Evaluation of the Safety and Toxicological Profile of RheoSpray™ on the Vaginal Mucosa

Abstract: The vaginal mucosa is a common site for local and systemic delivery of medication. This study aims to compare the safety and toxicological profile of RheoSpray, a proprietary sprayable topical gel compounding base, to the profile of the positive control Gynoll II, using a 3D model of the human vaginal mucosa. Results have demonstrated that RheoSpray presented an ET$_{50}$ superior to 24 hr, as opposed to an ET$_{50}$ of less than 4 hr for the Gynoll II. Therefore, it may be concluded that RheoSpray does not cause toxicity to the vaginal tissues. Compounded medicines including this sprayable topical gel base may then be considered safe to be applied to the vaginal mucosa for over 24 hr.

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 RheoSpray, a proprietary sprayable topical gel compounding base, in comparison to the positive control Gynoll II [nonoxynol-9 (3%)], an irritant of the vaginal mucosa, using a 3-dimensional (3D) in vitro model of the human vaginal mucosa.

Vaginal Tissue Model

The EpiVaginal™ tissue model by MatTek Corporation (Ashland, MA) 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 EpiVaginal™ tissue containing epithelial VEC-100 cells was the model used in this study (Figure 1) [3].

Methodology:

Upon receipt of the EpiVaginal™ tissue model, the VEC-100 cells (Lot 25009) were maintained in the supplied culture media and stored in accordance to the manufacturer's protocol until the initiation of the study. Following preparation of the cells, the EpiVaginal™ tissues were treated in triplicate with 100 µL of the test product (RheoSpray, Lot #7542733) and another set of tissues were treated with Gynoll II for 1, 4 and 24 hr. A triplicate set of EpiVaginal™ tissues was also left untreated to serve as 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 (ET$_{50}$) assay.

The MTT ET$_{50}$ 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) [4].

The MTT solution was prepared in the provided medium and added to the basal side of each tissue, followed by an incubation period of the tissues for 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 left untreated), as follows:

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

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The greater the absorbency 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 [3].

At the start of the study (t=0 hr), the viability of the cells was 100% for both the tissues exposed to RheoSpray and the tissues exposed to the positive control Gynol II. Following 24 hr, the viability of the cells exposed to the positive control was less than 3%, 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 RheoSpray for 24 hr was superior to 80%, as shown in Table 1 and Figure 2.

Table 1. Safety and toxicological profiles of RheoSpray and Gynol II.

<table>
<thead>
<tr>
<th>Exposure time (hours)</th>
<th>Cell viability (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>RheoSpray (mean ± SD)</td>
</tr>
<tr>
<td>0</td>
<td>100.00±2.22</td>
</tr>
<tr>
<td>1</td>
<td>96.41±5.93</td>
</tr>
<tr>
<td>4</td>
<td>106.06±7.82</td>
</tr>
<tr>
<td>24</td>
<td>83.45±3.19</td>
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</table>

The toxic exposure time (ET_{50}) is the time when cell viability is reduced to 50% [3]. The ET_{50} is represented by a red dashed line in Figure 2. According to the results obtained, the ET_{50} of the positive control is less than 4 hr, as opposed to the ET_{50} of RheoSpray which is superior to 24 hr. The proprietary compounding base RheoSpray does not cause toxicity to the vaginal tissues and may then be considered safe to the vaginal mucosa for over 24 hr.

Conclusions:

Compounded medicines applied to the vaginal mucosa must be safe and non-toxic as vaginal toxicity can cause irritation and tissue damage, which weakens the natural defenses of the mucosa and increases the risk of infections such as HIV and herpes simplex [5].

This study has demonstrated that RheoSpray presented an ET_{50} superior to 24 hr and, therefore, has a good safety and toxicological profile on the vaginal mucosa. As a result, compounded medicines including this proprietary sprayable topical gel compounding base may be applied to the mucosa without causing any toxicity to the vaginal tissues.

References:


