5'-ACTCAGAGCGCAAGAAGC-3'; primer R: 5'-TCAGCAAAGGCGAAGGTG-3'), PPAR- γ (primer F: 5'-GACACAGAGATGCCATTC-3'; primer R: 5'-CAGGCTCCACTTTGATTG-3'), and glyceraldehyde 3-phosphate dehydrogenase (primer F: 5'-AATCCCATCACCATCTTC-3'; primer R: 5'-AG-GCTGTTGTCATACTTC-3').

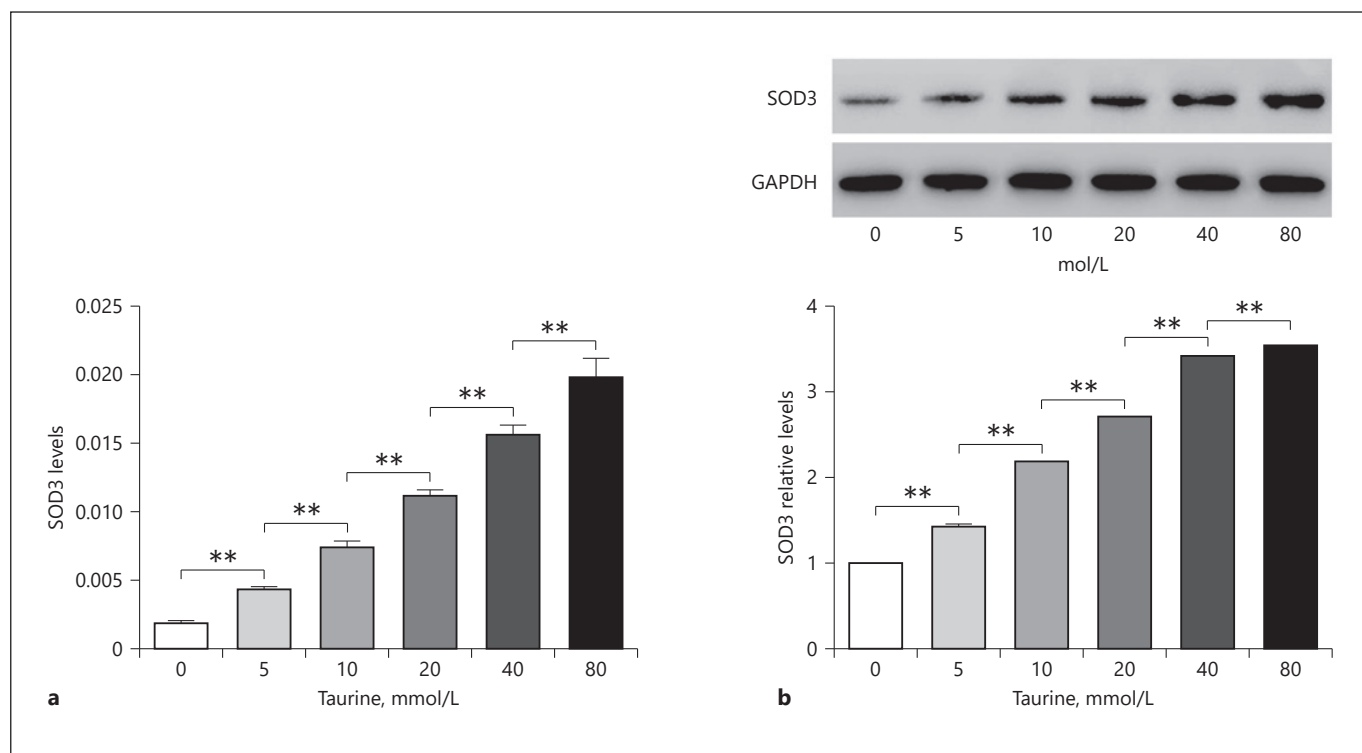


Fig. 2. Effect of taurine on SOD3 protein expression and mRNA transcription in HMC-1 cells. **a** Assessed by quantitative RT-PCR, SOD3 mRNA increased in HMC-1 cells treated with taurine in a dose-dependent manner. **b** Western blot analysis revealed taurine increased the protein level of SOD3 in HMC-1 cells in a dose-dependent manner. ** $p < 0.01$.

Ovalbumin-Induced AR Animal Model

Healthy C57BL/6 mice were 6–8 weeks old and free of murine-specific pathogens. Each mouse weighed 17–20 g. Male and female mice were raised in separate environments. All animals were handled according to protocols approved by the Animal Care Committee of Dahua Hospital (Shanghai, China). Systemic sensitization was performed with an intraperitoneal injection of 200 μ L of 0.1 mg/mL ovalbumin (OVA) in 20 mg/mL aluminum hydroxide on days 1, 8, and 15. Afterwards, local immunization was induced by intranasal instillation of 20 μ L of 10 mg/mL OVA 20 from day 22 to 29. Mice were divided into 5 groups ($n = 6$ for each group): (a) the negative control group that was challenged and treated with saline, (b) the AR group that was challenged with OVA and treated with saline, (c–e) the taurine treatment groups that were challenged with OVA and treated with 1, 3, and 5% taurine via intranasal instillation once a day after local immunization for 7–10 days.

Evaluation of Allergic Responses

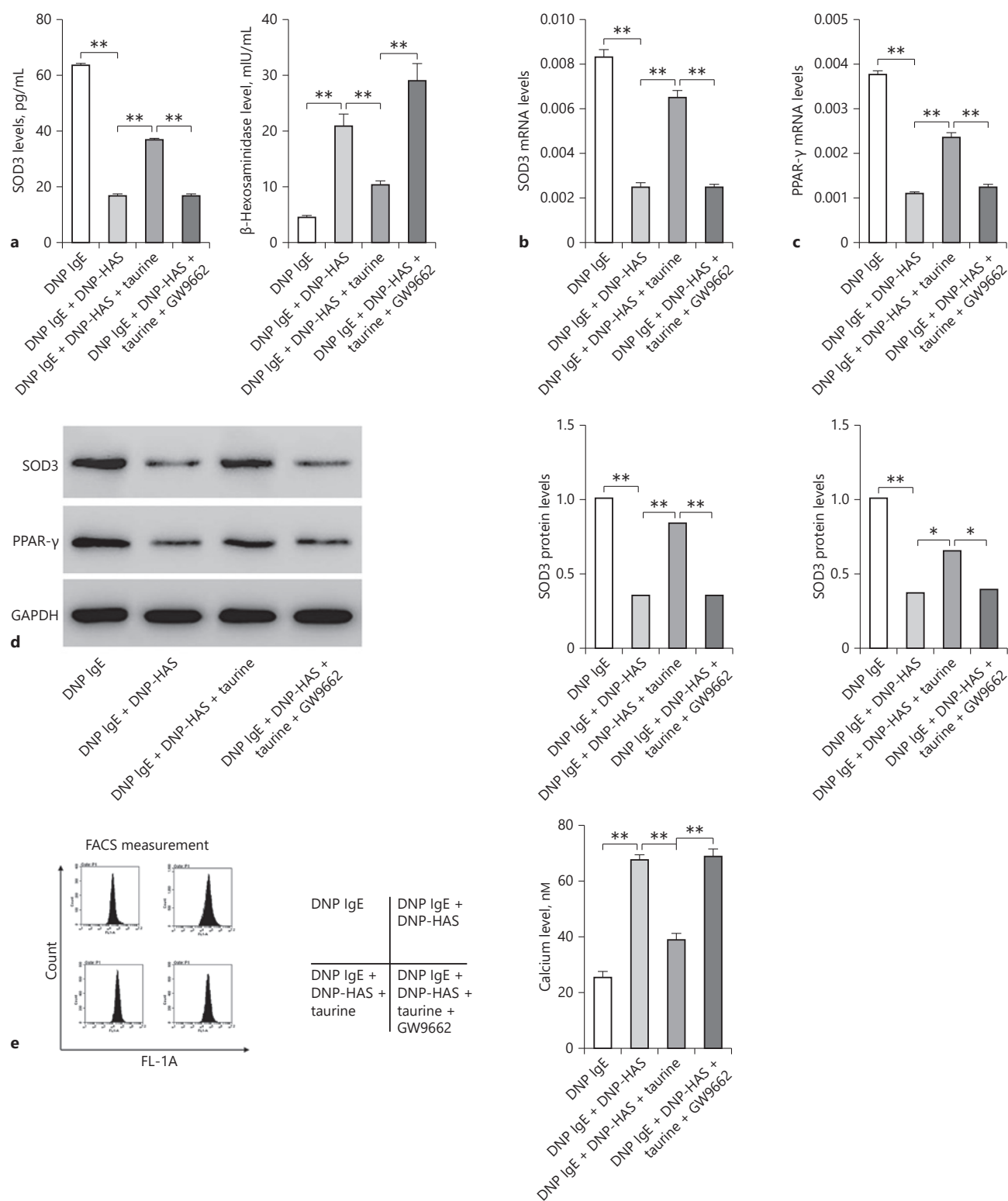
After the final intranasal OVA challenge, each animal was observed for 30 min to score the nasal symptom. The numbers of sneezes and nose rubbing were counted. The taurine-treated OVA-induced AR model was evaluated based on the score in comparison with the control group.

Measurement of SOD3 and Inflammatory Factors in Mouse Serum

Serum levels of SOD and cytokines were measured using ELISA kits according to the manufacturer's instruction (Cusabio, catalog #CSB-EL022399HU, USA). Absorbance at 450 nm was measured using a colorimetric microplate reader and the concentrations of SOD3, IFN- γ , IL-4, IL-6, and TNF- α were calculated using a standard curve (X-Y Biotechnology, Shanghai, China). All ELISA experiments were repeated in triplicates.

Western Blot Analysis

Murine nasal tissue was homogenized, and total protein was prepared by a detergent lysis procedure. Protein samples (25- μ g aliquots) were resolved by 10% SDS-polyacrylamide gel electrophoresis and electrotransferred onto a nitrocellulose membrane. The membrane was blocked with 5% bovine serum albumin at room temperature for 1 h or at 4 °C overnight. The membrane was incubated for 2 h at room temperature or at 4 °C overnight with primary antibodies against ERK1/2 (1:1,000), p-ERK1/2 (1:1,000), SOD (1:500), PPAR- γ (1:1,000), and glyceraldehyde 3-phosphate dehydrogenase (1:2,000) (CST, catalog #9101, #9102, #5174; Abcam, catalog #Ab83108, Ab59256). The membrane was washed with Tris-buffered saline/Tween-20 and developed by horseradish peroxidase-conjugated secondary antibodies. Proteins were visualized by enhanced chemiluminescence procedures.



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Histopathological Assessment

Nasal mucosa tissue was fixed in 10% paraformaldehyde for 48 h, washed with deionized water and embedded in paraffin. The tissue sections (4–7 μ m thickness) were subsequently stained with hematoxylin and eosin to visualize eosinophils (BASO, catalog #714094, #BA4099). The morphology of eosinophils was observed, and the numbers of eosinophils were counted. Toluidine blue O staining was used to visualize and count mast cells in the nasal mucosa tissue (Beijing Leagene Biotechnology).

Statistical Analysis

All data were presented as means $\pm$ SD. All statistical analysis was performed using GraphPad Prism. Student's *t* test and one-way ANOVA with post hoc Tukey test were employed for two groups and multiple comparisons, respectively. *p* values less than 0.05 were considered statistically significant.

Results

Serum SOD3 Level Was Reduced in Patients with AR

Serum concentrations of SOD3 were measured in healthy and AR patients by ELISA (Fig. 1). The SOD3 concentration was significantly lower in the AR patients ($p < 0.01$), indicating the association of the antioxidant enzyme SOD3 with the AR pathological state.

Increased Production and mRNA Expression of SOD3 in HMC-1 Cells Treated with Taurine

The mRNA and protein expression of SOD3 in HMC-1 cells in response to taurine treatment was determined by RT-PCR and Western blot, respectively. Taurine induced both mRNA (Fig. 2a) and protein production (Fig. 2b) of SOD3 in a dose-dependent manner. This result indicates that taurine affects the production and expression of SOD3 in human mast cells.

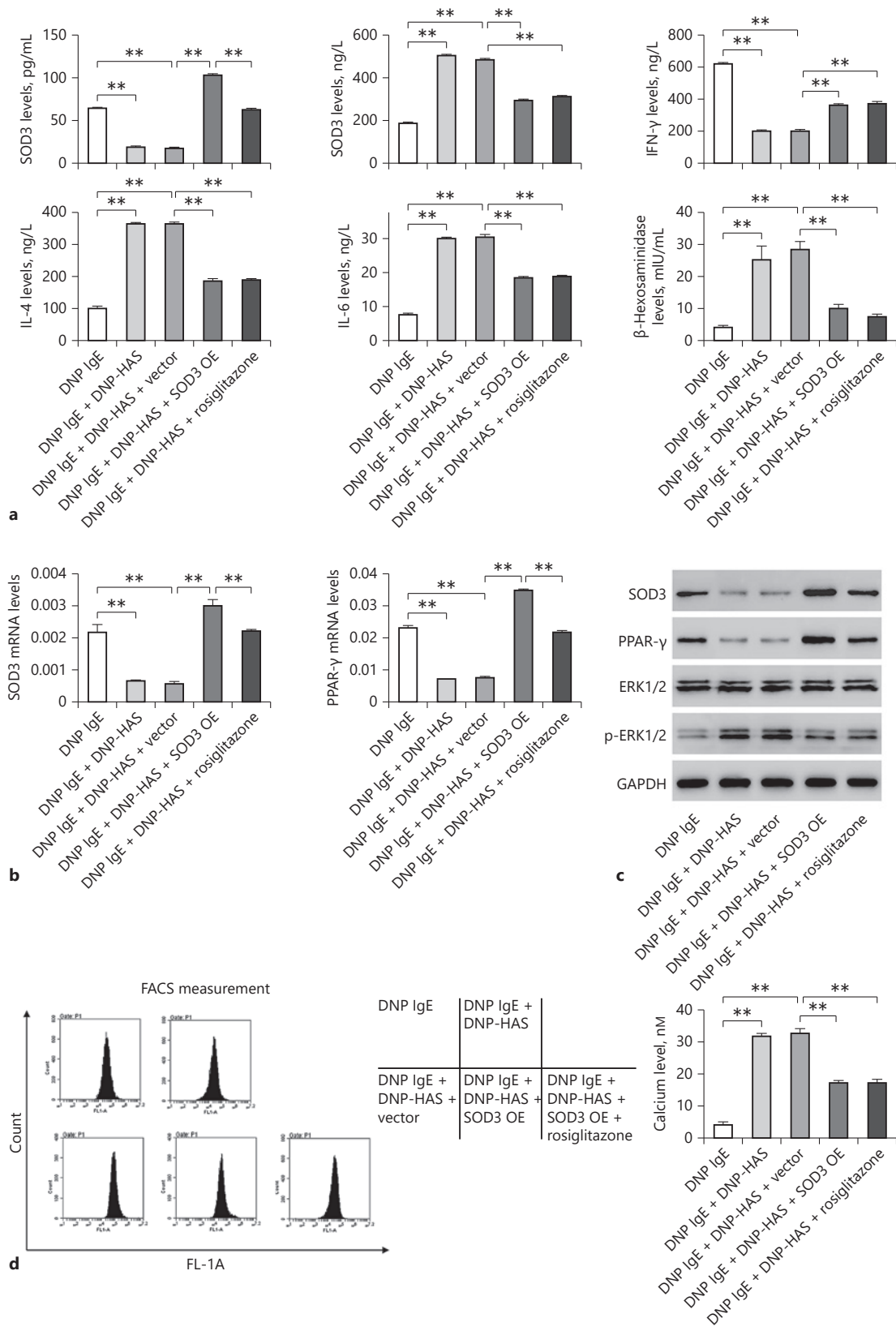
Increased Levels of SOD3 and PPAR- γ in DNP-HSA-Induced HMC-1 Cells Treated with Taurine

To mimic the AR condition in vitro, HMC-1 cells were sensitized with anti-DNP IgE, followed by immunization by adding antigen DNP-HSA. Both mRNA and protein expression of SOD3 and PPAR- γ were significantly decreased in the DNP-HSA-induced HMC-1 cells ($p < 0.01$, Fig. 3a–d). Treatment of 20 mmol/L taurine led to an increase in both SOD3 and PPAR- γ production in the DNP-HSA-stimulated HMC-1 cells, suggesting the anti-allergic effects of taurine are associated with its antioxidative function mediated by the production of SOD3 and PPAR- γ ($p < 0.01$, Fig. 3a–d). Furthermore, the administration of the PPAR- γ antagonist GW9662 reversed the effects of taurine by reducing the expression of both SOD3 and PPAR- γ , suggesting that PPAR- γ could play a role in regulating the transcription of SOD3 ($p < 0.01$, Fig. 3a–d). Additionally, mast cell degranulation occurred after the addition of DNP-HSA antigen as evidenced by a significant increase in the level of the degranulation indicator β -hexosaminidase. Taurine treatment greatly reduced the level of β -hexosaminidase, and the administration of the PPAR- γ antagonist GW9662 offset this effect ($p < 0.01$, Fig. 3a). Flow cytometric analysis showed an increase in intracellular calcium influx in HMC-1 cells after the stimulation of DNP-HSA antigen. Taurine treatment decreased the calcium signal significantly and GW9662 reversed the effect ($p < 0.01$, Fig. 3e).

Effect of SOD3 Overexpression on Mast Cell Degranulation and the Levels of Cytokines in DNP-HSA-Induced HMC-1 Cells

The effect of SOD3 overexpression on the production of cytokines in DNP-HSA-triggered HMC-1 cells was examined using ELISA (Fig. 4a). With the release of the mast cell degranulation indicator β -hexosaminidase, pro-inflammatory cytokines including TNF- α , IL-4, and IL-6 were increased in stimulated HMC-1 cells ($p < 0.01$, Fig. 4a). However, overexpression of SOD3 significantly reduced the release of β -hexosaminidase and these cytokines ($p < 0.01$, Fig. 4a). Secretion of IFN- γ was decreased in DNP-HSA-stimulated HMC-1 cells while overexpression of SOD3 restored IFN- γ release ($p < 0.01$, Fig. 4a). The PPAR- γ agonist rosiglitazone served as a positive control and exhibited a similar effect on changing cytokine release to SOD3 overexpression (Fig. 4a). The mRNA and protein levels of SOD3 and PPAR- γ was significantly increased in the SOD overexpression cells and rosiglitazone-treated cells ($p < 0.01$, Fig. 4b, c). These results indicate that SOD3 and PPAR- γ exhibited antiallergic ef-

Fig. 3. Effect of taurine on mast cell degranulation, SOD3 and PPAR- γ protein expression, and mRNA transcription in DNP-HSA-induced HMC-1 cells. HMC-1 cells were sensitized with anti-DNP IgE, then immunization was stimulated by DNP-HSA. Circulating SOD3 and β -hexosaminidase levels measured by ELISA (a), SOD3 and PPAR- γ mRNA levels measured by RT-PCR (b, c), and protein expression measured by Western blot (d) were reduced by DNP-HSA, but increased by 20 mmol/L taurine treatment, yet taurine's effect was reversed by the PPAR- γ antagonist GW9662. e Flow cytometric analysis of intracellular calcium levels in DNP-HSA-induced HMC-1 cells, treated with taurine and GW9662. ** $p < 0.01$, * $p < 0.05$.



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fects via inhibition of inflammatory cytokine production. Flow cytometric analysis showed an increase in intracellular calcium influx in HMC-1 cells after the stimulation of DNP-HSA antigen. Both overexpression of SOD3 and the PPAR- γ agonist rosiglitazone significantly decreased the calcium level ($p < 0.01$, Fig. 4d).

Effect of SOD3 Overexpression on the Phosphorylation of ERK1/2

Western blot analysis revealed a substantial decrease in SOD3 and PPAR- γ in DNP-HSA-stimulated HMC-1 cells relative to controls (Fig. 4c). However, overexpression of SOD3 and rosiglitazone increased not only SOD3,

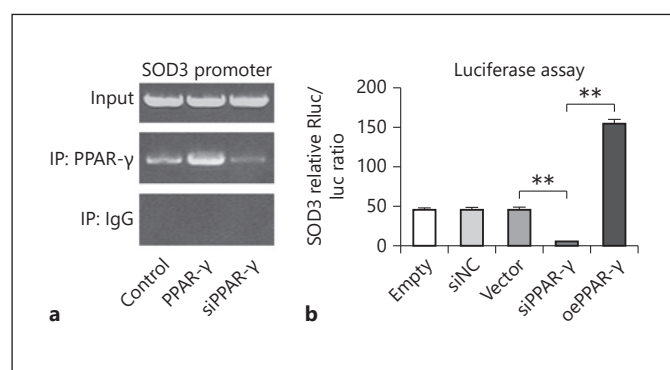


Fig. 5. Interaction between PPAR- γ and SOD3 detected by ChIP and luciferase assays. si, small RNA interference. **a** Using purified DNA as the template, the promoter fragments of SOD3 were immunoprecipitated by a PPAR- γ antibody. IP, immunoprecipitated. **b** PPAR- γ siRNA suppressed SOD3 expression while overexpressed (oe) PPAR- γ increased SOD3 expression as measured by reporter gene activity. siNC, control small RNA interference; Rluc, Renilla luciferase; luc, luciferase. ** $p < 0.01$.

Fig. 4. Effect of SOD3 overexpression on mast cell degranulation and the levels of cytokines in DNP-HSA-induced HMC-1 cells and the phosphorylation of ERK1/2. OE, overexpression; GAPDH, glyceraldehyde 3-phosphate dehydrogenase. **a** SOD3 was overexpressed in DNP-HSA-stimulated HMC-1 cells. The concentration of β -hexosaminidase and production of cytokines including IFN- γ , TNF- α , IL-4, and IL-6 in the supernatant was measured by ELISA. The PPAR- γ agonist rosiglitazone served as a positive control. **b** The mRNA levels of SOD3 and PPAR- γ significantly increased in the SOD overexpression cells and rosiglitazone-treated cells, as measured by quantitative RT-PCR. **c** Protein expression of SOD3, PPAR- γ , ERK1/2, and p-ERK1/2 in anti-DNP IgE-sensitized HMC-1 cells, DNP-HSA-stimulated HMC-1 cells, SOD overexpression cells and rosiglitazone-treated cells via Western blotting. **d** Flow cytometric analysis of intracellular calcium levels increased in DNP-HSA-induced HMC-1 cells, decreased in overexpression of SOD3 and rosiglitazone-treated cells. ** $p < 0.01$, * $p < 0.05$.

but also PPAR- γ protein expression. To define the role of mitogen-activated protein kinases on SOD3 expression, we determined the activation of ERK1/2 by evaluating its phosphorylation (p-ERK1/2) (Fig. 4c). The total amount of ERK1/2 did not change significantly in response to DNP-HSA, SOD3 overexpression, or rosiglitazone. However, ERK1/2 phosphorylation was drastically enhanced by DNP-HSA stimulation, which was likewise inhibited by overexpression of SOD3 as well as administration of rosiglitazone (Fig. 4c). Taken together, these results imply that SOD3 and PPAR- γ suppressed inflammatory cytokine production via regulation of the ERK pathway.

Identification of PPAR- γ -Mediated SOD3 Expression

To investigate the role of PPAR- γ in the regulation of SOD3, we performed a ChIP assay using an antibody against PPAR- γ (Fig. 5a). The promoter fragments of SOD3 were immunoprecipitated by the PPAR- γ antibody. PPAR- γ siRNA suppressed SOD3 expression while PPAR- γ overexpression increased SOD3 expression as measured by reporter gene activity ($p < 0.01$, Fig. 5b). This result suggests that the binding of PPAR- γ to the SOD3 enhancer may contribute to the induction of SOD3 gene transcription.

Effect of Taurine on Allergic Symptoms and Histopathology of OVA-Induced AR Animal Model

To test the therapeutic effect of taurine in vivo, we treated an OVA-induced AR animal model with different doses of taurine. Behavioral assessment of the allergic responses was compared between taurine-treated mice and controls (online suppl. Fig. S1, Table S1; see www.karger.com/doi/10.1159/000505209 for all online suppl. material). Scores of allergic reactions including the frequencies of nasal rubbing and sneezing were significantly elevated in mice with OVA systemic sensitization; taurine treatment reduced the instances of allergic reactions regardless of its concentration. Histopathological changes in the nasal mucosa were observed in AR mice such as multilayered epithelia, irregularity of the epithelial surface and prominent accumulation of eosinophils and mast cells (Fig. 6). Treatment of taurine alleviated some of the damage of mucosal epithelial cells, the detachment of epidermal cells, and the infiltration of inflammatory cells.

Effect of Taurine on the Serum Levels of Cytokines in the OVA-Induced AR Animal Model

The concentration of SOD3, TNF- α , IFN- γ , IL-4, and IL-6 in the peripheral serum was evaluated by ELISA

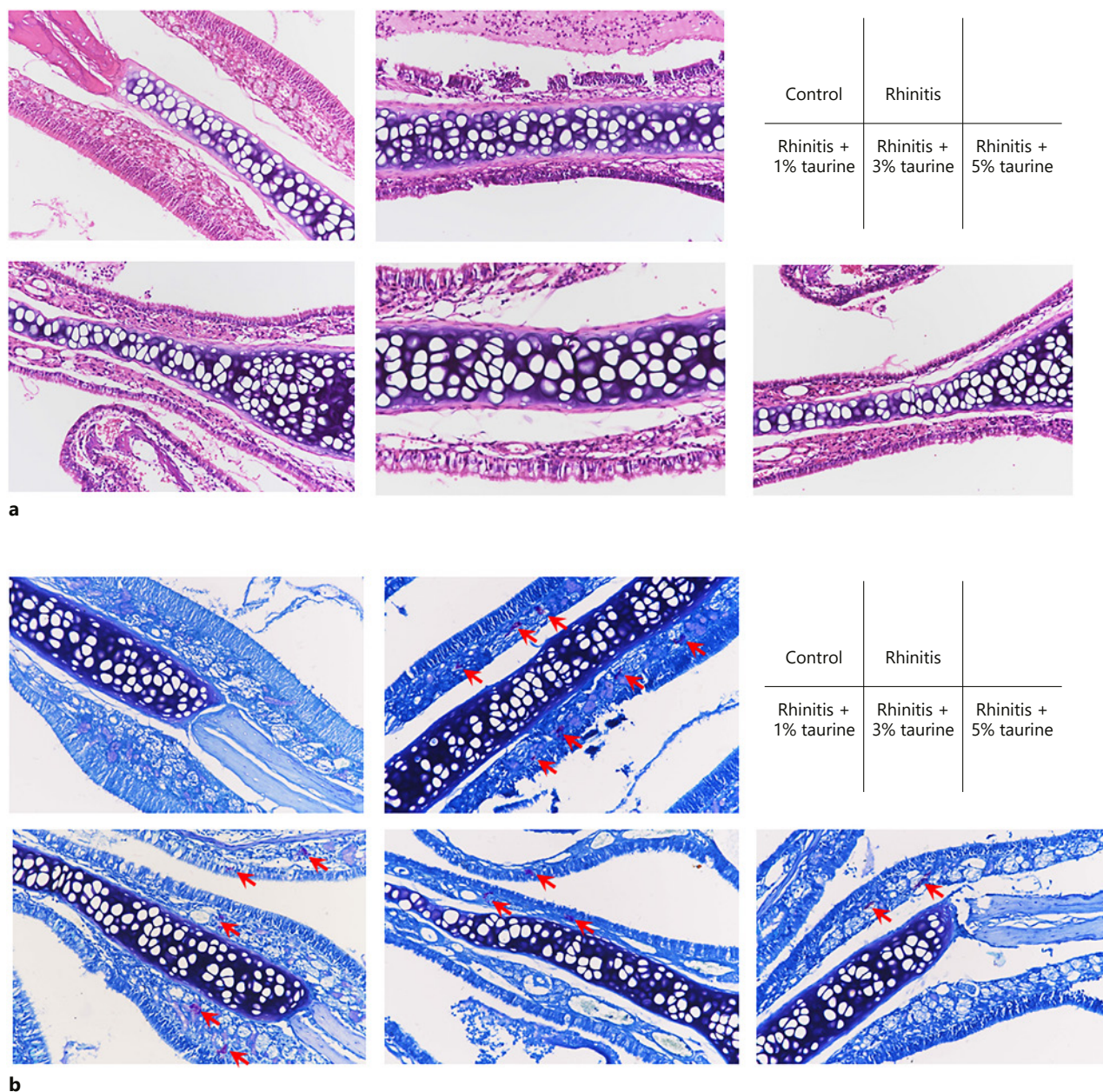


Fig. 6. Histopathological effect of taurine on the mouse nasal tissue. **a** Infiltration of eosinophils was visualized by hematoxylin and eosin staining. The number of eosinophils was decreased in the taurine-treated groups. **b** Infiltration of mast cells was visualized by toluidine blue O staining. The number of mast cells was decreased in the taurine-treated groups.

(Fig. 7). The concentration of TNF- α , IL-4, and IL-6 was significantly elevated in AR mice compared with controls. Taurine treatment resulted in the reduction of serum concentrations of TNF- α , IL-4, and IL-6 in a dose-

dependent manner ($p < 0.01$, Fig. 7). Conversely, peripheral serum concentrations of SOD3 and IFN- γ were significantly decreased in AR mice. Taurine increased concentrations of SOD3 and IFN- γ in a dose-dependent

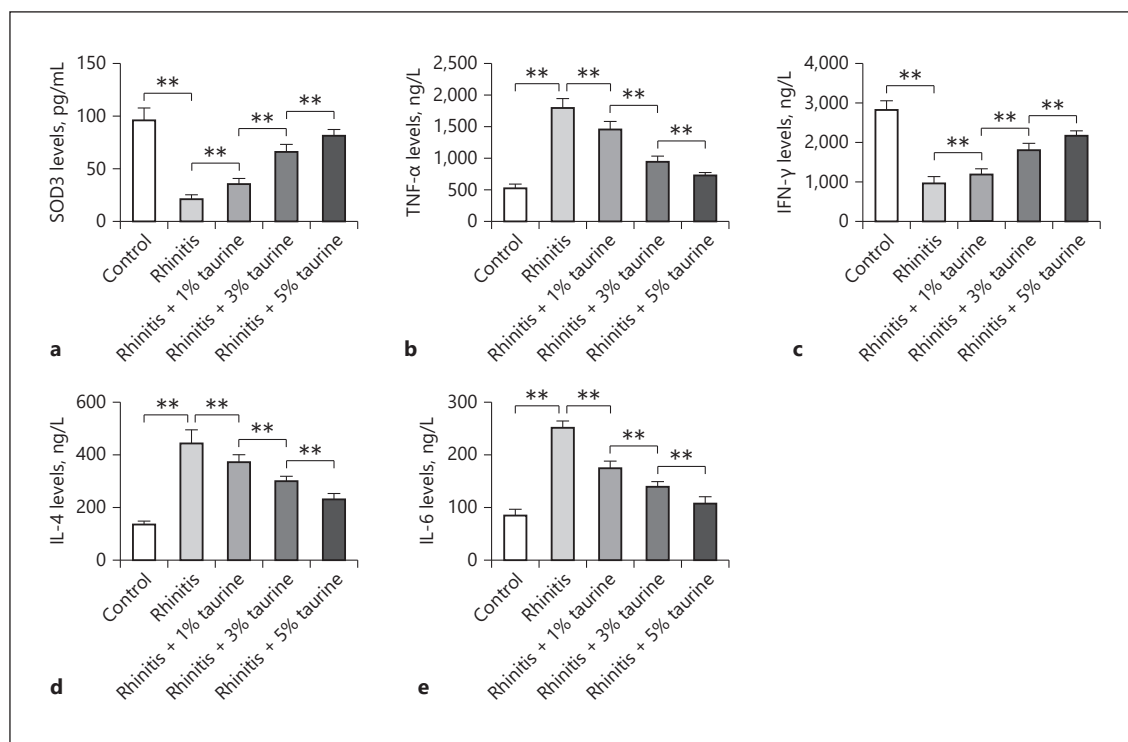


Fig. 7. Effect of taurine on serum cytokine concentration in an OVA-induced AR animal model. Measured by ELISA, the concentrations of SOD3, TNF- α , IFN- γ , IL-4, and IL-6 in the peripheral serum were changed by taurine treatment in a dose-dependent manner. ** $p < 0.01$.

manner ($p < 0.01$, Fig. 8). These results suggest that taurine exhibited anti-inflammatory effects via inhibition of inflammatory cytokine production in vivo.

Effect of Taurine on Expression of SOD3, PPAR- γ , and ERKs in the OVA-Induced AR Animal Model

Similar to the in vitro HMC-1 cell study results, protein expression of SOD3 and PPAR- γ substantially decreased in the OVA-induced AR mice relative to controls ($p < 0.01$, Fig. 8, online suppl. Fig. S1). Treatment with taurine enhanced the levels of SOD3 and PPAR- γ in a dose-dependent manner. ERK1/2 phosphorylation was substantially enhanced by OVA sensitization and was attenuated by administration of taurine ($p < 0.01$, Fig. 8). However, no obvious change in the total amount of ERK1/2 was observed. Taken together, these in vivo results imply that the ERK pathway is involved in the anti-allergic effects of taurine.

Discussion

The present study investigated the therapeutic effect and underlying mechanism of taurine in human mast cells (HMC-1) and in an OVA-induced AR animal model. We observed a reduction in the serum concentration of SOD3 in AR patients. Our study using HMC-1 cells showed that taurine increased the production and expression of SOD3 in a dose-dependent manner. Further in vitro results in DNP-HSA-stimulated HMC-1 cells indicated that SOD3 production was regulated by PPAR- γ in response to taurine. SOD3 overexpression inhibited the release of proinflammatory cytokines including TNF- α , IL-4, and IL-6 and ameliorated the loss of IFN- γ . SOD3 and PPAR- γ influenced inflammatory cytokine production via regulation of the ERK1/2 phosphorylation. The ovalbumin (OVA) induced-AR animal model in vivo study showed that taurine had efficacy in alleviating the allergic inflammatory reactions by relieving behavior symptoms of AR mice and reducing eosinophilic and mast cell infiltration into the nasal cavity. In addition, taurine treatment increased production of SOD3 and

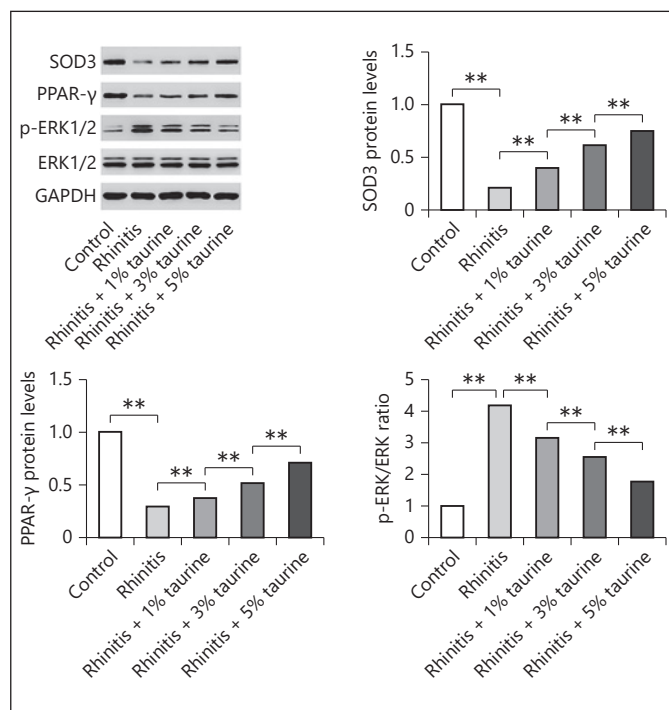


Fig. 8. Effect of taurine on the expression of SOD3, PPAR-γ, and ERKs in an OVA-induced AR animal model. Western blotting showed protein expression of SOD3, PPAR-γ, ERK1/2, and p-ERK1/2 in OVA-induced AR mice with and without treatment of different doses of taurine. GAPDH, glyceraldehyde 3-phosphate dehydrogenase. ** $p < 0.01$.

PPAR-γ which suppressed expression of proinflammatory cytokines through phosphorylation of ERK1/2.

Ample evidence points out that allergic disorders such as AR are mediated by oxidative stress, which occurs not only as a result of inflammation, but also from environmental exposure to allergens. SOD is the most important antioxidant enzyme system against free radicals and the decomposition of superoxide radicals into H_2O_2 in the human lung [23]. SODs are composed of three isoforms of intracellular copper-zinc SOD, mitochondrial manganese SOD, and extracellular SOD (ECSOD or SOD3). SOD3 mRNA is abundant in airway epithelium, and its protein is found primarily in connective tissue around airways [24]. The role of oxidative stress in AR is not well studied. Ozone exposure exacerbates allergy symptoms such as sneezing, nasal secretions, eosinophil infiltration, and hyperresponsiveness in guinea pigs [25]. The strategies for treating oxidative stress allergic disorders are twofold: reduce exposure to reactive oxygen species and augment antioxidant defenses. Therefore, increasing the expression of SOD3 could be a viable strategy for antial-

lergic treatments. In the present study, the taurine-treated group showed significantly upregulated SOD3 expression, supporting the hypothesis that the mechanism of the antiallergic effects of taurine is through enhanced antioxidant effects. Due to the lack of detailed clinical information, the correlation of SOD3 levels with clinical parameters was not analyzed, which is a main limitation of the present study. Taurine is present at high concentrations in most cells from both endogenous and diet sources. Particularly, high levels of taurine in phagocytes and leukocytes indicate its role in innate immunity [26]. In addition, high concentrations of taurine in tissues exposed to elevated levels of oxidants suggest its role in attenuating oxidative stress [27]. Taurine has been shown to regulate allergic inflammatory responses in an OVA-induced AR animal model [28]. Our results confirm its therapeutic effect against allergic inflammation and efficacy in ameliorating AR reactions. Besides behavioral and histopathological assessments, we also discovered that taurine attenuated the increasing serum concentration of TNF-α, IL-4, and IL-6 in OVA-induced AR mice. Conversely, taurine partially restored peripheral serum levels of SOD3 and IFN-γ in OVA-induced AR mice. Furthermore, ERK1/2 phosphorylation was substantially enhanced by OVA sensitization, which was attenuated by administration of taurine. All these results support the hypothesis that taurine increased production of SOD3 and PPAR-γ which, in turn, suppressed expression of proinflammatory cytokines through phosphorylation of ERK1/2. The previous study by Nam et al. [28] reported that taurine regulated allergic inflammatory responses through caspase-1/NF-κB/JNK/p38 pathways and suppressed OVA-induced AR. The results from our study, and theirs, suggested various signaling pathways were involved in the therapeutic effects of taurine in AR.

