

Immune responses to different patterns of exposure to ovalbumin in a mouse model of allergic rhinitis

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Abstract Allergic rhinitis (AR) has been a significant healthcare burden on individuals and society. However, the detailed effect of different patterns of allergen exposure on the development of AR remains controversial. A mouse model of AR was established to address the complex relationships between allergen exposure and the development of AR. Allergic symptom, OVA-specific IgE in serum and nasal lavage fluid, allergic inflammation in nasal tissues were evaluated after intranasal sensitization and challenge of ovalbumin (OVA) in mice treated with two different doses of allergen for different sensitized durations. Exposure to different doses and sensitized durations of OVA were capable of inducing allergic nasal response. Repetitive OVA exposure in the sensitization phase induced the recruitment of eosinophils and goblet cell hyperplasia. The level of OVA-specific IgE in serum depended on OVA exposure and was mediated in a duration-related manner. In addition, mice treated with low-dose OVA for prolonged duration manifested the major features of human local allergic rhinitis. There were dose- and duration-related effects of allergen exposure on the development of AR. LAR was associated with repetitive exposure to low-dose allergen. Thus, allergen avoidance should be an important aim of AR management.

Keywords Allergen exposure · Allergic rhinitis · Dose · Duration · Local allergic rhinitis

Introduction

Allergic rhinitis (AR) clinically expressed by sneezing, rhinorrhea, nasal itching and congestion has been a worldwide health problem. 10–20 % of the population around the world suffer from AR [1]. Moreover, the prevalence of AR is quickly increasing [2, 3], placing a significant healthcare burden on individuals and society.

AR is found to be dependent on exposure to inhalant allergen. It is now commonly accepted that AR is elicited by an interaction between environmental allergen and sIgE [4]. Considering allergen in the environment, the intensity and persistence of its exposure is likely to influence the progress of AR [5]. On one hand, the concentration of allergen varies greatly from one environment to another [6]; on the other hand, exposure to allergen for AR patients could last for months or years in their early lives, these variances may contribute to different manifestations of AR and have impact on its progress. However, the detailed effect of intensity and persistence of allergen exposure in AR remain unclear.

Recently, it has been demonstrated that there was a dose–response relationship between allergen level in the environment and sensitization to allergen in human studies [7–9]. We therefore, hypothesized that the detailed effect of allergen exposure on allergic inflammation might be related to the pattern of contact of the immune system with allergen—the concentration and/or duration. Accordingly, in the present study, we developed a mouse model of AR to address the complex relationships between allergen exposure and the development of AR, by serial nasal

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sensitization and challenge of ovalbumin (OVA) as an example of allergen [10]. The roles of different patterns of exposure to OVA in allergic symptom, OVA-sIgE in serum and nasal lavage fluid (NALF), allergic inflammation in nasal tissues were evaluated.

Materials and methods

Animals and experimental schedule

Female BALB/c mice (4 weeks of age) were purchased from Guangdong Medical Laboratory Animal Center (Guangzhou, China) and kept under specific pathogen-free condition. All animal experiments were performed with approval of, and under the guidelines of the Institutional Animal Care and Use Committee, Sun Yat-sen University (Approval No. IACUC 2014-0808).

To evaluate the concentration and duration-related effects, the animals were randomly divided into either the control or the treatment group on the basis of the following: (a) high-dose (400 µg) OVA exposure mice sensitized for 5 days (OVA/400-5); (b) high-dose (400 µg) OVA exposure mice sensitized for 14 days (OVA/400-14); (c) low-dose (25 µg) OVA exposure mice sensitized for 5 days (OVA/25-5); (d) low-dose (25 µg) OVA exposure mice sensitized for 14 days (OVA/25-14); (e) phosphate-buffered saline (PBS) control mice sensitized for 5 days (PBS-5); (f) PBS control mice sensitized for 14 days (PBS-14).

The mice received repeated sensitization by instillation in the nostril without anesthesia either with high-dose or low-dose OVA (Sigma) diluted in PBS (10 µL of 40 or 2 mg/mL) and then both emulsified in .4 mg of alum (Pierce) in a total volume of 20 µL. Control mice were intranasally sensitized with PBS instead of OVA. One week after the last sensitization, the mice were intranasally challenged with high-dose or low-dose OVA in 10 µL PBS or PBS alone for 5 days, respectively (Fig. 1).

