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Use of an *in vitro* human skin permeation assay to assess bioequivalence of two topical cream formulations containing butenafine hydrochloride (1%, w/w)

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ABSTRACT

The primary objective of this work was to investigate, using an *in vitro* human skin permeation study, whether changes in the excipients of butenafine hydrochloride cream would have any effect on bioperformance of the formulation. Such *in vitro* data would be a surrogate for any requirement of a bioequivalence (BE) study to demonstrate formulation similarity. A LC-MS/MS method for quantitation of butenafine in various matrices was developed and validated. A pilot study was performed to validate the *in vitro* skin permeation methodology using three cream formulations containing butenafine hydrochloride at concentrations of 0.5, 1.0 and 1.5% (w/w). Finally, a definitive *in vitro* human skin permeation study was conducted, comparing the extent of butenafine hydrochloride permeation from the new formulation to that from the current formulation. The results of the study comparing the two formulations showed that there was no statistically significant difference in the extent of butenafine permeation into human skin. In conclusion, these *in vitro* data demonstrated that the formulation change is likely to have no significant impact on the bioperformance of 1% (w/w) butenafine hydrochloride cream.

1. Introduction

Butenafine is an antifungal agent with primary fungicidal activity against dermatophytes such as Trichophyton mentagrophytes, Microsporum canis and Trichophyton rubrum which cause tinea infections (McNeely and Spencer, 1998). Butenafine selectively inhibits the growth of fungi by interfering with the synthesis of ergosterol in the cell wall, which results in the intracellular accumulation of squalene and rapid cell death (Syed and Maibach, 2000). Preclinical and clinical studies have shown that after topical application of butenafine, high concentrations were maintained in the *stratum corneum* and epidermis, resulting in significantly higher mycological cure rate as compared to vehicle control (Syed and Maibach, 2000). The currently approved formulation is a cream (Lotrimin Ultra®) containing 1% w/w butenafine hydrochloride and 0.3% w/w diethanolamine (DEA) as a pH adjuster. DEA, an alkalizing ingredient and FDA approved inactive ingredient (FDA IID), has been declared a possible carcinogen by the International Agency for Research on Cancer (IARC diethanolamine monograph). DEA became a listed substance on California's Proposition 65 in June of 2013 (State of California EPA Hazard Assessment). As a result, a decision was made to reformulate Lotrimin Ultra® by replacing DEA with trolamine (triethanolamine (TEA)). Currently no information is available on the carcinogenic effects of TEA in human or animals, and as such EPA has not classified TEA as a potential carcinogen (EPA Health Hazards Notebook). Like DEA, TEA is an alkalizing ingredient that has similar structural and physicochemical properties as DEA. The replacement of DEA (0.3% w/w) with TEA (0.43% w/w) involves the substitution of a molar equivalent amount of TEA for DEA so that the two formulations maintain equivalence in terms of their neutralizing/alkalinizing capacity. The higher molecular weight of TEA requires a slightly greater amount of TEA than DEA in the formulation, which is balanced by an equal reduction in purified water content. Apart from these changes, both formulations were identical.

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An *in vitro* permeation study in human skin was conducted, to assess the impact of this formulation change on butenafine hydrochloride absorption through skin. The purpose of this study was to use it as a surrogate for any requirement of a bioequivalence (BE) study between the two formulations and was designed following discussions with the United States Food and Drug Administration (FDA). It is well known that establishing BE of topical dermatological products is complicated and expensive. This is primarily because these products elicit pharmacological responses by locally acting at the site of application and hence plasma concentration profiles of these products are not always appropriate surrogates of their pharmacological activity (Kanfer, 2000; Narkar, 2010; Yacobi et al., 2014). For many topical products, bioequivalence between formulations is demonstrated by conducting comparative clinical end-point trials in a patient population; which are both lengthy and expensive (Kanfer, 2000; Narkar, 2010; Yacobi et al., 2014). The only alternative method approved to date by the United States Food & Drug Administration (FDA) is the vasoconstrictor activity (VCA) assay for topical glucocorticoids (FDA, 1995). An FDA Critical Path Initiative (CPI) identified that demonstrating BE of locally acting drugs (including topical dermatological drugs) represents a significant challenge in development of such products (Lionberger, 2008), and suggested several surrogate methods to demonstrate BE. Recently, a workshop organized by the Product Quality Research Institute (PQRI) also discussed the issues with establishing BE for topical products and identified several alternative methods to assess BE including *in vitro* skin permeation studies (Yacobi et al., 2014). In an effort to support the use of *in vitro* skin permeation assays as a surrogate for clinical studies, Franz et al. demonstrated that of seven topical drug products studied previously, *in vitro* data was able to predict bioequivalence for six products, in agreement with the clinical data (Franz et al., 2009).

