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Doxycycline exerts multiple anti-allergy effects to attenuate murine allergic conjunctivitis and systemic anaphylaxis

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ABSTRACT

Allergic diseases, which affect up to 20–30% of the world population, are still therapeutic challenge for allergists. Tetracyclines, which belong to an antibiotic drug family that possesses a striking variety of non-antibiotic properties, have been successfully applied to a wide range of diseases. However, their roles in allergic conjunctivitis and anaphylaxis and their underlying anti-allergy mechanisms remain elusive. Here, we reported that treatment with doxycycline significantly reduced IgE release from mouse B cells and the degranulation and inflammatory cytokines production of mouse mast cells (MCs) activated by IgE-dependent way. Furthermore, doxycycline treatment significantly inhibited histamine-induced vascular hyperpermeability *in vitro*. Mechanistically, the doxycycline-mediated inhibition of B cells, MCs and histamine may occur *via* modulation of the PI3K/Akt pathway. *In vivo*, our results demonstrated that treatment with doxycycline significantly attenuated clinical symptoms of mouse models of experimental allergic conjunctivitis (EAC) with a significant decrease in inflammatory cell frequency, IgE production, histamine release, and a decrease in TNF-α and IL-4 production. Using mouse models of MCs-dependent passive systemic anaphylaxis (PSA), we further confirmed anti-allergy effects of doxycycline and doxycycline-mediated inhibitory effects on MCs. Furthermore, our results showed that doxycycline significantly attenuate histamine-induced systemic anaphylaxis-like reaction (HISA) with a significantly downregulation of PI3K/Akt/eNOS/VE-cadherin pathway. The doxycycline-mediated anti-allergy effects during EAC, PSA and HISA were abrogated when an Akt activator, SC79, was administered. These findings suggest that doxycycline inhibits B cell, MC and histamine function and attenuates experimental allergic conjunctivitis and systemic anaphylaxis by possible modulating the PI3K/Akt pathway.

1. Introduction

Allergic diseases (ADs), which affect up to 20–30% of the world population, have become a major global health problem [1,2]. Allergic patients suffer from a broad variety of symptoms, including allergic conjunctivitis (AC), asthma, dermatitis, gastrointestinal symptoms, and systemic anaphylaxis (SA). These symptoms significantly affect patient health and can even threaten life. A number of treatment modalities have been undertaken to prevent or inhibit the development of ADs. However, current treatments for patients with ADs are variable, have limited clinical success and can lead to serious side effects caused by steroids [1,2]. Therefore, new safer and effective therapies are desirable.

Tetracyclines, an antibiotic drug family that includes tetracycline, doxycycline, minocycline and other derivative pharmaceuticals, also have a striking variety of non-antibiotic properties. Due to their multifunctional properties, there are currently over 200 ongoing clinical trials on tetracyclines for a wide range of diseases [3]. Recently, the anti-allergy properties of tetracyclines have

Abbreviations: ADs, allergic diseases; AC, allergic conjunctivitis; SA, systemic anaphylaxis; EAC, experimental allergic conjunctivitis; PSA, passive systemic anaphylaxis; HISA, histamine-induced systemic anaphylaxis like reaction; MC, mast cell; BMMCs, bone marrow derived mast cells; HUVEC, human umbilical vein endothelial cells; TNF-α, tumor necrosis factor-alpha; IL-4, interleukin-4; NO, nitric oxide; LPS, lipopolysaccharides; DNP, dinitrophenyl; OLF, Ophthalmic lavage fluid.

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received increasing attention [2]. Several studies have reported that the administration of doxycycline or minocycline significantly attenuates airway inflammation and hyperresponsiveness in a murine asthma model, and in humans with allergic asthma, these treatments improve asthma symptoms and reduce oral steroid requirements [4–6]. Therefore, tetracyclines may be a potential therapeutic option for ADs. However, the mechanisms by which tetracyclines mediate anti-allergy responses remain elusive. Moreover, the roles of tetracyclines in other ADs remain unclear. Thus, in this study, we investigated the anti-allergy mechanisms of doxycycline and the therapeutic effect of doxycycline on experimental allergic conjunctivitis (EAC) and passive systemic anaphylaxis (PSA).

2. Methods

2.1. Animals

BALB/c mice were supplied by the Guangzhou Animal Testing Center and were used at 4–6 weeks of age. All animal care and experiments were performed under institutional protocols approved by the Institutional Animal Care and Use Committee of Zhongshan Ophthalmic Center, Sun Yat-sen University. All procedures involving animal eye studies were conducted in accordance with the guidelines provided in the Association for Research in Vision and Ophthalmology statement for the use of animals in ophthalmic and vision research.

