SAFETY AND EFFICACY EVALUATION OF AN ISOTONIC MANGANESE-ENRICHED SEAWATER SOLUTION ON HUMAN NASAL EPITHELIUM RECONSTITUTED IN-VITRO

Barbara De Servi, PhD[1]; Marisa Meloni, PhD[1]; Josip Culig[2], Amina Saaid[3],
[1]VitroScreen, Milan
[2]Department of Pharmacology, Medical Faculty Osijek, University JJ Strossmayer, Croatia.
[3]Laboratoire Fumouze, Levallois, France

OVERVIEW

Nasal irrigation with saline solutions is frequently used for relief of rhinitis symptoms. In this study, safety and efficacy of Stérimar Allergic Nose (SAN) were evaluated in vitro. SAN stimulated the mucociliary clearance ($p<0.01$) and wound healing capacity without compromising the tissue integrity or exerting any cytotoxic or pro-inflammatory induction. The results support the use of SAN in relieving of rhinitis symptoms.

INTRODUCTION

Allergic rhinitis (AR) is one of the most common diseases affecting life of adults and children, which exerts a big impact on the quality of life (QoL) [1, 2, 3]. Current AR treatment options mainly focus on symptom management and are associated with adverse effects. Thus, alternatives to pharmacological remedies are of high interest[4].

One of these approaches is nasal irrigation. Used as an adjuvant to therapy, nasal irrigation has been shown to significantly reduce the intake of pharmacological drugs in AR patients [5,6].

Sterimar Allergic Nose (SAN) is an isotonic seawater-based formulation enriched with manganese. Manganese has been shown to inhibit the anaphylactic histamine release [7,8] and exert antioxidant activity which contributes to reduced inflammatory response [9, 10].

The main purpose of this study was to test the safety and efficacy of SAN in vitro.

METHODS

The in vitro experiments were performed in a 3D Reconstituted Human Nasal Epithelial model (RHNE, MucilAir™, Epithelix Sàrl, Geneva, Switzerland)(Figure 1). 3 days prior to testing, inserts were washed apically with MucilAir™ culture medium and were then incubated with 500µl MucilAir™ culture medium in a CO_2 incubator. 10µl SAN were applied apically to the inserts twice a day during 4 days. At the indicated time points, culture medium was collected and frozen at -80°C for further analysis.

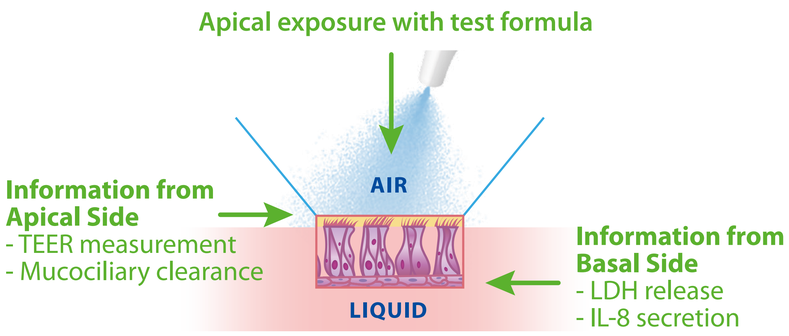

Figure 1: MucilAir™ 3D model. SAN or controls are applied on apical side. From the apical side, TEER levels and mucociliary clearance rates; and from the basal side, LDH and IL-8 levels were measured.

✔ **Tissue integrity** was monitored by measuring transepithelial/endothelial electrical resistance (TEER) between apical and basal compartments using a voltohmmeter (Millicell ERS, Millipore with range of 0-20kΩ), at D1 and D4 from untreated and SAN treated samples.

✔ **Lactate Dehydrogenase (LDH) secretion** was measured at D1-4, in untreated, SAN treated and 1% Triton X-100, Fluka Biochemika) treated tissues in saline solution (0.9% NaCl, 1.25mM $CaCl_2$, 10mM HEPES, Eurospital) using Cytotoxicity Detection Kit Plus (Roche).

✔ **IL-8 secretion** was measured at D1-4, in untreated, SAN treated and cytomix (positive control, 1% FCS, Amimed, Cat 2-01F36-I; 0.2mg/ml LPS, Sigma; 500ng/ml TNF-α, GeneTex; in duplicate) treated cells using an ELISA kit (BD OptEIA™ BD Bioscience).

✔ **Mucociliary clearance (MCC)** was evaluated at D1 and D4; 5µm microbeads were co-incubated at the apical compartment of untreated, SAN treated) and 50µM isoproterenol treated (positive control) MucilAir™ inserts. Following incubation, images were recorded every second for one minute by using a Ds-5mc camera (Nikon) connected to a DMIRE2 microscope (Leica). Particle velocity was calculated using Image Pro Plus (Mediacy).

✔ **In vitro wound healing assay.** RHNE inserts were treated with saline solution (0.9% NaCl) or with SAN for 30 minutes before generating an injury (30µL). The wound closure was assessed by image acquisition by a bright-field microscope at 0, 3, 6, 22 and 30h. Images were quantified using the Image J program.

RESULTS

ASSESSMENT OF SAFETY OF STÉRIMAR ALLERGIC NOSE

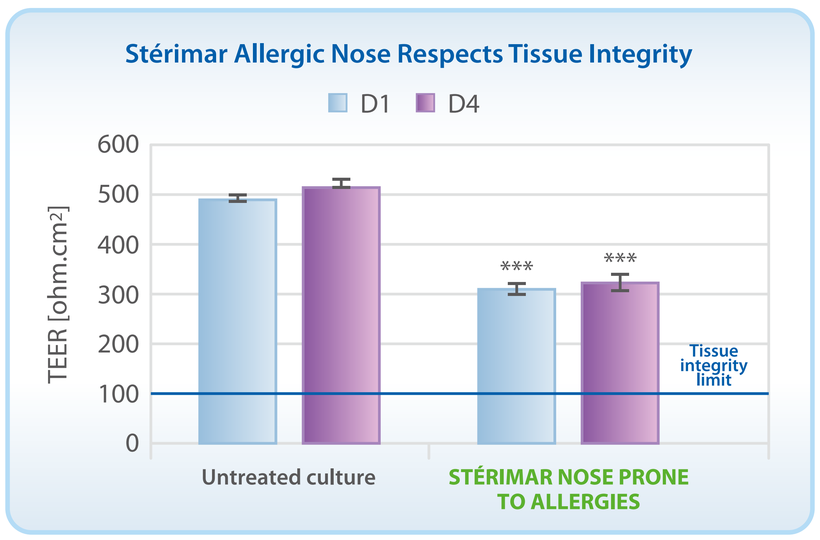

Figure 2: Barrier forming properties. TEER values after treatment at D1 and D4 of untreated and SAN treated RHNE cells (***$p< 0.001$, compared to untreated cells).

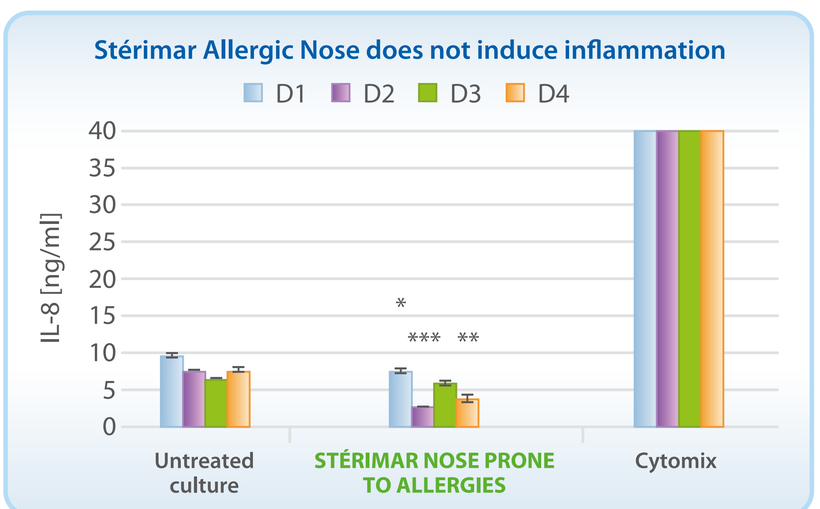

Figure 3: IL-8 secretion. Levels of IL-8 were measured in an ELISA assay in MucilAir™ epithelial cells untreated, treated with SAN and treated with Cytomix for 1-4 days (*$p<0.05$, **$p<0.01$ and ***$p<0.001$, compared to untreated cultures).

ASSESSMENT OF EFFICACY OF STÉRIMAR ALLERGIC NOSE

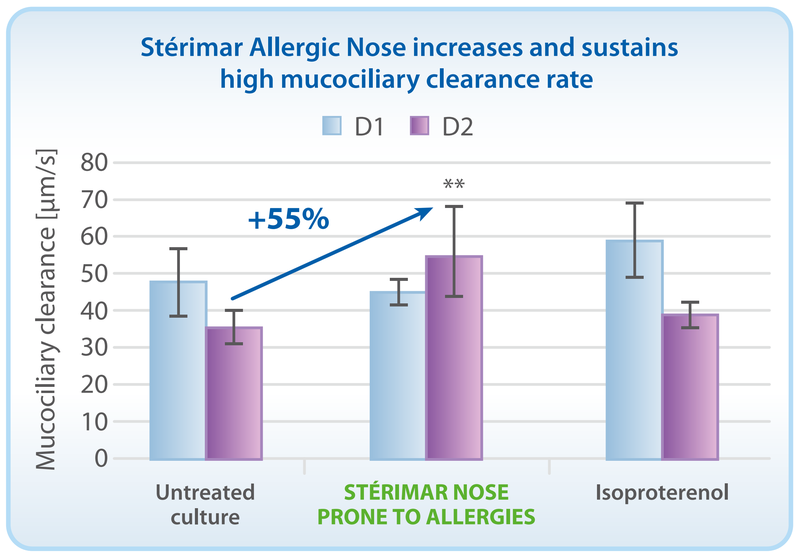

Figure 4: Mucociliary clearance. MCC rates of untreated, SAN treated and Isoproterenol treated MucilAir™ epithelial cells at D1 and D4 of treatment (** $p<0.01$, compared to untreated cultures).

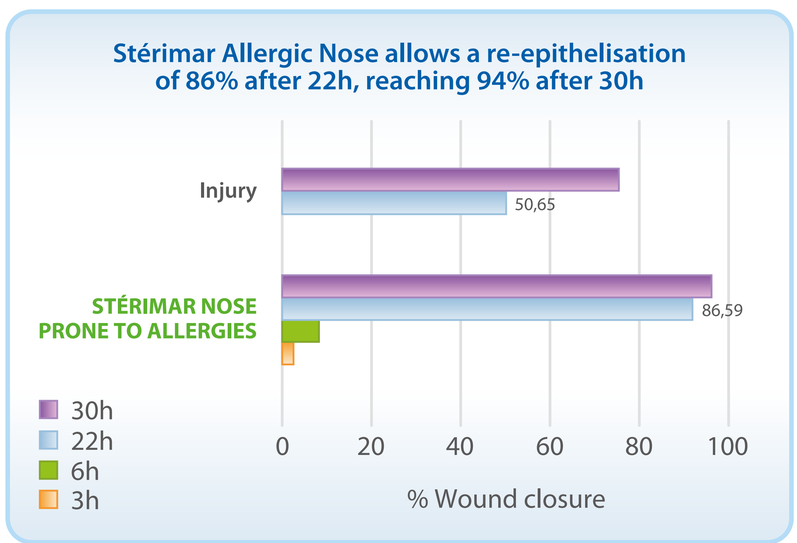

Figure 5: Wound healing capacity. (A) Saline and SAN treated wound 0, 3, 6, 22 and 30 h after injury. (B) Percentage of wound closure (re-epithelialization).

CONCLUSIONS

Current evidence indicates that SAN is well tolerated and effective for the improvement of symptoms related to allergic rhinitis. More specifically SAN improves MCC rates and accelerates wound repair in a 3D reconstituted nasal epithelium. In addition, improvement in MCC rates were maintained for longer period compared to controls. SAN preserves to tissue integrity, and does not exert any cytotoxic or inflammatory effects on the tissue. Thus, it can be concluded that Stérimar Allergic Nose is safe and efficient to use in humans. More studies would be necessary in the future in order to confirm these conclusions in vivo.

BIBLIOGRAPHY
1. Mattos, J.L., C.R. Woodard, and S.C. Payne, Trends in common rhinologic illnesses: analysis of U.S. healthcare surveys 1995-2007. Int Forum Allergy Rhinol, 2011. 1(1): p. 3-12.
2. Min, Y.G., The pathophysiology, diagnosis and treatment of allergic rhinitis. Allergy Asthma Immunol Res, 2010. 2(2): p. 65-76.
3. Meltzer, E.O., et al., Allergic rhinitis substantially impacts patient quality of life: findings from the Nasal Allergy Survey Assessing Limitations. J Fam Pract, 2012. 61(2 Suppl): p. S5-10.
4. Cingi, C., et al., Seawater gel in allergic rhinitis: entrapment effect and mucociliary clearance compared with saline. Ther Adv Respir Dis, 2010. 4(1): p. 13-8.
5. Hermelingmeier, K.E., et al., Nasal irrigation as an adjunctive treatment in allergic rhinitis: a systematic review and meta-analysis. Am J Rhinol Allergy, 2012. 26(5): p. e119-25.
6 Garavello, W., et al., Nasal rinsing with hypertonic solution: an adjunctive treatment for pediatric seasonal allergic rhinoconjunctivitis. Int Arch Allergy Immunol, 2005. 137(4): p. 310-4.
7. Foreman, J.C. and J.L. Mongar, The action of lanthanum and manganese on anaphylactic histamine secretion. Br J Pharmacol, 1973. 48(3): p. 527-37.
8. Tagen, M., et al., Mitochondrial uncoupling protein 2 inhibits mast cell activation and reduces histamine content. J Immunol, 2009. 183(10): p. 6313-9.
9. Coassin, M., F. Ursini, and A. Bindoli, Antioxidant effect of manganese. Arch Biochem Biophys, 1992. 299(2): p. 330-3.
10. Li, C. and H.M. Zhou, The role of manganese superoxide dismutase in inflammation defense. Enzyme Res, 2011. 2011: p. 387176.