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(Research Article)

Evaluation of experimental animal behaviors through establishment of an ovalbumin-induced experimental mouse model of allergic rhinitis

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Abstract

Allergic rhinitis (AR) was also called hay fever which was a type of nasal inflammation when the immune system overreacts to environmental allergen exposures. AR's clinical symptoms included a runny or stuffy nose, sneezing, red, itchy, watery eyes, and eye swelling. The fluid in the nasal cavity was usually clear. Patients with AR can affect sleep and work qualities. Seriously, the AR symptoms can also cause asthma, allergic conjunctivitis, or atopic dermatitis. Therefore, it is an important issue to attenuate AR symptoms and research the novel therapeutic drugs for AR patients. The purpose of this study was to introduce an easy-to-establish experimental mouse model of AR. In this study, the male BALB/c mice were divided respectively into as the Group A (n = 12) and the Group B (n = 12). Group A and Group B were designed as the normal control and RA, respectively. BALB/c mice in Group B were sensitized by intraperitoneal injection of ovalbumin (OVA) on day 0, day 4, day 13, and day 20, followed by continuous nasal administration of OVA solution once per day between day 21-43. BALB/c mice in Group A received sensitization of intraperitoneal injection of phosphate-buffered saline (PBS) on day 0, day 4, day 13, and day 20 and continuous nasal administration of PBS instead of OVA once per day between day 21-43. Before and after sensitization, the frequencies of nasal symptoms (sneezing, nasal rubbing) were recorded and counted. Results were showed that sneezing times in Group B were higher than Group A on D29, D30, D36, and D43 of the experiment. The sneezing times in Group A were significant higher on D29 and D30 of the experiment. However, the sneezing times in Group B were significant higher on D29, D30, D36, and D43 of the experiment. The rubbing times in Group B were higher than Group A on D29, D30, D36, and D43 of the experiment. The rubbing times in Group A were significant higher on D30 and D43 of the experiment. However, the rubbing times in Group B were significant higher on D29, D30, D36, and D43 of the experiment. Based on these results, a successful mouse model of AR has been established. We hope that this RA mouse model will provide a tool for the research of the novel AR therapeutic drugs and apply these novel AR therapeutic drugs to attenuate the AR symptoms in AR patients in the future.

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1. Introduction

Allergic rhinitis (AR) is an atopic disease and a common disease worldwide. AR symptoms characterized as nasal congestion, clear rhinorrhea, sneezing, postnasal drip, and nasal pruritis that approximately affects one in six individuals (the prevalence of AR was reported to be 15%-25%) and is associated with significant morbidity, loss of productivity, and healthcare costs. Children and adolescents, as well as young adults, were the age groups more affected by AR with comorbidities of asthma, sinusitis, conjunctivitis, and nasal polyposis [1-5].

Four typical symptoms of AR are nasal congestion, runny nose, sneezing, and itching of the nose after the nasal mucosa is exposed to allergens. Among them, nasal congestion is the most troublesome. Although the typical symptoms of AR are very similar to colds, colds generally do not have symptoms such as itchy nose, itchy eyes, or dark circles. However, how to diagnose AR? It is mainly based on the evaluation of clinical symptoms, and asking about allergy history (such as atopic dermatitis), family history (such as whether parents, siblings have allergic rhinitis or other allergic diseases). In addition, physical examination may reveal that the nasal mucosa is swollen, and the blood test for allergens and immunoglobulin E (IgE) is not a necessary condition for diagnosis. Even if the blood test shows allergies, if the patient does not have four typical symptoms, cannot be diagnosed AR [6-10].

There are hundreds of allergens. Generally, most allergens can be identified by using the allergen test. In addition, AR is mainly caused by air allergens or irritants such as second-hand smoke, dust mites, and pollen, although there are a few people may also induce allergic symptoms to certain foods such as peanuts, resulting in skin redness, AR, asthma, etc., but it is not common in clinical practice [11-18].

In fact, AR is also divided into mild and moderate to severe. Generally, if symptoms interfere with any daily routine such as sleep and work, it can be defined moderate-severe. According to the persistence of symptoms, it can be divided into intermittent type and continuous type. All in all, AR can be divided into four categories according to the severity and persistence of symptoms - mild intermittent, moderate to severe intermittent, mild persistent and moderate to severe persistent [19-23].

