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In Vivo Pharmacokinetics and Pharmacodynamics of Topical Ketoconazole and Miconazole in Human Stratum Corneum

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A direct study evaluating whether differential drug uptake of topical 2% miconazole and 2% ketoconazole from cream formulations into human stratum corneum correlated with differential pharmacological activity against *Candida albicans* was investigated in healthy human subjects. A single 24-h topical dose of 2% ketoconazole cream or 2% miconazole cream was applied unoccluded, at the same dose (2.6 mg of formulation per cm^2 of surface area), at four skin sites on both ventral forearms of six human subjects. At the end of the treatment, residual drug was removed with a tissue from all sites and the treated site was tape stripped 11 times, either 1, 4, 8, or 24 h later. The first tape disc was discarded. The remaining tape discs, 2 through 11, were combined and extracted for drug quantification by high-performance liquid chromatography and bioactivity against *C. albicans* growth in vitro. Topical 2% ketoconazole produced 14-, 10-, and 7-fold greater drug concentrations in stratum corneum than 2% miconazole at 1, 4, and 8 h after a single topical dose. Ketoconazole and miconazole concentrations in the stratum corneum were similar 24 h after drug removal. Tape disc extracts from 2% ketoconazole-treated skin sites demonstrated significantly greater bioactivity in the bioassay than 2% miconazole. The increased efficacy of 2% ketoconazole compared with that of 2% miconazole in vitro reflects their differential uptake into the stratum corneum and inherent pharmacological activity. Tape stripping the drug-treated site in conjunction with a bioassay is therefore a useful approach in the determination of bioavailability of topical antifungal agents.

Effective therapy of cutaneous disease requires that the active agent is delivered to the site of infection in adequate concentrations to produce a pharmacological effect. In the case of superficial dermatophyte infections, where the pathogen resides on or within the outermost layer of the skin, the antifungal therapeutic agent must be delivered to the stratum corneum in adequate concentrations to inhibit the growth of the fungal pathogen. A number of imidazole-derivative drugs have wide-spectrum activity against dermatophyte and yeast growth in culture (9, 15, 17). The principal mode of imidazole drug action is located at the level of the cell membrane (6, 27). Imidazoles inhibit cytochrome P-450-dependent lanosterol C_{14} demethylase, which is responsible for the production of ergosterol, a necessary component in fungal cell wall synthesis (1, 2). Changes in the cell membrane sterols may also affect the action of the membrane-bound enzymes, such as chitin synthase, thus ultimately slowing fungal cell growth (3).

Imidazole drug therapy is commercially available for a variety of routes of administration: parenteral, intravenous oral, and topical. The various imidazoles and routes of administration, however, have different indications and toxicities depending on the severity and type of dermatophyte infection. Two commercially available topical imidazole creams, 2% ketoconazole applied once a day and 2% miconazole applied twice a day, are indicated for superficial dermatophyte infections, including tinea corporis, tinea cruris, and tinea pedis caused by *Trichophyton* spp. and tinea versicolor caused by *Malassezia furfur*, as well as cutaneous candidiasis. The therapeutic efficacies in humans of topical miconazole versus topical ketoconazole have not been evaluated by direct comparison. MICs of miconazole necessary to inhibit dermatophytes and *Candida albicans* in vitro range from 0.2 to 10 μg/ml (14, 15), while the MIC of ketoconazole has been documented to be 100-fold less (0.005 to 0.48 μg/ml) in Eagle's minimal essential medium (15). In vitro susceptibility tests have been demonstrated, however, to reflect in vivo efficacy poorly (4, 12, 20, 25, 29). MICs obtained in vitro vary substantially, however, depending on the experimental conditions applied: the type of medium, temperature, presence or absence of serum, inoculum size, phase of fungal growth, and duration of incubation (19). The poor correlation between the model systems has been most rigorously evaluated with the oral or parenteral routes of administration but not with topical imidazole administration. Further, the previous in vitro MIC methods did not evaluate the drug activity in the target tissue, the skin, but rather with pure standards, which may not reflect the realities of that drug in the biological tissue. The use of clinical end points of efficacy without confirming the presence of drug at the target site in the human subjects might also produce poor correlations between the two model systems.

The current study was performed to quantitate the uptake and elimination of topical miconazole and ketoconazole into healthy human stratum corneum and investigate whether a differential drug content in stratum corneum would correlate with the differential bioactivity of the respective drugs in a quantitative in vitro bioassay.

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MATERIALS AND METHODS

Materials. A 2% miconazole cream (Ortho Pharmaceutical Corp., Dermatological Division, Raritan, N.J.) and 2% ketoconazole cream (Janssen Pharmaceutica Inc., Piscataway,

N.J.) were used as purchased from the University of Utah Health Sciences Center Pharmacy. Tape discs (1.4-cm diameter) were generated from a roll of Transpore tape (3M, St. Paul, Minn.) by using a number 8 cork borer. Degassed high-performance liquid chromatography (HPLC)-grade acetonitrile was purchased from EM Science Omnisolv (Van Walters Rogers). The 0.01 M monobasic phosphate buffer (Mallinckrodt Chemical Works, St. Louis, Mo.) was prepared in the laboratory. Miconazole nitrate and ketoconazole were used as purchased from Sigma (St. Louis, Mo.).

Human subject population. Five human subjects, three females and two males, ages 21 to 33 years, were tested in the topical 2% ketoconazole study. Seven human subjects, five females and two males, ages 21 to 48 years, five of whom were used in the topical ketoconazole study, were used in the topical 2% miconazole study. Both studies were approved by the University of Utah Institutional Review Board and were performed 3 months apart. Exclusion criteria for human subjects enrolled in either study included previous use of antimycotics or any other topical drugs for 3 months prior to the study and asthma or a previous history of allergy to sulfa drugs. Subjects were remunerated for their participation in the studies.

Application of the commercial formulation to human skin. One-milliliter disposable tuberculin syringes, without needles, were loaded with the respective commercial formulations.

A single 24-h topical application of commercial topical 2% ketoconazole cream or commercial topical 2% miconazole was applied unoccluded, at the same dose (2.6 μl of formulation per cm^2 of surface area), on four 3.8-cm^2 skin sites on both forearms of six human subjects. All demarked sites were located within the region on the ventral forearm that was 3 cm above the wrist and 3 cm below the antecubital fossa. Treatment sites were located on the skin surface such that demarked sites were 2 cm (center to center) apart. Ten microliters of formulation was dispensed from a previously loaded and centrifuged 1-ml tuberculin syringe to the demarked sites on the ventral forearm. The formulation was gently spread evenly over the 3.8-cm^2 area with a blunt conically tipped polypropylene microcentrifuge tube, such that >97% remained at the skin site. Protective but nonoccluding tape guards (23) were subsequently applied over the treated sites for the duration of the 24-h treatment period. The tape guards were prepared in our laboratory with a 2.2-cm-internal-diameter rubber O-ring (3 mm high) covered with a piece of single-ply open weave cotton gauze (3.5 by 3.5 cm) and aligned over a 2-cm-diameter hole in the midsection of a piece of paper tape (5.4 by 16 cm). To this assembly was attached, adhesive side to adhesive side, a smaller piece (4 by 4 cm) of paper tape (Johnson and Johnson, Inc.). In this way, the larger tape piece held the O-ring assembly in alignment over the drug-treated site, allowing air circulation to the drug-treated skin site and preventing occlusion. The smaller tape applied to the underside of the tape guard prevented the adhesion of the tape guard to the area surrounding the drug-treated site. Tape guards were prepared 24 h prior to the study and stored attached to wax paper.

Skin stripping the treated skin site. At the end of the dosing interval (24 h), the protective tape guard was removed. Residual drug on the skin surface was removed with a clean tissue from all treated sites. Each treated skin site was subsequently tape stripped with either 1.4-cm (miconazole)- or 0.8-cm (ketoconazole)-diameter tape discs 11 times, either 1, 4, 8, or 24 h after drug removal. In this tape-stripping procedure, a tape disc is applied to the center of the drug-treated skin site with a forceps. The round blunt end of the forceps is used to apply even pressure across the tape disc to increase tape tenacity to the skin surface. The tape disc is then removed from the skin surface with the forceps, removing a layer of about 100 μg of stratum corneum per cm^2 (24). Each subsequent tape disc applied to the same skin site removes another skin layer of 100 $\mu g/cm^2$. This tape-stripping procedure has been validated previously for consistent removal of stratum corneum weight per surface area of the tape disc with each sequential 10 tape strips (24). Accumulative stratum corneum weight removed with 10 sequential tape discs increases linearly with an increasing number of tape strippings. Further, increased stratum corneum weight removed is linearly related to number of stratum corneum cell layers removed with light microscopy. Previous data demonstrate that this skin-stripping technique removes >80% of the stratum corneum (23). The amount of stratum corneum removed in this manner is reproducible within a given subject regardless of vehicle type applied (23, 24). Data were therefore normalized as the amount of drug per surface area of the tape disc (micrograms per square centimeter). An untreated control site was also tape stripped from each individual and analyzed for possible skin interferences with the respective drugs. Larger diameter tape discs were utilized for the miconazole-treated sites to increase the amount of tissue collected, thereby improving the analytical sensitivity of drug quantification.

This method for quantifying drug uptake into treated stratum corneum following topical drug application has been used to differentiate among various vehicle formulations of a topical corticosteroid, betamethasone dipropionate (23, 24). In addition, those studies demonstrated that drug uptake into treated ventral forearm skin human stratum corneum correlates with drug pharmacological activity (skin blanching response) at that site (23). Of the 11 tape discs used in the tape stripping, the first tape disc was discarded because of potential contamination of residual drug on the skin surface. The remaining tape discs, 2 to 11, were combined into a 1.5-ml polypropylene microcentrifuge tube and subsequently chemically extracted for drug quantification by HPLC.