Previous studies have shown a protective role of PPAR-γ in the pathogenesis of asthma mediated by an IL-10-dependent mechanism [29]. The activation of PPAR-γ reduces human airway hyperresponsiveness and eosinophil activation induced by asthma [30, 31]. In diabetes mellitus, which is characterized by increased oxidative stress and impaired antioxidative capacity, interactions between SOD2 and PPAR-γ single-nucleotide polymorphisms was detected regarding the mortality rate [16]. Therefore, we hypothesized that SOD3 expression was mediated by PPAR-γ in the oxidative stress condition of AR. Indeed, our in vitro results in DNP-HSA-stimulated HMC-1 cells indicated that SOD3 production was regulated by PPAR-γ in response to taurine. SOD3 overexpression inhibited the release of proinflammatory cytokines

including TNF- α , IL-4, and IL-6 and ameliorated the reduction of IFN- γ . SOD3 and PPAR- γ influenced inflammatory cytokine production via regulation of the phosphorylation of ERK1/2. In addition, a ChIP assay demonstrated PPAR- γ protein binds to the SOD3 DNA sequence.

In conclusion, to our knowledge, we show for the first time that taurine can alleviate AR allergic inflammatory responses by increasing the production of the antioxidant enzyme SOD3 through activation of PPAR- γ , which, in turn, regulates the expression of cytokines through phosphorylation of ERK1/2. The present study provides evidence indicating that taurine can potentially be a therapeutic treatment for allergic disorders.

Statement of Ethics

This study has been approved by the Ethics Committee of Dahua Hospital (Shanghai, China). All experiments were performed in accordance with relevant guidelines and regulations. All participants provided written informed consent.

All animals were handled according to protocols approved by the Animal Care Committee of Dahua Hospital (Shanghai, China).

Disclosure Statement

The authors declare that they have no competing interests.

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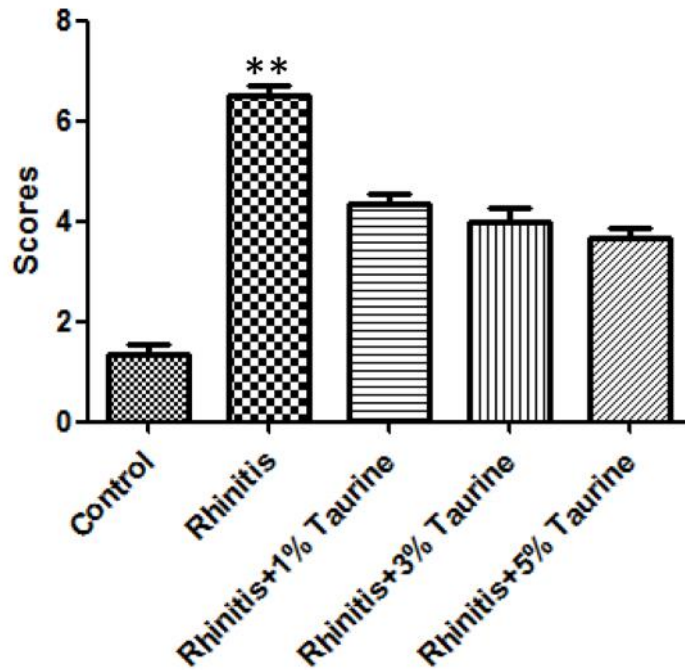
Author Contributions

J.Z. and Y.F. designed the study. J.Z. and Y.L. performed the experiments, analyzed the data, and wrote the manuscript. F.L. and W.W. performed the experiments. D.X. performed the data processing and statistical analysis. Y.F. supervised the experiments and wrote the manuscript.

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Supplementary Fig. S1: Behavioral assessment of the effects of taurine on allergic symptoms in OVA-induced AR mice. Scores of allergic reactions including the frequencies of nasal rubbing and sneezing were significantly elevated in mice with OVA systemic sensitization. Taurine treatment reduced the instances regardless of its concentration. ** $P < 0.01$.

Suppl. Table S1. Behavioral scores of taurine on allergic symptoms in OVA-induced

AR mice

Group	number	inchiness	watery nose	sneeze	score
Control	1	1	0	0	1
	2	0	0	1	1
	3	1	0	1	2
	4	0	0	1	1
	5	1	0	1	2
	6	1	0	0	1
Rhinitis	7	3	1	2	6
	8	3	2	2	7
	9	2	2	3	7
	10	2	2	2	6
	11	2	2	2	6
	12	3	2	2	7
Rhinitis + 1% taurine	13	2	1	1	4
	14	2	2	1	5
	15	1	2	1	4
	16	2	1	2	5
	17	0	2	2	4
	18	2	1	1	4
Rhinitis + 3% taurine	19	1	1	2	4
	20	2	1	1	4
	21	1	1	2	4
	22	2	2	1	5
	23	2	1	1	4
	24	1	1	1	3
Rhinitis + 5% taurine	25	2	1	1	4
	26	1	1	2	4
	27	1	1	1	3
	28	2	1	1	4
	29	2	1	1	4
	30	1	1	1	3