Steroid nasal sprays were also called the maintenance drugs which can effectively improve nasal mucosal inflammation and conjunctivitis. The decongestant nasal sprays can effectively improve nasal congestion symptoms. The disadvantage of the decongestant nasal sprays is rebound congestion may occur after stopping the drug after long-term use and will cause more severe nasal congestion and cause drug-induced rhinitis. Therefore, it is not recommended to use for more than five days in a row, even if AR symptoms do not improve, it should not be used again. Anti-histamine nasal sprays are not inferior to steroid nasal sprays in terms of AR symptom relief. However, when steroid nasal sprays were used to AR patients, the drugs sometimes flow back into the mouth and produce a bitter taste which is less acceptable to AR patients. Whether oral medication for AR are need? Generally, anti-histamines are still the mainstay, and steroids are used only when AR symptoms are very severe, but they are not often used clinically. So, oral drugs and nasal sprays, which one is more effective? The effect between the two is not too different. Some patients are very resistant to steroid nasal sprays. It is also possible to use oral antihistamines alone. If AR symptoms are severe, nasal sprays and oral drugs can be used in combination [1-5, 24-25].

In the *in vivo* AR studies, the animal studies on AR were adopted in various investigations. However, the establishment of an animal model for AR has been seldom seen. The purpose of this study was to introduce an easy-to-establish experimental mouse model of AR.

2. Material and methods

2.1. Chemicals and Reagents

Phosphate-buffered saline (PBS; Sigma-Aldrich, Cat. No. P3813), saline (Taiwan Biotech Co., LTD, Cat. No. 100-120-1101), Zoletil 50 (Virbac, Carros, France), Ovalbumin (OVA; Sigma-Aldrich, Cat. No. A7641), and Al $(OH)_3$ (Sigma-Aldrich, Cat. No. 239186) were applied in this experiment.

2.2. Preparation of AR Inducer

To weigh 5.0 g of $Al(OH)_3$ into 1,000 mL of PBS and mix evenly to make a 5 mg/mL $Al(OH)_3$ solution. Then, to adjust the pH with acetic acid stock solution to make pH = 6 and sterilize at 121°C for 30 minutes. After sterilization, the 5 mg/mL

Al $(OH)_3$ solution was cooled at room temperature. Following, to weigh 1 mg of OVA and add 1 mL of PBS to a 1.5 mL centrifuge tube and shake with vortex to completely dissolve OVA. Later, to pipette 200 mL of 5 mg/mL Al $(OH)_3$ solution into a beaker and add 1 mg OVA and place the beaker on an electromagnetic heating stirrer and stir with a magnet for 30 minutes until $Al(OH)_3$ can be dissolved and homogeneous adsorption of OVA. Finally, the preparation of AR inducer is finished [PBS + 40 μg/kg OVA + 40 mg/kg Al $(OH)_3$].

2.3. Video Recording System

The experiment was video-recorded at 5 time points, D19, D29, D30, D36, and D43 of the experiment. The cages, mirrors, and cameras were set up in the experiment (Figure 1).

