Skin Concentrations and Pharmacokinetics of Posaconazole after Oral Administration

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A randomized, single-center, open-label study of posaconazole (POS) was performed to determine the concentration of POS in the skin of 30 healthy adult human subjects receiving 400 mg POS oral suspension twice daily for 8 days with a high-fat meal. Blood samples for plasma POS level determination were collected at prespecified times on day 1 and day 8. From each subject, two 4-mm skin punch biopsy samples were obtained, one immediately before or after both the first and last doses of POS. A MIC₉₀ value of 250 ng/ml, which encompasses the majority of common dermatophytes, was used to calculate the time above the MIC₉₀ in plasma and skin. On days 1 and 8, POS attained peak plasma concentrations at median times of 8 and 5 h, respectively. On days 1 and 8, POS peak skin concentrations were attained at 12 and 3 h, respectively; peak skin concentrations were produced from a single composite profile. On day 8, POS concentrations in skin and plasma for the entire dosing interval were severalfold higher than the MIC₉₀. POS dosed at 400 mg twice daily per os was well tolerated in healthy subjects. Two subjects reported increased alanine aminotransferase (ALT) levels. The findings of this study demonstrate adequate skin penetration and have certain implications for the treatment of dermatophytic skin and nail infections.

Posaconazole, an extended-spectrum triazole antifungal, is approved in Europe and the United States as prophylaxis for invasive fungal infection (IFI) in high-risk patients and for the treatment of oropharyngeal candidiasis (15, 16); in Europe, it is also approved for the treatment of refractory IFI (16). Posaconazole has a large apparent volume of distribution (1,774 liters), suggesting extensive extravascular distribution and penetration into body tissues, and a long mean half-life of 35 h (9, 15). Pharmacokinetic data exist for the distribution of posaconazole within plasma, epithelial lining fluid, and alveolar cells (3, 6, 10–12), but there are no clinical data on the bioavailability of posaconazole for skin or its effectiveness against fungal pathogen that affect skin.

Newer azoles, such as itraconazole and fluconazole, have been used to treat superficial infections of the skin, hair, and nails that are caused by dermatophytes (20). Although topical and oral agents are available to treat these fungal infections, oral drugs such as terbinafine (an allylamine) and itraconazole (a triazole) (20) appear to be more efficacious. Posaconazole has potent in vitro activity against dermatophytes from the genera Trichophyton (T. rubrum and T. mentagrophytes), Epidermophyton, and Microsporum, which are primarily associated with dermatophytosis, and against yeast (Candida albicans) (7). Given the antidermatophyte and anticyeast activity of posaconazole in vitro and its characteristic tissue distribution and pharmacokinetic profiles in vivo, it is of clinical interest to determine the concentration of posaconazole in the skin after oral dosing.

The objectives of this study were to compare skin and plasma concentrations of posaconazole obtained after oral administration of 400 mg of posaconazole twice daily and to relate posaconazole levels in skin and plasma to the MIC required to inhibit 90% of relevant pathogenic dermatophytes (MIC₉₀). This is the first report of posaconazole levels in human skin following oral administration.

(Materials and methods)

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Study design. This was a single-center, phase 1, open-label, multiple-dose, randomized study of healthy subjects conducted in accordance with good clinical practices.

Eligible subjects received 400 mg of posaconazole (10 ml of a 40-mg/ml oral suspension) twice daily from day 1 until the morning of day 8, for a total of 15 doses. The subjects were allowed to return home and were instructed to self-administer posaconazole (400 mg) twice daily. The subjects were then allowed to return home and were instructed to self-administer posaconazole (400 mg) twice daily. The subjects returned to the study site on day 7, where they received the last dose of posaconazole on day 1. The subjects were then allowed to return home and were instructed to self-administer posaconazole (400 mg) twice daily. The subjects returned to the study site on day 7, where they received the evening dose of posaconazole, were confined overnight, and received the last dose of posaconazole on the morning of day 8.

Throughout the study, it was recommended that subjects take posaconazole 10 min after finishing a high-fat meal.

Study subjects. Healthy volunteers were screened up to 28 days before study initiation. The subjects were nonsmokers, of either gender and any race, aged 18 to 60 years, with a body mass index (BMI) of 18 to 30 kg/m². Female subjects of childbearing potential and male subjects agreed to use a medically accepted method of contraception while receiving study drugs and for 30 days following therapy; female subjects of childbearing potential were required to have a negative urine pregnancy test result before the first dose. The subjects could not consume alcohol from 48 h before the first dose until the last pharmacokinetic sample was drawn.

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The subjects were excluded if they had a positive result for drugs with a high potential for abuse, a history of drug or alcohol abuse in the past 2 years, or a history of allergy or intolerance to azole antifungal drugs. They were also excluded if they had previously been administered posaconazole. The subjects were not allowed to participate in any other clinical study either during the current study or within 30 days of enrollment. Use of prescription or over-the-counter drugs (other than ibuprofen, acetaminophen, hormone replacement therapy, and oral or injected hormonal contraceptives) was prohibited for a prespecified washout period of 1 to 30 days (depending on the class of drug) before posaconazole administration as well as during the current study. Subjects who tested positive for hepatitis B surface antigen, hepatitis C virus, or human immunodeficiency virus were not permitted in the study.

