Original Article

Effects of a Thermosensitive In Situ Gel Containing Mometasone Furoate on a Rat Allergic Rhinitis Model

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Abstract

Background: Mometasone furoate, one of the second generation intranasal corticosteroids, is currently used in suspension form due to its poor solubility. However, this is not favorable for nasal application because of the rapid elimination of the instilled drug from the nasal cavity by mucociliary clearance and delayed onset of action due to the slow dissolution of drug in suspension.

Objective: The aim of this study was to determine the antiallergic effects of mucoadhesive thermosensitive in situ gel containing mometasone furoate that we developed previously to prolong the contact between the drug and nasal mucosa and to prevent drainage of the formulation in an ovalbumin-induced rat model of allergic rhinitis.

Methods: An experimental allergic rhinitis model was developed in female Wistar albino rats by intraperitoneal injection of ovalbumin every 2 days for 14 days followed by its repeated intranasal instillation for 7 consecutive days. Intranasal instillation of ovalbumin was continued every other day for 14 days. Mometasone furoate in situ gel (5 μg/10 μl), mometasone furoate suspension (5 μg/10 μl), and physiological saline (10 μl) were administered into the bilateral nasal cavities from day 22 to day 35. Antiallergic effects were evaluated through histopathological evaluation, analysis of ovalbumin-specific serum immunoglobulin E, and a symptom score.

Results: Mometasone furoate in situ gel significantly decreased the nasal symptoms and ovalbumin-specific serum immunoglobulin E level as compared with mometasone furoate suspension and physiological saline. Additionally, inflammatory histological symptoms such as mucosal edema, vascular dilatation, eosinophil infiltration, and loss of cilia within the nasal mucosa of allergic rhinitis model rats were remarkably improved with the treatment of mometasone furoate in situ gel.

Conclusion: These results suggest that mometasone furoate in situ gel has a better therapeutic potential for the treatment of allergic rhinitis compared to mometasone furoate suspension.

Keywords

thermosensitive, in situ gel, mucoadhesive, allergic rhinitis, mometasone furoate, intranasal corticosteroids, suspension, rat model, ovalbumin, immunoglobulin, histopathological evaluation

Introduction

Allergic rhinitis (AR) is the most common immunoglobulin (Ig)E-mediated inflammatory disease seen in children, adults, and adolescents.[1,2] Intranasal corticosteroids (INCs) are considered the most effective and first choice option for the treatment of AR so far.[1] Mometasone furoate (MF) is one of a new generation of INCs which is topically effective with high affinity to glucocorticoid receptors and low systemic absorption.[1,3] Nasal sprays containing MF are presented as suspension in the market because of the poor water solubility. Due to the slow dissolution of

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MF in the suspension, the onset of action is delayed and the mucociliary clearance causes rapid elimination of MF from the nasal cavity.[1,4,5]

Thermosensitive hydrogels gelate by temperature change which are liquid at room temperature and undergo gelation when in contact with body fluids.[1,6] Recently, a thermosensitive mucoadhesive MF in situ nasal gel using 18% Pluronic® F-127 and 0.25% Carbopol® 974P NF (Tsol-gel: 30.1 ± 0.24°C) has been developed by our research group to extend the contact time between the drug and nasal mucosa and to prevent the formulation drainage by mucociliary clearance.[1]

In the present study, it was purposed to determine the potential antiallergic activity of repeated topical application of the in situ gel that was developed in our previous study in ovalbumin (OVA)-induced rat model through evaluating the histopathology of the nasal mucosa, analysis of OVA-specific serum IgE, and subjectively scoring the symptoms.

Materials and Methods

Animals

The study was conducted with 40 healthy female Wistar albino rats weighing 250–300 g at the Bezmialem Foundation University Experimental Application and Research Center after obtaining the approval of the Local Ethics Committee of Bezmialem Foundation University on Animal Studies (approval number: 2014-134). All rats were kept in cages ($n = 8$, each cage) under 12 h dark–light cycle in a temperature- and humidity-controlled room (22 ± 2°C, 55 ± 10% relative humidity). A standard commercial pellet diet and water were given to all animals ad libitum.

Experimental Design

Forty female Wistar albino rats were divided randomly into five groups ($n = 8$):

Group 1: Intranasal MF in situ gel (5 μg/10 μl). These rats had AR and MF in situ gel was administered for 14 days.

Group 2: Intranasal MF suspension (5 μg/10 μl). These rats had AR and MF suspension was administered for 14 days.

Group 3: Placebo group; Physiological saline (10 μl). These rats had AR and physiological saline was administered for 14 days.

Group 4: Control group. These rats were healthy and no treatment was administered.

Group 5: AR model group. These rats had AR but no treatment was administered.

Methods

Sensitization Protocol for Developing an AR Rat Model. Method described by Wen et al.[7] was modified to perform this study. In the first phase, the rats in Groups 1, 2, 3, and 5 were sensitized with OVA (0.3 mg intraperitoneal (i.p.), Grade V, Sigma Chemical, St. Louis, MO), applied with aluminum hydroxide (30 mg) in saline (1 ml i.p.) on alternate days for 14 days; 1 ml saline plus 30 mg aluminum hydroxide was administered to the rats in Group 4 (Control) intraperitoneally during the same days. In the second phase, 10 μL of 10% OVA was administered every day to both nostrils from day 15 to day 21. In the third phase, antigen challenges with 10 μL of 10% OVA were performed in all groups except the control group on alternate days from day 22 to 35 in order to maintain allergy symptoms (Figure 1).

Administration of Test Drugs. The test drugs were administered to the rats every day from day 22 to day 35 during inspiration at the same volume (10 μl) into each nostrils of the rats by micropipette 1 h before the nasal antigen challenge.[8] The composition of the test formulations is shown in Table 1.

Assessment of Nasal Symptoms in Sensitized Rats. Assessment of symptoms of AR was performed immediately after intranasal OVA administration on days 1, 14, 21, 28, and 35. After a 10-min acclimatization period, nasal symptoms such as sneezing and rubbing were

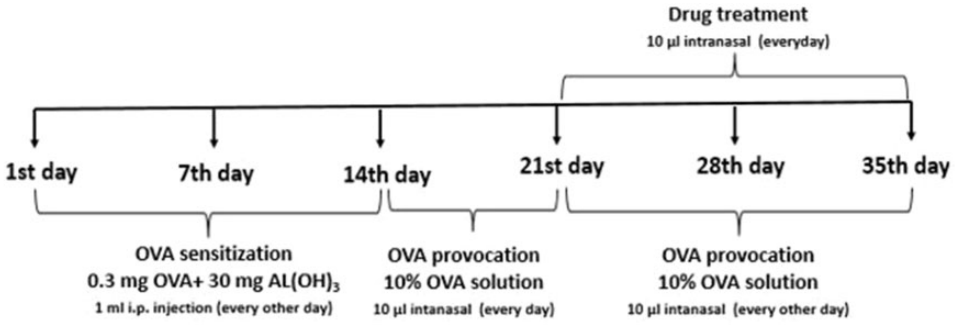

Figure 1. A schematic representation of AR rat model induced by OVA and treated with test drugs.
OVA: ovalbumin.

counted for 10 min by an observer blinded to experimental groups by placing each rat in an observation cage.[9]

Histopathological Assessment. Rats were sacrificed by an overdose (100 mg/kg i.p., Sigma Chemical, St. Louis, MO) of sodium pentobarbital at 24 h after the last intranasal challenge. Nasal tissues of each rat were removed and fixed in 10% neutral buffered formaldehyde (pH 7.2) for 72 h. The tissues were embedded in paraffin blocks for histological evaluation. Tissue sections (5 μm in thickness) were taken from paraffin blocks and they were left in xylene to deparaffinize.

Thereafter, samples were dehydrated by rinsing with 70%, 80%, 90%, 96%, and 100% ethanol, respectively. Tissue sections were stained with hematoxylin-eosin. The stained samples within the specified reference area were evaluated with a light microscope (Nikon Eclipse i5, Tokyo, Japan) and a video camera (Nikon, DS-Fi1c) attached to a light microscope.[10]

Table 1. Composition of the Test Formulations.

	Formulations	
Conc (w/v)	MF in situ gel	MF suspension
MF	0.05	0.05
Pluronic® F-127	18	–
Carbopol® 974P NF	0.25	–
Methylcellulose	–	1
Tween 80	–	0.25
Polyethylene glycol 400	5	5
Dexpanthenol	0.2	0.2
Sodium chloride	1	1
Benzalkonium chloride	0.02	0.02
Triethanolamine	q.s.	q.s.
Distilled water	q.s.	q.s.

MF: mometasone furoate.

Measurement of Ovalbumin-Specific IgE. Twenty-four hours after the last intranasal challenge, blood was withdrawn and centrifuged to isolate serum, which was then stored at −80°C until further analysis. OVA-specific serum IgE levels were determined with an enzyme-linked immunosorbent assay using a commercially available rat IgE enzyme-linked immunosorbent assay kit (Zymed laboratories Inc., South San Francisco, CA) according to the manufacturer's instructions.[10]

Statistical Analysis. All statistical analyses were performed using GraphPad Prim 5.0. Values were expressed as the mean ± standard deviation (SD). One-way analysis of variance was used to analyze differences among groups. The Dunnett's Multiple Comparison test was used for analyze the difference between variables. Differences between means were considered significant at $P < 0.05$.

Results

Changes in Allergic Rhinitis Symptoms

After 14 days of OVA injection, typical AR symptoms were significantly increased in all groups as compared with the control group ($P < 0.001$) (Figures 2 and 3). A significant reduction in the number of sneezing and nasal rubbing was achieved with MF in situ gel and MF suspension treatment, while a significant reduction in the number of nasal rubbing was achieved with physiological saline from day 22 to 35 as compared with the AR model group ($P < 0.001$). MF in situ gel was statistically more potent than MF suspension in the inhibition of AR symptoms during the period of treatment ($P < 0.001$).

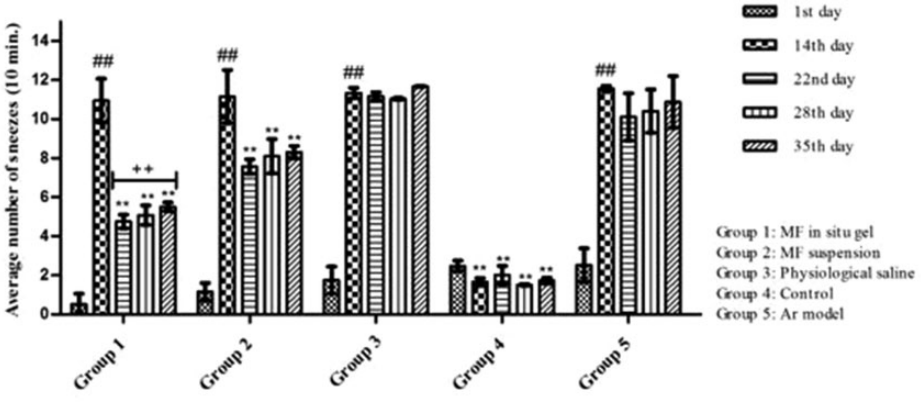

Figure 2. The number of sneezes of all groups. Values expresses the mean ± SD. ##There was a significant difference from the control group ($P < 0.001$). **There was a significant difference from the AR model group ($P < 0.001$). ++There was a significant decrease in the number of sneezes in the MF in situ gel group on the 22nd, 28th, and 35th days when compared to the MF suspension and the physiological saline group ($P < 0.001$).
AR: allergic rhinitis; MF: mometasone furoate.

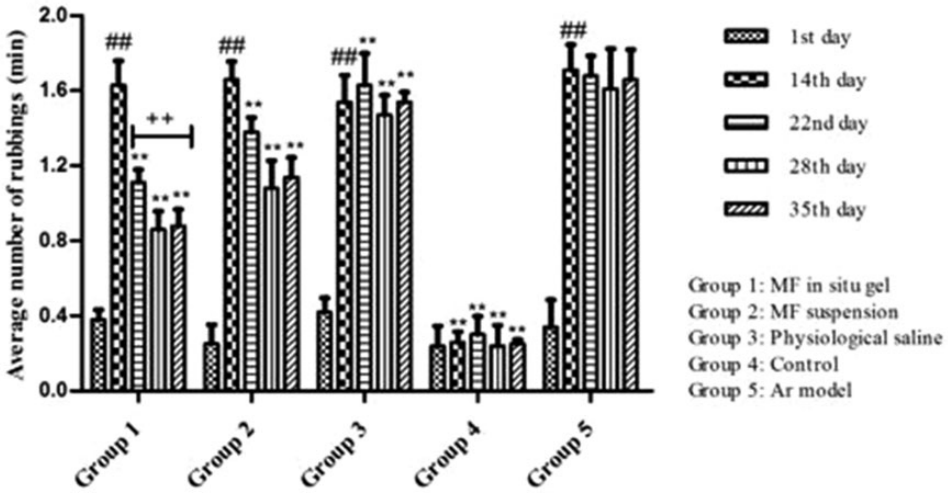

Figure 3. The number of nasal rubbing of all groups. Values expresses the mean ± SD. ##There was a significant difference from the control group ($P < 0.001$). **There was a significant difference from the AR model group ($P < 0.001$). ++There was a significant decrease in the number of rubbing in the MF in situ gel group on the 22nd, 28th, and 35th days when compared to the MF suspension and the physiological saline group ($P < 0.001$).
AR: allergic rhinitis; MF: mometasone furoate.

There was no significant decrease in the number of sneezing and nasal rubbing of the control group within 22–35 days. This indicated that the original levels for AR symptoms were still maintained to assess the efficacy of the test drugs ($P > 0.05$).

Histopathologic Changes

Photomicrographs of sample sections taken from nasal cavities are presented in Figure 4. It was seen that the pseudostratified columnar epithelium structure of the rats in Group 4 (control) was normal. The kinociliums located on the surface of the epithelium were regular. Mucosal edema or eosinophil infiltration was not detected. No dilatation was detected in the secretory ducts of lamina propria (Figure 4(a)). In Group 5 (AR model), vacuolization and dilatation in the pseudostratified columnar epithelium were determined. Mucosal edema, glandular hyperplasia, vascular dilatation, and eosinophil infiltration were detected in the lamina propria (Figure 4(b)). In Group 3 (physiological saline), dilatation in the pseudostratified columnar epithelium was determined. Vascular dilatation and vacuolization in the secretory ducts were detected in the lamina propria (Figure 4(c)). In Group 2 (MF suspension), dilatation in the pseudostratified columnar epithelium, vascular dilatation, hemorrhage, and vacuolization in the secretory ducts in the lamina propria were observed (Figure 4(d)). In Group 1 (MF in situ gel), the pseudostratified columnar epithelium structure of the rats was normal. No mucosal edema, vascular dilatation, eosinophil infiltration, or vacuolization in the secretory ducts in the lamina propria were observed (Figure 4(e)).

OVA-specific IgE Levels

The average OVA-specific serum IgE levels of rats from Groups 1–5 were presented in Figure 5. In the AR model group (155.502 ± 18.817 ng/ml), OVA-specific serum IgE level was found to be significantly higher than the control group (55.898 ± 11.194 ng/ml) ($P < 0.001$). The serum IgE levels in the MF in situ gel group (79.069 ± 31.141 ng/ml) and the physiological saline group (136.252 ± 34.516 ng/ml) both decreased significantly compared with the AR model group ($P < 0.001$).

Discussion

AR is a widespread chronic inflammatory disease of the upper respiratory tract caused by multiple environmental factors.[11–13] MF is a topically active, highly potent synthetic INCs commercially available in the form of aqueous suspension.[14,15] Because of its high affinity to the glucocorticoid receptor and its highly lipophilic nature, it shows minimal systemic absorption (<50 pg/ml) following oral and intranasal administration.[16,17]

In our previous study, we developed a thermoreversible mucoadhesive in situ nasal gel containing MF in order to extend the contact time of the drug with nasal mucosa, thereby preventing rapid elimination of MF.[1] In the present study, we aimed to clarify the effect of this new formulation in an OVA-induced rat model of AR.

The OVA antigen has been used extensively to develop an animal model to assess the efficacy of antiallergic

