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DRUG PERMEATION ASSESSMENT THROUGH RECONSTRUCTED VAGINAL EPITHELIUM: THE CASE OF ECONAZOLE

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INTRODUCTION

The new classification criteria introduced by the Medical Device Regulation EU n. 2017/745 (MDR) includes epithelia and mucosae as targets of substance based medical devices. There is an increasing interest and need to perform permeation studies not only on skin but also on epithelia such as vaginal, oral, nasal and ocular epithelia. Within this framework the use of animal explants presents practical (tissue dimensions and reproducibility) and ethical issues as mentioned in Directive EU n. 2010/63, which promotes the replacement of animal testing with alternative approaches.

When qualified, the *in vitro* testing approach will be used to substitute for the rabbit vaginal irritation (RVI) test and support clinical trials, pre-market approval and *de novo* application for personal lubrificants and vaginal moisturizers[(2)].

Up to now the OECD TG 428 principles, technical requirements and acceptance criteria for study validation represent the state of the art to conduct high quality, robust and biologically relevant *in vitro* cutaneous and epithelial penetration studies.

AIM OF THE STUDY

The aim of this research project was to explore the applicability and performances of 3D reconstructed Human Vaginal Epithelium (HVE-Episkin Laboratories, Lyon, France) tissue for adsorption and penetration studies to be conducted in realistic doses and exposure conditions and applied to products intended for gynaecological use. In this case study the permeation of econazole antifungal compound in a pharmaceutical formulation has been assessed to gain information on active permeation profile by dosing the molecule in the receptor compartment and on the impact of econazole formulation on barrier integrity, permeability and morphology by a multiple endpoint analysis (MEA) approach. The final goal being to demonstrate that the penetration study could be or not validated according to OECD TG 428 requirements in terms of barrier integrity and permeability modifications that could have an impact on the penetration kinetics.

ECONAZOLE: REFERENCE MOLECULE

Econazole (MW: 381.681 g/mole and LogKow: 5.5) is an imidazole with antifungal property that can be delivered intra-vaginally for the treatment of local fungal infections. Literature shows that vaginal administration leads to minimal systemic absorption: about 3-7% of the dose of econazole nitrate cream administered intravaginally is absorbed[(3)] and for commercially available econazole based cream and ovules a very poor percutaneous absorption (0.1-2%) is reported (e.g. Ecostatin).

ECONAZOLE EPITHELIAL PERMEATION ON 3D HUMAN RECONSTRUCTED VAGINAL EPITHELIUM - HVE

The transmucosal permeation has been assessed by homogenously applying a commercially available pharmaceutical formulation including 1% econazole (Pevaryl 1% solution from Janssen-Cilag SpA) on HVE (HVE/S/5, 0.5 cm^2) for a 24h exposure in standard culture conditions (37°C, 5% CO_2, saturated humidity). A total exposure time of 24h has been selected as worst case in the context of risk assessment. However, considering the estimated realistic contact time with the vaginal mucosa, the first quantification of its permeation in the receptor fluid has been performed after 8h. Thus, after 8h and 24h the receptor fluids (1 mL) in the basolateral compartment have been collected for HPLC-ULPC quantification as reported in **figure 1**. Three independent testing runs on a series of 6 biological HVE replicates of the same batch have been carried out.

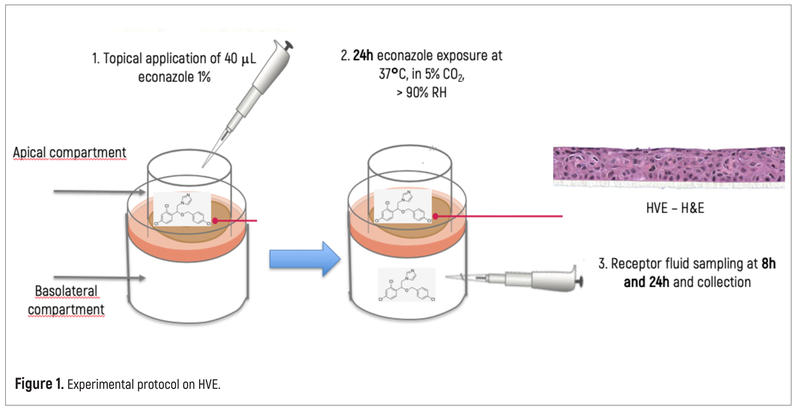


Figure 1. Experimental protocol on HVE.

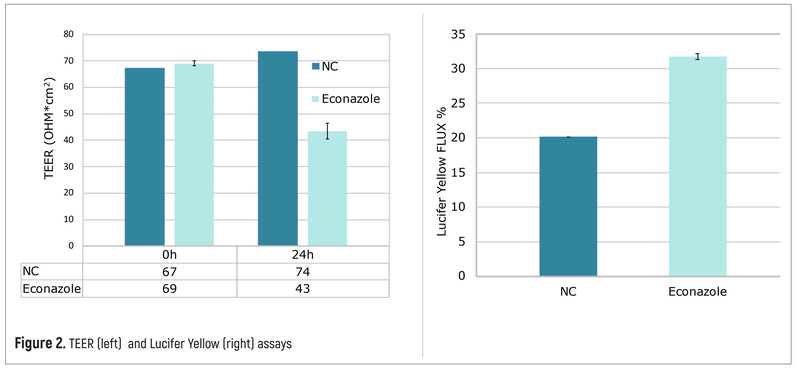
ANALYTICAL METHOD

The liquid chromatographic system employed was an Agilent 1290 infinity UPLC system (Santa Clara, USA) equipped with a reverse phase LUNA C18 column (Phenomenex, USA) for chromatographic separation and Empower 3 software (Waters, USA) for data acquisition and processing. The ULPC method has been developed on the Econazole formulation in presence or not of biological (HVE) matrix before performing the study. The method sensitivity was of 0.4 mg/L in solution.

