Evaluation of the Toxicity of Sodium Dodecyl Sulfate (SDS) in the MucilAir™ Human Airway Model *In Vitro*

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1 INTRODUCTION

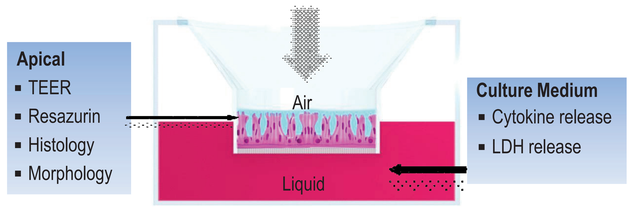
MucilAir™ is an *in vitro* airway model with morphology and functions mirroring the tracheo-bronchial epithelium. MucilAir™ units comprise cells derived from human airway biopsies cultured at the air interface on permeable membranes by Epithelix Sàrl. This model is increasingly used in inhalation toxicity and pharmaceutical lead optimisation development and testing to identify potential airway toxicants and facilitate *in vivo* dose range finding.

MucilAir™ was evaluated for use in predicting upper airway toxicity. Tissues were treated with increasing concentrations of sodium dodecyl sulphate (SDS). Monolayer integrity (trans-epithelial electrical resistance; TEER), membrane integrity (lactate dehydrogenase (LDH) release), metabolic competence (resazurin metabolism) and inflammatory mediator (IL-8) release were measured. Histology and scanning electron microscopy (SEM) were used to assess morphology.

2 MATERIALS AND METHODS

MucilAir™ units were obtained from 3 donors (1 unit per dose level from Donor 1 and 2 units per dose level from Donor 2 and Donor 3). SDS was obtained from Sigma-Aldrich, Dorset, UK. All other materials were obtained by Charles River and were analytical or tissue culture grade, as appropriate.

MucilAir™ was evaluated by treating units with SDS in saline solution (0-10 mM) for 24 h at a temperature of 37°C in a 5% CO_2 atmosphere. The units were maintained in culture until 168 h post-dose. The monolayer integrity was determined by measurement of trans-epithelial electrical resistance (TEER) using a Millicell® ERS meter at 0 h (pre-dose) and again at 24 h and 168 h post-dose. At 0, 24 and 168 h, the membrane integrity was assessed by measurement of lactate dehydrogenase (LDH) release using the Promega CytoTox ONE™ Homogeneous Membrane Integrity Assay in culture medium. At 0, 24 and 168 h, toxicity was assessed by determination of inflammatory mediator (IL-8) release using the R&D Quantikine® ELISA Human CXCL8/IL-8 Immunoassay in culture medium. At 168 h, metabolic competence was assessed by measuring resazurin metabolism in units incubated with resazurin solution for 1 h at a temperature of 37°C in a humidified 5% CO_2 atmosphere. Sample aliquots were assessed for the presence of the metabolite, resorufin, by measuring fluorescence (544_{em}/ 590_{em}). At 168 h post-dose, histology and scanning electron microscopy (SEM) were used to assess morphology. For histology, MucilAir™ was fixed in neutral buffered formalin, embedded in paraffin, sectioned, mounted, stained with haematoxalin-eosin and visualized using a Leica DM-2500 light microscope. For SEM analysis, MucilAir™ was fixed in glutaraldehyde buffer (pH 7.4) for 22 h at 5°C then rinsed for 30 min in sodium cacodylate buffer (pH 7.4) followed by 5 min in ultrapure water. Samples were dehydrated through ethanol, submerged in hexamethyldisilazane for 10 min, air dried and stored in a dessicator until analysis. Samples were then mounted on aluminium stubs, gold sputter-coated and viewed using a Philips XL30CP Scanning Electron Microscope.



3 RESULTS AND DISCUSSION

MucilAir™ displayed dose-dependent responses to SDS treatment as assessed by morphological appearance and membrane integrity (LDH release), monolayer integrity (TEER) and cytokine (IL-8) release. After 24 h SDS exposure, the most sensitive toxicity indicator was histology and SEM imaging (Figure 1) revealing damage at ≥0.63 mM SDS. TEER (Figure 2) and IL-8 release (Figure 3) demonstrated toxicity at ≥1.25 mM and LDH release (Figure 4) at ≥2.5 mM. Extensive cell damage explained the reduced IL-8 and LDH release at SDS concentrations of 5-10 mM.