Chromatography and tape strip extraction. Combined skin strippings in the capped 1.5-ml polypropylene microcentrifuge tubes were extracted with 500 μl of the 80:20 (vol/vol) acetonitrile–0.01 M KH_2PO_4 mobile phase for miconazole samples and 250 μl of the 65:35 (vol/vol) acetonitrile–0.01 M K_2HPO_4 (pH 6.0) mobile phase for ketoconazole. All samples were vortexed at high speed for 1 min to extract the drug from the tape discs. The tubes were subsequently centrifuged (Biofuge A; Baxter) at 8,000 rpm for 10 min. Thirty microliters of the extract was submitted to the in vitro bioassay, and the remainder was transferred directly into a 200-μl glass Polyspring insert (National Scientific, Lawrenceville, Ga.) and placed in a prelabeled 1.5-ml autosampler vial (National Scientific). Forty microliters of the extracts was injected via autosampler (Spectra Physics SP8000) onto an isocratic HPLC (Beckman model 234) fitted with a 5-μm reverse-phase C_{18} column (4.6 by 12.5 cm; Whatman). Analysis of the drugs utilized a flow rate of 0.7 ml/min with their respective mobile phases to provide retention times of 5.5 min for miconazole and 8.6 min for ketoconazole. Detection of miconazole was optimal at a 214-nm fixed UV wavelength, while ketoconazole was detected at a 254-nm UV wavelength. Extraction efficiency of the two antifungal drugs from the tape discs with a single extraction was >85%. Unknown ketoconazole and micon-

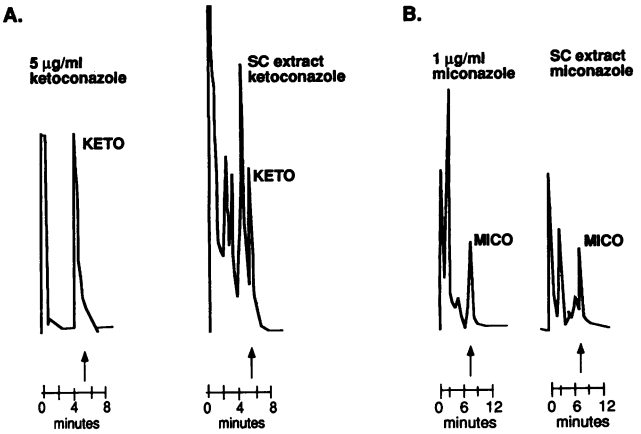

FIG. 1. (A) HPLC of a ketoconazole standard (5 μg/ml) and 10 extracted tape discs from human ventral forearm skin treated 24 h unoccluded with topical 2% ketoconazole. Retention time of ketoconazole (KETO) is 4.6 min. (B) HPLC of a miconazole standard (1 μg/ml) and 10 extracted tape discs from human ventral forearm skin treated 24 h unoccluded with a single topical application of 2% miconazole cream. Retention time of miconazole (MICO) is 8.6 min.

azole concentrations in the tape strip extracts were calculated against known standards via the method of area under the concentration-time curve. Both methods provided good interrun linearity of miconazole (r^2 = 0.9931 ± 0.0032 [mean ± standard deviation]; n = 9) and ketoconazole (r^2 = 0.9950 ± 0.0020; n = 10) over the concentration range of 0.05 to 25.0 μg/ml using a detector sensitivity of 0.001 absorbance unit, full scale. The intrarun coefficient of variation for both imidazole standard curves (0.0.5 to 25 μg/ml) was <5%. The interrun coefficients of variation for ketoconazole and miconazole over the concentration range 0.05 to 25 μg/ml were 3.2 and 2%, respectively. Assay sensitivity limitations for ketoconazole and miconazole were 0.05 μg/ml or 2 ng on the column.

Tape interference with the drug was noted in some of the tape-stripping samples. Ten blank tape discs were therefore also extracted and analyzed in the same manner with each HPLC run so that correction of the tape disc interferences from the drug could be performed when necessary. Extracted blank tape discs and drug-treated stratum corneum with tape discs are shown in Fig. 1A and B for ketoconazole and miconazole, respectively.