MULTIPLE ENDPOINT ANALYSIS (MEA) APPLIED TO ASSESS THE TISSUE INTEGRITY DURING THE EPITHELIAL PASSAGE

The assessment of integrity and permeability of the epithelial models after the penetration study has been performed in separate tissues (duplicate) treated in the same conditions but not used for the epithelial passage by evaluating different parameters:

- Transepithelial-electrical-resistance (TEER) measurements
- Lucifer Yellow (LY) assay
- Histomorphological analysis



	0h	24h
NC	67	74
Econazole	69	43

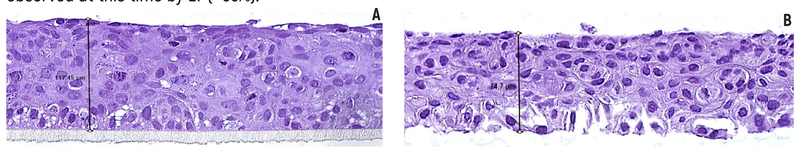
Figure 2. TEER (left) and Lucifer Yellow (right) assays

ECONAZOLE 1% formulation on the HVE tissues for 24h has significantly reduced the TEER values (-41%) compared to the negative untreated control.

The permeation of the LY as fluorescent probe on HVE tissues was increased after the 24h treatment with ECONAZOLE 1% solution (+ 65%) compared to the negative control (NC).

HISTOMORPHOLOGICAL ANALYSIS ON HVE MODEL

In **figure 3** are reported the images of the HVE tissue sections exposed for 24h to saline solution (negative control) and ECONAZOLE 1% formulation. The 24h exposure has modified the tissue morphology at basal level and determined a local toxicity (presence of few picnotic nuclei). The results are in agreements and support the functional measure of the epithelial barrier (TEER reduction of 41%) and the increased permeability observed at this time by LY (+65%).



HVE NEGATIVE UNTREATED CONTROL HVE TREATED CONTROL ECONAZOLE 1% 24H

Figure 3. Haematoxylin and eosin staining of HVE tissue sections after 24h treatment with saline solution (A) and ECONAZOLE 1% solution (B).

RESULTS

In **Table I** are reported the mean amount of econazole quantified in the receptor fluids at the selected time points and total amount permeated for each of the three testing runs (series) and expressed as µg. The Coefficient of Variability (CV%) has been calculated using the mean value of the three runs and the corresponding standard deviation. CV% among the 3 runs was found to be ≤ 15% in the 8h results and very low in the 24h. These data indicate low variability of the results within and between the three independent series confirming the good experimental reproducibility within the same tissue batch and with the same trained operator.

TABLE I	µg of econazole ± standard deviation		
	8 h	24 h	total
Media Run I	17.2 ± 0.11	92.06± 2.36	109.26 ± 2.40
Media Run II	17.54 ± 0.18	96.79 ± 1.47	114.35 ± 1.55
Media Run III	13.94 ± 0.10	95.06 ± 1.35	109.00 ± 1.42
CV%	12.24	2.53	2.72

In **figure 4** the cumulative penetration of ECONAZOLE expressed as % of the total amount topically applied (404 µg) is presented. The results indicate that 4% and 27.4% of ECONAZOLE were permeated through the tissue after 8h and 24h of exposure, respectively, and resulted available for the antifugal action. However, these results have to be interpreted taking into considerations the modifications occurred in the HVE at morphological level and in terms of permeability (+65% increase LY, -41% reduction of TEER).

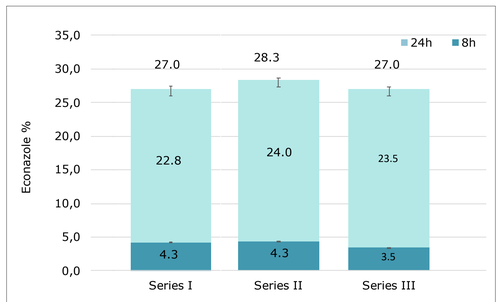


Figure 4. Cumulative Econazole expressed as % of the applied dose for each run.

CONCLUSION

The econazole permeation results through the HVE model (4% after 8h treatment) confirmed the low penetration rate of the econazole through this mucosa.

The assessment of barrier properties with the MEA approach on HVE allowed to understand the impact of the product on the living tissue and to correctly interpret the results of the permeation study: permeation values recorded after the 24h derived from econazole permeation through a damaged tissue as shown by TEER values reduction, LY passage increase and modified histo-morphology.

The advantages derived from the use of reconstructed mucosal models in combination with a multiple endpoint analysis (MEA) approach are:

- relevant and predictive assessment of molecule penetration kinetics
- application of realistic doses and exposure conditions
- flexibility in the adopted experimental conditions
- possibility to monitor the integrity of the tissue before and after the penetration study avoiding any type of experimental bias (including toxic effects) which could affect epithelial integrity and interfere with penetration kinetics
- easy handling of the *in vitro* models
- reproducibility of the measures
- overcoming scientific, regulatory and ethical concerns regarding the use of animals in toxicology.

References [1] De Jong W.H. et al., 2018 Round robin study to evaluate the reconstructed human epidermis (RhE) T model as an *in vitro* skin irritation test for detection of irritant activity in medical device extracts Poster at SOT 2017, Toxicology *in Vitro* 50: 439–449 | [2] Ayehunie S. et al., 2018 Hyper-osmolar vaginal lubricants markedly reduce epithelial barrier properties in a three-dimensional vaginal epithelium model Toxicology Reports 5: 134-140 | [3] Hell R.C. et al. 1978 Econazole: a review of its antifungal activity and therapeutic efficacy. Drugs, 16 (3), 177-201