VersaBase® – MucoLox™ – Estriol/Estradiol USP Dilutions™

Evaluation of the Safety and Toxicological Profile of Vaginal Gels on a 3D In Vitro Model of the Human Vaginal Mucosa

Abstract: The safety and toxicological profile of two vaginal gels (A and B) was evaluated using an in vitro model of the human vaginal mucosa. Both vaginal gels were composed of the proprietary bases MucoLox™ and VersaBase® Gel. The Placebo Hormone Solution, a proprietary solvent system, was part of vaginal gel B only. The viability of the tissues exposed to the vaginal gels and the controls was determined using the MTT ET50 assay. Following 24 hr of exposure, the viability of the cells was less than 5% for the positive control and superior to 60% for the vaginal gels, in comparison to the negative control. MucoLox, VersaBase Gel and the Placebo Hormone Solution are thus safe and non-toxic to the vaginal mucosa. Compounded medicines prepared with the PCCA Estriol/Estradiol USP Dilutions™ are likely to remain at the site of action for over 24 hr without causing damage to the vaginal tissue.

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 several conditions and also in hormone replacement therapy [2].

The aim of this study was to evaluate the safety and toxicological profile of two vaginal gels (A and B) in comparison to a positive control (Gynol II) and a negative control (purified water), using a 3-dimensional (3D) in vitro model of the human vaginal mucosa. The vaginal gel A is composed of the proprietary bases MucoLox 40% and VersaBase Gel 60%. The vaginal gel B is composed of MucoLox 40%, VersaBase Gel 50% and the Placebo Hormone Solution 10%.

Description of products

Gynol II (nonoxynol-9) is an irritant of the vaginal mucosa that was used in this study as the positive control, in accordance to the research protocol (MatTek Corporation).

MucoLox is a proprietary polymer base designed to improve mucoadhesion and prolong retention of medication at application sites such as the vaginal mucosa [3]. VersaBase Gel is a proprietary alcohol-free, aqueous base which allows for the incorporation of a variety of active pharmaceutical ingredients for topical and vaginal applications [4].

Placebo Hormone Solution was the name attributed in this technical report to the proprietary solvent system in the PCCA Estradiol/Estradiol USP Dilutions, which corresponds to a concentrated, non-ionicable, 100% bio-based, patent-pending solubilizing surfactant [5].

Vaginal tissue model

The EpiVaginal™ tissue model (MatTek Corporation) is a highly differentiated tissue cultured from normal, human-derived vaginal epithelial and dendritic cells. Its tissue structure and cellular physiology closely parallels in vivo vaginal epithelial tissue. It is therefore an ideal in vitro research tool for safety and toxicological testing of feminine products. The tissue containing epithelial VEC-100 cells was the model used in this study (Figure 1) [6].

Methodology:

Upon receipt of the EpiVaginal™ tissue model, the VEC-100 cells were stored in accordance with the manufacturer’s protocol until the initiation of the study. Following preparation of the cells, the EpiVaginal™ tissues were treated in duplicate with 100 µL of the test product (vaginal gel A, vaginal gel B or Gynol II) for 1, 4 and 24 hr. A set of EpiVaginal™ tissues was also treated (in duplicate) with 100 µL of purified water to serve as a negative control. Following the exposure period, the dosing solutions were removed and the cells were analyzed for cell viability by the MTT Effective Time 50 (ET50).

The MTT ET50 assay consists of measuring the reduction of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) by the cells. 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. MTT is therefore an indicator of cell viability as the tissues are evaluated for their ability to reduce soluble-MTT (yellow) to formazan-MTT (purple) [7].

The MTT solution was prepared in the provided medium and added to the tissues, followed by an incubation period of 3 hr at 37°C. The purple formazan product was then extracted using the provided extractant, which was previously applied to both the apical and basal side of the tissues. Sample absorbance was read at 570 nm and reference absorbance at 650 nm with CLARIOstar – BMG Labtech Plate reader.

Results and Discussion:

Viability of the vaginal cells following exposure to the test products is represented by the absorbance of the respective extracts and expressed in percentage relative to the negative control (tissues treated with purified water), as follows:

\[ \% \text{ Cell Viability} = 100 \times \frac{[\text{OD (test product)}]}{[\text{OD (negative control)}]} \]

The greater the absorbancy of the extracts, the greater the amount of MTT reduced by succinate dehydrogenase and, as a result, the higher the percent cell viability within the tissue [7]. At the start of the study (t=0 hr), the viability of the cells was 100% for all 3 tissues (exposed to vaginal gel A, vaginal gel B and the positive control, respectively). Following 24 hr, the viability of the cells exposed to the positive control was less than 5%, which means that the vaginal tissue was no longer functional and thus confirms the toxicity of Gynol II. On the contrary, the viability of the cells exposed to both vaginal gels for 24 hr was superior to 60%, namely: 79.50% for vaginal gel A and 62.34% for vaginal gel B (Table 1 and Figure 2).

The toxic exposure time (ET50) is the time when cell viability is reduced to 50% [7]. According to the results obtained, the ET50 of the positive control is 4 hr, as opposed to the ET50 of the vaginal gel A and the vaginal gel B which are superior to 24 hr. The vaginal gels may then safely bind to the vaginal mucosa for over 24 hr without causing toxicity to the tissues.

Figure 1. Illustration of the EpiVaginal™ tissue model. (adapted from MatTek Corporation, 2016)
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### Table 1. Safety and toxicological profiles of vaginal gels and Gynol II.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Vaginal gel A (mean ± SD)</th>
<th>Vaginal gel B (mean ± SD)</th>
<th>Gynol II (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.01±10.37</td>
<td>100.01±10.37</td>
<td>100.00±5.90</td>
</tr>
<tr>
<td>1</td>
<td>88.04±7.10</td>
<td>87.00±10.04</td>
<td>84.75±3.21</td>
</tr>
<tr>
<td>4</td>
<td>91.09±11.17</td>
<td>78.79±7.10</td>
<td>51.11±12.30</td>
</tr>
<tr>
<td>24</td>
<td>79.50±13.08</td>
<td>62.34±3.03</td>
<td>4.93±0.74</td>
</tr>
</tbody>
</table>

### Conclusions:

MucoLox, VersaBase Gel and the Placebo Hormone Solution are safe and non-toxic to the vaginal mucosa, as both vaginal gels presented an ET50 superior to 24 hr.

MucoLox is a proprietary polymer base that allows the medication to adhere to the vaginal tissue for a long period of time, despite the regular secretions of vaginal fluid. VersaBase Gel is a proprietary base that delivers the medication to the vaginal tissue. The ability of both bases to bind and deliver the Placebo Hormone Solution safely to the vaginal tissue is a very important characteristic as 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].

The PCCA Estriol/Estradiol USP Dilutions are composed of the hormones and the Placebo Hormone Solution. Both estriol and estradiol are known to be safe and non-toxic to the vaginal mucosa. This study has demonstrated that the Placebo Hormone Solution, a proprietary solvent system, is also safe and non-toxic to the vaginal mucosa. It is then concluded that compounded medicines prepared with the PCCA Estriol/Estradiol USP Dilutions are likely to remain at the site of action for over 24 hr without causing damage to the vaginal tissue.

### References:


