
STOP
Allergic Rhinitis
Nasaleze®
Treats the cause, not just the symptoms
Nasaleze
DRUG FREE • CLINICALLY PROVEN • FAST ACTING
Nasaleze®
ALLERGY BLOCKER
Relieves Sneezing, Runny Nose and Sinus Congestion
100% NATURAL
Nasale
Allergy Block

Nasaleze®

- **Natural prevention of allergy symptoms from dust mites, animal dander and pollen**
- **Clinically proven to be effective** [1,2,3,4,5]
- **Drug free, fast-acting and non-drowsy**
- **Safe for children (under supervision), pregnant and breast feeding women**
- **30-day supply (200 doses)**
- **Refereshing mint flavour**
- **Included in the UK National Health System (NHS) Reimbursable Prescription List in 2010**
- **Class A medical device in Malaysia and Class 1 medical device in Europe**

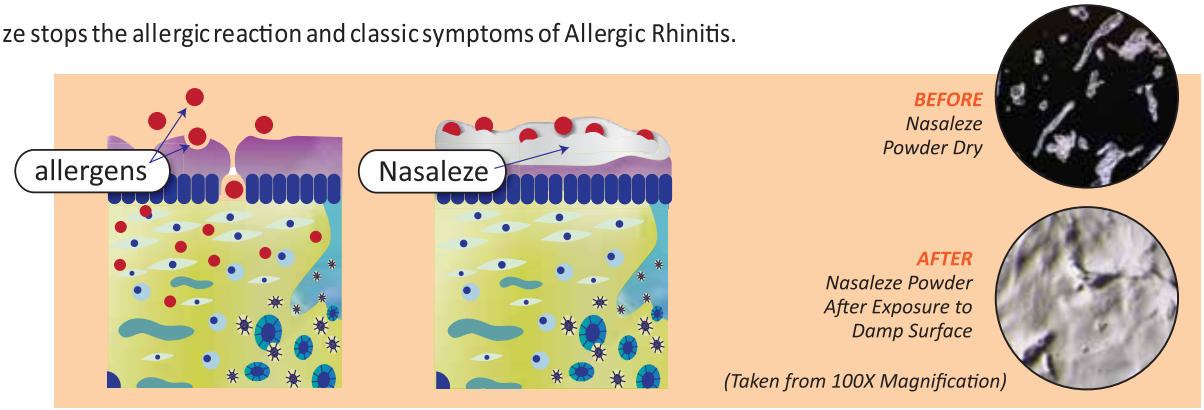
Nasaleze patented delivery system

Nasaleze Allergy Blocker

Nasaleze is an inert and natural micronized cellulose powder of vegetable origin which is applied to the inside of the nose via a patented delivery system. It prevents the initial allergic response and is clinically proven to relieve symptoms such as sneezing, runny nose and itchy/watery eyes within minutes.

Mode of action

Nasaleze stops the allergic reaction and classic symptoms of Allergic Rhinitis.



- *Nasaleze reacts with the moisture always found present within the nasal tract to form a protective gel-like barrier*
- *This barrier prevents contact between aggravating airborne allergens and the mucosa, thereby preventing mast cell degranulation and the release of histamine*

Nasaleze is suitable for all

- ✓ Young children (under supervision), pregnant and breastfeeding women where the use of allergy medication may not be suitable
- ✓ Teenagers/students and professionals where rapid relief is essential without the side effects of medication
- ✓ Anyone needing to drive or operate machinery
- ✓ Sufferers looking for a clinically effective but drug free approach
- ✓ Chronic allergy sufferers who want to avoid prolonged steroid/ antihistamine use, as even non-drowsy antihistamines can cause a 'hangover effect'

How to use

Nasaleze should be taken as soon as symptoms appear. The recommended dosage is three times a day but it can be taken as often as required. It can also be taken as a preventive measure before entering an environment where airborne allergens are likely to be present. The key to getting the best out of Nasaleze is to maintain a constant layer of powder across the lining of the nose.

Studies have shown that the use of Nasaleze alone reduces the need for rescue medication. However, chronic allergy sufferers may combine Nasaleze to their regular drug treatment for added relief.

For further information please refer to the product monograph.

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Nasaleze®
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1. Emberlin J, Lewis R. Curr Med Res Opin 2007:23(10): 2423-2431. 2. Emberlin J, Lewis R. Curr Med Res Opin 2006; 22(2):275-285. 3. Åberg, Ospanova, Niktin, Emberlin, Dahl. INT Arch Allergy Immunol 2014;163:313-318. 4. Zakharzhevskaya TV, Sidorenko IV, Treskunov VK, Karaulov AV. Sechenov Medical Academy, Moscow. Presented at Moscow XVI Congress for Men and drug April 06-10, 2009. 5. Aberg N, Benson M. Presented at EACCI June 2010 and accepted for publication in Int Arch Allergy & Immunol.


Nasaleze®
Fast acting, clinically proven,
natural nasal sprays

Nasaleze

The world's first powder nasal spray.

Nasaleze is now exported to over **50 countries** world wide from the production facility on the **Isle of Man**.

Over 14,000,000 bottles sold to date.

Taylor & Francis
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Expert Review of Respiratory Medicine

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Methyl-cellulose powder for prevention and management of nasal symptoms

Todor A. Popov , Nils Åberg, Jean Emberlin, Peter Josling, Natalia I Ilyina, Nikolai P Nikitin & Martin Church

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A review of **26 studies** with Nasaleze HPMC-powder, published in the Journal of **Expert Review of Respiratory Medicine**

bsaci

improving allergy care

through education, training and research

A formal and branded mention in BSACI Guidelines

British Society for Allergy & Clinical Immunology Guideline for the Diagnosis and management of Allergic and Non-Allergic Rhinitis 2017

(In the section under Treatment: Allergen Avoidance)

9.8 | Radiology

Radiology is not routinely recommended for simple rhinitis. However, when rhinosinusitis or nasal polyposis is suspected, especially non-responsive to medical therapy, CT scan is helpful.

9.9 | Nasal challenge

It is not routinely available outside specialist centres; there is no standardized methodology and asthmatic reactions can occur. It may be useful to confirm aspirin sensitivity or in occupational allergic rhinitis, where there is discrepancy between history and when there are potentially important occupational implications.

9.10 | Objective measures of nasal airway

Objective measurements of the nasal airway are not made in routine clinical practice but can be useful when allergen or aspirin challenges are undertaken and may be helpful when septal surgery or turbinate reduction are being contemplated.

season. For patients with house dust mite-sensitive AR the situation is complicated by the difficulties of reducing exposure to mites in the home. A systematic review of trials of mite allergen avoidance in rhinitis concluded that trials are generally small and of poor methodological quality and meta-analysis could not be performed.[140] Large studies of a combination of strategies to reduce exposure to dust mites have not been conducted but should probably include measures to reduce mites in cars, at school and work (see Figure 2).

Evidence from randomized studies is summarized in Table 3. For occupational AR complete avoidance of exposure to the causal agent is recommended.[115] Irritants such as smoke, traffic pollution can worsen rhinitis symptoms and should be avoided, where possible.

In a DBRPC study, the application of a cellulose powder (Nasaleze™) three times daily resulted in significant reductions in severity scores for sneezing, runny nose, stuffy nose and symptoms from eyes and lower airways with no clinically significant adverse effects (Grade B).[141]

Interventions that may help to reduce symptoms during the pollen season include patients wearing sunglasses (Grade C),[142]

AAIR
Allergy, Asthma & Immunology Research

Review

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Chinese Society of Allergy Guidelines for Diagnosis and Treatment of Allergic Rhinitis

8.2 Allergen avoidance

Individuals exposed to high concentrations of indoor allergens (*e.g.* HDMs and animal dander) may benefit from multifaceted avoidance measures after environmental counseling.[7,333] A Cochrane systematic review has shown that house dust mite avoidance measures can reduce allergen load and improve symptoms of perennial AR.[335] However, the authors of this review concluded that due to the small sample size of clinical trials and the poor quality of the evidence, it is still difficult to provide precise recommendations. A Chinese multicenter, randomized, placebo-controlled, crossover study has demonstrated that pollen blocker cream is effective in relieving nasal symptoms and improving QOL in both adults and children with perennial AR due to HDM.[336] Moreover, a systematic review and meta-analysis suggests that interventions to prevent and remediate indoor dampness and mold may reduce the risk of AR.[337]

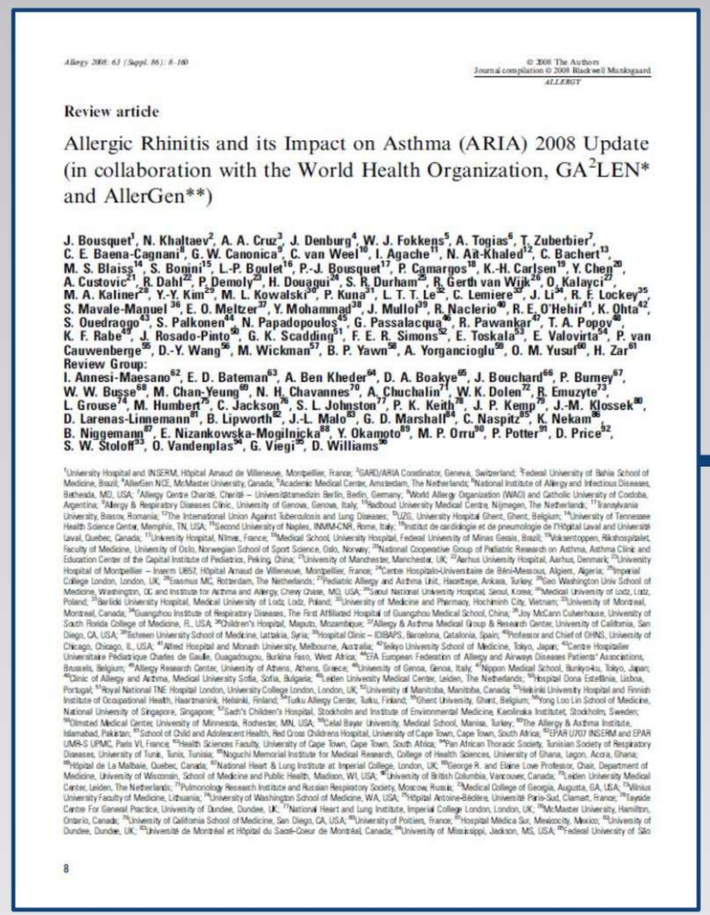
During outdoor activities in season with a high load of pollens, patients sensitive to pollen should avoid the peak of allergenic pollens spread in the air to reduce AR symptoms attack.

For individuals exposed to pollens in a natural environment, we recommend some allergen-controling tools (*e.g.* special masks, glasses, nasal filters, pollen blocker cream, nasal cellu-lose powder), which can reduce nasal inhalation or conjuncti-val contact of the allergenic pollen and relieve nasal and ocular symptoms.[7,338-341]

We have a formal mention in **Chinese Society of Allergy Guidelines** for Diagnoses and Treatment of Allergic Rhinitis.

Review article

Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 Update (in collaboration with the World Health Organization, GA^{2}LEN* and AllerGen**)



Allergy 2008: 63 (Suppl. 86): 8–160

ALLERGY

Review article

Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 Update (in collaboration with the World Health Organization, GA^{2}LEN* and AllerGen**)

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The cost-effectiveness of anti-IgE has been appreciated for its indication in severe asthma (1742, 1743) but not for rhinitis.

7.4.1. Subcutaneous immunotherapy combined with anti-IgE. Omalizumab pretreatment decreases acute reactions after rush immunotherapy for ragweed-induced seasonal allergic rhinitis (1744). The co-seasonal administration of omalizumab after preseasonal specific immunotherapy decreases ocular and nasal symptom scores and rescue medication use in grass-pollen allergic children (1745–1747). This combination might prove useful for the treatment of allergic rhinitis, particularly for polysensitized patients.

7.5. Complementary and alternative medicine

- Many patients who use complementary and alternative medicine appear to be satisfied.
- Evidence-based recommendations are difficult to propose for most complementary and alternative medicine interventions because of methodological problems.
- There is no evidence for the efficacy of most complementary and alternative medicines on allergic rhinitis and asthma.
- The safety of phytotherapy raises concerns.

Complementary/alternative medicines are extensively used in the treatment of allergic rhinitis and asthma (266), but evidence-based recommendations are difficult to propose due to methodological problems in many trials (e.g. not randomized, not controlled, not blinded and with no quantitative measurement; 25, 1748–1751). CAM is widely practised and many patients who use this treatment appear to be satisfied. From a scientific viewpoint, there is no definitive or convincing proof of efficacy for most CAMs in rhinitis or asthma.

Considering the RCTs, there is no clear evidence of the efficacy of acupuncture in rhinitis and asthma.

Some positive results have been described in rhinitis using homeopathy in good quality trials, but an equal number of negative studies counterbalance the positive ones (25). It is therefore impossible to provide evidence-based recommendations for the use of homeopathy in the treatment of allergic rhinitis, and further RCTs are needed.

Some herbal remedies have proved effective in the treatment of rhinitis (1076, 1752, 1753), but there are too few studies to make any firm recommendations. There are also safety and drug interaction concerns associated with these remedies. In fact, herbal remedies are not usually sufficiently standardized and can also contain harmful substances (1754–1756), such as the ephedrine-containing remedies that have been banned in the USA (1757). A mandatory prerequisite for evaluating herbal remedies/mixtures is that the method of preparation, doses, components and active ingredients should be clearly defined, according to the WHO guidelines (1758, 1759).

The therapeutic efficacy of CAM treatments is not supported by currently-available evidence (25). More data from randomized, double-blind, placebo-controlled trials are required. In addition, CAMs may not be devoid of side effects and some of these may interact with other medications (1754, 1756).

7.6. Other treatments

Saline douche is a simple and inexpensive treatment which was shown to bear some efficacy (228, 1760–1762).

Physico-chemical approaches have been proposed. Rhinophototherapy is effective (1763), but more data using simpler equipment are needed. Nasal filters (1764) or pollen-blocker creams (1765) during natural exposure to ragweed and grass pollen can reduce nasal symptoms. An inert cellulose powder has been on sale in the UK since 1994 as a remedy for hay fever and was found to reduce symptoms of pollen rhinitis (1766). In Japan, it is generic to wear a facemask and eyeglasses to prevent pollen inhalation. These masks are effective only if there is no strong wind or outside of the peak pollen season (1767).

Probiotics may influence symptoms of allergic diseases, but more data on large randomized trials are needed (1768, 1769).

7.7. Surgical treatment of rhinitis

As surgery cannot contribute to the treatment of allergic disease itself, it may only be used in certain precise conditions such as turbinate hypertrophy, cartilaginous or bony obstruction of the nasal airways or secondary and independent sinus disease. In patients who have been suffering from perennial allergic or nonallergic rhinitis for many years, a severe drug-resistant hypertrophy of the inferior turbinates may develop, which leads to constant nasal obstruction and watery secretion due to an increase in glandular structures. Consequently, the surgical reduction of the inferior turbinate body and mucosal surface, which should always be limited as much as necessary, reduces nasal obstruction and secretion (1770). Nowadays, endoscopically-controlled minimal-invasive techniques for the sinuses, but also for the turbinates, have replaced former procedures in most countries, and a range of new tools and instruments have been created to allow for more precise and less traumatic surgery. Laser surgery (1771) may also be used. Vidian neurectomy is not indicated for rhinitis because of side effects (1772) and the availability of medical treatment (1773). The indication for nasal and sinus surgery should always be based on a lack of effect of adequate drug treatment and the functional and clinical relevance of the anatomical variation or disease.

We have a formal mention in Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 Update.

1766. Emberlin JC, Lewis RA. A double blind, placebo controlled trial of inert cellulose powder for the relief of symptoms of hay fever in adults. Curr Med Res Opin 2006;22:275–285.

ALLERGY Nasaleze®

Scientific Brochure

July 2017 Issue 2

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Nasaleze Allergy:

- Nasaleze, an inert micronized cellulose powder, is composed of fine particles of hydroxypropyl methylcellulose (HPMC).
- Nasaleze is categorised as a Class 1 medical device in Europe
- The active ingredient HPMC, is combined with either peppermint or strawberry* flavoured powder depending on the product.

Characteristics:

- Protects allergy sufferers and strengthens their natural defences against airborne allergens such as pollen, dust mites and animal dander.
- Drug free, fast-acting and non-drowsy.
- No known contraindications or side effects.
- Nasaleze is suitable for all allergy sufferers including individuals with diabetes and asthma, pregnant and breast feeding women, the elderly and children over 18 months.

*Joint FAO/WHO Expert Committee on Food Additives **(JECFA)** and Food and Drug Administration **(FDA)** state that the constituents of the strawberry flavouring have been checked against various safety data bases.

Table of Contents

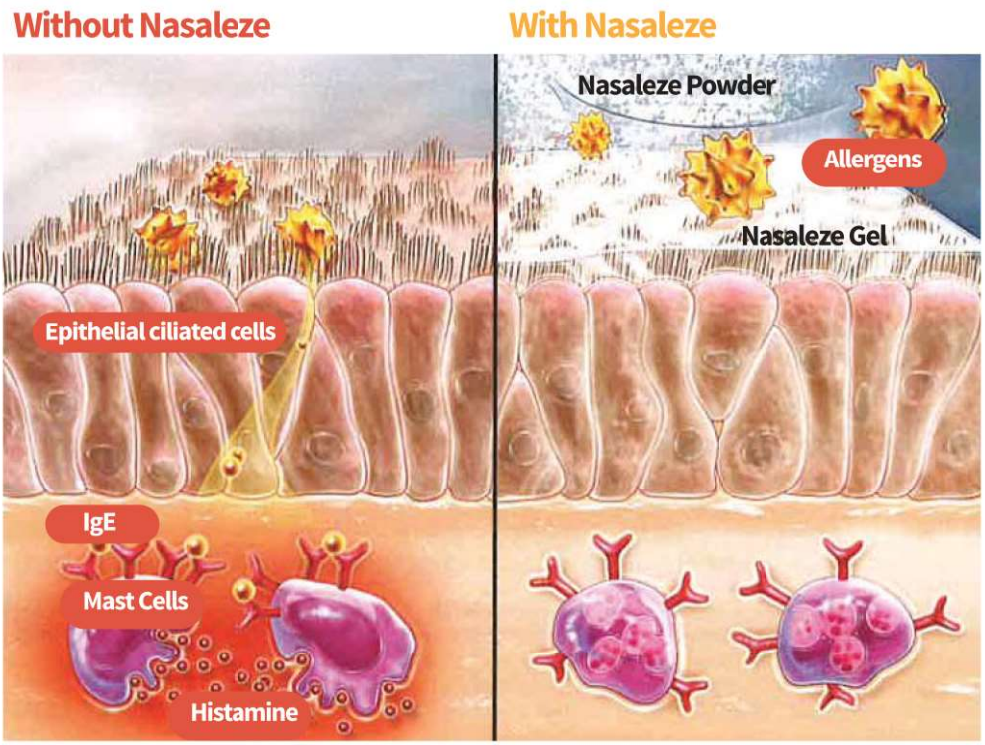
1 - Mechanism of Action

Nasaleze is applied to the inside of the nose via a novel patented method which ensures the delivery of an effective dose.

Cellulose particles absorb moisture from the nasal mucosa and swell to from a protective gel-like barrier in the nasal tract.

The gel barrier stops allergens from making contact with the mucosa. Thus halting the allergic reaction and stopping cell degranulation and the release of histamine from occurring.

The following studies demonstrate the mechanism of action of Nasaleze e.g. the formation of a mechanical barrier.



1 - Mechanism of Action

AIVAZIA – 2005

Aivazis V, Bourli E, Maratou E, Mavroudi A, Aivazi D, Foutzila E and Ilonidis G.

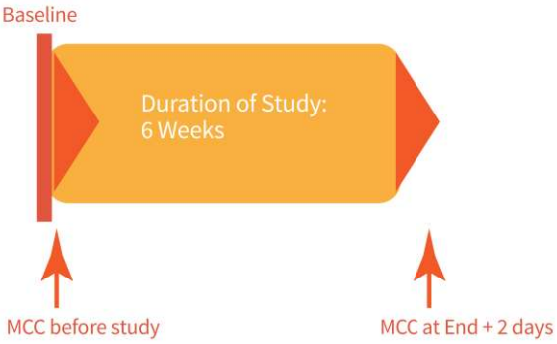
Study of mucociliary clearance in children with allergic rhinitis, before and after a six week therapy with natural cellulose powder.

Nea Pediatrica Chronica. 2005; 5(2)

Objective

To determine the nasal mucociliary clearance* rate before and after monotherapy with natural cellulose administrated in the form of inhaled powder in children with allergic rhinitis.

Design



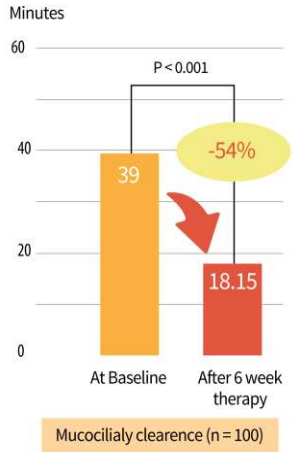
Mucociliary clearance (MCC) was determined in vivo by means of a non-invasive dye method**

Population

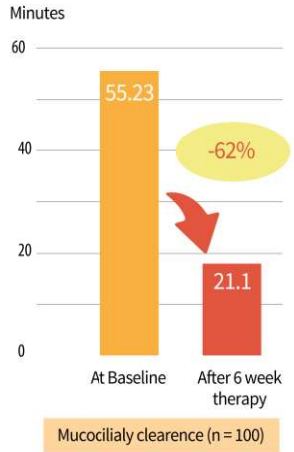
100 Children: 53 boys and 47 girls,
Mean age of the study group = 7.95 years (range 1.5 – 8 years)
All children had a positive medical history for allergic rhinitis.

Results

The MCC was reduced from 39 minutes to 18.15 minutes – a statistically significant reduction.



51% of the participants who had abnormally prolonged clearance (55.23 minutes) at the beginning of the trial reached a normal MCC (21.1 minutes) after treatment with Nasaleze.



Only 5 participants did not show significant improvement.

Conclusion

The significant decrease of MCC observed in participants is due to Nasaleze as the participants received no other therapy. Nasaleze enhances natural defences by improving the function of the nasal mucus. Effective filtration of allergens can now occur ensuring that only clean air reaches the lungs.

*Mucociliary clearance is an upper airway defence mechanism. Measuring the MCC provides a quantifiable measurement of cilial function and how diseases such as allergic inflammation can affect the mucociliary system.

**Dye Test: the dye (Edicol Orange 3% + CaHPO4 2H2O 97%) marks the infiltration area and the time it takes for its reappearance is recorded.

1 - Mechanism of Action

Diethart – 2010 (A)

Diethart B, Emberlin J. C, Lewis R. A.

Hydroxypropyl methylcellulose (Nasaleze) gel application delays Der p1 diffusion in vitro.

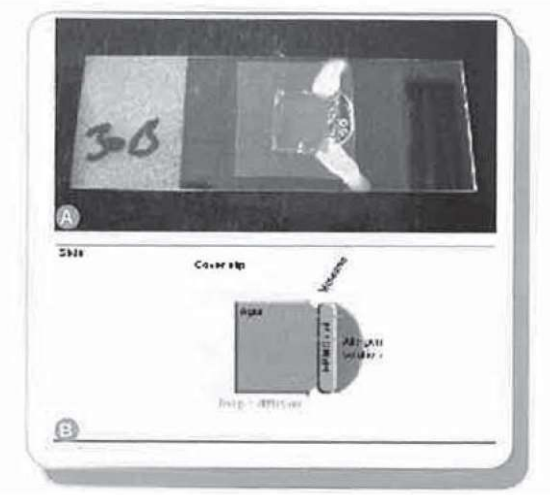
Natural Science. 2010; 2(2): p79-84

Objective

To investigate whether the HPMC gel acts as a mechanical barrier to Der p1 and prevents allergen diffusion towards the nasal epithelium.

Design

ELISA* was used to determine the amount of Der p1 which diffused through the cellulose gel and agar gel (imitation of nasal mucosa) *in vitro.*



Photograph (A) and diagram (B) of experimental setup for sample preperation for ELISA measurements of Der p 1 diffusion through HPMC gel.

Measurements were conducted at 15, 30, 45, 60, 180 and 360 minutes after application of the standard allergen solution.

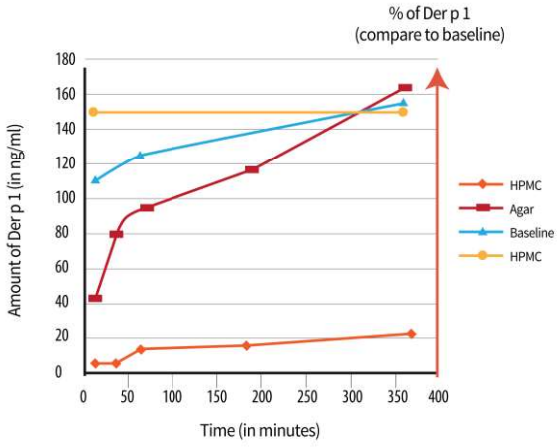
Results were compared to baseline reading (control) without a gel layer.

Results

Nasaleze significantly reduced the amount of diffused allergens in all tests.

After 15 minutes only 0.76% had diffused through the cellulose gel compared to the 28.1% of allergens which had diffused through the agar gel.

After 360 minutes the cellulose gel had only allowed 14.1% of the baseline allergens through while the agar gel had let 100%.



Conclusion

Nasaleze significantly delays Der p1 diffusion in vitro compared to both no barrier and the agar gel.

Nasaleze creates a polymer network with a small mesh size which inhibits the allergens diffusion to the nasal epithelium.

*ELISA (Enzyme-linked immunosorbent assay): an enzyme immunoassay which detects specific antigens in a wet sample.

1 - Mechanism of Action

Diethart – 2010 (B)

Diethart B, Emberlin J. C, Lewis R. A.

Nasal mucociliary clearance and mucoadhesion of hydroxypropyl methylcellulose powder used for alleviation of allergic rhinitis.

Poster presented at EAACI, 2010.

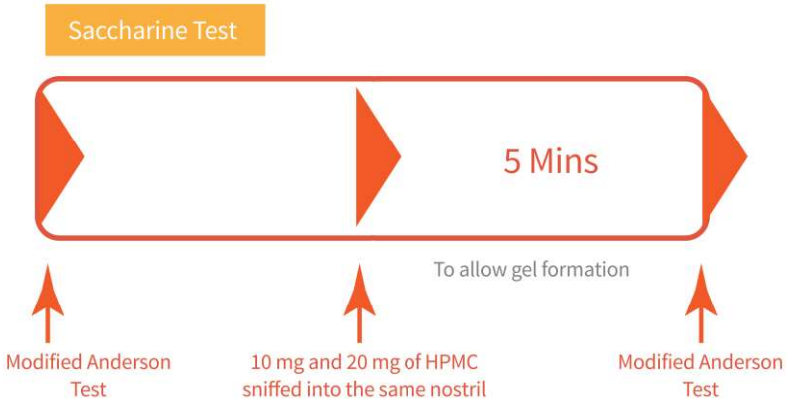
Objective

To investigate the effect of Nasaleze on mucociliary clearance in healthy participants.

Design

For this investigation a modified Andersen saccharine test was applied.

Modified Andersen Saccharine Test*: saccharine solution applied to interior of one nostril, participants were instructed not to sniff or sneeze and to report sweet taste. Time is measured from administration of saccharine solution to the sweet taste being detected.



Saccharine test (for mucociliary clearance): The upper respiratory tract is cleaned and small crystals of saccharin are placed on the inferior nasal mucosa. The time is measured until the patient has a sweet taste in the mouth. With normal ciliary transport the time should be 30 mins or less.

Population

12 healthy volunteers.

	Women	Men
Number of participants	9	3
Mean age (in years)	32.8	37
Age range (in years)	25-40	25-60
Allergic rhinitis during last two years	3 (33.3%)	1 (33.3%)
Smokers	1	1

Results

The mean mucociliary clearance time at baseline = 11.14 minutes.

Mean MCC with 10mg of HPMC = 35.45 minutes.

Mean MCC with 20mg of HPMC = 50.37 minutes.

Mean MCC with 20mg was statistically significant when compared to baseline and 10mg HPMC.

MCC with 20mg was 420% times longer than the baseline.

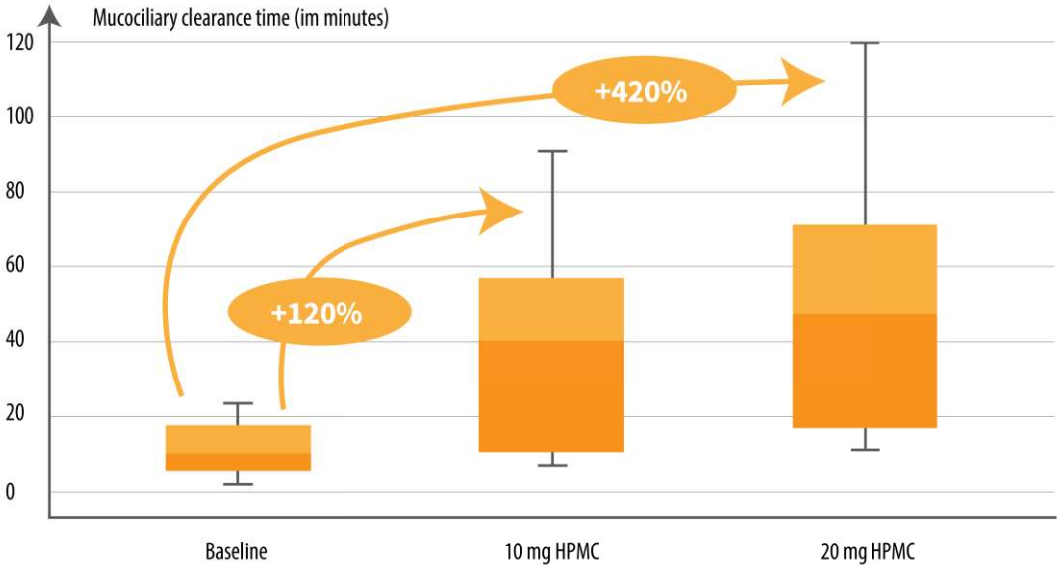


FIG 1: Boxplot of baseline MCT and MCT after nasal application of 10 mg and 20 mg of HPMC ($n = 12$, $p < 0.0005$)

Conclusion

The attachment of HPMC to nasal mucus (mucoadhesion) creates a barrier stopping allergy entry - this is demonstrated by the increase of MCC.

The increase in MCC demonstrates that the attachment of Nasaleze to the nasal mucus (mucoadhesion) creates a mechanical barriers stopping allergy entry.

Mucoadhesion also slows down nasal clearance, enabling longer residence time of Nasaleze in the nasal cavity. Nasaleze can now be an effective barrier for longer before it is cleared.

HPMC gel increase mucus viscosity, which might decrease the diffusion coefficient resulting in lower allergen diffusion.

*Saccharine Test: The upper respiratory tract is cleaned before small crystals of saccharin are placed on the nasal mucosa. The time taken for the patient to taste a sweet sensation is recorded. Normally it should take 30 minutes for the sweet taste to be detected.

2 - Clinical Studies

A selection of numerous international studies investigating the use, effects and safety of Nasaleze.

2 – Clinical Studies: Adult

Josling – 2003

Josling P, Steadman S.

Use of Cellulose Powder for the Treatment of Seasonal Allergic Rhinitis.

Advances in Therapy. 2003; 20(4): p213-219

Objective

To determine whether Nasaleze would be able to prevent an allergic rhinitis attack from occurring in participants who have suffered for many years.

Design

5-point scoring system to grade general well-being and serverity of any hay fever attacks.

Daily questionnaire was conducted assessing general well-being of the participant (5=well, 1=full hay fever attack).
Number and variety of symptoms were listed along with day or time elapsed when recovery began and time until symptoms were resolved.

Population

102 volunteers:
66 female, 36 male. Mean age = 44 years old.
All participants had previously used products for seasonal allergic rhinitis.

Results

Past Treatments

Treatment	Male Volunteers	Female Volunteers
Beconase® (steroid nasal inhaler) Glaxo Smith Kline, UK	3.0	3.1
Sodium cromoglycate (antihistamine nasal inhaler) - various generic manufacturers	1.3	2.1
Opticrom® (eyedrops) Aventis Pharma, UK	1.5	2.0
Clarityn® (oral tablets) Schering Plough, UK	2.0	2.2
Zirtech® (oral tablets) Glaxo Smith Kline, UK	1.1	1.8
Piriton® (oral tablets and liquid) Stafford Miller, UK	1.3	1.8
Telfast® (oral caplet) HMR, UK	2.0	1.8
Nasaleze	**3.8**	**3.9**

Nasaleze

On average the daily score with the Nasaleze treatment was over 4.0 in 35% of participants and above 3.0 (an occasional sneeze but no hay fever symptoms) in over 70% of participants.

After six weeks of using Nasaleze 70% participants rated the product as good or excellent.

Volunteers	Good	Excellent
Male	76	69
Female	80	75
TOTAL	78	72

Only 12% of participants had an average daily score of less than 2.9.

Participants were statistically likely to gain relief from symptoms within 0.1 to 3 hours of using Nasaleze.

6 women and 2 men required additional treatment with pharmaceutical products, however volunteers who took more than the recommended amount often perceived increased relief in their symptoms.

Conclusion

Nasaleze relieved classic hay fever symptoms, sometimes within minutes but often within 3 hours of inhalation.
Previous drug treatment had never alleviated patient's hay fever symptoms whereas upon treatment with Nasaleze there was resolution of symptoms.
Nasaleze treatment should be started as early as possible and continued throughout the pollen season, with number of applications increasing as appropriate to the individual.

2 – Clinical Studies: Adult

Vlahtsis - 2004

Vlahtsis K.

Clinical study of Nasaleze for relief of allergy symptoms including sneezing, runny nose, itchy and watery eyes.

Poster presented at Pan-Hellenic Conference of ENT Specialists, March 2004

Objective

To study how Nasaleze can benefit perennial or chronic allergy sufferers and protect from allergens such as dust mites, pet dander and smoke.

Design

One application of Nasaleze per nostril, mainly in the morning or shortly before the known time of day when symptoms usually appear. Duration of trial was 6 weeks with evaluations at time 0, 3 weeks and 6 weeks.

Scale used to measure symptoms (sneezing, runny nose, itchy and watery eyes):

5 = complete relief, without symptoms
4 = major relief, casual sneezing
3 = light, but noticeable allergy symptoms
2 = allergy symptoms apparent with periodic flare ups

Population

40 participants (24 women and 16 men).
All participants suffered from diagnosed allergic rhinitis diagnosed by radioallergosorbent test (RAST).
Previously used a pharmaceutical treatment either over-the-counter or prescribed.

Results

After three weeks of use, 85% of participants realized improvement in their allergy symptoms. This number increased to 90% after 6 weeks.

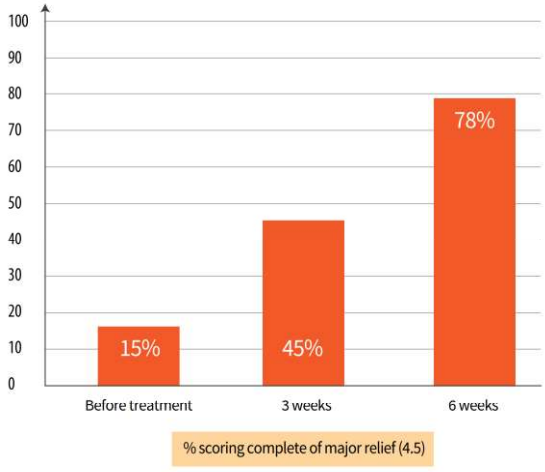


Table 1

78% have complete or major relief from symptoms after 6 weeks.

	3 SCALE IMPROVEMENT	2 SCALE IMPROVEMENT	1 SCALE IMPROVEMENT	0 SCALE IMPROVEMENT	MEAN IMPROVEMENT
	0%	5%	80%	15%	0.9 scales
6 weeks	7.5%	35%	47.5%	10%	1.4 scales

There were no side effects reported by any of the participants.
Participants reported that the product was simple and easy to use.

Conclusion

Nasaleze is able to decrease symptoms for participants who suffer from non-pollen induced allergic rhinitis.

2 – Clinical Studies: Adult

Emberlin – 2006 (A)

Emberlin J, Lewis R.

A double blind, placebo controlled trial of inert cellulose powder for the relief of symptoms of hay fever in adults.

Current Medical Research and Opinion. 2006; 22(2): p275-285

Objective

Principle Aim: to determine if there is a significant difference in the amount and type of rescue medication required for adult hay fever sufferers to control their symptoms.

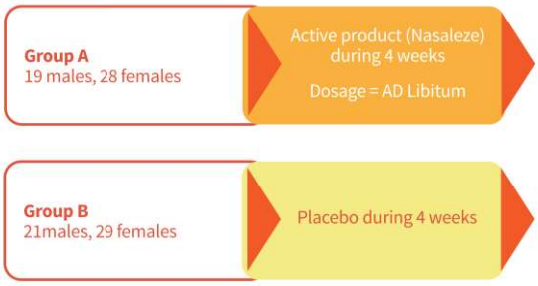
Secondary Aim: to determine whether the cellulose powder resulted in an improvement in symptom control.

Design

Double blind, randomized, placebo controlled study.

Participants were required to fill out daily diary cards for 4 weeks. The card include requests for:

- Likert scores for the following over the last 24hr: sneezing, runny nose, blocked nose, watering eyes
- How many times Nasaleze was used that day
- If any other allergic rhinitis medication or treatment was taken that day and if so what type and how much.
- Visit to GP or nursed related to their allergy rhinitis
- Whether they had cold or flu like symptoms. If so what were these?

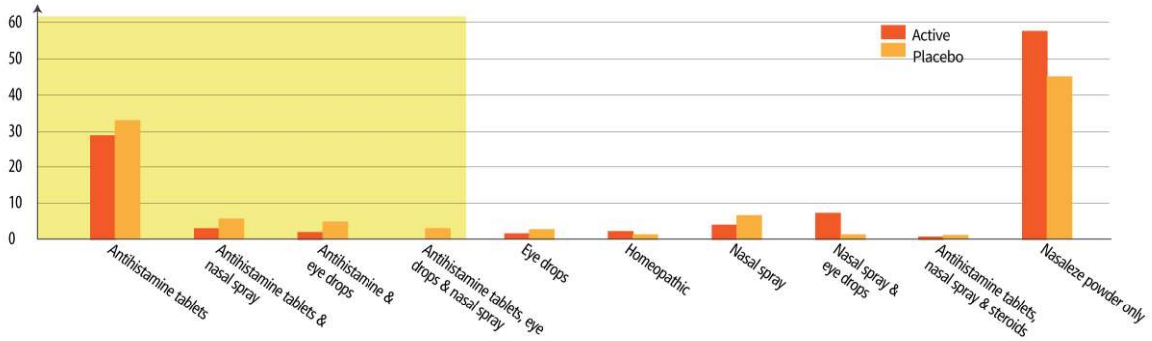


Population

The 97 participants who participated in the study were divided into two groups (A – active, B – placebo) matched by age by decades and gender.
All patients had symptoms of seasonal allergic rhinitis during June and July (grass pollen season) for at least 2 years.

Results

Significant differences were found in the overall amounts of rescue medication taken by the active and placebo groups. The placebo group took more rescue medication than the active group.



A significant difference was detected between the active and placebo group for the symptoms; running nose and blocked nose. 57% of participants in the active group only took Nasaleze with no rescue medicine compared with 44% in the placebo group. No adverse effect were reported during the study

Conclusion

This trial demonstrates that Nasaleze significantly reduced the need to take rescue medication for allergic rhinitis. Nasaleze has a positive effect on reducing common symptoms of allergic rhinitis such as runny and blocked nose.

2 – Clinical Studies: Adult

Emberlin – 2006 (B)

Emberlin J, Lewis R.

A double blind, placebo controlled cross-over trial of inert cellulose powder, by nasal provocation with grass pollen to assess efficacy of the product in controlling symptoms of hay fever.

Poster presented at EAACI, 2006

Objective

To explore the effects of Nasaleze in controlling symptoms when subjects are not taking any other medication.

Design

Double blind, placebo controlled, cross over trial.

After the powder (real or placebo) was placed in the nose, the equivalent of 350 grains per cubic metre air of grass pollen was placed in the nose.

Scores were taken for 6 symptom categories, nasal secretions were sampled for ECP* and nasal peak inspiratory (PIF) and expiratory flow (PEF) were measured at regular intervals for 4.5 hours.

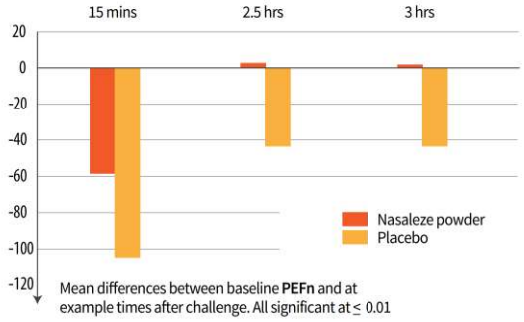
Population

11 adults

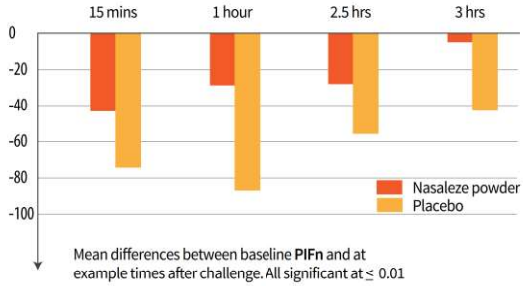
All participants were diagnosed as allergic to grass pollen but not to tree pollen by SPT.

Had suffered from symptoms in the two previous summers.

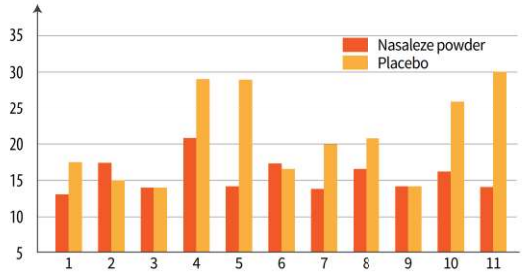
Results



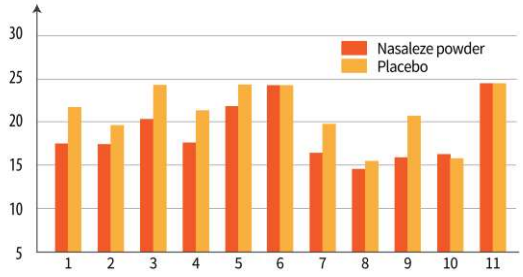
Mean differences between baseline **PEFn** and at example times after challenge. All significant at ≤ 0.01



Mean differences between baseline **PIFn** and at example times after challenge. All significant at ≤ 0.01



Total symptom scores for **itchy eyes** for each participant.
Differencs significant at p ≤ 0.01



Total symptom scores for **sneezing** for each participant.
Differencs significant at p ≤ 0.01

A significant reduction was found in nasal secretions and therefore ECP.

Results for other lung function tests and symptoms were slightly under the level of significance.

No adverse effect recorded.

Conclusion

Nasaleze has a significant effect in reducing the symptoms (sneezing and itchy eyes) of a grass pollen allergy.

Nasaleze also has a significant effect in reducing nasal inflammation, as shown with the reduction in nasal PEF, PIF and ECP.

Nasaleze is an effective treatment for allergic rhinitis due to its ability to alleviate symptoms.

***Eosinophil Cationic Protein (ECP)** are released from the eosinophil upon activation. They are attracted to the site of inflammation and become activated where they secrete several tissue-toxic mediators.

2 – Clinical Studies: Adult

Emberlin – 2007

Emberlin JC, Lewis RA.

Double blind placebo controlled cross-over trial of Nasaleze by nasal-provocation tests with Der p1 and Der f1.

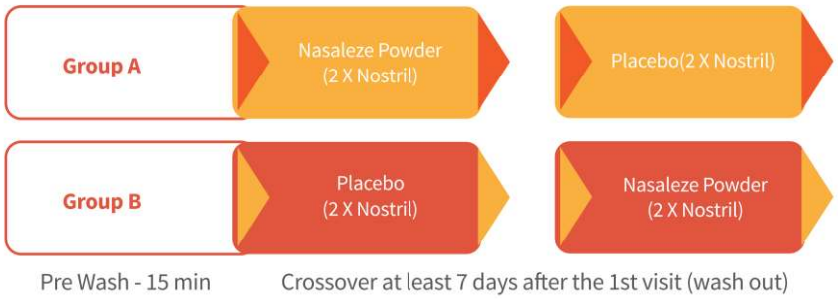
Current Medical Research Opinion. 2007; 23(10): p2423-2431

Objective

To assess whether Nasaleze would reduce the response to nasal challenge with house dust mite allergens.

Design

Double blind, placebo controlled, cross over trial.



Severity scores of symptoms (sneezing, nasal secretion, runny eyes, level ECP, nasal blockage, itching of the nose, throat and eye, PIF and PEF) were taken at regular intervals: 5 minutes after the challenge, every 15 minutes for the first hour after the challenge, then 30 minutes intervals until 4 hour, then at 6h and at 24h.

Symptoms were scored using the system below:

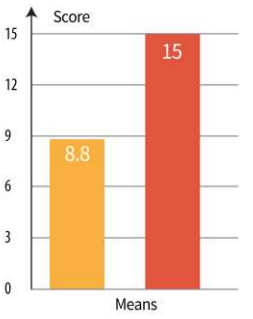
0 = absent.
1 = very mild, symptoms hardly noticeable.
2 = mild, symptoms noticeable all the time but do not interfere with any normal daily activities.
3 = moderate, symptoms noticeable all the time but do not interfere with any normal daily activities.
4 = severe, symptoms interfere with normal daily activities some of the time.
5 = very severe, symptoms interfere with normal everyday activities constantly.

Nasal secretions were sampled for ECPS and measures were taken of PIF and PEF at 5 min after challenge, 15 minutes later, then at 30 minute intervals for 2 hours and then again at 4 hours.

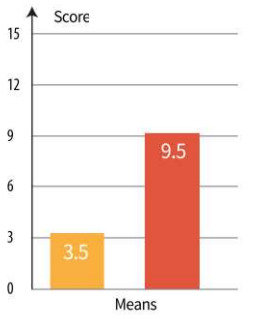
Population

15 adults (7 female and 8 male)
All persistent rhinitis sufferers – diagnosed positive to Der p1 and/or Der f1 by SPT.
All had symptoms for the previous two years.

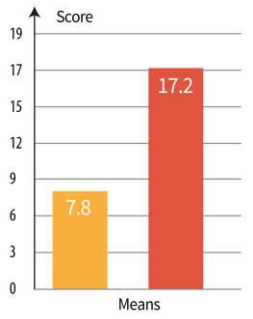
Results



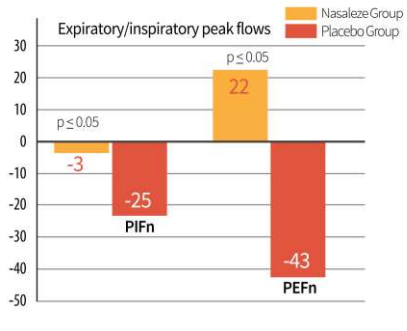
Mean score for runny nose
n = 15, p < 0.05



Mean score for sneezing
n = 15, p < 0.05



Mean score for itchy nose
n = 15, p < 0.05



Expiratory and inspriatory peak flows (n = 15).

There were no adverse reactions.

Conclusion

Nasaleze can have significant effects in reducing some symptoms of persistent rhinitis due to house dust mite allergy.

2 – Clinical Studies: Adult & Children

Zakharzhevskaya – 2009

Zakharzhevskaya TV, Sidorenko IV, Treskunov VK, Karaulov AV.

Efficacy and safety of medical device Nasaleze in prevention and treatment of persistent allergic rhinitis in adults and children.

Study presented at *Moscow XVI Congress for Man and Drugs,* 2009

Objective

To investigate the effectiveness and safety of Nasaleze as a medical device in prevention and treatment of allergic rhinitis.

Design

Participants received one puff of Nasaleze into each nostril 3 times a day for 4 weeks.

Once a week the participant would visit an investigator and their AR symptoms and the tolerability of Nasaleze was assessed.

A quality of life questionnaire and a visual analogue was filled out during initial and final visits. Al symptoms (sneezing, nasal and nasopharyngeal itching eyelid itching, nasal discharge, and impaired nasal breathing) were assessed using this following scale 0 = no symptoms to 5 = severe symptoms.

The effectiveness of treatment was assessed by investigator together with the patient during the final visit.

A daily diary was kept by all participants to record the severity of AR symptoms, any side effects and need for other medication.

Results

Improvement of AR symptoms was detected after just 1 week of treatment with Nasaleze and a significant decrease in the severity of all symptoms was detected by the end of the 4 weeks.

Population

48 patients: 25 adults and 23 children of both genders.

Age range 2 – 62 years old.

All had persistent allergic rhinitis.

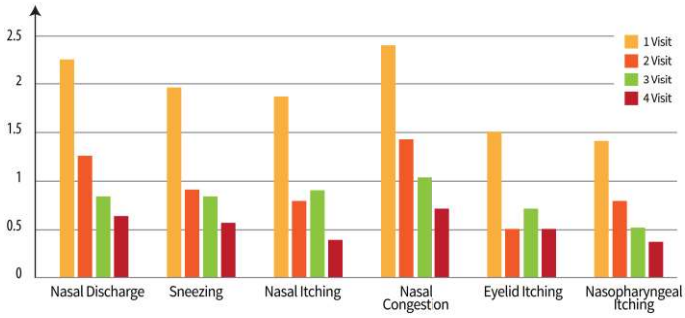


Fig 1: Evolution of AR symptoms in the course of 4 weeks treatment with Nasaleze in ADULTS.

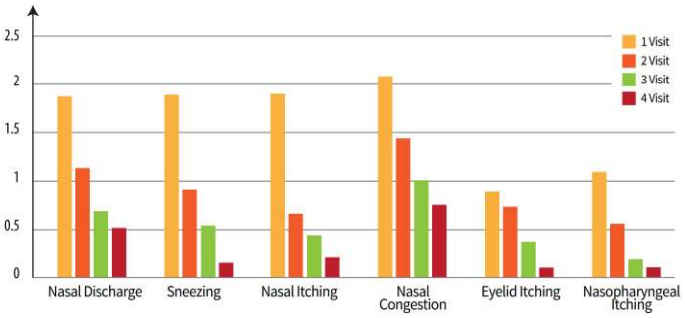


Fig 2: Evolution of AR symptoms in the course of 4 weeks treatment with Nasaleze in CHILDREN.

The majority of both adults and children assessed the efficacy of the product as good or very good. Only 2% of participants found Nasaleze moderately effect and none found it ineffective.

Assessment of the afficacy of Nasaleze.

EFFECTIVENESS	ADULTS (% OF ALL ADULT SUBJECTS	CHILDREN (% OF ALL PEDIATRIC SUBJECTS	TOTAL (% OF ALL SUBJECTS
Very good	45	38	41
Good	50	62	57
Moderate	5	-	2
No effect	-	-	-

Both children and adults reported good or very good tolerability of Nasaleze.

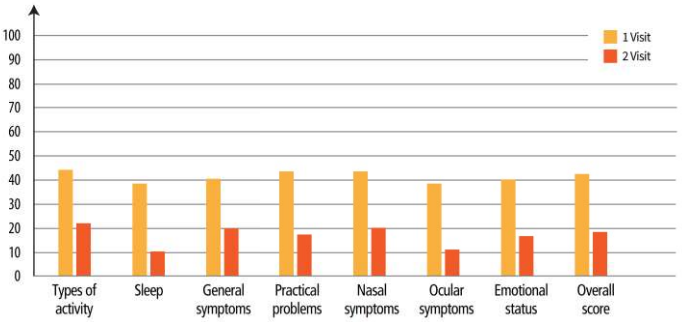


Fig 3: Assessment of quality of life by the patients before and after therapy with Nasaleze.

Conclusion

After one week of treatment Nasaleze reduces the severity of AR symptoms.

A two-fold improvement in the quality of life of the participants was reported after 4 weeks of treatment with Nasaleze.

Nasaleze is capable of creating a natural safe barrier protecting the airways from contact with allergens and oxidizing pollutants.

2 – Clinical Studies: Adult

Ilina – 2011

Ilina NI.

Open non-comparative study to evaluate the effectiveness of Nasaleze for patients with allergic rhinitis.

Russian Allergy Journal, 2011.

Objective

To evaluate the effectiveness of Nasaleze for patients with allergic rhinitis.

Design

Prospective open non-comparative study.

Nasal provocation tests increased until a positive reaction occurred.

Population

30 participants (18 women and 12 men) with an age range of 18 to 65 years old (mean age = 28.5).
Positive skin tests for dust and household or epidermal allergens.
Suffered from allergic rhinitis for no less than 2 years.

Results

28 out of the 30 participants found Nasaleze to be an effective therapy.
Nasal reactivity was shown to significantly decrease after treatment with Nasaleze.
The concentration of allergen needed to cause an allergic reaction increased from 1250 PNU/ml with no treatment to 5000 PNU/ml with Nasaleze.

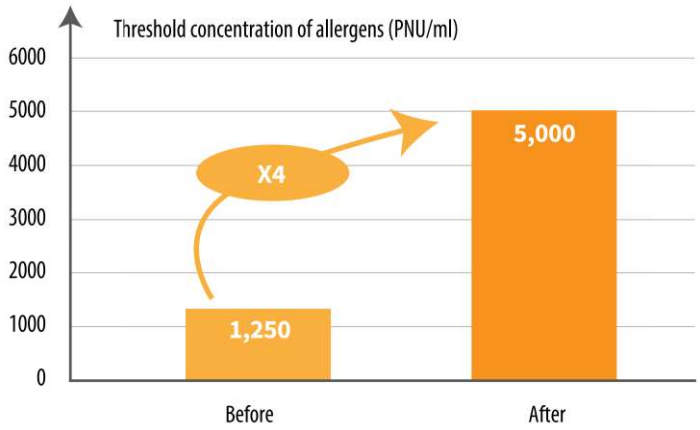


Fig 1: Threshold concentration of allergens (n + 30).

Conclusion

Under conditions of allergen provocation, Nasaleze has a prophylactic action and prevents the development of an allergic reaction.
For Nasaleze to be effective it must be applied before coming into contact with allergens and throughout the contact period.
Nasaleze has a high degree of safety due to the natural cellulose powder and has no systemic action in connection with the above, Nasaleze can be used by children and by pregnant or breast-feeding women.

2 – Clinical Studies: Children

Aberg – 2011

Aberg N, Benson M.

A nasally applied cellulose powder in Seasonal Allergic Rhinitis (SAR) in children and adolescents; reduction of symptoms and relation to pollen load.

Pediatric Allergy and Immunology. 2011; 22(6): p594-599

Objective

To assess the efficacy of Nasaleze in a common clinical setting along with an oral histamine in treating seasonal allergic rhinitis in children.

Design

A double blind, placebo-controlled study.

Duration of study = 4 weeks

ACTIVE TREATMENT 3 X/DAY:
NASALEZE 3X/DAY + DESLORATADINE (1 TABLET/DAY)

PLACEBO TREATMENT 3X/DAY +
DESLORATADINE (1 TABLET/DAY)

SMS was used to provide instructions, reminders and reporting of symptoms.
At the end of each day participants were asked to report the severity of their symptoms (sneezing, runny nose, blocked nose, eyes and lower airways) using the scale below:

1 - No trouble at all
2 - Little trouble
3 - Moderate trouble
4 - Rather much trouble
5 - Much trouble
6 - Very much trouble

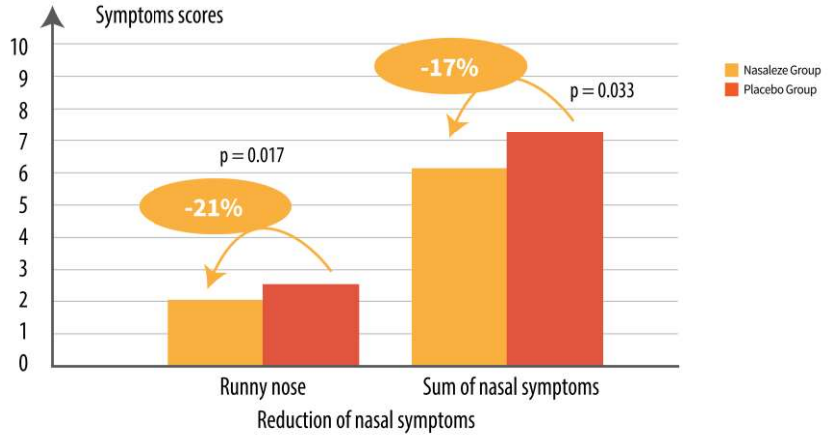
All reminder and reports were done using SMS on mobile phones.

Results

General tendency for reduction in symptom scores for all symptoms in the active group.
There was a significant reduction for sum of nasal symptoms and specifically running nose.
With a low to moderate pollen count sneezing is also significantly reduced.

Population

53 children participated in the study (age range 8-18 years old)
Must have tested positive for birch pollen allergy through a skin prick test.
They should not have used nasal steroids.



Conclusion

Nasaleze causes a significant alleviation of nasal symptoms in SAR in children and adolescents specifically runny nose and sneezing.
The best efficacy was seen after a low-moderate birch pollen load.
Nasaleze is effective in combination with oral antihistamine, the most common treatment of SAR.

2 – Clinical Studies: Adult

Aberg – 2014 (B)

Aberg N, Ospanova ST, Nikitin NP, Emberlin J, Dahl A.

A nasally applied cellulose powder in seasonal allergic rhinitis in adults with grass pollen allergy: A double-blind, randomized, placebo-controlled, parallel-group study.
International Archives of Allergy and Immunology. 2014; 163(1): p 313-318

Objective

To assess the efficacy of Nasaleze in grass pollen rhinitis in adults in Europe.

Design

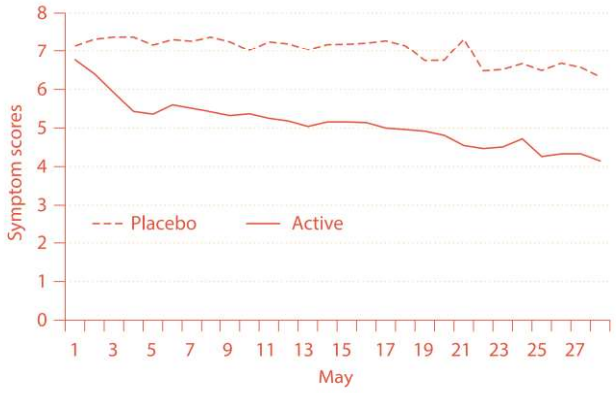
A double-blind, placebo-controlled study.
Patients were randomly assigned to the placebo or active group.
Patients had to puff the powder 3 times daily for 4 weeks.
Reminders were sent out by SMS to patients and SMS confirmation of powder application was sent back to the researchers.
In the evening, the severity of the patient's symptoms (nose: sneezing, running nose and blocked nose, eyes and lower airways) were scored from 1 (no symptoms) to 6 (strong symptoms).
The use of rescue medication was also recorded.

Population

108 patients, age range: 18-40 years old.
A positive test for timothy grass pollen was required for inclusion.

Results

A significant reduction was detected in the severity scores for sneezing, runny nose, stuffy nose and symptoms from eyes and lower airways, both separately and together.



87.1% of the active participants found the product had a good effect.

OPINION	PLACEBO, N	ACTIVE, N
No effect	28 (52.8%)	4 (7.4%)
Good effect	12 (22.6%)	32 (59.3%)
Very good effect	1 (1.9%)	15 (27.8%)
Don't know	12 (22.6%)	3 (5.6%)

Group differences, $p < 0.001$.

Only one patient in the active group received rescue medication – antihistamine tablets.

Conclusion

Nasaleze provided significant protection against all seasonal allergic rhinitis symptoms.
The magnitude and scope of efficacy support using Nasaleze as a preventative measure for allergic rhinitis.

2 – Clinical Studies: Adult

Valerieva – 2015

Valerieva A, Popov TA, Staevska M, Kralimarkova T, Petkova E, Mustakov T, Lazarova T, Dimitrov V, and Church M.

Effect of micronized cellulose powder on the efficacy of topical oxymetazoline in allergic rhinitis.
Allergy Asthma Proceedings. 2015; 36(1): p1-6

Objective

To assess the ability of Nasaleze to prolong and enhance the effectiveness of pharmaceutical therapies in the nasal cavity.

Design

Double-blind placebo-controlled study.

Peak inspiratory nasal flow was measured for 360 minutes after oxymetazoline and HPMC or placebo application on days 1 and 8 and at a single point on day 15.

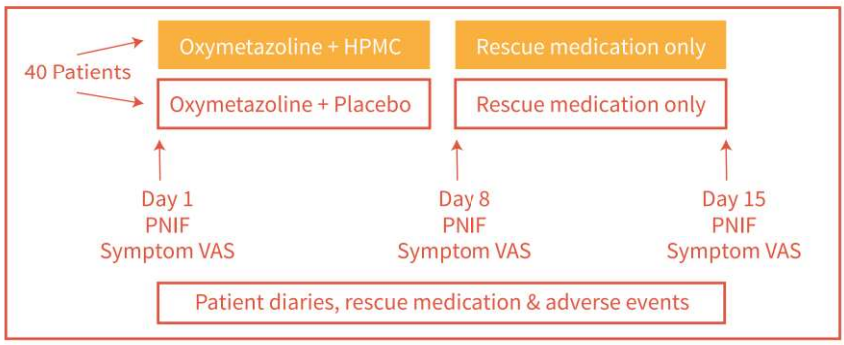


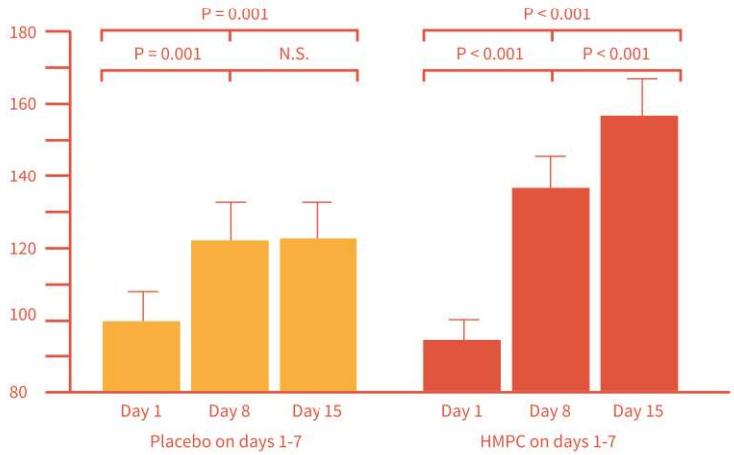
Figure 1. Study protocol

Population

40 participants (23 women and 17 men) with a mean age of 35 years old.
All had a clinical history of persistent moderate-to-severe allergic rhinitis and a positive skin prick diagnosis.

Results

Nasaleze significantly enhanced oxymetazoline, PNIF was higher at day 1 and 8.



Baseline PNIF values at days 1, 8, and 15. Each group contains results from 18 individuals. Significance values were calculated by using the student's t-test for paired data. *The baseline PNIF of the patients treated with HMPC at 15 days was significantly (p = 0.014) higher than that of patients treated with placebo. This value was calculated by using the Student's t-test for unpaired date.

Nasaleze reduces nasal congestion as PNIF is greater in the Nasaleze group than in the placebo.
By day 8 both groups had relieved nasal symptoms but only the active group continued to see improvements until day 15.
The active group used less rescue medication than the placebo group between days 8 and 15.

Conclusion

Nasaleze enhances the decongestant effect of oxymetazoline in allergic rhinitis patients.
The carryover efficacy of oxymetazoline for a week after its discontinuation may be due to Nasaleze aiding the mucosal barrier.

Conclusion

The clinical studies in this booklet provide a significant amount of data which prove that:

Nasaleze is an effective natural barrier that blocks airborne allergens, preventing the cause of allergic rhinitis.

Nasaleze effectively causes a significant reduction in allergic rhinitis symptoms.

When used in combination with pharmaceutical products, Nasaleze enhances and amplifies the effects of the pharmaceutical product.

Nasaleze is a safe product that can be used by adults, children over 18 months, pregnant and breast-feeding women.

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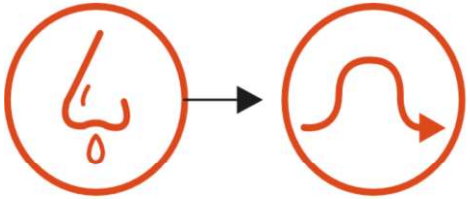
Allergic Rhinitis Treatment Algorithm



Allergic rhinitis

Allergen avoidance and patient education

Nasaleze – Forms a gel barrier that acts as an allergen avoidance

Mild intermittent symptoms

Nasaelze Allergy, first line of defence

Second- generation oral or intranasal antihistamine, as needed

Mild to moderate persistent symptoms

Nasaelze Allergy, first line of defence

Intranasal corticosteroids alone as first-line treatment

Consider nasal irrigation or decongestants for nasal congestion*

Consider ipratropium (Atrovent) or intranasal antihistamines for rhinorrhea

Consider oral or intranasal antihistamine for persistent nasal ocular symptoms

* Use of nasal decongestants for longer than three days is cautioned because of risk of rebound congestion.

Severe persistent symptoms

Intranasal corticosteroids plus oral or intranasal antihistamine, oral leukotriene receptor antagonist, or intranasal cromolyn (Nasalcrom)

Symptoms persist

Consider immunotherapy referral or alternative treatments (e.g. allergen avoidance,nasal irrigation, acupuncture, probiotics, herbal preparations)

Nasaleze – A valuable adjunct to standard treatment for Allergic Rhinitis

Study	Details	Results
1. **Emberlin (N=97, above 18yo); SAR** Current Medical Research & Opinion, 2006 (Study 4, pg.20)	Any medication (antihistamine/nasal spray/ eye drop) + Nasaleze / placebo	Nasaleze **significantly reduced the need to take rescue medications**
2. Aberg (N=53, from 8-18yo); AR Pediatric Allergy & Immunology, 2011 (Study 10, pg.72)	All on oral antihistamines + Nasaleze / placebo	Nasaleze showed **significant reduction in total symptom scores**, including running nose & sneezing. Nasaleze can be **effectively combined with oral antihistamines.**
3. Penechko (N=30, above 18yo); SAR Russian Allergy Journal, 2011 (Study 14, pg.97)	All on standard therapy (antihistamines & topical glucocorticosteroid) + Nasaleze / placebo	Nasaleze group showed **significant improvement in QoL** (sleep, types of activity, emotional state etc.) **and faster symptom alleviation**
4. Valerieva (N=40, mean age 35); AR Allergy Asthma Proceedings, 2015 (Study 17, pg.118)	All on intranasal oxymetazoline + Nasaleze/ placebo	Nasaleze **significantly improved nasal congestion** (higher PNIF rate) and **augments the effect of nasal oxymetazoline** for another 7 days after discontinuation
5. Popov T (N=25, mean age 31); SAR Presented at AAAAI, 2016 (Study 18, pg.126)	All on xylometazoline/azelastine/ mometasone, oral prednisolone/bilastine (PRN) + Nasaleze / placebo	Nasaleze **reduces symptoms and rescue medication use. Significantly better PNIF rates** (60% vs 31%) and better EBT rate; reduced nasal congestion and inflammation
6. Minov JB (N=74, 22-46yo); mild SAR J of Pulmonary & Respiratory Med, 2017 (Study 21, pg.136)	All on cetirizine + Nasaleze/placebo	Nasaleze group showed **significantly higher efficacy/improvement of symptoms**
7. Hristova (N=42, 18-55yo); SAR Presented at EAACI, 2017 (Study 22, pg. 140)	All on decongestant/antihistamine/ corticosteroid + Nasaleze / placebo	Nasaleze **augments local therapeutic effect in the nose; better PNIF & EBT (Exhaled breath temperature) rates.** A valuable adjunct to nasally applied drugs, enhancing their pharmacological effects.

Nasaleze

Invitro Studies

Nasaleze®

www.nasaleze.com

CONTENTS

Hydroxypropylmethylcellulose gel application delays Der p 1 diffusion in vitro.

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Natural Science

Hydroxypropylmethylcellulose gel application delays Der p 1 diffusion in vitro

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ABSTRACT

Background: A special hydroxypropylmethylcellulose powder (Nasaleze®) has been used for the alleviation of nasal symptoms of allergic rhinitis since 1994. The efficacy of the product has been recently proven but the mechanism of action was still largely unknown. The aim of the study was to investigate the hypothesis that the gel formed after moisture absorption in the nose might act as mechanical barrier that prevents allergen diffusion towards the nasal epithelium. Methods: The diffusion of Der p 1 through HPMC and agar gels was measured in vitro after 15, 30, 60, 180 and 360 minutes using ELISA. Agar blocks were used to simulate the nasal mucosa. Control samples without gel layer were obtained. Results: The control samples with no applied gel barrier absorbed 72.2 % of the Der p 1 solution after 15 minutes and 100 % after 60 minutes. In comparison, the HPMC and agar gel layers both significantly delayed Der p 1 diffusion. After 15 minutes 0.76 % had diffused through the HPMC gel layer compared to 28.1 % which diffused through the agar layer. After 360 minutes, 14.1 % of the baseline Der p 1 crossed the HPMC gel layer while 100 % had diffused through the agar layer. Conclusions: HPMC gel significantly reduces Der p 1 diffusion in vitro compared to no barrier and an agar gel layer. This is likely to be due to the small mesh size of the polymer network of HPMC and could have important implications for a preventative treatment of allergic rhinitis.

Keywords: Allergic Rhinitis; Der p 1; Diffusion Barrier; Hydroxypropylmethylcellulose

1. INTRODUCTION

Allergic rhinitis (AR) is a global health problem which affects up to 25 % of the adult population in industrialised countries and more than 40 % of children [1,2]. The rising prevalence of allergic rhinitis imposes a huge burden on the economy due to costs of treatment and loss of work productivity. Recent estimates of annual costs range from $2 to 5 billion in the U.S. alone [3-5]. The pathology of AR is associated with a severe impairment of the quality of life for those who suffer from it [6,7]. A reduction of quality-of-life impairment can be achieved by appropriate treatment of allergic rhinitis [7,8]. Modern medications such as antihistamines or corticosteroids can do a lot to help to alleviate symptoms and restore a normal lifestyle but many of them have unwanted adverse effects or are limited in their application [1,3,4]. Many people distrust these conventional medicines and therefore prefer to use complementary and alternative treatments. However, the therapeutic efficacy of many of these treatments is not supported by evidence and they might not be devoid of side effects [3,9].

A recent approach is offered by the use of an inert hydroxypropylmethylcellulose (HPMC) powder (Nasaleze ®) for allergy prevention and alleviation in the nose. Although the product has been registered as a class 1 medical device with the MHRA since 1991 and is sold over the counter in more than 50 countries worldwide, little work has been done on the effect of the powder on nasal symptoms. However, the efficacy of HPMC in decreasing symptoms of allergic rhinitis caused by grass pollen and house dust mite allergens was recently proven [10-12]. The investigators observed an improvement of symptoms when using HPMC for treatment of SAR and PAR. Nasal peak inspiratory flow (PIF) and peak expiratory flow (PEF) increased compared to placebo and some symptoms of allergic rhinitis including sneezing, itching and runny nose were alleviated significantly. Also the need to use rescue medication was found to be reduced. Considerable variance was observed in the results and some participants did not show any improvement. This was partly attributed to the application device which is suspected not to deliver constant doses [12,13].

The HPMC powder is applied to the nose using a specially designed dry powder dispenser bottle and forms a gel on the nasal lining by absorbing moisture from the nasal mucosa. It was hypothesised that this gel might act as a mechanical barrier preventing allergens from entering the mucosa [11,12]. However, no investigations on the mechanism of action of HPMC as an allergy treatment have been published as yet leaving the question how an inert cellulose derivative can offer relief to individuals affected by allergic rhinitis unanswered. Similar HPMC powders which also form hydrogels upon contact with liquids are widely used in controlled drug release formulations where they restrict the release of drug molecules through the tablet by serving as a barrier to drug diffusion [14]. Also, high-viscosity HPMC gels have been shown to limit glucose and cholesterol absorption in the gastrointestinal tract by creating a mechanical barrier [15,16]. Thus, it is assumed that HPMC gel might impede the passage of allergens in a similar manner.

The aim of this study was to investigate the possibility that HPMC gel might constitute a mechanical barrier to house dust mite allergen in vitro in order to gain information about the mechanism of action of HPMC in the alleviation of symptoms of allergic rhinitis.

2. METHODS

2.1. Materials

Hydroxypropylmethylcellulose powder was supplied by Nasaleze Limited, IOM. Der p 1 solution (in house reference, 7.5 µg Der p 1 per millilitre) was provided by Alk-Abello, Madrid.

2.2. Sample Preparation

Preparation of the samples took place in a cleanroom to minimise contamination by dust or allergens. All equipment needed for preparation was washed in isopropyl alcohol (70 %) for sterilisation and dried before each use. Ten ml of agar (1.5 %, prepared with 0.9 % saline solution) were cast into a petri dish. After cooling, small rectangles of equal dimensions (1 x 1 cm) were cut from the agar and then transferred to cleaned slides. Two lines of warm and therefore liquid Vaseline were drawn with a brush from the two edges of one side of the agar block to the edges of the slides to avoid diffusion of allergens through the side of the block (**Figure 1**). The position of the agar was marked on the bottom of the slide and the agar block was covered by a cover slip that sealed the upper surface of the agar. Allergen solution could therefore diffuse into the agar through only one free edge (**Figure 1**).

To test the barrier function of HPMC, a thin layer of HPMC gel was applied covering the edge of the agar which was used for allergen application. For this, 50 mg of HPMC powder were mixed with 1 ml physiological saline solution (0.9 %) to form a 5 % gel. Immediately after the mixing of the gel, 0.2 ml was applied to the open edge of the agar block using a 1 ml sterile syringe. The initial thickness of the gel layer was measured at 3 standard points. After covering with a cover slip, 20 µl of the allergen solution were applied to the HPMC gel covering the one side of the agar blocks limited by the Vaseline lines.

The slides were incubated at 35°C and 90 % relative humidity to simulate nasal conditions for 15, 30, 60, 180 and 360 minutes. After incubation the thickness of the HPMC layer was again measured. The agar blocks were then carefully removed from the slides and transferred to labelled microtubes containing 0.5 ml PBS-T as elution medium. Samples were shaken on an Autovortex for 20 seconds followed by shaking overnight on a lab shaker. Samples were stored frozen at -20°C.

2.3. Reference and Control Samples

To investigate the difference of diffusion through HPMC and agar, control samples were produced with an additional agar layer of 1.5 mm (average thickness of the HPMC gel layer calculated from measurements of HPMC samples using a digital caliper) to replace the HPMC gel and treated in exactly the same way as the HPMC samples.

Additionally, control samples with no allergen addi-

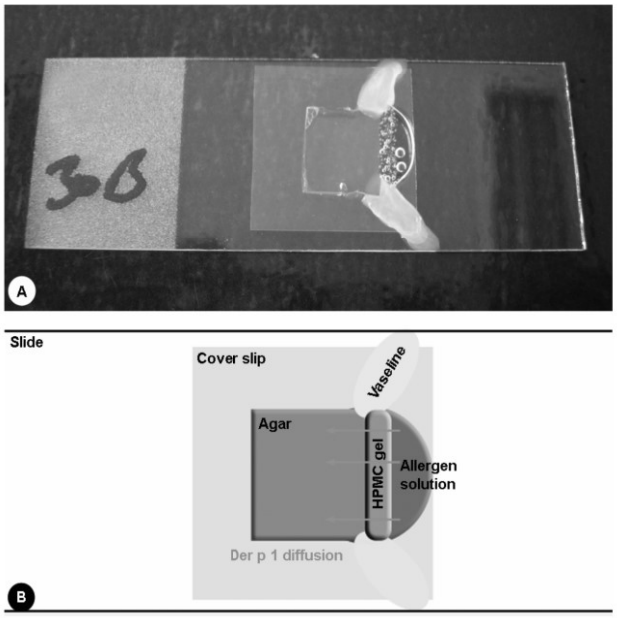

Figure 1. Photograph (A) and diagram (B) of experimental setup for sample preparation for ELISA measurements of Der p 1 diffusion through HPMC gel.

tion and no barrier addition, respectively were obtained.

Baseline measurements of the allergen amount in 20 µl of allergen solution were conducted by applying 20 µl of allergen solution directly to a microtube containing 0.5 ml of PBS-T. The microtubes were then treated in the same way as the microtubes containing the agar blocks.

2.4. ELISA Measurements

The monoclonal antibodies (mAbs) and Der p 1 allergen standards used in the assays were purchased from Indoor Biotechnologies, and the assays were performed according to the manufacturer's instructions.

2.5. Statistical Analysis

One-way ANOVA was applied for statistical analysis of the differences between Der p 1 diffusion in HPMC gel, agar gel and control samples, respectively. No serious violations of assumptions were observed. P values of 0.01 or less were considered to be statistically significant.

3. RESULTS

The mean baseline allergen content in 20 µl of the standard solution used was found to be 151.0 ng/ml (SD = 4.0 ng/ml). This is in good agreement with the calculated value of 150 ng/ml for the given dilution of a 7.5 µg/ml stock solution. All control samples with no allergen application were negative in the ELISA measurements.

The diffusion of Der p 1 molecules into the 1 x 1 cm agar blocks eluted for measurements was delayed with both gel barriers applied (**Table 1** and **Figure 2**). The amount of allergen diffused through 1.5 mm of 1.5 % agar gel was significantly different from the baseline values for the first 180 minutes ($p < 0.005$) but did not reach statistical significance after 360 minutes ($p = 0.628$). After 15 minutes of incubation, 28.1 % of the baseline allergen amount had diffused through the gel into the agar block (**Table 2**, $p < 0.0001$). The amount of allergen detected in the elutes of the agar blocks then steadily increased until it reached baseline level after 360 minutes of incubation (**Figure 2** and **Table 2**). The thickness of the agar layer applied as a barrier did not change during the measurement times from 15 to 360 minutes. In contrast, an initially 1.50 mm thick HPMC gel layer swelled to an average 3.34 mm in 360 minutes upon allergen solution application. Diffusion of Der p 1 molecules through 5 % HPMC gel showed a significant reduction of diffused allergen for all test times ($p < 0.001$). After 15 minutes 0.76 % of the baseline amount had diffused through the HPMC gel layer into the agar block compared to 28.1 % which diffused through the agar layer (**Table 2**). After 360 minutes, 14.1 % of the baseline Der p 1 crossed the HPMC gel layer while 100 % had diffused through the agar layer (**Table 2**). However, the HPMC data include several outliers and the standard deviation is high (**Table 1**). The mean coefficient of variation for all measurements for the HPMC gel was found to be 201.9 % which is very high compared to 37.8 % for agar.

Control samples with no barrier had absorbed 72.2 % of the baseline allergen content after 15 minutes and differences to baseline did not reach statistical significance after 60 minutes using a 99 % confidence interval ($p_{60min}=0.042$, $p_{360min}=0.990$).

4. DISCUSSION

Most of the commonly available treatments of allergic rhinitis affect the inflammatory processes (e.g. by abating mediator release or blocking receptors) initiated after

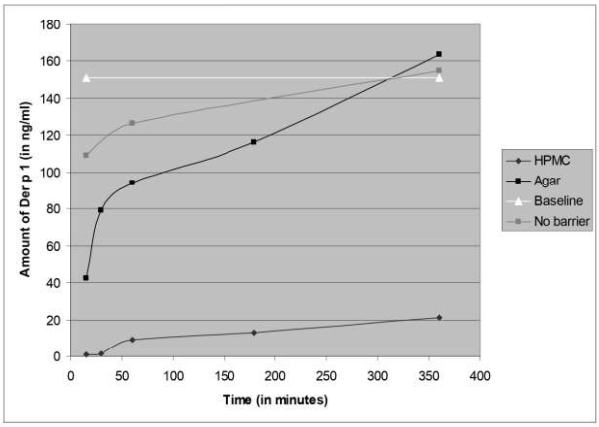

Figure 2. Amount of Der p 1 diffused through a 1.5 mm thick HPMC and agar gel layer, respectively compared to control (no barrier) and baseline allergen amount.

Table 1. Amount of Der p 1 diffused through a 1.5 mm thick HPMC and agar gel layer, respectively, amount of allergen absorbed without barrier (control) and baseline allergen amount in 20 µl of the applied solution.

	Amount of Der p 1 measured in samples (in ng/ml)				
Time (in min)	15	30	60	180	360
HPMC	1.15	1.57	8.98	13.17	21.34
Agar	42.46	78.98	93.92	116.46	163.59
No barrier	109.26	no value	126.62	no value	154.92
Baseline	151.04	151.04	151.04	151.04	151.04

Table 2. Fractions of allergen amount diffused through a 1.5 mm thick HPMC and agar gel layer, respectively and with no barrier compared to the baseline value of 151.04 ng/ml.

	Diffused fraction of Der p 1 (in % of baseline)				
Time (in min)	15	30	60	180	360
HPMC	0.76	1.04	5.94	8.72	14.13
Agar	28.11	52.29	62.18	77.11	108.31
No barrier	72.34	no value	83.83	no value	102.57
Baseline	100.00	100.00	100.00	100.00	100.00

allergen penetration into the mucosa and binding to IgE [1,17,18] and therefore represent symptomatic treatment. This means that inflammation and the associated damage of the mucosa are already established and the medication decreases signs of this inflammation while it is still on going. An ideal allergy treatment would inhibit the establishment of an allergic reaction altogether. Anti-IgE prevents binding of allergen to IgE antibodies and so inhibits a reaction while the allergens are already inside the epithelium [19]. HPMC might work at an earlier stage by preventing allergens from entering the mucosa in the first place by the generation of a mechanical gel barrier.

The present study aimed to investigate this possible barrier function of HPMC to allergens. The results obtained by ELISA-measurements show that HPMC significantly delays Der p 1 diffusion and that the amount of allergen diffused through the gel is even lower than indicated by preliminary tests [20]. This retardation might allow the mucosa to recover its physical integrity and the allergic reaction to decline. However, a complete barrier to Der p 1 diffusion could not be confirmed.

The retarded diffusion of solutes in hydrogels like HPMC gel or agar gel is well known and widely used for biotechnological separation methods such as electrophoresis or gel chromatography and in controlled release formulations [21,22]. The most comprehensible model developed to explain the diffusion delay of solutes in gels is the obstruction theory which assumes that the impenetrable polymer chains are obstacles that cause an increase in diffusional path length and additionally act as a sieve [21,24]. Therefore the mesh or pore size of the polymer network is a crucial parameter in the reduction of diffusion in hydrogels [25]. Hydrogels consist of high molecular weight molecules forming a threedimensional network which is dispersed in a continuous liquid medium [22,25]. Due to cross-links and entanglements of these molecules hydrogels can be described as a mesh with solvent filled spaces between the individual polymer chains which act as a filter for molecules larger than the spaces available [26,27]. Controlled release studies with FITC-dextran molecules of different molecular weights revealed that the critical molecular weight for diffusion in HPMC gels, which are characterised by a mesh size of 12 nm, lies between 65 and 66.5 kDa depending on the molecular weight of the polymer and the concentration of the gel [28]. Allergenic proteins usually have a molecular weight between 5 and 80 kDa [29,30]. This means that a great proportion of allergens theoretically are small enough to diffuse through the HPMC mesh spaces. Although Der p 1 (24 kDa) lies well below the mesh size of HPMC gels, a substantial delay in diffusion has been observed. Even though molecules larger than 65 kDa are stopped from diffusing through HPMC almost completely, all other smaller molecules will still be delayed by the longer diffusional path due to obstructions by the macromolecular chains and the slower water movement due to binding of water to the polymer. Furthermore, the mesh size and therefore the size of the spaces available for diffusion in weakly cross-linked homogenous gels is not stable but time-dependent and the size and location of the spaces change due to Brownian motion of the molecule chains [22,31].

In comparison to HPMC, the mesh size of a 1.5 % agar gel as used in this study has been observed to be between 70 and 800 nm [21,26]. Even the lowest of these values is almost six times larger than the mesh size of HPMC which explains the higher allergen diffusivity within agar gel.

The values obtained in the present study are valid for Der p 1 and allergens of the same or very similar molecular weight. It has been shown that the diffusion coefficient for globular proteins in agar decreases with increasing molecular weight and therefore radius of the proteins [21]. This leads to the assumption that allergens smaller than Der p 1 like Bet v 1 (17 kDa) or grass group 2/3 allergens (10-12 kDa) might be expected to diffuse faster whereas larger allergens like Amb a 1 (38-50 kDa) or Art v 1 (28-60 kDa) might exhibit slower diffusion velocities through the HPMC gel network.

The variability of the results of the measurements of Der p 1 diffusion through HPMC gel was high with a coefficient of variation (CV) of just over 200 %. In comparison, the CV of Der p 1 diffusion in agar gel was only about 37 %. For this reason the variation in the amount of allergen diffusing through the HPMC gel layer cannot solely be attributed to limitations in the methods that were applied. Similarly high variability of diffusion coefficients was obtained for mucus gels [32]. This was attributed to the heterogeneous nature of the

mucous gel producing uneven penetration profiles. Release from HPMC matrices for controlled drug release was found to be sensitive to alterations in the chemical composition and the polymer gel conformation and substantial batch-to-batch variations in release and swelling could be observed for a single type of HPMC [33,34]. The authors suspect that this might be due to aggregate formation in the gel causing transient cross-linking that could perturb diffusion in some places throughout the gel which cannot be predicted.

Due to its importance in controlled drug release, the effect of HPMC as a diffusion barrier for drugs has been studied extensively. However, no investigations of allergen diffusion in HPMC have been found in the accessible literature. It was confirmed in this study that HPMC gel delays Der p 1 diffusion in vitro. Other allergens need to be tested to extend the evidence for the efficacy of the product. Also many other factors will influence the efficiency of the product in vivo. For practicality reasons, the gel layer used in the experiments is thicker than the gel layer that can be expected to be established within the nasal cavity. Diffusion velocity is a crucial parameter needed to make assumption for in vivo conditions and should therefore be addressed in future research. A complete diffusion barrier is essential for the retardation of drug release [14] and similarly optimal coverage of the nasal mucosa is important since uncovered areas may allow free allergen entry and the provocation of an allergic response. Sub-optimum coverage is likely to reduce the efficiency of the product. The provision of a suitable powder delivery device therefore poses an important challenge for the maximisation of the efficacy of HPMC in the alleviation of allergic rhinitis.

In conclusion, a diffusion delay of Der p 1 in HPMC gel has been confirmed in vitro. This means that even though HPMC gel does not constitute an impermeable barrier to allergens, the significant delay of allergen entry into the mucosa could be beneficial to hay fever sufferers through the reduction of allergen exposure. This fairly novel way of treatment reduces the allergen load itself and not the symptoms caused after allergen entry into the mucosa. Thus, with the appropriate delivery device, HPMC could be a valuable, drug-free alternative for the treatment of allergic rhinitis. The efficacy of HPMC in hay fever treatment has been recently proven [10-12]. However, the research presented in this paper is the first to address the mechanism of action of HPMC in the alleviation of allergic rhinitis. This knowledge will allow improvements on the product to be made in order to increase its benefit to hay fever sufferers.

5. ACKNOWLEDGEMENTS

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To compare the abilities of Nasaleze gel and Nasalguard to limit/prevent the in vitro diffusion of pollen allergen.

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Contract Research Report

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Project Aims:
To compare the abilities of Nasaleze gel and Nasalguard to limit/prevent the *in vitro* diffusion of pollen allergen.

Date of report:
26/07/2012

Samples received:
Two packages were received by our office in Warminster on 16/07/2012 which were then forwarded to the lab in Cardiff and arrived on 17/07/2012. One contained 9 x 3g tubes of Nasalguard, AllergieBlock. The other contained 4 x 20g vials of HMPC powder (13/07/12, Barrel 161).

Summary of experiments performed:

Test products (HMPC/Nasaleze and Nasalguard) were used to create a barrier between absorbent agar gel and liquid allergen. For comparison the same set up with no product between the agar and allergen was also tested. The barrier functionality of the products was tested by incubating at 35°C

for 15, 30, 60, 120 and 360 minutes. The amount of allergen absorbed into the agar gel was determined by a pollen allergen (Phl p 5) specific ELISA.

Sample preparation / experimental set up:

The following accompanying document sent by Matt Duxbury, Nasaleze was used as a guide for the experimental procedures described below:

- Diethart, Emberlin and Lewis, 2010. Natural Science, Vol 2, No 2, 79-84. Hydroxypropylmethylcellulose gel application delays Der p 1 diffusion in vitro.

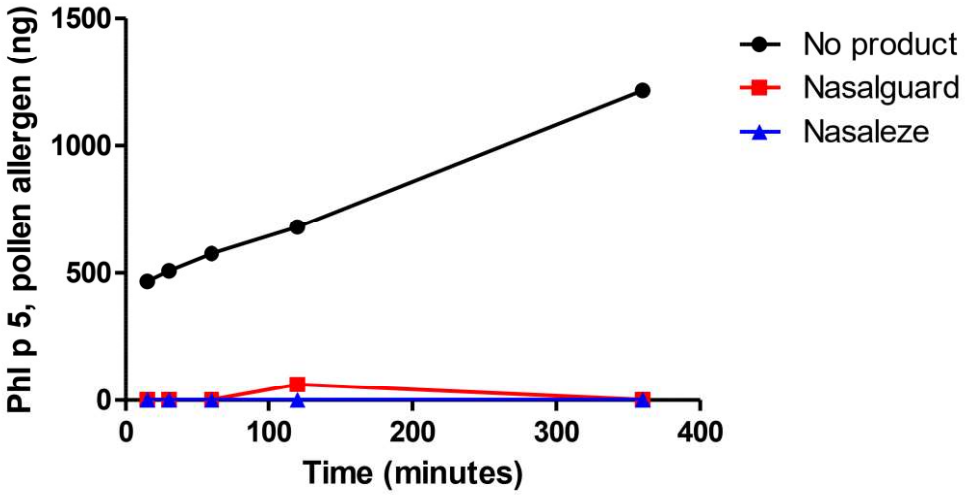
- Samples were prepared in a biosafety cabinet to minimise potential contamination by dust or allergens.
- All equipment was rinsed with 70% isopropanol for sterilisation.
- Small (~1cm x 1cm) rectangles of agar (1.5%, with phosphate buffered saline [PBS]) were cut and transferred to microscope slides.
- Using a brush, two lines of warm/liquid (60-70°C) Vaseline was drawn to prevent diffusion of allergen to the agar through the side as shown in Figure 1 of Diethart, 2010.
- A layer of each test product (1.Nasaleze, 2.Nasalguard and 3.no product for control purposes) was applied between the Vaseline walls to form a barrier to the agar as shown in Figure 1 of Diethart, 2010.
- Nasalguard was applied after transferring the product into a graduated syringe.
- Nasaleze was made up by mixing 0.25g of HMPC powder with 5ml of PBS and applied using a syringe. A new batch of Nasaleze was made up for each slide.
- Approximately 100µl of product was applied to each slide.
- To complete the barrier set up, a cover slip was carefully placed on top of the microscope slide and pressed down to ensure a seal had been made.
- The products tended to bulge out and form a semi-circle rather than a line after applying the cover slip.
- The thickness of the test product was not measured in the interest of saving time before applying allergen and starting the incubations.
- 20µl (2000ng) of recombinant pollen allergen solution Phl p 5 (100µg/ml) was pipetted between the cover slip and microscope slide adjacent to the test product.
- A slight settling down/absorption of liquid allergen was observed, particularly on the slides where no product had been applied.
- The slides were incubated at 35°C for 5 time periods (15, 30, 60, 120 and 360 minutes).
- Agar blocks were carefully removed from the slides and transferred to microcentrifuge tubes containing 0.5ml of PBS-tween.
- Samples were extracted by brief vortex (30 seconds) followed by gentle shaking overnight (14 hours) at room temperature.
- Extracted samples were stored at -20°C.
- The amount of allergen within extracted samples was measured using a pollen allergen (Phl p 5) ELISA according to the standard Indoor Biotechnologies protocol.
- For this 3 x 96 well microtiter plates were used, and each sample was diluted using 12 doubling dilutions from 1/10 to 1/20,480.
- The concentration of allergen detected was calculated for each sample by extrapolation from a standard curve generated from each plate.

Results:

The barrier capabilities of test products were tested using the set up described above. Over the time course of the experiment it appeared that Nasaleze expanded, perhaps pushing allergen away from the agar. The integrity of the barrier set up with both products was noticeably worse after 120 minutes and further degraded after 360 minutes. The amount of pollen allergen absorbed by and later extracted from the agar blocks incubated for various time periods with and without a product barrier in place was determined by ELISA. The results are shown in table 1 and graph 1 below.

	Amount of Phl p 5, pollen allergen (ng)				
Time (minutes)	15	30	60	120	360
No product	467	508	575	681	1216
Nasalguard	<2	<2	<2	63	<2
Nasaleze	<2	<2	<2	<2	<2

Table 1. Amount of pollen allergen (Phl p 5) absorbed by and later extracted from agar blocks following barrier set up experiment over various time periods.



Graph 1. Amount of pollen allergen (Phl p 5) absorbed by and later extracted from agar blocks following barrier set up experiment over various time periods.

When no product was placed between the liquid allergen and the agar block the amount of pollen allergen absorbed by and later extracted from the agar block steadily increased over time. However, when either Nasalguard or Nasaleze was placed between the agar block and liquid allergen very little allergen was absorbed by and later extracted from the agar block. Indeed, the level of allergen detected in the samples incubated with Nasaleze were consistently below the detection limit of the ELISA (4ng/ml, 2ng total) and therefore negligible even after 360 minutes. The same was true of Nasalguard except at one time point (120 minutes) where it appears the barrier function was not absolute and 63ng of allergen was detected.

Conclusions:

Nasaleze and Nasalguard significantly reduce the absorption of pollen allergen *in vitro* for up to 360 minutes.

Nasaleze®

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TRAVEL Nasaleze®

Clinical Trial Data

January 2020 Issue 2

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Contents

Nasaleze® Travel

- Natural protection from airborne germs and viruses
- Fast acting
- Carry with you and take before entering a crowded environment
- Clinically proven (Hillman and Josling, 'Preventing air-borne infections with an intra-nasal cellulose powder formulation.

What is Nasaleze Travel? - Nasaleze Travel is a natural nasal powder spray containing a blend of cellulose, peppermint and odour controlled wild garlic that delivers fast, continuous protection from airborne germs that are inhaled via the nose.

Why garlic? - the garlic used in Nasaleze Travel is odour controlled European wild garlic. This wild garlic extract contains copious amounts of ajoene and has been shown to possess excellent antiviral capabilities (Weber et al Planta Med 58 1992 417-423) outperforming all other garlic thiosulphinates in terms of anti-viral activity. As our European wild garlic is odour controlled there is little taste to it.

Why peppermint? - of all species of mint, peppermint contains the most menthol, a phytochemical that has antibacterial and antiviral effects.

The menthol in peppermint has long been used as a cough suppressant and decongestant. Even in the United States, where herbal medicine is not widely used, menthol is a common ingredient in cough drops, nasal spray, and mentholatum chest rubs. The FDA actually approved the marketing of peppermint as a cold remedy, as did a panel of experts in Germany that evaluates the safety and efficacy of herbs.

* www.vitaminstuff.com/herbs-peppermint.html

Garlic

Mint

Mechanism of Action

Nasaleze Travel coats your sensitive nasal membranes with a very fine layer of cellulose and peppermint. The cellulose powder reacts with the moisture in your nose to produce a thin gel like protective barrier over the nasal mucosa. The peppermint powder and garlic remains within the cellulose coating for added protection against airborne germs and viruses commonly found in our environment.

Indications

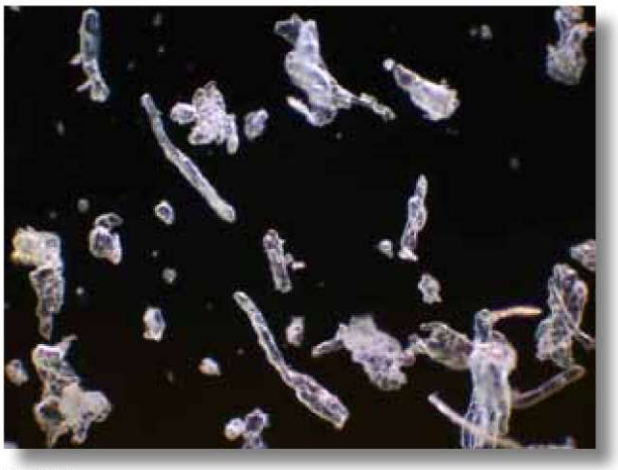
Nasaleze Travel is used for protection from and resistance to airborne germs and viruses. Frequent flyers, commuters, nurses and doctors, office workers and teachers would all be likely to benefit from using Nasaleze Travel to protect themselves from the high concentration of germs they encounter on a regular basis.

The Traveller's Unseen Journey

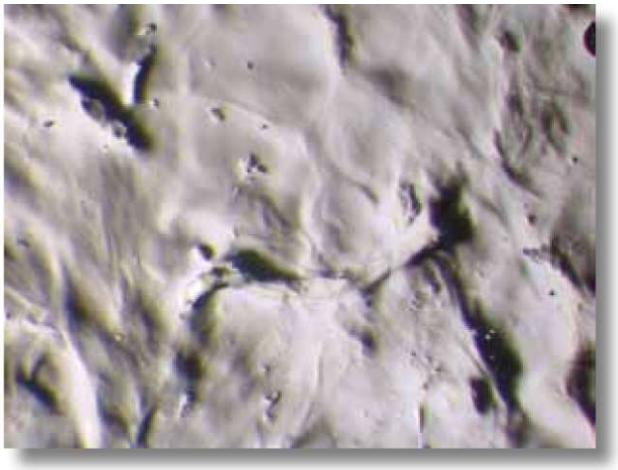
Crowded environments where the air is recirculated can often be heavily infected with unseen germs and viruses. For example, in an aeroplane the proximity of your fellow passengers, the low ceiling height and the multitude of international viruses boarding the flight with their hosts stack the odds of you completing the journey without picking up some unwanted illness against you.

Sometimes, the presence of germs is all too obvious. Many frequent flyers are able to recall an occasion where a passenger close to them was coughing and spluttering throughout the flight which led to them catching something during or soon after their trip.

People who work in large offices can face similar risks as the air conditioning system can spread viruses from one side of the building to the other. People who spend time in hotel rooms are also at risk as a recent report showed that rooms were never properly cleaned and the chance of picking up an infection was very high.



BEFORE
Nasaleze powder dry (taken from 100 x magnification)



AFTER
Nasaleze powder after exposure to damp surface (taken from 100 x magnification)

The main portal of entry for airborne germs and viruses is via the nose. Taking Nasaleze Travel daily or whenever you find yourself in a high risk environment has the potential to reduce your likelihood of catching a virus or another type of infection.

Clinical research on Nasaleze Travel

NO.	STUDY	POPULATION	DESCRIPTION	MEASUREMENTS AND RESULTS
1	Preventing Air-Borne Infections with an Intranasal Cellulose Powder Formulation *Hiltunen R, Josling PD, James MH* Advances in Therapy. 2007; 24(5):1146-53.	N=52 subjects	• Randomized = Nasaleze Travel vs. Nasaleze Allergy. • Determine whether Nasaleze Travel (with garlic) can reduce airborne infections. • Study period = 8 weeks in Finland & United Kingdom. • DOSE = 1 puff of Nasaleze Travel per day. Increase to 3 puffs if subject became sick with. infection. • Diary was kept recording 5-point symptoms scale.	Nasaleze Travel vs. Nasaleze Allergy: • Significantly fewer infections: 20 vs. 57 p<.001 • Far fewer days with infection: 126 vs. 240 days p<.05 • Number of serious infections > 7 days 6 vs. 12 p<0.05
2	Use of Nasaleze Travel as Prevention Method for Acute Respiratory Illnesses in Pediatrics *Geppe NA, Farber IM, Kozhevnikova TN, Andriyanova EV* Unpublished / Data on File	N=60 Children ▪ N=40 Treatment ▪ N=20 Control	• Randomized = Nasaleze Travel vs. No Treatment • Determine whether Nasaleze Travel can reduce incidence of Upper Respiratory Tract Infection (URTI) • Study period = 6 weeks in Moscow. • Use of 4-point symptoms scale • ENDPOINTS ▪ Incidence of Illness (URTI) ▪ Symptom Scores	Nasaleze Travel vs. No Treatment NZ Travel / Control / p-value • Did not fall ill at all 80% 0% p < 0.05 • Fell ill once 15% 55% NS • Fell ill twice 5% 45% p < 0.05 DEC 2008 - FEB 2009 (No Treatment) / DEC 2009 - FEB 2010 (Nasaleze Travel) • Incidence of URTIs 2.7 0.3 • Duration of URTIs 7.7 days 3.2 days Symptom Scores • In TREATMENT Group, there was definite reduction in URTI symptoms from Week 2 to Week 6
3	Viricidal Activity of Nasaleze and Nasaleze Travel in Cell Cultures Infected with Pathogenic Avian Flu Virus (H5N1) *Lvov DK, Deryabin PG* European Journal for Nutraceutical Research www.phytomedcentral.org | March 24, 2010	In vitro	TEST ARTICLES: Nasaleze Allergy; Nasaleze Travel CELL CULTURES: Porcine Embryo Kidney Cell Cultures (SPEV) INOCULUM: Flu A/H5N1 Virus ▪ High Dose = 10.0 TCID50 ▪ Low Dose = 1.0 TCID50 PROTOCOL: VARIATION 1 (Preventive; Before Infection with Flu Virus) / VARIATION 2 (Medical + Preventive; Immediately After Infection with Flu Virus) • Treatment • Nasaleze Administration CONTROLS: CONTROL 1 (Flu Virus ✓, Nasaleze X) / CONTROL 2 (Flu Virus X, Nasaleze ✓) / CONTROL 3 (Flu Virus X, Nasaleze X) EVALUATIONS • Timepoints: 2 days → 3 days → 4⅔ days • Assessments ▪ Viral Activity: Concentration of Flu Virus -- determined through titration for infectious activity ▪ Efficacy: % Survival of Infected Cells -- determined using optical microscopy to evaluate cells for morphology, vitality, cytoproliferative activity	VARIATION 1 -- PREVENTIVE Antiviral Activity BEFORE Infection with H1N1 Flu Virus ENDPOINT = CELL SURVIVAL FLU VIRUS HIGH / 2 days → 3 days → 4⅔ days DOSE Nasaleze Travel 100% 75% 0% Nasaleze Allergy 100% 20% 0% NO Test Product 80% 5% 0% FLU VIRUS LOW / 2 days → 3 days → 4⅔ days DOSE Nasaleze Travel 100% 100% 0% Nasaleze Allergy 100% 85% 0% NO Test Product 95% 30% 0% VARIATION 2 -- MEDICAL + PREVENTIVE Antiviral Activity IMMEDIATELY AFTER Infection with Flu ENDPOINT = CELL SURVIVAL FLU VIRUS HIGH / 2 days → 3 days → 4⅔ days DOSE Nasaleze Travel 100% 80% 0% Nasaleze Allergy 100% 25% 0% NO Test Product 75-80% 5% 0% FLU VIRUS LOW / 2 days → 3 days → 4⅔ days DOSE Nasaleze Travel 100% 90% 0% Nasaleze Allergy 100% 85% 0% NO Test Product 95% 25-30% 0% VARIATIONS 1 & 2 -- PREVENTIVE vs. MEDICAL + PREVENTIVE ENDPOINT = FLU VIRUS TITERS (at 3 Days) PREVENTIVE / MEDICAL + PREVENTIVE Nasaleze Travel 1.5 3.0 Nasaleze Allergy 3.0 4.0 NO Test Product 7.5 7.5
4	Nasaleze & Nasaleze Travel Safety Study Study of Effects of Inert Cellulose Powder on Nasal Mucosa *Angotoyeva IB and Sukhovetchenko YV* Russian Allergological Journal. N6; 2011.	N=60 ▪ N=30 GROUP I ▪ N=30 GROUP II	• GROUP I = Healthy Volunteers ⇒ Nasaleze Travel • GROUP II = Allergic Rhinitis ⇒ Nasaleze • ENDPOINTS ▪ Evaluation of Nasal Mucosa Condition ⇒ Assessed by Rhinoscopy + Endoscopy ▪ Mucociliary Clearance (MCC) ⇒ Assessed using Saccharine Test + Methylene Blue ▪ Ciliary Beat Frequency (CBF) ▪ Cytological Analysis of Mucosal Smears ▪ Signs of Inflammation ▪ Quality of Life (questionnaire)	• Rhinoscopy + Endoscopy + Cytology showed attenuation in nasal mucosa inflammation • Mucociliary transport was not inhibited • Nasaleze had no ciliotoxic effect on nasal mucosa • Nasal mucosa cell composition was unaffected Nasaleze Product Safety • No allergic reactions or significant side effects Quality of Life • QoL significantly improved in subjects with AR
5	Evaluation Biological Activity of Allicin + Nasaleze *Cutler RR, PhD* Unpublished / Data on File	In vitro	Bacteria: MRSA Clinical Isolate Test Samples: Allicin Powder; Nasaleze Cellulose Powder Control: Gum Acacia Powder Methods: Test sample is spread on 6mm well of plate / Incubated / Observed for Zone of Inhibition Zone of Inhibition / Biological Activity / Clinical Correlate No Zone: X: Bacterial Resistance Zone Size > 12mm: ✓: Bacterial Sensitivity	# / TEST SAMPLE / Allicin/Cellulose Ratio / 100 µg / 150 µg 1 Control: Control Powder Alone 0 0 2 Nasaleze: Cellulose Powder Alone 0 0 3 Allicin BN 2069: Allicin Powder Alone 14 19 4 Allicin + CPC 2102: 4:1 23 27 5 Allicin + CPC 2102: 6:1 28 28 6 Allicin + CPC 2069: 4:1 12 17 7 Allicin + CPC 2102: 8:1 22 26

TRAVEL Nasaleze®

Nasaleze patented delivery system

3 The nozzle delivers a fine mist of powder

2 The air and powder travel up the hollow delivery tube to the nozzle

1 When the bottle is squeezed, air forces Nasaleze powder up the hollow tube

Preventing Air-Borne Infections with an Intranasal Cellulose Powder Formulation

Hiltunen R, Josling PD, James MH

Advances in Therapy. 2007; 24(5):1146-53.

ABSTRACT

Fifty two volunteers were recruited to take part in a dual centred, randomized, blinded study to determine whether the level of airborne infections could be significantly reduced in patients receiving either plain Nasaleze cellulose extract or a combination of Nasaleze cellulose with PGE added (powdered garlic extract).

Volunteers were randomized to receive a plain cellulose extract delivered intra nasally or the same cellulose formulation with added PGE (powdered garlic extract). One puff into each nostril was recommended and if the volunteer caught an infection whilst travelling then at least 3 puffs per nostril were recommended until the symptoms reduced. The study took place over an 8 week period across Finland and England between November and March 2006/07. Volunteers were instructed to use a five-point scale to assess their health and record any common cold infections and symptoms in a daily diary. The active-treatment group (Nasaleze with PGE) had significantly fewer colds than the control group (20 vs 57, P<.001). The active treatment group also experienced far fewer days where a viral infection was obviously present (126 days in the active group vs 240 days in the control group p <0.05). Consequently, volunteers in the active group were less likely to pick up an airborne infection with the addition of PGE to this novel cellulose extract. Volunteers in the control were much more likely to get more than one cold over the treatment period or to suffer much longer periods of infection. This unique Nasaleze Travel formulation can significantly reduce the number of airborne infections that volunteers are exposed to whilst travelling throughout their respective countries.

Keywords: Nasaleze cellulose extract, Powdered garlic extract

INTRODUCTION

The common cold is the world's most widespread viral infection, with most people suffering approximately two to five colds per year. More than 200 different viruses are known to cause the symptoms of the common cold. Some, such as the rhinoviruses, seldom produce serious illnesses. Others, such as parainfluenza and respiratory syncytial virus, produce mild infections in adults but can precipitate severe lower respiratory infections in young children.

Rhinoviruses (from the Greek rhin, meaning "nose") cause an estimated 30 to 35 percent of all adult colds, and are most active in early fall, spring, and summer. More than 110 distinct rhinovirus types have been identified. These agents grow best at temperatures of about 91 degrees Fahrenheit, the temperature inside the human nose.

Scientists think coronaviruses cause a large percentage of all adult colds. They bring on colds primarily in the winter and early spring. Of the more than 30 kinds, three or four infect humans. The importance of coronaviruses as a cause of colds is hard to assess because, unlike rhinoviruses, they are difficult to grow in the laboratory.

Approximately 10 to 15 percent of adult colds are caused by viruses also responsible for other, more severe illnesses: adenoviruses, coxsackie viruses, echoviruses, orthomyxoviruses (including influenza A and B viruses, which cause flu), paramyxoviruses (including several parainfluenza viruses), respiratory syncytial virus, and enteroviruses.

The causes of 30 to 50 percent of adult colds, presumed to be viral, remain unidentified. The same viruses that produce colds in adults appear to cause colds in children. The relative importance of various viruses in pediatric colds, however, is unclear because it's difficult to isolate the precise cause of symptoms in studies of children with colds.

This is primarily an airborne infection, whose primary entry point in a human being is the nasal cavity. Touching your skin or environmental surfaces, such as telephones and stair rails, that have cold germs on them and then touching your eyes or nose or inhaling drops of mucus full of cold germs from the air are the most common methods of transmission.

Unfortunately airborne infections are commonplace all year round nowadays and although the chance of picking up an infection in the summer months is only 1 in 4 compared to winter there are some special factors that may increase the risk. Long haul jet flights appear to pose a special risk as there are no other periods when we are likely to be squeezed as tightly together with 400 potential sources of common cold infection. The chances are that any number of passengers will have the temerity to spread an airborne infection in the confined space of a jetliner making this an ideal environment for transmission of airborne disease. Experiments on exposing uninfected volunteers to others with common cold infections have shown that the chances of catching a cold are directly related to the number of hours of exposure to infection. Hence, you are much more likely to get a cold on a long haul

flight to the USA compared with a short hop to Europe. Our lifestyles often demand air conditioning which may contribute to infection. Although the lining of the nose is covered with a thin layer of mucus which protects against infection unfortunately air conditioners extract moisture from the air and therefore they may cause some drying of the protective mucous blanket in the nose and predispose to infection. This feature is one that our active test compound Nasaleze Travel® will significantly improve. The cold air may also help viruses to establish a hold in the nose as they reproduce better in a cold nose.

Travelling itself to different population areas, on public transport can significantly increase the risk of viral infection as we have probably already been exposed to all the current common cold viruses in our home environment but are likely to encounter quite new viruses, to which we have no immunity, as we circulate amongst our fellow human beings! We could ourselves be responsible for introducing new viruses into a foreign country if we arrive at a holiday or business destination with an active infection. With modern jet travel viruses are rapidly spread and this is why influenza spreads so rapidly around the world during an epidemic.

Sadly, since there are so many airborne infections available re-infection is prevalent.1 Published literature on the activity of garlic extracts (amongst others) against viral infections is sparse.2,3 but one report 4 describes that during an influenza epidemic, the former Soviet Union imported more than 500 tons of garlic cloves for acute treatment. Among the viruses thought to be sensitive to garlic extracts are the human cytomegalovirus, human rhinovirus type 2, herpes simplex types 1 and 2, and influenza B. Many consumers already take natural remedies including Echinacea, vitamin C, Zinc and garlic supplements as a preventive and report an absence of infection 5 colds or symptoms associated with viral replication.

Cellulose powder is used as a thickener in many liquid nasal sprays and is generally regarded as safe. The unique proprietary grade of micronized cellulose in this study (Nasaleze®) uses a patented device that ensures delivery into the nose of a suitable amount of material drawn from the container. Compared with liquid nasal sprays, which require preservatives, powdered cellulose inhibits bacterial and viral growth to a limited extent. While not a medicine, it is classified as a medical device that is safe to use throughout the year. This powdered cellulose product addresses the cause of allergic reactions, rather than the symptoms, because it works as a facial mask in preventing inhaled pollen, dirt, and allergens from reaching the lungs. This mechanism was also thought to help protect an individual from attack by airborne pathogens in particular viruses. In a healthy individual, the nose and nasal tract extract these materials from the inhaled air, including air that has been exposed to mucus membranes and therefore been stripped of allergens. Mucus has a low surface tension and can easily absorb allergens and infectious organisms from air as it passes down into the lungs.

Uniquely, the cellulose powder described herein turns into a gel on contact with the moisture always present in the nasal cavity. This gel is similar to normal mucus and helps to maintain delivery of a supply of clean air to the lungs.

This survey was designed to determine whether the addition of a simple garlic extract to Nasaleze® cellulose would enhance the capability of this formulation to trap airborne infections, disarm them and remove them safely into the stomach during normal mucociliary clearance. A randomized, blinded study design was incorporated in two countries, Finland and the United Kingdom to test whether the addition of PGE (powdered garlic extract) would increase the likelihood of preventing airborne infection amongst individuals travelling around locally and nationally during the cold winter period when airborne infections are at their peak.

METHODS

Following recruitment through advertisements in London and Helsinki daily newspapers, 52 participants were selected. A diary was designed in which each volunteer recorded general well-being for 8 weeks on a five-point scale as they travelled to and from work or on various other trips across the UK or Finland.

5 = well, no problems;

4 = quite well with occasional sneeze, not disruptive to normal routine;

3 = can feel a cold coming on, some minor symptoms;

2 = feeling low and beginning to exhibit symptoms;

1 = full cold symptoms [headache, sneezing, runny nose, tiredness].

If an infection occurred, volunteers noted the number and variety of symptoms, the day recovery began, and the day they felt completely better. The volunteers were separated into two groups of 26 participants each. A

simple random number generator assigned volunteers to the active or control group, and they were instructed to take one sniff up each nostril every day, according to the manufacturer's recommendation and if an infection was received then volunteers were instructed to take up to 3 sniffs per nostril every day that the infection was present to determine if the infectious period could be reduced in either group. Randomization codes were kept secure at the Herbal Research Centre and were not broken until all the diaries had been returned. Volunteers were contacted every 2 weeks to ensure that they were complying with the dosage regimen and that diary entries were made daily.

Diary Analysis

After diaries were returned, the number of infections experienced by volunteers was counted. An active infection was defined as a score of 3 or less that lasted for 4 days in succession. The duration of symptoms was the number of days with a recorded score of 3, 2 or 1, leading to an average recovery time that ended with a score of 4 or 5 taken across all recorded infections. The number of volunteers who did not experience a single airborne infection throughout the study period was recorded in each group.

Statistical Analysis

The average symptom length in days and the average number of days challenged by a cold were subjected to calculations of standard deviation, sample variance, and standard error of the difference of the means. Data were analysed by means of a Student's *t* test to gain a probability coefficient allowing for the calculated number of degrees of freedom.

RESULTS

No participants withdrew from the study and therefore an intention to treat analysis was performed on all completed diaries. At the end of the 56 - day study, 57 major infections were recorded in the control group, but the active group recorded a total of only 20 infections. This result is highly significant (P<.001) in favour of the addition of PGE to Nasaleze® as a preventative for airborne infections whilst travelling in daily lives.

The control group had 12 serious cases where an infection lasted for 7 days whereas the active group only had 6 such cases. Similarly the number of days reported with an active infection warranting a recorded score of 3 or less in the control group was 240 days whereas in the active group this was reduced to 126 days. This result is also highly significant at p<0.05.

During the study, the 11 volunteers taking the control experienced multiple infectious episodes but this was reduced to only 2 volunteers taking the active treatment suggesting that this was indeed a preventative option.

The details of our statistical analysis indicated that the sample variance and standard deviation was low and that although the two groups were composed of mostly female volunteers they were well matched statistically with a standard error for the difference of the means of just 0.76 for the number of active airborne infections suffered by each group so that the probability using a Students *t* test was p<0.01. Significance dropped to p<0.05 for both the number of volunteers with a serious infection lasting 7 days and the number of days reported with an active infection. However these figures clearly

	CONTROL GROUP (NASALEZE®)	**ACTIVE GROUP (NASALEZE TRAVEL®)**
Number of active infections during the study period	57	20 p<0.01
Number of volunteers without any infection	6	10
Number of volunteers with a serious infection lasting over 7 days	12	6 p<0.05
Number of days reported with an active infection	240	126 p<0.05
Number of volunteers experiencing multiple infections during the study period	11	2

Table 1 Results of randomized blinded comparison between 2 types of Nasaleze® cellulose extract administered intra nasally.

show a difference between the groups with the Nasaleze Travel® product proving superior to the plain Nasaleze® extract.

Volunteers were also asked to record in their diaries any other concerns they had during the study, such as comments about the acceptability of taking the product, side effects, taste, or other reason that might warrant discontinuation of treatment. Generally both groups were extremely well tolerated although in the active group several volunteers (3 in total) recorded that they could easily taste the PGE although this did not stop them from continuing with the treatment.

DISCUSSION

In this pilot investigation, two inert cellulose powder formulations, both dosed intra nasally using a novel, patented delivery system were compared in a pilot randomized and blinded study to see which formulation could provide the best protection against airborne infections of indiscriminate identity. Volunteers were encouraged to go about their normal daily lives travelling around their local and national boundaries. Some volunteers even ventured out internationally so this was a genuinely fair assessment of the relative dangers of picking up an airborne infection throughout the winter period and how that might be prevented.

The results were clearly in favour of the Nasaleze Travel® formulation now containing cellulose, mint and PGE (powdered garlic extract). Results indicate that a significant reduction in the number of airborne infectious pathogens picked up by volunteers was seen in this group when compared to plain Nasaleze® powder.

Examination of the volunteer diaries clearly shows that the control group suffered much more that the active group in terms of the number and duration of infectious episodes. Thus we can conclude that the addition of a potentially antiviral compound, in this case, a powdered garlic extract, can significantly reduce the number of infectious challenges that people meet during their travelling lives. The results also suggest that infection and reinfection may be effectively prevented by its daily use throughout the year, with an enormous potential savings to national industry in terms of reduced sick days. This product clearly exhibits excellent antiviral activity and warrants further investigation to determine the nature and method of its viral destruction.

ACKNOWLEDGMENTS

We should like to thank all the volunteers in both the United Kingdom and Finland, Charlotte Duxbury for her excellent assistance, Nasaleze International Limited for supplying the test materials.

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Use of Nasaleze Travel as Prevention Method for Acute Respiratory Illnesses in Pediatrics

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Use of Nasaleze Travel as a prevention method for acute respiratory illnesses in paediatric practice

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Acute viral respiratory infections are the most common childhood pathology. Every year, there are one to eight respiratory infections per child per year. The relevance of using prevention measures for viral respiratory infections is confirmed by the dynamics of incidence of the illness. Based on Rospotrebnadzor (Russian Federal Consumer Rights Protection and Human Health Control Service) data, the incidence of acute infections of the upper respiratory tract in May to December 2009 has grown by 3.5% compared with the same period in 2008 [1]. Children of all age groups are equally involved in the epidemic process. The average illness incidence in children from 0 to 2 years was 38.2% (for the 2008 epidemic season - 36.86%), three to six years - 43.5%, (41.9%), among schoolchildren - 27.3% (26.3%), and in persons over 18 years - 18% (15%) [2]. The highest incidence of the illness is noted among children of pre-school and primary school age. It is possible that adverse external factors also lead to an increase in the incidence of the illness (passive smoking, environmental pollution, living in industrial areas). The aetiology and clinical manifestations of URTI are varied, impeding the diagnosis and treatment of viral infections. Immunity after past URTI is type-specific, which results in repeat cases of illness. [3] The existing prevention methods are sufficiently well-developed but not always effective. Measures include: restricting the child's contact with people suffering from respiratory illnesses, ensuring good sanitation and hygiene, reduction in the use of public transport, extending the time the child spends in the fresh air and immunisation. However, children regularly attend formal establishments and it is possible to get infected at home, by parents, relatives and other children [4].

The high level of incidence, the severity of the diagnosis (especially in children of preschool and primary school age), the possible development of complications and the considerable socio-economic element of URTI result in a need to develop and put into practice effective preventive methods [5].

There are new opportunities for preventing respiratory infections through the use of the locally acting drug Nasaleze Travel. The drug consists of natural components - microdispersed cellulose powder and plant-derived wild garlic extract - which are sprayed from the vial onto the nasal cavity mucosa. A peppermint extract is also included as an auxiliary substance, giving a pleasant taste and odour to the powder. The preparation is a nasal powder spray acting as an "invisible mask", protecting the nasal mucosa from viruses and bacteria [6].

Upon contact with the nasal cavity mucus, the micronised cellulose (polysaccharide-cellulose obtained from plant cellular membrane) forms a gel-like coating that protects the body from microparticles that are inhaled in the air (viruses, bacteria, allergens, pollutants). [7] The wild garlic extract included in the drug composition has been used in medicine for over 5000 years, contains essential oils, a high amount of vitamin C and phytoncides. Phytoncides (from Greek *phytón* - plant and Latin *caedo* - to kill) are biologically active substances formed by plants, which detoxify or suppress the growth and development of microorganisms. The active substances in garlic are allicin and ajoenes, which have a proven anti-bacterial, fungicidal and anti-viral effect (the anti-viral effect is more pronounced in ajoenes). [8] As opposed to anti-biotics and anti-viral drugs, microorganism resistance does not develop for phytoncides.

The product is issued in the form of a dry spray in a special 500 mg bottle that dispenses the exact dose. A gel-like layer is formed on the nasal mucosa, acting as a natural barrier or filter against viruses and bacteria inhaled in the air, and breathing is not affected. Nasaleze Travel can be used prophylactically for daily defence against URTI during an epidemic season, for emergency protection before coming into contact with someone suffering from an infection, in places of mass public gathering or prior to journeys on public transport. Prescription is twice a day.

If required (after sneezing or blowing nose) it is recommended to repeat the spraying to restore the protective coating.

Aims and objectives.

An open comparative randomised study of the efficacy and safety of using microdispersed cellulose powder (Nasaleze Travel) for the prevention of respiratory viral infections in children was carried out over six weeks in the season from December 2009 to January 2010.

The study was based at the outpatient department of the Children's Diseases Clinic of the I.M. Sechenov Medical Academy, Moscow, as well as at the Tula Municipal Centre for Paediatric Respiratory Pathology. Parents of children included in the study were informed about the method of preventing respiratory infections. Monitoring included 63 patients aged three to 14 years who suffered from acute respiratory infections almost every month (from six to 12 times a year). 43 children were prescribed Nasaleze Travel. 20 children in the comparison group received symptomatic treatment. There were 28 girls (44%) and 35 boys (56%) and the average age was 6.8 ± 2.5 years.

Inclusion criteria for the programme were as follows: outpatients three to five years old and outpatients six to 12 years old; informed consent of the patient's parents for taking part in the study; no URTI symptoms; no heightened sensitivity to any of the product's ingredients.

Exclusion criteria for the study were as follows: hypersensitivity and/or contraindications for any ingredients of the investigative product; inability to follow medical recommendations; presence of somatic disorders that may worsen in the course of the patient's participation in the programme; no written consent for taking part in being monitored; patients suffering from severe forms of chronic illnesses; discontinuation of taking part in the programme. The reasons for patients' early withdrawal were: erroneous inclusion in the study; patient's desire to leave the study, deviation from the programme (non-observance of doctor's recommendations with regard to the investigative product); occurrence of severe adverse events calling for withdrawal of the investigative product.

Developing URTI symptoms during the period of observation was not an indication for discontinuing Nasaleze Travel. The patients were monitored for six weeks.

Throughout the observation period, the state of nasal breathing at night and during the day, discharge from the nasal cavity and its characteristics, sneezing and coughing were all evaluated daily on a 5-point scale (where 0 points - no symptoms; 1 point - symptoms appear but do not bother the patient significantly; 2 points - manifestations of the illness cause moderate discomfort, 3 points - symptoms are pronounced, they reduce the patient's activity and affect sleep, 4 points - manifestations of the illness are expressly pronounced, they significantly reduce the patient's activity and negatively affect sleep). Body temperature, intoxication symptoms (headache, lack of energy, drowsiness, restless sleep), tolerance of the drug based on presence/absence of allergic reactions and other side effects were also evaluated.

Parameters were monitored at weeks two and six after starting use of the drug. The Nasaleze Travel medical device was used in accordance with the recommended dosage: one spray into each nostril twice a day. Patients were recommended to re-spray Nasaleze Travel after each time they blew their nose or when likely to come into contact with someone suffering from URTI in order to restore the protective layer.

All patients taking part in the study belonged to the group of children who are frequently ill (URTI 6-10 times/year). The comparison group consisted of 20 children, (control group) comparable in age and gender, not receiving treatment with Nasaleze Travel spray.

Permissible therapy: vitamins and drugs that have to be taken for concurrent conditions, provided they are not included in the list of drugs not permitted for use during the study.

Prohibited therapy during the treatment was taking other nasal medical preparations as well as drugs for prevention of URTI (Grippferron, Viferon, Arbidol etc.)

Study group characteristics.

Data regarding objective and subjective URTI symptoms during and after use of Nasaleze Travel was evaluated. These indicators were compared with the same ones in the group of patients who did not receive preventive treatment with the product and with the same period in the previous year for patients receiving Nasaleze Travel. The results were recorded in the "Patient observation diary".

The average age of patients in the main group (1) and comparison group (2) was 6.9 ± 2.5 and 7.1 ± 3.2 years accordingly. By the start of the study the frequency of URTI for the past three months in both groups was 2.92 ± 1.3 and 2.84 ± 1.78. The frequency of URTI in the previous year in these groups was 2.72±1.11 and 2.79±1.7.

A similar number of children with concurrent allergic conditions and illnesses of the ENT organs was noted in both groups. (Table No. 1)

Table 1. Patient medical history characteristics

GROUPS	MAIN		CONTROL	
	%	Number of children	%	Number of children
Obstructive bronchitis	7.7%	3	10%	2
Bronchial asthma	23%	9	25%	5
Allergic rhinitis	31%	12	30%	6
Atopic dermatitis	10%	4	12.5%	2
Chronic tonsillitis	3.12%	8	5%	1
Adenoids	28.25%	11	25%	5
Chronic rhinopharyngitis	10.2%	4	10%	2

At the start of the study the patients had not received any other drugs for the prevention of URTI.

The patients visited the doctor three times every 2.5 weeks (Table No. 2).

Table 2. Case monitoring timetable for the patients per visit.

Evaluation of efficacy and safety variables was done in accordance with the observation schedule

STUDIES	Visit 1 (prior to starting therapy)	Visit 2 (after 2 weeks)	Visit 3 (after 4 weeks)
Informed consent	✘		
URTI frequency over the past 3 months, URTI frequency the previous year (December, January)	✘		
History of allergic reactions (presence of concurrent allergic conditions, ENT illnesses)	✘		
Patient examination	✘	✘	✘
Inclusion and exclusion criteria	✘		
Evaluation of URTI symptoms' intensity, should they occur (using a 5-point scale, where 0 means 'no symptom' and 4 means 'symptom has maximum intensity')	✘	daily	daily
ENT specialist consultation	✘		✘
Assessment of adverse events		✘	✘
General doctor and patient assessment		✘	✘

Results of the study and discussion:

Analysis of the observation cards has revealed that over the observation period, of the 43 children in the main group, individual intolerance of the drug was observed in three children (6%). All three had allergic conditions: bronchial asthma and perennial allergic rhinitis. In two patients, intensification of all URTI symptoms was observed, coupled with intensified bronchial asthma, which may have been connected with individual sensitivity. Nasal bleeding was noted in one patient on day four of using the drug. The drug was discontinued and the children were withdrawn from further observation. Thus, 40 children remained in the main group and continued to take the drug in accordance with the study protocol.

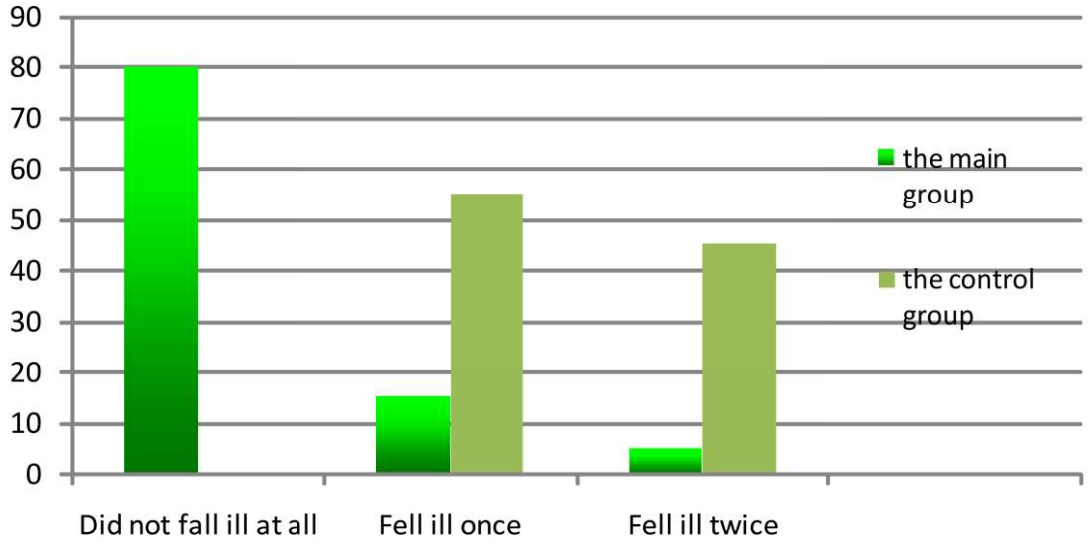
Of these 40 children:

- 32 children (80%) did not fall ill at all
- 6 children (15%) fell ill once
- 2 children (5%) fell ill twice

Table 3. Incidence of illness in children in the main and control groups for the observation period.

INCIDENCE OF ILLNESS	Nasaleze Travel	Control
Did not fall ill at all	32 *(80%)	0 (0%)
Fell ill once	6 (15%)	11 **(55%)
Fell ill twice	2 (5%)	9 *(45%)
TOTAL	40 (100%)	20 (100%)
* - differences are significant, (p<0.05)	** - differences are significant, (p<0.1)	

Fig. No. 1. Incidence of illness in children in the main and control groups for the observation period.



We have analysed the data about the incidence of illness among the children of the main group who received Nasaleze Travel for the same period the previous year. Table No. 2.

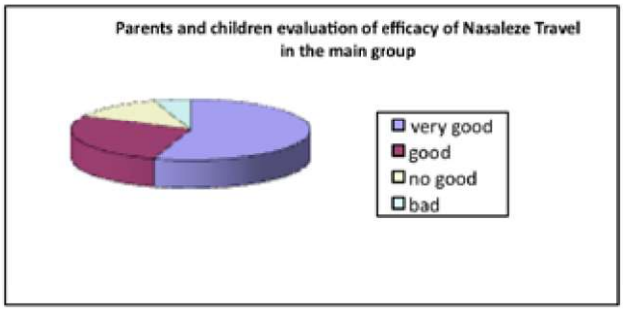
Table 4. Comparative analysis of incidence of URTI in 2008 and 2009 for children in the main group.

EVALUATION CRITERION	Number of children receiving Nasaleze Travel		
	2008 - 2009 (December, January, February)	2009 - 2010 (December, January, February)	
Number of instances of URTI	2.72 ± 1.11	0.25 ± 0.54	Decreased by 10 times
Duration of URTI (in days)	7.65 ± 3.54	3.24 ± 2.17	Decreased by 2.5 times

Thus, the number of children who did not fall ill in the main group was 80% (32 children); in 17.5% of children, the severity of illnesses decreased. Compared to the same period last year, taking Nasaleze Travel decreased incidence in 90% of patients.

Adverse effects associated with taking Nasaleze Travel were noted in four patients (10%). Five days after taking the drug, children experienced severe nasal discharge (rhinorrhea) and sneezing intensified; these decreased when antihistamines were added to the therapy. Three of these children had bronchial asthma coupled with perennial allergic rhinitis. One child had a medical history of chronic tonsillitis. These children had no catarrhal events registered over the whole observation period, their temperature did not go up, the children did not have URTI and continued taking Nasaleze Travel.

On the whole, the majority of parents (82.5%) and doctors (90%) considered the microdispersed cellulose powder Nasaleze Travel highly effective for the preventive treatment of acute respiratory infections **(Fig. No. 2, 3)**. Good tolerance of Nasaleze Travel was noted by 72.5% of parents and 87.5% of doctors **(Fig. No. 4, 5) .**



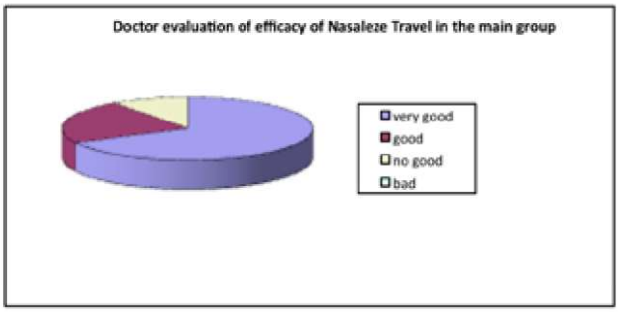
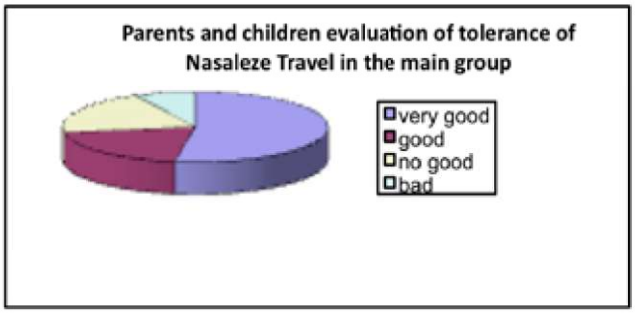
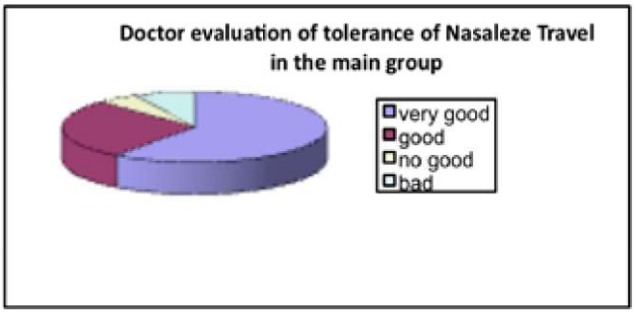


Fig. 2, 3. Parent and doctor evaluation of efficacy of Nasaleze Travel in the main group.

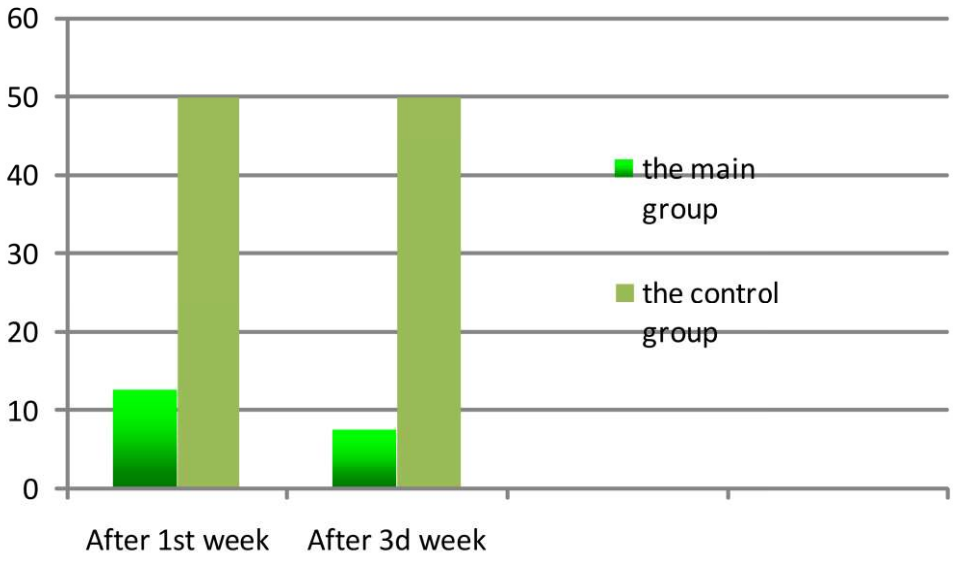
Fig. 4, 5. Parent and doctor evaluation of tolerance of Nasaleze Travel in the main group.





One week after the start of using microdispersed cellulose powder (Nasaleze Travel), five children (12.5%) had fallen ill in the main group, whereas 10 children (50%) had fallen ill in the control group. three weeks after starting use of the drug, in the main group three children – 7.5% (two of them had a repeat illness) fell ill in the main group, and in the control group, again 10 children fell ill – 50% (nine of them had a repeat illness). Fig. No. 6

Fig. No. 6. Illness incidence for children in the main and control groups towards the end of observation weeks one and three.



We have conducted a points-based evaluation of the URTI symptoms in children who fell ill in both groups, a week after the start of the URTI illness, i.e. weeks two and six after the start of observation. In two weeks, children in the main group who fell ill had a less marked manifestation of the main URTI symptoms, the points-based evaluation of which is shown in Fig. 1-7, as compared to the control group: nasal congestion in the daytime decreased from 0.91 ± 0.4 to 0.64 ± 0.6 points; nasal congestion at night decreased from 1.07 ± 0.5 to 0.67 ± 0.6; sneezing – from 0.62 ± 0.6 to 0.51 ± 0.6; headache, lack of energy and drowsiness decreased from 0.43 ± 0.5 to 0.25 ± 0.6; and restlessness during sleep decreased from 0.4 ± 0.5 to 0.23 ± 0.5 ($p<0.05$).

Dynamics of points-based evaluation of subjective URTI symptoms in children of the main and control groups in week two (visit two) and in week six (visit three) of the observation (OY axis – intensity of symptoms expressed in points) ($p<0.05$). Fig. No. 7-13.

Fig. 7. Nasal congestion in the daytime

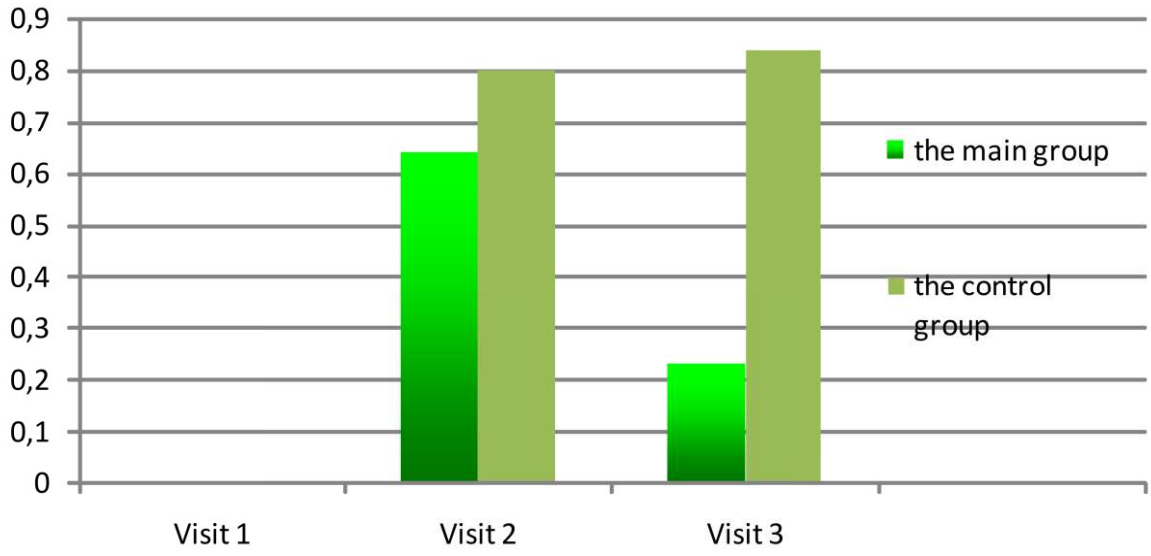


Fig. 8. Nasal congestion at night

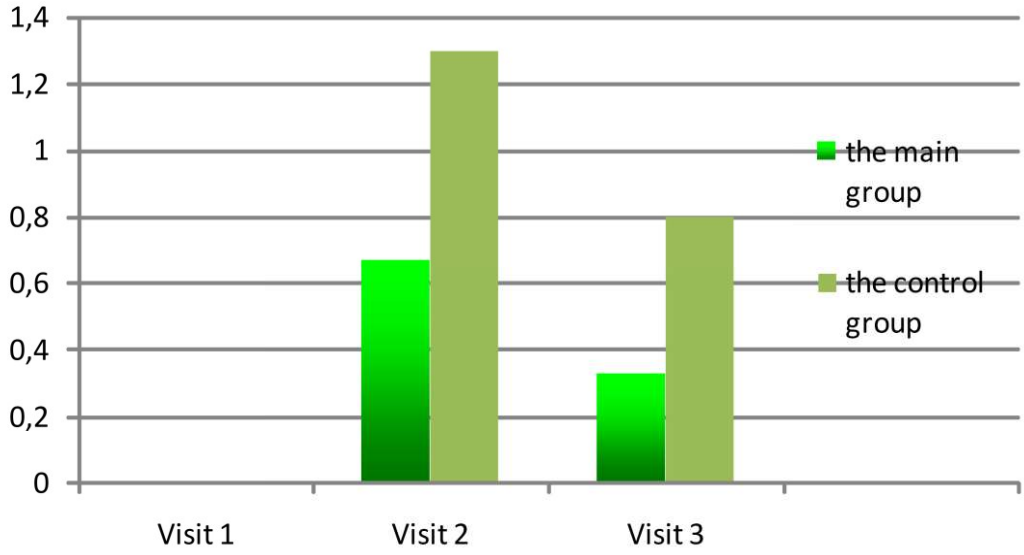


Fig. 9. Sneezing

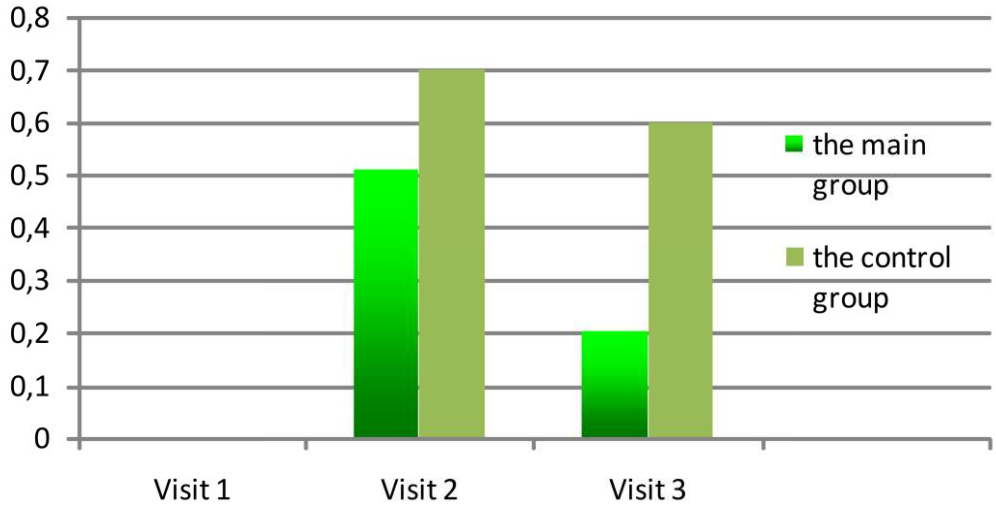


Fig. 10. Nasal discharge

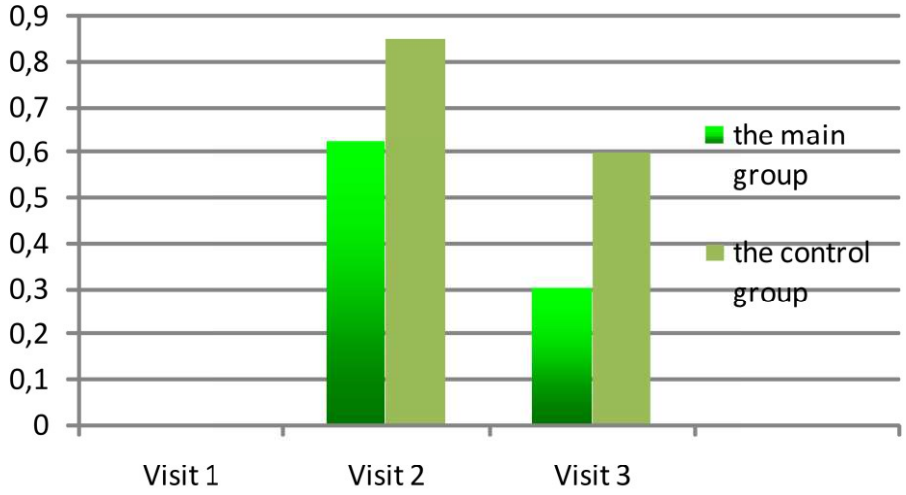


Fig. 11. Cough

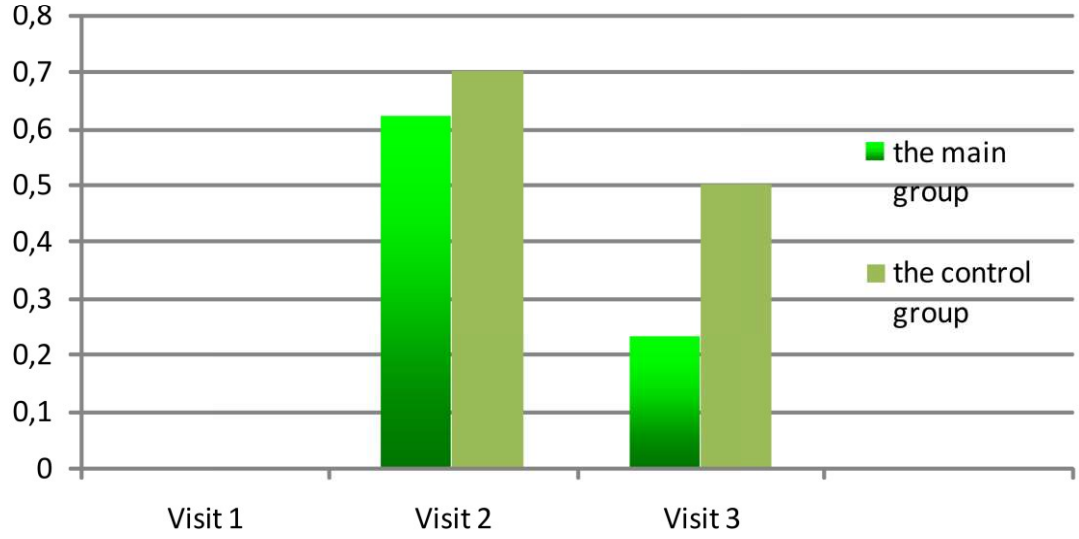


Fig. 12. Headache, lack of energy, drowsiness

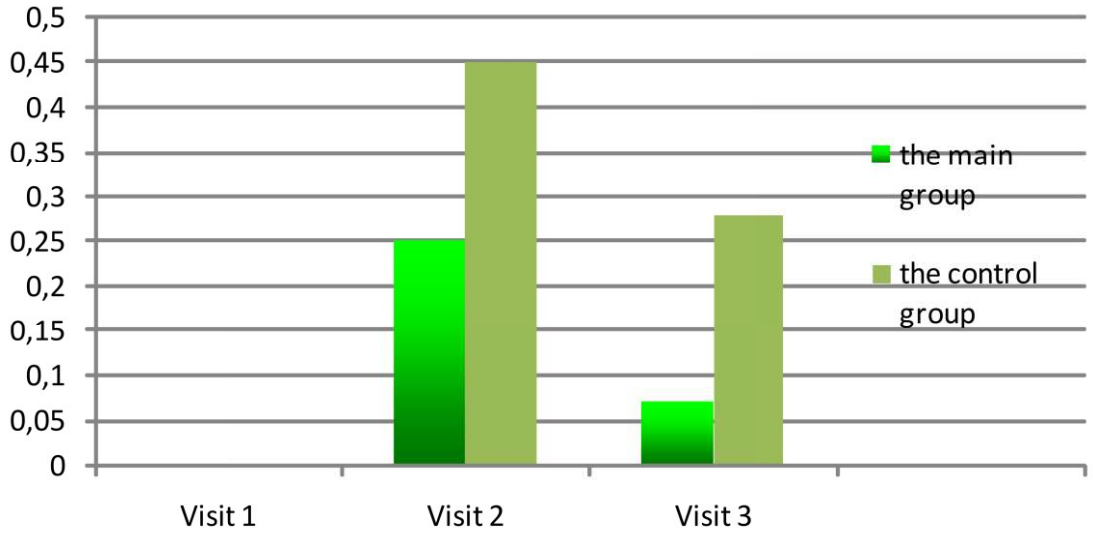
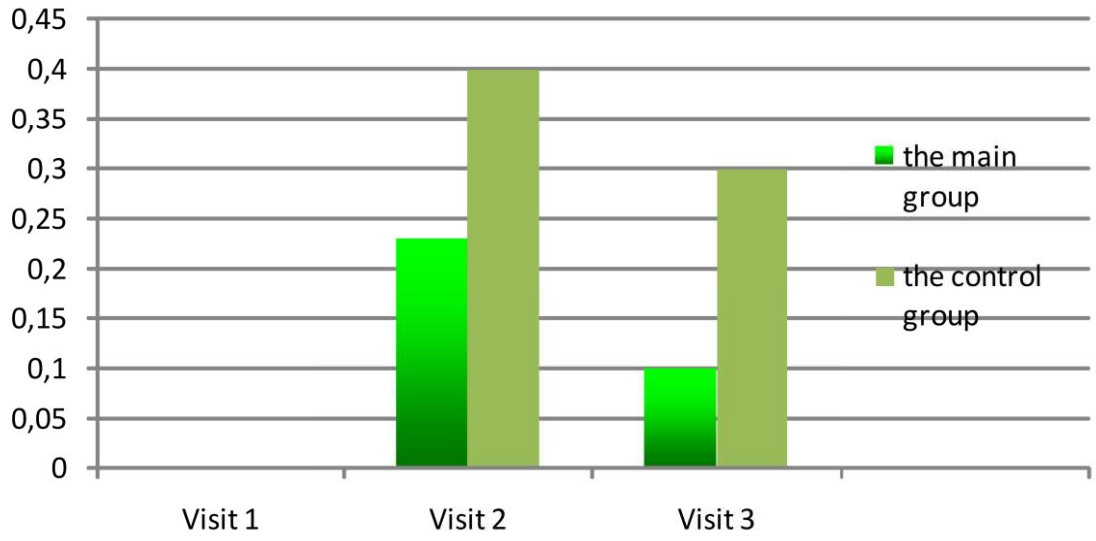


Fig. 13. Restlessness during sleep



In six weeks, a considerable reduction in objective and subjective URTI symptoms was noted as compared with the control group: nasal congestion in the daytime decreased from 0.91 ± 0.4 to 0.23 ± 0.37 points; nasal congestion at night - from 1.07 ± 0.5 to 0.33 ± 0.54; sneezing - from 0.62 ± 0.6 to 0.2 ± 0.44; nasal discharge - from 0.69 ± 0.5 to 0.3 ± 0.28; cough - from 0.64 ± 0.5 to 0.23 ± 0.4; headache, lack of energy and drowsiness - from 0.43 ± 0.5 to 0.07 ± 0.08; restlessness during sleep - from 0.4 ± 0.5 to 0.1 ± 0.09 ($p<0.001$). These data reflect the fact that fewer children had fallen ill by that time in the main group and their illnesses were less severe compared with those of the children in the control group.

Thus, the impact of the microdispersed cellulose powder Nasaleze Travel on objective and subjective URTI symptoms has been clearly demonstrated.

Conclusion:

1. When taking Nasaleze Travel:
   - did not fall ill during the observation period - 32 children (80%)
   - had one episode of URTI - six children (15%)
   - were ill twice - two children (5%).
2. Compared with the same period last year, the illness incidence decreased in 90% of patients, and the duration of URTI (in days) decreased by 2.5 times.
3. Whereas in the control group there were no children who did not fall ill at least once, 11 children (55%) fell ill once, and nine children fell ill twice (45%). Thus, the total number of children who fell ill in the main group is 80% less than in the control group.
4. Tolerance of the drug was noted as very good in the majority of cases; individual intolerance of the drug was observed in three children (6%). In two children, the start of taking the drug caused an intensification of bronchial asthma, of moderate to severe intensity, leading to withdrawal of the drug. In 1 patient, an instance of nasal bleeding was noted on day four of using the drug; this also led to withdrawal of the drug. Thus, Nasaleze Travel must be prescribed with care to children with moderate to severe bronchial asthma for the prevention of URTI. Moreover, presence of nasal bleeding in medical history should be a criterion for excluding patients from the study.

5. Many parents noted the ease of using the drug. The majority of parents (82.5%) rated the microdispersed cellulose powder Nasaleze Travel as a highly effective preventive agent against URTI. Good tolerance of the drug was noted by 72.5% of parents**.**

6. Also, when taking Nasaleze Travel, a clear effect on URTI symptoms in children who fell ill in the main group was noted as compared to control group children. A week from the start of illness, children experienced a definite reduction in such symptoms as nasal congestion in the daytime and at night, nasal discharge, cough, headache, lack of energy; and a tendency towards normal sleep was noted as compared to the control group. A definite reduction in objective and subjective URTI symptoms was also noted in week six of taking the drug.

7. Thus, the use of Nasaleze Travel as a means for preventing the development of respiratory illnesses in children must be recommended for a period of at least one month.

Nasaleze Travel can be recommended for carrying out preventive treatment of cold-related illnesses in children.

Discussion:

Thus, daily use of Nasaleze Travel with a preventive and protective aim: definitely prevents occurrence of respiratory infections (URTI); and protects against re-infection. Use of Nasaleze Travel during the active infection period helps to reduce the duration of the illness; and reduces the severity of URTI. It is important that Nasaleze Travel is not absorbed into the bloodstream, has no systemic action and does not affect immunity. It creates a double natural barrier, mechanical and biological, providing anti-bacterial and anti-viral protection. It is also known that Nasaleze Travel consists of only natural components and is safe for prolonged use throughout the season of cold-related diseases. Microdispersed cellulose powder is well-tolerated, easy to use and may be used in children of any age, starting from the very young. Regular use of inert cellulose powder in the nostrils may effectively prevent and alleviate the symptoms of URTI.

Nasaleze Travel is a modern, effective and safe natural spray for protecting the body against viruses, bacteria and other harmful external factors.

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Viricidal Activity of Nasaleze and Nasaleze Travel in Cell Cultures Infected with Pathogenic Avian Flu Virus (H5N1)

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Virucidal activity of Nasaleze (Nasaval) and Nasaleze Travel (Nasaval Plus) in cell cultures infected with pathogenic avian flu virus (H5N1)

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Abstract

This *in vitro* study determined the viral efficacy of two cellulose formulations presented as Nasaleze (Nasaval) and Nasaleze Travel (Nasaval PLUS) against Influenza A/Duck/Novosibirsk/56/05 (Avian Flu H5N1) at concentrations that did not exhibit toxicity. Both test substances were used at sub-optimal dosing levels. The virucidal activity of both formulations was measured at 48, 72 and 112 hour periods after incubation. Results showed that both formulations were able to reduce the viral titre of Influenza A/Duck/Novosibirsk/56/05 (Avian Flu H5N1) significantly when compared to the control virus titre. The extract Nasaleze Travel (Nasaval PLUS) showed greater activity and both formulations showed potential to be used as preventative agents. These data reinforce the established antiviral activity of these formulations acting as barrier prevention and disruption of viral replication.

Introduction

In recent years a number of countries in East and South-East Asia have seen an outbreak of avian flu A (H5N1). The infection mainly affects poultry (chickens and ducks) which are then wiped out in their hundreds of thousands. But there have also been cases where the virus has affected people. The total number of people killed by the infection has been low but the fatality rate has been astonishing: around 70% of those infected have died, even when given treatment. The highly pathogenic avian flu virus arrived in Russia in July 2005 and to date the H5N1 flu virus has been recorded in many parts of the Russian Federation: in Western Siberia, in the Urals and in the Astrakhan province.

As we know, flu is primarily an infection which affects birds, mainly waterfowl, and all of the strains of the human flu virus come from avian bird flu viruses. The genome of any human virus contains genes from avian viruses.

Avian flu is extremely dangerous for humans, but fortunately it cannot be transmitted between people and can only be caught from infected birds. Human flu is easily transferred between people but the strains we are familiar with have become manageable on account of their joint evolution. However, some animals, primarily pigs, are easily infected with this and other types of flu. When the avian flu epizootic combines with a human flu epidemic (and they normally occur during the same months), both viruses can be found in pigs. The simultaneous reproduction of the two viruses in pigs may lead to reassortment and to the emergence of a new "hybrid" virus, in which the "avian" proteins and antigens of the avian flu A virus will combine with the ability to be transferred from person to person. At this point, a disaster is almost inevitable: the new agent will be infectious like human flu and lethal like bird flu. There is therefore a real threat of a new pandemic strain appearing.

We therefore need to develop new treatments and preventive measures for flu. At the D.I. Ivanovsky Scientific Research Institute of Virology we carry out research into the avian flu virus, developing diagnosis methods and treatment and preventive measures for the infection. Practically all known strains of avian and human flu viruses are held at the State Virus Collection at the Institute. It is precisely these viruses which could serve as the building blocks for a future pandemic virus. In particular, during the first outbreak of the H5N1 flu virus, we isolated the first highly pathogenic strains of this virus from patients and poultry (ducks and chickens) that had died from the disease, which were then deposited at the State Virus Collection. We are currently researching the decoding of the epizootics amongst birds in different parts of the country including the Republic of Kalmykia and the Astrakhan Province. Moreover, the research at the Institute is aimed at improving diagnosis methods, preventive measures and the treatment of this infection.

The D.I. Ivanovsky Scientific Research Institute of Virology at the Russian Academy of Medical Sciences, is licensed to carry out pre-clinical trials of different products, received commercial samples of two products to be studied from Pharmaval Inc. Nasaleze (Nasaval in Russia) and Nasaleze Travel (Nasaval PLUS in Russia), manufactured by Nasaleze Ltd, in Ramsey, Isle of Man. The aim of the research was to study the activity of these unique cellulose powder extracts against infection with the pandemic flu A/H5N1 virus in cell cultures, which we isolated during the poultry epizootic in July 2005 in the Novosibirsk province.

Materials and Methods

The virus

Our observations were carried out on both test substances and we determined the anti-viral activity against strains of the flu A/Duck/Novosibirsk/56/05 virus which was isolated in summer 2005 from infected ducks in the Novosibirsk province and deposited at the State Virus Collection. The virus multiplies in Madin-Darby canine kidney (MDCK) cell cultures (embryonic canine kidney cell cultures), in SPEV cell lines (porcine embryo kidney) and in many other cell cultures.

Cell cultures

Porcine embryo kidney cell cultures (SPEV) were used as the substrate for studying antiviral activity. This virus multiplies and accumulates in a titer of up to 4.5 lg TCD50 in these cultures. SPEV cell cultures were cultivated in medium 199 with the addition of 10% foetal bovine serum and antibiotics. As the support medium for the cells which have been infected with the flu virus we used the same nutrition medium composition without adding the serum. The cells were cultivated in single-use 24-hole sterile plastic culture plates. .

Test Samples

Nasaleze (Nasaval) and Nasaleze Travel (Nasaval PLUS) were used in the form of a ready-prepared nasal spray in 500mg bottles providing 200 doses, which we received from the Pharmaval Inc. During our trials we used one dose of each of the products which was the equivalent of one spray, equal to 2.5mg of the product.

Trial protocol
1st variant

On the second day after planting the SPEV cell cultures in 24-hole plastic plates, a cell monolayer had formed in the holes. The nutrient medium was removed from each of the holes, the holes were washed with 0.4 ml of the support medium, after which the holes were drained off, leaving around 0.1 ml of the medium in the hole. The spray containing each test substance was sprayed into each hole with a cell monolayer, with 1 spray of each of the products in 8 of the holes with the cell cultures. 10 minutes after the cells had been treated with the powder spray 20 µl of the flu A virus was added to 4 of the holes in a dose of 10.0 $TCID_{50}$, and 20 µl of the flu A virus was added to another 4 holes in a dose of 1.0 TCD_{50}. The 8 holes with a cell culture monolayer were infected with the flu virus in doses of 10.0 $TCID_{50}$ and 1.0 $TCID_{50}$ (4 holes for each dose), but were not treated with the products. The remaining 8 holes with a SPEV cell culture monolayer were not infected with the virus but were treated with the test substances in the same doses. After 30 minutes contact between the virus and the cells, 0.4 ml of the support medium (medium 199 with added antibiotics but without foetal bovine serum) was added to each of the holes and they were left in a germinator at 36.7° C. The percentage of healthy cells was determined towards the end of the experiment using methylene-blue.

2nd variant

On the second day after planting the SPEV cell cultures in 24-hole plastic plates, a cell monolayer had formed in the holes. The nutrient medium was removed from each of the holes, the holes were washed with 0.4 ml of the support medium, and the support medium was then drained off. Then 20 µl of the flu A virus was added to 8 holes in a dose of 10.0 $TCID_{50}$, and 20 µl of the flu A virus was added to another 8 holes in a dose of 1.0 $TCID_{50}$. After 30 minutes of contact for the virus to be adsorbed onto the cells, the powder spray containing Nasaleze (Nasaval) and Nasaleze Travel (Nasaval PLUS) was sprayed into each of the holes with the infected cell monolayer, with 1 spray of each of the products for the 4 holes with the cell cultures. The remaining 4 holes with the monolayer of infected SPEV cell cultures were not treated with the products. 0.4 ml of the support medium (medium 199 with added antibiotics but without foetal bovine serum) was then added to each of the holes and they were left in a germinator at 36.7° C. The infected cultures were observed over 4-5 days, and cytopathic changes were observed in the infected control cell cultures which were not treated with the test substances.

Determining the ability of the infected cells to produce the infectious flu A/H5N1 virus

48 hours after the cells were infected, 40 µL of the nutrient media was removed from the holes containing the infected SPEV cell cultures and the concentration of the infectious virus in the samples was determined through titration for infectious activity using a 2-day-old SPEV cell culture monolayer cultivated in 96-hole plates. After reaching the maximum display of cytopathic action, infectious titers were found in all of the test variants. The percentage of healthy cells was determined towards the end of the experiment using methylene-blue.

Results

The results are shown in tables 1 – 3.

Cytotoxic properties of the test substances

Upon visual observation under an optical microscope we were able to see that, in terms of morphological properties, vitality and cytoproliferative activity, the SPEV cell cultures did not differ from similar cells cultivated without treatment by the test substances over a period of 7-8 days' cultivation. On the first day after treatment with the test substances we were able to use the microscope to see a semi-transparent film covering the cell monolayer which disappeared after the 2nd day of observation and which had no effect on the vitality of the SPEV cells for the entire observation period.

Antiviral activity of Nasaleze (Nasaval) and Nasaleze Travel (Nasaval PLUS)

The information shown in table 1 shows that the test substances when treating the cell cultures before infection with the flu H5N1 virus (preventive application) in a dose of 2.5 mg per hole, are able to protect most of the

Table 1 : Antiviral properties of the products Nasaleze (Nasaval) and Nasaleze Travel (Nasaval PLUS) with regard to infection with the flu A/H5N1 virus in SPEV cell cultures. Effect on the vitality of infected cells when used for preventive purposes.

Dose of the virus (TCD50)	Products	Percentage of infected cells in the monolayer					
		SPEV+product+virus			SPEV without the product+virus		
		48 hours after infection	72 hours after infection	112 hours after infection	48 hours after infection	72 hours after infection	112 hours after infection
10.0	Nasaleze (Nasaval)	100±0	20±5	0	80±10	5±5	0
	Nasaleze Travel (Nasaval Plus)	100±0	75±10	0	80±10	5±5	0
1.0	Nasaleze (Nasaval)	100±0	85±10	0	95±15	30±5	0
	Nasaleze Travel (Nasaval Plus)	100±0	100±0	0	95±15	30±5	0

SPEV cell monolayer against the cytopathogenic effect of the flu A virus within 72 hours after infecting the cells. It was found that up to 85% - 100% of the cells in the monolayer survive when treated with the product Nasaleze Travel (Nasaval PLUS), while a total of 30% of the SPEV cells infected with the flu virus which are not treated with the product survive. It was also found that Nasaleze Travel (Nasaval PLUS) has a slightly greater antiviral effect than original Nasaleze (Nasaval).

At 112 hours after infection, most of the cells in the control and experimental test variants had been killed.

We received similar data when using the test substances immediately after infecting the SPEV cell cultures (table 2). We also found that this depended on the characteristics of the product which was used. So, when infecting the SPEV cells with the flu A virus in a dose of 10.0 $TCID_{50}$ under the effect of the test substance Nasaleze (Nasaval) at 72 hours after infection, 25% of the infected cells survived (in the control samples which were not treated with the product 5% of the cells survived in these conditions).

Table 2 : Antiviral properties of the products Nasaleze (Nasaval) and Nasaleze Travel (Nasaval PLUS) with regard to infection with the flu A/H5N1 virus in SPEV cell cultures. Effect on the vitality of infected cells when used for medical and preventive purposes.v

Dose of the virus (TCD50)	Products	Percentage of infected cells in the monolayer					
		SPEV+product+virus			SPEV without the product+virus		
		48 hours after infection	72 hours after infection	112 hours after infection	48 hours after infection	72 hours after infection	112 hours after infection
10.0	Nasaleze (Nasaval)	100±0	25±5	0	75±10	5±5	0
	Nasaleze Travel (Nasaval Plus)	100±0	80±10	0	85±10	5±5	0
1.0	Nasaleze (Nasaval)	100±0	85±10	0	95±15	25±5	0
	Nasaleze Travel (Nasaval Plus)	100±0	90±0	0	95±15	30±5	0

If the cell cultures were treated with the product Nasaleze Travel (Nasaval PLUS), 80% of the cells survived after 72 hours. However, in these conditions cells in all of the test variants had died at 112 hours after infection. Multiple treatments of the cells with the products would most probably be needed in order to achieve a stable antiviral effect.

It was interesting to learn about the effect of these test substances on the ability of the infected SPEV cells to produce the flu A virus in the medium. The results of titration of the samples of the medium collected from the infected cell cultures at 72 hours after infection are shown in table 3.

Table 3 : Antiviral properties of the products Nasaleze (Nasaval) and Nasaleze Travel (Nasaval PLUS) for the flu A/H5N1 virus in SPEV cell cultures. Effect on the concentration of the infectious virus produced by the cells (during preventive use of the products). Virus dose of 1.0 lg TCID50.

Route of administration	Products	Flu A virus titers (lg TCID50/ml) 72 hours after infection	
		SPEV+product+virus	SPEV without the product+virus
		72 hours after infection	72 hours after infection
Preventive	Nasaleze (Nasaval)	3.0±0.5	7.5±0.5
	Nasaleze Travel	1.5±0.5	7.5±0.5
Medical and preventive	Nasaleze (Nasaval)	4.0±0.5	7.5±0.5
	Nasaleze Travel (Nasaval Plus)	3.0±0.5	7.5±0.5

These show that at 72 hours after infection, the Nasaleze (Nasaval) test substance was able to reduce the production of the virus by the cells by 10,000+ times when compared with the production of the virus by untreated cells (table 3). In these conditions Nasaleze Travel (Nasaval PLUS) significantly reduced the infectious activity of the virus (to 6.0 lg $TCID_{50}$). Significant but somewhat lower levels of antiviral activity of the products were shown when using them for medical and preventive purposes (table 3).

These data sets indicate that the test substances Nasaleze (Nasaval) and Nasaleze Travel (Nasaval PLUS) are able to protect the cells from the cytopathogenic effect of the highly pathogenic flu A/H5N1 virus. The factors involved in the antiviral effect of theses natural compounds require further research. At the same time, we should point out the known viricidal qualities (ability to inactivate the infections properties of virions) of phytoncides in the composition of Nasaleze Travel (Nasaval PLUS) would suggest that it is superior to Nasaleze (Nasaval). However, the data generated clearly shows the antiviral effect of Nasaleze (Nasaval) without adding phytoncides. Here we should point out that the test substances, which are presented as microcellular powder, after treatment of the cell monolayer in combination with culture fluid, form a gel-like film layer which is often used in virological research to limit the reproduction of viruses. It is possible that this film may protect the cells against the adsorption of viruses onto their membrane.

Furthermore if the virus still penetrates the cell where it is not protected by the film, the virus which has multiplied and left the cell cannot be passed on to healthy cells which are protected by the film. Therefore, the infection process is significantly slowed down and could even be stopped with multiple applications of the test substances. It is also likely that the toxins and proteins which are formed as a result of the death of the infected cells will be used by the film, swept down into the stomach by normal muco-cilliary clearance mechanisms and will not cause intoxication or allergisation, which are observed during the normal infection process.

Conclusion and Discussion

The test substances Nasaleze (Nasaval) and Nasaleze Travel (Nasaval PLUS), provided by Pharmaval Inc, are able to protect most cell cultures from the cytopathogenic effect of the flu A/H5N1 virus. Our results indicate the Nasaleze Travel (Nasaval PLUS) product has more pronounced antiviral properties than the Nasaleze (Nasaval) formula. Both substances are however capable of significantly reducing the production of the flu A/H5N1 virus by infected cells over a period of 72 hours after the cells are infected using the equivalent of just 1 daily dose. Moreover, neither test substance showed any cytotoxic properties for SPEV cell cultures.

It is clear that these simple patented natural formulations have some interesting virucidal properties that warrant further investigation and that they could certainly be utilized as an alternative in preventing and perhaps treating active viral infections including the currently well described "avian flu". Our data indicate very strongly that Nasaleze (Nasaval) and particularly Nasaleze Travel (Nasaval PLUS) could be used both as a preventative measure and a treatment option for this pernicious and persistent viral infection.

Nasaleze & Nasaleze Travel Safety Study

Study of Effects of Inert Cellulose Powder on Nasal Mucosa

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Study of the Effects of Inert Cellulose Powder on Nasal Mucosa

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Key Words: inert cellulose powder, allergic rhinitis, study of the efficacy and safety of Nasaleze and Nasaleze Travel, mucociliary clearance.

This article describes the results of the study of therapeutic efficacy of inert cellulose powder in allergic rhinitis (AR), its safety and effect on the nasal mucosa.

The purpose of this open-label prospective trial was to study new treatment options able to reduce clinical symptoms of AR.

Materials and Methods Two groups were enrolled in the study (30 healthy volunteers and 30 patients with AR). Quality of Life assessment using a questionnaire, evaluation of nasal mucosa, mucociliary clearance rate, ciliary movement frequency of columnar epithelium cells, inflammation signs in mucosal smears prior to and after the treatment with inert cellulose powder (Nasaleze and Nasaleze Travel) were performed.

Results. After administration of the medication, quality of life significantly improved in patients with AR, rhinoscopy and endoscopy as well as cytological findings showed attenuation in inflammation signs in the nasal mucosa. It was shown that the medication had no ciliotoxic effect on nasal mucosa. During the whole study period, there were no allergic reactions or significant side effects associated with the medication which demonstrates its safety.

Conclusion. Inert cellulose powder is a therapeutically effective and safe agent for AR treatment and has no negative effect on nasal mucosa.

Introduction

Allergic rhinitis (AR) is a widespread disease with steadily rising prevalence. This leads to increasing social and economic costs. Various prescription and non-prescription medications and treatments are currently available; however, many of these agents have side effects, and patients are reluctant to use them [2]. The existing medications cannot guarantee 100% safety during their administration, especially in such populations as children, pregnant and breast-feeding women. Therefore, there is still a significant unmet need for a safe and effective agent for AR prevention and treatment in the urban environment.

Cellulose powder is used as a filler in a variety of liquid nasal sprays and is very safe. There is a patented method for grinding fine-dispersed (micronized) cellulose particles, which provides delivery of an optimal dose of substance to the nasal cavity. As opposed to liquid nasal sprays, in which preservatives are used, cellulose powder suppresses bacterial growth. Not being a drug, cellulose powder, nevertheless, is classified as a medical device, which can be safely used for a year. Ground cellulose directly prevents the cause rather than the consequences of allergic reactions, since it acts as a face mask and prevents dust, pollutants and allergens from getting into the lungs. Respiratory mucosa is characterized by a low surface tension and can readily adsorb allergens from air flowing to lungs [3]. Every day up 20 billion particles enter the nasal passage, deposit on the posterior nasal wall, are swallowed and finally destroyed by gastric fluid. This process is completed by the wave activity of nasal ciliary cells [4]. Properly functioning mucociliary clearance is the first barrier on the way of infectious agents and allergenic particles to the lower respiratory tract, playing a key role in the protective function of the nose [2, 5]. Consequently, the absence of ciliotoxic effect of the drug is the most important criterion of its safety.

The purpose of this study was to assess new treatment options able to reduce clinical symptoms of AR.

The main trial objectives were: to assess the ciliotoxic effect of inert cellulose powder, to determine the mucociliary transport rate prior to and after inert cellulose powder administration, and to assess safety of inert cellulose powder administration.

Materials and methods

This prospective open-label study was performed in healthy volunteers (urban residents) and patients with AR. 30 volunteers in general good health and 30 patients with perennial or seasonal AR were enrolled in the study. The inclusion criteria were: age 15 to 70 years; males and non-pregnant, non-breast feeding females; patients with perennial and seasonal AR, earlier diagnosed in an allergy clinic.

The exclusion criteria were: patients with chronic sinusitis; patients on systemic antibacterial therapy; patients with severe nasal septum deviation; patients involved in other clinical studies. The exclusion of a patient from the study could occur on patient's or the investigator's decision. The reasons for exclusion were documented the Patient's Case Report Form (CRF).

The inert cellulose powder Nasaleze Travel (group of healthy volunteers) and the inert cellulose powder Nasaleze (group of patients with AR) were used in the study. Group I (healthy volunteers) were recommended to receive the medication twice a day for 7 days. Group II (patients with AR) were recommended to receive the medication prior to the contact with an allergen, if possible, but not less than twice a day for 40 days.

To evaluate patients' condition the following tests were performed:

1. Physician's assessment of nasal mucosa condition according to the results of anterior rhinoscopy and endoscopic examination (colour and moisture level of nasal mucosa, severity of turbinate oedema, amount of discharge, severity of nasal obstruction) using visual analogue scale.
2. Measurement of mucociliary clearance time using polymer films with methylene blue and saccharin.
3. Determination of ciliary beat rate (CBR) of nasal ciliated epithelium.
4. Cytological analysis - nasal mucosa smears.
5. Patient's subjective assessment of life quality (filling in the modified Quality of Life Questionnaire for Rhinological Patients followed by the statistic processing of data).

CBR and mucociliary transport rate as well as nasal mucosa smears prior to and after the drug administration were evaluated in group I (healthy volunteers). The quality of life was also assessed by the subjects (filling in the modified Quality of Life Questionnaire for Rhinologic Patients followed by the statistic processing of data); side effects occurring during the administration of this medicinal product were registered.

In group II consisting of patients with AR, the investigator evaluated the intensity of clinical symptoms of AR, assessed the nasal mucosa with the use of anterior rhinoscopy and endoscopic examination (colour and moisture level of nasal mucosa, severity of turbinate edema, discharge properties) using a visual analogue scale. The patients assessed their quality of life (filling in the modified Quality of Life Questionnaire for Rhinological Patients followed by the statistic processing of data) and recorded side effects occurring during the administration of this medicinal product.

Allergic reactions and side effects were assessed for the safety profile. Adverse events (allergic reactions, anaphylaxis) were also recorded. If any side effects associated with the study drug arose, it was documented in CRF. The details concerning adverse events (nature, severity, actions taken and their outcomes) were recorded in Adverse Event Report Forms. A subject was asked to discontinue taking the investigational product if any clinical adverse event, or if another medicinal condition or complication occurred making their ongoing participation in the study not in best interests of the subject. The study drug was stopped if any exclusion criterion became apparent.

Monitoring regimen:

On day 1 of the study the following procedures were performed in groups 1 and 2:

1. Assessment of inclusion/exclusion criteria.
2. Physician's assessment of nasal mucosa using anterior rhinoscopy and endoscopic examination (colour and moisture level of nasal mucosa, severity of middle and lower turbinate edema, amount of discharge and severity of nasal obstruction). The data were recorded in the form of a table using quantitative

values (0, 1, 2), reflecting sign intensity prior to and after the drug administration with the subsequent statistical analysis of the data.

3. Measurement of mucociliary clearance time using polymer films with methylene blue and saccharin.
4. Measurement of CBR of nasal ciliated epithelium prior to and after the administration of inert cellulose powder. CBR was assessed without drug administration and 10 min after its administration.
5. Cytological analysis - nasal mucosa smears, in which epithelium composition and the presence of inflammation elements were assessed. Percentages of cells with cilia (functional activity of cells) and without cilia (loss of functional activity) in cell composition of columnar epithelium were estimated, as well as the presence of metaplastic epithelium (manifestation of the reaction to inflammation) was registered as «+», «++» and «+++». Inflammation elements were assessed semi-quantitatively («+» – few, «++» – moderately, «+++» – many) and according to the contents (in percentage): neutrophilic leukocytes (manifestation of acute inflammation) and lymphoid-histiocytic elements (monocytes, lymphocytes, histiocytes) - manifestation of productive inflammation.
6. Subjective assessment of the drug effects by a patient. The modified Quality of Life Questionnaire for Rhinological Patients with a maximum score of 140 and a possibility of separate assessment of nasal breath, olfaction, nasal secretion, pain, attitude to treatment, productivity etc. was used for this purpose.

On day 7 in group I (healthy volunteers) all the above parameters were re-evaluated and documented in the patient's Case Report Form. Determination of CBR of nasal ciliated epithelium prior to and after the administration of Nasaleze Travel. At this stage CBR was determined in nasal cavity without drug administration and 30 min after its administration.

Patients in group II (patients with AR) were re-examined on day 40 of the study. All the above listed parameters were re-evaluated. CBR was determined prior to and 30 min after its administration.

Statistical analysis was carried out using program Microsoft Excel and STATISTICA Computer Software (version 6.0). The level of significance was 0.05.

Study Results

The parameters (CBRs, questionnaire scores, mucociliary clearance times, the physician's subjective assessment of nasal cavity) prior to and after the treatment in all the groups were compared using the Wilcoxon test for normal distribution (the number of subjects in each group was 30) with Yates' continuity correction and the threshold value of 1.96 for normal distribution according to the corresponding table at significance level of 5%.

When study parameters were evaluated in group I, the following results were obtained:

1. There was no deterioration in quality of life measurements in volunteers treated with Nasaleze Travel, since the differences in scores were not statistically significant.
2. The physician's endoscopic examination prior to and after Nasaleze Travel administration showed no negative nasal mucosal alterations, which was confirmed by the statistical processing of the scores.
3. Nasaleze Travel did not inhibit mucociliary transport. The difference in mucociliary clearance rates in healthy volunteers prior to and after Nasaleze Travel usage was not statistically significant.
4. Nasaleze Travel did not show ciliotoxic effect. CBR did not change significantly 10 and 30 minutes after a single dose of the drug or on day 7 after its repeated twice-daily dosing.
5. Nasaleze Travel did not affect cell composition of nasal mucosa. Cytological analysis of smears from nasal mucosa prior to and one week after the drug administration revealed no statistically significant reduction in the number of functionally active cells (cells with cilia) relative to the total number of columnar epithelial cells. No changes in the numbers of metaplastic epithelial cells, inflammation elements, percentages of neutrophilic leukocytes and lymphoid-histiocytic elements were observed either.
6. No allergic reactions or significant side effects were observed. 20% of patients complained of a garlic smell, 8% of a tickling sensation in the nose for the first 10-15 minutes after dosing.

When study parameters were evaluated in group II (patients with AR), the following results were obtained:

1. Nasaleze-treated patients with AR reported an improvement in their quality of life. Analysis of the data of the modified Quality of Life Questionnaire for Rhinologic Patients prior to and 40 days after Nasaleze administration showed statistically significant [standard deviation 2.072>1.96 (threshold t value on 5% significance level)] increase in the patients' quality of life scores after the treatment (by a mean of 13.5 points).
2. Comparing mucosa condition scores as assessed by the physician prior to and after 40-day treatment, revealed a statistically significant positive therapeutic effect, by a mean of 2 points. Standard deviation was 2.32>1.96 (threshold t value on 5% significance level).
3. Nasaleze did not slow mucociliary transport even after 40-day continuous usage. The saccharin test showed no statistically significant changes in mucociliary clearance rates for this period.
4. Nasaleze did not exert ciliotoxic effect during its 40-day continuous usage, which was confirmed by the absence of statistically significant changes in CBRs 10 and 30 min after the drug dosing or after 40 days of its twice-daily dosing.
5. Nasaleze administration caused a reduction in inflammation elements in nasal mucosa. Cytological analysis of nasal mucosa smears prior to and 40 days after the drug administration revealed no statistically significant reduction in the number of functionally active cells (cells with cilia) relative to the total number of columnar epithelial cells. No changes in the numbers of metaplastic epithelial cells were observed either. A statistically significant decrease in inflammation elements (standard deviation 2.13>1.96 on 5% significance level) owing to neutrophilic leukocytes was noted in smears with a concomitant increase in the relative counts of lymphoid-histiocytic elements to neutrophilic leukocytes (standard deviation 1.99>1.96 on 5% significance level).
6. There were no drug-related allergic reactions or side effects in this group. 80% of patients estimated the effect of the drug administration as "good", 5% - as "excellent", 15% - as "insufficiently pronounced". 25% of patients reported slight irritation of nasal mucosa ("tickling") within first few minutes after drug dosing.

The results of the study suggest that Nasaleze and Nasaleze Travel did not slow mucociliary clearance neither in healthy volunteers, nor in patients with AR, i.e. both medications have no ciliotoxic effect. They also do not affect CBR which was demonstrated in both groups of subjects during the whole period of monitoring.

The attenuation of inflammation signs in the cellular composition of nasal mucosa smears owing to the reduction in the relative counts of neutrophilic leukocytes was observed in patients with AR after 40-day usage of inert cellulose powder At the same time there was no reduction in the number of ciliary epithelial cells. In healthy volunteers, drug administration did not influence the cellular composition of nasal mucosa smears.

Forty-day Nasal administration in patients with AR was accompanied by an improvement in quality of life (based on the data of the modified Quality of Life Questionnaire for Rhinologic Patients) and the positive therapeutic effect confirmed by the results of the physician's assessment of nasal mucosa. For the whole period of study no allergic reactions or side effects associated with the medications were reported, showing their safety.

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Evaluation Biological Activity of Allicin + Nasaleze

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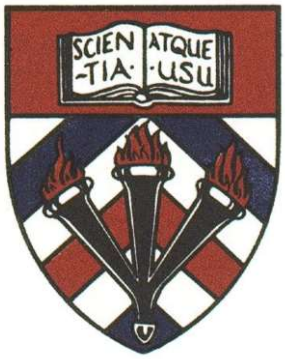

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Evaluation of the biological activity of Allicin powder + Nasaleze powder

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Introduction

The quality of garlic formulations is related to the content of marker compounds or suspected active compound groups.

In an attempt to produce a novel method to deliver allicin to the nasal cavity mixtures of allicin powder and the cellulose powder mixture of Nasaleze were investigated for antistaphylococcal activity

The biological activity of allicin against bacteria is well established, we have further shown that certain species of methicillin resistant *Staphylococcus aureus* (MRSA) are exceptionally susceptible to allicin. Using a susceptible strain of MRSA, we have developed a novel method whereby we can determine whether or not different batches of allicin capsules possess biological activity.

There are a number of tests available to determine the anti-microbial activity of selected agents. Diffusion tests determine the susceptibility of isolates by measuring the zones of inhibition around a measured amount of the anti-microbial agent. Zones of inhibition not more than 6mm smaller than those of a known control strain indicate that the test bacterium is sensitive to the anti-microbial agent. Zone sizes of 12mm or less usually indicate antibiotic resistance. There is also an intermediate antibiotic resistant group between with susceptibilities between these levels and zone sizes greater than 12mm

Materials and methods

Bacteria: MRSA clinical isolate Uel301 was used. Overnight broth cultures in isosensitest broth were prepared.

Media: Isosensitest agar (Oxoid Ltd) were used.

Powders: supplied by Allicin International, Nasaleze powder + allicin powder

Method:

- A broth containing 10^5 cfu/ml was prepared in peptone water.
- 0.2ml was spread over each isosensitest plate.
- Plates were air dried and a 6mm well cut in the centre of the plate.
- A volume of 100ug or 200ug of each powder was added to each well.

- Plates were incubated overnight at 37oC
- The presence of zones of inhibition around a well is indicative of biological activity being present. No zone around the 6mm well, (as with the negative control) represented no biological activity.

Results

Comparative zone sizes in mm, 0 represents 6mm well size.

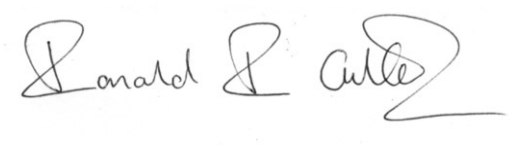
number	Preparation	100ug	Bioactive	150ug	Bioactive
1	Negative control	0 (6mm)	-	0 (6mm)	-
2	Nasaleze	0	-	0	-
3	Allicin BN 2069/03	14	+	19	+
4	Allicin CPC 2102 4-1	23	+	27	+
5	Allicin + CPC 2102 6-1	28	+	28	+
6	Allicin CPC 2069/03 4-1	12	+	17	+
7	Allicin CPC 21028-1	22	+	26	+

Conclusion:

- The method was shown to effectively demonstrate biological activity present in a number of powder mixtures.
- The most active powder was Allicin + CPC 2102 6-1

Recommendations:

- Allicin powder mixes well with Nasaleze and produces reasonable zones of inhibition
- Further work is required to optimise the mixtures to be used especially to determine the balance between activity and gelatinisation.
- Some cellulose must be present in the mixture for gelatinisation to occur



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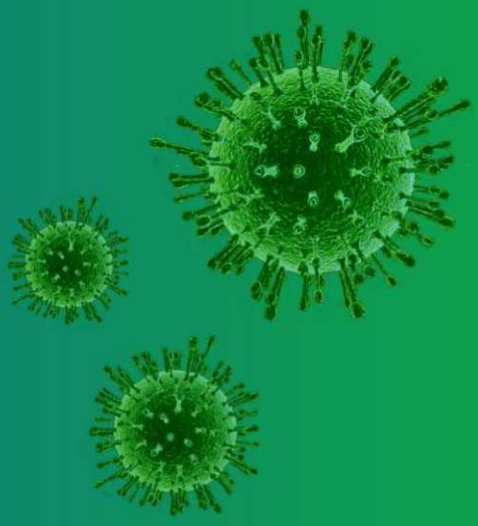
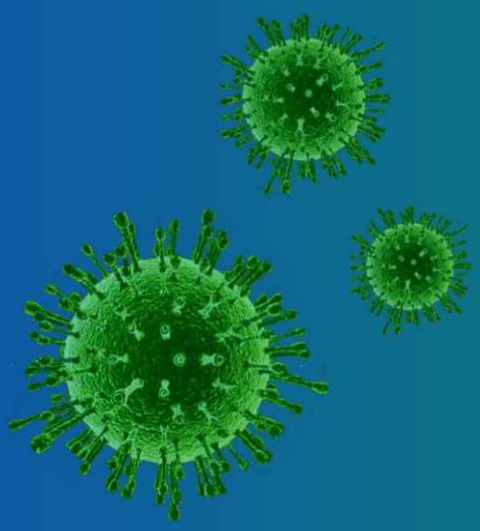
TRAVEL Nasaleze®



Nasaleze® & The Coronavirus

The Invisible Mask



A message from Paul Duxbury, CEO of Nasaleze International

Introduction

2020 has indeed been a challenging year for all. We were busy putting our year plans into place in the 1st quarter with the focus on our Allergy products as usual but as the corona-crisis got underway we found ourselves with enormous demand on our Cold & Flu Blocker and Travel products.

The whole world went crazy for Vitamin C, Zinc, hand sanitisers, face masks and, well Nasaleze – The Invisible Mask.

Nasaleze Cold & Flu Blocker and Nasaleze Travel are marketed in the UK with the same formula. The differentiation initially was point of sale; we were trying to put the Travel product into airport pharmacies and keep Cold & Flu Blocker in the high street pharmacy.

As the UK went into lockdown and the high streets emptied it was the online sales that erupted – we had a 9000% increase online!

The bulk of demand in the UK at least went towards our Travel product. With people being concerned about leaving the house a Travel product offering protection against airborne germs & viruses in general, if not COVID-19, became a very attractive proposition.

At one point we were selling over 3,000 units per day on Amazon UK – 125 per hour on average. Amazon were in complete chaos and it became increasingly difficult to get the stock booked into their warehouse and made available for sale. We estimate sales would have been over the 5,000 unit per day level had the Amazon booking-in system kept pace.

As the virus went around the world the demand from our export markets followed it, with Russia in particular seeing a massive spike and being the 1st Nasaleze territory to order in excess of 1,000,000 bottles in single year (a feat achieved within 8 months actually, year to date).

We did not make any COVID-19 claims unlike some unscrupulous products – the demand was fuelled by the desire for prevention & protection – something Nasaleze has been promising since its birth in the early 2000's.

However, with our previous clinical trial data on rhinovirus and H5N1 virus we found ourselves the right product, at the right place at the right time.

As always we have very much been a company heavily reliant on proving ourselves so didn't lose much time in setting up an initial in vitro study to see if Nasaleze products could help with the crisis.

We found a suitable partner to fulfil the brief…
Perfectus Biomed. A company specialising in viral assays, including virucidal efficacy and viral barrier testing.

PERFECTUS BIOMED GROUP

The protocol was agreed, testing samples prepared and once restrictions had lifted sufficiently for us to get to the Post Office and received by Perfectus we were in business.

Over the next few pages you can see the outcome of the Perfectus study, written in conjunction with the Nasaleze Scientific Advisory Board.

In addition to the study there is also a press release from our PR Agency, Twelve.

Who with our own design team have created some suitable material to responsibly promote the benefits of Nasaleze for virus prevention, as a sensible add on to the WHO & various Government endorsed self-care strategy against COVID-19.

We wish to be clear and state we do not consider ourselves the silver bullet for COVID-19, but we do believe we can prove from the new and old data we have that our products are useful tools against viruses and hope we can play a part in helping people.

Best regards from all at Nasaleze.

Virucidal activity of Nasaleze® Cold & Flu Blocker and Nasaleze® Travel in cell cultures infected with human pathogenic coronavirus 229-E

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Abstract

This *in vitro* study determined the anti-viral efficacy of a unique blend of powder cellulose supplemented with powdered garlic extract (PGE) and a signalling agent. The composition, presented as Nasaleze® Cold & Flu Blocker/Nasaleze® Travel, was assessed against Human Coronavirus 229E, CoV 229E {ATCC VR-740} in an *in vitro* experiment. The test substance was used at sub-optimal dosing levels to explore its prevention and treatment capabilities. The virucidal activity of this novel formulation was measured at 48, 72 and 112 hour periods after incubation. Results showed strong reductions in viral titre of Coronavirus 229E compared to a control, while no toxicity to human cells from the test formulation was noted. The extract Nasaleze® Cold/Travel showed potential to be used as a therapeutic and preventive agent.

The data reconfirms the established anti-viral activity of this formulation acting as a barrier preventing the virus from accessing the nasal mucosa and disrupting its replication.[1,2,3]

Introduction

The **COVID-19 epidemic in the United Kingdom** is part of the worldwide pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus reached the country in late January 2020. As of 30th August 2020, there have been 334,467 confirmed cases and 41,499 deaths of confirmed cases, the world's fourth-highest death rate. Worldwide more than 27 million cases and over 891,000 deaths have been recorded, with the United States, Brazil and India recording the highest number of cases. More than 90% of those dying had underlying illnesses or were over 60 years old.

In March 2020 , the UK government imposed an order, dubbed "Stay Home, Protect the NHS, Save Lives", banning all non-essential travel and contact with people outside one's home (including family and partners), and shutting almost all schools, businesses, venues, facilities, amenities and places of worship. Those with symptoms, and their households, were told to self-isolate 14 days, while those at higher risk due advanced age and accompanying comorbidities were told to shield themselves. People were told to keep apart in public. Police were empowered to enforce the measures, and the Coronavirus Act 2020 gave the government emergency powers not used since the Second World War.

The lengthy restrictions severely damaged the UK economy, lead to millions of job losses, worsened mental health and suicide rates, and caused "collateral" deaths due to isolation and decline of living standards.

In recent years, a number of countries in East and South-East Asia including China had seen an outbreak of various types of infectious flu including SARS Cov 1, MERS, H5N1 avian flu and now Coronavirus described as COVID-19. The infection mainly affected poultry (chickens and ducks) or bats, which were then wiped out in their hundreds of thousands.

The highly pathogenic avian flu virus arrived in Russia in July 2005 and to date the H5N1 flu virus has been recorded in many parts of the Russian Federation: in Western Siberia, in the Urals and in the Astrakhan province. This prompted the conduct of *in vitro* tests using Nasaleze® Cold/Travel which proved very successful at both destroying H5N1 and preventing its replication in human cell lines. This data was published in the European Journal for Nutraceutical Research[3]. Subsequently, a similar *in vitro* test against Coronavirus 229E which is part of the corona virus and common cold virus families with similar characteristics and structures were carried out. With the agent picked for this

evaluation we already had a history of success in controlling the viral agent H5N1, so our aim was to check if this Nasaleze® Cold/Travel formulation could be successful in both reducing viral load of Covid 229E and preventing its replication.

Material and methods

The test viral organism chosen was Human Coronavirus 229E and the utilised cell type was MRC-5. This Medical Research Council cell strain 5 is a diploid human cell culture line composed of fibroblasts, originally developed from lung tissue.

Cell maintenance and assay set-up

MRC-5 cells were used as the host cell line for human coronavirus 229E (CoV 229E) propagation. MRC-5 cells were maintained in Eagle's Minimum Essential Medium (EMEM) supplemented with 20% Foetal Bovine Serum (FBS) and 1% penicillin-streptomycin (complete EMEM) at 37 ± 2 °C and 5% CO2. In preparation for the cytotoxicity screening and anti-viral assays, MRC-5 cells were seeded into 24 well plates at 1.0 x 105 cells/mL and incubated at 37 ± 2 °C and 5% CO2 for 24 hours, or until they reached 80-90% confluency. In preparation for tissue culture infectivity dose 50 (TCID50) testing, MRC-5 cells were seeded into 96 well plates at 2 x 105 cellsmL-1 and incubated at 37 ± 2 °C and 5% CO2 for 24 hours.

Phase 1: Checking for potential cytotoxic effects of the Nasaleze® Cold/Travel formulation **on the selected MRC-5 cell line**

Nasaleze® Cold/Travel was diluted to 3.2 mg/0.1mL, 6.4 mg/0.1mL and 12.8 mg/0.1mL in EMEM supplemented with 2% FBS and 1% penicillin-streptomycin (assay medium). Complete EMEM was aspirated from the test plates and 100 µL of each test concentration was added to duplicate wells. Following a 10-minute incubation period at 20 ± 2 °C an additional 400 µL of assay medium was added to the test wells. Plates were incubated for 24 hours at 37 ± 2 °C and 5% CO2. Following incubation, visual scoring was performed on a scale of 0 to 4 according to ISO 10993-5 guidelines (Table 1). Cytotoxic effects were assessed based on a variety of morphological changes to the MRC-5 cells such as cell rounding, detachment and cell lysis.

Visual Score	Cells with cytotoxic effects (%)	Reactivity classification
0	0	None
1	0 – 20	Slight
2	20 – 50	Mild
3	50 – 70	Moderate
4	70 – 100	Severe

Table 1. Cytotoxicity visual scoring and reactivity classifications.

Phase 2: Assessment of the preventative and virucidal capabilities of Nasaleze® Cold/Travel

MRC-5 cells were treated with Nasaleze® Cold/Travel according to two methods to determine the preventative and treatment capabilities of the formulation. The assays were performed in 24-well plates utilising duplicate wells for each experimental condition.

Preventive treatment of MRC-5 cells using Nasaleze® Cold/Travel before infection with high and low doses of human coronavirus 229E

To assess the preventative capabilities of Nasaleze® Cold/Travel against CoV 229E, MRC-5 cells were pre-treated with 3.2 mg of the formulation for 10 minutes before infection with CoV 229E multiplicity of infections (MOIs) of 1 (high dose) and 0.01 (low dose). Complete EMEM was aspirated from the test plates and washed once in Dulbecco's phosphate buffered saline (DPBS) before application of 3.2 mg Nasaleze® Cold/Travel in 100 µL assay media. Following a 10 minute incubation at 20 ± 2 °C, cells were inoculated with 100 µL CoV 229E, pre-diluted to achieve the high and low MOI infection, and incubated at 35 ± 2°C and 5% CO2 for 30 minutes. Infected cells were then supplemented with an additional 300 µL of assay medium and incubated at 35 ± 2 °C and 5% CO2 for four days. The cytopathic effect (CPE) of the virus on the MRC-5 cells was scored on days 2, 3 and 4 to the criteria described in Table 1. On days 3 and 4, 100 µL of media was harvested from each well to determine the viral titre before replacing with 100 µL of fresh assay medium. Harvested samples were stored at -80 °C until required for viral titre determination. It should be noted that 3.2mg of the test substance is sub optimal dosing and represents only 1 puff into only 1 nostril, whereas the product instructions indicate multiple dosing into BOTH nostrils to prevent or treat any type of airborne infection.

Treatment of human coronavirus 229E infected MRC-5 cells with Nasaleze® Cold/Travel

To assess the treatment capabilities of Nasaleze® Cold/Travel against CoV 229E, MRC-5 cells were first infected with high and low CoV 229E MOIs, 1 and 0.01 respectively, before treatment with the formulation. Complete EMEM was aspirated from the test plates and washed once in DPBS before being inoculated with 100 µL of pre-diluted CoV 229E to achieve high and low MOI infections and incubated at 35 ± 2 °C and 5 % CO2 for 30 minutes. Following incubation, viral inoculum was removed and a sub optimal 3.2 mg dose of Nasaleze® Cold/Travel in 100 µL assay media was added to the cells and incubated for 10 minutes at 20 ± 2 °C to allow the formation of the gel barrier. Treated cells were then supplemented with an additional 300 µL of assay medium and incubated at 35 ± 2 °C and 5% CO2 for four days. The CPE of the virus on the MRC-5 cells was scored on days 2, 3 and 4 to the criteria described

in Table 1. On days 3 and 4, 100 µL of media was harvested from each well to determine the viral titre before replacing with another 100 µL of fresh assay medium. Harvested samples were stored at -80 °C until required for viral titre determination.

Viral infectivity quantification by TCID50

To determine the viral titre of harvested samples, 10-fold serial dilutions were performed in assay medium. Medium was aspirated from the wells of the cell plate and cells were washed with DPBS. One hundred microlitres of each dilution of the samples were added to the corresponding test wells. Test plates were incubated at 35 ± 2 °C and 5% CO_2 for 7 days. There were four replicate wells for each test condition. After incubation, viral CPE was determined using an Olympus CK2 inverted microscope. The viral titre was calculated using the Spearman-Kärber method.

Results

Phase 1: MRC-5 cytotoxicity screen

There was no observable cytotoxicity in MRC-5 cells exposed to Nasaleze® Cold/Travel following a 24-hour contact time (Table 2). When visual scoring was performed, the gel barrier formed by Nasaleze® Cold/Travel was visible on top of the cell monolayer. Additionally, a residue was visible on treated cells (Appendix I).

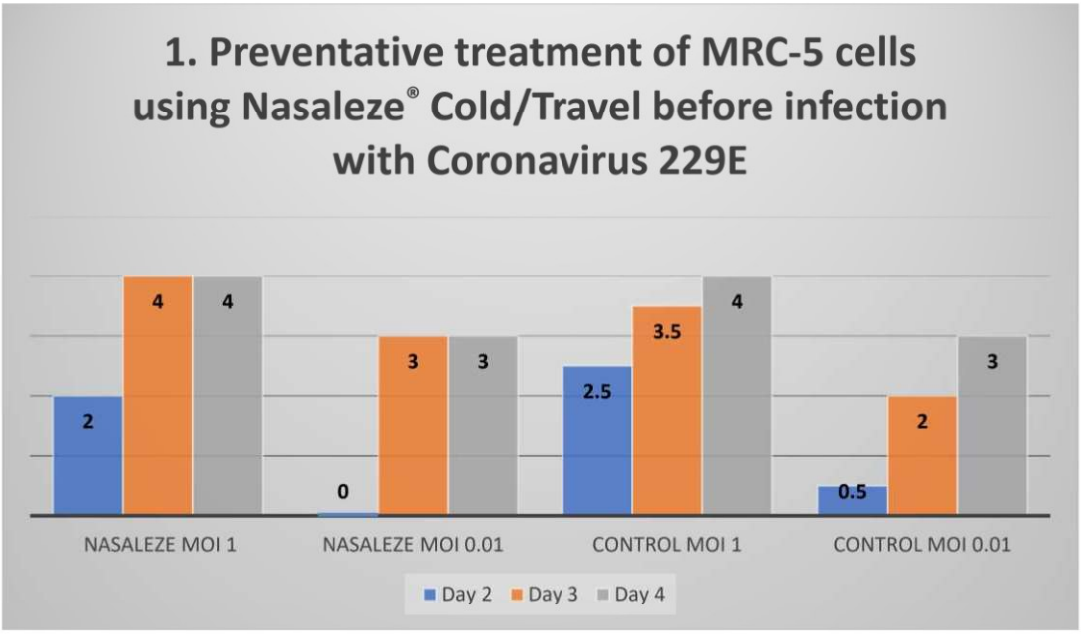
Treatment	Visual score	Reactivity classification
Nasaleze® Cold/Travel	0	No cytotoxicity

Table 2. Cytotoxicity of Nasaleze® Cold/Travel using visual scoring.

Preventive treatment of MRC-5 cells using Nasaleze® Cold/Travel before infection with coronavirus 229E

Cytopathic effect of CoV 229E on MRC-5 cells pre-treated with Nasaleze® Cold/Travel

Following a 2, 3 and 4 day or 48, 72 and 112 hours incubation period, the CPE of the test plate was scored (Chart 1). Representative images of the CPE observed are presented in Appendix II. Duplicate cells treated with Nasaleze® Cold/Travel with a high MOI of CoV 229E showed slight CPE on day 2 and severe CPE on days 3 and 4. Duplicate cells treated with Nasaleze® Cold/Travel with a low MOI of CoV 229E showed no CPE on day 2 and moderate CPE on days 3 and 4

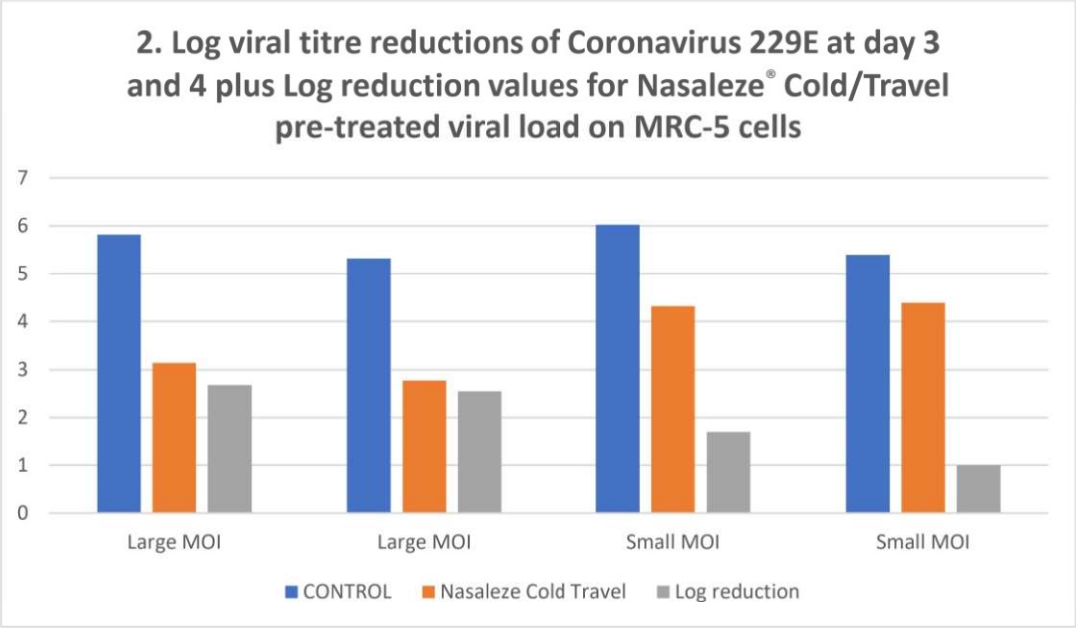


Following a 3 and 4 day incubation period with a high MOI of CoV 229E the negative control resulted in an average viral titre of 5.82 ± 0.35 $Log_{10}TCID_{50}$/mL and 5.32 ± 0.35 $Log_{10}TCID_{50}$/mL, respectively. Pre-treatment of MRC-5 cells with Nasaleze® Cold/Travel resulted in a 2.68 $Log_{10}TCID_{50}$/mL and 2.55 $Log_{10}TCID_{50}$/mL reduction in viral titre on day 3 and day 4 post-infection, respectively, when compared to the negative control showing an average of 3.14 ± 0.18 and 2.77 ± 0.53 Table 3 and 4 - Chart 2.

Large viral titre

Product	Average Viable CoV 229E ± SD ($Log_{10}TCID_{50}$/mL)		Log Reduction ($Log_{10}TCID_{50}$/mL)	
	Day 3	Day 4	Day 3	Day 4
Negative Control	5.82 ± 0.35	5.32 ± 0.35	N/A	N/A
Nasaleze® Cold/Travel	3.14 ± 0.18	2.77 ± 0.53	2.68	2.55

Table 3. Log TCID50 and Log reduction values for human coronavirus 229E (CoV 229E) following treatment with Nasaleze® Cold/Travel before infection at a high multiplicity of infection and incubated for 3 and 4 days. N/A = not applicable, SD = standard deviation.



Small viral titre

Following a 3 and 4 day incubation period with a low MOI of CoV 229E the negative control resulted in an average viral titre of 6.02 ± 0.53 $Log_{10}TCID_{50}$/mL and 5.39 ± 0.18 $Log_{10}TCID_{50}$/mL, respectively. Pre-treatment of MRC-5 cells with Nasaleze® Cold/Travel resulted in a 1.70 $Log_{10}TCID_{50}$/mL and 1.00 $Log_{10}TCID_{50}$/mL reduction in viral titre on day 3 and day 4 post-infection, respectively, when compared to the negative control (Table 4, Chart 2).

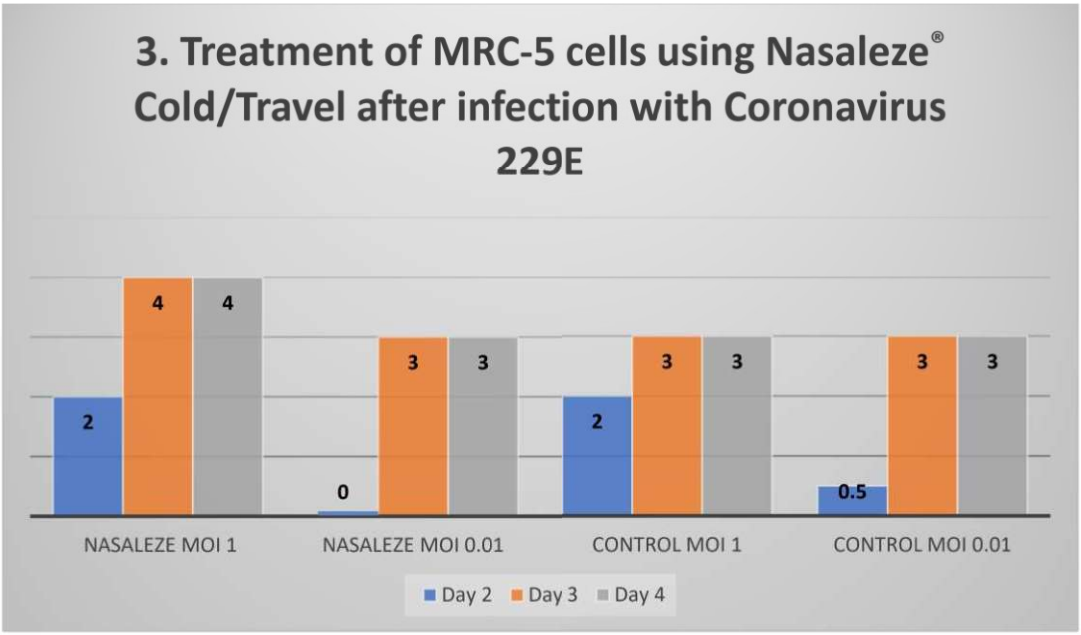
Product	Average Viable CoV 229E ± SD ($Log_{10}TCID_{50}$/mL)		Log Reduction ($Log_{10}TCID_{50}$/mL)	
	Day 3	Day 4	Day 3	Day 4
Negative Control	6.02 ± 0.53	5.39 ± 0.18	N/A	N/A
Nasaleze® Cold/Travel	4.32 ± 0.35	4.39 ± 0.18	1.70	1.00

Table 4. Log TCID50 and Log reduction values for human coronavirus 229E (CoV 229E) following treatment with Nasaleze® Cold/Travel before infection at a low multiplicity of infection and incubated for 3 and 4 days. N/A = not applicable, SD = standard deviation.

Treatment capabilities of Nasaleze® Cold/Travel

Cytopathic effect of CoV 229E on MRC-5 cells treated with Nasaleze® Cold/Travel after viral infection

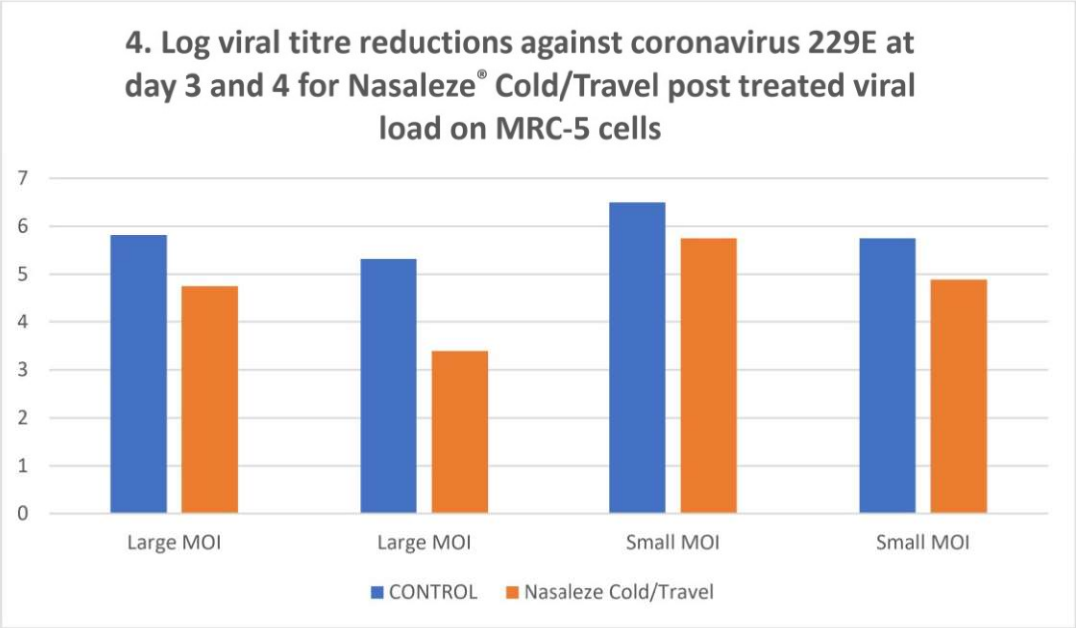
Following a 2, 3 and 4 day incubation period, the CPE of the test plate was scored. Representative images of the CPE observed are presented in Appendix II. Duplicate cells treated with Nasaleze® Cold/Travel after infection with a high MOI of CoV 229E showed mild CPE on day 2 and severe CPE on days 3 and 4 post-infection. Duplicate cells treated with Nasaleze® Cold/Travel after infection with a low MOI of CoV 229E showed no CPE on day 2 and moderate CPE on days 3 and 4 post-infection.



Viral titration of samples treated with Nasaleze® Cold/Travel Blocker after viral infection Chart 4

Following a 3 and 4 day incubation period with a high MOI of CoV 229E the negative control resulted in an average viral titre of 5.82 ± 0.35 $Log_{10}TCID_{50}$/mL and 5.32 ± 0.35 $Log_{10}TCID_{50}$/mL, respectively. Treatment with Nasaleze® Cold/Travel after infection resulted in a strong log reduction on days 3 and 4 at 4.75 ± 0.00 and 3.39 ± 0.18 respectively.

Furthermore a 3 and 4 day incubation period with a low MOI of CoV 229E the negative control resulted in an average viral titre of 6.50 ± 0.00 $Log_{10}TCID_{50}$/mL and 5.89 ± 0.18 $Log_{10}TCID_{50}$/mL, respectively. Treatment with Nasaleze® Cold/Travel after infection resulted in a strong log reduction on days 3 and 4 at 5.75 ± 0.00 and 4.89 ± 0.18 respectively.



Discussion

The dissemination of potentially pathogenic viruses increases infection risk in both healthy and immunocompromised individuals. Coronaviruses are enveloped, single stranded RNA viruses responsible for a variety of upper-respiratory tract illnesses in humans. The severity of these illnesses ranges from mild as in common cold to severe acute respiratory syndrome as seen in the recent COVID-19 pandemic. Coronaviruses are thought to be predominantly transmitted through respiratory droplets with some evidence to suggest the virus can remain active on fomites for several days. Interventions, both preventative and curative, are essential to slowing and/or stopping the spread of coronaviruses.

The assessment of interventions against coronavirus surrogate strains allows for the safe evaluation of product efficacy. Coronavirus 299E is structurally and genetically similar to the SARS-CoV-2 virus. Since the COVID-19 pandemic, the Australian regulatory body, Therapeutic Goods Administration (TGA), is the first regulatory body to announce that **Coronavirus 229E as a suitable coronavirus surrogate strain for biocide coronavirus claims.**

Two different experiments were performed to investigate the anti-viral efficacy of Nasaleze® Cold/Travel. In the first experiment, MRC-5 cells were pre-treated with Nasaleze® Cold/Travel before infection with high and low doses of CoV 229E. in the second experiment MRC-5 cells were infected with a high and low dose of CoV 229E before treatment with Nasaleze® Cold/Travel. Treatment with Nasaleze® Cold/Travel did not damage the experimental MRC-5 cell line, but yielded substantial reductions in viral titre indicating a high level of anti-viral potential. Although the reduction in CPE was not large or maintained it should be noted that a sub optimal dose was used representing only one dose into a single nostril, whereas real life clinical data accumulated thus far has shown that a three times daily dose into each nostril can significantly reduce airborne infection.[1,2,3]

Future work could investigate the optimal dosing of Nasaleze® Cold/Travel, simulating the real-life intended use of the product. Additionally, similar experiments could be performed using other respiratory viruses such as influenza, adenovirus and rhinovirus. Finally, as this formulation shows such promise in both preventing and treating viral infection, a 3D primary nasal cell culture model could be considered for use to obtain a more translatable result as well as clinical evaluations in human subjects to add to the existing database

for this unique powder cellulose, signalling agent and garlic extract, marketed as Nasaleze® Cold & Flu Blocker and Nasaleze® Travel.

Key take away points from the report

We asked co-author Peter Josling for his comments on the results…

"This is a very interesting *in vitro* study that clearly shows Nasaleze® Cold & Flu Blocker and Nasaleze Travel are unique active formulations in the fight to both prevent and treat coronavirus infections.

It is clear that pre-treatment reduces viral replication and may therefore stop Coronavirus 229E in its tracks when used at optimum dosing levels.

Even when viral replication is already infecting healthy human cells Nasaleze® Cold/Travel can attack and disable viral replication.

These results are from a SINGLE dose of Nasaleze® Cold/Travel and we would expect multiple doses to be even more effective.

Nasaleze® Cold/Travel do not have any negative cytopathic effect on human cells.

This is a step forward in the prevention and management of coronavirus infection."

Dr Peter Josling
Herbal Research Centre

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Project Start Date 20th April 2020

Project Completion Date 3rd July 2020