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Original Research Article

Lysozyme addition to slightly hypertonic nasal spray in the treatment of acute nasopharyngitis: a prospective post-marketing study

Zumreta Pintol[1], Aziz Sukalo[2], Meliha Mehic[2], Amna Tanovic Avdic[2], Rusmir Baljic[3], Una Glamoclija[2,4]*

[1]Dr. Abdulah Nakas General Hospital, Sarajevo, Bosnia and Herzegovina
[2]Bosnalijek JSC, Sarajevo, Bosnia and Herzegovina
[3]University Clinical Centre Sarajevo, Sarajevo, Bosnia and Herzegovina
[4]University of Sarajevo, Sarajevo, Bosnia and Herzegovina

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***Correspondence:**
Dr. Una Glamoclija,
E-mail: una.glamoclija@bosnalijek.com

ABSTRACT

Background: Acute nasopharyngitis is often treated with hypertonic saline that can be combined with additional compounds, such as lysozyme. The aim of this study was to compare efficacy and safety of hypertonic saline solution with or without lysozyme in the treatment of acute nasopharyngitis.
Methods: Non-interventional, prospective, multicentre, observational, parallel study was conducted on adult subjects with symptoms of acute nasopharyngitis. Subjects were divided into hypertonic saline or lysozyme group (receiving slightly hypertonic nasal spray with addition of lysozyme). Time until the patency of both nasal passages was measured after the first application of therapy. The congestion severity was assessed by using a visual analogue scale before the therapy application, after 30 minutes, and after seven days. Adverse reactions were monitored and evaluated.
Results: The total number of included subjects was 252 (60 in the hypertonic saline group and 192 in the lysozyme group). In both groups, a significantly better assessment of the severity of the nasal passages' obstruction was recorded after 30 minutes and seven days from therapy start (for all compared time intervals $p<0.001$). The lysozyme group had a significantly lower nasal congestion score compared to hypertonic saline 30 minutes after therapy ($p<0.001$) and seven days from the therapy start ($p=0.001$). In the hypertonic saline group, a significantly shorter time was observed to establish the patency of the nasal passages after the first therapy application ($p<0.001$). All adverse events were mild.
Conclusions: Addition of lysozyme to slightly hypertonic nasal spray brings added value in the pharmacotherapy of acute nasopharyngitis.

Keywords: Acute nasopharyngitis, Comparative study, Hypertonic saline, Lysozyme, Nasal congestion, Pharmacotherapy

INTRODUCTION

Acute nasopharyngitis is an upper respiratory infection usually caused by viruses with nasal congestion present in about 80% of patients.[1] Reduction of inflammation and nasal edema is crucial for successful treatment of nasal congestion characterized by swelled mucosa and airflow blockage. Treatment options include topical and systemic preparations. Various compositions of saline for nasal application are used. Those include isotonic and hypertonic solutions that are often enriched with specific compounds to achieve additional benefits.[2] Among those,

molecules that are naturally a part of nasal mucosa immune defense could play a significant role. Nasal secretions contain numerous compounds, including antimicrobial proteins where lysozyme and lactoferrin are among the most abundant ones. Lysozyme is secreted by nasal submucosal glands under control by parasympathetic nervous system. It plays important roles in immune response of nasal mucosa.[3] Together with secretory immunoglobulins, lysozyme is component of mucosal blanket involved in removal of particles from the nose.[4] Also, lysozyme has direct anti-pathogen effects in nasal secretions.[5] Hen egg white lysozyme directly promotes the ciliary beats in human nasal mucosa.[6] Certain conditions and treatments can decrease lysozyme activity in nasal secretions. Although Tewfik et al found that precursor of lysozyme is downregulated in subjects with chronic rhinosinusitis[7], there are still controversies regarding the concentrations of lysozyme in those patients.[5] High variations in lysozyme mRNA expression between healthy controls indicate interaction of different factors influencing lysozyme levels.[8] Woods et al found that isotonic saline irrigation can cause short decrease in lysozyme and lactoferrin levels that is reverted within six hours.[9] However, Harcourt-Smith did not find changes in lysozyme levels after initial application of isotonic or low salt irrigations, but found increased levels of lysozyme after 14 days of daily irrigations with low salt solutions.[10]

Due to its physiological roles and variations in nasal fluids, lysozyme addition to nasal sprays brings added value in the therapy of nasal congestion in acute nasopharyngitis. Although there are commercially available nasal sprays containing lysozyme, additional research is needed to explore the full potential of those compositions.

The aim of this study was to evaluate the efficacy and safety of hypertonic saline solution compared to slightly hypertonic nasal spray with lysozyme in the treatment of acute nasopharyngitis.

METHODS

Study population and data collection

The non-interventional, multicenter, observational, parallel, cohort study of efficacy and safety of hypertonic saline solution compared to slightly hypertonic nasal spray with lysozyme was conducted on adult subjects of both sexes treated in health institutions in Bosnia and Herzegovina between October 2019 and December 2020. The study was approved by the Agency for Medicinal Products and Medical Devices of Bosnia and Herzegovina. Inclusion criteria were symptoms of acute nasopharyngitis that did not last longer than 7 days and a congestion symptom score of 1 or more (on a scale from 0 to 3, where 0 is a completely open nose for breathing, and 3 is a complete blockage of both nasal passages with mouth breathing). Exclusion criteria were allergy to any ingredient of applied nasal spray, cardiovascular, endocrinological, respiratory diseases, severe septal deviation or nasal polyps, previous surgery on the nasal passages or sinuses, taking medications that may affect congestion (such as systemic steroids, intranasal medications), symptoms of nasopharyngitis that lasted longer than 7 days, recently used nasal decongestants, pregnancy and breastfeeding.

After signing the informed consent, subjects were included into the study and divided into two groups: hypertonic saline (receiving hypertonic saline of various producers in the osmolality range 750 to 1000 mOsm/kg) or lysozyme group (receiving Lynase® nasal spray, producer Bosnalijek JSC, Bosnia and Herzegovina, containing 0.5 mg/ml hen egg white lysozyme hydrochloride and 0.9% NaCl but slightly hypertonic with osmolality 400 mOsm/kg due to other ingredients). Application of nasal sprays was performed according to approved instructions for use. Subjects received therapy as part of their routine medical care. The doctors who normally treat the respondents recommended therapy at their own discretion independently of the study. Monitoring of subjects lasted 7 days, during which there were two visits: at the baseline and after 7 days. On baseline demographic data and comorbidities were recorded. On both visits side effects and serious side effects were recorded. Side effects were coded according to Standardized Medical Dictionary for Regulatory Activities® (MedDRA®) version 26.1. The severity of congestion was assessed based on a visual analogue scale (VAS) before the application of the medicinal product, 30 minutes after application, and 7 days after the therapy start. The time until the patency of both nasal passages after application of the nasal spray was measured with the help of a stopwatch.

Statistical analysis

Descriptive statistics (absolute and relative numbers) was used for the presentation of results. Normality of data distribution was evaluated with the Kolmogorov-Smirnov test. Similarity of variances was assessed by Levene's test for equality of variances, and then a t-test was used to compare BMI between groups. Age, nasal congestion score, and time required for complete nasal decongestion were compared by Mann-Whitney U test. Sex and comorbidities between groups were compared by the Pearson’s chi-square test. To assess the difference in the nasal congestion score in three-time intervals, the Friedman test was used, followed by the comparison between the two measurement times using Wilcoxon signed-rank test with Bonferroni correction of the p value ($p<0.017$ was calculated as a statistically significant value). $P<0.05$ was a value that was accepted as a statistically significant difference, except in the case of tests where the Bonferroni correction was applied. Statistical analysis was performed using the SPSS (Statistical Package for Social Sciences) program, version 23.0.

RESULTS

Study population and data collection

The total number of included subjects was 252, of which 60 were in the hypertonic saline group and 192 in the lysozyme group. Age (U=5086.5, p=0.230), sex ($\chi2(1)=0.221$, p=0.658) and BMI (t (237)=1.029, p=0.304) were similar between groups. A higher percentage of subjects in the hypertonic saline group had significant organic disease ($\chi2(1)=8.592$, p=0.004) (Table 1).

Table 1: Measured parameters before the use of the therapy, 30 minutes after the therapy and 7 days after the start of the therapy.

Parameter	All subjects (n=252)	Hypertonic saline group (n=60)	Lysozyme group (n=192)	P value hypertonic saline vs lysozyme group
	N (%)	N (%)	N (%)	
Age in years (interquartile range)	40 (29-54)	43 (31-56)	40 (28-53)	0.230
Sex, male	114 (45)/ 138 (55)	26 (43)/ 34 (57)	88 (46)/ 104 (54)	0.658
Body mass index (BMI) (±SD)	25.4 (±3.5)	25.0 (±3.4)	25.5 (±3.5)	0.304
Significant organic disease	44 (17)	18 (30)	26 (14)	0.004
Cardiovascular	18 (7)	11 (18)	7 (4)	
Respiratory	5 (2)	1 (2)	4(2)	
Hepatobiliary	0 (0)	0 (0)	0 (0)	
Gastrointestinal	3 (1)	0 (0)	3 (1)	
Another disease	17 (7)	6 (10)	11 (6)	
Unknown	1 (0)	0 (0)	1 (1)	
Nasal congestion score on a scale from 0 (no obstruction) to 3 (nose completely blocked)				
Initial visit, Before using the therapy	2.0 (2.0-3.0)	2.0 (2.0-2.3)	2.0 (2.0-3.0)	0.200
Initial visit, 30 minutes after using the therapy	1.0 (1.0-1.8)	1.0 (1.0-2.0)	1.0 (0.0-1.0)	<0.001
Seven days after the initial visit	0.0 (0.0-1.0)	0.0 (0.0-1.0)	0.0 (0.0-0.0)	0.001
The time required for the complete unclogging of the nose after the first application of the therapy (minutes)	5.0 (3.0-10.5)	3.5 (1.0-10.0)	6.0 (4.0-14.0)	<0.001

Efficacy of therapies

Although both groups had a similar nasal congestion score before therapy (U=5189, p=0.200), the lysozyme group had a significantly lower nasal congestion score 30 minutes after therapy (U=3542, p<0.001) and 7 days from the start of therapy (U=4417, p=0.001) when compared to hypertonic saline group. In both groups, a significantly decreased nasal passages' obstruction was recorded 30 minutes after the application of the therapy compared to the period before the application of the therapy, and seven days after the therapy start compared to both periods: before the application of the therapy and 30 minutes after the therapy application (for all compared time intervals p<0.001). In the hypertonic saline group, a significantly shorter time to establish the patency of the nasal passages after the first application of the therapy was observed (U=3987, p<0.001) (Table 1).

Safety of therapies

Not a single patient had a deterioration in the condition of the nasal passages, pharynx and oral cavity, larynx, external ear canal and eardrum during the examination. Adverse events were mild. In hypertonic saline group one adverse reaction of epistaxis – SOC respiratory, thoracic, and mediastinal disorders was recorded. In lysozyme group one adverse reaction of nose burning during and after first application of nasal spray (Nasal burning – SOC Respiratory, thoracic, and mediastinal disorders) was recorded.

DISCUSSION

Both evaluated nasal sprays (hypertonic saline and slightly hypertonic solution enriched with lysozyme) were effective and safe in the treatment of nasal congestion in acute nasopharyngitis. In both groups, an improvement in the nasal congestion score was observed 30 minutes and 7 days after the start of therapy. However,

nasal spray with lysozyme showed better efficacy 30 minutes after therapy application and subjects had significantly better nasal congestion score compared to hypertonic saline. Also, 7 days after the start of therapy, subjects in the lysozyme group had a better nasal congestion score compared to subjects who used hypertonic saline. Those results could be due to the role of lysozyme in natural immunity of nasal mucosa and anti-inflammatory effects of this compound.[11,12] Also, lysozyme promotes colonization of nasopharynx with healthy microbiota leading to long-term beneficial effects.[13,14] When hen egg white lysozyme is administered to experimental animals, antibacterial activity results in production of peptidoglycans that induce strong immune response. Activity of lymphocytes is modulated by lysozyme, an enzyme considered as "alternative antibiotic."[15] Antimicrobial activity of this compound could lead to better nasal decongestion, especially 7 days after the therapy start.

The hypertonic saline had a faster effect and the median time required for nasal decongestion was 3.5 minutes (IQR 1.0-10.0 minutes) in contrast to lysozyme group where the median was 6.0 minutes (IQR 4.0-14.0 minutes). Hypertonic saline exerts fast effects of nasal decongestion by mechanical and physiological activities. When hypertonic solution is applied it promotes transport of the water to nasal mucosa resulting in increased mucociliary clearance and reduction of swelling in submucosal tissue.[16] Besides NaCl concentration, osmolality of solution depends on presence of other ions.[16] The lysozyme group in this study received a slightly hypertonic solution containing 0.9% NaCl but also other ions that are resulting in osmolality of 400 mOsm/kg. Although this is slightly hypertonic compared to normal serum osmolality which is in the range 275 to 295 mOsm/kg, this is lower osmolality compared to hypertonic saline solutions used in this study that were in the range between 750 and 1000 mOsm/kg.[17] Higher osmolality decreased the time to nasal decongestion in hypertonic saline compared to lysozyme group.[18] However, several minutes longer decongestion in lysozyme group should not present significant delay in everyday practice especially considering the long-term beneficial effects of lysozyme in acute nasopharyngitis treatment.

In the hypertonic saline group, a greater number of subjects had significant comorbidities, primarily cardiovascular diseases. Since this study did not randomly divide subjects into groups, this result could be due to the tendency of treating investigators to include more subjects with comorbidities into hypertonic saline group. This approach is not justified taking into consideration that lysozyme is natural compound with excellent safety profile.[19] In our study, two adverse reactions were recorded. In the lysozyme group nose burning sensation was recorded during and after the first application of nasal spray. This is one of the most common adverse reactions for hypertonic saline nasal sprays which promote release of substance P and stimulate nociceptive nerves.[16,20] One case of epistaxis during seven days follow-up was recorded in hypertonic saline group. This is known, rare adverse reaction for hypertonic saline.[16]

This study had several limitations. Therapies were not included randomly so the bias could be introduced through selection of subjects into groups. However, nasal congestion score at the initial visit, before using the therapy, was similar between groups. Also, efficacy was measured subjectively using the nasal congestion score on a scale from 0 (no obstruction) to 3 (nose completely blocked). Since this was observational study, no laboratory parameters were measured. A larger randomized study focused on exploration of mechanisms of activity for nasal spray containing lysozyme and hypertonic saline should be performed.

CONCLUSION

A good efficacy and safety of both hypertonic nasal saline spray and slightly hypertonic nasal saline spray enriched with lysozyme in treating nasal congestion in acute nasopharyngitis was found. After the first application, a longer time for nasal decongestion was needed in the lysozyme group (median 6.0 minutes) compared to the hypertonic saline group (median 3.5 minutes). The lysozyme group had a better decongestion score 30 minutes and 7 days after therapy introduction. This study brings valuable results about the application of lysozyme in slightly hypertonic nasal spray in the treatment of acute nasopharyngitis.

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Conflict of interest: Aziz Sukalo, Meliha Mehic, Amna Tanovic Avdic, and Una Glamoclija disclose the following relationships. Employees of Bosnalijek d.d., a pharmaceutical company producing lysozyme-based medicines. Bosnalijek d.d. had a role in the design of the study, in the collection, analyses, and interpretation of data, in the writing of the manuscript, and in the decision to publish the results
Ethical approval: The study was approved by the Agency for Medicinal Products and Medical Devices of Bosnia and Herzegovina

REFERENCES

1. Grief SN. Upper Respiratory Infections. Prim Care. 2013;40:757-70.
2. Cox DR, Wise SK. Medical Treatment of Nasal Airway Obstruction. Otolaryngol Clin North Am. 2018;51:897-908.
3. Raphael GD, Jeney EV, Baraniuk JN, Kim I, Meredith SD, Kaliner MA. Pathophysiology of rhinitis. Lactoferrin and lysozyme in nasal secretions. J Clin Invest. 1989;84:1528-35.

4. Settipane GA. Chronic Rhinitis: An Updated Clinical Review. Am J Rhinol. 1991;5:199-206.
5. Stevens WW, Lee RJ, Schleimer RP, Cohen NA. Chronic Rhinosinusitis Pathogenesis. J Allergy Clin Immunol. 2015;136:1442-53.
6. Hisamatsu K, Yamauchi Y, Uchida M, Murakami Y. Promotive effect of lysozyme on the ciliary activity of the human nasal mucosa. Acta Otolaryngol. 1986;101:290-4.
7. Tewfik MA, Latterich M, DiFalco MR, Samaha M. Proteomics of Nasal Mucus in Chronic Rhinosinusitis. Am J Rhinol. 2007;21:680-5.
8. Woods CM, Lee VS, Hussey DJ et al. Lysozyme expression is increased in the sinus mucosa of patients with chronic rhinosinusitis. Rhinology. 2012;50:147-56.
9. Woods CM, Tan S, Ullah S, Frauenfelder C, Ooi EH, Carney AS. The effect of nasal irrigation formulation on the antimicrobial activity of nasal secretions. Int Forum Allergy Rhinol. 2015;5:1104-10.
10. Harcourt-Smith EA, Krstic ET, Soekov-Pearce BJ, et al. The Nasal Innate Immune Proteome After Saline Irrigation: A Pilot Study in Healthy Individuals. Am J Rhinol Allergy. 2023;37:419–28.
11. Bergamo A, Sava G. Pharmacological Modulation of Host Immunity with Hen Egg White Lysozyme (HEWL)- A Review Molecules. 2023;28:5027.
12. Lee M, Kovacs-Nolan J, Yang C, Archbold T, Fan MZ, Mine Y. Hen egg lysozyme attenuates inflammation and modulates local gene expression in a porcine model of dextran sodium sulfate (DSS)-induced colitis. J Agric Food Chem. 2009;57:2233-40.
13. Gurov AV, Yushkina MA, Muzhichkova AV. Microbiocenosis-regulating therapy of inflammatory pathology of the oropharynx. Vestn Otorinolaringol. 2021;86:51-6.
14. Karpova EP, Gurov AV, Burlakova KY. Possibility of treatment chronic adenoiditis and otitis media with effusion in children, into account the role of the microbiota of the mucous of the nasopharynx. Vestn Otorinolaringol. 2019;84:100-7.
15. Ferraboschi P, Ciceri S, Grisenti P. Applications of Lysozyme, an Innate Immune Defense Factor, as an Alternative Antibiotic. Antibiotics (Basel). 2021;10:1534.
16. Štanfel D, Kalogjera L, Ryazantsev SV, Hlača K, Radtsig EY, Teimuraz R, et al. The Role of Seawater and Saline Solutions in Treatment of Upper Respiratory Conditions. Marine Drugs. 2022;20:330.
17. Najem O, Shah MM, De Jesus O. Serum Osmolality. In: Stat Pearls. Treasure Island (FL): Stat Pearls Publishing; 2023.
18. Liu L, Pan M, Li Y, Tan G, Yang Y. Efficacy of nasal irrigation with hypertonic saline on chronic rhinosinusitis: systematic review and meta-analysis. Braz J Otorhinolaryngol. 2020;86:639-46.
19. Lambre C, Barat Baviera JM, Bolognesi C et al. Safety evaluation of the food enzyme lysozyme from hens eggs. EFSA J. 2023;21:e07916.
20. Baraniuk JN, Ali M, Yuta A, Fang SY, Naranch K. Hypertonic saline nasal provocation stimulates nociceptive nerves, substance P release, and glandular mucous exocytosis in normal humans. Am J Respir Crit Care Med. 1999;160:655-62.

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