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Inhibition of ovalbumin-induced allergic rhinitis by sumatriptan through the nitric oxide pathway in mice

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ABSTRACT

Aims: Allergic rhinitis is a global cause of disability, characterized by airway inflammation. Sumatriptan is a 5-hydroxytryptamine 1B/1D (5HT1B/1D) agonist used as a treatment for migraine headaches. Activation of 5HT1B/1D receptors can inhibit the release of neuropeptides and inhibit the inflammation cascades. This study investigated the effect of sumatriptan on ovalbumin-induced allergic rhinitis model in mice and the role of nitric oxide.

Methods: Female Balb/c mice were sensitized by intraperitoneal ovalbumin and challenged by intranasal ovalbumin. Mice received sumatriptan in doses 3, 10, 30 μg/kg intraperitoneally, 30 min before the last ovalbumin challenge.

Key findings: Intraperitoneal injection of sumatriptan significantly decreased the nasal scratching, IL-4 and serum IgE levels of allergic mice, but it increased IFNγ levels. Histopathological analysis showed that the number of eosinophils was significantly elevated in nasal mucosa of ovalbumin-induced allergic mice, while sumatriptan treatment significantly reduced the number of eosinophils. GR-127935, a selective 5-HT1B/1D-receptor antagonist, reversed the anti-allergic effects of sumatriptan. Acute administration of L-NAME, a non-specific inhibitor of nitric oxide synthase, along with sumatriptan attenuated the anti-allergic effects of sumatriptan but chronic administration of L-NAME did not affect the influences of sumatriptan. Furthermore, sumatriptan decreased the inducible nitric oxide synthase (iNOS) protein expression in allergic mice, but it did not change the concentration of eNOS protein.

Significance: This study shows that sumatriptan administration is associated with anti-allergic effects which are through 5HT1B/1D receptors. Decrease in iNOS expression and changes in T-helper 1&2 cytokines levels may indicate the involvement of inducible NOS and inflammation.

1. Introduction

Allergic rhinitis is a chronic condition that affects the upper airway. About 500 million people in the world suffer from this disorder [1] which leads to decreased quality of life in both adults and children not only in physical aspects but also in mental function, besides it increases societal expenses [2,3]. The hallmark of allergic reactions is determined by antigen-specific immunoglobulin E (IgE) production; T helper-1& 2 cells, eosinophils, mast cells increase; and allergic mediators release [4]. After an increase in IgE production and its binding to receptors on mast cells, eosinophils, and basophils; the release of inflammatory mediators occurs. These events are associated with vasodilation, increased vascular permeability, and acute functional changes in affected organs as airway mucus secretion [5]. The treatment of this disease now includes avoiding allergens and medications including corticosteroids, antihistamines, anticholinergics, decongestants, leukotriene receptor antagonists, mast cell stabilizers and immunotherapy [6] which most of them cause various side effects [7–10].

Sumatriptan is a 5-hydroxytryptamine 1B/1D (5HT1B/1D) receptors agonist which has been used as a treatment for migraine headaches to control the pain and associated symptoms [11]. 5HT1 receptors are a subfamily of serotonin receptors that couple to Gαi (2) and decrease intracellular cyclic adenosine monophosphate (cAMP) due to inhibition of adenylyl cyclase [12]. Sumatriptan mechanism of action

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can be described as the following: it causes vasoconstriction at cerebral arteries via 5-HT1B receptors leading to decreased blood flow [13]. It also inhibits pro-inflammatory neuropeptides release in trigeminal fibers via 5-HT1D receptors, prevents neurologic inflammation by blocking plasma protein leakage, and decreases plasma calcitonin gene-related peptide (CGRP) levels [14,15]. In previous studies, 5HT1B receptors are detected around blood vessels in human nasal mucosa [16]. A previous experiment demonstrated that intranasal serotonin has caused sneezing and nasal discharge in normal people [17]. Also, high levels of serotonin is detected in the nasal mucosa and nasal discharge of allergic patients [18,19]. Previous studies documented that 5HT1B/1D receptors activities were along with other receptors and transmitters, as it was shown that nitric oxide (NO) is involved in various effects of sumatriptan in the experimental models [20].

Nitric oxide (NO) is a signaling molecule, generating from arginine with roles in many physiological functions [21].It has been detected in animal and human exhaled air [22]. Nasal NO concentration is elevated in allergic rhinitis patients [23]. In addition, the interaction of NO and sumatriptan has been revealed in some studies including chloroquine-induced scratching, morphine-induced antinociceptive tolerance and pentylenetetrazole-induced seizures models [45–47].

Therefore, the aim of this experiment is to assess the possible role of sumatriptan, a 5HT1B/1D receptors agonist, in allergic rhinitis model in mice and the role of the nitric oxide pathway.

2. Methods

2.1. Animal

Female balb/c mice of 8–10 weeks of age and free of murine-specific pathogens were randomly used. All animals had free access to food and water, had ovalbumin free diet and were maintained in facilities with 12 h light and dark cycle and temperature of 23 ± 2 °C. Groups of 8 mice were used in this experiment. All procedures were conducted in compliance with guidelines of the US National Institute of Health (NIH Publications No. 8023, revised 1978) and institutional Guideline for the Care and Use of Laboratory Animals with the approval of Tehran University Research and Medical Ethics Committees (IR.TUMS.MEDICINE.REC.1397.766). All behavioral experiments carried out at the same time of the day. Each mouse was used only once in this study.

2.2. Chemical

The following drugs were used throughout this study: Sumatriptan, a 5HT1B/1D receptor agonist (Sigma, USA); GR127935, a selective 5-HT1B/1D-receptor antagonist (Sigma, USA); L-NAME [L-NG-Nitro-L-arginine methyl ester hydrochloride], a non-specific inhibitor of NOS (Sigma, USA); Ovalbumin, a drug used to trigger T cell activation (Sigma, USA), Al(OH)3[aluminum hydroxide] (Sigma, USA). All chemicals, including aluminum hydroxide gel, sumatriptan, L-NAME, and GR127935 were dissolved in normal saline and administered intraperitoneally (i.p.). Ovalbumin injected intraperitoneally (i.p.) in day 1, 5, 14 and 21, then it was administered intranasally in day 22–35.

2.3. Paradigm

To induce allergic rhinitis, mice were sensitized with ovalbumin 40 μg/kg and hydroxide aluminum 40 mg/kg by intraperitoneal injection on days 1, 5, 14, 21. Then they were challenged for 14 days by intranasal administration of 20 μl ovalbumin in a dose of 25 mg/ml [24–26]. Vehicle-treated group received only saline during the mentioned time. A single injection of sumatriptan (3, 10, 30 μg/kg, i.p.) was done 30 min before the last ovalbumin nasal challenge in 35th day. Mice received GR127935 (10 μg/kg, i.p.) 10 min before vehicle or sumatriptan (3 μg/kg, i.p.) and 40 min before the last ovalbumin nasal challenge in 35th day. L-NAME (10 mg/kg, i.p.) was administered 15 min before vehicle or sumatriptan (3 μg/kg, i.p.) and 45 min before the last ovalbumin nasal challenge in 35th day. Finally, mice received a chronic injection of L-NAME (10 mg/kg, i.p.) before allergen on days 1, 5, 14, 21, and 15 min before vehicle or sumatriptan (3 μg/kg, i.p.) on the 35th day.

2.4. Nasal scratching behavior evaluation

Immediately after the last nasal challenge, each mouse was recorded for 10 min for the evaluation of nasal scratching behavior. The videos were independently observed by two blind expert observers. After that, mice were anesthetized with ketamine (80 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.); their blood samples were gathered, and nasal cavities were removed.

2.5. Nitric oxide synthase protein expression measurement

We measured iNOS (inducible nitric oxide synthase) and eNOS (endothelial nitric oxide synthase) protein expression in nasal tissue of mice using Western blot method using manufacturer's protocols. [NOS2 (C-11): sc-7271 (SANTA CRUZ BIOTECHNOLOGY, INC.) and NOS3 (A-9): sc-376751 (SANTA CRUZ BIOTECHNOLOGY, INC.)].

2.6. Immunoglobulin E, IL-4 and IFNγ measurement

After anesthesia cardiopuncture was done to collect mice blood. Blood samples were centrifuged to obtain serum. The samples were kept at −80 °C. Serum total IgE, IL-4 and IFNγ levels were measured by Mouse IgE, IL-4 and IFNγ ELISA (enzyme-linked immunosorbent assay) Kits (Sigma, USA) following the manufacturer's protocol.

2.7. Histopathology

After anesthesia with ketamine and xylazine on day 35, all nasal cavities were removed, fixed in 10% formalin and placed in ethylene diamine tetra-acetic acid (EDTA, (ReAgent, Runcorn, England)] solution, until decalcification. The sections of 4 μm were obtained from paraffin-embedded sinonasal structures followed by routine hematoxylin and eosin staining. Eosinophils identified as bi-lobed cells with bright red eosinophilic cytoplasm were counted in 3 non-overlapping high-power-fields (× 40 field area: 0.24 mm^2) of the respiratory mucosa by two pathologists under a double-headed light microscope (BX51, Olympus Co, Tokyo, Japan). In each field, the mean counts of the 2 observers were recorded.

2.8. Statistics

Data are presented as the mean ± standard error of the mean (S.E.M). The one-way analysis of variance (ANOVA) followed by Tukey multiple comparisons and Bonferroni post hoc test were used to indicate the statistical significance of differences between the experimental means. P value < 0.05 was considered significant for all analyses. Graph-pad prism software version 6 was used to analyze the data.

3. Results

3.1. Effects of sumatriptan on ovalbumin-induced allergic rhinitis in mice

Fig. 1 shows the effects of ovalbumin, aluminum hydroxide gel, and sumatriptan in mice. The group that were sensitized and challenged by ovalbumin showed significantly more scratching behavior compared to the vehicle group that was challenged with normal saline only ($P^{<}0.001$). Sumatriptan as a treatment in doses of 3, 10, 30 μg/kg decreased the scratching events significantly compared to allergic group ($P^{<}0.001$; Fig. 1). Interestingly, the dose of 3 μg/kg sumatriptan

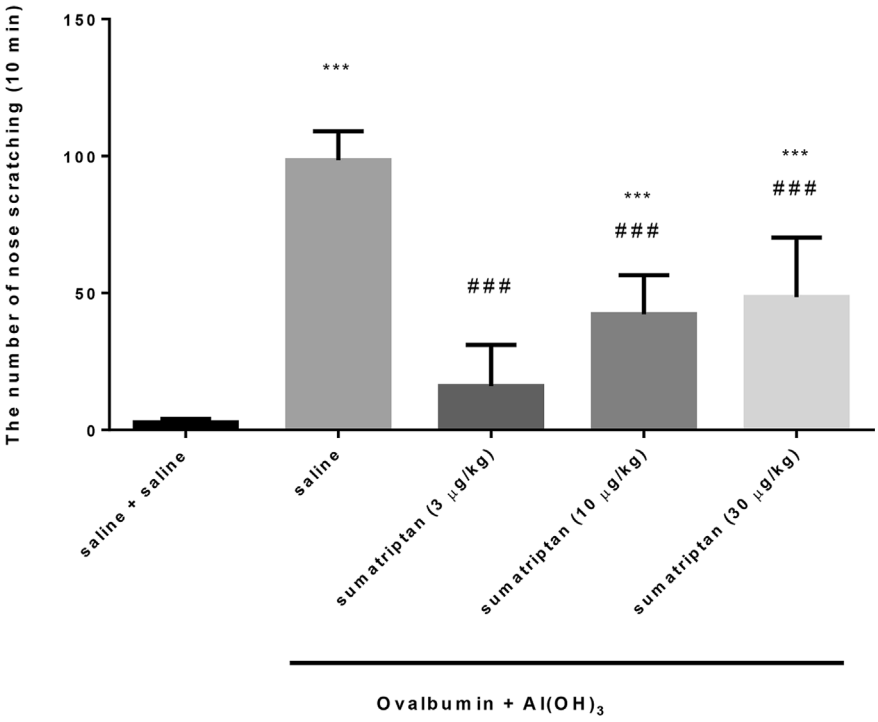

Fig. 1. Sumatriptan blocks ovalbumin, aluminum hydroxide induced allergies in female balb/c mice. Sumatriptan was administrated in doses 3, 10, 30 μg/kg intraperitoneally 30 min before the last ovalbumin nasal challenge on the 35th day. Data are presented as means ± S.E.M (n = 8). ***P < 0.001 compared with saline + saline group, ###P < 0.001 compared with saline + ovalbumin & Al $(OH)_3$ treated group.

eliminated the scratching behavior remarkably; in which there was no significant difference with saline-treated animals. This dose of sumatriptan was chosen as the most effective dose for the following experiments.

3.2. Effects of GR-127935 on ovalbumin-induced allergic rhinitis in mice

Fig. 2 illustrates the effect of GR-127935, as a selective 5-HT1B/1D-receptor antagonist, on ovalbumin-induced allergic rhinitis in mice. Mice received GR-127935 (10 μg/kg) 10 min before normal saline or sumatriptan (3 μg/kg). While GR-127935 (10 μg/kg) injected along with sumatriptan 3 μg/kg it significantly reversed the effect of sumatriptan treated group (P<0.001; Fig. 2); it was not effective when it was injected before normal saline.

3.3. Effects of acute L-NAME injection on ovalbumin-induced allergic rhinitis in mice

Fig. 3 shows the effect of acute injection of L-NAME on ovalbumin-induced allergic rhinitis in mice. L-NAME (10 mg/kg, i.p.) was administered 15 min before vehicle or sumatriptan (3 μg/kg, i.p.) in allergic mice. Acute injection of L-NAME along with sumatriptan attenuated the sumatriptan protective effect on ovalbumin-induced scratching in mice (P < 0.001), but it was not effective when it was

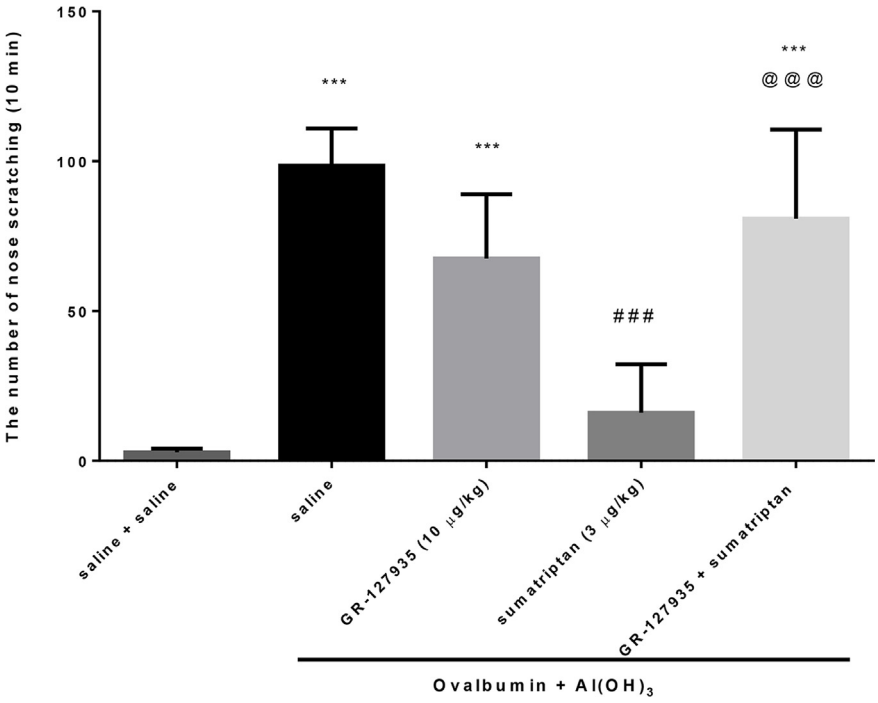

Fig. 2. GR127935 reverses the anti-allergic effect of sumatriptan in ovalbumin-induced allergic rhinitis in mice. GR127935 (10 μg/kg) was injected intraperitoneally 10 min before ovalbumin or normal saline on the 35th day. Data are presented as means ± S.E.M (n = 8). ***P < 0.001 compared with saline + saline group, ###P < 0.001 compared with saline + ovalbumin & Al $(OH)_3$ treated group, @@@ P < 0.001 compared with sumatriptan (3 μg/kg) treated group.

