TECHNICAL REPORT

MucoLox™

Evaluation of the Safety and Toxicological Profile of MucoLox: Human Oral Mucosa, Nasal Mucosa and Vaginal Mucosa (Part 1/3)

Abstract: Mucoadhesive polymers are delivery systems designed to overcome low mucosal retention associated with buccal delivery of medication. Due to the prolonged contact between the delivery system and mucosal tissues, irritancy and toxicity potentials of these polymers should be considered. This study was designed to compare the safety and toxicological profile of MucoLox, a mucoadhesive polymer gel, to that of Triton™ X-100 (positive control) and distilled water (negative control), using a three-dimensional (3D) model of the human oral mucosa. Results show that MucoLox is potentially as safe as distilled water following 4.5 hr of exposure (98% mean cell viability). MucoLox is less toxic than Triton X-100 as the amount of time required to reduce cell viability to 50% was 6 times longer for MucoLox than that of Triton.

Introduction: The buccal mucosa, which contains non-keratinized epithelial cells lining the inner cheeks of the mouth, is a site for local and systemic delivery of medication. This region is highly vascularized and relatively immobile [1]. Buccal delivery allows for the active ingredients to exert rapid onset of action, bypassing liver first pass-metabolism, and avoiding pH fluctuations and degradation within the gastrointestinal tract [2]. Due to the small absorptive surface of the buccal mucosa, mucoadhesive polymers have been developed to prolong mucoadhesion and increase residence time (time at the site of action) [3]. Mucoadhesive polymers are often used to deliver medication for the treatment of local diseases and conditions of the oral mucosa such as mucositis (inflammation of the mucous membrane), ulcers, infections, and candidiasis [4]. The prolonged intimate contact between the delivery system and the tissue require these polymers to be non-irritating and non-toxic in order to minimize adverse effects and patient discomfort [2]. The purpose of this study is to evaluate the safety and toxicological profile of MucoLox, in comparison to Triton X-100 (positive control) and distilled water (negative control), using a 3-dimensional (3D) model of the human oral mucosa. MucoLox is a proprietary polymer gel that acts as a delivery system to improve mucoadhesion and prolong retention of medications at application sites within the oral mucosa [5]. Triton X-100 is a nonionic surfactant, not approved for oral use, used in this study as a positive control [6].

Methodology: The materials used in this study include EpiOral™ tissue samples, MucoLox (Lot: 6481454) at 50% (diluted in distilled water), and Triton X-100 at 1%. The EpiOral (ORL-200) tissue model (Figure 1) comprises of normal human-derived non-keratinized oral epithelial cells, cultured and differentiated to resemble the native buccal tissue of the human oral mucosa [7]. Following tissue preparation, 40 µL of MucoLox 50% and Triton X-100 1% were applied onto separate EpiOral tissue samples and left to incubate at 37°C. Incubation intervals were 1, 4.5, and 20 hr. Application of distilled water on an EpiOral tissue served as the negative control. After the allotted exposure time, each tissue was rinsed twice with phosphate buffer saline (PBS) and excess liquid was removed. Afterwards, 300 µL of MTT solution (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide) was added to the EpiOral tissues and left to incubate for 3 hr. MTT was used as an indicator of cell viability. Succinate dehydrogenase enzymes within the mitochondria of viable cells have the ability to reduce soluble yellow tetrazonium salt of MTT to an insoluble purple formazan derivative [8]. After 3 hr of exposure to MTT, the tissues were immersed in 2 mL of extraction solution, sealed in a plastic bag, and stored at room temperature overnight. The excess liquid was then decanted and the remaining extractant solution was agitated. A 200 µL aliquot of each extract was evaluated using a Molecular Device SpectraMax® M5 Microplate Reader. This device quantifies the absorbance potential of the samples at 570 nm, a wavelength absorbed by reduced MTT [8]. This experiment was repeated two months following initial testing using identical methodology and the Electrolyte Saliva Gel (PCCA Formula #11150), which consists of sodium chloride, potassium chloride, calcium chloride, and a flavor extract in MucoLox (Lot: 6481454) 50% (diluted in distilled water).

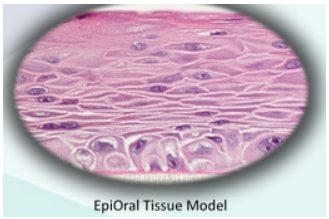

Figure 1. Illustration of the EpiOral tissue model.

Results and Discussion: Toxicological profiles for MucoLox and the positive control, Triton X-100, were compared by examining absorbancy potentials of the extracts collected from both experiments. The greater the percent absorbancy, the greater the amount of MTT reduced by succinate dehydrogenase within the extract, and the higher the percent cell viability within the tissue [8]. Mean percent cell viabilities were calculated for the two experiments and are illustrated in Figure 2. Percent viability for tissues treated with distilled water (negative control) was 100% throughout this study.

For tissues treated with MucoLox 50%, mean percent viabilities were 97%, 98%, and 85% following 1, 4.5, and 20 hr of exposure, respectively. For EpiOral tissues treated with Triton X-100 1%, mean percent viabilities were 117%, 30%, and 6% following 1, 4.5, and 20 hr of exposure, respectively. At 4.5 hr, the difference in percent cell viability between MucoLox and distilled water was not significant. Cell viability at 98% after 4.5 hr with MucoLox exposure is promising data to suggest a favorable safety profile similar to that of distilled water. From the data collected, using a semi-log scale, percent viabilities were plotted and ET_{50}, the time at which percent viability would be 50%, was estimated [9]. ET_{50} for MucoLox was approximately 6-folds higher than that of Triton X-100 with ET_{50} > 20 hr for MucoLox and ET_{50} of 3.2 hr for Triton X-100. This result indicates that MucoLox can bind to the tissue 6 times longer than Triton X-100 before 50% cell viability is reached.

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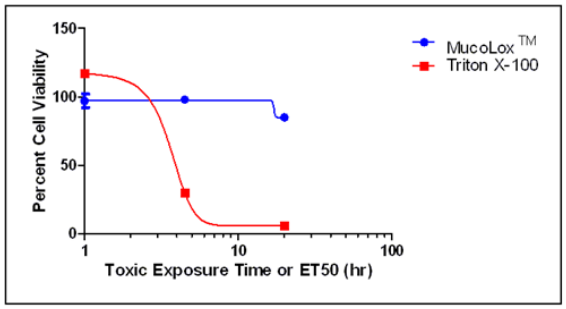

Figure 2. Mean percent cell viability of MucoLox and Triton X-100.

The intimate contact between the mucoadhesive delivery system and the mucosal tissue is an important factor to consider when examining toxicity results. According to this study, after 4.5 hr of exposure to MucoLox, cell viability was preserved at 98% in comparison to 100% for distilled water, which suggests that delivery of medication with MucoLox is potentially as safe as distilled water. This study was conducted *in vitro*, without the impact of physiological factors such as mucosal cell turnover, food intake, tongue movements, and continuous washing of the inner cheek with saliva. In normal *in vivo* conditions, buccal delivery of medication typically last for about 4 to 6 hr before the delivery system is removed from site of application [3].

However, even at 20 hr of exposure to MucoLox, which is longer than the amount of time the delivery system would typically remain within the buccal mucosa, cell viability was still at 85%.

Depending on the nature of the patient's buccal mucosa and the amount of MucoLox applied, by the time the delivery system is naturally removed from the tissue surface, percent cell viability would potentially still be in the high 90s, indicating very minimal toxicity and irritancy potential with MucoLox.

Conclusions: MucoLox improves mucoadhesion and prolongs retention of medications at application sites within the oral mucosa. The evaluation of the safety and toxicological profile of MucoLox is very important taking into account the prolonged intimate contact between the delivery system and the oral mucosa. An ideal mucoadhesive should facilitate healing without causing damage and irritation to surrounding tissues. MucoLox can bind to tissues 6 times longer than Triton X-100 (before 50% cell viability) which indicates that MucoLox is not toxic when compared to Triton X-100. In addition, cell viability at 98% after 4.5 hr with MucoLox exposure suggests that MucoLox is potentially as safe as distilled water. MucoLox may then be used in pharmaceutical compounding as a safe option in the treatment of diseases and conditions of the oral mucosa such as oral mucositis, candidiasis and mouth ulcers.

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