Comparison of Dermatopharmacokinetic *vs.* Clinicial Efficacy Methods for Bioequivalence Assessment of Miconazole Nitrate Vaginal Cream, 2% in Humans

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Purpose. To compare the dermatopharmacokinetic vs. clinical trial methods for bioequivalence assessment of two miconazole nitrate vaginal cream, 2% products.

Methods. The dermatopharmacokinetic method determined the bioequivalence of two products simultaneously in 24 healthy subjects, as a function of C_{max} and AUC_{0-t} parameters using miconazole nitrate content in harvested volar forearm stratum corneum. The clinical trial method determined bioequivalence as a function of clinical, mycological culture and therapeutic cure(s) after 7 days of product use and 30 days after therapy cessation in 106 female subjects with positive signs and symptoms of vaginitis, KOH vaginal smears and *Candida* cultures, randomly assigned to test or reference product.

Results. The dermatopharmacokinetic method demonstrated that the two products were not bioequivalent, while the clinical trial method concluded bioequivalence.

Conclusion. The dermatopharmacokinetic method allowed simultaneous evaluation of both products in the same subject, within the same study period, and was more sensitive and discriminating in the assessment of bioequivalence between the two miconazole nitrate vaginal cream, 2% products than the clinical trial method.

KEY WORDS: skin stripping; anti-fungal; bioequivalence; skin; human.

INTRODUCTION

Bioequivalence assessment between two sources of a topical drug product can be evaluated by either of four methods in order of preference: pharmacokinetic, pharmacodynamic, comparative clinical trials or *in vitro* studies (1). Pharmacokinetic evaluation of the active drug in the circulating blood of healthy human subjects is the method of choice for bioequivalence assessment of two oral solid dosage products. Dermatological and vaginal drug products however, are designed to target the local tissue to which they are applied and as such, have limited systemic absorption. Thus, traditional pharmacokinetic evaluation of the locally acting drug products using systemic blood samples has historically proved fruitless. Hence, determination of bioequivalence of two similar topical vaginal targeted drug products has therefore relied on comparative clinical trials studies as the method of bioequivalence assessment.

Bioequivalence between two products is assumed in a comparative clinical trial method if no significant difference in clinical outcome and fungal culture negative samples from the enrolled diseased subject is measured. While such comparative clinical trials studies do insure that two products have similar therapeutic outcomes, this method for bioequivalence testing is fraught with high variability of the target disease severity, disease onset and duration before treatment, and tissue status of the enrolled subjects. Further, clinical studies provide no mechanistic basis of understanding for the nonresponders of the drug therapy. Thus, clinical outcome studies require large number of patients over extended periods of time (4 weeks) to increase the statistical power to achieve appropriate discrimination between products.

Miconazole nitrate is a wide spectrum synthetic antifungal agent formulated as a 2% cream in a water miscible base to treat tinea corporis, tinea cruris, tinea pedis in skin caused by *Trichophyton rubrum, Trichophyton mentagrophytes* and *Epidermophyton floccosum* or tinea versicolor caused by *Malessezia furfur* as well as vaginal candidiasis. Effective locally acting antifungal therapy requires that applied drug be delivered to the target tissue to exert its pharmacological activity and produce a therapeutic effect. Thus, drug must leave its vehicle and partition into the target tissue. For cutaneous fungal infections, the target tissue is the stratum corneum. In the case of vaginal candidiasis, the target tissue is the superficial stratified epithelium. The stratified epithelium in both tissues is dynamic and acts as a "rate-limiting barrier" to all drug absorption into and across the respective tissue (2–6). Ideally, pharmacokinetic bioequivalence assessment methods measure the drug concentration after application of both products in the target tissue at multiple time points in healthy human subjects after a single dose of a test and reference product, which are qualitatively (Q1) and quantitatively (Q2) similar (7). For locally acting vaginal-targeted products, vaginal tissue collection would be required. Collection of this tissue at multiple time points however, is challenged by the invasive nature of the biopsy procedure, lack of a validated method for tissue collection and the unlikelihood of obtaining informed consent from human subjects. In contrast to vaginal tissue, the stratified epithelium of skin is easily accessible. It can be harvested noninvasively in a reproducible manner using adhesive discs and subsequently analyzed for drug content by a validated analytical a1ssay (8–10).

The two epithelial tissues share common, yet unique properties. The stratified stratum corneum in skin is anucleate and highly keratinized into a "horny layer' which has a high 70% protein composition, 15% water and 15% lipid (5). Vaginal tissue is also a keratinized, stratified epithelium containing ceramide lipids important to barrier function, in addition to high glycogen concentration, the latter which varies as a function of plasma estrogen concentration (11–13). Both epithelial tissues are mildly acidic (pH 5 and 4, respectively) (6,12) and have a cellular turnover rate of 21–28 days. Growth and differentiation of both tissues is mediated by keratinocyte growth factor (14–15), calcium (16–17) and retinoids (18–19). Vaginal tissue differs from skin in its chemical composition of

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ABBREVIATIONS: mcg, micrograms; sq cm, square centimeters; mcl, microliter.

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the percutaneous absorption barrier and the presence of mucus, a complex mixture of glycoprotein, lipid, salts and cellular debris that protects the underlying tissue and can adversely affect drug penetration into this tissue (20).

The current study evaluates whether bioequivalence assessment of the of two miconazole nitrate vaginal cream, 2% products by the dermatopharmacokinetic method in human volar forearm stratum corneum *in vivo* agrees with the assessment using a comparative clinical trial results in females positively diagnosed with vaginal candidiasis.

METHODS AND MATERIALS

Materials

Stratum corneum harvesting, also known as skin stripping, was performed using D-squame® adhesive system (CuDerm Corp., Dallas, Texas), which has ten 1.3 cm diameter discs mounted on a polymer backing sheet. One sheet of ten discs was used for each skin site. Skin stripping samples were stored in 1.7 ml polypropylene capped conical tipped microcentrifuge tubes at −70°C until extracted and analyzed by HPLC.

The HPLC assay utilized a 5 micron, 4.6 mm × 12.5 cm C_{18} reverse phase analytical column (5 micron particle, Partisil® Whatman), HPLC grade acetonitrile (HPLC Grade, Fisher Scientific Co.), monobasic potassium phosphate (EM® Science, St. Louis, Missouri) and in-house double distilled deionized water, Labnet Shaker 20 (National Labnet Co., Woodbridge, New Jersey), 1.7 ml borosilicate, T/S septa lined, screw capped autosampler vials (National Scientific Co.) and a binary HPLC system (Thermo Separations) with a UV detector (Spectral Focus®, Thermo Separations). Testosterone (Sigma) was used as purchased for the internal standard.

The miconazole nitrate vaginal cream, 2% products evaluated are described in Table I. The two products contained similar concentrations of miconazole nitrate (2%) but differed in the vehicle composition, in terms of both the qualitative (Q1) and quantitative (Q2) inactive excipients (7). The products were used as purchased.

Human Subjects

Studies using human subjects followed the tenets of the Declaration of Helsinki promulgated in 1964 and was approved by the Institutional Review Board. Informed consent was obtained from all human subjects before study commencement. Subjects were remunerated for participation in the studies.

Table I. Miconazole Nitrate Vaginal Cream, 2% Study Products

Name	Test	Reference[a]
Tube size (gm)	30	30
Inactive Excipients	Benzoic acid	Benzoic acid
	Butylated hydroxyanisole	Cetyl alcohol
	Mineral oil	Isopropyl myristate
	Peglicol-5 oleate	Polysorbate 60
	Pegoxol-7 stearate	Potassium hydroxide
	Purified water	Propylene glycol
		Purified water
		Stearyl alcohol

[a] Monistat 7® vaginal cream, 2%.

Reproducibility of Stratum Corneum Harvesting Procedure

Reproducibility of the stratum corneum harvesting procedure was validated by weight using an ultramicro balance (Sartorius 4504 MP8-1) with a sensitivity of 0.1 mcg. Nine adhesive discs were measured for weight 0.5–1 h before and within 1 h after the stratum corneum harvesting procedure at two anatomic regions on each volar forearm. Two skin sites, located side-by-side were placed in each anatomic region on each forearm. In this way, variability in the stratum corneum harvesting procedure could be assessed in a single individual within as well as between anatomic regions on the right and left forearms as well as between subjects.

Dermatopharmacokinetic Study

Subjects

Subjects represented both males (n = 7) and females (n = 17), with a mean ± SD age of 39.0 ± 12.9 years. All subjects were right handed. Fifteen of the subjects were Caucasian and ten were of Asian ethnicity, representing Fitzpatrick skin types (21): I (n = 1), II (n = 1), III (n = 20) and IV (n = 2). Subjects were healthy as established by the investigator with no existing medical conditions or concomitant medication use.

Study Design

The study was designed to quantify and compare the uptake and elimination profile of miconazole nitrate vaginal cream, 2% products from two manufacturers. One hour before drug application, all subjects forearms were cleansed with 1 drop of Purpose® Gentle cleansing wash (Johnson and Johnson) worked into a lather with a wetted cotton ball and scrubbed along the full length of the ventral forearm (wrist to antecubital fossa) 3 times. Forearms were rinsed in tepid water and gently blotted with a nonabrasive paper towel. Five minutes before drug product application, the designated untreated control skin sites were harvested for stratum corneum. Both miconazole nitrate vaginal cream, 2% products were applied synchronously to all designated skin sites at 0 h. Residual product removal was staggered at various time points after a 4 h dose. Stratum corneum harvesting was performed either immediately after product removal (0.5, 1, 2, and 4 h), to quantify drug uptake, or at various time points after drug removal of a 4 h treatment (0, 4, 8, 20, and 28 h), to quantify drug elimination from the skin. Investigators were unaware of the randomization of the particular drug product application.

Product Application on the Volar Forearms

A template was prepared to contain ten 1.2 cm diameter circles that insured that the ten skin sites on a ventral forearm in all subjects were located at similar discrete intervals (2.5 cm apart, center-to-center), a minimum of three cm above the wrist and three cm below the antecubital fossa. The ten skin sites were demarcated on both ventral forearms of all subjects immediately after arm washing and 1 h before product appli-

cation using an indelible marker. Each ventral forearm was divided into five anatomic regions: I -V on the right forearm and VI - X on the left forearm. Product application was randomized between subjects with respect to application duration on the right arm and the time point(s) of elimination on the left arm. Each anatomic region contained two 1.2 cm diameter skin sites. Anatomic regions I–V were assigned to either a 0 (untreated control), 0.5, 1, 2, or 3 h application duration. Anatomic regions VI–X all received a 4 h treatment duration.

Each anatomic region contained 2 skin sites (#1 and #2), each spaced 2.5 cm center-to-center apart. All skin sites within an anatomic region were treated for the same application duration. Among the two skin sites within each region, site #1 or # 2 was assigned to the test product A or the reference product B. The randomization of product assignment to a particular skin site (#1 or #2) was consistent within a subject on both forearms, but differed between subjects.

Following application of the 5 mcl dose of each drug product to the center of the skin site, the product was evenly distributed to the entire surface area of the skin site (1.13 sq cm) with a disposable, conical-bottom 1.7 ml polypropylene microcentrifuge tube. No product was applied to the designated untreated skin sites. A non-occluding protective guard, prepared in the laboratory was used to cover both product-treated sites within an anatomic region, to protect the drug treatment from the environment and/or accidental removal. Subjects were allowed non-caffeinated, non-alcohol drinks and food *ad libitum* and were not confined to the study facility.

Single Dose Dispensing Syringe

A 5 mcl dose of each product was dispensed from an individual preloaded 1725 LT Hamilton syringe. Reproducibility of a single dose by this delivery method had a mean (± SD) weight of 4.5 ± 0.4 mg for the reference product and 4.6 ± 0.4 mg for test product. The coefficient of variation of both products with this dosing methodology was similar, ~8.9% and acceptable according to manufacturer specifications. Product dispensing was performed by a single investigator independent of a different investigator harvesting the stratum corneum.

Stratum Corneum Harvesting Procedure

Stratum corneum was harvested from each skin site on only one occasion using ten adhesive discs. A blunt ended forceps was used to sequentially apply an individual adhesive disc to the skin site, rub the disc 3 times with the rounded blunt end and carefully removed the adhesive disc from the skin site. The first adhesive disc applied and removed was discarded due to potential residual product contamination. The remaining nine adhesive discs were stacked together, placed into a prelabeled 1.7 ml polypropylene capped microcentrifuge tube and stored at −70°C until extracted and analyzed by HPLC for drug content. Therefore, each time point in the uptake/elimination profile represents a single randomly designated skin site on the volar forearms of each subject evaluated. In this way, the uptake and elimination profile for both products were evaluated simultaneously in a subject in the same study period. Stratum corneum harvesting was performed by a single investigator independent of a different investigator applying / removing the drug product.

HPLC Assay

Miconazole nitrate in the adhesive disc-stratum corneum samples collected from the product-treated and untreated control skin sites were quantified in a validated HPLC assay. The latter nine adhesive discs collected frm each skin site were combined and extracted with 1 ml of extraction solution (30/70 (v/v) acetonitrile/0.1 M KH_2PO_4 pH 2.5, containing 10 mcg/ml of the internal standard testosterone). Samples were shaken at high speed (1400 rpm) for 2 h on a tabletop shaker (Labnet Shaker 20; National Labnet Co., Woodbridge, New Jersey). Recovery of miconazole nitrate was greater than 90%. The extracts were then transferred into prelabeled, borosilicate glass, T/S septa lined screw-topped autosampler vials (National Scientific Co.). Miconazole nitrate concentration was quantified from a 50 mcl injection volume using a 205 nm wavelength (Spectra-focus® detector, Thermal Separations Products) and C_{18} RF column (4.6 mm × 12.5 cm, 5 micron particle: Partisil®, Whatman) at 23°C with a mobile phase of 46.5/52.5/1 (v/v/v) acetonitrile/0.1 M KH_2PO_4 pH 2.5/H_3PO_4 and a 1.25 ml/min. flow rate. These HPLC conditions separated miconazole nitrate from testosterone with retention times of 5.76 m and 4.49 m, respectively.

The HPLC miconazole nitrate assay was linear over the concentration range of 0.100 to 8.000 mcg/ml. The mean linear regression parameters for the 24 subjects evaluated was r^2 = 0.9979. The limit of quantification was 0.100 mcg/ml. Precision was less than 11% and accuracy was >93% for all extracted, miconazole nitrate-spiked adhesive disc calibration standards. Unknown contents of miconazole nitrate in the adhesive disc-stratum corneum harvested samples were calculated against extracted, analyte-spiked adhesive disc standards ranging from 0.100 to 8.000 mcg/ml. All drug contents were normalized to mcg of miconazole nitrate per adhesive disc surface area of 1.33 sq cm.

Pharmacokinetic Parameters

All the available data from 24 subjects who participated in the study were used in the pharmacokinetic analyses. Peak content (C_{max}) was the observed maximum value during the absorption period 0–4 h. The time to peak content (T_{max}) was the collection time at which C_{max} was observed, or first observed, if the peak value was reported at more than one sampling time. Area-under-the content-time curve (AUC) from 0–4 h (uptake phase) and to the time of the last non-zero content (C_t) was calculated by the linear trapezoidal method. The apparent first-order elimination rate constant (Ke), was estimated as the negative value of the slope of the regression line for the terminal, log-linear content-time values. A minimum of three terminal values was used to obtain an estimate. When the terminal data were not log-linear, no elimination estimate was obtained. Elimination half-life was calculated as log (2)/Ke (22).

Statistical Analysis

Statistical analyses were performed using the General Linear Models (GLM) procedure of the SAS statistical program (PC version 6.12) with all of the available data from 24

subjects. The calculated pharmacokinetic parameters, as well as the contents at each of the individual collection times, were compared statistically. The statistical model contained main effects of subject and treatment in the analysis of variance conducted at $\alpha = 0.05$. Confidence intervals (90%) for pairwise area and peak content comparisons were calculated by the *t*-test approach (two, one-sided) at $\alpha = 0.10$ overall, $\alpha = 0.05$ each side (23). The intervals were computed for the mean treatment differences, expressed as a percent of the reference treatment mean, and for the geometric mean test-to-reference ratios using log-transformed results. Bioequivalence was assumed if the 90% confidence interval of the log transformed C_{max} was within 70–143% and log transformed $AUC_{0\text{-}t}$ was within 80–125% using the two, one-sided test (22–23). Stratum corneum harvesting reproducibility using the adhesive discs was analyzed by ANOVA (Fishers PLSD) using Statview™ version 4.1.

Bioassay

Briefly, the *in vitro* bioassay used 30 mcl of extracted drug-spiked adhesive disks pipetted onto a 6 mm diameter paper disc (TAXO discs; VWR) and air dried at room temperature (25°C). Five hundred microliters of a *Candida albicans* suspension in YM broth (Difco, Detroit, Michigan) was pipetted onto a 60 mm diameter Sabouraud dextrose agar plate (MicroBio Products, Inc., Tucson, Arizona) and spread evenly over the surface area of the plate using a hockey stick. Immediately following inoculation of the yeast on the agar plates, an air-dried paper disc previously loaded with a known drug concentration was place on one quadrant of the inoculated plate. Drug activity was quantified as the average diameter (cm) of the zone of growth inhibition of *Candida albicans* measured in two directions 48 h after inoculation (10). All data was normalized to that zone associated with 0 mcg/sq cm. Six calibration standard curves, representing miconazole nitrate contents ranging from 0.25 to 25.00 mcg/sq cm, were evaluated. The linear portion of the calibration curve was between 0.25–2.50 mcg/sq cm concentrations (Fig. 1).

Miconazole nitrate content measured in the stratum corneum harvested samples collected from human subjects was related to the drug content in the bioassay by the equation: $B = (0.040 * D)$, where B represents the miconazole nitrate content in the bioassay (mcg) and D represents the drug content (mcg/sq cm). For example, 30 mcl of a harvested stratum corneum sample extract containing 1.25 mcg / sq cm drug content or 1.66 mcg/ml miconazole nitrate (1.25 mcg × 1.33 sq cm skin surface area) is equivalent to 0.05 mcg in the bioassay. The bioassay was able to statistically differentiate contents that differ by at least 1.25 mcg/sq cm or 0.05 mcg (ANOVA, Fisher's PLSD, $P < 0.05$) (28). Linear regression analysis of the extracted, miconazole nitrate-spiked adhesive disc calibration drug content curve over the range of 0.25 to 2.50 mcg/sq cm and the resulting anti-fungal activity in the bioassay demonstrates the mathematical relationship from which percent inhibition can be derived as $I = 0.1603 + (0.2657 * D)$, where I is inhibition and D is drug concentration (mg/ml). Thus, discrimination in the biologic response was ± 0.332 cm zone of inhibition and the corresponding discrimination in drug content was ± 1.25 mcg/sq cm over the 0.25–2.50 mg/sq cm concentration range.

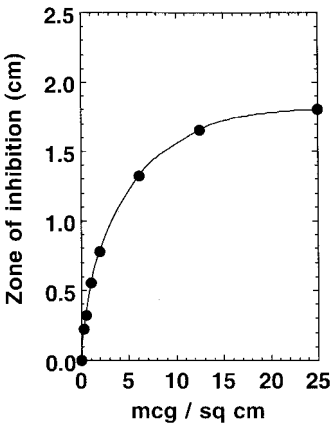

Fig. 1. Bioassay Standard Calibration Curve. Zone of growth inhibition of *Candida albicans* as a function of miconazole nitrate concentration in extracted, miconazole nitrate spiked adhesive discs used for stratum corneum harvesting. Mean of six replicate standard curves.

Comparative Clinical Trials Study

One hundred and six females with positive signs and symptoms of vaginitis, positive KOH vaginal smear and positive *Candida* culture were enrolled in the study after providing informed consent. Subjects were randomly assigned to the test or reference product. Fifty-one subjects received the test product and 55 subjects received the reference product. Enrolled subjects were instructed to use the assigned product twice daily for 7 days. Three clinical end point parameters; clinical scores of signs and symptoms for vaginitis, KOH vaginal smears and *Candida* mycology cultures, were evaluated 7 days after product use and 30 days after cessation of product use (7 and 37 days, respectively). Data is presented as percent responders in terms of clinical cure, mycological cure and therapeutic cure. Clinical cure is defined as the lack of signs and symptoms of vaginosis. Mycological cure is defined as a negative *Candida* culture and KOH smear. Therapeutic cure is defined as the lack of clinical signs and symptoms of vaginosis and a negative KOH smear and *Candida* culture. Clinical and mycological cures were assessed at 7 days and 37 days. Therapeutic cure was assessed at the 37 day time point only.

RESULTS

Stratum Corneum Harvesting Reproducibility

Mean stratum corneum weight harvested at multiple sites was 354.2, 284.9, and 197.8 mcg on the right arm and 272.7, 223.2, and 253.7 mcg on the left arm of subjects #1, 2, and 3, respectively (Table II). The variability in stratum corneum weight harvested at the multiple sites on the same arm, or intra-arm variability, ranged from 8.93% to 35.26% on the right arm and 25.5% to 40.2% on the left arm of the individual subjects (Table II). Between-arm variability (%) or that variability in all skin and on both arms was of 27.29%, 17.85%, and 26.84% in subjects #1, #2, and #3, respectively. These data demonstrate that variability in stratum corneum removal using the current stratum corneum harvesting methodology was subject dependent but less than 30% in all subjects evaluated.

Table II. Reproducibility in Stratum Corneum Weight Harvested

Subject	Region	Location	Right arm mcg harvested[a]	Region	Location	Left arm mcg harvested[a]	mcg harvested[a] both arms
1	I	inner	236.1	III	inner	366.1	
		outer	428.6		outer	208.9	
	II	inner	336.8	IV	inner	233.1	
		outer	415.0		outer	282.7	
	mean		354.1			272.7	313.4
	SD		88.5			69.4	85.5
	CV%		25.0%			25.5%	27.3%
2	I	inner	302.4	III	inner	275.6	
		outer	260.3		outer	181.5	
	II	inner	266.1	IV	inner	230.4	
		outer	310.9		outer	205.5	
	mean		284.9			223.2	254.1
	SD		25.4			40.2	45.4
	CV%		8.9%			18.0%	17.9%
3	I	inner	235.9	III	inner	290.1	
		outer	175.4		outer	242.6	
	III	inner	269.0	IV	inner	280.2	
		outer	110.8		outer	201.7	
	mean		197.8			253.7	225.7
	SD		69.7			40.2	60.6
	CV%		35.3%			15.9%	23.8%

[a] as weighed on a Sartorius 4504 MP8-1 Ultramicro scale, with sensitivity to 0.1 mcg.
[b] $P < 0.05$ ANOVA (Fishers PLSD) at sites within an arm.
[c] $P < 0.05$ ANOVA (Fishers PLSD) at right vs left arms.
NA = not available or not applicable.

Dermatopharmacokinetic Study

The mean dermatopharmacokinetic profiles of the two miconazole nitrate vaginal cream 2% products are shown in Fig. 2. Mean dermatopharmacokinetic profiles for the test product were significantly less than ($P < 0.05$) the reference product at all time points of assessment except the 4.0 h Data demonstrate a pseudo-steady state of miconazole nitrate uptake achieved within 4 h of product application and an elimination half-life of 7 h consistent with a two compartment, open elimination model (24). Median T_{max} of the test and reference products in human volar forearm skin was 3 h and 2 h, respectively confirming that both products achieve maximal uptake into the same stratum corneum at a similar time after product application.

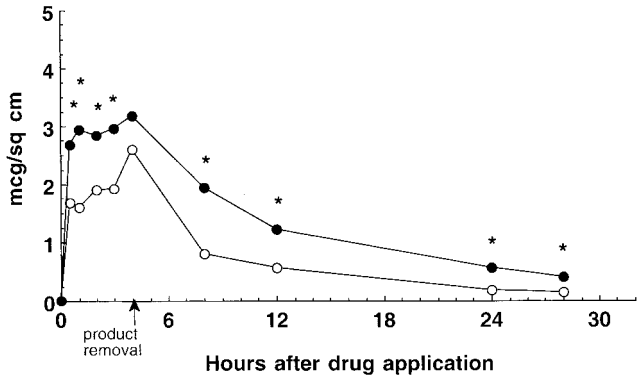

Fig. 2. Mean Untransformed Miconazole Nitrate Dermatopharmacokinetic Profiles Of Test And Reference Vaginal Cream, 2% Products. (●) = reference product, (○) = generic product. Residual product(s) removed at 4 h. Mean of n = 24 subjects, *$P < 0.05$ miconazole nitrate content in reference vs. test products.

Bioequivalence Assessment of Test and Reference Products

The ratio of geometric means of log-transformed C_{max} for the test-to-reference products was 0.666 and demonstrated a statistically significant difference ($P < 0.05$)(Table III). The 90% confidence interval was 54.0%–79.2%, which is outside the proposed acceptance criteria (80.0%–125.0%) for documentation of bioequivalence between dermatological

Table III. Statistical Evaluation Of Log-Transformed Miconazole Nitrate Dermatopharmacokinetic Parameters in N = 24 Subjects

	Test[a]	Reference[a]	Ratio[c]	90% Confidence interval[b] Lower	Upper
C_{max}	3.023	4.537	0.666[f]	54.0%	79.2%
T_{max}[d]	3.000	2.000	1.000	NA	NA
AUC[e]					
0–4	7.460	10.924	0.655[f]	57.6%	73.5%
0–t	21.864	41.791	0.523[f]	40.2%	64.5%

[a] Least-squares geometric means for log-transformed data.
[b] Confidence interval on the ratio.
[c] Ratio calculated as Test product least-squares mean divided by reference product least-squares mean.
[d] median.
[e] Area under the miconazole nitrate content-time curve.
[f] Comparison was detected as statistically significant by ANOVA ($\alpha = 0.05$).
NA = not applicable.

drug products (7). Therefore, these two products would not be considered to be bioequivalent based on the C_{max} parameters.

The ratio of population geometric means of the test-to-reference miconazole nitrate vaginal cream, 2% products for $AUC_{0\text{-}4}$ and $AUC_{0\text{-}t}$ were 0.655 and 0.523, respectively. The 90% confidence intervals for the log-transformed $AUC_{0\text{-}4}$ and $AUC_{0\text{-}t}$ were outside the proposed acceptance criteria (80–125%) for bioequivalence and reflect the inferior miconazole nitrate contents attained with the test product compared with the reference product. These two miconazole nitrate vaginal cream, 2% products are therefore not bioequivalent using the dermatopharmacokinetic method.

Bioassay

The bioassay statistically discriminates between paired harvested stratum corneum samples that have miconazole nitrate content differences greater than ± 1.25 mcg / sq cm. The majority of paired samples collected at the various time points in the dermatopharmacokinetic profile have Test-Reference (T-R) miconazole nitrate content values less than ± 1.25 mcg / sq cm and therefore, do not demonstrate any statistical significance between the products. Thus, while the dermatopharmacokinetic method demonstrates significant differences between the miconazole nitrate vaginal cream, 2% products, the bioassay does not.

Comparative Clinical Trials

After 7 days of twice daily treatment, 92% and 87% of the enrolled women with positive signs and symptoms of vaginitis demonstrated clinical cure with the test and reference miconazole nitrate vaginal cream, 2% products, respectively (Table IV). Ninety-six percent of the enrolled women showed mycological cure with both products on day 7. The 90% confidence interval on clinical and mycological cure on day 7 was 93%–117% and 91–107%, respectively. Both 90% confidence intervals were within the proposed acceptance criteria (80–125%) for bioequivalence and therefore support bioequivalence of the two miconazole nitrate vaginal cream, 2% products.

Thirty days after cessation of therapy (Day 37), 86% and 84% of the enrolled women continued to demonstrated clinical cure with the test and reference products, respectively, with a 90% confidence interval for bioequivalence of 89%–116%. Mycological cure was achieved in 86% and 78% of the women treated with the test and reference products, respectively, with a 90% confidence interval for bioequivalence of 94%–122%. Therapeutic cure with the test product was 76%, while the reference product was 67% producing a 90% confidence interval of 93%–126%. The 90% confidence interval for clinical cure and mycological cure at both 7 and 37 days are within the proposed 80–125% acceptance criteria for bioequivalence. The 90% confidence interval for therapeutic cure (93%–126%) was just outside the upper limit of the 80–125% acceptance criteria for bioequivalence, but accepted as evidence for bioequivalence in conjunction with the other clinical endpoints. Thus, by the comparative clinical trials trial method, the two miconazole nitrate vaginal cream, 2% products are bioequivalent.

DISCUSSION

Recently, a draft guidance was issued by the Food and Drug Administration (7) which describes a pharmacokinetic approach in which drug content in the rate limiting barrier to skin absorption is quantified. This method uses adhesive discs to harvest stratum corneum, also known as skin stripping. Quantification of drug in harvested stratum corneum samples from product-treated skin sites over various periods of time is used to characterize the uptake / elimination profiles of the applied drug thereby assessing the bioequivalence of two products. The current study evaluated whether bioequivalence assessment between two miconazole nitrate vaginal cream, 2% products using the dermatopharmacokinetic method would concur with the assessment from a previously evaluated comparative clinical trial.

The dermatopharmacokinetic test product demonstrated that the two products were not bioequivalent as a function of C_{max} and $AUC_{0\text{-}t}$ parameters. In contrast, the comparative clinical trial method found the same products bioequivalent, as a function of clinical and mycological cure. Discordance in product bioequivalence assessment by the two methods likely reflects differences in study design, single and multiple doses, tissue barrier function, presence / absence of mucus, healthy vs. diseased tissue, subjective vs. objective assessments of drug activity / concentration, as well as, the sensitivity and dose responsiveness of the detection method used. The dermatopharmacokinetic method evaluated both products as a single dose simultaneously in healthy subject forearm skin, while the clinical trial method evaluated each product in independent groups of vaginal candidiasis patients using a multiple dosing regimen over 1 week, and follow up assessment 4 weeks later. Parameters used for assessing bioequivalence were largely subjective in the comparative clinical trial method, but objective in the dermatopharmacokinetic method. Despite many similarities between vaginal and skin tissue (10–15), drug uptake into vaginal tissue may be addi-

Table IV. Comparative Clinical Trial Bioequivalence Study

Event	Parameter	Test product[a]	Percent responders	Reference product[a]	Percent responders	90% CI[b]
Day 7	Clinical cure	47/51	92%	48/55	87%	93%–117%
	Mycological cure	49/51	96%	53/55	96%	92%–107%
Day 37	Clinical cure	44/51	86%	46/55	84%	89%–116%
	Mycological cure	44/51	86%	43/55	78%	94%–122%
	Therapeutic cure	39/51	76%	37/55	67%	93%–126%

[a] # cures/total subjects evaluated.
[b] 90% confidence interval calculated with $\alpha = 0.05$.

tionally complicated by the changing chemical composition, histology, and the presence of mucus in the vaginal tissue throughout the estrus cycle (11–13,20).

It is imperative to establish the limits of detection, variability and the linearity of the dose response relationship for any analytical method used to assess bioequivalence, While these parameters are easily determined in the analytical assay used to quantify miconazole nitrate in stratum corneum, they are not generally established using the comparative clinical trial method. The critically relevant question "how much different can two products be and still be "bioequivalent", remains elusive due to the lack of information on these issues in the comparative clinical trial method and the paucity of pharmacokinetic / pharmacodynamic relationships with topical products.

Ideally, the bioequivalence method used should be sensitive to differences in the product chemical composition. Evaluation of the vehicle composition of the two current products reveals that they are qualitatively (Q1) and quantitatively (Q2) different (7). Chemical composition differences between the products produced statistically different drug content profiles in human stratum corneum by the dermatopharmacokinetic method, but not the clinical trial method. The mechanistic basis of those differences may reflect the histologic, chemical composition and therefore barrier permeability differences in the two tissues, as well as the linear vs. curvilinear dose responses of the pharmacokinetic vs. bioassay methods.

In a curvilinear, or E_{max} dose response model common to many physiological systems, increasing the drug concentration produces increasing responses up to a maximum concentration, after which increasing the concentration further produces no additional response. The specific growth inhibition bioassay evaluating miconazole nitrate activity against *Candida albicans* growth demonstrated that drug concentration must differ by ±1.25 mcg/sq cm in the harvested stratum corneum samples to demonstrate significant growth inhibition differences in the bioassay. The mean difference in C_{max} values for the two products in human stratum corneum however, was less than 1.25 mcg/sq cm and therefore would not produce significantly different responses. The bioassay data support the conclusions of the comparative clinical trial, in which the test and reference products were determined to be bioequivalent. The bioassay and likely the diseased subject models have limited dose response ranges for miconazole nitrate and therefore lack sensitivity to discriminate between drug concentrations. Thus, bioequivalence between two products can be attained pharmacodynamically in the current bioassay and likely the clinical efficacy study by merely delivering excess drug to the target tissue. In contrast, the dermatopharmacokinetic method was able to discriminate between the drug products that differ qualitatively (Q1) and quantitatively (Q2) in vehicle composition due to its dose responsiveness over a larger concentration range. Thus, while the dermatopharmacokinetic method may fail some products that would otherwise pass a bioequivalence assessment in a clinical efficacy trial, it errors on the side of consumer and regulatory confidence that two products are indeed bioequivalent by the most discriminating method. Thus, the dermatopharmacokinetic method may be beneficial in providing a mechanistic understanding of bioequivalence and in some cases, bioavailability issues concerning locally acting drug products targeted to vaginal tissue.

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