Allergic symptom

Immediately following the last nasal challenge, the frequency of sneezing event was recorded for each mouse for 10 min by blinded observers. The mice were then sacrificed 24 h after the last challenge for further analyses.

Nasal histology

The skulls of mice were skinned, and the nasal portion was excised from the head along the line of both eye sockets after the lavage. The nasal tissues were fixed in 4 % paraformaldehyde for 3 days at 4 °C and decalcified in 10 % EDTA solution for 10 days at room temperature.

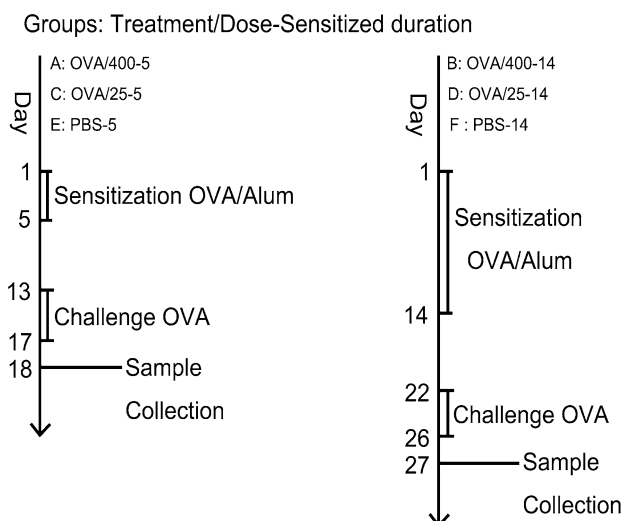


Fig. 1 Schematic diagram showing the procedures and timelines

After decalcification, the tissues were embedded in paraffin and sliced into 5 µm section, followed by hematoxylin and eosin staining to examine eosinophil infiltration and periodic acid-Schiff staining to evaluate hyperplasia of goblet cells. Under high magnification ($\times 400$), the number of infiltrating eosinophils were evaluated in the whole nasal mucosa by microscopy. Goblet cells were counted as the cells stained positive by periodic acid-Schiff and the numbers of positive cells per millimeter of basement membrane in the nasal septal mucosa were determined.

Preparation of serum samples and nasal lavage fluid (NALF)

After collecting blood from the mice at the time of sacrifice, blood samples were left for 30 min at room temperature. Serum was obtained after centrifugation at $2500\times g$ for 10 min. NALF was collected following partial tracheal resection. A 22-gauge catheter was inserted into the nasopharynx from the tracheal opening and the nasal passages were gently perfused with 150 µL of cold PBS from the choana to the nostril for three times. NALF was collected from the nares and centrifuged at $2500\times g$ for 10 min at 4 °C. All samples were stored at -80°C until the measurements.

Data analysis

Statistical analysis was performed using IBM SPSS Statistics 20. All values are shown as mean $\pm$ SEM. Statistical analysis was performed using a one-way analysis of variance followed by Bonferroni test for multiple comparisons of the data with Gaussian distribution. If data were abnormally distributed, Kruskal–Wallis rank sum tests followed by pairwise comparisons for multiple

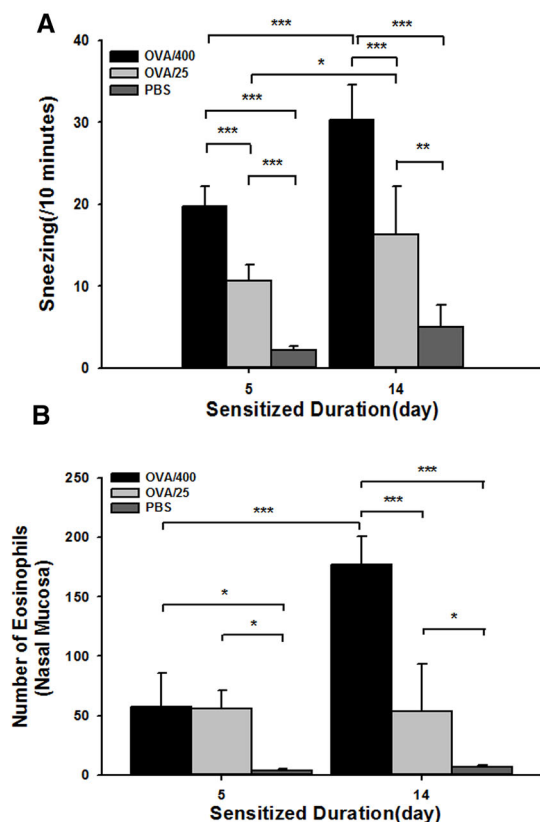
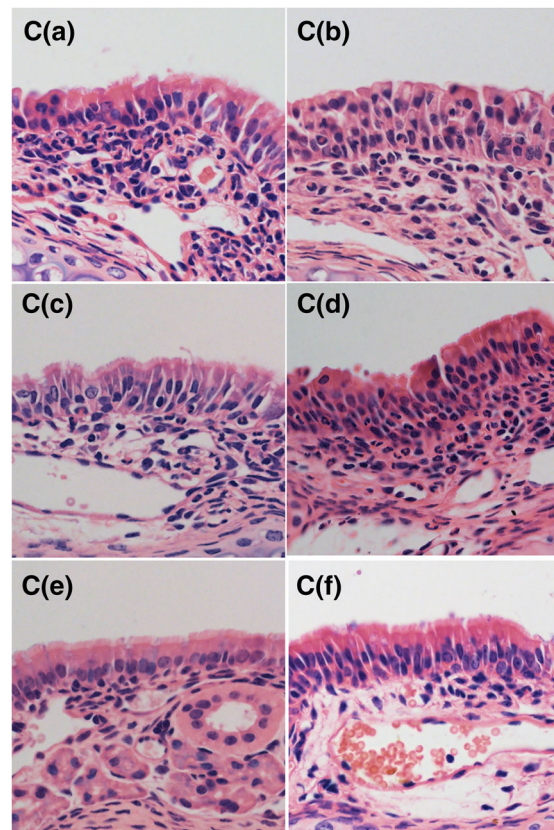


Fig. 2 Effects of OVA exposure with different patterns on allergic response. **A** The frequency of sneezing. **B** Presence of eosinophils in nasal mucosa. Expressed are the means $\pm$ SEMs. * $P < .05$; ** $P < .01$; *** $P < .001$. OVA ovalbumin, OVA/400 400 μ g OVA exposure mice, OVA/25 25 μ g OVA exposure mice, PBS PBS control mice. **C** Representative photomicrographs of HE-stained nose



sections from **a** 400 μ g OVA exposure mice sensitized for 5 days, **b** 400 μ g OVA exposure mice sensitized for 14 days, **c** 25 μ g OVA exposure mice sensitized for 5 days, **d** 25 μ g OVA exposure mice sensitized for 14 days, **e** PBS control mice sensitized for 5 days, and **f** PBS control mice sensitized for 14 days. Bar 20 μ m

comparisons were performed. Pairs of groups of samples distributed parametrically were compared by unpaired 2-tailed Student's *t* test, and those samples distributed nonparametrically were compared by Mann–Whitney *U* test. Differences with a *P* value less than .05 were considered to be statistically significant.

Results

Establishment of OVA-sensitized OVA-challenged AR

To mimic patients who were initially sensitized with inhalant allergen through the nasal route, a mouse model of OVA-specific AR without systemic immunization was generated by means of sequential nasal administration of OVA/alum. We first investigated whether OVA exposure induced the occurrence of nasal response. We counted the frequency of sneezing over a 10-min period immediately

after the final nasal challenge. Both OVA/400-5 ($P < .001$) and OVA/400-14 mice ($P < .001$) displayed significant higher sneezing frequency compared to control mice. OVA/25-5 ($P < .001$) and OVA/25-14 mice ($P < .01$) also significantly increased sneezing relative to control mice. Moreover, there were significant differences between 400 and 25 μ g OVA-treated mice in both the sensitized for 5 days group ($P < .001$) and 14 days group ($P < .001$). OVA/400-14 mice showed significant higher sneezing compared to OVA/400-5 mice ($P < .001$). This significant difference also could be observed in mice treated with 25 μ g OVA ($P < .05$) (Fig. 2A).

OVA dose-dependently increased nasal accumulation of eosinophils

We observed prominent eosinophilic accumulation in the nasal septal mucosa, nasal lateral mucosa and nasal turbinate of OVA-treated mice but not of control mice (all $P < .05$). On one hand, OVA/400-14 mice displayed

significant higher accumulation of eosinophils compared to OVA/400-5 mice ($P < .001$). However, there were no significant increases in eosinophils between OVA/25-5 and OVA/25-14 mice. On the other hand, OVA/400-5 mice significantly increased the number of eosinophils, comparable with those seen in OVA/25-5 mice. Moreover, OVA/400-14 mice showed a markedly augmented accumulation of eosinophils when compared to OVA/25-14 mice ($P < .001$) [Fig. 2B, C(a–f)].

Prolonged sensitized duration resulted in the damages at nasal mucosa

Excessive mucus secretion from hyperplastic goblet cells occurred after prolonged allergen exposure [Fig. 3A, B(a–f)]. Although both OVA/400-5 and OVA/25-5 mice exhibited no significant increases in goblet cells as compared to control mice, OVA sensitization with longer duration significantly induced hyperplasia of goblet cells in nasal septal mucosa in both 25 and 400 μ g OVA-treated mice (both $P < .05$). In addition, we observed that nasal epithelium gradually dropped away, and some cilium of the epithelium disappeared after longer duration of allergen exposure. Submucosal vessels were increased, and dilatation of vessels occurred (data not shown).

Prolonged sensitized duration decreased circulating level of OVA-sIgE

Allergen sIgE in serum is a typical feature of allergies, giving an index of the extent of sensitization to an allergen. Compared with control mice, mice treated with different doses and sensitized durations of OVA showed significant increases in serum OVA-specific IgE (all $P < .05$), except for OVA/25-14 mice. Interestingly, we found OVA-sIgE was down-regulated as sensitized period was increased in both OVA treatment at 400 μ g mice and 25 μ g mice (both $P < .01$). The level of OVA-specific IgE in serum, however, was not significantly affected by OVA dose either in the sensitization for 5 days group or 14 days group. Notably, OVA/25-14 mice displayed increased allergic nasal response but exhibited no significant increases in serum OVA-sIgE as compared to control mice (Fig. 4A).

OVA exposure increased OVA-sIgE level in NALF

We next examined the secretion of OVA-sIgE that was present in the NALF in response to nasal challenge. Here, the existence of OVA-sIgE represents allergic inflammation in local nasal mucosa. OVA exposure of all of these mice evidenced significant increases of OVA-sIgE in

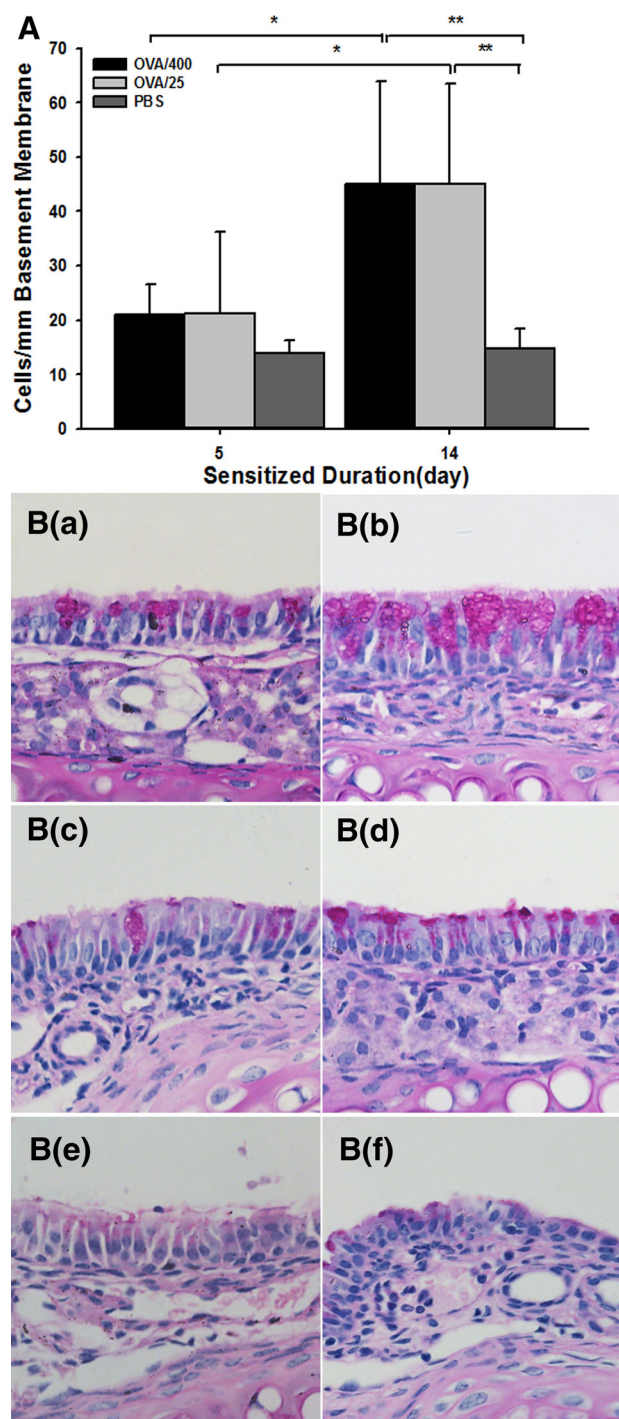


Fig. 3 Hyperplasia of goblet cells. **A** Number of goblet cell per milliliter of basement membrane in nasal septal mucosa. Expressed are the means $\pm$ SEMs. $*P < .05$; $**P < .01$; $***P < .001$. OVA ovalbumin, OVA/400 400 μ g OVA exposure mice, OVA/25 25 μ g OVA exposure mice, PBS PBS control mice. **B** Representative photomicrographs of PAS-stained nose sections from **a** 400 μ g OVA exposure mice sensitized for 5 days, **b** 400 μ g OVA exposure mice sensitized for 14 days, **c** 25 μ g OVA exposure mice sensitized for 5 days, **d** 25 μ g OVA exposure mice sensitized for 14 days, **e** PBS control mice sensitized for 5 days, and **f** PBS control mice sensitized for 14 days. Bar 20 μ m

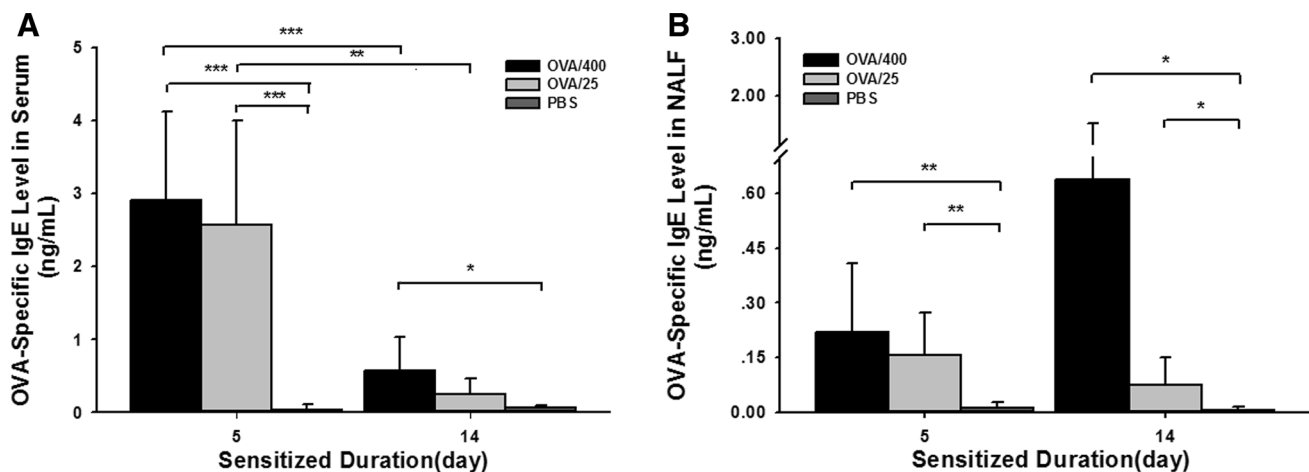


Fig. 4 Effects of OVA exposure with different patterns on sIgE response. **A** OVA-specific IgE in serum. **B** OVA-specific IgE in NALF

NALF (all $P < .05$) in comparison to PBS treated controls. No dose- and duration-related effects on OVA-sIgE in NALF were found. Surprisingly, although there were no significant changes in OVA-sIgE level in serum for OVA/25-14 mice, OVA-sIgE could be detected in the NALF (Fig. 4B). These mice manifested the major features of local allergic rhinitis (LAR) in human.

Discussion

In this study, we used two different allergen doses and periods of allergen sensitization to induce nasal airway inflammation to try to mimic different patterns of allergen exposure in AR. We then investigated allergic nasal response, sIgE in serum and NALF, and nasal histology in mice. These mice evidenced dose- and duration-related effects of OVA exposure on inducing allergic inflammation. We first demonstrated that both 400 and 25 μ g OVA, except PBS control, could induce allergic nasal symptoms and eosinophils accumulation, suggesting that both high-dose and low-dose allergen are capable of developing AR. Both dose and sensitized duration had significant effects on nasal symptoms. Histologic examinations revealed that the dose of OVA played an important role in the recruitment of eosinophils under the circumstances of prolonged sensitization. In addition, the sensitized duration of OVA had an effect on the recruitment of eosinophils in regard to high-dose sensitization. These results suggest that there may be dose $\times$ duration interactions of allergen in nasal symptoms and eosinophils recruitment. Moreover, we found that persistence of OVA exposure induces goblet cell hyperplasia and that nasal epithelium and its cilium disappeared, sub-mucosal vessels were increased and dilated. Although our mouse model has shown that repetitive allergen exposure in

sensitization phase reduced sensitization and promoted tolerance in the immune system, repetitive allergen exposure could change in structural cells, resulting in the damaged functions at the affected nasal mucosa. The pattern of allergen exposure appeared to be directly associated with allergic nasal symptoms and histologic damages.

The level of OVA-sIgE in serum depended on OVA exposure. Importantly, the circulating level of sIgE was mediated in a sensitized duration-related manner, where prolonged sensitization decreased sIgE level in serum. It is somewhat surprising that mice with obvious decreases in serum sIgE antibody levels showed higher frequency of sneezing. For the most part, serum sIgE has been considered a major component of the AR response. However, only several types of serum sIgE results were strongly associated with allergy-related outcomes in a large cross-sectional analysis [11]. Furthermore, in this study, prolonged low-dose allergen exposure in sensitization phase could induce localized sIgE and prominent accumulation of eosinophils in nasal mucosa without the detectable level of serum sIgE. Allergic inflammation tended to be a focal lesion after repetitive exposure with low-dose allergen. These results suggest that serum sIgE may contribute to, but is not essential for, the allergic inflammation in AR. Therefore, local sIgE may be better qualified than serum sIgE to represent the allergic inflammation at local nasal mucosa. The underlying mechanism responsible for the reduced circulating sIgE is not clear. We proposed the possibility that increasing allergen exposure in sensitization phase might induce tolerance, shifting a Th2 response from IgE to IgG. The IgG antibodies then acted as blocking antibodies.

In recent findings, some patients, whose symptoms are similar to AR, with localized but not systemic sIgE are classified as LAR [12–15]. Surprisingly, we found that

repeatedly exposed to low-dose allergen might contribute to the development of LAR. As demonstrated in our study, mice receiving low-dose allergen for prolonged duration exhibited the major features of human LAR, including nasal symptom and localized sIgE. This finding is consistent with a cross-sectional study in which the majority of LAR patients had a long history of rhinitis [16]. Additionally, four parts of patients with LAR appeared positive SPT responses and serum sIgE during immunotherapy [17]. It is possible that serum sIgE in LAR patients may be boosted by administration with increasing doses of the allergen during specific immunotherapy.

The concentration of allergen in the environment is not immutable; it may change over time. A limitation in this study is that we only investigated the effect of fixed level of environmental allergen. Whether allergen level alters during the sensitization or challenge phase affect the development of AR needs to be further elucidated.

Conclusions

In summary, this report demonstrated that there were allergic symptom, allergen sIgE and allergic nasal inflammation in AR that were affected to some extent by the pattern of allergen exposure. Since allergen exposure can contribute to long-lasting changes in immunologic responses, structural cells and even functions at local nasal mucosa, allergen avoidance should be an important aim of AR management.

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Compliance with ethical standards

Ethical standards All procedures performed in this study were in accordance with the ethical standards of the Animal Ethical and Welfare Committee.

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

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