IN VITRO SAFETY, EFFICACY AND MECHANISM OF ACTION OF AN ISOTONIC SEAWATER SOLUTION FOR NASAL HYGIENE

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SUMMARY & INTRODUCTION

Nasal hygiene is critical to overall nasal health and provides protection against airborne contaminants including pathogens and allergens[1,2]. Nasal irrigation is generally well tolerated, often used for managing various sinonasal conditions and has been shown to improve nasal hygiene[3]. This study demonstrates the ionic balance resemblance of an isotonic seawater solution, Stérimar Nose Hygiene Baby and Adults (SNH) to human plasma in comparison to electrodialysed seawater (EDS). In addition, SNH has longer-lasting effect than EDS on recovery from hypotonicity-induced stress. This work also shows the safety and efficacy of SNH in maintaining good nasal hygiene which can contribute to allergy prevention, consistent with SNH benefits demonstrated in clinical trials[4-7].

METHODS

- Safety and efficacy assays were performed using **MucilAir™ 3D Reconstituted Human Nasal Epithelial model[8]**. At the indicated time points, culture medium was collected and frozen at -80ºC for further analysis.
- **Ion concentration determination** of Ag, Ba, Cd, Co, Cu, Fe, I, Mn, Mo, Ni, Pb, Se, U, V and Zn were determined by ICP-MS. B, Ca, K, Mg, Na, S, Si and Sr were determined by ICP-AES. Cl concentration was determined by IC.
- **Hypotonic stress:** Tissues were treated with hypotonic solution (300 µl, 1mM $CaCl_2$ and 1mM $MgCl_2$) for 5 min, and immediately replaced with 100 µl saline solution, SNH or EDS (for 5 and 15 min). For stainings, tissues were rinsed with saline solution, fixed, and embedded in paraffin blocks to obtain 5 µm sections.
- **ATP release** was quantified by CellTiter-Glo® Luminescent Cell Viability Assay kit (Promega) (n=3). The plates were incubated at 37°C for 20 min and read (2 measurements each).
- **AQP3 staining:** Slides were incubated o/n with primary rabbit polyclonal AQP3 antibody, 30 min with Histofine Simple Stain AP Multi, and with New Fuchsin chromogen and examined under the microscope (40x).
- **Alcian Blue staining:** Slides were stained with alcian blue for 30 min, and counterstained with nuclear fast red solution (5 min). Tissues were dehydrated, mounted with resinous mounting medium and analysed under microscopy (40x).
- **Lactate dehydrogenase secretion** was evaluated in untreated (n=2), SNH treated (n=3), and 1% Triton X-100 (n=1) in saline solution treated tissues on Days 1-4, with Cytotoxicity Kit Plus (LDH) following manufacturer's instructions.
- **IL-8 secretion** was evaluated by ELISA in untreated (n=2), SNH treated (n=3) and Cytomix [1% FCS, 0.2mg/ml LPS, 500ng/ml TNF-α; n=2) treated tissues on Days 1-4.

RESULTS

1. ISOTONIC DILUTED SEAWATER RESEMBLES HUMAN PLASMA IN IONIC BALANCE

SNH has been naturally obtained by diluting seawater guaranteeing an ionic balance similar to that of human plasma[9], thus respects human physiology. Its profile clearly resembles human plasma in ionic balance compared to EDS.

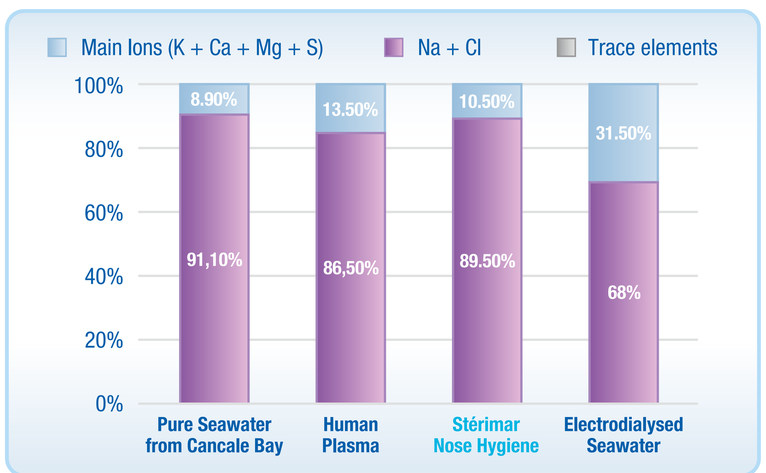

Figure 1: Ionic balance of SNH compared to other solutions (represented as % of all quantified 24 elements). Concentration of elements is determined by ICP-MS, ICP-AES or IC.

2. SNH HELPED TISSUE RECOVERY FROM THE HYPOTONIC STRESS-ASSOCIATED ATP RELEASE, AND MAINTAINED TISSUE MORPHOLOGY

SNH has more sustained effect in reversing hypotonic-stress-induced ATP release, compared to EDS, possibly owing to its resemblance to human plasma in terms of ionic balance. SNH treatment re-established the normal morphology and localization & levels of AQP3 expression indicating a recovery in tissue morphology. EDS treatment only partially recovered from stress.

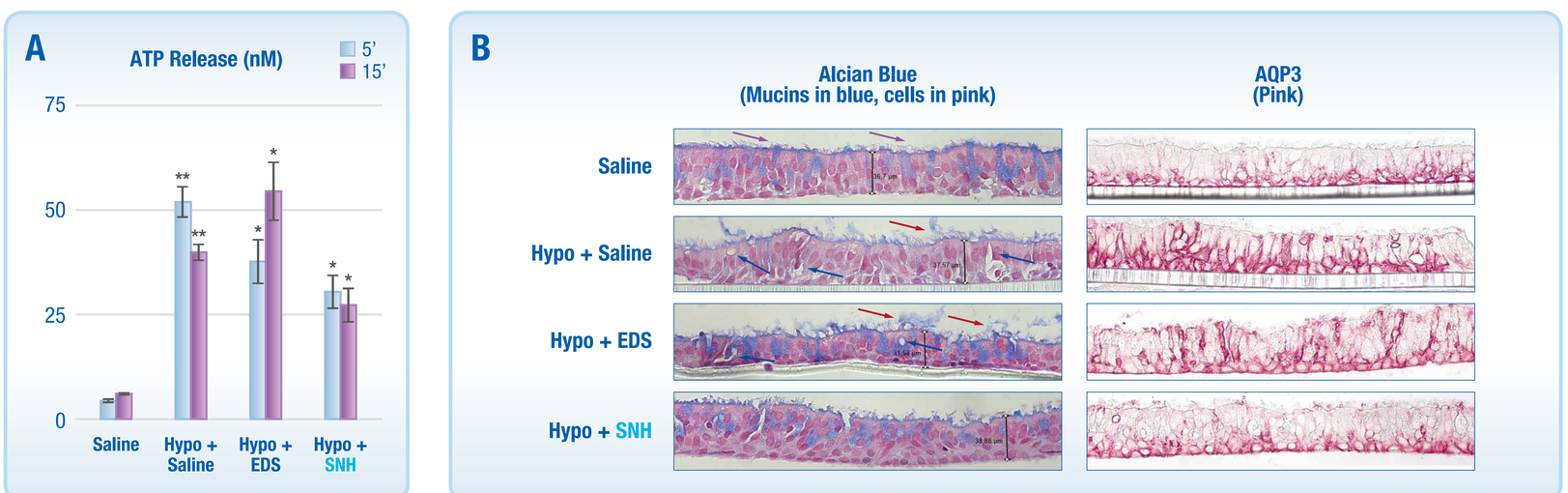

Figure 2. Effect of SNH on recovery from hypotonic-stress compared to EDS. (A) ATP release (nM) 5 and 15 min post-treatment after 5 min of hypotonic saline solution application. *p<0.05, **p<0.01, hypo + saline was compared to saline only, EDS and SNH were compared to the hypo + saline. (B) Histochemical staining of the treated tissues by alcian blue and AQP3 antibody, 15 min post-treatment. Violet arrows: ciliated epithelial cells; blue arrows: protrusions or blebs; red arrows: damaged cilia.

3. EFFECT ON LDH RELEASE AND IL-8 SECRETION

Treatment with Triton X-100 was used as a positive control for cell lysis corresponding to 100% cytotoxicity. These results indicate that SNH is not cytotoxic, as cells treated with SNH had a similar LDH release profile to untreated cells. IL-8 secretion assay also showed that SNH does not cause a pro-inflammatory response. Cytomix was used as a positive control.

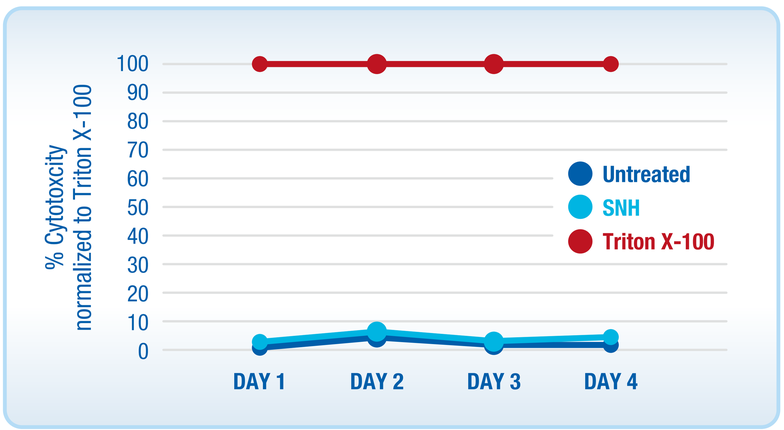

Figure 2: Effect of SNH on LDH Release. Secreted lactate dehydrogenase profile of tissues after treatment on consecutive Days 1-4.

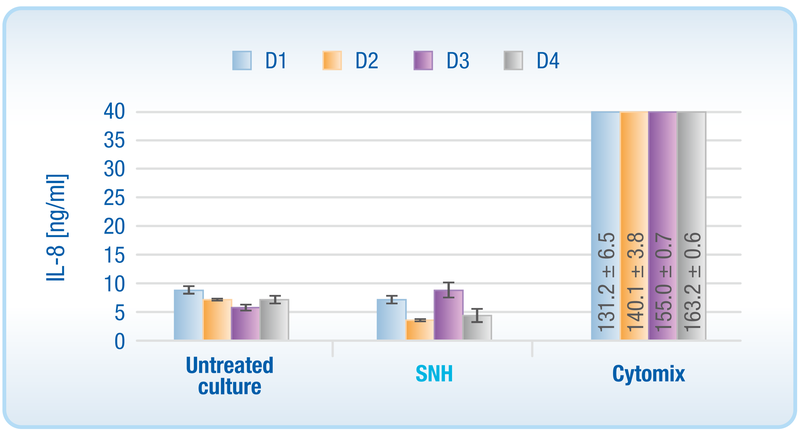

Figure 3: Effect of SNH on IL-8 Secretion. Secreted IL-8 levels in untreated, SNH-treated and Cytomix-treated cells for 4 days. *p<0.05 and ***p<0.001 compared to untreated cultures.

CONCLUSIONS

- SNH is highly similar to human plasma in terms of ionic balance.
- SNH is superior to EDS in restoring homeostasis after hypotonicity-induced stress conditions for longer periods (15 minutes).
- SNH has a homeostatic interaction with the ciliated epithelium, thus safe: it does not disrupt epithelial structure nor induce cytotoxicity or inflammation.
- **As a conclusion,** this work shows the safety and efficacy of SNH in maintaining good nasal hygiene which can contribute to allergy prevention, consistent with SNH benefits demonstrated in previous clinical trials[4-7].

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