Dexmedetomidine alleviates airway hyperresponsiveness and allergic airway inflammation through the TLR4/NF-κB signaling pathway in mice

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Abstract. Dexmedetomidine (DEX) suppresses inflammatory responses and protects against organ injury. The aim of the present study was to investigate the effect of DEX on airway hyperresponsiveness (AHR) and allergic airway inflammation, as well as its underlying mechanism of action in a murine model of ovalbumin (OVA)-induced asthma. A total of 30 female BALB/c mice were divided into 6 groups (n=5 mice/group): Control, OVA, OVA + DEX (20, 30 or 50 µg/kg) and OVA + TAK-242 [a toll-like receptor 4 (TLR4) inhibitor]. The mice were intraperitoneally injected with 20, 30 or 50 µg/kg DEX 1 h before OVA challenge. AHR to inhaled methacholine (Mch) was measured, and the mice were sacrificed 24 h after the last challenge. AHR following Mch inhalation was measured using the FlexiVent apparatus. Hematoxylin and eosin, periodic acid-Schiff and Wright-Giemsa staining was performed to evaluate inflammatory cell infiltration in the lung tissue. The levels of IL-4, IL-5 and IL-13 in the bronchoalveolar lavage fluid were analyzed using ELISA, and their mRNA expression levels in the lung tissue were examined using reverse transcription-quantitative PCR. The protein expression of TLR4, NF-κB and phosphorylated (p) NF-κB in the lung tissue was also detected using immunohistochemistry. In the murine OVA-induced asthma model, DEX decreased AHR following Mch inhalation and reduced the infiltration of inflammatory cells. IL-4, IL-5 and IL-13 levels in the bronchoalveolar lavage fluid were significantly lower following DEX treatment. Furthermore, DEX treatment inhibited the expression of TLR4, NF-κB and p-NF-κB in the lung tissue and exhibited a similar effect to TAK-242 treatment. In conclusion, DEX may attenuate AHR and allergic airway inflammation by inhibiting the TLR4/NF-κB pathway. These results suggested that DEX may represent a potential anti-inflammatory agent for the treatment and management of patients with asthma.

Introduction

Asthma is one of the most common chronic airway inflammatory conditions in adults and children (1), affecting 5-10% of the global population (2). It is predominantly mediated by T helper (Th) 2 cells and characterized by eosinophilic airway inflammation, mucus hypersecretion and airway hyperresponsiveness (AHR) (1). Asthma is an important public health issue owing to its increasing incidence, with the incidence increasing from 9.3 to 11.5% in young adults between 2008 and 2016 (3) and substantial economic burden (4). Patients with asthma undergoing surgery are at great risk of perioperative morbidity and mortality due to bronchospasms and hypoxemia (5). Although medical treatment that can relax bronchial smooth muscle (6) or inhibit leukotriene production (7) is available, the development of additional approaches to relieve these life-threatening symptoms during the perioperative period may be beneficial for patients with asthma.

Allergic airway inflammatory responses are characterized by eosinophil and Th2 cell infiltration, secretion of type-2 cytokines and elevated immunoglobulin E levels (8). Cytokines derived from Th2 cells, including IL-4, IL-5 and IL-13, enhance airway eosinophilia, mucus production and AHR, and are involved in the pathogenesis of asthma (9). Therefore, targeting these cytokines could represent an effective therapeutic approach, as previous studies have demonstrated that reduced levels of these cytokines can relieve the typical symptoms of asthma (10,11).

Various signaling pathways have been studied with respect to the pathophysiology of asthma. Among them, the toll-like receptor (TLR) family of conserved pattern-recognition receptors, has been demonstrated to serve a pivotal role in asthma (12,13). TLR4, in particular, can be activated by...
bacterial lipopolysaccharide, which is the main component of the cell wall of Gram-negative bacteria (14). Activation of TLR4 can initiate inflammatory responses by targeting myeloid differentiation primary response gene 88 and NF-κB. The TLR4/NF-κB pathway has been demonstrated to trigger airway inflammation and AHR by promoting cytokine production (15-17).