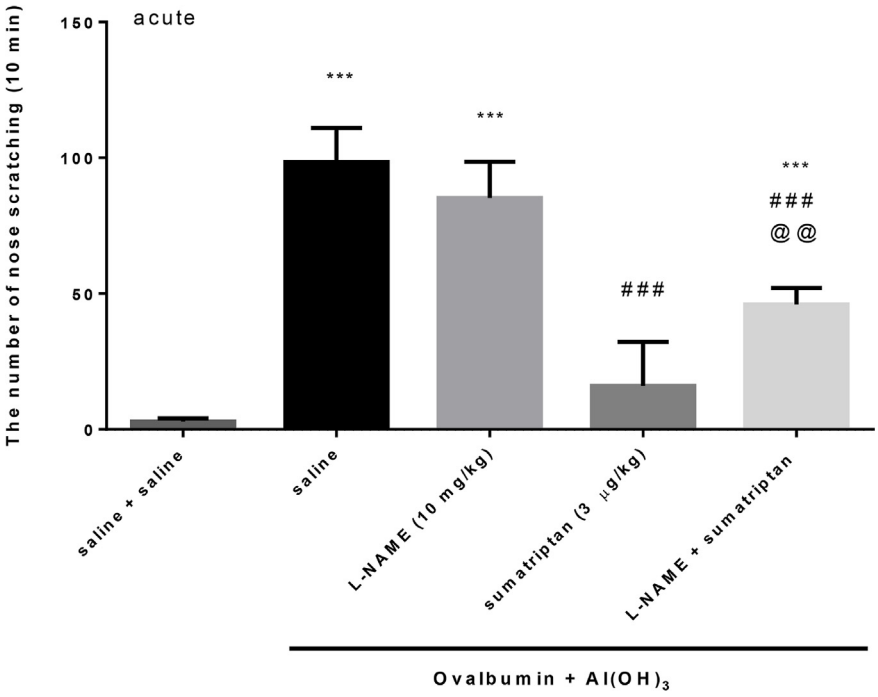

Fig. 3. Acute injection of L-NAME attenuates the anti-allergic effect of sumatriptan in ovalbumin-induced allergic rhinitis in mice. L-NAME (10 mg/kg, i.p.) was injected 15 min before normal saline or sumatriptan (3 μg/kg, i.p.) on the 35th day. Data are presented as means ± S.E.M (n = 8). ***P < 0.001 compared with saline + saline group, ###P < 0.001 compared with saline + ovalbumin & $Al(OH)_3$ treated group, @@ P < 0.01 compared with sumatriptan (3 μg/kg) treated group.

injected before normal saline in vehicle group compared to vehicle (P > 0.05).

3.4. Effects of chronic L-NAME injection on ovalbumin-induced allergic rhinitis in mice

Fig. 4 illustrates the effect of chronic L-NAME injection on ovalbumin-induced allergic rhinitis in mice. Mice received a chronic injection of L-NAME (10 mg/kg, i.p.) before allergen on days 1, 5, 14, 21 and 35; these animals received vehicle or sumatriptan (3 μg/kg, i.p.) only in the last day (35th day). Chronic injection of L-NAME alone did not show significant alteration in scratching behavior in mice compared to vehicle and sumatriptan-treated animals (P > 0.05). Chronic administration of L-NAME along with sumatriptan, did not change the sumatriptan effect on the scratching behavior compared to allergic group (P > 0.05).

3.5. Western blot analysis of iNOS and eNOS proteins in nasal tissue

Fig. 5 shows the iNOS (inducible nitric oxide synthase) and eNOS (endothelial nitric oxide synthase) proteins expression in nasal tissue of saline + saline group, allergic-rhinitis induced animals, and sumatriptan (3 μg/kg) treated groups. Western blot analysis of revealed that nasal tissue iNOS protein concentration was significantly higher in allergic-rhinitis group compared to saline + saline group (P < 0.0001). Interestingly, sumatriptan (3 μg/kg) significantly reduced this elevated

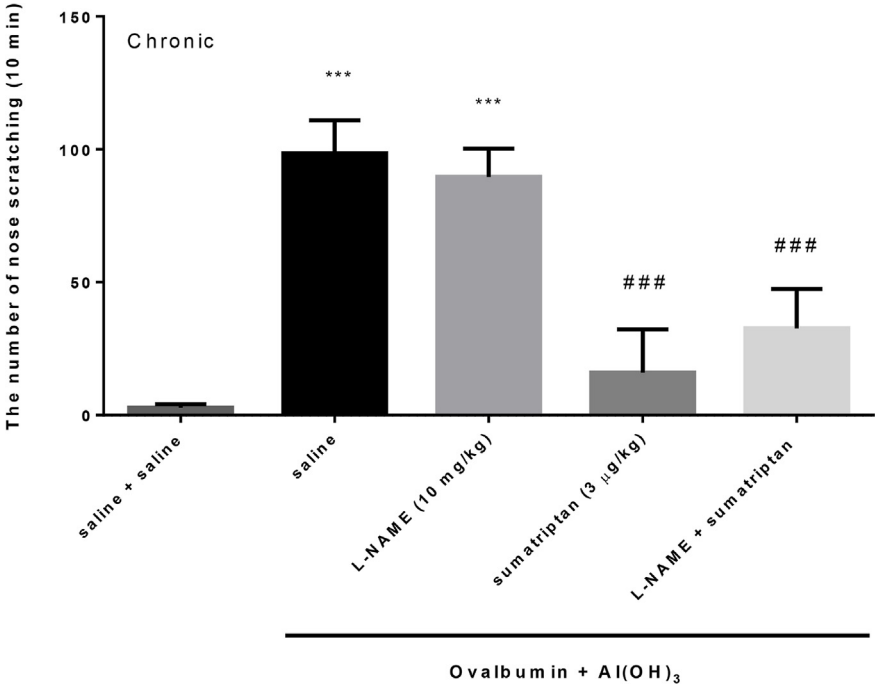

Fig. 4. Chronic injection of L-NAME has no significant effect on anti-allergic effect of sumatriptan in ovalbumin-induced allergic rhinitis in mice. Mice received a chronic injection of L-NAME (10 mg/kg, i.p.) before allergen on days 1, 5, 14, 21 and 35; these animals received saline or sumatriptan (3 μg/kg, i.p.) in the last day (35th day). Data are presented as means ± S.E.M (n = 8). ***P < 0.001 compared with saline + saline group, ###P < 0.001 compared with saline + ovalbumin & $Al(OH)_3$ treated group.

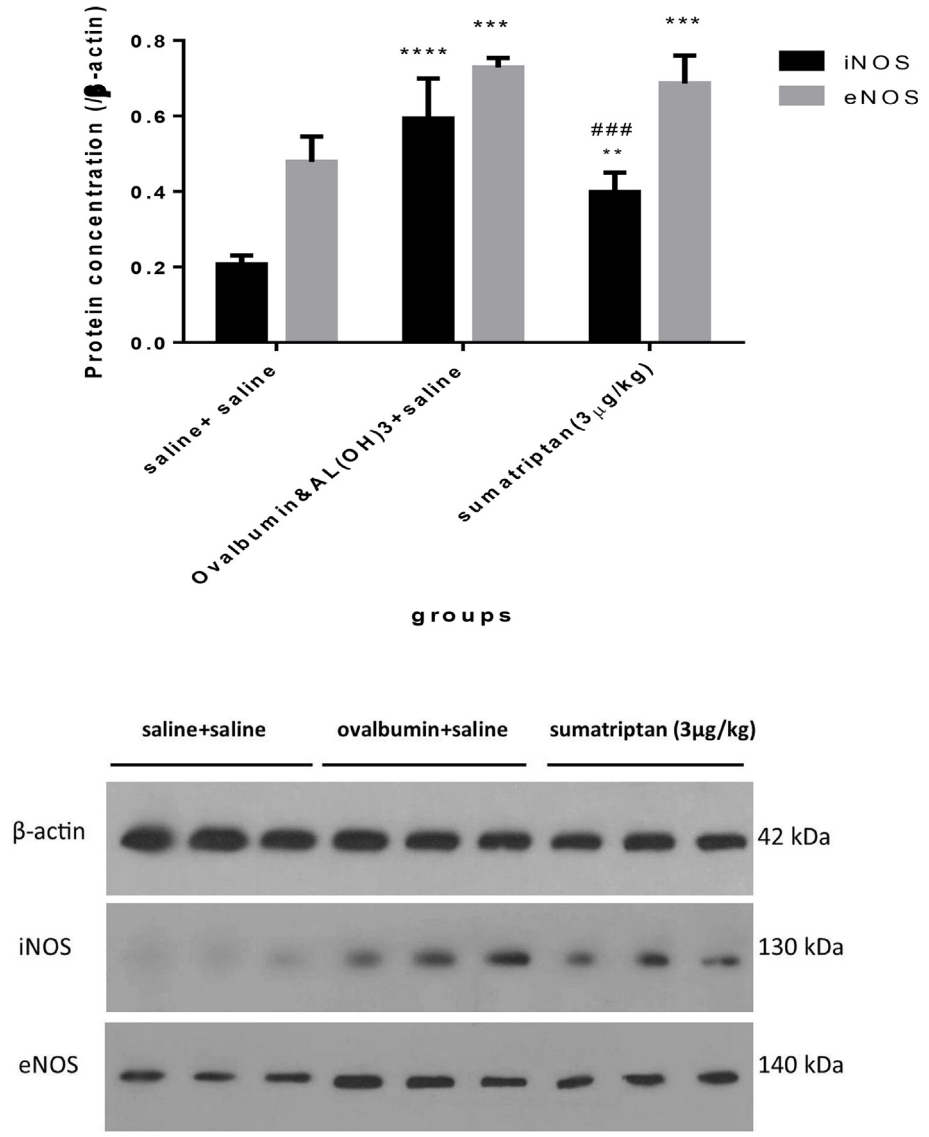

Fig. 5. The effects of sumatriptan (3 μg/kg) on expression of iNOS- and eNOS-proteins in the nasal tissue of allergic-rhinitis induced mice. Data are presented as means ± S.E.M. 3 mice were used in each experiment. **P < 0.01 vs saline + saline group, ***P < 0.001 vs saline + saline group, ###P < 0.001 vs saline + allergen (ovalbumin + Al $(OH)_3$).

concentration of iNOS compared to allergic-rhinitis group (P < 0.0001).

The level of eNOS was also significantly higher in allergic-rhinitis group compared to saline + saline group (P < 0.001) assessed by western-blotting technic, but sumatriptan did not have any significant effect on eNOS expression compared to allergic-rhinitis group (P > 0.05).

3.6. IgE assessment

Table 1 shows the measured IgE levels in mice serum. The IgE level in the group that were sensitized and challenged with ovalbumin was significantly higher than the vehicle group that was sensitized and challenged with normal saline (P < 0.001). Sumatriptan in doses of 3 and 10 μg/kg significantly decreased the IgE levels compared to the allergic group (P < 0.001). IgE level in the group that received GR127935 (10 μg/kg) along with sumatriptan (3 μg/kg) was significantly higher than sumatriptan treated group (P < 0.001); however GR127935 injection in vehicle group did not significantly change the IgE level (P > 0.05). Acute injection of L-NAME (10 mg/kg) along with sumatriptan (3 μg/kg) significantly increased the IgE level compared to sumatriptan (3 μg/kg) + allergen group (P < 0.001), but acute L-NAME (10 mg/kg) injection in vehicle group did not affect the IgE level (P > 0.05). Chronic injection of L-NAME (10 mg/kg) along with sumatriptan (3 μg/kg) also significantly increased the IgE level compared to sumatriptan (3 μg/kg) + allergen group (P < 0.001), but it was not effective in the vehicle group (P > 0.05).

3.7. Serum interlukin-4 and interferon gamma measurement

Fig. 6 represents interlukin-4 (IL-4) level (a TH2 marker) in mice serum. The allergic group had significantly higher IL-4 level compared to saline + saline group (P < 0.0001). Sumatriptan (3 μg/kg) significantly decreased the IL-4 level compared to allergic group (P < 0.0001). Acute administration of L-NAME (10 mg/kg) along with sumatriptan (3 μg/kg) significantly increased the IL-4 level compared to the group that received sumatriptan (3 μg/kg; P < 0.01). Whereas, chronic administration of L-NAME (10 mg/kg) along with sumatriptan did not change the sumatriptan effect on IL-4 levels.

Fig. 7 shows serum interferon gamma (IFNγ) level as a TH1 marker.

Table 1
The measured Immunoglobulin E levels in mice serum. The immunoglobulin E levels were measured using enzyme-linked immunosorbent assay. Data are presented as means ± S.E.M. 8 mice were used in each experiment. ***P<0.001 vs saline + saline. ###P<0.001 vs saline + allergen (ovalbumin + Al $(OH)_3$). @@@ P<0.001 vs sumatriptan (3 μg/kg) + allergen treated group.

groups	IgE level (IU/ml)	Statistical difference
Saline + saline	7 ± 0.0	
Saline + allergen (ovalbumin + Al$(OH)_3$)	87.5 ± 9.81	***P<0.001
Sumatriptan (10 μg/kg) + allergen	34 ± 2.30	***P<0.001 ###P<0.001
Sumatriptan (3 μg/kg) + allergen	23.5 ± 4.04	***P<0.001 ###P<0.001
GR-127935 + allergen	41 ± 5.47	***P<0.001
GR-127935 + Sumatriptan + allergen	85.5 ± 6.02	***P<0.001 @@@P<0.001
Acute L-NAME + allergen	31.5 ± 4.92	***P<0.001
Acute L-NAME + Sumatriptan + allergen	49 ± 3.28	***P<0.001 @@@P<0.001
Chronic L-NAME + allergen	40 ± 14.24	***P<0.001
Chronic L-NAME + Sumatriptan + allergen	89.66 ± 0.51	***P<0.001 @@@P<0.001

Serum IFNγ was significantly lower in allergic group compared to saline + saline group ($P < 0.0001$). Sumatriptan (3 μg/kg) significantly increased IFNγ levels compared to allergic group ($P < 0.0001$). Acute injection of L-NAME along with sumatriptan (3 μg/kg) significantly decreased IFNγ level compared to the group that received sumatriptan (3 μg/kg; $P < 0.0001$). But chronic administration of L-NAME did not show any significant impact.

3.8. Histopathology assessment

Table 2 shows the average number of eosinophils counted in 3 non-overlapping high-power-fields (× 40 field area: 0.24 mm^2) of the sinonasal mucosa. The number of eosinophils in the group that received ovalbumin and Al $(OH)_3$ was significantly higher than saline + saline group ($P < 0.0001$). Sumatriptan in doses 3 and 10 μg/kg significantly reduced the number of eosinophils compared to the ovalbumin + Al $(OH)_3$ group ($P < 0.0001$). Mice that received GR-127935 (10 μg/kg) along with sumatriptan (3 μg/kg) had significantly higher eosinophils compared to the sumatriptan treatment group ($P < 0.01$). Injection of L-NAME (10 mg/kg) in acute form along with Sumatriptan (3 μg/kg) significantly increased the eosinophils numbers compared to the group that was treated with sumatriptan (3 μg/kg; $P < 0.01$). Chronic L-NAME administration, however, did not significantly affect the number of eosinophils in sinonasal mucosa compared to sumatriptan (3 μg/kg) treated group ($P > 0.05$). Also, the histopathology pics are shown in Fig. 8.

4. Discussion

This study demonstrates the effect of sumatriptan on allergic rhinitis model induced by ovalbumin in mice and the role of nitric oxide. According to the findings of this study, intraperitoneal administration of sumatriptan, a 5HT1B/D agonist, attenuated the nasal scratching behavior in allergic mice. In our study, the serum IgE and the number of

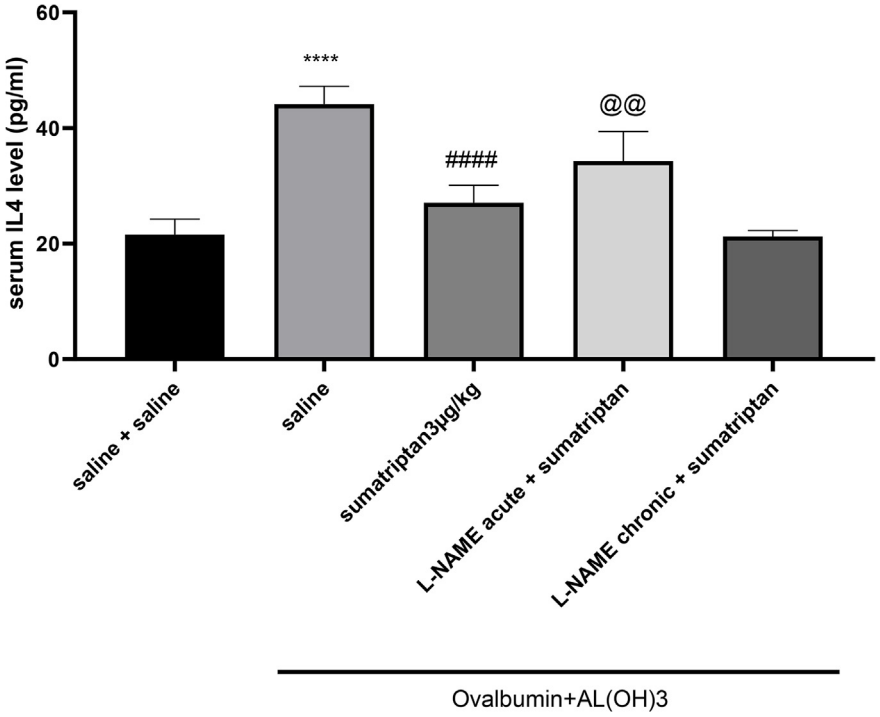

Fig. 6. Measured interlukin-4 (IL-4) levels in mice serum. The serum IL-4 level was measured using enzyme-linked immunosorbent assay. Data are presented as means ± S.E.M. 8 mice were used in each experiment. ****P < 0.0001 vs saline + saline group, ####P < 0.0001 vs saline + allergen (ovalbumin + Al $(OH)_3$), @@ P<0.01 vs sumatriptan (3 μg/kg) + allergen group.

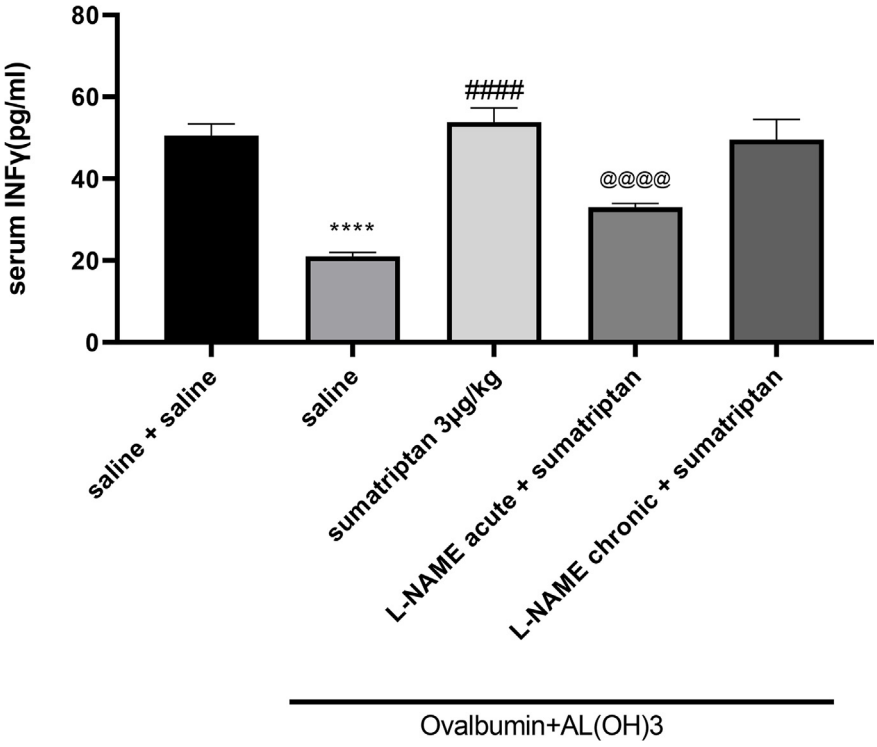

Fig. 7. Measured interferon gamma (IFNγ) level in mice serum. The serum IFNγ level was measured using enzyme-linked immunosorbent assay. Data are presented as means ± S.E.M. 8 mice were used in each experiment. ****P < 0.0001 vs saline + saline group, ####P < 0.0001 vs saline + allergen (ovalbumin + Al $(OH)_3$), @@@@ P<0.0001 vs sumatriptan (3 μg/kg) + allergen group.

Table 2
The average number of eosinophils counted in 3 non-overlapping high-power-fields (× 40 field area: 0.24 mm^2) of the sinonasal mucosa. Data are presented as means ± S.E.M .8 mice were used in each experiment.*P<0.05, **P<0.01, ***P<0.001 vs saline + saline. @@ P<0.01 vs sumatriptan (3 μg/kg) + allergen treated group.### P<0.001 vs Saline + allergen (ovalbumin + $Al(OH)_3$).

groups	Number of eosinophils in sinonasal mucosa of mice (Mean ± S.E.M)	Statistical significance
Saline + saline	0.8889 ± 0.7817	
Saline + allergen (ovalbumin + $Al(OH)_3$)	17.33 ± 5.087	***P<0.001
Sumatriptan (10 μg/kg) + allergen	6.000 ± 3.295	###P<0.001
Sumatriptan (3 μg/kg) + allergen	3.444 ± 1.509	###P<0.001
GR-127935 + allergen	12.33 ± 9.913	***P<0.001
GR-127935 + Sumatriptan + allergen	14.00 ± 4.336	***P<0.0001 @@ P<0.01
Acute L-NAME + allergen	10.44 ± 4.246	**P<0.01
Acute L-NAME + Sumatriptan + allergen	12.44 ± 5.525	@@ P<0.01
Chronic L-NAME + allergen	10.00 ± 5.060	*P<0.05
Chronic L-NAME + Sumatriptan + allergen	8.667 ± 3.386	–

eosinophils in histopathological assessments were significantly decreased after sumatriptan administration. Sumatriptan decreased serum IL-4, but it increased IFNγ levels compared to allergic group and reversed the TH2/TH1 ratio. The anti-allergic effect of sumatriptan was reversed by GR-127935, a selective 5HT1B/D receptors antagonist. Acute treatment with L-NAME, non-selective NOS inhibitor, eliminated all impacts of sumatriptan in allergic rhinitis induced with ovalbumin, whereas the chronic administration of L-NAME did not change the sumatriptan protective attitude. Sumatriptan also significantly reduced nasal tissue iNOS protein expression compared to allergic mice, but it did not have any significant effect on eNOS protein level. These results can suggest that the anti-allergic effect of sumatriptan could be through 5HT1B/D receptors and nitric oxide pathway due to its anti-inflammatory effect.

Allergic rhinitis can significantly decrease the quality of individual life with severe consequences on social expenses. The most important parts of allergic reactions are T helper-2 cells, eosinophils, mast cells, IgE production and allergic mediators release which result in acute functional changes in affected organs [27].Serotonin has been suggested as a mediator in allergic rhinitis [28] and previous studies showed that human mast cells, which are one of the characteristic cells in allergic rhinitis, can release serotonin [29]. Neuropeptides have a role in the mechanism of allergic rhinitis by increasing nasal airway resistance. They can also stimulate T cells, mast cells, eosinophils, and induce chemotaxis of inflammatory cells [30]. Substance P and neurokinin A, as sensory neuropeptides, and their receptors have been detected in human airways [31]. 5-HT1B/1D receptors decrease cyclic adenosine monophosphate (cAMP) due to inhibition of adenylyl cyclase [12]. As a result activation of these receptors can inhibit the release of many neuropeptides [32]. 5-HT1B/1D receptors can inhibit neurogenic inflammation in peripheral nerves [33].

Sumatriptan activates 5HT1B/1D receptors located on sensory nerves and inhibits neuropeptides release via a pre-synaptic action. This mechanism leads to inhibition of neurogenic plasma extravasation [34]. Administration of sumatriptan reduces CGRP in animal and human studies [35]. It also inhibits the release of substance P [36].

There are many resemblances between migraine headaches and allergic rhinitis. A previous study reported that more than 50% of patients with migraine have a history of allergic rhinitis [37]. Some mediators including IgE, histamine, tumor necrosis factor-α (TNF-α), CGRP, prostaglandins, interleukin–1, tryptase, and also activation of mast cells and NO are important in both allergic rhinitis and migraine headaches [38]. NO can cause neurogenic inflammation, vasodilatation and increased vascular permeability and has an important role in pathophysiology of allergic rhinitis. There is amplified evidence that shows NO has a vital role in vasodilation and neurotransmission pathways of nociceptive impulses in migraine headaches [39]. Sinus headaches are common in allergic rhinitis patients. After binding of IgE to mast cells and basophils, the release of inflammatory mediators can cause acute symptoms. Activation of mast cells and basophils leads to sinus headaches in individuals with allergic rhinitis. In addition, one of the symptoms observed in late phase of allergic rhinitis, which occurs

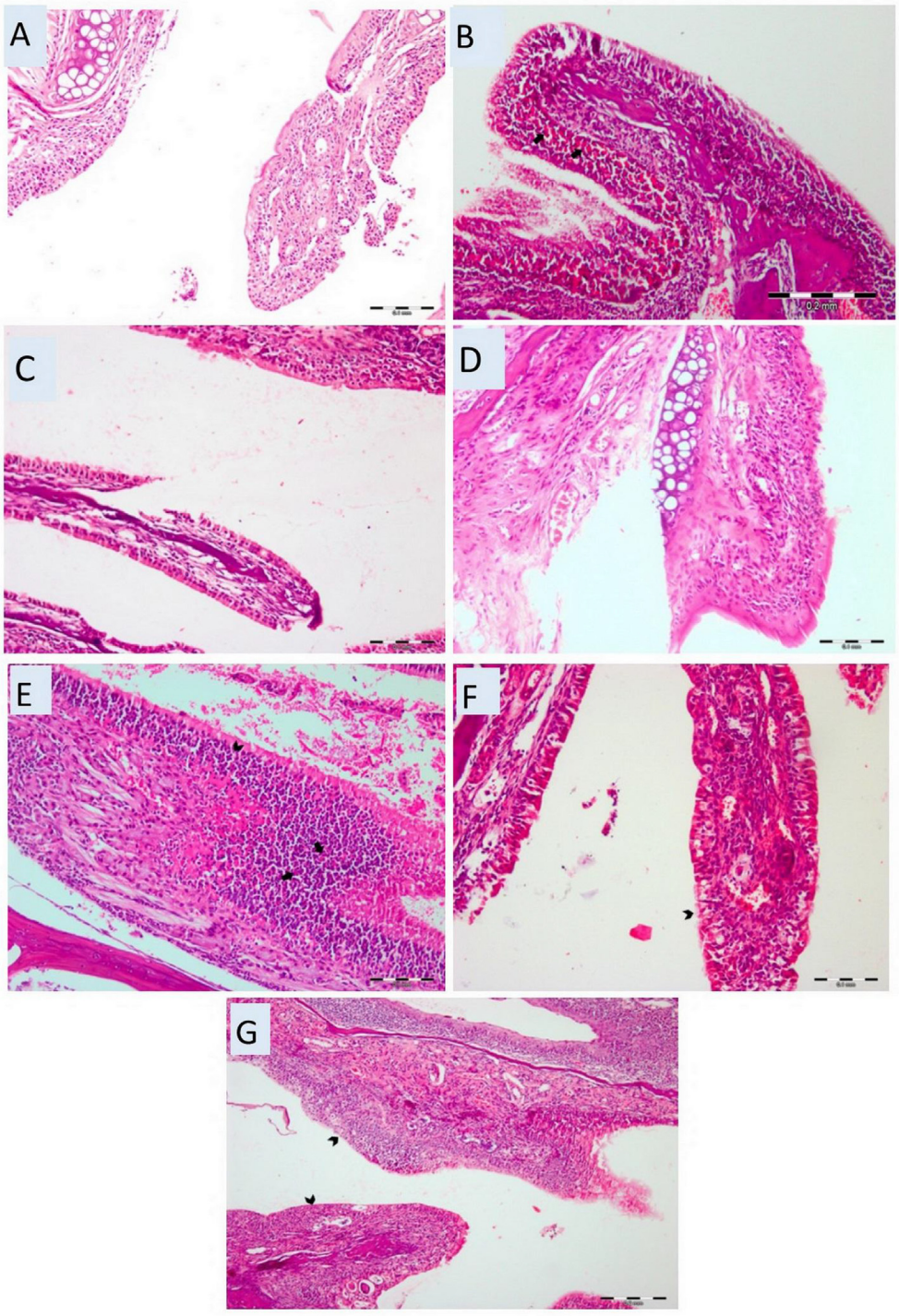

Fig. 8. Represents sections of eosinophilic infiltration in nasal mucosa tissue (Hematoxylin and Eosin staining; scale bars represent 0.1 and 0.2 mm): (A) Saline + saline group. (B) Saline + Ovalbumin & Al $(OH)_3$ group. (C) Sumatriptan (3 μg/kg) group. (D) Sumatriptan (10 μg/kg) group. (E) GR-127935 (10 μg/kg) + Sumatriptan (3 μg/kg) group. (F) L-NAME (10 mg/kg; acute) + Sumatriptan (3 μg/kg) group. (G) L-NAME (10 mg/kg; chronic) + Sumatriptan (3 μg/kg) group. Chevrons and arrows indicate acute + chronic inflammation and eosinophilic infiltration, respectively.

about 8 h after the exposure to allergen, is headache. Some patients who have symptoms of sinus headache response to sumatriptan [40].

