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Strontium chloride hexahydrate as a candidate molecule for long-term treatment of allergic rhinitis

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***Background & objectives*: Neurogenic inflammation plays a role in the pathophysiology of allergic rhinitis (AR). Strontium salts are highly effective in reducing the sensory irritation. This study was aimed to investigate the efficacy of strontium chloride (SC) on AR symptoms based on the duration of SC use before the symptoms begin.**

***Methods:* Wistar albino rats (n=18) were randomly divided into three groups: Group 1, received 1µg mometasone furoate (MF); Group 2, three per cent SC; and Group 3 received five per cent SC (2 µl/site). Drugs were administered to the each nasal cavity for three weeks every morning. On the days 7, 14 and 21, histamine dihydrochloride (HD) 5 µmol (2 µl/site) was administered and the frequencies of nasal rubbing and sneezing were counted for 15 min.**

***Results:* After 7, 14 and 21 day medication period, the groups were compared in terms of the frequency of sneezing and nasal rubbing following HD. There was a significant difference among the groups in terms of the frequency of sneezing on the day 7 ($P<0.05$). Intragroup comparisons for the nasal rubbing showed significance ($P<0.05$). In Group 3, there was a decrease in the number of nasal rubbings on the day 14 and 21; however, the difference was not significant.**

***Interpretation & conclusions*: Our results showed that three and five per cent SC were less effective than MF for sneezing during the first week, but the efficiency was equal to that of MF after the first 14 days. Long-term use of SC was as effective as MF on nasal rubbing. SC can be as effective as MF on both sneezing and nasal rubbing on regular use over three weeks.**

Key words Allergic rhinitis - long-term usage - mometasone furoate - nasal rubbing - sneezing - strontium chloride

Allergic rhinitis (AR), caused by allergens, is a chronic nasal mucosa inflammation impairing the quality of life, sleep and work efficiency[1,2]. Sneezing, rhinorrhoea, nasal itching and nasal congestion are among the clinical symptoms of AR. The mechanism leading to the development of symptoms associated with AR is complex, including activation and infiltration of inflammatory cells, oedema, increased and altered gland activity, nerve terminal activation, triggering of neurogenic inflammation and morphologically detectable remodelling processes in the mucous membrane[3]. Neurogenic inflammation has been known

to play a role in the pathogenesis of AR[4,5]. Corticosteroids having anti-inflammatory effects are the essential, first-line therapy in AR patients[6]. Mometasone furoate (MF), which is a nasal spray, is an effective treatment for AR and has anti-inflammatory, anti-pruritic, anti-hyperproliferative actions[7,8]. In long-term use, MF appears to reduce the extent of inflammatory cell infiltration by attenuating the inflammatory process, particularly of eosinophils[9]. Activation of the central and peripheral nervous system plays a major role in the pathophysiology of itching and sneezing. Sensory nerves of the afferent trigeminal system including myelinated Aδ-fibres and thin, non-myelinated C-fibres of the nasal mucosa transmit signals that generate sensations, including itching and motor reflexes, such as sneezing[6]. Lee *et al*[10] reported that itching was not only associated with sensorial stimulation but also the perception level and they showed that strontium chloride (SC) increased the perception threshold. They suggested that strontium selectively blocked the activation of both nociceptors (Aδ and C fibre) that respond to chemical stimuli. Hahn[11] has reported that strontium salts are highly effective in reducing the sensory irritation caused by some substances and hypothesized that strontium salts directly affect the type C nerve.

We hypothesized that SC, which has a positive effect on neurogenic inflammation in AR, might be efficacious in AR treatment. In our preliminary study we found that administration of SC before and after the symptoms occur, was as effective as MF on sneezing (unpublished data). In the present study, the aim was to investigate the efficacy of SC on AR symptoms based on the duration of SC use before the symptoms begin.

Material & Methods

The study was carried out in department of Otolaryngology, Faculty of Medicine, Cumhuriyet University, Sivas, Turkey between August 2014 and September 2014; with 18, 16-18 wk old male Wistar albino rats with an average body weight of 200-220 g. The rats were housed in standard laboratory conditions (12 h light/dark cycles, 24°C±2°C, 35-60% humidity) and fed with standard pellet chow and water *ad libitum*. The study protocol was approved by the Institutional Review and Animal Ethics Use Committee of Cumhuriyet University School of Medicine, Sivas, Turkey, and followed the accepted guidelines for the care and use of laboratory animals for research.

Wistar albino rats (n=18) were randomly divided into three groups: group 1 received 1µg MF; group 2 three per cent SC; and group 3 received five per cent SC (2 µl/site).

Drug and chemicals: Histamine and SC were obtained from Sigma Chemical Co. (St. Louis, MO, USA). These reagents were dissolved in saline. The following reagents were also purchased: sefiyol nasal spray (Delta Vital, İstanbul, Turkey) and nasonex aqueous nasal spray (l8 g/140 dose, Schering- Plough, USA). The rats were given a nasal instillation of the drugs in volume of 2 µl into the bilateral cavities using a micropipette.