In this paper, the results of an *in vitro* human skin permeation study comparing extent of butenafine hydrochloride permeation from the new formulation containing TEA to that from the current formulation containing DEA are presented. The purpose of this study was to use it as a surrogate for any requirement of a bioequivalence (BE) study between the two formulations and was designed following discussions with the FDA. The results of the *in vitro* permeation study demonstrating similarity of the two formulations was accepted by FDA and resulted in authorization to market the new Lotrimin Ultra® cream. In addition, the development of a validated LC-MS/MS method for quantifying butenafine in various matrices and a pilot study demonstrating the validity of the *in vitro* skin permeation methodology employed are described.

2. Materials & methods

2.1. Materials and reagents

Butenafine hydrochloride was supplied to Charles River by Merck & Co. Testosterone propionate, methanol (Chromasolv grade) and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich (UK). Bovine serum albumin (BSA) and formic acid (analytical grade) were obtained from Fisher Scientific (Loughborough, UK). Simple soap was obtained from Unilever (London, UK).

2.2. Preparation of calibration standards and quality control samples

Working calibration standards, quality control (QC) solutions of butenafine and the internal standard testosterone propionate were prepared by diluting a solution (1 mg/mL) with methanol and were stored at *ca* 4 °C in glass vials, when not in use. Calibration standards for receptor fluid and skin wash were fortified with butenafine at concentrations of 2.00, 3.50, 7.50, 10.0, 50.0, 75.0, 100, 200, 450 and 500 ng/mL. QC samples were prepared at 2.00, 5.00, 50.0 and 400 ng/mL.

2.3. Sample preparation

2.3.1. Receptor fluid and skin wash

An aliquot (50 μL) of control matrix, either BSA in PBS (5%, w/v) for receptor fluid or Simple Soap diluted in water (2%, v/v) for skin wash, was fortified with 10 μL of the appropriate calibration standard or QC solution. For test samples, an aliquot (50 μL) was used and methanol (50 μL) was added to all tubes. Internal standard was added (10 μL), followed by methanol (700 μL). The samples were vortex mixed and centrifuged at 2740 *g* for 5 min at *ca* 4 °C.

2.3.2. Skin, tape, tissue swabs, cling film and pipette tips (methanol extracts)

Skin, tape, tissue swab, cling film and pipette tip samples were extracted in methanol (10 mL). The samples were then flatbed shaken for 10 min and placed in a sonic batch for 10 min. An aliquot (50 μL) of sample was then removed for further processing. Receptor fluid was fortified with the appropriate calibration standard or QC solution (10 μL) and then methanol (50 μL) was added. For test samples methanol extract (50 μL) was added to receptor fluid (50 μL). Internal standard (10 μL) was added, followed by methanol (700 μL). The samples were vortex mixed and centrifuged at 2740 *g* for 5 min at *ca* 4 °C.

2.4. LC–MS/MS analysis

For LC–MS/MS analysis, the chromatographic system consisted of Perkin Elmer Series 200 HPLC pumps (Beaconsfield, UK), a HTS-PAL CTC Analytics Autosampler (Zwingen, Switzerland) and an Applied Biosystems API3000 Sciex mass spectrometer (Warrington, UK) operated in positive ionization mode. The separation was performed on an Agilent Poroshell EC-120 C_{18} 50 × 2.1 mm, 2.7 μm column (Crawford Scientific, UK). A gradient was performed using mobile phases of methanol/formic acid (100/0.1, v/v) and water/formic acid (100/0.1, v/v) at a flow rate of 0.3 mL/min. A linear gradient was applied as follows: increase from 5 to 95% methanol/formic acid (100/0.1, v/v), 0–2.0 min; hold at 95%, 2.0–4.5 min; decrease from 95% to 5%, 4.5–4.6 min and hold at 5%, 4.6–6.0 min. The column and autosampler tray temperatures were 60 °C and 4 °C, respectively. The analytical run time was 6.0 min. Multiple-reaction-monitoring (MRM) mode was used for the quantification by monitoring the transitions: m/z 318.5 → 141.2 for butenafine and m/z 345.2 → 97.0 for testosterone propionate. Peak areas for all components were integrated using Analyst version 1.4.2 software (Sciex, UK).

2.5. Method validation

The method was validated for selectivity, sensitivity, linearity of the calibration curve, precision and accuracy, recovery, stability and dilution integrity according to the US FDA guidance document for bioanalytical method validation (FDA, 2001), the EMA guidelines on bioanalytical validation (EMA, 2012a,b) and the VICH GL1 and VICH GL2 guidelines for validation of analytical procedures ((VICH GL1 (Validation Definition) and VICH GL2 (Validation Methodology), October 1998; effective October 1999)).

2.6. Validation of in vitro human skin permeation methodology

The study described in this section was conducted according to