In a previous study, 5HT1B receptors were detected around blood vessels in the human nasal mucosa.[16]Since 5HT receptors are known to cause vasoconstriction, it could be possible that 5HT1B receptors are involved in nasal secretions and airway resistance. It is also possible that the effect of sumatriptan in alleviating allergic rhinitis symptoms could be due to activation of 5HT1B receptors in the nasal mucosa.

Eosinophils have an important role in pathophysiology of allergic rhinitis, and the number of eosinophils is increased in nasal mucosa of allergic patients [41]. There is a correlation between the symptoms severity and inflammatory markers [42].In our study, the serum IgE and the number of eosinophils in nasal mucosa were elevated after induction of allergic rhinitis via ovalbumin and AL (OH_3). Injection of sumatriptan decreased serum IgE levels and airway eosinophils.

The balance between T Helper 2 and T Helper 1 cells has an

important role in many inflammatory processes. It is known that TH2 cytokines (including IL-4, IL-5 and IL-13) are involved in allergic inflammations. TH1 cytokines (including IL-12 and IFNγ) can inhibit TH2 response and play a role in inhibiting allergic cascades [43].In our study administration of sumatriptan decreased serum IL-4 level, while it increased IFNγ levels and therefore reversed the TH2/TH1 mediators' ratio in allergic mice.

Nitric oxide (NO) is a signaling molecule which is generated by 3 isoforms of nitric oxide synthase (NOS) including inducible (iNOS), endothelial (eNOS) and neuronal (nNOS). Sumatriptan can reduce NO level in cats and rats brain and this mechanism may have a role in anti-migraine effect of sumatriptan [44]. Read et al. reported that in a rat model of migraine a single sumatriptan pre-treatment modulated the cortical NO concentrations when compared to basal [45]. In a study on electrical induced vasodilation in rats, sumatriptan inhibited the NO overproduction by inhibiting CGRP release; NO overproduction was observed in patients with severe headache [46]. sumatriptan has been shown to decrease chloroquine-induced itching behavior through the nitric oxide pathway [47]. Another study demonstrated that sumatriptan in a dose of 1 mg/kg has an anti-convulsive effect, and it decreases the brain nitrate level in the mentioned dose [48].Also, inhibition of nitric oxide synthase blocks the effect of sumatriptan in morphine-induced antinociceptive tolerance in mice [49].In addition, studies indicated that Nitric oxide synthase (NOS) inhibitors are effective in the treatment of acute migraine [46,50].

Previous studies showed that NO generated by iNOS has inflammatory features, whereas NO produced by eNOS is anti-inflammatory [51,52]. In some studies iNOS level was higher in allergic patients but eNOS and nNOS were not significantly different compared to controls [53]. Another study showed that iNOS and eNOS were higher in allergic patients [54]. Similar to mentioned articles, in our experiment sumatriptan reduced iNOS level in nasal tissue of allergic mice. It did not have any significant effect on eNOS level. Our results also showed that acute administration of L-NAME, a non-specific inhibitor of NOS, along with sumatriptan decreased the inhibitory effects of sumatriptan in scratching behavior, serum IgE level, TH2/TH1 balance and nasal mucosa eosinophils numbers compared to sumatriptan treated mice, Whereas the chronic L-NAME injection did not affect the inhibitory influence of sumatriptan, which could be related to remodeling mechanisms.

It would be wonderful if we could perform this experiment on knock out mice for allergic rhinitis to get rid of all possible intervening variations of this assessment. The next suggested step following this experiment would be investigating the effects of sumatriptan in an allergic rhinitis model of cell culture, and then on clinical trial studies.

5. Conclusion

The signs of allergic rhinitis, including nasal scratching behavior, elevated serum IgE levels, TH2/TH1 ratio alteration, and airways tissue eosinophils rise may be eliminated with acute sumatriptan treatment through its impact on inflammatory mechanisms via 5HT1B/1D receptors and nitric oxide pathway.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2019.116901.

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

[1] J.L. Brożek, J. Bousquet, C.E. Baena-Cagnani, et al., Allergic rhinitis and its impact on asthma (ARIA) guidelines: 2010 revision, Journal of Allergy and Clinical Immunology 126 (3) (2010) 466–476.
[2] R.A. Nathan, The burden of allergic rhinitis, Paper Presented at: Allergy and Asthma Proceedings, 2007.
[3] M. Valls-Mateus, F. Marino-Sanchez, K. Ruiz-Echevarría, et al., Nasal obstructive disorders impair health-related quality of life in adolescents with persistent allergic rhinitis: a real-life study, Pediatric Allergy and Immunology 28 (5) (2017) 438–445.
[4] D.P. Skoner, Allergic rhinitis: definition, epidemiology, pathophysiology, detection, and diagnosis, Journal of allergy and clinical immunology 108 (1) (2001) S2–S8.
[5] S.J. Galli, M. Tsai, M. Adrian, Piliponsky AM the development of allergic inflammation, Nature 454 (7203) (2008) 445–454.
[6] P. Van Cauwenberge, C. Bachert, G. Passalacqua, et al., Consensus statement* on the treatment of allergic rhinitis, Allergy 55 (2) (2000) 116–134.
[7] P. Wojciechowski, M. Dziewonska, P. Rys, M.L. Kowalski, Adrenal suppression by inhaled corticosteroids in patients with asthma: a systematic review and quantitative analysis, Paper Presented at: Allergy & Asthma Proceedings, 2016.
[8] B. Thomas, R. Stanhope, D. Grant, Impaired growth in children with asthma during treatment with conventional doses of inhaled corticosteroids, Acta Pædiatrica. 83 (2) (1994) 196–199.
[9] M.W. Marcus, R.P. Müskens, W.D. Ramdas, et al., Corticosteroids and open-angle glaucoma in the elderly, Drugs & aging 29 (12) (2012) 963–970.
[10] G. Philip, A.S. Nayak, W.E. Berger, et al., The effect of montelukast on rhinitis symptoms in patients with asthma and seasonal allergic rhinitis, Current medical research and opinion 20 (10) (2004) 1549–1558.
[11] E. Brown, C. Endersby, R. Smith, J. Talbot, The safety and tolerability of sumatriptan: an overview, European neurology 31 (5) (1991) 339–344.
[12] J.D. McCorvy, B.L. Roth, Structure and function of serotonin G protein-coupled receptors, Pharmacology & therapeutics 150 (2015) 129–142.
[13] A.H. Ahn, A.I. Basbaum, Where do triptans act in the treatment of migraine? Pain 115 (1–2) (2005) 1.
[14] P.P. Humphrey, W. Feniuk, Mode of action of the anti-migraine drug sumatriptan, Trends in pharmacological sciences 12 (1991) 444–446.
[15] M.G. Buzzi, M.A. Moskowitz, Evidence for 5-HT1B/1D receptors mediating the antimigraine effect of sumatriptan and dihydroergotamine, Cephalalgia 11 (4) (1991) 165–168.
[16] R. Uddman, J. Longmore, L.O. Cardell, L. Edvinsson, Expression of 5-HT1B receptors in human nasal mucosa, Acta oto-laryngologica. 121 (3) (2001) 403–406.
[17] P. Tønnesen, N. Mygind, Nasal challenge with serotonin and histamine in normal persons, Allergy 40 (5) (1985) 350–353.
[18] Y. Habib, A. Belal, A. El-Garem, M.E. Lozy, H.M. El-banna, H. Toppozada, Serotonin in nasal allergy, Allergy 26 (1) (1971) 39–48.
[19] P. Tønnesen, I. Hindberg, Serotonin in nasal secretion, Allergy 43 (4) (1988) 303–309.
[20] S.J. Read, P. Manning, C.J. McNeil, A.J. Hunter, A.A. Parsons, Effects of sumatriptan on nitric oxide and superoxide balance during glyceryl trinitrate infusion in the rat. Implications for antimigraine mechanisms, Brain research 847 (1) (1999) 1–8.
[21] L. Zhou, D.-Y. Zhu, Neuronal nitric oxide synthase: structure, subcellular localization, regulation, and clinical implications, Nitric oxide 20 (4) (2009) 223–230.
[22] L.E. Gustafsson, A. Leone, M. Persson, N. Wiklund, S. Moncada, Endogenous nitric oxide is present in the exhaled air of rabbits, Guinea pigs and humans, Biochemical and biophysical research communications 181 (2) (1991) 852–857.
[23] J.F. Arnal, A. Didier, J. Rami, et al., Nasal nitric oxide is increased in allergic rhinitis, Clinical & Experimental Allergy 27 (4) (1997) 358–362.
[24] Y. Kim, T. Yang, C.S. Park, et al., Anti-IL-33 antibody has a therapeutic effect in a murine model of allergic rhinitis, Allergy 67 (2) (2012) 183–190.
[25] H. Saito, K. Matsumoto, A.E. Denburg, et al., Pathogenesis of murine experimental allergic rhinitis: a study of local and systemic consequences of IL-5 deficiency, The Journal of Immunology 168 (6) (2002) 3017–3023.
[26] T.Y. Jang, C.-S. Park, K.-S. Kim, M.-J. Heo, Y.H. Kim, Benzaldehyde suppresses murine allergic asthma and rhinitis, International immunopharmacology 22 (2) (2014) 444–450.
[27] K.D. Stone, C. Prussin, D.D. Metcalfe, IgE, mast cells, basophils, and eosinophils, Journal of Allergy and Clinical Immunology 125 (2) (2010) S73–S80.
[28] P. Tønnesen, N. Mygind, Nasal challenge with serotonin and histamine in normal persons, Allergy 40 (5) (1985) 350–353.
[29] N.M. Kushnir-Sukhov, J.M. Brown, Y. Wu, A. Kirshenbaum, D.D. Metcalfe, Human mast cells are capable of serotonin synthesis and release, Journal of Allergy and Clinical Immunology 119 (2) (2007) 498–499.
[30] G. Joos, P. Germonpre, R. Pauwels, Neurogenic inflammation in human airways: is it important? Thorax 50 (3) (1995) 217.
[31] J.N. Baraniuk, J.D. Lundgren, M. Okayama, et al., Substance P and neurokinin A in human nasal mucosa, Am J Respir Cell Mol Biol 4 (3) (1991) 228–236.
[32] M. Moskowitz, M. Buzzi, Neuroeffector functions of sensory fibres: implications for headache mechanisms and drug actions, Journal of neurology 238 (1) (1991) S18–S22.
[33] M.A. Moskowitz, Neurogenic versus vascular mechanisms of sumatriptan and ergot

alkaloids in migraine, Trends in pharmacological sciences 13 (1992) 307–311.
[34] M.D. Ferrari, P.R. Saxena, Clinical effects and mechanism of action of sumatriptan in migraine, Clinical neurology and neurosurgery 94 (1992) 73–77.
[35] P.P. Humphrey, P.J. Goadsby, The mode of action of sumatriptan is vascular? A debate, Cephalalgia 14 (6) (1994) 401–410.
[36] S.J. Tepper, A.M. Rapoport, F.D. Sheftell, Mechanisms of action of the 5-HT1B/1D receptor agonists, Archives of neurology 59 (7) (2002) 1084–1088.
[37] Eric Eross, David Dodick, Michael Eross, The sinus, allergy and migraine study (SAMS) CME." headache, The Journal of Head and Face Pain 47 (2) (2007) 213–224.
[38] A. Saberi, S. Nemati, R.J. Shakib, E. Kazemnejad, M. Maleki, Association between allergic rhinitis and migraine, Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences 17 (6) (2012) 508.
[39] M. Ku, B. Silverman, N. Prifti, W. Ying, Y. Persaud, A. Schneider, Prevalence of migraine headaches in patients with allergic rhinitis, Annals of Allergy, Asthma & Immunology 97 (2) (2006) 226–230.
[40] C. Schreiber, R. Cady, C. Billings, Oral sumatriptan for self-described 'sinus' headache, Cephalalgia 21 (4) (2001).
[41] M. Bakhshaee, M. Fereidouni, M. Farzadnia, A.-R. Varasteh, The nasal smear for eosinophils, its value, and its relation to nasal mucosal eosinophilia in allergic rhinitis, Iranian Journal of Otorhinolaryngology 22 (3) (2010) 73–78.
[42] L. Winther, C. Reimert, P. Skov, L. Kærgaard Poulsen, L. Moseholm, Basophil histamine release, IgE, eosinophil counts, ECP, and EPX are related to the severity of symptoms in seasonal allergic rhinitis, Allergy 54 (5) (1999) 436–445.
[43] Cengiz Kirmaz, et al., Effects of glucan treatment on the Th1/Th2 balance in patients with allergic rhinitis: a double-blind placebo-controlled study, European cytokine network 16 (2) (2005) 128–134.
[44] J. Olesen, The role of nitric oxide (NO) in migraine, tension-type headache and cluster headache, Pharmacology & therapeutics 120 (2) (2008) 157–171.
[45] S.J. Read, P. Manning, C.J. McNeil, A.J. Hunter, A.A. Parsons, Effects of sumatriptan on nitric oxide and superoxide balance during glyceryl trinitrate infusion in the rat. Implications for antimigraine mechanisms, Brain research 847 (1) (1999) 1–8.
[46] S. Akerman, D. Williamson, H. Kaube, P. Goadsby, Nitric oxide synthase inhibitors can antagonize neurogenic and calcitonin gene-related peptide induced dilation of dural meningeal vessels, British journal of pharmacology 137 (1) (2002) 62–68.
[47] N.S. Haddadi, S. Ostadhadi, S. Shakiba, et al., Pharmacological evidence of involvement of nitric oxide pathway in anti-pruritic effects of sumatriptan in chloroquine-induced scratching in mice, Fundamental & clinical pharmacology 32 (1) (2018) 69–76.
[48] M. Gooshe, K. Ghasemi, M.M. Rohani, et al., Biphasic effect of sumatriptan on PTZ-induced seizures in mice: modulation by 5-HT1B/D receptors and NOS/NO pathway, European journal of pharmacology 824 (2018) 140–147.
[49] M. Hassanipour, N. Rajai, N. Rahimi, et al., Sumatriptan effects on morphine-induced antinociceptive tolerance and physical dependence: the role of nitric oxide, European journal of pharmacology 835 (2018) 52–60.
[50] M.D. Ferrari, K.I. Roon, R.B. Lipton, P.J. Goadsby, Oral triptans (serotonin 5-HT1B/1D agonists) in acute migraine treatment: a meta-analysis of 53 trials, The Lancet 358 (9294) (2001) 1668–1675.
[51] Y. Xiong, G. Karupiah, S.P. Hogan, P.S. Foster, A.J. Ramsay, Inhibition of allergic airway inflammation in mice lacking nitric oxide synthase 2, J Immunol 162 (1999) 445–452.
[52] R. Ten Broeke, R. De Crom, R. Van Haperen, et al., Overexpression of endothelial nitric oxide synthase suppresses features of allergic asthma in mice, Respir Res 7 (2006) 58.
[53] Hasan Yuksel, et al., Nasal mucosal expression of nitric oxide synthases in patients with allergic rhinitis and its relation to asthma, Annals of Allergy, Asthma & Immunology 100 (1) (2008) 12–16.
[54] Y. Li, Y. Wang, Q. Zhang, Expression of cell adhesion molecule and nitric oxide synthase in nasal mucosa in allergic rhinitis, Lin chuang er bi yan hou ke za zhi= Journal of clinical otorhinolaryngology 20 (7) (2006) 315–318.