Before any study-related activities, written informed consent was obtained from all subjects using a study-specific informed consent form. The clinical study protocol and consent form were reviewed and approved before study initiation by the Mid Lands Institutional Review Board, Leawood, KS.

Pharmacokinetic assessment. Blood samples (4 ml each) taken to determine the level of posaconazole in plasma were collected on day 1 (first dose) and on day 8 (last dose) immediately before the dose (0 h) and 3, 5, 8, and 12 h after the dose. Each sample was collected into a prechilled EDTA-containing tube and centrifuged at 4°C at 1,500 x g for approximately 15 min. Plasma was collected into labeled, prechilled cryovials and immediately frozen at −20°C until analysis.

The subjects were randomized to 1 of 5 groups (n = 6 per group) so that 2 skin samples (one each on days 1 and 8) were obtained from subjects at 5 different times. The skin sampling time was determined by group assignment; skin samples were taken either immediately before or 3, 5, 8, or 12 h after the dose on the mornings of days 1 and 8. Skin punch biopsy specimens (4 mm) were taken from an area of the upper arm over the triceps area (or other site with no obvious hair growth) with no local skin lesions after injection with local anesthetic (1% lidocaine with 1:200,000 epinephrine). Skin samples were then placed into labeled, prechilled cryovials and immediately frozen at −20°C. Weighed skin samples (20 to 40 mg) were placed in a clear, screw-cap vial with 100 µl of methanol-water (50:50) and 900 µl of 1.0 M NaOH-isopropl alcohol (70:30) per 10 mg of skin. The vials were sealed with a polytetrafluoroethylene-lined screw cap and vinyl tape and placed in a preheated 70°C reciprocating water bath at approximately 100 rpm. After approximately 1.5 to 2 h, the digested skin was removed and allowed to cool to room temperature before analysis to determine posaconazole levels using the same validated method that was used to analyze plasma samples (17).

Posaconazole plasma and skin concentrations were assayed using a validated liquid chromatography with tandem mass spectrometry (LC-MS-MS) method (17). For plasma and skin, the assays had a lower limit of quantitation of 5.00 ng/ml and 0.250 ng/mg, respectively, a calibration range of 5.00 to 5,000 ng/ml and 0.250 to 100 ng/mg, respectively, precision (coefficient of variation [CV]) of 6.0 to 10.7% and 1.5 to 4.2%, respectively, and accuracy (mean percent difference) of −0.3 to 5.3% and −2.7 to 0.4%, respectively.

Pharmacokinetic noncomparative analyses were conducted using the Pharsight Knowledgebase Server, version 2.0.1 with WinNonlin version 4.0.1 (Pharsight Corporation, Cary, NC). Individual plasma posaconazole concentration-time data were analyzed. Skin concentrations at each collection time were averaged, and the resulting single composite concentration value at each collection time was used to construct a composite concentration-time profile, which was then used to analyze data. The primary pharmacokinetic parameters derived from the posaconazole concentration data for plasma and skin were the maximum observed concentration (Cmax), time to maximum observed concentration (Tmax), area under the concentration-time curve from 0 h to 24 h (AUC0–24), area under the concentration-time curve from 0 h to 12 h (AUC0–12), and trough level immediately before a subject received the last dose (Cmin).

A Cmax value of 250 ng/ml—a value that encompasses the majority of common dermatophytes—was used to calculate the time above MIC90 in plasma and skin (7).

Safety assessment. Safety variables assessed included adverse events, clinical laboratory tests (hematology, clinical chemistry, and urinalysis), vital sign measurements (systolic and diastolic blood pressures, respiration rate, heart rate, and oral body temperature), and electrocardiograms. Adverse events were summarized according to Medical Dictionary for Regulatory Activities (MedDRA) version 10.0 preferred terms with adverse event severity and relationship to the study medication presented. Adverse events were graded according to the National Cancer Institute’s Common Terminology Criteria (CTC) grading system (13) or as mild, moderate, severe, or life threatening for adverse events not covered by the CTC grading scale. The investigator assessed the relationship of any adverse event to study drug use as unlikely related, possibly related, or probably related.

RESULTS

Subjects. Thirty healthy subjects were enrolled in this study to receive 400 mg of posaconazole orally twice daily, and 27 subjects completed the treatment phase. Two subjects were withdrawn from the study because of adverse events, and 1 subject did not wish to continue participating in the study and withdrew from the study. Table 1 shows the demographic characteristics of the subjects. The mean age of the subjects was 37.7 years. Twenty subjects were male, 16 subjects were white, 13 were black or African American, and 1 was Asian.