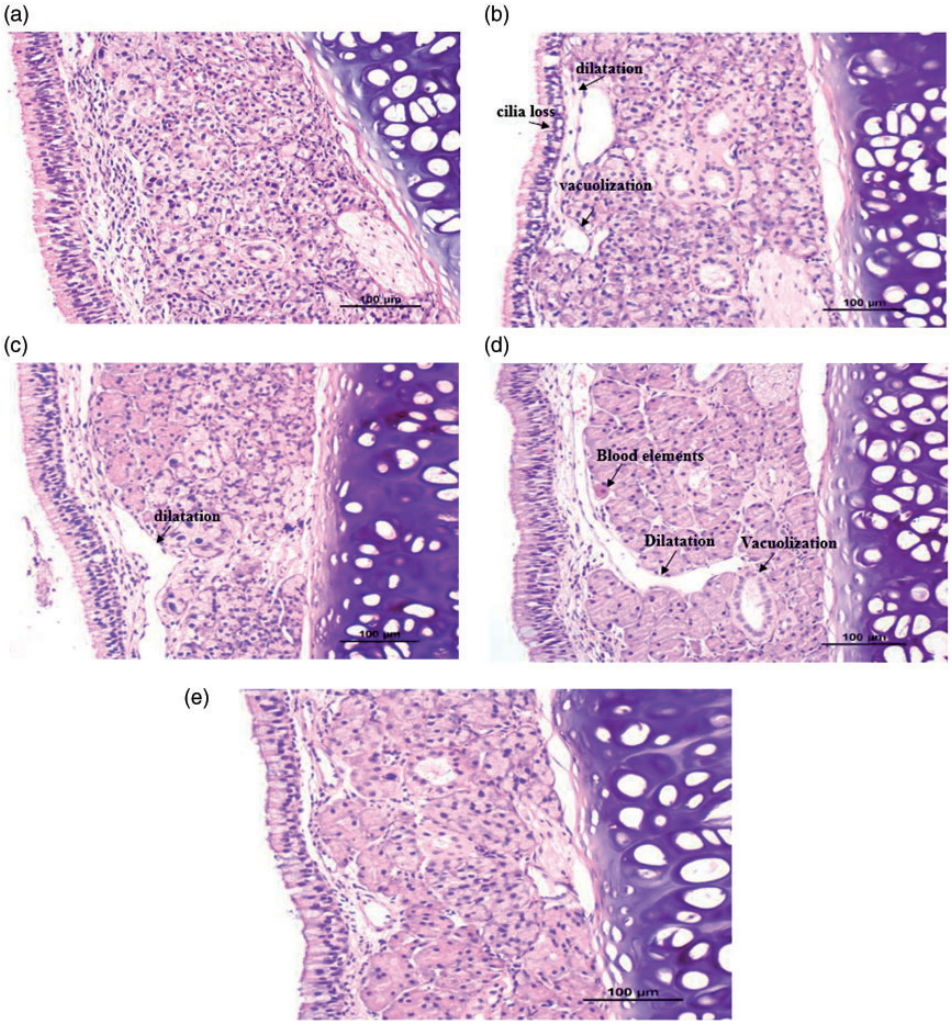

Figure 4. Photomicrographs of sample sections taken from nasal cavities following 35-day exposure (a) Control; (b) AR model; (c) Physiological saline; (d) MF suspension; (e) MF in situ gel (Hematoxylin & Eosin, 200×, bar: 100 μm).

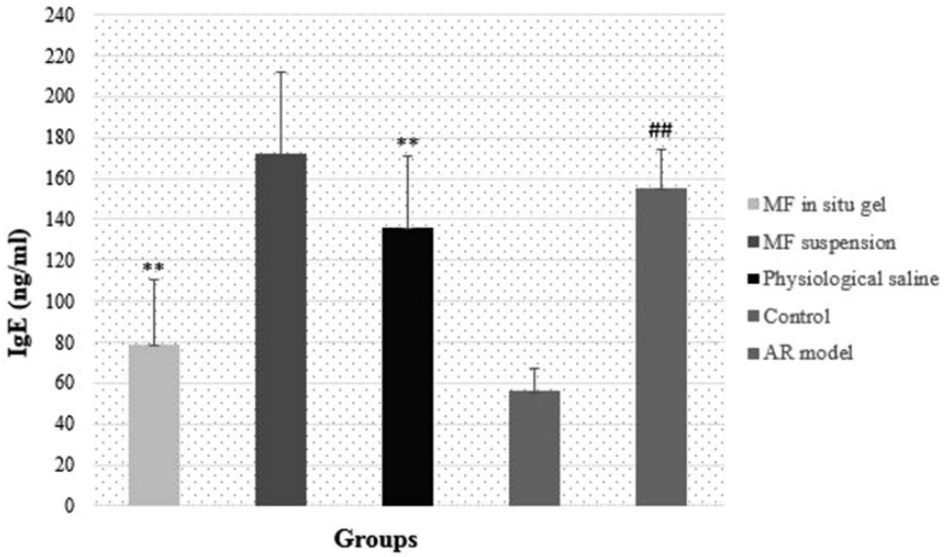

Figure 5. The average OVA-specific serum IgE levels in the blood of rats. Values expresses the mean ± SD. ##There was a significant difference from the control group ($P < 0.001$). **There was a significant difference from the AR model group ($P < 0.001$).
AR: allergic rhinitis; MF: mometasone furoate.

drugs as it induces similar nasal allergic symptoms to human AR.[18–22] In our study, antigen-induced nasal symptoms were remarkably increased in the AR model group by OVA administration (Figures 2 and 3) being in accordance with the previous reports.[18,23–25] Therefore, it was considered that the AR model was successfully developed in this study.

In a study conducted by Sugimoto et al.,[26] the topical application of MF dose-dependently inhibited the increase of nasal symptoms in rats. Furthermore, MF was found more potent than that of fluticasone propionate in inhibiting nasal symptoms. In our study, test drugs were applied intranasally in a volume of 10 µl which is the maximum convenient volume for administration to rats via nasal route.[8] After the treatment with MF in situ gel, the symptoms of AR were significantly decreased compared with the AR model group ($P < 0.001$). Furthermore, although the dose of MF in situ gel and MF suspension was the same, it was demonstrated that MF in situ gel was obviously more potent than MF suspension in the inhibition of nasal symptoms ($P < 0.001$). This can be attributed to the fact that in situ gel with mucoadhesive properties extend the contact time between drug and nasal mucosa compared to the suspension form and thus prevents rapid elimination of the drug due to mucociliary clearance.[27]

Increasing levels of IgE in response to environmental allergens are an important finding in investigating the presence of allergic diseases.[23] Yman[28] reported that analysis of the presence of IgE antibodies as a specific method can be used for the diagnosis of allergic diseases. Intranasal OVA sensitization and provocation in animal models causes OVA-specific IgE increase in the plasma and infiltration of inflammatory cells in the nasal mucosa.[29] In our study, in the AR model group, OVA-specific serum IgE level was found to be significantly higher than the control group ($P < 0.001$) (Figure 5). This finding suggests that the methodology we designed in our study was successful in creating a chronic AR model in rats. OVA-specific serum IgE level was significantly reduced by the application of the MF in situ gel repeatedly as compared with the MF suspension and the physiological saline ($P < 0.001$). These results indicated that MF in situ gel has a suppression effect on antibody production. Similar results were obtained in a study conducted by Tsumuro et al.[8] In this study, the IgE antibody titers in the MF-treated group were found to be significantly lower when compared to the control group. This result was attributed to its ability to immunosuppressive effect and direct allergic reaction inhibitory effect.[8]

Infiltration of the nasal mucosa with eosinophils and other inflammatory cells and pathological changes are characteristic features of an allergic reaction due to allergen–organism interaction.[7,30–32] Histopathological examination is an objective analysis that can be used to determine anti-inflammatory activity of drugs.[31] In our study, histopathological findings of AR such as increased inflammation, mucosal edema, glandular hyperplasia, vascular dilatation, and eosinophil infiltration in the lamina propria and loss of cilia, vacuolization, and dilatation in the pseudostratified columnar epithelium on the surface of the nasal mucosa were detected in the AR model group (Figure 4(b)). It was detected that there was a remarkable decrease in histopathological changes in rats receiving MF in situ gel compared with the AR model group in our study (Figure 4(e)). In addition, none of local side effects such as irritation, epithelial necrosis, or hemorrhage were determined in any of the rats, suggesting that 2-week repeated application of the developed in situ gel maintained nasal mucosal integrity. Significant difference was not found in histopathological findings in rats receiving physiological saline and MF suspension compared to the AR model group. Furthermore, hemorrhage which may occur due to 2-week repeated application of corticosteroids was detected in the lamina propria in rats treated with MF suspension.

Conclusion

In conclusion, our study demonstrated that MF in situ gel exhibits a better antiallergic effect by alleviating the symptoms of AR, decreasing the OVA-specific serum IgE levels and improving the pathophysiological findings of AR as compared with MF suspension in the OVA induced AR model. These results suggest that intranasal administration of MF in situ gel can offer safety and efficacy advantage in long-term usage over intranasal MF suspension and can be further developed for the AR therapy.

Authors' Note

This work was presented as a poster at the *22nd Biomedical Science and Technology Symposium*, Ankara, Turkey, 12–14 May 2017.

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.

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