Dexmedetomidine (DEX) is a selective α2 adrenoceptor that is widely used in the clinic, as it can induce sedation and analgesia, as well as reduce anxiety, without respiratory depression (18). In addition, the anti-inflammatory properties of DEX have also been examined, since they may attenuate acute multiple organ injury (19). Several studies have reported that DEX may have a protective effect on pulmonary dysfunction (20,21). However, whether DEX could alleviate AHR and allergic airway inflammation in asthma is unclear, and the potential underlying mechanism remains unknown. Therefore, the aim of the present study was to evaluate the effect of DEX on airway inflammation and AHR in allergic asthma and to examine its potential effect on the TLR4/NF-κB pathway.

Materials and methods

Animals. In the present study, 7-week-old specific pathogen-free female BALB/c mice (weight, 18-22 g) purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. Mice were housed under standard laboratory conditions at 25°C with 50±5% humidity and 12-h light/dark cycles in the experimental animal center of the Plastic Surgery Hospital (Chinese Academy of Medical Sciences and Peking Union Medical College; Beijing, China) for 1 week prior to the start of the experiments. The animals were provided with sterilized food and water ad libitum. All experimental procedures were performed according to the People’s Republic of China Animal Protection Law. Experimental animals were handled under a protocol approved by the Institutional Animal Care and Use Committee of Plastic Surgery Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College.

Experimental protocols. A total of 30 female BALB/c mice were divided into six groups (n=5 mice/group): Control, OVA, OVA + DEX (20, 30 or 50 µg/kg) and OVA + TAK-242 (an inhibitor of TLR4) groups. All mice except those in the control group were sensitized on days 1, 7 and 14 with an intraperitoneal (i.p.) injection of 100 µg ovalbumin (OVA; Sigma-Aldrich; Merck KGaA), 10 mg aluminum hydroxide (Sigma-Aldrich; Merck KGaA) in saline (200 µl). The mice were sensitized with 3 mg/kg DEX (Jiangsu Hengrui Medicine Co., Ltd.) groups received daily i.p. injections of 20, 30 or 50 µg/kg DEX, and those in the OVA + TAK-242 (MedChemExpress) group received 3 mg/kg TAK-242 daily i.p. injection; these mice were also challenged by intranasal administration of 200 µg OVA in 30 µl saline 60 min after the i.p. injection. From day 21 to 29, the control group was intranasally administered 30 µl saline once a day, and each OVA group received a daily intranasal administration of 200 µg OVA in 30 µl saline. The mice were sacrificed 24 h after the last challenge. After airway resistance measurement, the mice were euthanized using an i.p. administered overdose of 2% pentobarbital sodium (150 mg/kg); bronchoalveolar lavage fluid (BALF) and lung tissue were then collected.

Histological analysis of lung tissue. Following BALF collection, lung tissue samples were fixed at room temperature with 4% paraformaldehyde for 48 h, then embedded in paraffin. A series of 5-µm thick lung sections were prepared for hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) staining (Beijing Solarbio Science & Technology Co., Ltd.). The slides were deparaffinized in xylene and rehydrated in descending alcohol solutions. H&E staining was performed at room temperature to assess inflammatory cell infiltration. Briefly, cells were stained with hematoxylin for 1 min, washed with tap water five times, incubated with blue nuclei in 1X PBS for 1 min, washed three times with distilled water, counterstained in alcoholic-cosin for 1 min and then dehydrated using an ascending alcohol series. Subsequently, xylene clearing was performed. PAS staining was performed at room temperature to assess goblet cell hyperplasia. Following oxidation in 0.5% periodic acid solution for 6 min and rinsing with distilled water, the slides were covered by Schiff regent for 15 min, washed with tap water for 5 min, stained with hematoxylin for 50 sec, rinsed with running water for 2 min and then differentiated with hydrochloric acid for 3 sec. The percentage of PAS-stained cells in the airway epithelium is indicative of the production of mucus (22). A total of five fields of view were examined by two experienced pathologists blindly at ×200 magnification using a BX53 upright fluorescence microscope (Olympus Corporation) under the mode of bright-field imaging. The evaluation of peribronchial inflammation was based on a modified six-point scoring system (23) as follows: 0, normal; 1, a few cells; 2, a ring of inflammatory cells consisting of one cell layer; 3, a ring of inflammatory cells consisting of two-four cell layers; 4, a ring of inflammatory cells consisting of five-seven cell layers; 5, a ring of inflammatory cells consisting of eight-ten cell layers; 6, a ring of inflammatory cells consisting of >10 cell layers. A modified scoring system (24) was used for the abundance of PAS-positive mucus-containing cells in each airway as follows: 0, <2%; 1, ≥2% and <20%; 2, ≥20% and <40%; 3, ≥40% and <60%; 4, ≥60% and <80%; and 5, ≥80% PAS-positive cells.

Analysis of BALF. The mice were anesthetized with 2% pentobarbital sodium (150 mg/kg) 24 h after the last challenge. After inserting a catheter into the trachea, the lungs were flushed with 0.8 ml cold PBS three times, and 85-95% of the lavage volume was collected through the catheter. BALF was centrifuged at 187 x g at 4°C for 10 min. The supernatant was collected and stored at -80°C until analyzed by ELISA. The total count of inflammatory cells in the BALF was determined using a chamber slide and the eosinophil counts were determined using ImageJ software (version 1.8.0; National Institutes of Health) by Wright-Giemsa staining (Beijing Solarbio Science & Technology Co., Ltd.) at room temperature for 1.5 min.

ELISA. Commercial ELISA kits (IL-4, cat. no. EK0405; IL-5, cat. no. EK0408; and IL-13, cat. no. EK0425; all from Boster Biological Technology) were used to detect the levels of IL-4, IL-5 and IL-13 in BALF according to the
manufacturer's protocol. The optical density was spectrophotometrically measured at 450 nm using a multimode plate reader (PerkinElmer, Inc.).

Assessment of AHR. AHR was detected 24 h after the last challenge. The mice were anesthetized with 2% pentobarbital sodium (50 mg/kg) and the airway resistance was measured using FlexiVent (SCIReq). Aerosolized methacholine (Mch) at different concentrations (0, 6, 12, 24 and 48 mg/ml) was continuously nebulized through a catheter in each animal for 6 min. The airway resistance was collected every minute and the final result of each concentration for each animal was presented as the average of 6 min. The airway resistance is presented as respiratory resistance (Rrs) in cmH2O/ml/sec. The results are presented as the percentage increase in Rrs at each concentration of Mch over the baseline (Rrs in the control mice at different concentrations of Mch).

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from homogenized lung tissue (50 mg) using TRIzol® (Ambion; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The RNA pellet was resuspended in DNase/RNase-free water (Beijing Solarbio Science & Technology Co., Ltd.). RNA concentration was measured at a wavelength of 260 nm using a NanoDrop® 2000 ultraviolet spectrophotometer (Thermo Fisher Scientific, Inc.), and the quality of RNA was evaluated by verifying that the ratio at 260/280 nm was 1.8-2.0. RT was carried out with 5 µg RNA using the TransScript® First-Strand cDNA Synthesis SuperMix (Beijing Transgen Biotech Co., Ltd.) in 20-µl reaction volumes, according to the manufacturer's protocol. The qPCR primers (Invitrogen; Thermo Fisher Scientific, Inc.) were as follows: IL-4 forward, 5'-CTCAGACGAACGAAAGACACC-3' and reverse, 5'-CTCCTCGACACTCTCTC TT-3'; IL-5 forward, 5'-AGATCAACTGTTCCGCTTGGG-3' and reverse, 5'-TCTCTGCCACACTCTCTTTT-3'; IL-13 forward, 5'-CTCTGTTCTGGCTTGGTGTCT-3' and reverse, 5'-TGTGATGTTGCTCAGCTCCTC-3'; TLR4 forward, 5'-TCATCACTGGATCGGTGTCAG-3' and reverse, 5'-TTTCCATCAACAGGGCTT-3'; NF-κB forward, 5'-GGGGCC TGCAAAGGTATC-3' and reverse, 5'-TGCTGTACCGGT GCATACC-3'; and β-actin forward, 5'-CTCTTTTCCCAGCT CTTCCCTCTT-3' and reverse, 5'-AGGTCTTACCGATG CAACGT-3'. The relative expression levels of the IL-4, IL-5, IL-13, TLR4 and NF-κB were quantified using the 2^ΔΔCq method (25) and LightCycler® 96 Instrument (Roche Diagnostics GmbH). The experiments were performed independently three times. Quantitative data are presented as the mean ± SD and were analyzed using one-way ANOVA followed by Bonferroni's multiple comparisons test. Ordinal data are presented as the median + interquartile range and were analyzed using Kruskal-Wallis followed by Dunn's multiple comparisons test. Graphs were generated and data were analyzed using GraphPad Prism (version 6; GraphPad Software, Inc.). P<0.05 was considered to indicate a statistically significant difference.

Results

DEX reduces AHR and inflammatory cell infiltration in BALF. To assess the effect of DEX on inflammatory cell infiltration in the airway, the number of inflammatory cells in BALF was analyzed. Compared with the control group, the number of total cells and eosinophils in BALF were significantly increased in the OVA group (Fig. 1A and B). However, the number of total cells and eosinophils in the OVA + DEX (30 and 50 µg/kg) groups were reduced compared with the OVA group, whereas pretreatment with DEX at 20 µg/kg did not decrease the number of eosinophils (Fig. 1B). The dose of 30 µg/kg DEX (DEX30) resulted in the largest decrease.

Compared with the control group, the mice that received OVA alone exhibited increased Rrs in response to inhaled Mch at all concentrations. Compared with the OVA group, the various DEX treatments significantly reduced Rrs. The OVA + DEX30 group exhibited the largest reduction in AHR (Fig. 1C).

These results suggested that treatment with DEX30 inhibited the recruitment of inflammatory cells in BALF and reduced AHR in a murine OVA-induced asthma model.

DEX alleviates airway inflammation and mucus overproduction. Since inflammatory cell recruitment and mucus overproduction are the main characteristics of allergic
asthma (9), the subsequent experiments aimed to determine whether treatment with DEX could attenuate these symptoms. Inflammatory cell infiltration was assessed using H&E staining (Fig. 2A). Compared with the control group, lung tissue from mice in the OVA group exhibited a significantly higher number of inflammatory cells (Fig. 2B). However, treatment with DEX30 significantly inhibited inflammatory cell infiltration compared with the OVA group, and DEX at 20 and 50 µg/kg showed inhibitory effects on inflammatory infiltration, but these effects were not significant.

Mucus production in goblet cells was assessed using PAS staining. The percentage of PAS-positive cells in the OVA group was significantly higher than that of the control group, and treatment with DEX30 reduced the production of mucus in the airway compared with the OVA group, while pretreatment with DEX at 20 and 50 µg/kg displayed an inhibitory effect on mucus production that was not statistically significant (Fig. 2C and D). Thus, 30 µg/kg DEX could attenuate airway inflammation and mucus overproduction in the murine OVA-induced asthma model.

**DEX inhibits the production of inflammatory cytokines in the murine OVA-induced asthma model.** The levels of pro-inflammatory cytokines were detected in BALF from mice in the Control, OVA and OVA + DEX30 groups. As presented in Fig. 3, IL-4, IL-5 and IL-13 levels in the OVA group were significantly higher compared with those in the control group. However, treatment with DEX30 significantly reduced the levels of these cytokines compared with the OVA group.

Furthermore, the mRNA expression levels of IL-4, IL-5 and IL-13 in the lung tissue were also examined (Fig. 4). IL-4, IL-5 and IL-13 mRNA expression levels in the lung tissue of the OVA group were significantly higher compared with those of the control group. Treatment with DEX30 significantly decreased the levels of IL-4, IL-5 and IL-13 mRNA compared with the OVA group.

**TAK-242 attenuates airway inflammation and AHR in asthma model mice.** It was examined whether AHR and airway inflammation in the OVA-induced asthma model could be ameliorated by TAK-242. The i.p. injection of TAK-242 or DEX before OVA challenge reduced airway resistance in response to inhaled Mch (Fig. 1C), and reduced the total number of cells and eosinophils in the BALF (Fig. 5A). To examine the effect of TAK-242 on airway inflammation, H&E and PAS staining were performed to detect inflammatory cell infiltration and the production of mucus in the lung, respectively. Administration of TAK-242 or DEX attenuated OVA-induced airway inflammation by...
reducing the number of infiltrating inflammatory cells in the airway (Fig. 5D and F) and the production of mucus (Fig. 5E and G). In addition, ELISA and RT-qPCR were used to assess pro-inflammatory cytokine levels in BALF and mRNA levels in the lung tissue, respectively. Both TAK-242 and DEX30 treated groups exhibited decreased levels of inflammatory cytokines in BALF (Fig. 5B) and lower expression levels of IL-4, IL-5 and IL-13 mRNA (Fig. 5C) in the lung tissue. These results indicated that the inhibitor of TLR4 (TAK-242) and DEX could alleviate the asthmatic symptoms in the murine OVA-induced asthma model to a similar extent.
**DEX inhibits the TLR4/NF-κB pathway.** TLR4 and NF-κB serve an important role in inflammatory responses by promoting the transcription of various pro-inflammatory cytokines (26). Immunohistochemistry was performed to...
detect the expression of TLR4, NF-κB and p-NF-κB in the lung tissue. As shown in Fig. 6A and B, the expression of TLR4, NF-κB and p-NF-κB was increased in the OVA group compared with the control group, whereas DEX at 30 µg/kg significantly downregulated the expression level of TLR4, NF-κB and p-NF-κB in the lung tissue of the asthmatic mice. The expression levels of TLR4 and NF-κB mRNA were also determined by RT-qPCR (Fig. 6C). The mRNA expression of TLR4 and NF-κB in the lung tissue from the OVA group was elevated, and DEX significantly downregulated their levels. The aforementioned results suggested that DEX may inhibit the activation of the TLR4/NF-κB pathway.

**Discussion**

AHR is a characteristic of asthma that has emerged as a major challenge in the perioperative management of asthmatic patients (27). AHR can be a life-threatening symptom for patients with asthma during the perioperative period, as it can result in hypoventilation, hypoxemia and cardiac arrhythmia. The perioperative management of asthmatic patients includes the following: i) Detection of upper airway infection prior to surgery; ii) appropriate choices of anesthesia; and iii) β-2 adrenergic agonist treatment in the case of acute attacks (28). Certain anesthetic agents can dilate the airway, whereas others can also induce bronchoconstriction (29). Therefore,
it is essential to select appropriate anesthetic agents that will benefit the patient.

DEX is an anesthetic adjuvant reported to attenuate perioperative inflammation and improve the immune function of patients who have undergone surgery (18). Groebeen et al. (30) reported that intravenous administration of DEX attenuated bronchoconstriction in dogs treated with histamine. It was clinically observed that patients who had received DEX exhibited reduced airway resistance during mechanical ventilation (31) in surgery and hemodynamic stability during extubation (32). In the present study, an OVA-induced murine asthma model was used to examine the effect of DEX on allergic asthma. TAK-242, a TLR4 inhibitor, was used to determine whether DEX could affect TLR4 signaling. To the best of our knowledge, the present study is the first to demonstrate the inhibitory effect of DEX on allergic airway inflammation in mice.

According to the dose choices of previous studies on the role of DEX in inflammatory diseases, most working doses in mice were in the 20-50 µg/kg range [5, 10 and 20 µg/kg (33); 25 µg/kg (34); and 50 µg/kg (21)], and the safety of DEX at 200 µg/kg i.p. in BALB/c mice has been demonstrated (35). To further narrow down the range, a dose-response experiment with different concentrations of DEX (10, 20, 30, 40, 50 and 60 µg/kg) was performed (data not shown). According to the cell counts and Rs results, 20, 30 and 50 µg/kg were used in the present study.

Increasing infiltration of leukocytes around the airway, particularly eosinophils, is one of the main factors in the pathogenesis of asthma (36). The present study demonstrated that DEX effectively decreased inflammatory cell infiltration in the airway and inhibited mucus overproduction in OVA-induced asthmatic mice. This indicated that DEX may suppress inflammatory responses in the lungs of asthmatic patients.

The imbalance of Th1/Th2 cells is pivotal in promoting the development of asthma; indeed, the Th2 phenotype is predominant, and the activity of Th1 cells is suppressed in allergic asthma (37). Activated Th2 cells can release type-2 cytokines, such as IL-4, IL-5 and IL-13, which promotes the development of asthma, including AHR, mucus overproduction and eosinophil infiltration (9). The present study revealed that treatment with DEX reduced the levels of IL-4, IL-5 and IL-13 in BALF of asthmatic mice. Thus, it was demonstrated that DEX may attenuate airway inflammation in asthmatic mice by suppressing the Th2 immune response.

AHR is a typical symptom of asthma that can also be associated with behavioral changes in mice (38). In the present study, following intranasal administration of OVA, mice in the OVA group were more irritated and exhibited an elevated respiratory rate, compared with the OVA + DEX group. In the present study, cyanosis was also observed in the OVA group (data not shown). These behavioral changes may be associated with AHR owing to a limited airflow (39). The mechanism of AHR remains unclear, although a previous study demonstrated a close association between inflammatory cytokines and the development of AHR (40). The results of the present study demonstrated that DEX may effectively relieve inflammation in the lungs and attenuate OVA-induced AHR in response to inhaled Mch.

It has been suggested that the activation of the TLR4/NF-κB pathway can promote the infiltration of inflammatory cells in the airway and trigger airway inflammation (41). Although strong evidence that the α-adrenergic receptor is associated with TLR4/NF-κB signaling is still lacking, several studies have indicated that DEX can protect organ function by inhibiting TLR4 signaling (14,30,31). The TLR4/NF-κB signaling pathway has also been associated with the anti-inflammatory effects of DEX (42). Activated NF-κB p65 is phosphorylated, then translocated into the nucleus to promote the transcription of target genes encoding inflammatory cytokines, which contribute to the pathogenesis of asthmatic airway inflammation (43). The present results indicated that DEX inhibited the activation of NF-κB by reducing the expression of NF-κB p65 and TLR4 in OVA-induced asthmatic mice. Thus, DEX may attenuate airway inflammation in the OVA-induced asthma model by suppressing the activation of the TLR4/NF-κB pathway.

How DEX affects the TLR4 pathway has not been fully determined, although it has been demonstrated that microRNA (miRNA/miR) and long non-coding (lnc)RNAs may serve a role in this context. miRNAs serve crucial roles in regulating various signaling pathways, such as miR-30 regulates the MAPK/KRAS pathway (44), miR-486 regulates the PI3K/AKT pathway (45) and miR-340-5p regulates the PI3K/AKT pathway (46). Administration of DEX alters the level of several miRNA molecules in certain murine disease models, such as neuroinflammation (47), myocardial ischemia/reperfusion (48) and postoperative cognitive dysfunction (49). In addition, it has been reported that DEX can inhibit the NF-κB pathway by regulating the MAPK/KRAS pathway via the regulation of miR-129 (49). In addition to miRNAs, lncRNAs are also involved in several diseases through DEX, such as chronic obstructive pulmonary disease (50), postoperative cognitive dysfunction (51) and oxygen-glucose deprivation/reperfusion injury (52). Thus, it may be hypothesized that miRNAs or lncRNAs could mediate the effect of DEX on TLR4/NF-κB signaling.

In the present study, DEX treatment at 30 µg/kg resulted in an improved therapeutic effect compared with treatment with a higher concentration (50 µg/kg), suggesting that there may be a dose limit to the therapeutic effect of DEX in the murine OVA-induced allergic asthma model. Doses higher than the 30 µg/kg limit may result in reduced therapeutic effect causing unexpected results, such as worsening symptoms or other side effects. This dose limit may also differ between mouse strains, administration mode and other factors.

DEX may be a safer choice as a sedative and an anesthetic adjunct in patients with acute asthma. Acute asthma attack causes anxiety and agitation in patients, which hinders treatment and adversely affects patients (53). DEX allows patients to rest and increase their tolerance to treatment without respiratory depression. It is also used as adjunctive treatment for acute and severe asthma (53,54). DEX has been used to facilitate the induction of noninvasive positive pressure ventilation for acute respiratory failure in patients with severe asthma (53) and in monitoring anesthesia care for bronchial thermoplasty (54).

In conclusion, the present study demonstrated that DEX attenuated AHR and airway inflammation by decreasing...
the production of type-2 cytokines through the inhibition of TLR4/NF-κB signaling in a murine OVA-induced asthma model. These findings suggested that DEX may represent an alternative choice for the perioperative management of patients with asthma and a potential anti-inflammatory anesthetic.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions
QW analyzed and interpreted the data and designed the study. SX drafted the manuscript and critically revised it for important intellectual content. QW, SX, XZ and HG performed the experiments. JZ and DY made substantial contributions to the study conception. QW and SX confirmed the authenticity of experiments. JZ and DY made substantial contributions to the study conception. QW and SX confirmed the authenticity of experiments. JZ and DY made substantial contributions to the study conception.

Ethics approval and consent to participate
Experimental animals were handled under a protocol approved by the Institutional Animal Care and Use Committee of Plastic Surgery Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (approval no. 2021(201); Beijing, China).

Patient consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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