At 168 h, metabolic activity was compromised at ≥5 mM SDS (Figure 5). Although histology demonstrated that pseudo-stratified morphology did not recover, recovery of basic cellular functions was observed in moderately damaged units (1.25-2.5 mM) at 168 h post-dose. Predose levels of TEER and LDH and IL-8 release were consistent within and between donors. Predose, 24 h and 168 h TEER, IL-8 and LDH release were unaffected up to 0.63 mM SDS. Therefore, NoEL was considered to be an SDS concentration of *ca* 0.63 mM.

4 CONCLUSIONS

In conclusion, MucilAir™ displayed dose-dependent responses when treated with SDS, with cytotoxicity visualised by histology at ≥0.63 mM and measured chemically at ≥1.25 mM. Monolayer integrity (TEER) and marker release (LDH and IL-8) patterns were comparable between equivalently dosed units. These data support the use of MucilAir™ as a relevant model for airway toxicity studies.

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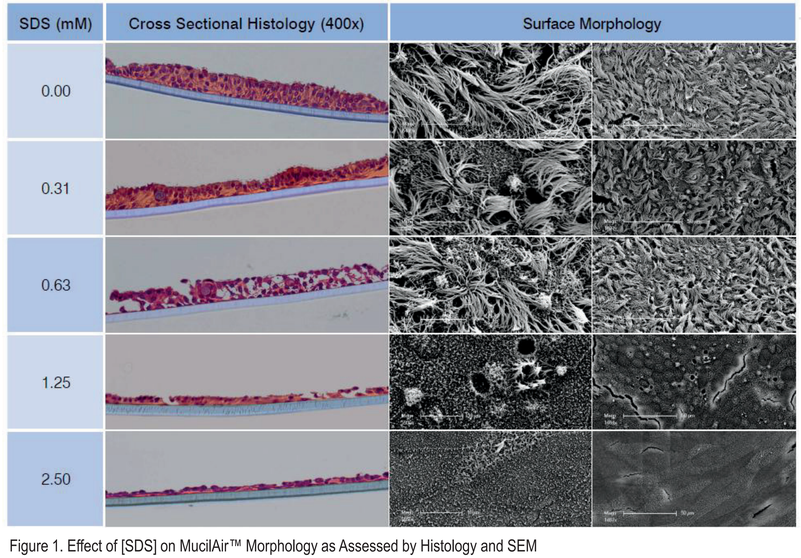

Figure 1. Effect of [SDS] on MucilAir™ Morphology as Assessed by Histology and SEM

Data not shown for SDS at concentrations of 5 and 10 mM as only the plate matrix was visible.

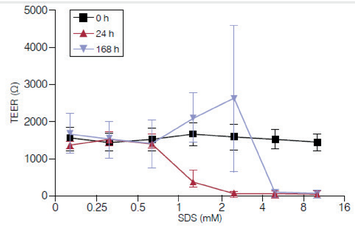

Figure 2. Effect of [SDS] on MucilAir™ Monolayer Integrity

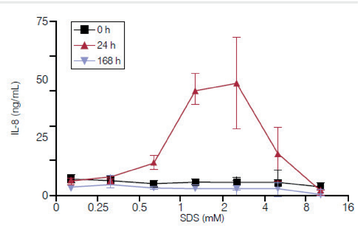

Figure 3. Effect of [SDS] on MucilAir™ IL-8 Release

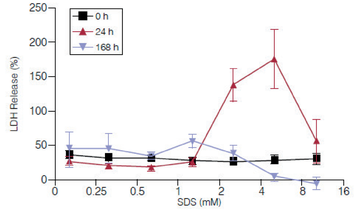

Figure 4. Effect of [SDS] on MucilAir™ Membrane Integrity *(% Maximal Damage)*

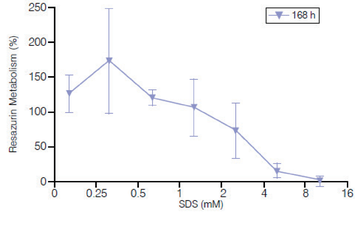

Figure 5. Effect of [SDS] on MucilAir™ Metabolic Activity (% Metabolism vs Untreated Units)