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Preliminary report

Q1 Nasal sodium cromoglycate (Lomusol) modulates the early phase reaction of mild to moderate persistent allergic rhinitis in patients mono-sensitized to house dust mite: A preliminary study

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ABSTRACT

We evaluated the clinical improvement of patients with mild to moderate persistent allergic rhinitis (AR) due to mono-sensitization to house dust mite (HDM) allergen, by sodium cromoglycate nasal spray (Lomusol 4%). Lomusol was used as a single agent treatment, and its anti-inflammatory effects, in the early phase reaction were evaluated. Herein we showed that Lomusol significantly improved the subjective nasal symptom scores especially nasal obstruction. This was associated with significant and specific reduction in neutrophils influx in nasal cytology but had no effect on other cell types. This selective anti-inflammatory effect on nasal cytology was associated with significant reduction in the levels of platelet activating factor (PAF) and histamine in nasal secretions but had no effect on PGD₂, LTC₄ or CysLt levels. Lomusol was also able to induce significant reduction in eosinophil cationic protein (ECP) levels in nasal secretions without altering the percentage of eosinophil influx in nasal cytology. Taken collectively, we showed the first evidence that nasal sodium cromoglycate possesses a selective inhibition on neutrophil recruitment into nasal cytology in the early phase reaction of AR patients mono-sensitized to HDM. This may be attributed to the ability of Lomusol to significantly reduce the amount of PAF recovered in nasal secretion. These results were associated with significant improvement in subjective symptom scores especially nasal obstruction that may in addition, be due to the ability of Lomusol to down-regulate eosinophil degranulation activity as well.

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1. Introduction

The prevalence of AR increased over the last 3 decades significantly and reached figures of 40% affection of the world wide population. This entails alternation of these patients quality of life with large socio-economic impacts [1–3]. The latest classification of allergic rhinitis and its impact on asthma (ARIA) classified AR as intermittent (<4 days/week or <4 weeks) or persistent (>4 days/week and >4 weeks). The severity of symptoms, sleep pattern, daily activities and impact on work and school, further define the classification as mild or moderate to severe [1,4].

The International Primary Care Respiratory Group (IPCRG), the British Society for Allergy and Clinical Immunology (BSACI) and the American Academy of Allergy Asthma and Immunology (AAAAI) recommend pharmacotherapy with non-sedating H1 blocker antihistamine and to add nasal corticosteroid, nasal decongestants, cromones or anti-leukotrienes in the more severe cases [5–7]. Although nasal corticosteroids have effective control over the symptoms and the nasal inflammation, the long use of nasal corticosteroids is controversial in the

pediatric population and has its limitation in certain conditions. For example, a substantial amount of patients stops its use because of epistaxis that is a recognized complication of nasal corticosteroids. Patients with glaucoma cannot take nasal corticosteroids and the long term users may have atrophy effects on their nasal mucosa. Therefore, AR patients with mild to moderate symptoms may benefit from nasal sodium cromoglycate that has better safety profile and its use is ideal for pregnant women and children.

The majority of rhinologists use nasal sodium cromoglycate for seasonal AR where they start the therapy a few weeks before the beginning of the pollen season and continue throughout the season to stabilize mast cells and prevent the allergic symptoms. However, to our best knowledge, no report is available on the efficacy of nasal sodium cromoglycate as a first line therapy, in mild to moderate persistent AR against HDM allergen that is a major trigger for persistent AR and asthma.

Since the discovery of disodium cromoglycate over 50 years ago [8], other cromoglycate like agents (chromones) have been developed and showed ability to inhibit both the early and late phase reaction of asthma [9–12]. Although early studies concentrated on the histamine and cytokines release from mast cells [13–17] other studies demonstrated anti-allergic activity unrelated to mast cell activation [18].

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Herein, we show the first evidence that nasal sodium cromoglycate was able to improve the symptom scores significantly in patients with mild to moderate persistent AR due to mono-sensitization to HDM. The subjective symptom scores improvement was associated with objective selective and significant reduction of nasal neutrophilia, PAF, histamine and ECP release in the early phase reaction following allergen challenge.