In vitro bioassay. Drug activity in known standards or extracts of the skin strippings collected from the drug-treated skin sites was quantitated with a growth inhibition assay in vitro using *C. albicans* (ATCC 36232). Thirty microliters of each extracted skin stripping or known tape disc standard was pipetted onto a 6-mm-diameter blank paper disc (TAXO discs; VWR) standing on its edge in a V-shaped 96-well microtiter plate and allowed to air dry at room temperature (25°C). Five hundred microliters of a medium suspension of *C. albicans* in YM broth (Difco, Detroit, Mich.) was pipetted onto the 60-mm Sabouraud dextrose agar plate (MicroBio Products, Inc., Tucson, Ariz.) and spread evenly over the surface area with a glass hockey stick, providing a dense confluent growth of yeast within 48 h. Immediately following inoculation of the yeast on the agar plates, an air dried paper disc previously loaded with 30 μl of a known drug standard solution or tape disc extract was placed on one quadrant of an inoculated plate. Each inoculated plate therefore contained one known extracted tape disc drug standard for quality assurance and three extracted unknown tape disc samples from the human subjects. Limiting the maximum number of samples analyzed per agar plate to four was necessary to adequately measure the zone of inhibition associated with high drug concentrations while maintaining a minimum of 0.5 cm between those large zones from different samples. Each tape strip sample collected was analyzed once. A total of five samples were analyzed per subject (before drug treatment and 1, 4, 8, and 24 h after removal of the drug).

Drug activity was quantitated as the average diameter (centimeters) of the zone of growth inhibition of *C. albicans* measured in two directions 48 h after inoculation. The zone of inhibition measured in this assay was that zone, edge to edge across the disc, in which the otherwise dense confluent growth was inhibited. In most cases, this was visualized by a complete clearing in that area, with dense yeast growth around the zone. In some extracted samples, however, a few (<10) small isolated colonies of yeast growth were present within the cleared area with both ketoconazole and miconazole. The location and distribution of those isolated colonies were random and were subsequently ignored. All measurements of the zone of growth inhibition were therefore collected from the gross edges of the cleared growth areas. The zone of growth inhibition was measured in two directions to check for nonconcentric growth patterns that might suggest inadequate culture growth conditions or inappropriate placement of the paper disc impregnated with drug extracted from the tissue-tape disc samples. The zone of inhibition in the top-bottom direction was 85 to 110% of that measured in the left-right direction for all drug concentrations. For example, a 1-μg ketoconazole standard produced a zone of inhibition of 2.1 cm in the top-bottom direction but a 1.9-cm zone in the left-right direction. Those measurements are 85 to 110% of each other, the average being 2.0. The average value was used as the datum point for the sample. All data were normalized to that zone associated with 0 μg/ml.

Statistical analysis. Statistical analyses of the data were performed with StatView II (Abacus Concepts, Casabassa, Calif.) using one-way analysis of variance and the nonparametric Mann-Whitney U test for unpaired data and the Wilcoxon signed rank test for paired data.

RESULTS

Drug activity in the growth inhibition assay. Drug activities of various concentrations of ketoconazole and miconazole extracted from 10 spiked blank tape strips (0.01 to 1 μg) in a growth inhibition assay of *C. albicans* in vitro are shown in Fig. 2. Drug concentrations less than 0.01 μg were not detected in the bioassay. The relationship between ketoconazole concentration (micrograms) and drug activity (zone of growth inhibition in centimeters) against *C. albicans* fit a logarithmic equation, $y = 2.59 + 0.62*\ln(X)$. Drug concentrations greater than 1 μg produced zones of inhibition similar to that of the 1-μg standard. Maximal activity in this assay was 2.6 cm, and the concentration at which 50% of the drug activity was demonstrated was 0.124 μg. The relationship between miconazole concentration and drug activity also fit a logarithmic equation, $y = 1.9 + 0.45*\ln(X)$. Maximal miconazole activity in this assay was 1.9 cm, and the concentration at which 50% of the drug activity was demonstrated was 0.12 μg.

Pharmacokinetics of topical ketoconazole and miconazole in

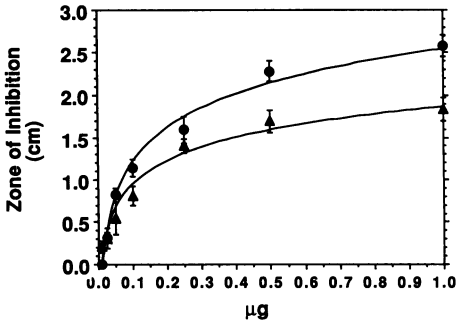

FIG. 2. Ketoconazole and miconazole dose response curve in an in vitro bioassay of *C. albicans* growth inhibition. Filled circle, ketoconazole [$y = 2.59 + 0.62*\ln(X)$], mean ± SEM for $n = 17$; filled triangle, miconazole [$y = 1.9 + 0.45*\ln(X)$], mean ± SEM for $n = 6$.

human skin in vivo. Application of a single unoccluded dermatological dose (2.6 μl of formulation per cm^2) of commercial 2% ketoconazole or 2% miconazole to the ventral surface of the forearms of human subjects for 24 h produced 14-, 10-, and 7-fold greater ($P < 0.05$) stratum corneum ketoconazole concentrations than miconazole concentrations at 1, 4, and 8 h after drug removal, respectively (Fig. 3, solid lines). Ketoconazole concentrations in the treated stratum corneum decreased linearly ($r = -0.97$) from 1 to 8 h after drug removal at a rate of ~2 $\mu g/cm^2/h$. Miconazole concentrations in the rate-limiting barrier did not significantly change over the same period.

Pharmacodynamic activity of topical antifungal agents in drug-treated extracted human skin. Drug activity from the extracted tape discs of in vivo ketoconazole-treated and miconazole-treated human skin collected 1, 4, 8, and 24 h after drug removal of a single unoccluded dose produced bioactivity against the growth of *C. albicans* in vitro (shown as the dashed lines in Fig. 3A and B, respectively). Bioactivity (centimeters) in the extracted tape discs collected from the 2% ketoconazole-treated ventral forearm skin was 10-fold greater ($P < 0.02$) than 2% miconazole-treated skin at all time points investigated. Ketoconazole activity in the extracted human stratum corneum tape strippings decreased only 1.5-fold ($P < 0.05$) from 1 to 24 h (1.73 ± 0.09 to 1.16 ± 0.34 cm [mean ± standard error of the mean {SEM}], respectively). In contrast, miconazole activity in the extracted human stratum corneum tape strippings decreased eightfold ($P < 0.05$), from 0.25 ± 0.10 to 0.03 ± 0.04 cm, over the same period.

Increasing ketoconazole uptake to greater than 7 $\mu g/cm^2$ of surface area (0.55 μg in the bioassay) did not further increase the pharmacological activity in the bioassay ($P < 0.05$). For example, ketoconazole bioactivities in the tape-stripped treated drug sites were similar at 1, 4, and 8 h after drug removal (1.96 ± 0.27, 1.71 ± 0.2, and 1.67 ± 0.16 cm, respectively) despite differences in drug content in the treated ventral forearm skin tape strips (21.5 ± 11.6, 12.2 ± 5.4, and 7.4 ± 2.8 $\mu g/cm^2$, respectively). In contrast, miconazole bioactivity decreased 10-fold from 1 to 24 h (0.25 ± 0.1 and 0.3 ± 0.4 cm, respectively) despite similar drug contents in the extracted tape strips (1.49 ± 0.15 and 1.58 ± 0.15 $\mu g/cm^2$, respectively).

The relative potency of the two imidazole drugs in human stratum corneum against yeast growth in vitro can be calculated from the ratio of bioactivity and amount of drug in the tape-stripped stratum corneum (centimeters per microgram) (Fig. 4). Topical 2% ketoconazole produced a constant bioactivity-per-microgram of drug ratio of ~5.5 from 1 to 24 h after removal of a single dose. In contrast, topical 2% miconazole produced a bioactivity-per-microgram of drug ratio that decreased from 1.73 ± 0.58 (mean ± SEM; $n = 7$)

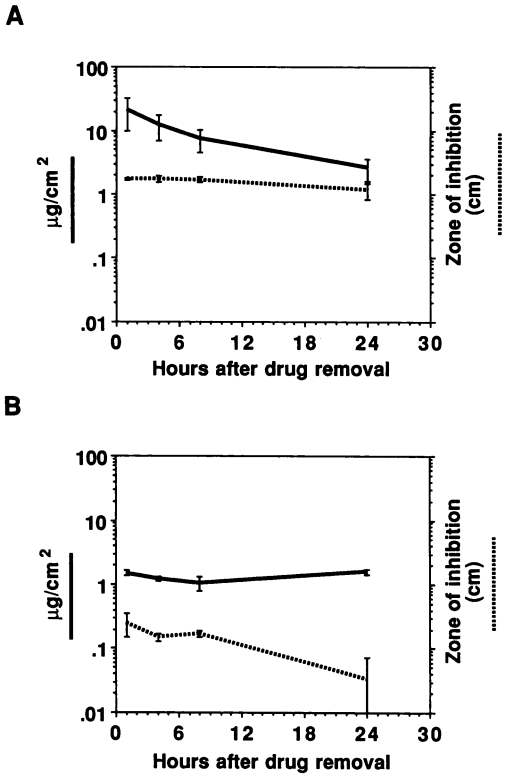

FIG. 3. (A) Ketoconazole content and bioactivity in 10 tape discs collected 1, 4, 8, and 24 h after drug removal of a single topical unoccluded application of 2% ketoconazole cream applied for 24 h to the human ventral forearm. Solid line, drug content; dashed line, bioactivity. Values are means ± SEM; $n = 5$. (B) Miconazole content and bioactivity in 10 tape discs collected 1, 4, 8, and 24 h after drug removal of a single topical unoccluded application of 2% miconazole cream applied for 24 h to the human ventral forearm. Solid line, drug content; dashed line, bioactivity. Values are means ± SEM; $n = 7$.

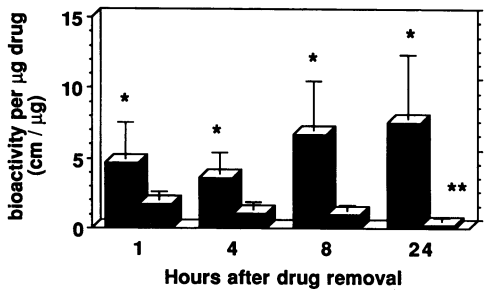

FIG. 4. Relative bioactivity per microgram of drug of ketoconazole versus miconazole in the extracted tape discs collected from the treated ventral forearm skin over time. Solid bar, ketoconazole ($n = 5$); hatched bar, miconazole ($n = 7$). Values are means + SEM. *, $P < 0.05$ from miconazole; **, $P < 0.05$ 1 h after removal of miconazole.

to 0.24 ± 0.22 from 1 to 24 h after drug removal, respectively.

DISCUSSION

Mechanisms of failed topical drug therapy often are not understood clinically. Effective topical drug therapy requires drug uptake into skin at sufficient concentrations over a particular period of time for maximal pharmacological activity. Differential efficacy may result from inadequate drug delivery to the skin, rapid elimination from the skin, or the inherently weak pharmacological activity of a particular drug within the skin. The current study was designed to investigate whether two drugs from the same class of therapeutic agents, imidazole antifungal agents, commonly prescribed for the treatment of tinea pedis, could be differentiated in terms of drug uptake into the skin and/or drug activity against a susceptible strain of yeast.

Both ketoconazole and miconazole demonstrate growth inhibition activity against *C. albicans* in vitro in a dose-dependent manner with the use of known spiked extracted tape disc standards. Overall, maximal activity of ketoconazole was 1.5-fold greater ($P < 0.05$) than miconazole, demonstrating the inherent differential pharmacological activities between these imidazoles. However, the concentration at which 50% of drug activity was demonstrated was similar for both imidazoles at approximately 0.12 μg in the bioassay or 0.5 μg/cm^2 of skin surface area. These data demonstrate that while miconazole may be equipotent to ketoconazole at concentrations less than 0.25 μg, it is significantly less potent at concentrations greater than 0.5 μg in the bioassay. This differential pharmacological activity of the two imidazoles offers an avenue by which to further investigate the mechanisms of altered efficacy with these drugs in terms of inherent pharmacological activity as well as the inadequate drug delivery to the skin.

The skin-stripping method developed in our laboratory offers an objective and relatively noninvasive evaluation of drug uptake into human stratum corneum in vivo. The skin-stripping method is relatively noninvasive, removing only the stratified anucleate epidermis, and is reproducible (25 to 30% coefficient of variation) within an individual day to day (23). Quantitation of drug uptake into treated human stratum corneum in vivo with the tape-stripping method demonstrates that a single dose of 2% ketoconazole partitions into human stratum corneum 15-fold greater than 2% miconazole. Commensurate with the greater ketoconazole uptake into the stratum corneum than miconazole is an approximately sevenfold greater activity (centimeters) against *C. albicans* growth in vitro. These data also demonstrate that increasing ketoconazole uptake into skin up to 7 μg/cm^2 increases the drug bioactivity at the treatment site. A further increase in stratum corneum drug concentration is not commensurate with bioactivity.

Tape-stripping-treated human stratum corneum at various intervals over time post-drug removal also allows for the quantitation of drug elimination from the skin. While ketoconazole is eliminated from the stratum corneum at a rate of ~2 μg/cm^2/h from 1 through 8 h after drug removal, miconazole concentrations do not change. The prolonged retention of topically applied miconazole in the skin is consistent with previous observations that miconazole remains in the skin after topical application for up to 4 days (5, 14). Whether the constant concentration of miconazole in human stratum corneum represents tissue binding of the drug in skin or the inability of the drug to partition from this skin layer to the deeper epidermis and dermis in human skin has not been previously investigated. Skin disposition of topical 2% miconazole in human skin orthotopically grafted onto nude mice (21) demonstrates, however, that a concentration profile exists through all of the skin layers after a single unoccluded application of topical drug for 24 h, with stratum corneum concentrations fourfold greater than epidermal or dermal concentrations. Miconazole was not eliminated from any of the human skin layers over 24 h post-drug removal (data not shown). These data suggest that the lack of significant drug clearance from any of the skin layers results from tissue binding of the drug in the skin and not the inability of the drug to partition between the skin layers.

Comparison of the relative potencies of the two imidazole drugs, calculated as bioactivity against *C. albicans* per microgram of drug in the stratum corneum (centimeters per microgram), demonstrates a greater potency of ketoconazole than miconazole at all time points of investigation. Topical 2% ketoconazole produced a bioactivity-per-microgram of drug ratio of ~5 at 1 h after drug removal and remained constant over the following 24 h despite the 1.5-fold reduction in stratum corneum drug concentration over time. Maximal ketoconazole activity in the bioassay is ~0.5 μg or ~7 μg/cm^2 of skin surface area. Ketoconazole concentrations greater than 7 μg/cm^2, measured at the 1-, 4-, and 8-h time points, do not produce statistically greater bioactivities in the bioassay.

