RheoSpray™

Evaluation of the Safety and Toxicological Profile of RheoSpray™ on Reconstructed Human Epidermis

Abstract: The human epidermis 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 Triton X-100, using a 3D model of the human epidermis tissue. Results have demonstrated that RheoSpray presented an ET<sub>50</sub> superior to 24 hr, as opposed to that of Triton X-100. Therefore, it may be concluded that RheoSpray does not cause toxicity to the epidermis tissue. Compounded medicines including this sprayable topical gel base may then be considered safe to be applied to the human epidermis for over 24 hr.

Introduction:

The epidermis is the outermost skin layer and it is increasingly used as a route of drug administration. Following topical application, medications may act locally on the skin surface or may penetrate the skin by percutaneous absorption into the microcirculation [1]. Topical compounded medications must be non-toxic and non-irritant to the skin and, therefore, it is important to guarantee the safety of the bases used in compounding.

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 Triton X-100, a nonionic surfactant that can be used as a solubilizer, stabilizer, and emulsifier [2], using a 3-dimensional (3D) in vitro model of the human epidermis.

Reconstructed Human Epidermis: EpiDerm™

The reconstructed human epidermis tissue model – EpiDerm™ – by MatTek Corporation (Ashland, MA) is a highly differentiated 3D model which consists of human-derived epidermal keratinocytes, cultured and differentiated to resemble the human epidermis. Its tissue structure and cellular physiology closely parallels in vivo human epidermis tissue. It is therefore an ideal in vitro research tool for safety and toxicological testing of topical products. (Figure 1) [3].

Methodology:

Upon receipt of the standard EpiDerm™ kit, the EPI-200 cells (Lot 24956) were maintained in the supplied culture media and stored in accordance to the manufacturer's protocol until the initiation of the study [4]. Following preparation of the cells, the EpiDerm™ tissues were treated in triplicate with 100 µL of the test product (RheoSpray, Lot #7542733) and another set of tissues were treated with Triton X-100 (1%) for 1, 4 and 24 hr. A triplicate set of EpiDerm™ 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<sub>50</sub>) assay.

The MTT ET<sub>50</sub> 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) [5].

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 epidermis tissue 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)}}
\]
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 [4].

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 Triton X-100. Following 24 hr, the viability of the cells exposed to the positive control was less than 5%, which means that the epidermis tissue was no longer functional and thus confirms the toxicity of Triton X-100. On the contrary, the viability of the cells exposed to RheoSpray for 24 hr was superior to 95%, as shown in Table 1 and Figure 2.

### Table 1. Safety and toxicological profiles of RheoSpray and Triton X-100.

<table>
<thead>
<tr>
<th>Exposure time (hours)</th>
<th>Cell viability (%)</th>
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<tbody>
<tr>
<td></td>
<td>RheoSpray (mean ± SD)</td>
</tr>
<tr>
<td>0</td>
<td>100.03±5.29</td>
</tr>
<tr>
<td>1</td>
<td>99.17±1.84</td>
</tr>
<tr>
<td>4</td>
<td>98.91±7.99</td>
</tr>
<tr>
<td>24</td>
<td>95.07±9.60</td>
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</table>

The toxic exposure time (ET<sub>50</sub>) is the time when cell viability is reduced to 50% [4]. The ET<sub>50</sub> is represented by a red dashed line in Figure 2. According to the results obtained, the ET<sub>50</sub> of the positive control is less than 15 hrs, as opposed to the ET<sub>50</sub> of RheoSpray which is superior to 24 hr. The proprietary compounding base RheoSpray does not cause toxicity to the epidermis tissue and may then be considered safe for over 24 hr.

### Conclusions:

Compounded medicines applied to the human epidermis must be safe and non-toxic in order to avoid irritation and potential skin damage. The general guideline for correlation of in vitro and in vivo results states that products with an ET-50 of 24hr are expected to be non-irritant [4]. RheoSpray presented an ET<sub>50</sub> superior to 24 hr and, therefore, has a good safety and toxicological profile. As a result, compounded medicines including this proprietary sprayable topical gel compounding base may be applied to the skin without causing any toxicity to the epidermis tissue.

### References:


