Original Research

In vivo visualization and analysis of ciliary motion in allergic rhinitis models induced by ovalbumin

Chen Liu[1,2,3,4], Chuan Pang[5], Dai-shi Chen[6], Jin Wang[1,2,3,4], Wen-qi Yi[1,2,3,4], Ning Yu[1,2,3,4] and Lei Chen[1,2,3,4]

[1]Senior Department of Otolaryngology-Head & Neck Surgery, The Sixth Medical Center of PLA General Hospital, Beijing 100048, China; [2]National Clinical Research Center for Otolaryngologic Diseases, Beijing 100048, China; [3]State Key Lab of Hearing Science, Ministry of Education, Beijing 100048, China; [4]Beijing Key Lab of Hearing Impairment for Prevention and Treatment, Beijing 100048, China; [5]Department of General Surgery, First Medical Center of Chinese PLA General Hospital, Beijing 100853, China; [6]Department of Otorhinolaryngology, Shenzhen People's Hospital, The Second Clinical Medical College, Jinan University and The First Affiliated Hospital, Southern University of Science and Technology, Shenzhen 518020, China
Corresponding author: Lei Chen. Email: chen301@aliyun.com

Impact Statement

Allergic rhinitis (AR) influences the structure and function of ciliated cells due to different factors. However, the image of *in vivo* nasal ciliary motion of AR has never been captured because of the lack of an effective method. In this study, we rely on the method established by our team last year to observe and analyze the nasal ciliary function *in vivo* in AR rats. Such images of ciliary motion from AR living animals have not been reported to date. Our study first visually clarifies that AR reduced ciliary motion *in vivo*. And, our data suggest that ciliary motion tends to show tolerance to the inhibition of ovalbumin (OVA) and histamine in OVA-sensitized rats. We adopt the assessment approach of our group to observe and analyze the ciliary function in AR animals. It lays a good foundation for further *in vivo* study on AR.

Abstract

Due to the lack of an assessment approach, the image of *in vivo* nasal ciliary motion of allergic rhinitis (AR) has never been captured and analyzed to date. Here, we have used an optimized approach to analyze the nasal ciliary function *in vivo* in AR rats. The digital microscopy system, a method for direct observation of ciliary motion in a living AR rat model, was applied to visualize and measure ciliary motion *in vivo*, including ciliary beat frequency (CBF) and ciliary beat distance (CBD). The AR rat model was established by ovalbumin sensitization. Comparisons of nasal ciliary motion *in vivo* between the experimental group (ovalbumin sensitization, allergen, or histamine) and the control group were analyzed. In the living rat model of allergic rhinitis, CBF and CBD decreased to 57.8 and 73.1% of the control group, respectively, but were restored after administration of chlorpheniramine maleate. Ovalbumin (OVA) significantly inhibited the ciliary motion of normal mucosa *in vivo*. However, responding to the OVA challenge, the ciliary motion of OVA-sensitized mucosa would not decrease further and stay at a stable level. Histamine stimulated *in vivo* ciliary motion quickly within 30 min, but afterward, the ciliary motion gradually decreased below the baseline. These results have clarified that *in vivo* ciliary motion was impaired by nasal mucosal sensitization, and this impairment was most likely related to allergen challenge and histamine. In addition, the short-term stimulation and long-term inhibition effects of histamine on *in vivo* ciliary motion were first reported in this study.

Keywords: *In vivo*, ciliary motility, microscopy, allergic rhinitis, histamine, nasal mucosa

***Experimental Biology and Medicine* 2022; 247: 1287–1297. DOI: 10.1177/15353702221088781**

Introduction

Allergic rhinitis (AR) is one of the most common diseases of the nasal mucosa that is induced by immunoglobulin E (IgE)-mediated inflammation and affects over 10–40% of the population worldwide with an increasing prevalence in recent years.[1–3] High concentrations of inflammatory mediators and neuropeptides in the nasal mucosa were involved in AR development.[4–6] At the same time, these factors are very likely to influence the structure and function of ciliated cells. Previously many studies focused on the relationship between cilia and sinusitis, whereas evidence of the relationship between AR and cilia is insufficient and controversial. Some studies[7,8] found a decline of the AR patients' ciliary beat frequency (CBF); the longer the disease course, the lower the CBF. But in an animal experiment, the allergen could induce a slight increase of the CBF in the trachea of sensitized sheep. However, the results above were collected from ciliated cells *in vitro*, detached from the mucus, and highly sensitized internal environment *in vivo*. Therefore, it is doubtful whether these *in vitro* results of ciliary motion accurately reflect their actual physiological state *in vivo*.

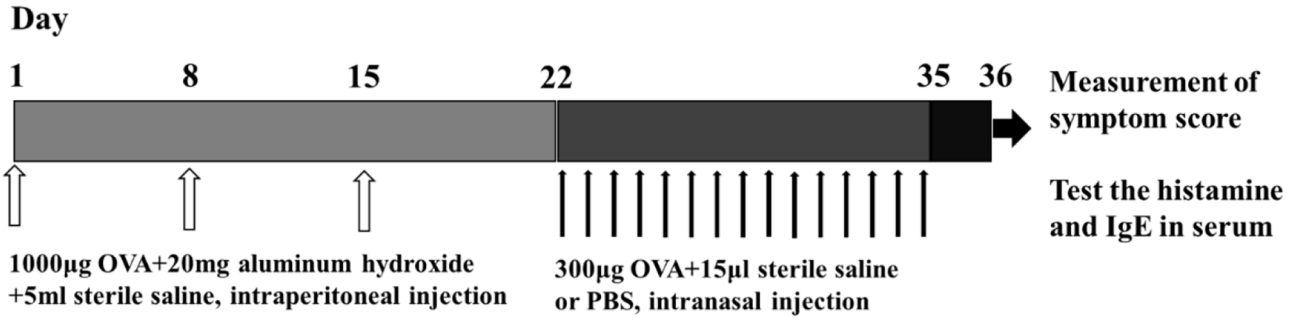

Figure 1. The protocol of the ovalbumin sensitization on SD rats and the overall experimental process of this study.

We have previously demonstrated significant differences in ciliary motion and its response to environmental stimuli *in vitro* and *in vivo*.[9]

It is generally believed that immune cells, inflammatory mediators, cytokines, chemokines, neuropeptides, and adhesion molecules cause the inflammation of the mucosa, which affects the coordinated ciliary activity in a complex synthetic work.[4–6,8,10,11] Among inflammatory mediators, histamine is recognized as one of the most important and plays a vital role in developing AR. However, the dispute over the effect of histamine on the ciliary motion has not been definitively concluded yet. Some studies reported that histamine could stimulate cilia with an increase in CBF.[12–15] In contrast, other studies found that a high concentration of histamine had cilia-inhibiting or cilia-static effect.[12,16] A few studies found no significant effect of histamine on the ciliary motion.[17,18] Nevertheless, these controversial results were all based on experiments *in vitro* or cell cultures. The ciliary response to histamine could be different from that *in vivo*, which remains unknown.

Therefore, we established an AR experimental animal model and analyzed the effect of nasal mucosal sensitization, allergen, and histamine challenge on the function of ciliary epithelium *in vivo* using a digital microscopy system.[9]

Materials and methods

Animals and preparation

Three-month-old SD rats, weighing 200–300 g, provided by the Medical Animal Center of Chinese PLA General Hospital (Beijing, China), were used in this study. The procedures of animal experiments were conducted and approved by the Ethics Committee of the PLA General Hospital under laboratory animal protection guidelines. The overall experimental process of this study is summarized as shown in Figure 1.

Ovalbumin-sensitized rat model of AR

All the rats were injected intraperitoneally (IP) with antigenic adjuvant suspension ((ovalbumin, OVA), 1000 μg; aluminum hydroxide, 20 mg; sterile standard saline 5 mL) three times on the 1st, 8th, and 15th day, and then sensitized intranasally with OVA (300 μg/15 μL) or phosphate-buffered saline (PBS) 15 μL for control per nostril daily from day 22 to 35, according to the protocol depicted in Figure 1.

The rats were subdivided into two groups ($n = 20$ per group): (1) PBS-treated rats (control group), (2) OVA-sensitized rats (AR group). After the final intranasal provocation on day 35, the number of nasal sneezing and scratching events were observed during a 20 min period as the symptom score to confirm the AR phenotype. On day 36, we collected the blood from the tail tip of rats and tested the histamine and IgE in serum using enzyme-linked immunosorbent assay (ELISA).

Histological and morphometric analysis

Five rats from each group were selected, and the nasal septum mucosa was harvested and stained with hematoxylin and eosin (HE staining) for histological and morphometric analysis.

In vivo visualization and analysis of ciliary motion

The detailed protocol of *in vivo* visualization and analysis of ciliary motion was previously described by our group.[9] To be brief: the rats were anesthetized by intraperitoneal injection of sodium pentobarbital (60 mg/kg; Merck Company, New Jersey, USA) and then maintained anesthesia with intravenous infusion of pentobarbital (6 mg/h). A stethoscope monitored heart rates. Next, the unilateral nasal wall was excised to expose the nasal septum mucosa. A custom-made platform with a heating pad was used to hold the animal secured to reduce image instability caused by respiratory and cardiac motions. The animal on the platform was then placed on the digital microscopy system (VHX6000; Keyence Corporation, Osaka, Japan) with the observation lens (VH-Z100R; Keyence Corporation) set perpendicular to the mucosal surface. The images were recorded with a high-speed microcamera (VW-9000; Keyence Corporation) at 150 frame/s (fps) for 5 s. During the overall experiments, the room temperature was maintained at 24°C with relative humidity at 40–50% by an air conditioner.

The images were recorded at 150 frame/s(fps) for 5 s each. Five ciliary motion areas were randomly selected from the nasal mucosa of each animal. During measurement, we randomly selected 5 points in a region of interest (ROI) for measurement and averaged them to represent the ciliary motion in this region.

CBF(Hz) = Ps/Pw (one pixel = one frame, Ps = pixels per second, Pw = pixels of each wave). CBD (ciliary beat distance) is defined as the distance traveled by the front edge of a ciliary wave between maximum recovery stroke and maximum power stroke *in vivo*. CBD was compared by using normalized CBD (CBD ratio), namely, the ratio of the measured

CBD value to the CBD value baseline at the beginning of the experiment. The method of how to measure the CBF and CBD in detail can be found in the precious research results of our team.[12] The CBFs and CBDs of every region were measured by the software of ImageJ as described previously.[12] The recordings were separately analyzed twice by Liu Chen and Pang Chuan, who were blinded to each other's results.

Changes of *in vivo* ciliary motion in OVA-sensitized rats

Live imaging of *in vivo* ciliary motion was recorded with a high-speed microcamera at the beginning of the experiment from two groups as the baseline. Then the nasal mucosal surface of the two groups was administered with 1 mmol/L chlorpheniramine maleate (15 μL), and the ciliary motion was recorded again 60 min later.

Changes of *in vivo* ciliary motion to OVA

CBDs and CBFs of control and OVA-sensitized rats were measured as a baseline before the administration of OVA. We first examined the effect of OVA on the ciliary motion. Then, OVA (300 μg in 15 μL PBS) was administered on the nasal mucosal surfaces of five rats each in control and AR groups. PBS (15 μL) was administered on another five rats in each group as control. The live imaging of ciliary motion was recorded continuously for 60 min *in vivo*.

Effects of histamine on *in vitro* ciliary motion

Mucosa specimens were obtained from the nasal septum mucosa and placed in a Petri dish, submerged in the Leibovitz L15 medium (Gibco™, USA).The specimens were examined under an inverted phase-contrast microscope (DMi 8; Leica Microsystems GmbH, Wetzlar, Germany).The beating cilia were recorded with a high-speed digital camera (EoSens-4CXP; Mikrotron GmbH, Unterschleissheim, Germany) and the CBF was analyzed with ImageJ software. The effect of the 10^{-1} mol/L, 10^{-3} mol/L, and 10^{-5} mol/L histamine (15 μL) on CBFs was observed, respectively, for 60 min *in vitro* on the nasal mucosal specimens of five rats each in control and AR group. We randomly selected 5 points in a specimen for measurement and averaged them to represent ciliary movement in this specimen.

Effects of histamine on *in vivo* ciliary motion

We further examined the effect of histamine on the ciliary motion in OVA-sensitized rats. A high concentration of 10^{-1} mol/L histamine (15 μL) was administered to observe the trend of the histamine effect; 15 μL histamine (10^{-1} mol/L) and 15 μL PBS were, respectively, administered on the nasal mucosal surfaces of five rats each in control and AR groups. The ciliary motion was recorded for 60 min *in vivo*. Then, the effect of the 10^{-3} and 10^{-5} mol/L histamine on CBFs and CBDs was observed in the same protocol above. In addition, CBFs and CBDs were measured after administering 15 μL chlorpheniramine maleate (0.1 mol/L) on the nasal mucosal surfaces of five rats each in control and AR group. The ciliary motion was measured for 60 min *in vivo*. Then 15 μL histamine (0.1 mol/L) or PBS was administered on the nasal mucosal surfaces of both groups, respectively. The ciliary motion was observed for another 60 min *in vivo*.

Statistical analysis

Continuous variables were expressed as median (Upper/Lower limits), and normality of the distribution was checked using the Shapiro–Wilk test. Comparisons between AR and control groups of symptoms scores, serum histamine and IgE levels, and CBF and CBD data *in vivo* were made with Student's t test. In addition, OVA and histamine response over time and grouped comparison were evaluated using the Mann–Whitney U test and Wilcoxon rank-sum test. The p values less than 0.05 were considered statistically significant. All statistical analyses were performed with GraphPad Prism 8.0 software (GraphPad, San Diego, CA, USA).

Results

Symptoms, serum histamine and serum IgE of AR rats

On the 35th day of the sensitization, behavioral observations were performed to evaluate the symptoms of AR, and blood tests were performed at the same time. The symptom behaviors (the number of sneezing and scratching) significantly increased in OVA-sensitized rats (AR group) compared with the control group (Figure 2(a) and (b)). In addition, the levels of histamine and IgE in serum increased in OVA-sensitized rats (Figure 2(c) and (d)).

Histomorphology of nasal mucosa in OVA-sensitized rats

The effect of OVA sensitization on mucosal histology was examined by using HE staining. In the control group, intact cilia, a small number of goblet cells, and none of the obvious tissue edema, vascular congestion, or glandular hyperplasia were found (Figure 3(a)). On the contrary, the OVA-sensitized rats (AR group) exhibited ciliated epithelial lesions, including ciliary adhesion, lodging, and loss. Meanwhile, increased goblet cells and hyperplasia of glands in the lamina propria were found (Figure 3(b)).

Effect of OVA-sensitization on the *in vivo* ciliary motion

Video 1 and video 2 (Supplementary File), respectively, showed a region of ciliary motion on the normal and AR rat's nasal septum magnified 1000×. Compared to the control, the *in vivo* CBFs and CBDs of AR rats decreased to 57.8 and 73.1%, respectively (Figure 4(a) and (b)). These results indicated that allergic reaction induced the decline of ciliary motion *in vivo*.

The ciliary motion was measured after administrating the histamine antagonist (chlorpheniramine maleate) 60 min later. Again, the CBF obviously increased in the AR group, which recovered to 79% of the normal level (Figure 5(a)). However, there was no significant change in CBD after antihistamine administration (Figure 5(b)).

These two experiments suggested that OVA sensitization lowered ciliary motion, and the CBF could recover to a

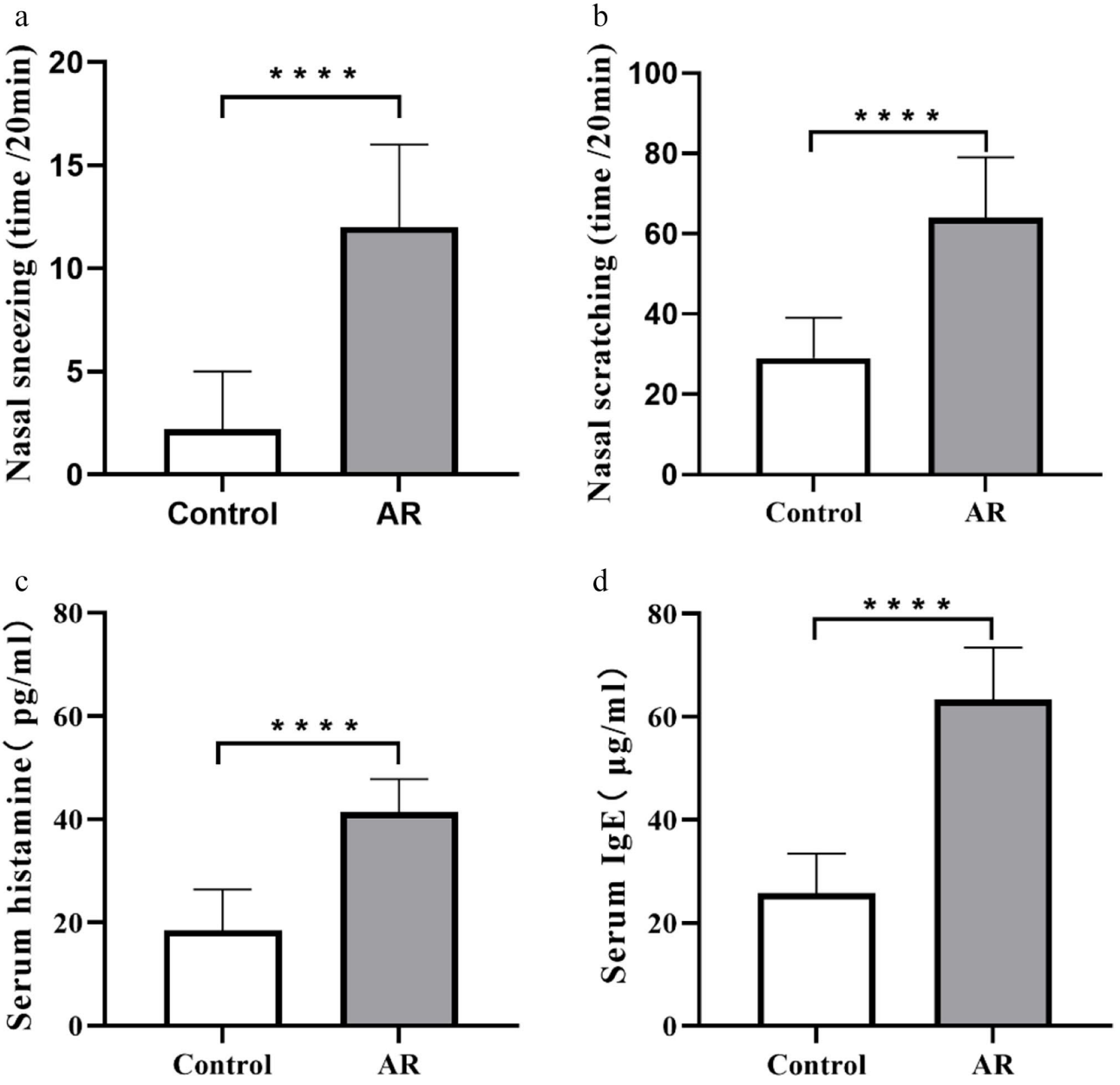

Figure 2. In OVA-sensitized rats (AR group) ($n = 20$), symptom scores were determined by the incidence of (a) nasal sneezing and (b) nasal scratching in 20 min. The amount of serum (c) histamine and (d) IgE after the intranasal provocation of OVA.
****$p < 0.0001$.

certain level after administration of antihistamine (chlorpheniramine maleate), but the CBD could not.

Effects of OVA challenge on ciliary motion *in vivo*

To further explore the allergen challenge effects on the nasal mucosal cilia, we administrated the OVA solution on the nasal mucosal surface of the control and AR group, and observed for 60 min, using PBS solution as control. The results showed that the CBF and CBD of normal nasal cilia decreased after OVA administration (Figure 6(a) and (c)). Although the baseline of CBF and CBD was relatively low in OVA-sensitized rats, they could maintain a stable level after the OVA challenge (Figure 6(b) and (d)), which implied OVA tolerance of sensitized cilia. These results suggested that the allergen OVA solution significantly inhibited the nasal mucosal ciliary motion *in vivo*. However, responding to OVA challenge, the ciliary motion after OVA sensitization would not decrease further and maintain a stable level.

Effects of histamine on *in vitro* ciliary motion

To study the effect of histamine on *in vitro* nasal ciliary motion in both the control group and AR group, we, respectively, administered histamine solution and PBS to the nasal mucosa specimens in the two groups. The results showed that CBFs in both groups decreased rapidly after exposure to 10^{-1} mol/L histamine and complete ciliostasis was observed within 10 min (Figure 7(a) and (b)). But the

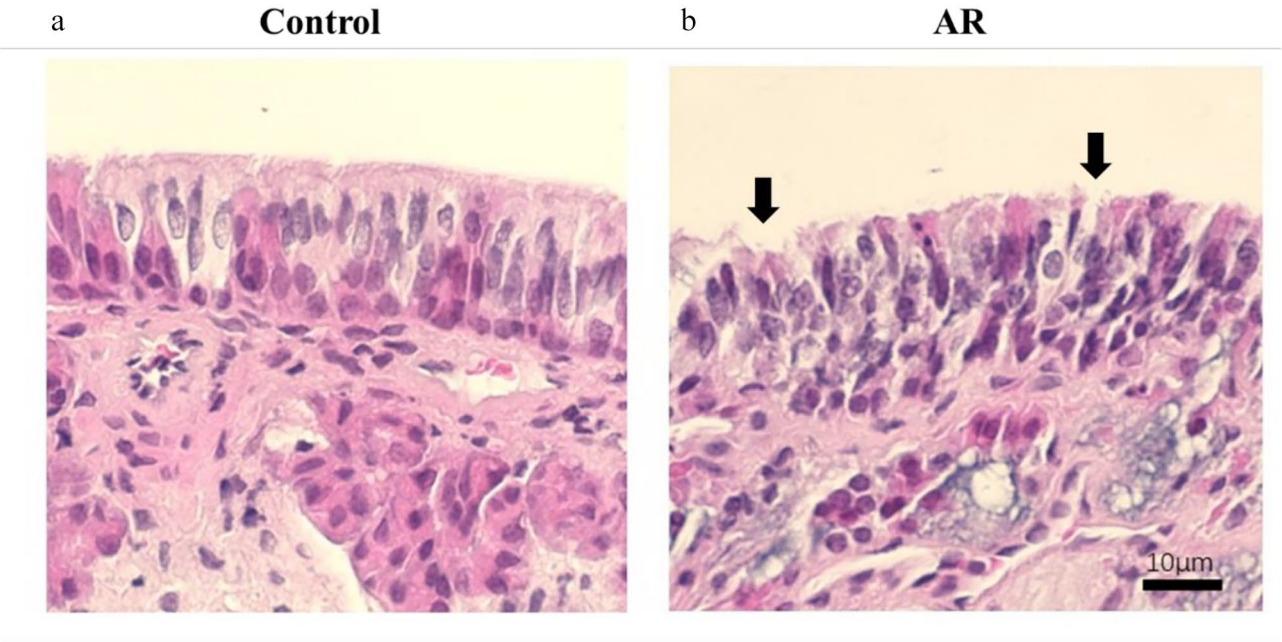

Figure 3. Histological changes in the nasal septal mucosa after the OVA sensitization. (a) Normal septal mucosa and (b) ciliated epithelial lesions in OVA-sensitized rats. The two black arrows indicate the ciliary adhesion, lodging, and loss. Scale bar, 10 μm. (A color version of this figure is available in the online journal.)

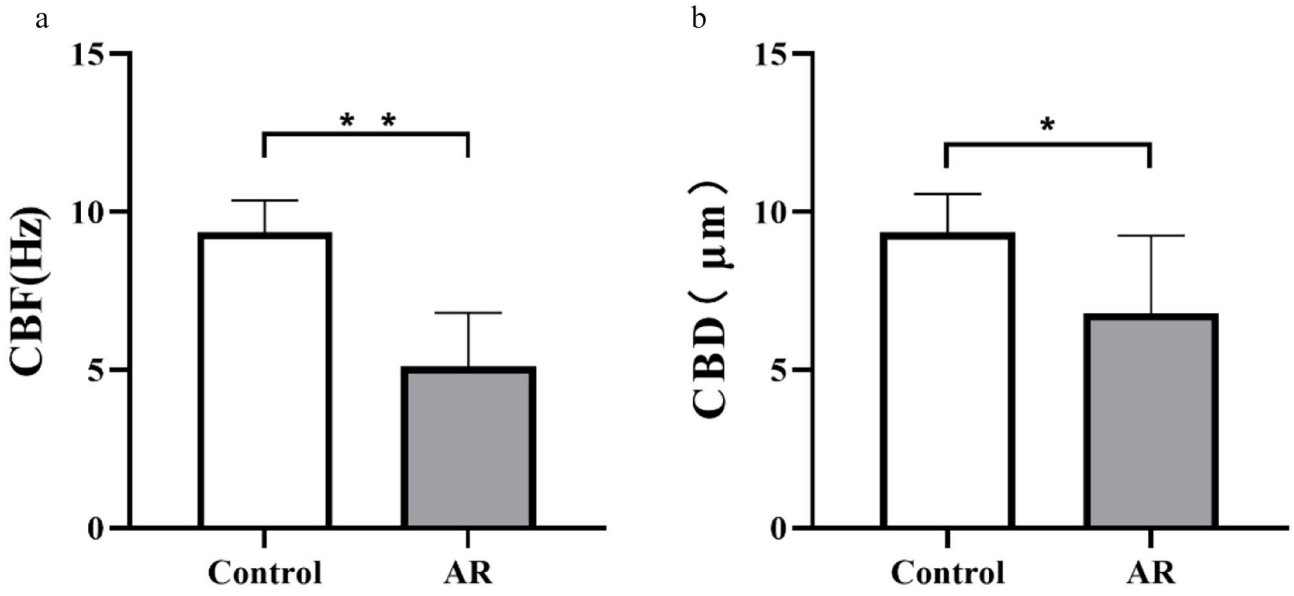

Figure 4. *In vivo* CBF (a) and CBD (b) were significantly decreased by OVA sensitization in rats ($n = 5$).
$^{**}p(\text{CBF}) = 0.0060$; $^{*}p(\text{CBD}) = 0.0268$.

lower concentrations (10^{-3} and 10^{-5} mol/L) of histamine had no obvious effect on CBFs of the mucosa specimen in both groups *in vitro* (Figure 7(c) to (f)).

Effects of histamine on *in vivo* ciliary motion

To study the effect of histamine on *in vivo* nasal ciliary motion in both the control group and AR group, we, respectively, administered histamine solution and PBS to the nasal mucosa of the two groups. The results showed that CBFs in both groups increased rapidly after exposure to 10^{-1} mol/L histamine (Figure 8(a) and (b)). The CBF in the control group rapidly increased within 2 min and gradually decreased to a level lower than the baseline 30 min later (Figure 8(a)). However, CBF in the AR group increased first and maintained a relatively high level under the effect of histamine (Figure 8(b)). The CBDs in both groups were inhibited by histamine (Figure 9(a) and (b)). Lower concentrations (10^{-3} and 10^{-5} mol/L) of histamine had no effect on CBFs (Figure 8(c) to (f)) and CBDs (Figure 9(c) to (f)).

These results revealed that histamine stimulates *in vivo* cilia in a short time and has potential ciliotoxicity in the long term. In addition, OVA sensitization attenuated the histamine-induced inhibition to ciliary motion.

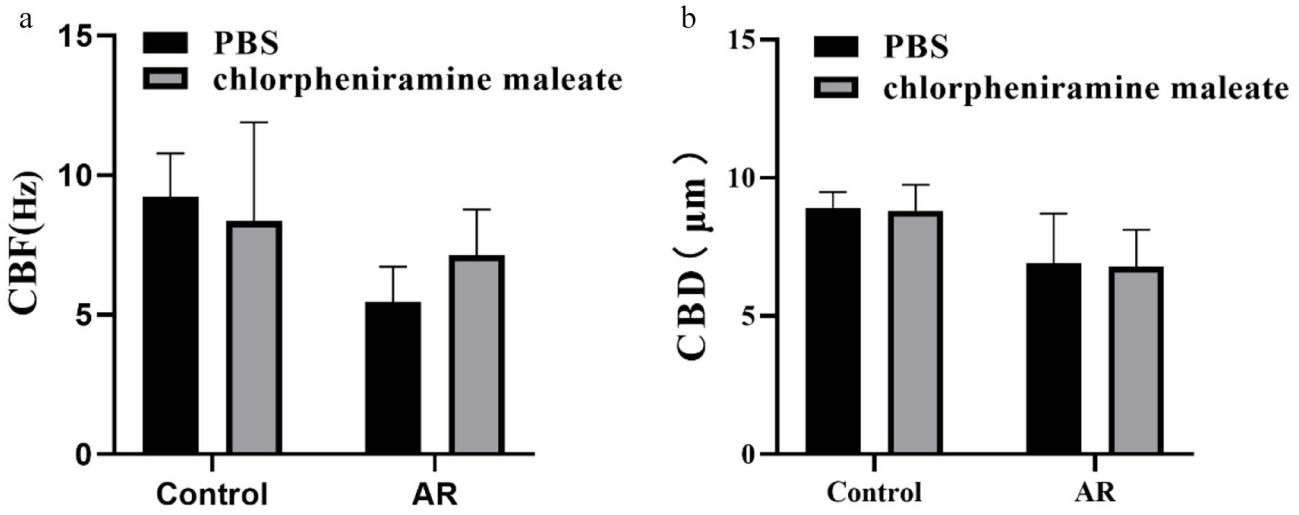

Figure 5. Changes of CBF (a) and CBD (b) after the administration of the chlorpheniramine maleate in the AR and control group ($n=5$). $^{**}p=0.0073$.

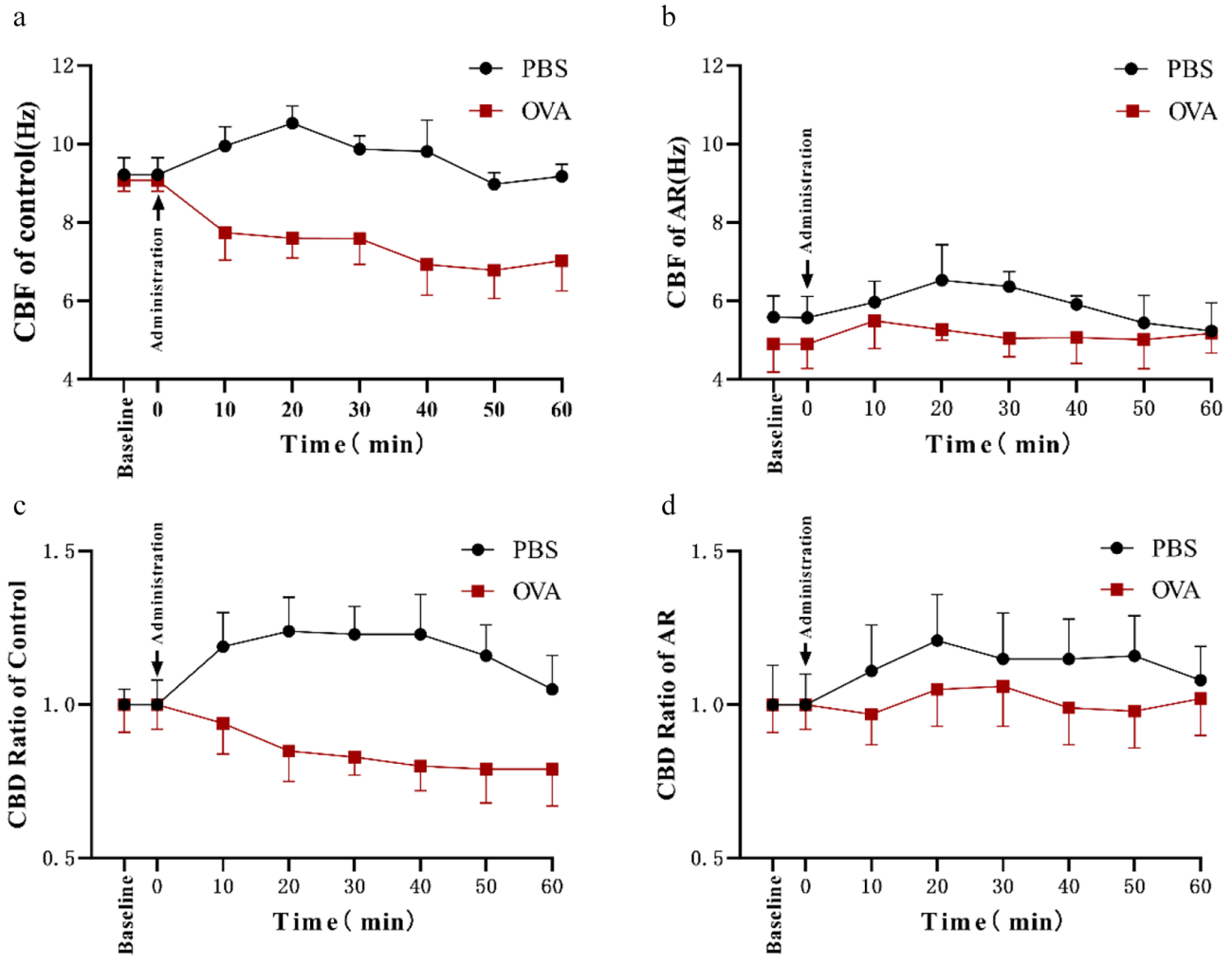

Figure 6. Effects of OVA challenge on ciliary motion in AR and control rats ($n=5$). Normal nasal mucosal CBF was significantly inhibited by OVA solution (a). Although the baseline of AR nasal mucosal CBF was relatively low, it showed a somewhat tolerance and could maintain at the baseline level (b). Changes of CBD responses in each group within 60 min after OVA stimulation: Normal CBD was inhibited by OVA solution (c), while AR CBD was also able to maintain at the baseline level (d). (A color version of this figure is available in the online journal.)

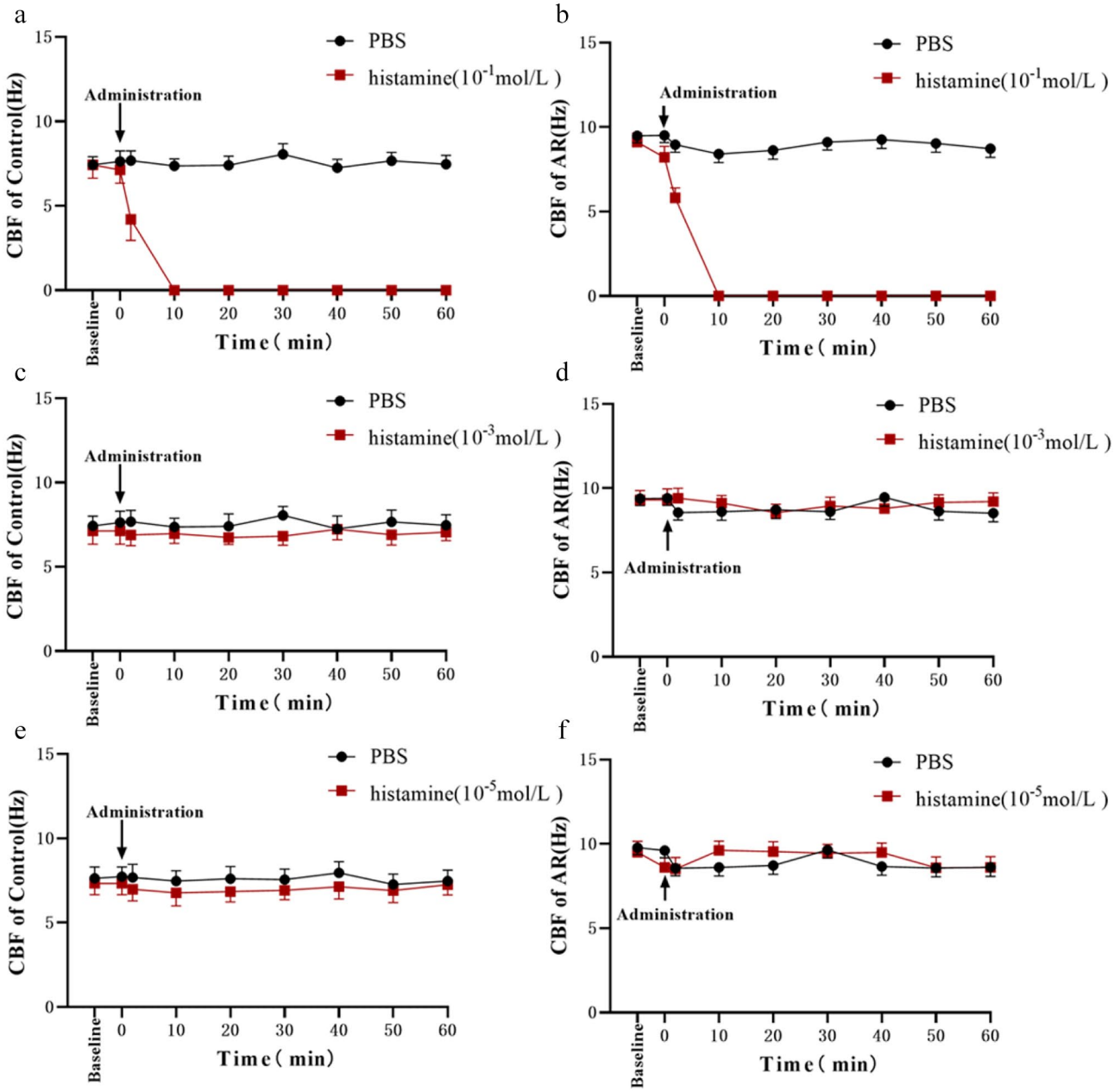

Figure 7. Effects of histamine on ciliary motion *in vitro* in 60 min. The red squares showed that normal CBF was inhibited as administration of 10^{-1} mol/L histamine and complete ciliostasis was observed within 10 min in both control and AR group ($n = 5$) (a and b). The lower concentrations 10^{-3} mol/L (c and d) and 10^{-5} mol/L (e and f) of histamine had no obvious effect on CBFs of the mucosa specimen in both groups *in vitro* PBS instead of histamine serves as control (black circle). (A color version of this figure is available in the online journal.)

To further verify the effect of histamine on nasal ciliary motion, we administered an H1 receptor antagonist (chlorpheniramine maleate) for 60 min and then administrated histamine and PBS to the nasal mucosa in both groups. In both groups, either stimulation or inhibition of histamine to the ciliary motion was absent (Figure 10(a) to (d)). The baseline of CBF in the AR group increased after the administration of chlorpheniramine maleate ($p < 0.05$) (Figure 10(b)). These results indicated that chlorpheniramine maleate could antagonize the changes of ciliary motion caused by histamine and restore the impaired ciliary motion caused by OVA sensitization.

Discussion

In this study, we explored the changes of ciliary motility in the OVA-sensitized rat model with a novel approach[9] *in vivo* for the first time. It is notable that the ciliary motion of the OVA-sensitized rats significantly attenuates *in vivo* when compared with the control. The effects of AR on ciliary motion in previous studies based on biopsy[19,20] or culture of ciliated cells *in vitro*[12,21,22] were controversial. Consistent with our study, Ohashi *et al.*[7] obtained ciliary cells from patients with AR for analysis *in vitro* and found that ciliary motility was impaired, CBF decreased, and got worse with the

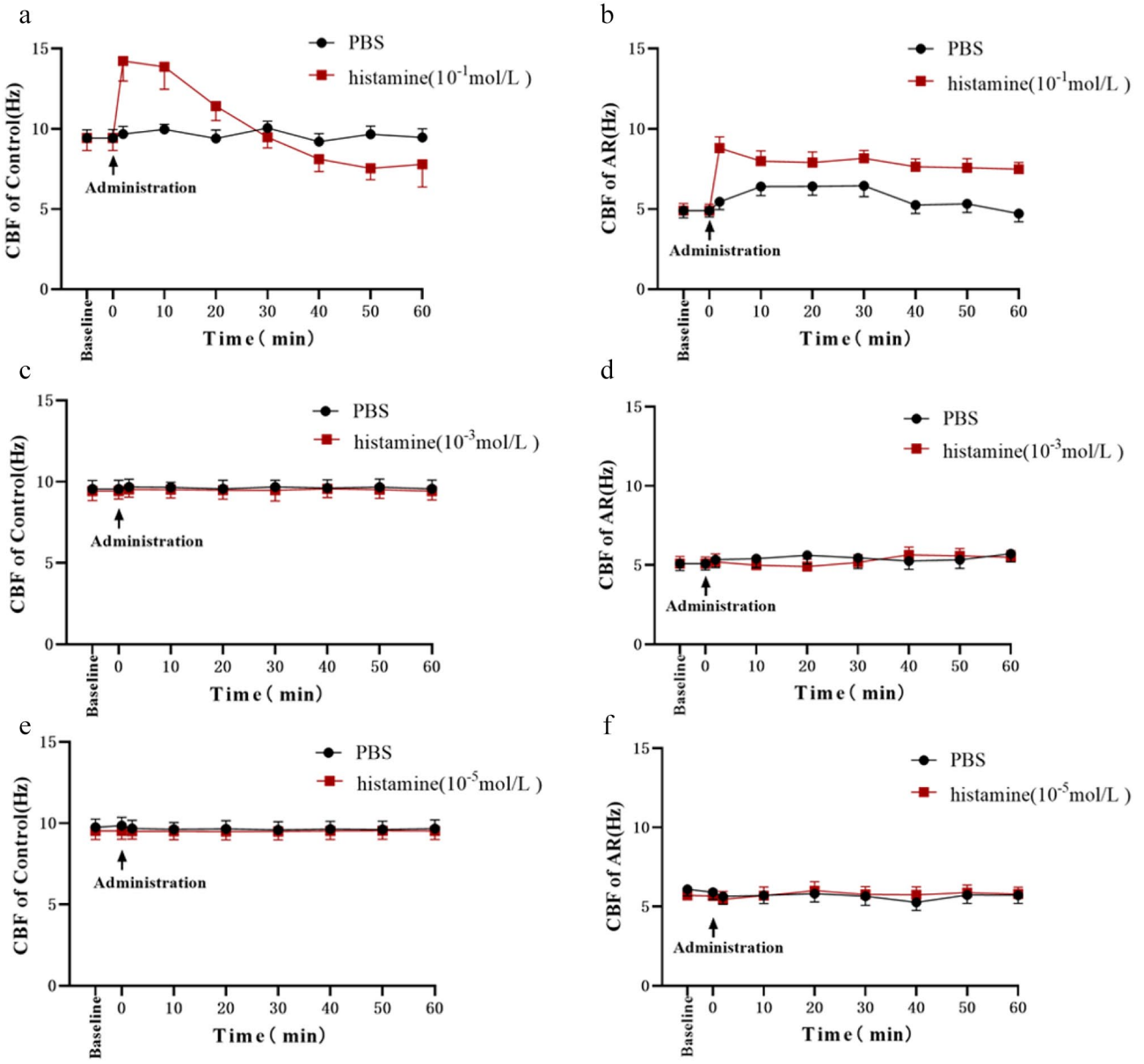

Figure 8. Effects of histamine on CBFs *in vivo* in 60 min ($n=5$). The red squares showed that normal CBF increased first and then inhibited when stimulated by the 10^{-1} mol/L concentration of histamine (a). *In vivo*, the cilium of AR has a certain tolerance to histamine inhibition and can maintain a high level of motion (b). PBS instead of histamine serves as control (black circle). The concentrations (10^{-3} and 10^{-5} mol/L) of histamine had no obvious effect on CBFs of the mucosal cilium in both groups *in vivo* (c to f). (A color version of this figure is available in the online journal.)

prolongation of the disease course. One possible reason is the destruction of the ciliary structure, including ciliary adhesion and loss, from the effect of AR.[23,24]

However, the results of some experiments[16] *in vitro* were just the opposite; CBF increased in the mouse model of AR, which indicated that other mechanisms might also control CBF in the phase of AR. A reason for that could be the *in vitro* state of ciliary motion is likely to change when the original mucus on the surface of the airway was replaced by buffer solution or culture solution. Compared with the experiments *in vitro*, the results of our study *in vivo* can better reflect the actual motion state of nasal mucosal cilium in the early phase of OVA sensitization. In addition, the ciliary motion recovered after administration of antihistamines (chlorpheniramine maleate), which indicated that the ciliotoxicity of the high concentration of histamine might play a key role in the ciliary impairment.[16]

Our study is the first to directly observe the changes of nasal ciliary motion under allergen challenge in living animals. We found that normal ciliary motion *in vivo* was significantly inhibited by OVA. This finding is quite different from the previous experiments *in vitro* which reported the stimulative effect of allergen challenge on the ciliary motion. These studies[16] adopted nasal allergen provocation *in vivo*, and ciliated cells were harvested and observed *in vitro*. This process most likely led to structural and functional alteration of cilia. Also, they were unable to measure immediate *in vivo* changes of ciliary motion when allergen provocation was performed. A possible reason why OVA solution inhibited *in vivo* ciliary motion is that the OVA solution itself has

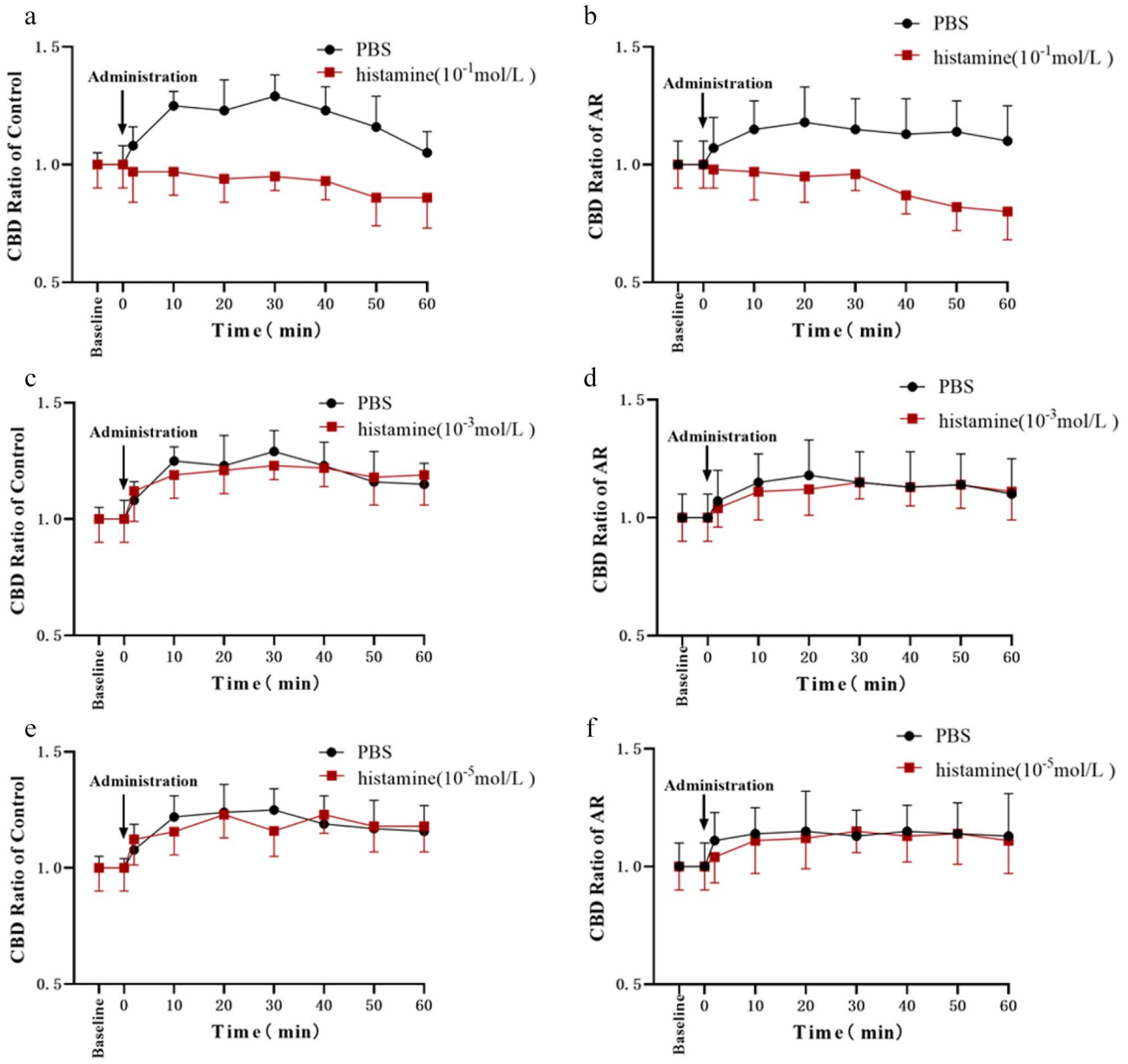

Figure 9. Effects of histamine on CBDs *in vivo* in 60 min. Histamine inhibited ciliary CBDs in both groups ($n=5$) within 60 min (red square) when stimulated by the 10^{-1} mol/L concentration of histamine (a and b). PBS instead of histamine serves as control (black circle). The concentrations (10^{-3} and 10^{-5} mol/L) of histamine had no obvious effect on CBDs of the mucosal cilium in both groups *in vivo* (c to f). (A color version of this figure is available in the online journal.)

high viscoelasticity, resulting in inhibition to CBF, as is well known that the higher the viscoelasticity of periciliary liquid (PCL), the lower the CBF.[25]

However, in OVA-sensitized rats, the ciliary motion was not suppressed and maintained a stable level under the OVA challenge. One hypothesis is that the nasal mucosa sensitized by OVA developed an allergic reaction to OVA, releasing inflammatory mediators (such as histamine, leukotriene, and prostaglandin E2) and neuropeptides (such as acetylcholine, substance P(SP)), which might promote ciliary motion.[18,26,27] Another possible reason is that ciliary motion had fallen to a "lower limit" that is difficult to decrease further. But its mechanism remains unclear and we will study further.

As one of the most important inflammatory mediators in the development of AR, histamine has been proved to decrease epithelial barrier integrity.[28] To verify the effect of histamine on nasal mucosal cilia *in vivo*, this study administrated a high concentration of histamine solution (10^{-1} mol/L) to the nasal mucosa. We found histamine stimulated CBF in a short time of 30 min while showed certain ciliotoxicity as time went on and gradually inhibited CBF. Lee *et al.*[16] previously reported that high concentration of histamine (10^{-1} mol/L) had significant inhibitory effects on CBF of normal and AR mice *in vitro*. The ciliary motion was gradually impaired and completely stopped within 15 min, but a low concentration of histamine ($<10^{-1}$ mol/L) did not affect CBF. The ciliotoxicity of high-concentration histamine found in this study is somewhat consistent with our research.

The short-term stimulative effect of histamine on cilia may be due to the increase of intracellular calcium ions caused

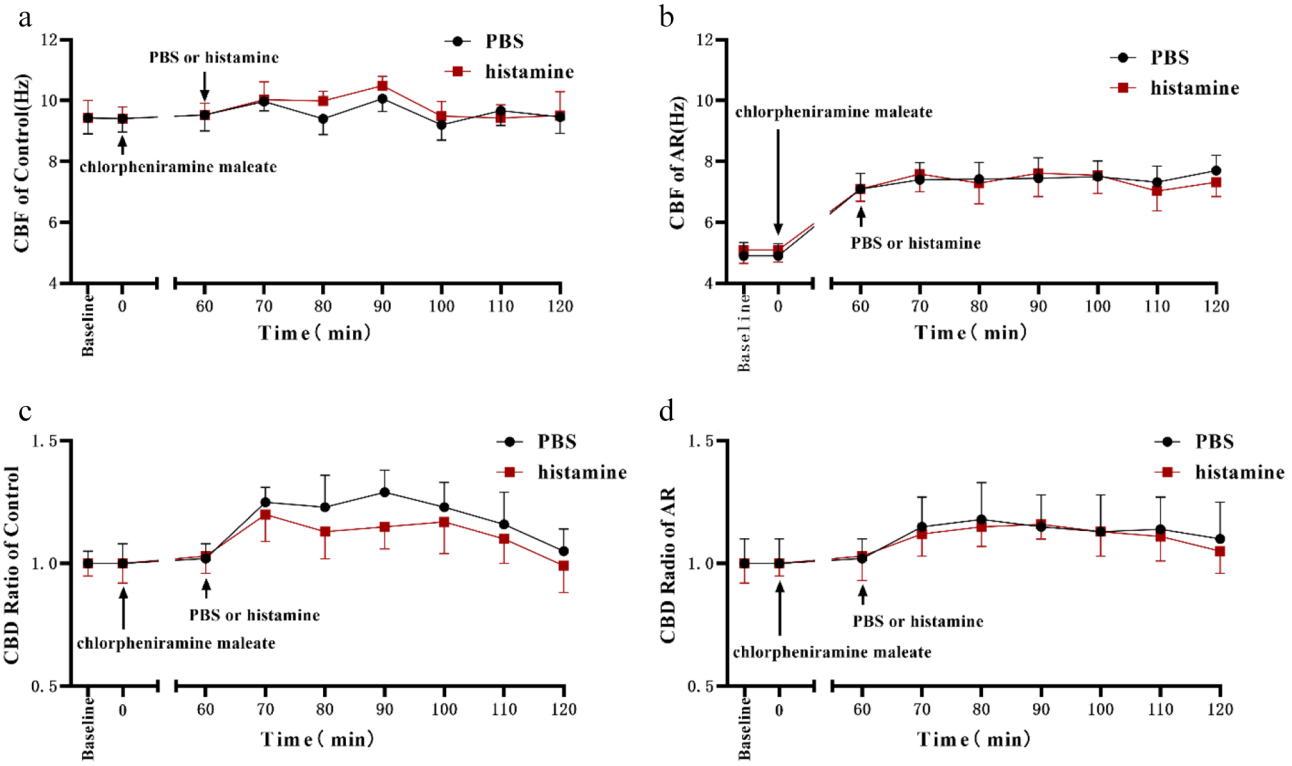

Figure 10. Effects of histamine on ciliary motion in control and AR group (n=5) after the administration of chlorpheniramine maleate for 60 min. Pre-application of antagonist chlorpheniramine maleate for 60 min antagonized the stimulation and inhibition of CBF by histamine (red square), which showed no difference compared with PBS control (black circle) (a) and increased basal CBF in AR group (b). The effect of histamine on CBD was not different from that of PBS (black circle) after 60 min pretreatment in both groups (c and d). (A color version of this figure is available in the online journal.)

by the binding of histamine and histamine receptors.[29] The increase of intracellular calcium ions will lead to the increase of CBF.[13] In the previous study,[13] the CBF changes were not recorded after histamine treatment 20 min later. Our study extended the visualization time to 60 min and found that the long-term (more than 30 min) effect of histamine would inhibit the ciliated epithelium. The potential mechanism is that the motion of cilia is dependent on the release of ATP,[30] and prolonged high-speed ciliary motion leads to excessive consumption of adenosine-triphosphate (ATP). There was a study[14] on ciliated cells *in vitro* found that CBF was stimulated by histamine and then gradually decreased back to the baseline in 20 min, and the administration of ATP could lead to a second spike of CBF, implying that excessive consumption of ATP may be one of the reasons for the decrease of CBF in the later period after administration of histamine.

This study first found that the *in vivo* CBF and CBD in OVA-sensitized rats could stay relatively high after histamine-mediated stimulation. The later inhibition effect did not appear within 60 min, a potential ciliary "tolerance" to the histamine inhibition effect, which has not been reported before. Based on the findings of an earlier study,[16] a high concentration of histamine had an apparent inhibitory effect on ciliary cells of both control and AR mice *in vitro*. It suggests that histamine tolerance of cilia in OVA-sensitized rats *in vivo* may not be related to the cells themselves but *in vivo* environments such as mucus, inflammatory mediators, or neuropeptides secreted from other cells in the nasal mucosa after histamine provocation, which may promote ciliary motion to maintain the original level of motive intensity.[18,26,27]

One limitation of this study is that the OVA and histamine experiments only lasted for 60 min. Although the duration of observation was longer than that in previous studies, it was significantly shorter than the actual clinical course in AR patients, which lasted for several years or even decades. Although the potential histamine tolerance of AR cilia was observed, under the continuous influence of long-term high histamine, ciliary damage appeared to be unavoidable. That was why the baseline of ciliary motion in OVA-sensitized rats found in this study is significantly lower than that in control. Extensive studies remain to be done to understand the questions in this regard further. In addition, limitations of the novel *in vivo* ciliary motion analysis method used in this study still exist and were mentioned at its first reporting.[12] Briefly, the exposure of mucosa somewhat influenced its original physiological environment, and the complex *in vivo* experimental condition is more difficult to control than *in vitro*.

Conclusions

In summary, an animal model of AR sensitized by OVA was established to investigate the changes of the ciliary motion *in vivo* and the effects of OVA and histamine on ciliary motion. First, our study clarified that AR reduced ciliary motion *in vivo*. Second, our data suggested that short-term histamine could induce the enhancement of ciliary motion *in vivo*, while OVA and prolonged histamine inhibit the ciliary motion. Ciliary motion tends to show tolerance to the inhibition of OVA and histamine in OVA-sensitized rats.

AUTHORS' CONTRIBUTIONS

All authors participated in the design, interpretation of the studies. CL and CP carried out the experiments, analyzed the data, and reviewed the manuscript; CL is the main author of this publication and CP is the equal contributor. NY, DSC, JW, WY contributed reagents/materials/analysis tools. LC is the corresponding author of this manuscript.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by Beijing Natural Science Foundation [7222185], the National Key Research and Development Project [2020YFC2005203] and the National Key Research and Development Project [2020YFC2005206].

ORCID IDS

Chen Liu https://orcid.org/0000-0001-5204-8143
Lei Chen https://orcid.org/0000-0001-9759-982X

SUPPLEMENTAL MATERIAL

Supplemental material for this article is available online.

REFERENCES

1. Drazdauskaite G, Layhadi JA, Shamji MH. Mechanisms of allergen immunotherapy in allergic rhinitis. *Curr Allergy Asthma Rep* 2020;**21**:2
2. Wang XD, Zheng M, Lou HF, Wang CS, Zhang Y, Bo MY, Ge SQ, Zhang N, Zhang L, Bachert C. An increased prevalence of self-reported allergic rhinitis in major Chinese cities from 2005 to 2011. *Allergy* 2016;**71**:1170–80
3. Wang XY, Ma TT, Wang XY, Zhuang Y, Wang XD, Ning HY, Shi HY, Yu RL, Yan D, Huang HD, Bai YF, Shan GL, Zhang B, Song QK, Zhang YF, Zhang TJ, Jia DZ, Liu XL, Kang ZX, Yan WJ, Yang BT, Bao XZ, Sun SH, Zhang FF, Yu WH, Bai CL, Wei T, Yang T, Ma TQ, Wu XB, Liu JG, Du H, Zhang L, Yan Y, Wang DY. Prevalence of pollen-induced allergic rhinitis with high pollen exposure in grasslands of northern China. *Allergy* 2018;**73**:1232–43
4. Do BH, Ohbuchi T, Wakasugi T, Koizumi H, Yokoyama M, Hohchi N, Suzuki H. Acetylcholine-induced ciliary beat of the human nasal mucosa is regulated by the pannexin-1 channel and purinergic P2X receptor. *Am J Rhinol Allergy* 2018;**32**:217–27
5. Mikolajczyk M, Janukowicz K, Majewska E, Baj Z. Impact of allergic rhinitis on nasal mucociliary clearance time in children. *Int Arch Allergy Immunol* 2019;**179**:297–303
6. Workman AD, Carey RM, Chen B, Saunders CJ, Marambaud P, Mitchell CH, Tordoff MG, Lee RJ, Cohen NA. CALHM1-mediated ATP release and ciliary beat frequency modulation in nasal epithelial cells. *Sci Rep* 2017;**7**:6687
7. Ohashi Y, Nakai Y, Kihara S, Ikeoka H, Takano H, Imoto T. Ciliary activity in patients with nasal allergies. *Arch Otorhinolaryngol* 1985;**242**:141–7
8. Kirtsreesakul V, Somjareonwattana P, Ruttanaphol S. The correlation between nasal symptom and mucociliary clearance in allergic rhinitis. *Laryngoscope* 2009;**119**:1458–62
9. Pang C, An F, Yang S, Yu N, Chen D, Chen L. In vivo and in vitro observation of nasal ciliary motion in a guinea pig model. *Exp Biol Med* 2020;**245**:1039–48
10. Wong LB, Park CL, Yeates DB. Neuropeptide Y inhibits ciliary beat frequency in human ciliated cells via nPKC, independently of PKA. *Am J Physiol* 1998;**275**:C440–8
11. Antunes MB, Cohen NA. Mucociliary clearance—a critical upper airway host defense mechanism and methods of assessment. *Curr Opin Allergy Clin Immunol* 2007;**7**:5–10
12. An F, Xing L, Zhang Z, Chen L. Effects of histamine on ciliary beat frequency of ciliated cells from guinea pigs nasal mucosa. *Eur Arch Otorhinolaryngol* 2015;**272**:2839–45
13. Winters SL, Davis CW, Boucher RC. Mechanosensitivity of mouse tracheal ciliary beat frequency: roles for Ca2+, purinergic signaling, tonicity, and viscosity. *Am J Physiol Lung Cell Mol Physiol* 2007;**292**:L614–24
14. Dolata J, Lindberg S, Mercke U. Histamine stimulation of mucociliary activity in the rabbit maxillary sinus. *Ann Otol Rhinol Laryngol* 1990;**99**:666–71
15. Cima F, Franchi N. Histamine stimulates ciliary beat frequency via the H2 receptor in the protochordate *Botryllus schlosseri*. *J Exp Zool B Mol Dev Evol* 2016;**326**:176–92
16. Lee MC, Kim DW, Kim DY, Rhee CS. The effect of histamine on ciliary beat frequency in the acute phase of allergic rhinitis. *Am J Otolaryngol* 2011;**32**:517–21
17. Schuil PJ, van Gelder JM, ten Berge M, Graamans K, Huizing EH. Histamine and leukotriene C4 effects on in vitro ciliary beat frequency of human upper respiratory cilia. *Eur Arch Otorhinolaryngol* 1994;**251**:325–8
18. Khan R, Dolata J, Lindberg S. Effects of inflammatory mediators on ciliary function in vitro. *Rhinology* 1995;**33**:22–5
19. Thomas B, Rutman A, O'Callaghan C. Disrupted ciliated epithelium shows slower ciliary beat frequency and increased dyskinesia. *Eur Respir J* 2009;**34**:401–4
20. Peng Y, Chen Z, Guan WJ, Zhu Z, Tan KS, Hong H, Zi X, Zeng J, Li Y, Ong YK, Thong M, Shi L, Yang Q, Qiu Q, Wang DY. Downregulation and aberrant localization of forkhead box J1 in allergic nasal mucosa. *Int Arch Allergy Immunol* 2018;**176**:115–23
21. Park DY, Kim S, Kim CH, Yoon JH, Kim HJ. Alternative method for primary nasal epithelial cell culture using intranasal brushing and feasibility for the study of epithelial functions in allergic rhinitis. *Allergy Asthma Immunol Res* 2016;**8**:69–78
22. Inui TA, Murakami K, Yasuda M, Hirano S, Ikeuchi Y, Kogiso H, Hosogi S, Inui T, Marunaka Y, Nakahari T. Ciliary beating amplitude controlled by intracellular Cl(-) and a high rate of CO2 production in ciliated human nasal epithelial cells. *Pflugers Arch* 2019;**471**:1127–42
23. Altuntaş E, Yener G, Doğan R, Aksoy F, Şerif Aydın M, Karataş E. Effects of a thermosensitive in situ gel containing mometasone furoate on a rat allergic rhinitis model. *Am J Rhinol Allergy* 2018;**32**:132–8
24. Peng Y, Guan WJ, Zhu ZC, Tan KS, Chen Z, Hong HY, Zi XX, Andiappan AK, Shi L, Yang QT, Wang DY, Qiu QH. Microarray assay reveals ciliary abnormalities of the allergic nasal mucosa. *Am J Rhinol Allergy* 2020;**34**:50–8
25. Kuyper LM, Pare PD, Hogg JC, Lambert RK, Ionescu D, Woods R, Bai TR. Characterization of airway plugging in fatal asthma. *Am J Med* 2003;**115**:6–11
26. Schuil PJ, Ten Berge M, Van Gelder JM, Graamans K, Huizing EH. Effects of prostaglandins D2 and E2 on ciliary beat frequency of human upper respiratory cilia in vitro. *Acta Otolaryngol* 1995;**115**:438–42
27. Inoue D, Furubayashi T, Ogawara K, Kimura T, Higaki K, Shingaki T, Kimura S, Tanaka A, Katsumi H, Sakane T, Yamamoto A, Higashi Y. In vitro evaluation of the ciliary beat frequency of the rat nasal epithelium using a high-speed digital imaging system. *Biol Pharm Bull* 2013;**36**:966–73
28. Steelant B, Seys SF, Van Gerven L, Van Woensel M, Farre R, Wawrzyniak P, Kortekaas Krohn I, Bullens DM, Talavera K, Raap U, Boon L, Akdis CA, Boeckxstaens G, Ceuppens JL, Hellings PW. Histamine and T helper cytokine-driven epithelial barrier dysfunction in allergic rhinitis. *J Allergy Clin Immunol* 2018;**141**:951–63.e8
29. Hill SJ, Ganellin CR, Timmerman H, Schwartz JC, Shankley NP, Young JM, Schunack W, Levi R, Haas HL. International Union of Pharmacology. XIII. Classification of histamine receptors. *Pharmacol Rev* 1997;**49**:253–78
30. Droguett K, Rios M, Carreno DV, Navarrete C, Fuentes C, Villalon M, Barrera NP. An autocrine ATP release mechanism regulates basal ciliary activity in airway epithelium. *J Physiol* 2017;**595**:4755–67

(Received October 19, 2021, Accepted March 1, 2022)