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Levels of Terbinafine in Plasma, Stratum Corneum, Dermis–Epidermis (without Stratum Corneum), Sebum, Hair and Nails during and after 250 mg Terbinafine Orally Once per Day for Four Weeks

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The distribution of terbinafine in stratum corneum dermis–epidermis (without stratum corneum), sebum, hair, nails and plasma was studied in human male volunteers during and after 250 mg orally once daily for 28 days. The highest concentration was seen in sebum, 56.07 μg/g, after 14 days of therapy. The concentration was still 1.0 μg/g 44 days after stop of medication. In stratum corneum the highest concentration, 14.4 μg/g, was seen 1 day after the last day of therapy, and it was 2.1 μg/g 44 days after stop of medication. The concentrations in hair and nails were lower with a maximum of 2.36 and 0.39 μg/g respectively, 1 day after stop of therapy, and still 0.21 μg/g in hair and 0.09 μg/g in nails 55 days after the last day of medication. With the exception of nails, all other tissue levels were at all times above the plasma concentrations. For nails, tissue levels exceeded that of plasma as early as 1 day after stop of medication, and this difference continued to increase until the last day of tissue sampling, 55 days after the last tablet. These results indicate that terbinafine is delivered to the stratum corneum through sebum and to a minor extent by direct diffusion through dermis–epidermis. Probably short-term therapy with terbinafine may be effective in the treatment of several dermatomycoses, due to the strong binding of terbinafine to stratum corneum for a long time after stop of medication. Details of skin distribution of orally active antifungal agents should provide the basis for a more rational, pharmacodynamic-oriented approach to antifungal therapy than the use of traditional blood levels. ***Key words: Skin pharmacokinetics; Antimycotics; Volunteers.***

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Terbinafine (Lamisil®, Sandoz) is a new synthetic antifungal agent of the allylamine class (1). It is highly active against dermatophytes such as *Trichophyton* spp., *Microsporum* spp. and *Epidermophyton floccosum*, with minimum inhibitory concentrations (MIC) from 0.0015 to 0.01 μg/ml (2). Terbinafine is also active against molds, dimorphic fungi and many yeasts of the genera *Pityrosporum, Candida* and *Rhodotorula* (3). The dose for patients with dermatophytosis is 250 mg once daily.

The antimycotic activity of terbinafine is due to its interference with ergosterol biosynthesis, specifically its inhibition of fungal squalene epoxidase. Squalene is accumulated within the cell, leading to concentrations that are toxic for the fungal cell (4).

In humans, terbinafine is rapidly absorbed following oral administration with maximal plasma concentrations of approximately 0.8–1.5 μg/ml measured 2 h after a single 250 mg oral dose (5). The absorption half-life is calculated to be 0.8–1.2 h. The total plasma clearance is approximately 1250 ml/min if a bioavailability of 80% is assumed.

In an earlier study, we determined terbinafine levels in serum, stratum corneum, dermis–epidermis (without stratum corneum), hair and sebum during and after 250 mg terbinafine orally once daily for 12 days in 12 volunteers (6). We found a very high concentration of terbinafine (up to 45 μg/g) in sebum. In stratum corneum the concentrations continued to increase up to 9.1 μg/g of tissue, never reaching a plateau.

In the present study the levels of terbinafine were studied in plasma, stratum corneum, dermis–epidermis (without stratum corneum), sebum, clipped hair and nails during and after 250 mg terbinafine orally once daily for 4 weeks.

MATERIAL AND METHODS

Volunteers

Twelve healthy male volunteers (mean age 30 years; range 22–49) received terbinafine 250 mg once daily for 28 days. Informed consent was provided and the study was approved by the Ethics Committee of the University of Gothenburg.

Drug administration

Terbinafine was administered as one 250 mg tablet once daily for 28 days.

Collection of samples

Samples were taken on days 0, 7, 14, 28, 29, 34, 41, 52, 63, 72 and 83. Day 1 was the first day and day 28 was the last day of medication. Samples were always taken 2 h after the intake of the tablet.

Plasma. Blood was drawn from all volunteers on the days of sampling into a glass tube containing heparin. Plasma was kept at −70°C.

