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Sinupret® oral drops protect against respiratory epithelium atrophy in experimental acute rhinitis

Svitlana Yaremchuk[1*], Dmytro Zabolotny[1], Igor Vareniuk[2], Nikolay Makarchuk[2] and Stanislav Veselsky[3]

Abstract

Background: More than 90 % of uncomplicated upper respiratory tract infections are caused by viruses, with a risk of developing subsequent bacterial infections in some patients. The risk of development of microbial complications depends on the degree of viral damage of the structure of the respiratory mucosa and its functions.
The objective of this work was to study the effect of Sinupret oral drops on nasal mucosa atrophic changes in a rat model of experimental rhinitis.

Methods: 60 female rats Wistar were allocated for the experimental study. To study the effect of phytopreparation on atrophy of the nasal epithelium the animals were divided into 3 groups. The 1-st group ($n = 20$) was without experimental acute rhinitis. The 2-nd group ($n = 20$) was with experimental acute rhinitis and without treatment with Sinupret®. The 3-rd group ($n = 20$) with experimental acute rhinitis and treatment with Sinupret®. Experimental rhinitis was induced by placing rats in a swimming pool with cold water, followed by a draught of cool air. The effect of oral Sinupret treatment on histopathological changes in nasal mucosa was assessed on 3 and 14 days post challenge. The results were analyzed using normal or modified Student's t-test for independent groups.

Results: Histological evaluation demonstrated that Sinupret® oral drops applied during acute rhinitis attenuate atrophic and destructive changes of the ciliated epithelium.

Conclusions: Oral administration of the herbal medicinal product Sinupret oral drops weakens atrophy of the nasal mucosal ciliated epithelium in a rat model of acute rhinitis.

Keywords: Acute rhinitis; Treatment; Nasal mucosa; Herbal medicinal product

Background

Acute rhinitis is a typical feature of upper respiratory tract infections (URTI), and is the most common inflammatory disorder of the upper air passages. More than 90 % of URTI are caused by viruses, with a risk of developing subsequent bacterial infections in some patients [1]. The initial response of the nasal mucosa to the presence of a pathogen is an inflammatory reaction accompanied with mucus hypersecretion [1, 2], resulting in increased mucus viscosity and eventually an impairment of mucociliary clearance. Mucus accumulation in the air passages promotes an extended contact of viruses or bacteria with the mucosal lining of the sinuses facilitating pathogen colonization. Further sinuses and nasal congestion due to a mucosal edema provokes a drop in the tissue oxygen tension, and impairs the barrier functions of the mucosa. Impaired mucus clearance and a decrease in the partial pressure of oxygen are optimal conditions for bacterial development and infections, thus creating as a vicious circle of pathological processes [1, 2]. In the case of chronic inflammation, a diffuse metaplasia turning functional columnar ciliated epithelium into stratified multilayer epithelium occurs whereby the mucociliary transport is impaired [2–5]. The herbal medicinal product Sinupret®, composed of *Gentianae radix, Primulae flos cum calycibus, Sambuci flos, Rumicis herba* and *Verbenae herba*, is frequently used for the treatment of acute or chronic rhinosinusitis [6, 7] due to its pleiotropic

* Correspondence: yaremchuk191269@mail.ru
[1]State Institution «A.S. Kolomiychenko Institute of Otolaryngology, NAMS of Ukraine», 3, Zoologichna street, Kiev 04114, Ukraine
Full list of author information is available at the end of the article

effects directed against viral replication [8], bacterial infection, and inflammation [9]. In addition, its beneficial impact on mucociliary clearance was demonstrated *in vivo* in mice and in *in vitro* experiments using respiratory epithelial cells [10–12].

The aim of the current work was to investigate the effect of Sinupret® on the nasal mucosa, in particular the ciliated epithelium, in a model of experimentally induced acute rhinitis in rats.

Methods

Sixty female Wistar rats (body weight, 150 ± 20 g at study start) were kept in the animal housing facility under standard conditions in the Kiev National University. All the procedures were performed according to Ukrainian and International guidelines for the use of animals in research [13, 14].

After 1-week of acclimatization, the animals were randomized into 3 groups. 1st group Control (CON) (n = 20; no induction of experimental acute rhinitis (AR) and without Sinupret® (SIN) treatment); 2nd group - AR (n = 20; induction of experimental acute rhinitis without SIN treatment); 3rd group AR + SIN (n = 20; induction of experimental AR and treatment with SIN). Commercially available Sinupret® Oral Drops were used throughout the study. 100 g of Sinupret® Oral Drops (19 % ethanol (V/V)) contain 29 g of an aqueous-ethanol extract (extracting agent: ethanol 59 % (V/V; drug/extract ratio 1:11) from *Gentianae radix, Primulae flos cum calycibus, Sambuci flos, Rumicis herba* and *Verbenae herba* in the fixed ratio of 1:3:3:3:3.

SIN was orally administered to animals of the AR + SIN group at a dose of one drop corresponding to a human dose equivalent of 1.3 fold the recommended human dose. Upon allocation of the animals, experimental AR was induced by placing the rats in a swimming pool with cold water (+9 °C, 3 minutes), followed by an exposure to a draught of cool air (+15 °C) for 5 minutes. Nasal secretion began after 12 hours. Sinupret® was administered directly after inducing AR in the animals of AR and AR + SIN groups.

The animals were sacrificed by decapitation 3 and 14 days after study start ($n = 10$ group per occasion). For morphological studies at day 3 and 14, nasal epithelium was fixed in 10 % formalin solution. The samples were embedded in paraffin, cut into 5 μm-paraffin sections and stained with iron hematoxylin and hematoxylin-eosin. Digital section micrographs were obtained by a morphometric setup consisting of a microscope (PrimoStar, Carl Zeiss) and digital camera (Tucsen). Cross-sectional area of ciliated cell nuclei and goblet mucous cells and height of the olfactory epithelium were assessed.

The data of each measured parameter in all experimental groups was compared with the values from naïve control group. In addition, data of the experimental rhinitis groups (AR and AR + SIN) were compared.

Results were checked for normal distribution (Shapiro-Wilk test) and for equality of variances (Levene's test). The results were analyzed using normal or modified Student's t-test for two independent groups. Non-normally distributed data were analyzed with the Mann–Whitney U-test for two independent groups. Differences were considered statistically significant for $P < 0.05$. Data are presented as means ± standard error of the mean (SEM).

Results

The histopathological sequelae of experimental acute rhinitis in rats were assessed on days 3 and 14 by cell morphometric analysis of ciliated epithelial cells, intercalary epitheliocytes and goblet cells (Fig. 1, Table 1).

The results for the cell morphometric parameters are listed in Table 1. At day 3, animals with experimental AR without Sinupret® treatment displayed significantly reduced respiratory epithelium height compared to control animals. Sinupret® treatment (SIN + AR) significantly attenuated loss of epithelial height due to acute rhinitis induction ($p < 0.05$ vs. AR) (Table 1, Fig. 1a-c). The cross-sectional area of the nuclei of the ciliated cells was significantly reduced following induction of acute rhinitis compared to controls (Table 1, Fig. 1a-c). The nuclei in the micrographs appeared dark and hyperchromic (Fig. 1b). No significant differences were observed between groups on day 3 on the cross-sectional area of the goblet cells (Table 1, Fig. 1a-c).

After 14 days, the height of the respiratory epithelium in the AR group was still reduced and the protective effect on epithelial cells by Sinupret continued though this parameter did not reach statistical significance (not significant vs. control) (Table 1, Fig. 1d-e). Concerning the cross-sectional area of the nuclei of the ciliated cells, a significant reduction was still present in the AR group, which was less pronounced in the AR + SIN group (not significant vs. control) (Table 1). The nuclei of the AR group appeared dark and hyperchromic. The cell nuclei in the AR + SIN group were brighter, i.e. less hyperchromic (Fig. 1d-e).

In contrast to 3 day, at day 14 the cross-sectional area of the goblet cells was significantly increased in animals with acute rhinitis, independently of concomitant Sinupret® treatment (Table 1).

Discussion

We employed a rat model of experimentally induced (AR) to assess the effect of Sinupret treatment. Our data demonstrate that the clinical symptoms such as sneezing observed after the cool water/cool air protocol were associated with histopathological changes to the nasal mucosal epithelium, mainly a reduction of epithelial height and Goblet cell hypertrophy. Treatment with Sinupret