2.2. Cell culture

BALB/c splenic B cells were purified (purity >95% as determined by flow cytometry analysis of B220 cell surface expression) using a B cell isolation kit (Miltenyi Biotec, Auburn, CA, USA). B cells (1×10^6 ml^{-1}) were stimulated with IL-4 (50 ng/ml; Peprotech, Rocky Hill, NJ, USA) and LPS (10 μg/ml; Sigma, St Louis, MO, USA) for 5 days in RPMI 1640 containing 10% FBS (Hyclone, Logan, UT, USA), L-glutamine (Invitrogen, Carlsbad, CA, USA) and 50 μM β-mercaptoethanol (Sigma, St Louis, MO, USA). Day-6 supernatants were collected for IgE and IgG1 analysis. Mouse bone marrow-derived mast cells (MCs) were obtained as previously reported [7,8]. In brief, bone marrow cells were obtained by flushing bone marrow cells from the femurs of BALB/c mice. The cells were cultured in RPMI 1640 medium supplemented with 10% FBS (Hyclone, Logan, UT, USA), 100 U/ml penicillin, 100 mg/ml streptomycin (Invitrogen, Carlsbad, CA, USA), 25 mM HEPES, 1.0 mM sodium pyruvate, non-essential amino acids (BioSource International, Camarillo, CA, USA), 0.0035% β-mercaptoethanol and 30 ng/ml recombinant mouse IL-3 (PeproTech, Rocky Hill, NJ, USA). After 4 weeks, mast cell purity was evaluated by toluidine blue staining and CD117 (ebioscience, San Diego, CA, USA) surface staining by flow cytometry. The purity of the mast cells used in this study was greater than 95% (data not shown). The MCs were used following 4–6 weeks of culture at 37 °C and 5% CO_2. The HUVEC line was purchased from American Type Culture Collection (Manassas, VA, USA). A specific Akt activator [9] (4 μg/ml, SC79; Sigma, St Louis, MO, USA) was used in several experiments.

2.3. IgE-mediated activation of MCs

IgE-mediated activation of MCs was performed as described in our previous study [8]. In brief, MCs (1×10^6 ml^{-1}) were sensitized with 1 μg/ml of DNP-specific IgE (Sigma, St Louis, MO, USA) in medium for 4 h and challenged with DNP-BSA (100 ng/ml, Sigma, St Louis, MO, USA) for 30 min in Tyrode's buffer [10 mM HEPES buffer (pH 7.4), 130 mM NaCl, 5 mM KCl, 1.4 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM glucose, and 0.1% BSA]. BMMCs cultured alone with and without IgE sensitization and subsequent DNP challenge were used as positive and negative controls, respectively.

A β-hexosaminidase substrate assay was used to quantitate MC degranulation. For the β-hexosaminidase substrate assay, IgE-presensitized MCs were challenged in Tyrode's buffer with DNP-BSA for 30 min. Then, the β-hexosaminidase substrate assay was performed. In brief, the β-hexosaminidase substrate, p-nitrophenyl-N-acetyl-β-D-glucosaminide (50 ml/well, 1.3 mg/ml in 0.08 M sodium citrate pH 4.5; Sigma, St Louis, MO, USA), was added to 10 ml of supernatant or lysate from mast cells and incubated at 37 °C overnight. Sodium hydroxide (50 ml/well, 1 N; Sigma, St Louis, MO, USA) was added to stop the reaction, and the release of the product 4-p-nitrophenol was detected by the absorbance at 405 nm. The percent of maximum release was calculated compared to the total amount of β-hexosaminidase from the control cell lysate after treatment with 0.2% Triton X-100 using the following formula: % maximal β-hex release = [(sample–background)/(total cell lysate–background)] × 100.

For the histamine analysis, IgE-presensitized MCs were challenged in Tyrode's buffer with DNP-BSA for 30 min. Then, the supernatants were collected for histamine analysis. For cytokine analysis, IgE-sensitized BMMCs were cultured for 16 hr in the presence of DNP-BSA. Then, the supernatants were collected for cytokine analysis.

2.4. Vascular permeability assay

Vascular permeability assays were performed as described previously [10]. In brief, HUVECs (2×10^5) were grown in a transwell plate (BD Biosciences, San Jose, CA, USA) in 500 μl of medium until a monolayer was formed. HUVEC monolayer permeability was tested 8 h later by the addition of 7.5 μl streptavidin-HRP (1.5 μg/ml; R&D, Minneapolis, MN, USA) to the upper chamber. The monolayers were stimulated with histamine (100 μM) for 30 min prior to the addition of streptavidin-HRP. The media (50 μl) in the lower chamber was collected 5 min after the addition of streptavidin-HRP and was assayed for HRP activity by the addition of 100 μl TMB substrate. Color development was detected using a microplate reader at 450 nm.

2.5. Treatment of EAC by doxycycline

EAC was developed using the following protocol: a mixture of 10 μg OVA (Sigma, St Louis, MO, USA) and 0.5 ml aluminum hydroxide gel (Pierce, Rockford, IL, USA) was administered intraperitoneally on the first day. On day 5, the sensitization procedure was repeated to enhance the allergic reaction. From days 10 to 14, 10ul of 2 mg OVA solution was applied to each eye daily to induce EAC. The mice in the blank group were only subjected to PBS. Mice with EAC were treated with topical doxycycline (0.1% by instillation four times/day) on days 5–14 or were treated with the vehicle control.

Slit lamp checking was performed throughout the course of the study. After 30 min of challenge, the clinical reactions were recorded and evaluated by two observers who were blinded to the treatment groups. The allergic symptoms were graded using a previously published system [11]. Conjunctival edema, lid swelling, tearing and conjunctival redness were graded from 0 to 4 based on the criteria. The clinical score was the sum of the four parameters. The scratching times of each animal 15 min after the 30 min challenge were also counted by observers who were blinded to the treatment protocol. The scratching response was defined as rapid movements of the hind paws that were precisely directed toward the eye.

Ophthalmic lavage fluid (OLF) was collected after the last OVA exposure. PBS (10 μl) was applied to the eye using a micropipette