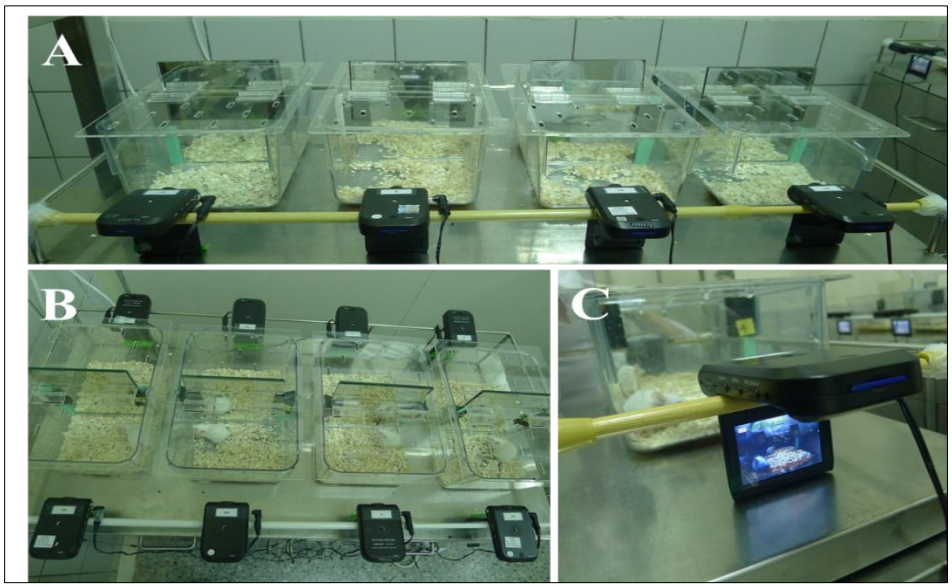

Figure 1 Video recording system. (A) Lateral photo. (B) Front photo. (C) Cameras

2.4. Experimental Animals and Experimental Design

Adult male 24 BALB/c mice [8 weeks old; BW between 24-25 g)] with specific pathogen-free conditions were used for this study, were purchased from BioLASCO Taiwan Co., Ltd. (Yilan, Taiwan). The environment was maintained room temperature (24-27°C) and 60%-70% humidity with a photoperiod of 12-hr light/12-hr dark cycle. The study will begin after a week acclimation. The Institutional Animal Care and Use Committee (IACUC) of Agricultural Technology Research Institute inspected all animal experiments and this study comply with the guidelines of protocol IACUC 103055 approved by the IACUC ethics committee. The male BALB/c mice were divided respectively into as the Group A (n = 12) and the Group B (n = 12). Group A and Group B were designed as the normal control and RA, respectively. All BALB/c mice were fed with standard laboratory diet (No. 5053, LabDiet®; PMI Nutrition International, St. Louis, MO, USA) and were administrated with distilled water ad libitum during the experimental period. The clinical behaviors of BALB/c mice were monitored during the experiment (Figure 2).

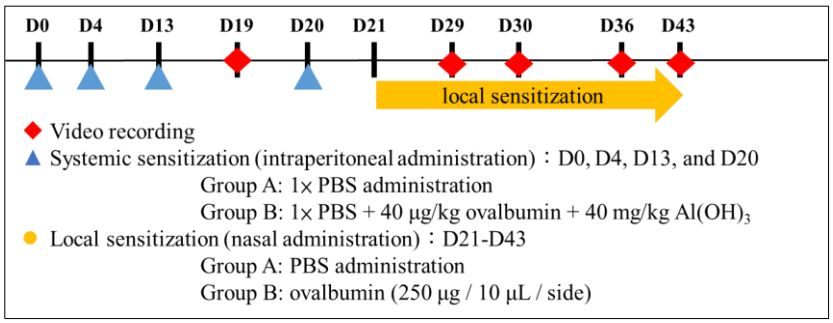

Figure 2 Experimental group and design

2.5. The Number of Sneezing and Nasal Rubbing

The experiment was video-recorded at five time points, D19, D29, D30, D36, and D43. Before video-recording BALB/c mice' behavioral observation, the BALB/c mice must be placed in the observation cage to adapt to the environment for 10 minutes, and a video camera was placed in front to record BALB/c mice' behavior within 15 minutes after local sensitization. After completing the video record, the experimenter must check BALB/c mice' behavior recorded in the video, and count the number of the sneezing and nose grinding times for each mouse within 15 minutes at 5 experimental time points.

2.6. Statistical Analysis

SPSS (Statistical package for the social sciences) statistical software (version 28.0) and MedCalc statistical software (version 20.113) were used for statistical analysis. Measurement data were expressed as mean ± standard error of mean (SEM). All comparisons were made by one-way ANOVA (Analysis of Variance) and Duncan's multiple range test. All significant differences are reported at $^{*}p < 0.05$.

3. Results

3.1. The Counts of the Number of Sneezing

The experiment was video-recorded at 5 time points, D19, D29, D30, D36, and D43 of the experiment. Data were showed that sneezing times in Group B were higher than Group A at D29, D30, D36, and D43 of the experiment. The sneezing times in Group A were significant higher ($p < 0.05$) at D29 and D30 of the experiment. However, the sneezing times in Group B were significant higher ($p < 0.05$) at D29, D30, D36, and D43 of the experiment (Figure 3 and Table 1).

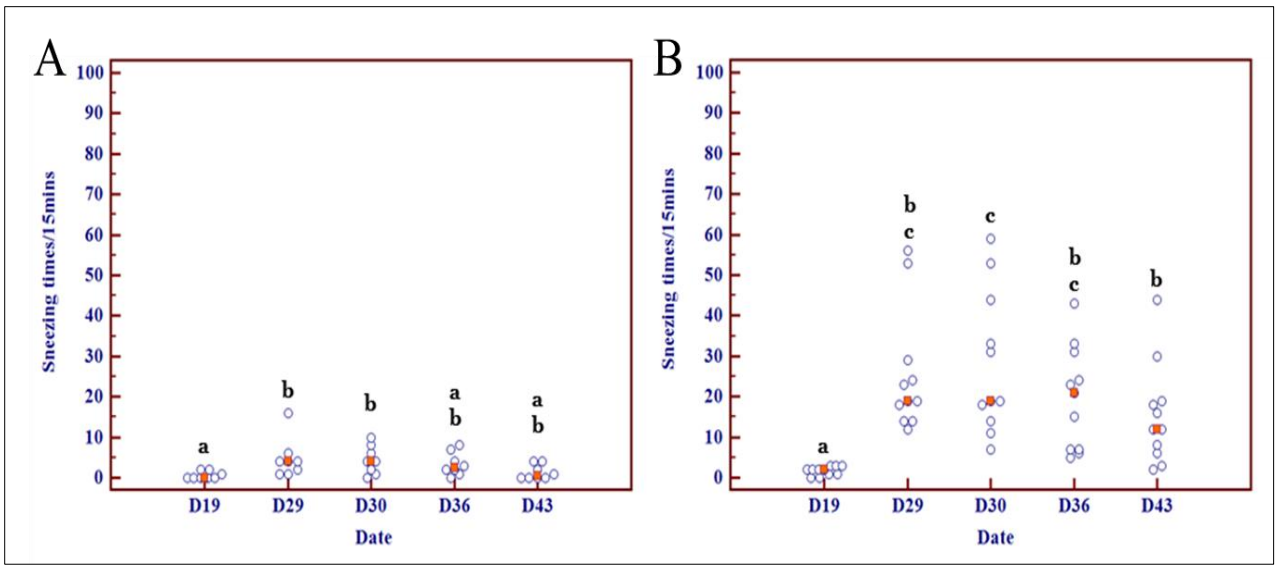

Figure 3 The counts of the number of sneezes. The experiment was video-recorded at 5 time points, D19, D29, D30, D36, and D43 of the experiment. (A) Group A: the normal control. (B) Group B: RA induction. The significant difference presented the different superscript letter

Table 1 The counts of the number of sneezes. The experiment was video-recorded at 5 time points, D19, D29, D30, D36, and D43 of the experiment. (A) Group A: the normal control. (B) Group B: RA induction. The significant difference presented the different superscript letter

	RA-induced time (day; D)				
	D19	**D29**	**D30**	**D36**	**D43**
Group A	0.63 ± 0.32^{a}	4.75 ± 1.72^{b}	4.38 ± 1.22^{b}	3.38 ± 1.00^{ab}	1.38 ± 0.63^{ab}
Group B	$1.73 \pm 0.33a$	25.55 ± 4.57^{bc}	28.00 ± 5.28^{c}	19.55 ± 3.85^{bc}	15.45 ± 3.75^{b}

3.2. The Counts of the Number of Nasal Rubbing

The experiment was video-recorded at 5 time points, D19, D29, D30, D36, and D43 of the experiment. Data were showed that rubbing times in Group B were higher than Group A at D29, D30, D36, and D43 of the experiment. The rubbing times in Group A were significant higher ($p < 0.05$) at D30 and D43 of the experiment. However, the rubbing times in Group B were significant higher ($p < 0.05$) at D29, D30, D36, and D43 of the experiment (Figure 4 and Table 2).

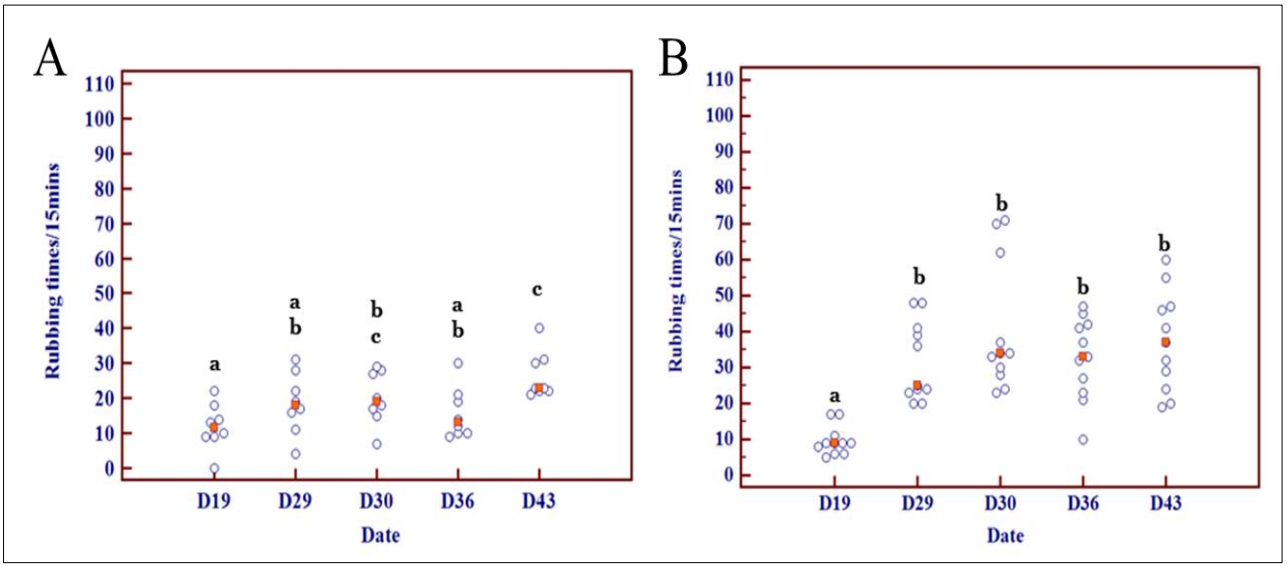

Figure 4 The counts of the number of nose grinding. The experiment was video-recorded at 5 time points, D19, D29, D30, D36, and D43 of the experiment. (A) Group A: the normal control. (B) Group B: RA induction. The significant difference presented the different superscript letter

Table 2 The counts of the number of nose grinding. The experiment was video-recorded at 5 time points, D19, D29, D30, D36, and D43 of the experiment. (A) Group A: the normal control. (B) Group B: RA induction. The significant difference presented the different superscript letter

	RA-induced time (day; D)				
	D19	**D29**	**D30**	**D36**	**D43**
Group A	11.88 ± 2.34^{a}	18.50 ± 3.09^{ab}	20.13 ± 2.68^{bc}	15.63 ± 2.57^{ab}	26.50 ± 2.35^{c}
Group B	9.64 ± 1.22^{a}	31.64 ± 3.31^{b}	40.55 ± 5.44^{b}	32.55 ± 3.45^{b}	37.27 ± 4.18^{b}

4. Discussion

Typical allergic diseases include asthma, AR, atopic eczema, conjunctivitis, food allergy, drug allergy, insect allergy, and anaphylactic shock, among which the most common are AR and allergic asthma. AR is a type I allergic disease of the nasal mucosa mediated by IgE. The symptoms of AR include nasal itching, nasal congestion, clear nasal discharge, runny nose, and sneezing as the main clinical manifestations and often accompany by itchy eyes, conjunctival congestion, or tearing [26-28].

In recent years, AR research has also received more and more attention. With the development of science and technology, disease animal models have become an important method for medical research due to their advantages of small ethical and moral restrictions, short experimental periods, and few interference factors. There are various animal modeling methods for AR. However, to sum up, the most commonly used modeling method of AR is intraperitoneal injection and nasal stimulation method. The selection of model animal species include guinea pig, rat, mouse and rabbit. Recently, it was found that the mouse model is more suitable for AR studies and CBA/J and BALB/c inbred mouse strains are the most commonly used. Experimental mice are rich in sources, relatively cheap, easy to raise and breed indoors, have a developed lymphatic system, and are very sensitive to external stimuli, which are favorable conditions for mice to be used to study AR [29-30].

The allergens that can be successfully modeled currently mainly include toluene diisocyanate (TDI), OVA, ragweed pollen, cedar pollen, etc. OVA has become the most commonly used allergen in the establishment of AR animal models due to the persistence of antibodies produced after OVA sensitization and has been widely used in AR research. Adding various adjuvants can enhance the immunogenicity of OVA, which can not only induce AR symptoms rapidly, but also produce high titers of IgE and IgG. Al (OH) $_3$ is generally used as an immune adjuvant. Al (OH) $_3$ is non-toxic and has good adsorption capacity, which can wrap the antigen at the injection site to prevent it from being eliminated by the body. Additionally, the main function of Al (OH) $_3$ is to induce humoral responses and stimulate the body to produce Th_2-type responses, thereby rapidly producing durable IgE antibodies. Al (OH) $_3$ has high safety and is a good immune adjuvant. However, it must be noted that if Al (OH) $_3$ is used in excess, it may lead to immunosuppression [31-35].

The optimal AR mouse model was applied with intraperitoneal injection for systemic sensitization and nasal challenge method for local sensitization. Systemic sensitization stage included as 75 μg OVA and 2 mg Al (OH) $_3$ into 200 μL PBS were sensitized by intraperitoneal injection to mice on day 1, 7, 14, and 21. Local sensitization stage included as 22-30 days later, mice were challenged with 500 μg of OVA and 20 μL of PBS in the nasal cavity, and the control mice were given PBS instead of OVA in the same way [36-37]. In this study, AR systemic sensitization inducer is a mixed solution PBS + 40 μg/kg OVA + 40 mg/kg Al (OH) $_3$ and PBS instead of OVA in the control mice. AR local sensitization inducer is 250 μg / 10 μL / side OVA and PBS instead of OVA in the control mice. Systemic and local sensitization methods were respectively applied intrapertoneal and nasal cavity administration. Animal species and strain is BALB/c inbred mice. Systemic sensitization by intraperitoneal injection to BALB/c mice on day 0, 4, 13, and 22. Local sensitization by nasal cavity administration to BALB/c mice on day 21-43. According to our results, AR mouse modeling can be successfully induced.

How to evaluate a successful AR animal model and its behaviors. Immediately, 30 mins and 2 h after the sensitization, the AR-induced mice were respectively observed and scored for the symptoms as nasal itching [score 1-3; score 1: scratch the nose lightly 1-2 times; score 2: frequently scratching the nose and face; score 3: constantly scratching the nose and face (or rubbing the nose against the rat cage)], sneezing (score 1-3; score 1: 1-3 times; score 2: 4-10 times; score 3: over 11 times), runny nose (score 1-3; score 1: snot into the front nostrils; score 2: snot through the front nostrils; score 3: snot on all face), and asthma (score 1-3; score 1: shortness of breath; score 2: significant wheezing; score 3: death). A total score greater than 5 scores indicates successful modeling. Although the behavioral score or symptom score is affected by subjective factors, the accuracy is slightly poor, but the method is simple, convenient, easy to operate, and can reflect the success of modeling to a certain extent, so it is accepted by many scholars [36-37]. In this study, the number of sneezing and nasal rubbing were counted. The experiment was video-recorded at 5 time points, D19, D29, D30, D36, and D43 of the experiment. Data were showed that sneezing times in AR mice were higher than the control mice at D29, D30, D36, and D43 of the experiment. The sneezing times in AR mice were significant higher on D29, D30, D36, and D43 of the experiment. The rubbing times in AR mice were higher than the control mice on D29, D30, D36, and D43 of the experiment. The rubbing times in AR mice were significant higher on D29, D30, D36, and D43 of the experiment. According to our results, AR mouse modeling can be successfully induced.

5. Conclusion

To summarize our study, BALB/c mice sensitized with intraperitoneal and nasally administrated OVA showed increased frequencies of AR symptoms (sneezing and nasal rubbing). OVA is a widely accepted and easily available allergen in animal study. The intraperitoneal and nasally administrated OVA might be a useful technique for AR animal models. Our method is easily reproducible and cost effective. We hope that this RA mouse model will provide a tool for the research of the novel AR therapeutic drugs and apply these novel AR therapeutic drugs to attenuate the AR symptoms in AR patients in the future.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

The Institutional Animal Care and Use Committee (IACUC) of Agricultural Technology Research Institute inspected all animal experiments and this study comply with the guidelines of protocol IACUC 103055 approved by the IACUC ethics committee.

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