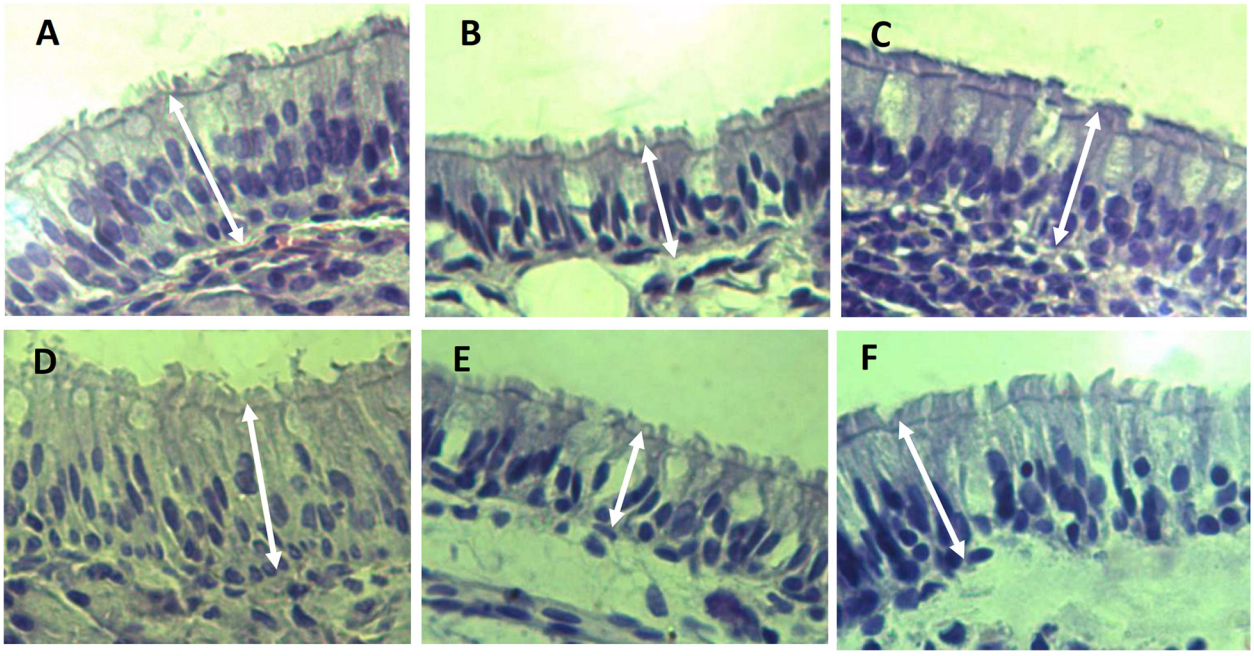

Fig. 1 REPRESENTATIVE MICROGRAPHS OF NASAL MUCOSAL EPITELIUM at day 3 (**a**: control; **b**: acute rhinitis, **c**: acute rhinitis + Sinupret) and day 14 (**d**: control; **e**: acute rhinitis; **f**: acute rhinitis + Sinupret) after induction of acute rhinitis or sham treatment. White arrows indicate the height of the epithelial layer

oral drops attenuated the decrease in epithelial height at day 3 suggesting a protective effect of treatment against the atrophy of the olfactory epithelium seen in the AR group. After 14 days, there was still a marked difference in epithelium height between control group and the AR group. However the treatment group (AR + SIN) showed no significant difference to the control at this time point. The nuclei of the ciliated cells were of normal appearance in the control group. In the AR group with induced rhinitis, the nuclei appeared dark and hyperchromic at day 3, suggesting reduced functionality. Interestingly at day 14, the nuclei remained hyperchromic. In the active treatment group (AR + SIN), the nuclei were brighter than in the AR group, suggesting a partial reversal of the acute rhinitis by the Sinupret® treatment.

The sectional area of the goblet cells increased both in the AR and AR + SIN group compared to the control. This mucosal thickening indicates hypertrophy of the cells due to the acute experimental rhinitis. The use of the herbal drug did not prevent or change the degree of hypertrophy of the mucous-producing goblet cells. Mucosal thickening has been described after experimental acute sinusitis (bacterial Staphylococcus aureus induction) in an animal model (rabbits) [15]. However, potential therapeutic effects of Sinupret on mucociliary clearance via the recently described mechanisms of hydration of airway surface liquid and stimulation of ciliary beat frequency [10, 12] were not assessed in our model which focused on histological alterations.

Table 1 Cell morphometric parameters of nasal mucosal epithelium in control (CON), acute rhinitis (AR) and acute rhinitis plus Sinupret treatment (AR + SIN) groups at day 3 and day 14 post challenge

Day	Experimental group	Height of olfactory epithelium [μm]	Cross-sectional area of ciliated cell nuclei [μm2]	Cross-sectional area of goblet cells [μm2]
Day 3	Control	40,11 ± 1,99	24,60 ± 1,32	126,52 ± 13,00
	Acute rhinitis	27,59 ± 1,13*	17,45 ± 0,75*	138,28 ± 20,34
	Acute rhinitis + Sinupret	35,84 ± 1,60$^{\#}$	18,68 ± 0,85*	164,36 ± 13,70
Day 14	Control	40,08 ± 1,87	26,49 ± 1,07	130,50 ± 11,51
	Acute rhinitis	28,82 ± 0,95*	21,66 ± 0,99*	175,26 ± 12,42*
	Acute rhinitis + Sinupret	34,77 ± 2,07	23,79 ± 1,13	174,30 ± 13,05*

Data are presented as means ± SEM from $n = 10$ animals/group
*$p < 0,05$ vs. control; $^{\#}p < 0,05$ vs. AR, Student´s t-test

It can therefore be concluded that the herbal medicinal product Sinupret® partially prevents atrophy of the olfactory epithelium present in the course of acute experimental rhinitis in rats.

Abbreviations
URTI: Upper respiratory tract infections; SIN: Sinupret®; CON: Control; AR: Acute rhinitis.

Competing interests
The authors report no conflicts of interest. The authors alone are responsible for the study design; collection, analysis and interpretation of data; content and writing of the paper; and the decision to submit the report for publication.

Authors' contributions
Conceived and designed the study and experiments: SY; DZ. Carried out the experiments: IV; SV. Contributed preparations/materials/analysis tools: IV; NM. Participated in the design of the study and performed the statistical analysis: SY; IV. Wrote the manuscript: SY; DZ. All authors read and approved the final manuscript.

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Author details
[1]State Institution «A.S. Kolomiychenko Institute of Otolaryngology, NAMS of Ukraine», 3, Zoologichna street, Kiev 04114, Ukraine. [2]Department of Cytology, Histology and Developmental Biology, Taras Shevchenko National University of Kyiv, 2, Glushkova avenue, Kyiv 03187, Ukraine. [3]Department of Human and Animal Physiology, Taras Shevchenko National University of Kyiv, 2, Glushkova avenue, Kyiv 03187, Ukraine.

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References
1. Fokkens WJ, Lund VJ, Mullol J, Bachert C, Alobid I, Baroody F, et al. EPOS 2012: European position paper on rhinosinusitis and nasal polyps 2012. A summary Otorhinolaryngol Rhinol. 2012;50 Suppl 23:1–298.
2. Eloy P, Poirrier AL, De Dorlodot C, Van Zele T, Watelet JB, Bertrand B. Actual concepts in rhinosinusitis: a review of clinical presentations, inflammatory pathways, cytokine profiles, remodeling, and management. Curr Allergy Asthma Rep. 2011;11:146–62.
3. Yan Y, Gordon WM, Wang DY. Nasal epithelial repair and remodeling in physical injury, infection, and inflammatory diseases. Curr Opin Otolaryngol Head Neck Surg. 2013;21:263–70.
4. Mynatt RG, Do J, Janney C, Sindwani R. Squamous metaplasia and chronic rhinosinusitis: a clinicopathological study. Am J Rhinol. 2008;22:602–5.
5. Soler ZM, Sauer DA, Mace J, Smith TL. Relationship between clinical measures and histopathologic findings in chronic rhinosinusitis. Otolaryngol Head Neck Surg. 2009;141:454–61.
6. Melzer J, Saller R, Schapowal A, Brignoli R. Systematic review of clinical data with BNO-101 (Sinupret) in the treatment of sinusitis. Forsch Komplementmed. 2006;13:78–87.
7. Jund R, Mondigler M, Steindl H, Stammer H, Stierna P, Bachert C, et al. Clinical efficacy of a dry extract of five herbal drugs in acute viral rhinosinusitis. Rhinology. 2012;50:417–26.
8. Glatthaar-Saalmüller B, Rauchhaus U, Rode S, Haunschild J, Saalmüller A. Antiviral activity in vitro of two preparations of the herbal medicinal product Sinupret® against viruses causing respiratory infections. Phytomedicine. 2011;19:1–7.
9. Rossi A, Dehm F, Kiesselbach C, Haunschild J, Sautebin L, Werz O. The novel Sinupret® dry extract exhibits anti-inflammatory effectiveness in vivo. Fitoterapia. 2012;83:715–20.
10. Kreindler JL, Chen B, Kreitman Y, Kofonow J, Adams KM, Cohen NA. The novel dry extract BNO 1011 stimulates chloride transport and ciliary beat frequency in human respiratory epithelial cultures. Am J Rhinol Allergy. 2012;26:439–43.
11. Virgin F, Zhang S, Schuster D, Azbell C, Fortenberry J, Sorscher EJ, et al. The bioflavonoid compound, Sinupret, stimulates transepithelial chloride transport in vitro and in vivo. Laryngoscope. 2010;120:1051–6.
12. Zhang S, Skinner D, Hicks SB, Bevensee MO, Sorscher EJ, Lazrak A, et al. Sinupret activates CFTR and TMEM16A-dependent transepithelial chloride transport and improves indicators of mucociliary clearance. PLoS One. 2014;9:1–8.
13. Law of Ukraine of 21.02.2006 № 3447-IV «On protection of animals from cruelty» // Supreme Council of Ukraine. 2006;27:230
14. European convention for protection of vertebrate animals used for experimental and other scientific purpose: Council of Europe. 18.03.1986. – Strasbourg, 1986; 1–52
15. Ozcan KM, Ozcan I, Selcuk A, Akdogan O, Gurgen SG, Deren T, et al. Comparison of histopathological and CT findings in experimental rabbit sinusitis. Indian J Otolaryngol Head Neck Surg. 2011;63:56–9.