TECHNICAL REPORT

MucoLox™

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

Abstract: The vaginal mucosa is a common site for local and systemic delivery of medication. Mucoadhesive polymers have been developed to increase residence time and prevent leakage of medication often seen with conventional vaginal delivery systems. The intimate contact between the mucoadhesive polymer and the vaginal mucosal tissue requires the delivery system to be non-irritating and non-toxic. This study aims to compare the safety and toxicological profile of MucoLox, a polymer gel, to that of Triton™ X-100, a positive control, using a 3D model of the human vaginal mucosa. Results have demonstrated that MucoLox was less toxic as it can bind to the vaginal tissue approximately 14 times longer than Triton X-100 before cell viability is reduced to 50%. Compounded medicines prepared with MucoLox are then likely to remain at the site of action for a long period of time without causing damage to the vaginal tissue, potentially reducing the need for frequent dosing and increasing the effectiveness of each dose administration.

Introduction: Vaginal delivery of medication is advantageous in allowing for the medication to avoid first-pass metabolism and gastrointestinal degradation [1]. Lined with non-cornified, stratified squamous epithelium, the vaginal mucosa offers a large surface area and rich blood supply, making it a promising site for delivery of medication in the treatment of conditions such as vaginitis (inflammation), bacterial, fungal, and viral infections. The vagina has self-cleansing potential with large secretions of vaginal fluid, often limiting the residence time (time at the site of action) of conventional vaginal dosage forms such as tablets, creams, gels, and foams. For this reason, the use of mucoadhesive polymers as a delivery system is preferred. Mucoadhesive polymers can prevent leakage by prolonging the contact time between the medication and the mucosal tissue. Due to the prolonged contact with the vaginal mucosa, an ideal mucoadhesive polymer should be non-toxic and non-irritating [2].

The aim of this study was to evaluate the safety and toxicological profile of MucoLox, in comparison to that of Triton X-100 (positive control), using a 3-dimensional (3D) model of the human vaginal mucosa. MucoLox is a proprietary polymer gel designed to improve mucoadhesion and prolong retention of medication at application sites within the vaginal mucosa [3]. Triton X-100, which served as a positive control in this study, is a nonionic surfactant that can be used as a solubilizer, stabilizer, and emulsifier [4].

Methodology: The EpiVaginal™ tissue model (MatTek Corporation) is a multilayered tissue produced from human-derived vaginal-ectocervical epithelial cells (Figure 1). Comprising of a basal layer and multiple non-cornified layers, this tissue is highly differentiated to resemble the growth and morphological characteristics of the human vaginal mucosa [5].

Within a 6-well plate, 100 µL of MucoLox 100% and Triton X-100 1% were applied onto separate EpiVaginal tissue samples and left to incubate at 37˚C for 45 min to 20 hr. Following incubation, tissues were rinsed twice with Phosphate Buffer Saline (PBS) and excess liquid was removed. A 300 µL of MTT (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide) solution was then added to the tissues and left to incubate for 3 hr. MTT served 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 [6]. Tissues were then immersed in 2 mL of extraction solution and sealed in a plastic bag to soak overnight at room temperature. Excess liquid was decanted the following day, the remaining extractant solution was agitated, and 200 µL aliquot of each extract was evaluated with a Molecular Device SpectraMax® M5 Microplate Reader. This device quantifies the absorbance potential of the samples at 570 nm, a wavelength absorbed by the formazan derivative [6].

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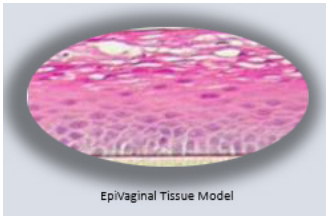

Figure 1. Illustration of the EpiVaginal tissue model.

Results and Discussion: Percent viability within the tissue is represented by the absorbance potential of each extract (reduced MTT). 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 [6]. Percent cell viabilities for the tissue treated with MucoLox were 87%, 78%, and 79% following exposure at 1, 4.5, and 20 hr, respectively (Figure 2).

For the tissue treated with Triton X-100 (positive control), percent cell viabilities were 97% and 26% at 45 min and 2 hr of exposure, respectively. Via a semi-log scale, percent viabilities were plotted and the ET_{50} was estimated. ET_{50} is the time when percent cell viability is reduced to 50% [7].

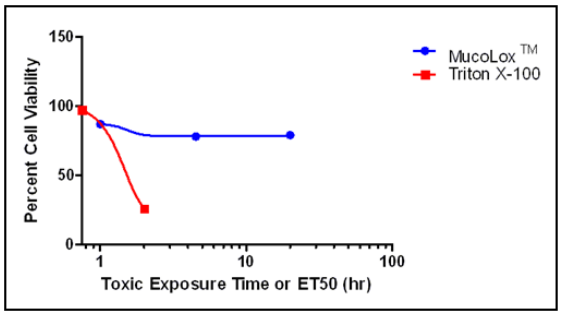

Figure 2. Toxicological profiles of MucoLox and Triton X-100.

ET_{50} for MucoLox was approximately 14-folds higher than that of Triton X-100 with ET_{50} > 20 hr for MucoLox and 86.4 min for Triton X-100. This result indicates that MucoLox can bind to the vaginal mucosa 14 times longer than Triton X-100 before 50% cell viability is reached. Being non-toxic and non-irritating to the vaginal mucosa, in comparison to the positive control, MucoLox may therefore be considered a safe delivery system in its ability to prolong contact between the medication and the vaginal tissue.

Conclusions: MucoLox is a mucoadhesive polymer that allows the medication to adhere to the vaginal tissue for a long period of time, despite the regular secretions of vaginal fluid. The ability of mucoadhesive polymers to bind to the vaginal tissue without causing toxicity is a very important characteristic to be considered and, therefore, the safety and toxicological profile of MucoLox was evaluated. Vaginal toxicity can cause irritation and tissue damage, which weaken the natural defenses of the vaginal mucosa, increasing the risk of infections such as HIV and herpes simplex [8]. Study results have demonstrated that MucoLox exerts minimal toxicity on the vaginal mucosa following over 20 hr of exposure.

Compounded medicines prepared with MucoLox are then likely to remain at the site of action for a long period of time without causing damage to the vaginal tissue, potentially reducing the need for frequent dosing and increasing the effectiveness of each dose administration.

References:

1. Shaikh, R., Singh, T., Garland, M.J., Woolfson A.D. & Donnelly R.F. 2011, 'Mucoadhesive drug delivery systems', *Journal of Pharmacy BioAllied Sciences*, vol. 3, no. 1, pp. 89–100.
2. Pereira, R. & Bruschi, M. 2012, 'Vaginal mucoadhesive drug delivery systems', *Drug Development and Industrial Pharmacy*, vol. 38, no. 6, pp. 643–52.
3. *MucoLox* 2014, PCCA, viewed 13 January 2015, http://www.pccarx.com/pcca-products/pcca-exclusives/bases/mucolox.
4. Oberle, R.L., Moore, T.J. & Krummel, A.P. 1995, 'Evaluation of mucosal damage of surfactants in rat jejunum and colon', *Journal of Pharmacological and Toxicological Methods*, vol. 33, pp. 75.
5. *EpiVaginal Tissue Model* 2015, MatTek Corporation, viewed 14 January 2015, http://www.mattek.com/epioral/applications/drug-delivery.
6. Wang, H., Cheng, H., Wang, F., Wei, D. & Wang, X. 2010, 'An improved 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide reduction assay for evaluating the viability of Escherichia coli cells', *Journal of Microbiological Methods*, vol. 82, pp. 330–3.
7. Ayehunie, S., Cannon, C., Gimondo, J., Hayden, P., Kandárová, H. & Klausner, M. 2007, 'Human vaginal-ectocervical tissue model for testing the irritation potential of vaginal-care products', *Toxicology Letters*, vol. 172, pp. S73.
8. Ayehunie, S., Cannon, C., LaRosa, K., Pudney, J., Anderson, D.J. & Klausner, M. 2011, 'Development of an in vitro alternative assay method for vaginal irritation', *Toxicology*, vol. 279, no. 1–3, pp. 130–8.