Miconazole demonstrated 2- to 10-fold less potency than ketoconazole at all of the time points of evaluation. Maximal miconazole bioactivity per microgram of drug (0.26 cm/μg) was twofold less than ketoconazole at 1 h and fivefold less at 24 h after drug removal (0.24 cm/μg). The decreasing mean bioactivity of miconazole in human skin over time was not significant ($P > 0.05$); however, constant drug concentrations may reflect increasing tissue binding of the drug in the skin or the generation of an unknown inactive miconazole metabolite(s) that was not chromatographically distinct from the parent compound with the current HPLC methods. Specific or nonspecific tissue binding of miconazole will decrease the amount of free pharmacologically active drug, thereby decreasing bioactivity. While the skin samples are extracted with acetonitrile before submission to the bioassay, which should release tissue-bound drug, it is plausible that some of the drug could remain bound to some cellular or tape adhesive constituent, which may not alter the chromatography but may decrease its activity in the bioassay. The presence of tape adhesive in the extracted tape strip pure standards, however, did not cause any significant difference in bioactivity versus the bioactivity of pure standards. The presence of inactive metabolites that cochromatograph with the parent drug could also result in overestimation of the parent compound and produce less bioactivity. While imidazoles are known to be extensively metabolized systemically and to be hepatic cytochrome P-450 inhibitors (22, 28), which is the basis for many drug interactions (7, 8, 10, 11, 13, 15, 16, 18, 26), neither inactive metabolites nor miconazole-cytochrome P_{450} complexes have been investigated in the skin. Regardless of the metabolic fate of miconazole in the skin, the overall result is a decreased pharmacological activity in the bioassay and a decreasing bioactivity-per-microgram of drug ratio over time compared with ketoconazole. Further investigation of miconazole tissue binding and metabolism in human skin is necessary to elucidate the mechanism for the decreasing miconazole bioactivity measured over time.

In summary, the data demonstrate a pharmacokinetic-

pharmacodynamic relationship with topical miconazole and ketoconazole in human skin. Increasing the amount of imidazole delivered into skin results in an increased drug bioactivity up to its maximal inherent pharmacological activity. The inferior miconazole bioactivity per amount of drug in the stratum corneum compared with that of ketoconazole is the result of decreased uptake into human stratum corneum, its lesser inherent pharmacological activity, and possibly tissue binding of miconazole within the stratum corneum. The good correlation between drug uptake into skin and the resulting bioactivity combined with the dose response of pharmacological activity of miconazole against *C. albicans* in the bioassay suggests that improving miconazole uptake into human stratum corneum should result in better bioactivity and therefore better clinical efficacy. The pharmacokinetic and pharmacodynamic methods presented offer a new more comprehensive approach with which to identify optimized topical vehicle formulations for antifungal delivery to skin and to determine bioequivalence between topical antifungal products.

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REFERENCES

1. **Borgers, M.** 1984. Structural changes in *Candida albicans* after ketoconazole treatment, p. 43–49. *In* B. W. Eliot (ed.), Oral therapy in vaginal candidosis. The Medicine Publishing Foundation, Oxford.
2. **Borgers, M., and J. Van Cutsem.** 1985. Ketoconazole induced morphological changes in yeasts and dermatophytes, p. 51–60. *In* W. Meinhof (ed.), Oral therapy in dermatomycoses: a step forward. The Medicine Publishing Foundation, Oxford.
3. **Borgers, M., H. Van den Bossche, and M. De Brabander.** 1983. The mechanism, of action of the new antimycotic ketoconazole. Am. J. Med. **74**(Suppl. 1B):2–8.
4. **Brass, C., J. Z. Shainhouse, and D. A. Stevens.** 1979. Variability of agar dilution-replicator method of yeast susceptibility testing. Antimicrob. Agents Chemother. **15**:763–768.
5. **Brugmans, J. P., J. M. Van Cutsem, and D. C. Thienpont.** 1970. Treatment of long-term tinea pedis with miconazole. Double-blinded clinical evaluation. Arch. Dermatol. **102**:428–432.
6. **Cohen, J.** 1982. Antifungal chemotherapy. Lancet **ii**:532–537.
7. **Dieperink, H., E. Kemp, P. P. Leyssac, H. Starklint, M. Wanscher, J. Nielsen, K. A. Jorgensen, V. Faber, and H. Flachs.** 1986. Ketoconazole and cyclosporine A: combined effects on rat renal function and on serum and tissue cyclosporine A concentration. Clin. Nephrol. **25**(Suppl. 1):s137–s143.
8. **Dieperink, H., and J. Moller.** 1982. Ketoconazole and cyclosporin. Lancet **ii**:1217.
9. **Dixon, D., S. Shadomy, H. J. Shadomy, A. Espinel-Ingroff, and T. M. Kerkering.** 1978. Comparison of the in vitro antifungal activities of miconazole and a new imidazole, R 41,400. J. Infect. Dis. **138**:245–248.
10. **D'Mello, A. P., M. J. D'Souza, and T. R. Bates.** 1985. Pharmacokinetics of ketoconazole-antipyrine interaction. Lancet **ii**: 209–210.
11. **Engelhard, D., H. R. Stutman, and M. I. Marks.** 1984. Interaction of ketoconazole with rifampicin and isoniazid. N. Engl. J. Med. **311**:1681–1683.
12. **Galgiani, J. N., and D. A. Stevens.** 1976. Antimicrobial susceptibility testing of yeasts: a turbidimetric technique independent of inoculum size. Antimicrob. Agents Chemother. **10**:721–726.
13. **Glynn, A. M., R. L. Slaughter, C. Brass, R. D'Ambrosio, and W. J. Jusko.** 1986. Effects of ketoconazole on methylprednisolone pharmacokinetics and cortisol secretion. Clin. Pharmacol. Ther. **39**:654–659.
14. **Harvey, S. C.** 1980. Antiseptics and disinfectants: fungicides; ectoparasiticides, p. 976. *In* A. G. Gilman, L. S. Goodman, T. W. Rall, and F. Murad (ed.), Goodman and Gilman's the pharmacological basis of therapeutics, 7th ed. Macmillan Publishing Co., Inc., New York.
15. **Heel, R. C., R. N. Brodgen, A. Carmine, P. A. Morley, T. M. Speight, et al.** 1982. Ketoconazole: a review of its therapeutic efficacy in superficial and systemic fungal infections. Drugs **23**:1–36.
16. **Henry, H. L.** 1985. Effect of ketoconazole and miconazole on 25-hydroxyvitamin D3 metabolism by cultured chick kidney cells. J. Steroid Chem. **23**:991–994.
17. **Lefler, E., and D. A. Stevens.** 1984. Inhibition and killing of *Candida albicans* in vitro by five imidazoles in clinical use. Antimicrob. Agents Chemother. **25**:450–454.
18. **Long, E.** 1983. Warfarin-miconazole interaction. Arch. Intern. Med. **143**:2214–2215.
19. **McIntyre, K. A., and J. N. Galgiani.** 1989. In vitro susceptibilities of yeasts to a new antifungal triazole, SCH 39304: effect of test conditions and relation to in vivo efficacy. Antimicrob. Agents Chemother. **33**:1095–1100.
20. **Odds, F. C.** 1980. Laboratory evaluation of antifungal agents: a comparative study of five imidazole derivatives of clinical importance. J. Antimicrob. Chemother. **6**:749–761.
21. **Pershing, L. K., and J. Corlett.** 1991. In vivo cutaneous kinetics of ketoconzaole in human skin following oral and topical administration. J. Invest. Dermatol. **96**:580A.
22. **Pershing, L. K., M. R. Franklin, and J. K. Ritter.** 1986. Clotrimazole. Just another cytochrome P-450 inducer? Pharmacologist (Basel) **28**:240.
23. **Pershing, L. K., L. D. Lambert, V. P. Shah, and S. Y. Lam.** 1992. Variability and correlation of chromameter and tapestripping methods with the visual skin blanching assay in the quantitative assessment of topical 0.05% betamethasone dipropionate bioavailability in humans. Int. J. Pharm. **86**:201–210.
24. **Pershing, L. K., B. S. Silver, G. G. Krueger, V. P. Shah, and J. P. Skelly.** 1992. Feasibility of measuring the bioavailability of topical betamethasone dipropionate in commercial formulations using drug content in skin and a skin blanching assay. Pharm. Res. **9**:45–51.
25. **Richardson, K., R. J. Andrews, M. S. Marriott, M. H. Tarbit, and P. F. Troke.** 1986. Correlation of in vitro and in vivo activity of azole antifungals, p. 147–149. *In* K. Iwata and H. Van den Bossche (ed.), In vitro and in vivo evaluation of antifungal agents. Elsevier Science Publishers, Amsterdam.
26. **Rolan, P. E., A. A. Somogyi, M. J. R. Drew, W. G. Cobain, D. South, et al.** 1983. Phenytoin intoxication during treatment with parenteral miconazole. Br. Med. J. **287**:1760.
27. **Sud, I. J., and D. S. Feingold.** 1981. Mechanisms of action of the antimycotic imidazoles. J. Invest. Dermatol. **76**:438–441.
28. **Testa, B., and P. Jenner.** 1981. Inhibitors of cytochrome P-450s and their mechanism of action. Drug Metab. Rev. **12**:1–117.
29. **Van Cutsem, J., F. Van Gerven, and P. A. J. Janssen.** 1986. The in vitro evaluation of azoles, p. 51–64. *In* K. Iwata and H. Van den Bossche (ed.), Proceedings of a symposium on in vitro and in vivo evaluation of antifungal agents, Tokyo, 19 to 22 June 1985. Elsevier Science Publishers, Amsterdam.