2. Materials and methods

2.1. Patients

Twenty one patients were recruited to this study; 14 for the Lomusol treatment group and 7 for the placebo treatment group. All of them were men except one female. Their mean age was 29.7 years old. They received either the Lomusol spray 4% or the placebo spray in a blinded fashion to the patient and the principal investigator. For the nasal cytology experiments, the placebo treated group consisted of 6 patients (one patient developed acute rhinosinusitis and had to be excluded).

2.1.1. Inclusion criteria

- AR patients mono-sensitized to HDM allergen proved by allergy skin test and radioallergosorbent test (RAST).
- AR patients mono-sensitized to HDM and were not taking any anti-allergy pharmacotherapy at least 2 months before the study.
- AR patients mono-sensitized to HDM allergen and presented with mild to moderate persistent AR (nasal obstruction, sneezing and rhinorrhea that does not affect sleep pattern, daily activities or absence from work).

2.1.2. Exclusion criteria

- Pregnant women.
- Children.
- AR patients sensitized to other allergens than HDM.
- Patients with severe AR symptoms that affect sleep pattern, daily activities or absence from work.
- AR patients who were taking already nasal corticosteroids, antihistamine or anti-leukotrienes.
- AR patients with associated asthma.
- AR patients with acute infective rhinosinusitis.

2.2. Symptom scores evaluation

Patients were handed sheets for symptom scores evaluating the severity of the cardinal symptoms of allergic rhinitis; nasal obstruction, sneezing and rhinorrhea. The score was from 0–10, where 0 indicates no such symptom and 10 maximum troubles from the symptom. Patients were asked to mark their scores before the beginning of treatment and after 4 weeks from the start of the treatment. The treatment consisted of Lomusol spray 4% given as a single puff each nostril 4 times a day for 4 weeks. The placebo group used isotonic saline spray given as a single puff each nostril 4 times a day for 4 weeks.

2.3. Nasal provocation test

AR patients who presented to our rhinology clinic with clinical symptoms of AR and were allergic to house dust mites participated in the allergen nasal challenge. None of them were taking antihistaminic or nasal/systemic cortisone therapy. After obtaining their consent, first they were asked to blow their nose and their nasal secretions were collected during 30 min (pre-challenge). The challenge test started with 10 µl of the diluent being introduced into the nose to the anterior part of the inferior turbinate unilaterally on impregnated discs. After

15 min and after excluding the nonspecific hyperresponsiveness to diluent, nasal lavage with saline was done and then the purified and standardized allergen dilution (stallorgenes 100 IR/ml) was introduced into the nose in a similar fashion. After 1–2 min the patients were asked to blow their nose. The nasal secretions were collected during 30 min. Saline nasal irrigation was then done. Patients' vital signs were monitored for at least 30 min after challenge before being discharged from the clinic.

2.4. Preparation of nasal secretions for cytology and ELISA

The collected nasal secretions were first weighted and diluted three times with DPBS (Lonza BioWhittaker, Belgium). After centrifugation at 872 g for 8 min at 4 °C, the supernatant was collected and stored at –80 °C until they were tested by ELISA for the contents of different mediators. The cells pellet was then treated with 5 mM Sputolysin (DTT) (calbiochem, USA) during 20 min at room temperature to remove mucus. Cells were then washed twice with DPBS and then centrifuged (872 g for 8 min at 4 °C). The cells were then suspended in DPBS at a final concentration of 0.5×10^6 cells/ml and were stained with diff-Quick set (Atom Scientific-UK). 500 cells per slide were then examined under light microscopy for the percentage of different cell types.

As for ELISA the following kits were purchased and ELISA was performed according to the manufacturer protocol: PAF was purchased from Bio-Connect-Netherlands; ECP was from Emelca Bioscience-Belgium; PGD2, CysLT, LTC4 & histamine were all purchased from Bertin pharma-France. Exact equal amounts of nasal secretions (diluted $\times 3$ and homogenized) were then analyzed to ensure actual amounts of mediators released and to overcome the problem of too little or too concentrated samples.

2.5. Analysis of data

The data were analyzed as $\pm$ SEM. Statistical analysis was performed by paired t-test and Wilcoxon test. A *p* value of less than 0.05 was considered significant.

3. Results

3.1. Symptom scores before and after Lomusol treatment

Fig. 1 demonstrates the $\pm$ SEM of symptom scores of studied patients. As expected the subjective base line symptom scores (before treatment) varied between patients especially sneezing. This was further reflected by the difference of patients' number in each treatment group due to the difficulty of recruiting large number of patients to clinical studies. Nonetheless, there was no significance difference between the patients treated by placebo and those treated by Lomusol for the base line symptom scores when compared and analyzed by paired t-test (Supplementary Fig. 1). Accordingly the statistical analysis of symptom scores improvement of each sample group was carried separately by paired t-test. As can be seen in Fig. 1B, after treatment for 4 weeks with nasal sodium cromoglycate as a single therapeutic agent, there was a significant improvement in all symptoms with highly significant improvement on nasal obstruction when compared to the placebo group who did not show significant difference in their symptom scores (Fig. 1A). In the Lomusol treatment group, the symptom scores improved from 7 ± 0.6 to 3.1 ± 0.6 for nasal obstruction, from 4.2 ± 1.2 to 1.3 ± 1 for sneezing and from 4.7 ± 1.4 to 1.1 ± 0.3 for nasal discharge. On the other hand, the placebo group showed no significant symptom scores improvement. The score values variation was from 5.8 ± 1.2 to 5.4 ± 1.1 for nasal obstruction, 1.8 ± 0.8 to 0.8 ± 0.5 for sneezing and from 5.2 ± 1.7 to 3.8 ± 1.3 for nasal discharge. These results indicate potency for nasal sodium cromoglycate as a single therapeutic agent, in improving at least subjectively the symptoms in AR patients with mild to moderate severity. To further confirm and validate

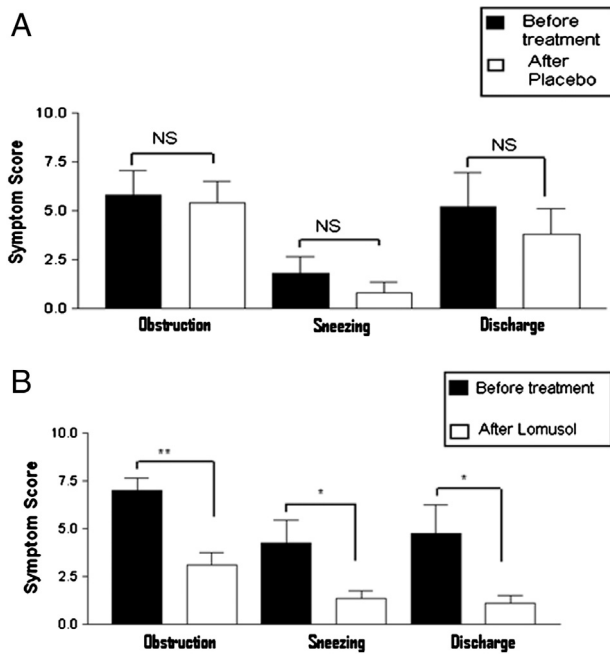


Fig. 1. Symptom scores of AR patients' mono-sensitized to HDM before and after treatment with (A) placebo (n = 7) & (B) Lomusol (n = 14) for 4 weeks. Asterisk indicates $p^* = 0.02$ & $p^{**} = 0.002$, by paired t-test.

the results of symptom scores improvement by Lomusol, the following objective experiments were performed at the cellular and molecular levels.

3.2. Effect of Lomusol on inflammatory cells in nasal cytology

The mean volume of nasal secretions was $829 \mu\text{l} \pm 1100$, and the number of cells was $5.6 \times 10^5 \pm 6.4 \times 10^5$. As can be seen in Fig. 2, before challenge the base line cellular infiltrate showed a predominant granulocytes infiltration among all inflammatory cells recovered (compare Fig. 2A & B with C & D). Eosinophils and neutrophils ratio was $12.7\% \pm 8.3.4\% \pm 5$, respectively of all cellular infiltrate recovered. Other inflammatory cells such as lymphocytes and macrophages were almost negligible $0.01\%:0.2\% \pm 0.4$ (C & D). Other recovered cells were ciliated epithelial cells ($34.1\% \pm 5$), squamous cells ($31.9\% \pm 5$) and atypical cells $<0.1\%$. After challenge there was no change in the dynamics of cellular infiltrate in nasal secretion during the early phase (30 min) as seen in Fig. 2 (A–F). However, interestingly re-challenging the same patients with HDM allergens after 4 weeks treatment with nasal sodium cromoglycate, demonstrated a significant reduction in neutrophils from $3.4\% \pm 5$ to $0.3\% \pm 0.1$ ($p < 0.05$) but not in other cell types as demonstrated in Fig. 2A–E. The placebo group did not alter inflammatory cells influx in nasal cytology as seen in Fig. 2A–G.

3.3. Effect of Lomusol on allergic mediators release in nasal secretions

In order to have further insight on the anti-inflammatory effects of sodium cromoglycate on the nasal cytology of AR patients mono-sensitized to HDM, we next compared the amount of different allergic mediators closely related to granulocytes nasal infiltration, in the early phase reaction, before and after exposure to HDM. As summarized in Table 1, there was significant increase in the mean $\pm$ SEM of the amounts of PGD2, LTC4 and CysLT after challenge from $330 \text{ pg/ml} \pm 461$ to $3732 \text{ pg/ml} \pm 591$ ($**p = 0.003$), from $17.3 \text{ pg/ml} \pm 5$ to $45.5 \text{ pg/ml} \pm 45$ ($*p = 0.02$) and from $193 \text{ pg/ml} \pm 86$ to $>1000 \text{ pg/ml}$ ($**p = 0.007$) respectively, while histamine and PAF augmented in a non-significant manner. However, re-challenging the same patients

with HDM but after treatment with nasal sodium cromoglycate for 4 weeks, we witnessed a significant reduction in PAF and histamine but no effect on PGD2, LTC4 or CysLT as seen in Table 1. Taken collectively, these results may explain at least in part the selective reduction in neutrophilia but not in eosinophilia seen in Fig. 2. To see whether nasal sodium cromoglycate stabilized eosinophil degranulation without affecting its chemotaxis, we measured the amount of ECP released in nasal secretions. As seen in Table 1 ECP was not changed in the early phase reaction after challenge. However, after treatment with nasal sodium cromoglycate for 4 weeks re-challenging AR patients' monosensitized to HDM, demonstrated significant reduction in the amount of ECP secreted in nasal secretions.

4. Discussion

Sodium cromoglycate is a mast cell stabilizer that prevents histamine release and is used in atopic asthmatics to prevent the allergic attacks and before exercise. It is also indicated in seasonal allergic rhinitis where it is started before the season to prevent mast cells degranulation during the pollen season. Although several reports indicated the efficacy of nasal sodium cromoglycate in relieving rhinitis symptoms (the reader is referred to the review by Ratner et al. [19]), no reports studied its anti-inflammatory properties during the early phase reaction of the immunological response, in allergic rhinitis patients mono-sensitized to HDM. These patients suffer from chronic allergic inflammation all over the year with season variation in the symptoms severity. This seasonal variation in symptoms severity was recently studied by Gelardi et al. [20] who demonstrated that nasal cytology from these patients demonstrate neutrophils as the predominant cell type recovered with augmentation of both neutrophils and eosinophils from October to April. Our study was conducted early during the same period (April–May) and showed also granulocytes to be present as the dominant inflammatory cells. This indicates effector roles for granulocytes infiltrating the nasal mucosa in allergic inflammation of these patients. Interestingly, Lomusol taken 4 times daily for 4 weeks was able to reduce significantly neutrophils in the nasal cytology but had no effect on eosinophils and other inflammatory cells. This reduction in nasal neutrophilia was associated with significant improvement in nasal symptom scores especially nasal obstruction. Since the nasal obstruction is claimed to be due to nasal hyperresponsiveness against eosinophil activation, we next studied the ability of Lomusol in modulating eosinophil degranulation. Although Lomusol did not modulate eosinophil influx into nasal cytology it did reduce significantly the amount of ECP secreted in nasal secretions. This may further explain the clinical improvement of nasal obstruction in our studied patients.

We next studied the effect of Lomusol on the allergic mediators that play effector roles in attracting granulocytes to the allergic site that thence promote the chronicity of inflammation. We found that after nasal provocation with HDM allergen the amounts of histamine and PAF augmented in nasal secretions and CysLT, PGD2 & LTC4 were significantly increased in nasal secretions. This indicates that mast cells are already activated in these patients due to the chronic exposure to the allergen and that in the early phase reaction following allergen exposure the histamine and PAF further increase in amount with arachidonic acid derivatives being significantly induced. Among these mediators PAF is well known to act as a powerful chemoattractant to neutrophils, while the same role for histamine is controversial. On the other hand, PGD2 is a powerful chemoattractant for eosinophil through its CRTH2 receptor. CysLTs and LTC4 that are also from the same family of arachidonic acid derivatives as PGD2, are also capable to activate the migratory function of eosinophils. Interestingly, Lomusol was able to significantly and specifically reduce PAF and histamine release, while had no effect on PGD2, CysLT or LTC4. These further support our results of selective inhibition of neutrophils but not eosinophils in the nasal cytology of AR patients mono-sensitized to HDM allergen by Lomusol. It also may provide the mechanism by which Lomusol inhibits nasal

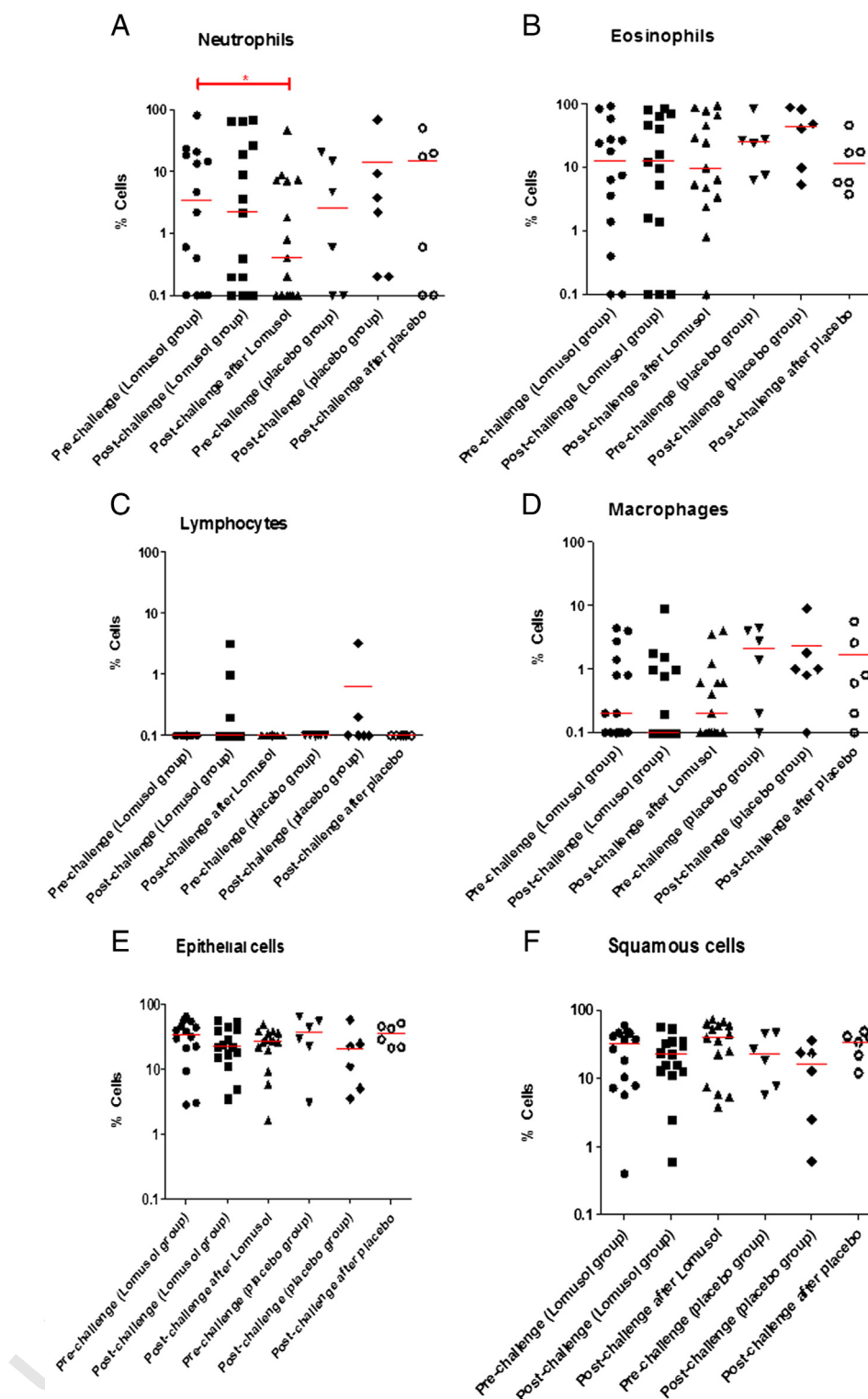


Fig. 2. Nasal cytology of AR patients mono-sensitized to HDM. Percentage of different cell types recovered in nasal cytology (A–F). There was a significant reduction of neutrophils in nasal cytology after treatment with Lomusol when compared to placebo (A). No effect of Lomusol was witnessed with other inflammatory cell types (B–D); epithelial cells (E) or squamous cells (F). Asterisk indicates $p < 0.05$ by Wilcoxon test. $N = 14$ for the treatment group (Lomusol 4%) and $N = 6$ for placebo group.

neutrophilia in these patients. It remains mandatory to carry out further studies to see whether sodium cromoglycate has also direct effects on neutrophils such as altering PAF receptors expression levels or other molecules involved in neutrophil signal transduction of chemotaxis. Other mediators that chemoattract neutrophil, and are involved in the allergic inflammation such as IL-8 should be also studied.

