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Short communication

3D human foreskin model as an alternative method for testing topical formulations

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Introduction

Sildenafil citrate is an effective and approved drug used for the treatment of erectile dysfunction and premature ejaculation. Local transdermal drug delivery of this drug is being explored as an interesting and noninvasive alternative administration method that avoids adverse effects arising from peak plasma drug concentrations (Atiparin et al.2020). Although human and animal skin represent the most reliable models for performing penetration studies, they involve a series of ethical issues and restrictions. For these reasons new *in vitro* approaches based on artificially reconstructed human skin or "human skin equivalents" are being developed as possible alternatives for transdermal testing. The validation and implementation of these experimental models as alternative methods in the evaluation of the molecule permeation, therefore, are strongly promoted, representing a promising scientific innovation.

Materials and methods

Preparation of three topical formulations of sildenafil citrate

Sildenafil citrate was incorporated in three preformed transdermal vehicles. Specifically, the tested creams are: formulation A (Pentravan®), formulation B (Liposomial Heavy®) and formulation C (HRT Heavy®).

3D Human foreskin preparation

The 3D fully-human skin equivalent was generated by successively fabricating a dermal compartment consisting of fibroblasts and a multi-layered, well differentiated epidermal compartment on top of the dermis following the protocol described by (Zoio 2022).

Permeation assay of sildenafil citrate through 3D skin model

3D skin equivalents (200 μm) were transferred into a new six-well plate, where skin penetration study was conducted. The receptor (basolateral)/surrounding compartment was filled with 4.5 mL of physiological solution. At time 0, 0.5 mL of each formulation (corresponding to 38.6 mg of sildenafil citrate) was carefully applied on the surface of the skin equivalent. The plate was subsequently covered, horizontally shaken and maintained at 32°C, 5% CO_2, throughout the experiment (4 h). At pre-specified time intervals (0, 20, 40, 60, 120, 180, and 240 min) 1.0 mL of receptor solution was withdrawn and immediately replaced with an equal volume of fresh buffer. Samples were analyzed by high performance liquid chromatography (HPLC). Experiments were conducted in three replicates.

Analysis of sildenafil citrate by High Performance Liquid Cromatography (HPLC)

For each test, the concentration of sildenafil citrate was obtained using an Agilent 1260 chromatograph equipped with a diode array (DAD). The mobile phase was composed of acetonitrile (A) and water 0.1% formic acid

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(B) at a flow rate of 0.4 mL/min, in isocratic phase: 30% A, 70% B.

Results and discussion

It can be observed that the amount of drug in the basolateral medium increased over time among all the formulations at the end of the contact time (4 hours). Notably, the amount of sildenafil citrate was particularly high in formulation A and formulation C after 4 h of contact through 3D full-thickness skin samples (217 ± 17.8 µg/cm^2 and 165 ± 13.3 µg/cm^2, respectively), while the maximum concentration of the drug reached in the formulation B was 53.9 ± 28.3 µg/cm^2. Formulation A showed the highest skin permeation of sildenafil citrate (Fig.1) related to the liposomal structure of such formulation that has been already demonstrated to improve transdermal absorption of drugs (Egbaria et al. 1990).

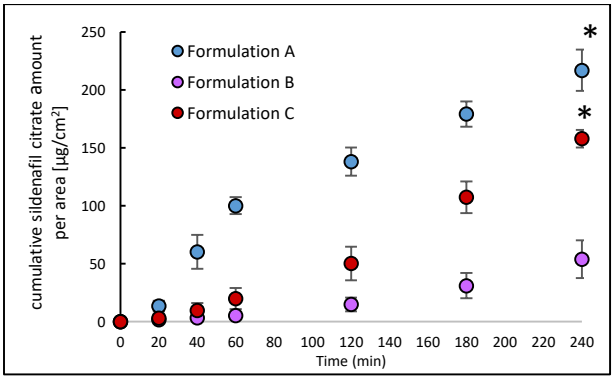

Fig. 1: Sildenafil citrate amount (µg/cm^2) from topical formulations that permeated in the receptor fluid at specific extraction times. Asterisk (*) indicates statistically significant differences between formulation B and the other two tested formulations (formulation A and formulation C; $p < 0.05$).

A similar permeation rate was observed when sildenafil citrate was dissolved in the oil-in-water (o/w) emulsion formulation C, allowing the penetration of hydrophilic substances through the *stratum corneum* of the skin. Conversely, the skin permeation of sildenafil citrate from formulation B was much lower compared to the other two tested creams. This may be attributed to the fact that formulation B is a liposomial organogel (lipophilic gel) representing not an optimum vehicle for hydrophilic molecules such as sildenafil citrate. Figure 2 shows that the 3D model formed a skin complex architecture with a complete epidermis, mimicking the normal process of epidermal stratification. Moreover, the expression of involucrin, critically involved in the formation of the cornified cell envelope, was evaluated to analyze the epidermal barrier formation in the skin equivalent. Our results reveal the presence of such typical epidermal tissue protein in the skin equivalent, confirming the epidermal differentiation (Fig. 2).

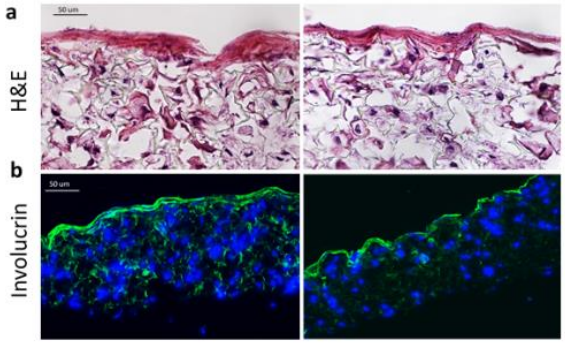

Fig. 2**:** Hematoxilin and Eosin staining of the 3D full thickness skin equivalent. (a): The morphology of the developed skin equivalent. (b): Expression of involucrin. The corresponding bright field image. Scale bar: 50 µm

Conclusion

The results reported in this study confirmed the importance of the formulation on the dermal permeation of the sildenafil citrate. Although further studies need to be performed, including an expanded panel of substances, the 3D skin equivalent adopted in this work is able to provide a rapid initial estimation of the amount of sildenafil citrate permeated through the skin, with the potential of being a valid alternative to *ex-vivo* animal skin for skin penetration measurements of new dermal formulations of such important drug.

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