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Histomorphological Study of a New Nasal Spray with Anti-inflammatory Properties Efficacy in Rabbits with Rhinosinusitis

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ABSTRACT

Introduction: The high world prevalence of rhinosinusitis (RS) initiates the ways of a favorable search for effective and safe medicines for its pathogenetic treatment. The important part of this process is the choice of the most comfortable dosage form, which will enhance therapeutic compliance and ensure the appropriate medicine efficacy and safety. **Aim:** To substantiate the efficacy of a new nasal spray with anti-inflammatory properties containing Enisamium Iodide (EI) at a concentration of 10 mg/mL by histomorphological study of the nasal cavity and paranasal sinuses mucosal in rabbits with experimental rhinosinusitis (ERS). **Methods:** EI (nasal spray) was a test object. Sinupret® was a reference drug. ERS was induced in rabbits on the first day of the study by tamponade of the right half of the nasal cavity under general anesthesia. The study was performed using 24 rabbits (4 groups, 6 rabbits in each group). The histomorphological examination was performed on the 25th day of the study by the standard light microscopy methods. **Results:** The histomorphological examination of EI 10 mg/mL (nasal spray) impact on RS in rabbits, which administered during 10 days intranasally, revealed the significant therapeutic effect presented by reduced inflammation signs in the epithelium of the nasal cavities and paranasal sinuses mucosal. Besides, the EI impact was not inferior to the reference drug Sinupret® in tablets. The study of the pharmacological properties of the EI (nasal spray) on ERS showed the high rate of onset of EI actions when used intranasally which was superior to the rate of actions of the reference drug Sinupret® (tablets) administered intragastrically. **Conclusion:** The EI (nasal spray) is a promising drug for a pathogenetic therapy of acute RS, which demands further pre-clinical and clinical studies aiming to substantiate its implementation to the clinical practice.

Keywords: Enisamium Iodide (nasal spray), Experimental Rhinosinusitis, Histomorphological Study, Anti-Inflammatory Effect.

1. INTRODUCTION

The enhancement of acute upper airways inflammatory disease therapy, particularly, acute rhinosinusitis (ARS) is an important issue of modern clinical medicine. The global prevalence of rhinosinusitis (RS) is 6-15 %. Over the last years, the nose and paranasal sinuses diseases incidences have been increasing which results in the growth of both absolute (morbidity and prevalence) and relative (% of total ENT pathologies) indices (1, 2). The Center for Health Statistics of the Ministry of Health (Ukraine) reported that the prevalence of ARS, RS, and rhinopharyngitis in Ukraine approached 489.9 cases per 10 000 persons and the prevalence is 5–15 cases per 10 000 persons depending on the season. These pathologies are showed

by 60-65 % outpatient treated by otolaryngologists.

According to the epidemiological data as well as expert opinions of the prominent world clinicians and scientists, ARS remains to be the leading disease in the outpatient practice, which significantly decreases the quality of life as well as makes a great socio-economic impact in society (3-6).

The choice of the best medicine is only one component of ARS treatment efficacy. It should be accompanied by providing a patient with complete information about the mechanism of action and rules of chosen drug use (route of administration, single and daily doses), the potential side effects, the interactions with other drugs and meals. There are no «good» or «bad» med-

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icines. Even the most effective drug might do not show the required effect because of inappropriate choice and poor competence regarding the rules of administration. Addressing this issue will enhance not only the efficacy but also the safety of ARS treatment.

Farmak JSC (Ukraine) has developed a new original dosage form of a nasal spray containing an aqueous solution of a well-known pharmaceutical substance Enisamium Iodide (EI) that is a derivate of an isonicotinic acid and belongs to the group of non-steroid anti-inflammatory drugs. The pre-clinical studies showed the anti-inflammatory (35.3 %) and analgesic (43.4 %) effects of the EI spray, which were the most pronounced at the concentration of 10 mg/mL (7-9). The analysis of the pre-clinical study results justifies that the efficacy of the EI 10 mg/mL (nasal spray) is based on the local anti-inflammatory action caused by the specificity of its pharmacodynamics and the intranasal route of administration. Thus, EI 10 mg/mL (nasal spray) is a promising medicine for pre-clinical studies and clinical trials aiming to substantiate the reasonability of its use for ARS treatment and prevention of its complications well as further implementation of this medicine to the clinical practice.

2. AIM

The aim of our study was to substantiate the efficacy of a new nasal spray containing EI at a concentration of 10 mg/mL by the histomorphological study of the nasal cavity and paranasal sinuses mucous membrane in rabbits with experimental rhinosinusitis (ERS).

3. MATERIAL AND METHODS

The test object was a new nasal spray containing EI (Farmak JSC, Ukraine) with active substance concentration 10 mg/mL. This concentration was chosen considering the results of the previous stage of the EI (nasal spray) experimental study (7, 9). The nasal spray was used at the dose of 0.1 mL intranasally (i.n.), which corresponds to the dose 0.03 mL/kg considering an average weight of a rabbit at the beginning of the study (around 3.0 kg). According to the FDA recommendations on dose extrapolation (10), this dose corresponds to 0.6 mL for a human, i.e. 0.2 mL (twice pressing of the dosing valve) per 3 times a day. EI 10 mg/mL (nasal spray) was administered in an unchanged state into the right rabbit nasal passage using Biohit Proline fixed volume pipettor 200 µl with Biohite pipettor tips (11). 0.9% saline in an equivalent dose and route of administration was used in the intact control and control pathology groups.

The reference drug was Sinupret® («Bionorica SE», Germany) in coated tablets for oral use containing 78 mg of native herbal extract in each tablet (Rumex acetosa, Sambucus nigra, Primula veris, Verbena Officinalis, Gentiana lutea). The Sinupret® was used at a dose of 25 mg/kg, which under FDA recommendations (10) corresponds to the 480 mg dose for human or 6 tablets, i.e. 2 tablets 3 times a day that is equal to the recommended dosage regime indicated in the drug label (12). The Sinupret® was administered to rabbits orally (intragastrical-

ly) as a water suspension via a special catheter with an elastic cannula.

Twenty-four white New Zealand rabbits of both sexes aged around 90-110 days weighing 2.5–3 kg were used to induce the experiment. The test animals were kept in the Vivarium of the Central Research Laboratory, National University of Pharmacy (Kharkiv, Ukraine) following the sanitary standards and the required diet. All researches were conducted in accordance with the Directive 2010/63/EU on compliance with the laws, directives and administrative regulations of the EU countries on the protection of animals used for scientific purposes (13). The Bioethics Commission of the National University of Pharmacy (Kharkiv, Ukraine) approved study design (Protocol No 3 of 15 March 2017).

All animals were randomly allocated into four experimental groups (6 rabbits in each group) as follows:

- Intact control group – healthy animals treated with 0.9% saline 0.1 mL i.n.;
- Control pathology group – rabbits with ERS treated with 0.9% saline 0.1 mL i.n.;
- EI treated group – rabbits with ERS treated with EI 10 mg/mL (nasal spray) 0.1 mL i.n.;
- Sinupret treated group – rabbits with ERS treated with Sinupret® (coated tablets) at 25 mg/kg intragastrically.

ERS was induced in rabbits on the first day of the study by tamponade of the right half of the nasal cavity under general anesthesia with Ketamine/Xylazine (35/5 mg/kg, intramuscularly) (14) which is a well-known model of RS in experimental rhinology (15, 16). After 15 days of pathology inducement, sponges were removed and RS manifestation was determined by the endoscopic method (17). Starting from the 15th day and during the further 10 days, animals received the research medicines.

At the end of the study on the 25th day, animals were euthanized under general anesthesia in compliance with bioethical standards of conducting experiments with laboratory animals (18). In addition, the nasal cavity and paranasal sinuses tissues were obtained for further macroscopic and histomorphological examination.

The histomorphological examination of the right half of the nasal cavity was performed on the 25th day of the study by the standard light microscopy methods (19-22). The microscopic examination, the histomorphological analysis and image taking were performed using bright-field microscope B-1000BF (Optika, Italy) with a digital camera Optikam HDMI Pro (Optika, Italy). Digital image processing of the slide photos, as well as the measurement of various elements, was performed using the software designed by Optika (Italy) – Optika Is View and Optika Vision Lite. During the morphometrical analysis of the microscope slides, the thickness of the epithelium was measured in µm at x200 magnification. The average number of goblet exocrinocytes (goblet cells) was calculated in the one mm² area on the four different sectors of each slide stained by Periodic Acid-Schiff.

The scale for the histomorphological assessment and the method of semi-quantitative score assessment of certain parameters was used to ensure the objectivity of the

A histological sign	Points
Inflammatory cell infiltration	
Physiological state (0-10 goblet cells in FoV1 with an increase in $\times 400$)	0
Focal infiltration (10-30 goblet cells in FoVa with an increase in $\times 400$)	1
Moderate infiltration (30-50 goblet cells in FoVa with an increase in $\times 400$)	2
Severe infiltration (more than 50 goblet cells in FoVa with an increase in $\times 400$)	3
Mucous oedema	
No changes (physiological state)	0
Focal subepithelial oedema	1
Diffuse subepithelial oedema	2
Diffuse large oedema	3

Table 1. The scale for the histomorphological assessment of the nasal cavity and paranasal sinus mucosal damage in rabbits with experimental rhinosinusitis Note. aFoV – field of view.

study results. A range of total scores was assessed which represented the damage extent of the nasal cavity and paranasal sinuses mucosal basic structures (Tables 1, 2).

Statistical analysis of the results was performed using the Kruskal-Wallis test and Mann-Whitney U test for a posteriori pairwise comparisons (23, 24). Utilized computer software included IBM SPSS Statistics v. 22 (IBM Corp., USA) and MS Excel 2016 (Microsoft Corp., USA). The level of statistical significance was considered as $p < 0.05$.

4. RESULTS

The results of the morphometrical analysis of the microscope slides obtained from the right half of the nasal cavity and paranasal sinus mucosal are listed in Table 2.

The intact control group

The intact control group microscopic slides examination revealed the morphology that corresponds to the modern concepts regarding of the nasal cavity and paranasal sinuses mucosal structure. The maxillary sinus mucosal has been lined with the respiratory-type (i.e. multilayered ciliated) epithelium of varied thickness. The maxillary sinus ostium has been lined with the epithelial cells of the greatest height with the remarkable although not always

pronounced brush border. This epithelium gradually has turned to the lamina propria of the mucosal with a few numbers of glands (Figure 1 A).

The epithelium thickness varied from 10 to 50 μm and averaged 20.59 μm . The number of goblet exocrinocytes was a few – 5.34 cells per 1 mm^2 (Table 2).

The total score calculated for the intact group on the 25th of the study was 0 (0 ÷ 0) which corresponds to the physiological state (Table 3).

Control pathology group

The histomorphological examination of the control pathology group revealed significant morphological changes in the right maxillary sinus, which underwent a tamponade of the respective nasal passage.

Firstly, the changes have been observed in the respiratory-type epithelium which state expectedly deteriorated after air exchange impairment. Pathological changes of varied significance have been shown in the epithelium line along its whole length.

In some areas of the slides, the epithelium though has been observed but has poor phenotypic traits as a dystrophy result. It has become swollen, nuclei have been lined up, the brush border has been almost lacking, and the connection with the basement membrane has been impaired. There has been shown the tendency to the desquamation of the whole epithelium (Figure 1 B).

Animal groups	Epithelium thickness, μm	Goblet exocrinocytes density, cells / mm^2
Intact control (n=6)	20.59 $\pm$ 0.86	5.34 $\pm$ 0.28
Control pathology (n=6)	30.45 $\pm$ 1.34 ^c	18.13 $\pm$ 1.10 ^c
Ele treated group (n=6)	21.54 $\pm$ 0.71 ^d	6.58 $\pm$ 0.44 ^d
Sinupret treated group (n=6)	24.62 $\pm$ 1.04 ^{c, d}	12.11 $\pm$ 0.89 ^c

Table 2. The morphometric parameters of the nasal cavity and paranasal sinus mucosal in rabbits with experimental rhinosinusitis (nb=24). Notes: an – number of the animals in experimental group; b – total number of the experimental animals; c – differences are significant compared to the intact control group ($p < 0.05$); d – differences are significant compared to the control pathology group ($p < 0.05$); e – Enisamium Iodide.

Experimental groups	Number of the experimental animals / relative percentage of micropreparations								The total score (the median)
	Inflammatory cell infiltration of mucosal points				Mucous edema points				
	0	1	2	3	0	1	2	3	
Intact control group (na=6)	6 / 100%	0	0	0	6 / 100%	0	0	0	0 (0 ÷ 0)
Control pathology group (na=6)	0	1 / 17%	4 / 66%	1 / 17%	0	2 / 33%	3 / 50%	1 / 17%	4.0c (2 ÷ 6)
Ele treated group (na=6)	2 / 33%	3 / 50%	1 / 17%	0	3 / 50%	3 / 50%	0	0	1.5c, d (0 ÷ 3)
Sinupret treated group (na=6)	1 / 17%	2 / 33%	3 / 50%	0	1 / 17%	4 / 66%	1 / 17%	0	2.0c, d (1 ÷ 4)

Table 3. The semi-quantitative assessment of the nasal cavity and paranasal sinus mucosal state in rabbits with experimental rhinosinusitis according to the histomorphological evaluation (nb=24) Notes: an – number of the animals in experimental group; b – total number of the experimental animals; c – differences are significant compared to the intact control group ($p < 0.05$); d – differences are significant compared to the control pathology group ($p < 0.05$); e – Enisamium Iodide.

Figure 1. Histomorphological structure of the right half of the nasal cavity mucosal (experimental rhinosinusitis, 25th day of the study): A – intact control group; B – control pathology group; C – EIa treated group; D – Sinupret treated group. Note: a – Enisamium Iodide.

Notably, the mean epithelium thickness was 1.5 times as much as the one of the control group with a significant difference (Table 2) and the brush border was lacking over the sections of varying length. According to the results of the semi-quantitative assessment, the total score was 4.0 ($2 \div 6$) on the 25th day (Table 3). All of this is significantly greater than in the intact control group ($p < 0.05$ vs. intact control group).

EI treated group

The histomorphological study of microscope slides of the nasal cavity tissues of rabbits with ERS receiving EI 10 mg/mL (nasal spray) i.n. has shown that the test drug significantly reduces ERS progression and had a positive therapeutic effect. Epithelial cells have been orderly. Neither dystrophic nor necrotic processes have affected the process (Figure 1 C).

There was a significant decrease in goblet cell numbers and the mucus-producing amount. All of this is significantly greater than in the control pathology group ($p < 0.05$ vs. intact control group) (Table 2). According to the results of the semi-quantitative assessment, the total score on the 25th day was 1.5 ($0 \div 3$) (Table 3). All of this is significantly greater than in the intact control and control pathology groups ($p < 0.05$ vs. intact control and control pathology groups).

Sinupret treated group

Microscope slides histomorphological study of the nasal cavity tissues of rabbits with ERS received Sinupret® tablets (orally) has revealed that the reference drug also influences positively the course of pathology, reduces ERS progression, and has a positive therapeutic effect. However, this effect was inferior to EI impact in the dosage form of a nasal spray.

The thickness of sinuses epithelium in the Sinupret treated group although did not approach the control parameters but was lower compared to the respective parameters of the intact control group ($p < 0.05$ vs. intact control group) (Table 2).

Over the quite long sections, there have been shown the complete epithelium and the evident brush border. However, over the numerous sections, the morphological structure of the epithelial layer differed significantly from the normal state.

Mucous cysts, tissue depletion (goblet exocrinocytes number was 2.2 times as much as the one of the intact control group with a statistical difference), the brush border was not seen, desquamation – all these processes did not differ significantly from the control pathology group (Figure 1 D).

According to the results of the semi-quantitative assessment, the total score on the 25th day was 2.0 ($1 \div 4$) (Table 3). All of this is significantly greater than in the

intact control and control pathology groups ($p < 0.05$ vs. intact control and control pathology groups).

5. DISCUSSION

The histomorphological examination has justified that tamponade of the right rabbits' nasal passage results in the development of ARS on the 15th day of the study accompanied by all morphological signs of inflammatory and destructive damages of the epithelium in the nasal cavity and paranasal sinus mucosal. Evaluation of the microscope slides of intact animals has shown the morphology that corresponds to the modern concepts regarding the structure of the nasal cavity and paranasal sinus mucosal (19-22).

The intranasal administration of EI 10 mg/mL (nasal spray) for 10 days caused a significant positive effect on the course of the induced pathological process and showed reducing signs of the inflammatory infiltration, swelling and destruction of epithelial cells. Besides, the EI impact was not inferior to the reference drug Sinupret®, which is the well-known medicine with evidence-based efficacy used for RS treatment in the national and global clinical practice (25).

The analysis of the obtained data showed that anti-inflammatory action of the test drug and the intranasal route of its administration cause the high efficacy of EI. This specific feature is an advantage of EI compared to Sinupret®, which has a lower anti-inflammatory effect and the lack of local action because of the oral route of administration. This drug has systemic, mostly secretolytic activity. Moreover, the obtained results show a higher rate of onset of EI actions when used i.n. compared to the use of the reference drug.

6. CONCLUSIONS

The histomorphological examination of Enisamium Iodide 10 mg/mL (nasal spray) efficacy in rabbits with ERS, revealed the significant effect presented by reduced inflammation signs in the epithelium of the nasal cavities and paranasal sinus mucosal. Besides Enisamium Iodide's impact was not inferior to the reference drug Sinupret® in tablets.

The study results analysis showed that the efficacy of the Enisamium Iodide 10 mg/mL (nasal spray) for RS treatment is based on anti-inflammatory action caused by its pharmacodynamics and the intranasal route of administration. This is an advantage of Enisamium Iodide compared to Sinupret® (tablets) which has a lower anti-inflammatory effect and the lack of local action because it was administered orally.

The Enisamium Iodide (nasal spray) is a promising drug for further pre-clinical and clinical studies aiming to substantiate the reasonability of its use for ARS treatment and prevention of ARS complications and as well as its further implementation to the clinical practice.

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- **Author's contribution:** I.Z., T.Zh., and S.Sh. gave substantial contribution to conception and design of the research study; T.Zh., and S.Sh. gave substantial contribution to acquisition of data. T.Zh. and I.O. have drafted the article. All authors had a substantial contribution to analysis and interpretation of data, revising the article critically for important intellectual content and final approval of the version to be published. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
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REFERENCES

1. Fokkens WJ, Lund V, Mullol J. European position paper on rhinosinusitis and nasal polyps 2012. *Rhinology*. 2012; 50 (SUPPL. 23): 1-298.
2. Chow AW, Benninger MS, Brook I, Brozek JL, Goldstein EJC, Hicks LA, et al. Executive Summary: IDSA Clinical Practice Guideline for Acute Bacterial Rhinosinusitis in Children and Adults. *Clin Infect Dis*. 2012; 54 (8): 1041-1045. doi: 10.1093/cid/cis370.
3. Passali D, Cambi J, Bellussi LM, Passali FM. Phytoneering: A new way of therapy for rhinosinusitis. *Acta Otorhinolaryngol Ital*. 2015; 35(1): 1-8. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4443571/>
4. Koch AK, Klose P, Lauche R, Cramer H, Baasch J, Dobos GJ, et al. A systematic review of phytotherapy for acute rhinosinusitis. *Forsch Komplementarmed*. 2016; 23(3): 165-169. doi:10.1159/000447467.
5. Zupanets IA, Zhulai TS, Sakharova TS, Andrieieva OO. The significance of selecting phytopreparations for individualized therapy of acute rhinosinusitis. *Klinična Farm*. 2017; 21(4): 52-58. doi: 10.24959/cphj.17.1444.
6. Zupanets IA, Popovich VI, Zhulai TS, et al. Pharmaceutical care of patients in acute rhinosinusitis treatment. *Method. recommend. Zolde pages*, Kharkiv 2019; 1-36. [in Ukrainian]
7. Zhulai TS, Shebeko SK, Zupanets IA. The study of the dose-dependent antiexudative effect of a new anti-inflammatory nasal spray. *Klinična Farm*. 2017; 21(4): 11-17. doi: 10.24959/cphj.17.1446.
8. Zhulai TS. Preclinical study of a new nasal spray with anti-inflammatory properties: the effect on the leukotriene-induced inflammation. *Klinična Farm*. 2018; 22(4): 27-33. doi: 10.24959/cphj.18.147-
9. Zhulai T, Shebeko S, Goy A. Perspectives for use of the new nasal spray with anti-inflammatory action in treatment of acute rhinosinusitis. *Rhinology*. 2018; 56(27): 585. Available from: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:C:2012:326:TOC%0Ahttp://eur-lex.europa.eu/legal-content/EN/ALL/?uri=OJ:L:2009:300:TOC>.
10. Guidance for industry. Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. FDA. 2005; 1-30.
11. Turner PV, Pekow C, Vasbinder MA, Brabb T. Administration of Substances to Laboratory Animals: Equipment Considerations, Vehicle Selection, and Solute Preparation. *Journal of the American Association for Laboratory Animal Science*

- ence 2001; 50 (5): 614-627.
12. Ministry of Health of Ukraine, Order No 509 of 12 May, 2017. On state registration (re-registration) of medicinal products (medical immunobiological preparations) and amendments to the registration materials. Available from: http://search.ligazakon.ua/l_doc2.nsf/link1/MOZ27507.html [in Ukrainian]
13. Parliament, European and Council, European. (2010). DIRECTIVE 2010/63/EU on the protection of animals used for scientific purposes. EU Official Journal. L276.
14. Flecknell PA. Laboratory Animal Anesthesia, 4th ed. Oxford: Academic Press 2015; 1-350. doi: 10.1016/C2013-0-13494-0
15. Perez A.C, Buzatto G.P, de Picolo Dantas I, Vicente J. Review of experimental models: sinusitis in rabbits. *Braz J Otorhinolaryngol.* 2014; 80(5): 435-440.
16. Costa HO, Ruschi e Luchi GE, Guilherme Augusto, Castro M, Coelho de Souza F. Comparative study of several sinusitis experimental modelling techniques in rabbits. *Rev Bras Otorrinolaringol* 2007; 73(5): 627-631.
17. Colley P, Mace JC, Schaberg MR, Smith TL, Tabaee A. Impact of educational intervention on the interrater agreement of nasal endoscopy interpretation. *Laryngoscope.* 2015; 125(10): 2259-2265.
18. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring No 20. Guidance Document for Receiving Authorities on the Review of the GLP Status of Non-Clinical Safety Studies. ENV/JM/MONO 2019; 25.
19. Hem A, Kormak D. Histology. Moscow: Mir. Publ. 1983; V. 3. [in Russian].
20. Yushkantseva SI. Histology, Cytology and Embryology - A Short Atlas. SPb: P-2 Publ. 2007. [in Russian].
21. Kuznetsov SL. Histology, cytology and embryology. Moscow: Med. News Agency. 2007. [in Russian].
22. Sarkisova DS, Perova YuL. Mikroskopicheskaya tehnika . Mosc.: Med. 1996. [in Russian].
23. Islam MA, Al-Shiha A. Foundations of Biostatistics. Springer Nature Singapore Ltd. 2018.
24. Quirk TJ, Quirk MH, Horton HF. Excel 2016 for Biological and Life Sciences Statistics: A Guide to Solving Practical Problems. Springer International Publishing Switzerland, 2016.
25. Palm J, Steiner I, Abramov-Sommariva D. et al. Assessment of efficacy and safety of the herbal medicinal product BNO 1016 in chronic rhinosinusitis. *Rhinology.* 2017; 55(2): 142-151. doi: 10.4193/Rhin16.103.