Evaluation of the nasal symptoms in rats: This study was based on a mouse model of AR[12]. It has been shown that nasal administration of histamine dihydrochloride (HD) at a dose of 5 µmol (2 µl/site) significantly elicits nasal symptoms[12]. As this study was performed for the first time on rats, for rat modelling, Kusaka *et al*'s[12] mouse model was administered step by step. For acclimatization, animals were placed in an observation cage (30 × 28 × 20 cm) for 10 min before starting the experiment.

On the first day of the experiment, 2 µl of sefiyol nasal spray was administered via micropipette to each nasal passage of every rat, and nasal symptoms were counted and recorded. On the second day of the experiment, HD (2 µl, 5 µmol/site) was administered via micropipette to each nasal passage of each rat and, again, nasal symptoms were counted. Symptoms occurring on the first and second days of the experiment were compared. After approving the accuracy of mouse model of AR for rats[12], the test protocol was initiated.

In the present study, 1µg MF (2 µl/site) was applied to group 1, three per cent SC (2 µl/site) was applied to group 2 and five per cent SC (2 µl/site) was applied to group 3 with a micropipette to each nasal cavity for three weeks every morning at 0900 h. On days 7, 14 and 21, before the administration of HD (5 µmol, 2 µl, nasal instillation into bilateral cavities), the animals were placed back into the observation cage (1 animal per cage). The frequencies of nasal rubbing and sneezing were counted for 15 min. During the study, one rat each in groups 1 and 3 died on the 12th and 19th days, respectively.

Statistical analysis: Data were analyzed using Statistical Package of Social Science (SPSS Inc., Chicago, IL, USA) for Windows version 22.0. As the parametric test assumptions were not met, Kolmogorov–Smirnov,

Kruskal–Wallis (KW) test, Friedman test and Wilcoxon test were used to evaluate the data obtained.

Results

The mean age of rats was 17.00±0.89 wk in group 1, 17.16±0.98 wk in group 2 and 17.33±0.81 wk in the group 3 (range 16-18 wk). The mean body weight was 210.00±6.60 g in group 1, 209.33±7.78 g in group 2 and 209.83±7.65 g in group 3 (range 200-220 g).

After 7, 14 and 21 day medication period, the groups were compared in terms of the frequency of sneezing following HD but no significant difference was found between the groups on the days 14 and 21, while there was a significant difference on the 7th day (P<0.05). Groups 1 and 2 when compared in terms of the frequency of sneezing showed significant differences on the 7th day (P<0.05). Further analysis confirmed that the first group was different from the other groups. Intragroup comparison did not yield any significant difference (Table I).

After 7, 14 and 21 day medication period, the groups were compared in terms of the frequency of nasal rubbing, and no significant difference was found among the groups. Intragroup comparisons revealed significant differences in groups 1 and 2 on day 7 compared to days 14 and 21. Nasal rubbing gradually decreased with medication in the first and second groups. In group 3, there was a decrease in the number of nasal rubbings on the 14th and 21st days; however, the difference was not significant (Table II).

Discussion

The results of the present study showed that three and five per cent SC were less effective than MF during the first week, but the efficacy was equal to that of MF after the first 14 days. Long-term use of SC yielded a significant decrease in sneezing symptom and was as effective as MF on nasal rubbing.

Allergy is caused by an IgE-driven overreaction of the immune system to what would otherwise be

Table I. Frequency of sneezing in the study groups at days 7, 14 and 21

Group	Sneezing								
	First week (seven days)			Second week (14 days)			Third week (21 days)		
	Minimum	Maximum	Mean±SD	Minimum	Maximum	Mean±SD	Minimum	Maximum	Mean±SD
Group 1 (n=6)	3	10	6.66±2.58	1	25	7.50±9.12	0	13	3.16±4.96
Group 2 (n=6)	7	30	15.66±7.94*	1	42	11.83±15.84	0	22	6.40±9.02
Group 3 (n=6)	5	28	14.16±10.24*	2	41	12.60±16.26	3	14	7.40±4.56

*P<0.05 compared to group 1. The rats were given an intranasal administration of 1 µg mometasone furoate (2 µl/site), 3 per cent strontium chloride (2 µl/site) and 5 per cent strontium chloride (2 µl/site) for seven, 14 and 21 days. After the administration of histamine dihydrochloride (5 µmol, 2 µl/site), the frequency of sneezing was counted for 15 min.

Table II. Frequency of nasal rubbing at days 7, 14 and 21 in the study groups

Group	Nasal rubbing								
	First week (seven day)			Second week (14 days)			Third week (21 days)		
	Minimum	Maximum	Mean±SD	Minimum	Maximum	Mean±SD	Minimum	Maximum	Mean±SD
Group 1 (n=6)	6	14	8.16±3.06**	1	5	3.33±1.63	0	6	2.50±2.50
Group 2 (n=6)	5	11	8.12±2.23*	0	7	2.80±2.43	0	7	2.80±3.42
Group 3 (n=6)	1	17	10.60±6.62	2	7	4.40±2.07	0	10	3.40±3.91

P *<0.05, **<0.01 compared to values in the same group at second and third week. The rats were given an intranasal administration of 1 µg mometasone furoate (2 µl/site), 3 per cent strontium chloride (2 µl/site) and 5 per cent strontium chloride (2 µl/site) for 7, 14 and 21 days. After the administration of histamine dihydrochloride (5 µmol, 2 µl/site), the frequency of nasal rubbing was counted for 15 min.

a relatively innocuous stimulus. Clinically, allergy is characterized by symptoms that, by in large, are secondary to an altered nervous system. Sneezing and persistent itching of the nasal mucosa are distressing symptoms of AR. It has been shown that itchy sensations and sneezing reflexes can be associated with hyperinnervation of sensory neurons and/or increased expression of neuropeptides in nerve terminals[13,14]. Sensory nerve fibres in the nasal mucosa are afferent connections from the trigeminal nerve and these are classified into two categories, myelinated thick Aδ-fibres and non-myelinated thin C-fibres. In AR, some polymodal nociceptive type C-fibres convey itching and sneezing stimuli from the nasal mucosa[6,15,16]. Neuropeptides, such as substance P (SP) and calcitonin gene-related peptide (CGRP), are expressed in trigeminal sensory fibres and have been shown to induce allergic symptoms[17]. In AR patients, nerve fibre density in the nasal epithelium, lamina propria (subepithelium) and glandular/vascular regions is also increased[13,14]. Therefore, allergy can be categorized as an immune-neuronal disorder[18]. The target of the current therapeutic approaches of AR treatment is the inhibition of inflammatory molecules and suppression of the immune reaction[19] through the use of anti-histamines, leukotriene antagonists and steroids. As no appropriate attention is given to the neuronal aspect of this immune-neuronal disorder, the neurological approach to AR treatment has not been investigated yet.

Histamine, prostaglandin D2, cysteine leukotriene D4, serotonin and bradykinin, which are allergy-associated mediators, may decrease the activity of certain potassium channels and lead to an increase in the resistance of the afferent nerve and a subsequent increase in electrical excitability[20]. Cysteine leukotriene D_4-stimulating cysteine leukotriene 1 receptors in C-fibre neurons of the trigeminal ganglion does not overtly activate the nerve but increases its excitability such that the action potential discharge frequency in response to another activating stimulus is substantially enhanced[20]. The kinetics of the increase in afferent nerve excitability secondary to allergen activation of mast cells can be quite persistent and last for several hours after acute mast cell activation. Products from activated eosinophils might also contribute to the increases in afferent nerve excitability at sites of allergic inflammation[21]. Furthermore, if the allergic mediators have made the sensory nerve hyperexcitable, then subthreshold stimuli and even non-noxious routine stimuli might evoke these nociceptor-associated reflexes[18]. In a study conducted on a murine model of AR, Sawaki *et al*[13] showed that intranasal administration of recombinant Sema3A alleviated sneezing and nasal rubbing and decreased nasal mucosa innervation in the AR model mice.

In organs at the interface between our body and the environment, the sensory neuropeptide SP is one key mediator of an acute local stress response through neurogenic inflammation but may also alter cytokine balance and dendritic cell function[22]. In AR patients, the numbers of SP and CGRP-positive fibres invading the epithelial and subepithelial regions are increased[14]. Strontium can substitute calcium to trigger neurotransmitter release and it has been used as an experimental tool to study this process[23]. Studies on the squid giant synapse[24], the neuromuscular junction[25,26] and at central synapses[27] have shown that strontium triggers a 'desynchronized' neurotransmitter release with a reduced peak and prolonged duration. Therefore, SC was suggested to be less effective than Ca2+ in triggering exocytotic transmitter release[28]. In a study where the authors claimed that itching was not only associated with sensorial stimulation but also the perception level; Lee *et al*[10] showed that SC increased the perception threshold.

The limitations of our study included the following: (*i*) the sample size was small, (*ii*) showing the effectiveness of the drug in different animal models, such as ovalbumin-induced AR rat model was necessary, (*iii*) standardized therapeutic doses were not known in the research model, and (*iv*) systemic or local adverse events were not determined.

In conclusion, our study showed that SC was as effective as MF on both sneezing and nasal rubbing in long-term use in the rat model of AR. The results also suggest that intranasal administration of SC may provide a novel approach to alleviate the allergic symptoms for AR.

***Conflicts of Interest*:** None.

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