Pharmacokinetic results. Table 2 provides a summary of posaconazole pharmacokinetic parameters on days 1 and 8. The median posaconazole Tmax values in plasma were 8 h on day 1 and 5 h on day 8. As generated from a single composite profile, the posaconazole Tmax values in skin were 12 h on day...
1 and 3 h on day 8. The AUC ratios for skin to plasma were 0.49 on day 1 and 1.26 on day 8.

On day 8, the mean posaconazole concentrations in plasma and skin were similar (Fig. 1), and both were severalfold higher than the MIC$_{90}$ value of 250 ng/ml for the entire 12-hour dosing interval.

**Safety results.** No deaths or serious adverse events occurred during the study. Seventeen subjects (57%) reported treatment-emergent adverse events (TEAEs) during the study. All TEAEs were mild or moderate, and those reported by more than 1 subject included: pale feces ($n = 3$), fatigue ($n = 3$), headache ($n = 3$), increased alanine aminotransferase (ALT) ($n = 2$), flatulence ($n = 2$), acne ($n = 2$), and allergic rhinitis ($n = 2$). Eleven subjects (37%) had treatment-related TEAEs during the study, all of which were mild or moderate. Treatment-related TEAEs reported by more than 1 subject included: fatigue ($n = 3$), pale feces ($n = 3$), increased ALT ($n = 2$), and acne ($n = 2$). Of the 2 subjects who withdrew because of adverse events: 1 withdrew because of elevated ALT and aspartate aminotransferase (AST), and 1 because of elevated ALT, all of which were considered treatment related. There were no clinically significant changes in any other safety parameters.

**DISCUSSION**

We report the total concentration of posaconazole in skin and plasma, measured after oral administration of 400 mg twice daily, and compare it with the MIC$_{90}$ (250 ng/ml), which encompasses the majority of common dermatophytes.

The findings of this study demonstrated clinically significant skin distribution of posaconazole based on AUC. The posaconazole skin and plasma concentration-time profile was virtually flat, and concentrations remained severalfold above the MIC$_{90}$ value of 60 ng/ml and 250 ng/ml for common infection-causing yeast (C. albicans) and dermatophytes over the entire dosing period (7, 14). A virtually flat plasma concentration-time profile provides sustained levels with minimum fluctuation around the mean. The AUC ratios for skin to plasma of 1.26 on day 8 indicated that after oral administration, skin posaconazole levels similar to the levels in plasma were achieved. In another report, posaconazole treatment was shown to result in sustained pulmonary concentrations in epithelial lining fluid and alveolar cells above the MIC$_{90}$ for Aspergillus spp. (3). The results presented here further support the wide distribution of posaconazole into body tissues. The use of in vivo microdialysis catheters has been reported to provide a more accurate assessment of drug distribution in tissue than tissue biopsy specimens (2); this method was beyond the scope of this study but may be useful for future studies. Although not performed in the current study, it would also have been interesting to determine posaconazole levels in nail clippings during and after treatment.

Dermatophytes cause a number of superficial infections, including *Trichophyton unguium* (nail infection [onychomycosis]), *T. capitis* (hair infection), *T. pedis* (athletes’ foot), and *T. cruris* (jock itch). Terbinafine, an allylamine, is the preferred choice for treatment of dermatophytic infections, although the triazole antifungal drugs fluconazole and itraconazole are also effective at treating such mycoses (8). Compared with terbinafine, however, itraconazole and fluconazole appeared to have lower clinical efficacy (7). An in vitro study showed that posaconazole was more active than both itraconazole and fluconazole against the dermatophytes *T. rubrum*, other *Trichophyton* spp., *Epidermophyton floccosum*, and *Microsporum* spp., as well as against 25 yeast strains and 28 non-dermatophytes (7). Another in vitro study reported that itraconazole and posaconazole were equally effective against fungal isolates from the genus *Trichophyton* but that posaconazole was more potent against *Microsporum* spp. (1). However, in vitro posaconazole was not as potent against dermatophytes as terbinafine was and had higher MICs (7).

Posaconazole administered in 400-mg doses twice a day was safe and well tolerated during 8 days of treatment in this study of healthy volunteers. A favorable safety profile had previously
been reported for posaconazole in healthy volunteers and patients (4, 5, 18, 19). Two subjects in this study withdrew because of treatment-related adverse events (elevated ALT and AST). Mild to moderate elevations in ALT and AST had been reported in previous posaconazole clinical trials but were generally reversible on discontinuation of therapy and in some cases normalized without drug interruption (15).

Conclusions. The degree of skin distribution exhibited by posaconazole in this study, along with its favorable safety and tolerability profile and potent in vitro activity against fungi (dermatophytes) and yeasts (1, 7), suggests that oral posaconazole may be useful in the treatment of dermatophytic infections, such as onychomycosis. It remains to be determined whether favorable pharmacokinetics, as well as in vitro susceptibility, will translate into clinical efficacy. Future studies should focus on determining the clinical efficacy of posaconazole in skin, hair, and nail infections.

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