Taken collectively, we show the first evidence that nasal sodium cromoglycate possesses a selective inhibition on neutrophils recruitment in nasal cytology of AR patients mono-sensitized to HDM that may be attributed to the significant reduction of PAF. These results were associated with significant improvement in symptom scores especially nasal obstruction that may be also attributed at least in part to the ability of

Table 1

Effect of Lomusol on the amounts of different mediators recovered in nasal secretions, from AR patients participated in the current study who received Lomusol 4% nasal spray, as a single therapeutic agent for 4 weeks. Asterisks indicate statistical significance by Wilcoxon test.

Mediator	Pre-challenge	Post-challenge before treatment	Post-challenge after treatment	Statistical analysis
PAF	1139 pg/ml $\pm$ 164	1882 pg/ml $\pm$ 212	<370 pg/ml***	(***) $p = 0.0007$ (Between post-challenge before treatment and post-challenge after treatment)
Histamine	>50 nM	>50 nM	45.1 nM $\pm$ 12*	(*) $p = 0.03$ (Between post-challenge before treatment and post-challenge after treatment)
ECP	1.1 ng/ml $\pm$ 0.08	0.9 ng/ml $\pm$ 0.04	0.25 ng/ml $\pm$ 0.02***	(***) $p = 0.0002$ (Between post-challenge before treatment and post-challenge after treatment)
PGD2	330 pg/ml $\pm$ 461	3732 pg/ml $\pm$ 591	1886 pg/ml $\pm$ 707	$p = 0.06$ (Between post-challenge before treatment and post-challenge after treatment)
LTC4	17.3 pg/ml $\pm$ 5	45.5 pg/ml $\pm$ 45	195.4 $\pm$ 65 pg/ml	$p = 0.06$ (Between post-challenge before treatment and post-challenge after treatment)
CysLT	193 pg/ml $\pm$ 86	De >1000 pg/ml	>1000 pg/ml	$p = 0.6$ (Between post-challenge before treatment and post-challenge after treatment)

sodium cromoglycate to down-regulate eosinophil degranulation measured herein as ECP release.

In conclusion,

1. Sodium cromoglycate as a single therapeutic agent is able to improve the mild to moderate symptom scores of AR patients' mono-sensitized to HDM. This was through significant inhibition of neutrophils nasal recruitment in cytology and probably through inhibition of PAF release in nasal secretions.
2. Sodium cromoglycate could be used as a single therapeutic agent during the months of nasal neutrophilia with mild to moderate symptoms and combined with other medication that controls eosinophilic inflammation in the months of severe inflammation with mixed granulocytes nasal infiltrate.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.intimp.2015.02.004>.

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