Stratum corneum collection. The back of the subject was divided into 6 sections. Sampling on days 41–83 was in the same sections as on days 0–29. The stratum corneum was scraped with a curette down to the stratum lucidum into a tared Petri dish. The Petri dish was weighed again and the weight of the stratum corneum determined by subtracting the tare from the total weight. The dish was sealed and stored at −70°C.

Dermis–epidermis (without stratum corneum) collection. In the same section a 3-mm punch biopsy was taken immediately after the scraping of stratum corneum. The subcutaneous tissue was separated and the biopsy transferred to a tared tube. The weight of the biopsy was determined and the tissue stored at −70°C.

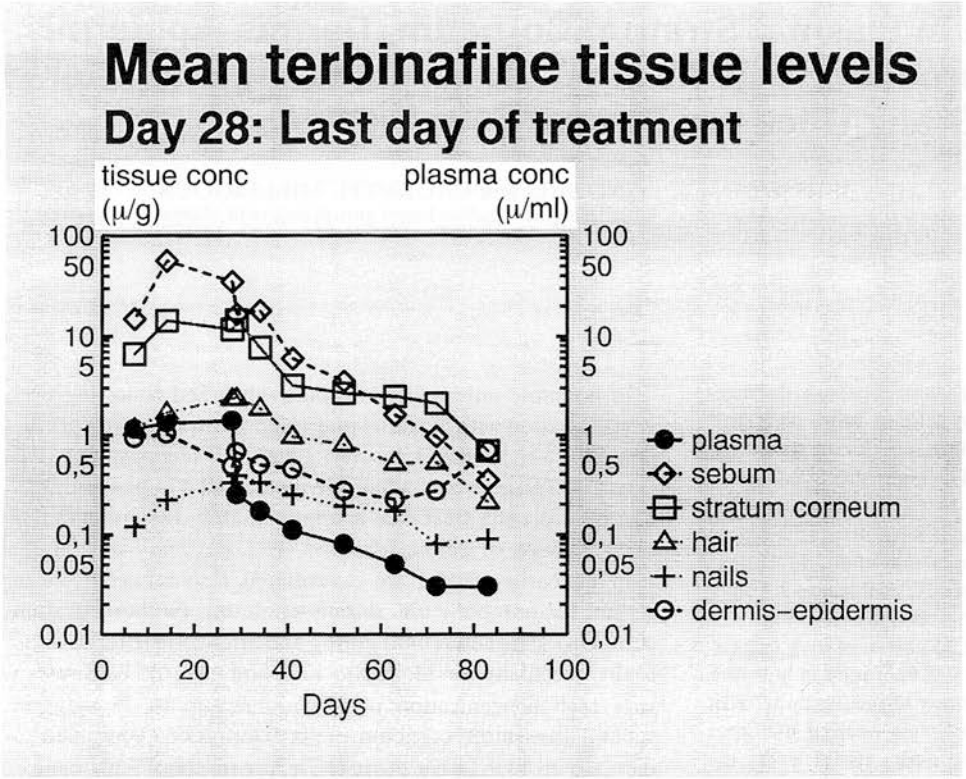

Fig. 1. Distribution of terbinafine in various skin compartments during and after 28 days of daily administration of 250 mg.

Hair collection. At each sampling time, approximately 20 strands of hair were clipped from the scalp. The clipping was as close to the scalp skin as possible. The hairs were transferred to a tared tube and stored at −70°C.

Nail collection. Every time one toe nail, of nails number 2–4, was clipped at the free border, transferred to a tared tube and stored at −70°C.

Sebum collection. Sebum was collected with the cigarette paper method from forehead skin. One piece of cigarette paper measuring 5 cm^2 was placed on the forehead. This was covered by aluminium foil, and then a gauze band was wound around the head and fastened with tape. The bandage was left in place for 3 h. The cigarette paper was transferred to a tared glass tube. The lipids were extracted by ethyl acetate, weighed and stored at −70°C.

Assay for terbinafine

With minor modifications, the same method as described earlier was used (6).

Plasma. Plasma was extracted with 8 ml of hexane. 0.1 ml internal standard (IS) solution (2.5 ng/µl IW 85190) and 1 ml 0.2 M borate buffer, pH 9.0, were added to 1.0 ml of plasma. The extraction was for 25 min. Then, after 5 min of centrifugation (3000 rpm), an aliquot of 7 ml of hexan was re-extracted by 1 ml of 85% 0.5 M H_2SO_4 and 15% 2-propanol (v/v) mixture for 15 min. After 5 min of centrifugation (3000 rpm) and after the upper phase (hexane) had been discarded, 250 µl of the hydro-alcoholic phase was injected for HPLC analysis.

Stratum corneum. Stratum corneum was transferred from Petri dishes into thick-walled stoppered glass tubes by flushing the dishes several times with 7.5 ml of ethyl acetate/methanol (50:50, v/v). Following evaporation to apparent dryness, hydrolysis of the samples was achieved by adding 1.5 ml of 5 N NaOH and heating for 1.5 h in a water bath at 90°C to hydrolyze the stratum corneum. After neutralization (addition of 0.8 ml H_3PO_4 40%) to about pH 6, IS (50 µl = 50 ng IS/sample) was added, and the extraction of terbinafine and IS into water-saturated ethyl acetate was performed by shaking the hydrolysates horizontally for 10 min with 1.5 ml of water-saturated ethyl acetate on a LAB-shaker (250 r.p.m.). The tubes were centrifuged for 10 min to improve phase separation. Thereafter, the organic phase was transferred into glass tubes and evaporated to apparent dryness. Each of the residues was redissolved in 1.5 ml of ethyl acetate. Aliquots of 500 µl were injected for HPLC analysis.

Dermis–epidermis (without stratum corneum). The biopsies were transferred into thick-walled stoppered glass tubes, 1.5 ml of 5 N NaOH and 0.1 ml of methanol were added to each tube and the tubes were heated for 1.5 h in a water bath at 90°C to hydrolyze the skin piece. The hydrolysate was neutralized by the addition of 0.45 ml H_3PO_4 85% to each tube. IS (50 µl = 500 ng IS/sample) and pH 5 buffer (2 ml) were added before the hydrolysate was extracted with

Table I. *Compilation of the mean terbinafine concentrations in different tissue compartments (µg/g or for plasma µg/ml)*

Time[a] (days)	Mean of plasma	Mean of sebum	Mean of str cor	Mean of hair	Mean of nails	Mean of dermis–epidermis
0	0.0	0.0	0.0	0.0	0.0	0.0
7	1.15	14.78	6.64	1.16	0.12	0.93
14	1.33	56.1	14.24	1.69	0.22	1.03
28	1.39	34.76	11.67	2.34	0.33	0.48
29	0.25	16.50	14.40	2.36	0.39	0.67
34	0.17	17.87	7.69	1.86	0.33	0.50
41	0.11	5.97	3.21	0.96	0.25	0.45
52	0.08	3.54	2.67	0.80	0.19	0.27
63	0.05	1.61	2.47	0.52	0.17	0.22
72	0.03	0.96	2.09	0.53	0.08	0.27
83	0.03	0.35	0.69	0.21	0.09	0.69

[a] Day 28 is the last day of treatment.

hexane (6.5 ml). After agitation 10 min at 250 CPM, and centrifugation 10 min at 2620 g, the organic phase was evaporated to apparent dryness, and the residue was redissolved using 0.1 ml of methanol and 0.1 ml of a mixture of 0.1 M pH 7/acetonitrile/triethylamine/water (100/450/0.5/449.5). The solution was transferred into glass vials and an aliquot of 20 µl injected for HPLC analysis.

Hair. The procedure was identical to that performed for dermis–epidermis.

Nail. The procedure was identical to that performed for dermis–epidermis.

Sebum. 1.5 ml of ethyl acetate and 50 ng/ml of IS were added to the tubes containing the lipid extract of sebum and the tubes were vortexed. Aliquots of 500 µl were injected for HPLC analysis.

Calibration and calculation

Calibration and quali control samples were spaced in blocks throughout every batch and analyzed in at least duplicate. The calculation of the concentration of terbinafine was related to the peak area of the calibration samples and corrected by the peak area of the internal standard. Calibration factors were established for each batch and calibration lines were obtained from extended least squares linear regression. All samples within a batch were evaluated with the same calibration parameters.

Safety

All volunteers were questioned for the occurrence of adverse events. Blood samples were taken for analysis of hemoglobin, erythrocytes, white blood cell count, platelets, liver function tests (SGOT, SGPT, alkaline phosphatase, bilirubin), renal function test (urea and creatine), cholesterol, triglycerides and blood glucose. The tests were performed at day 0 and after 28 days of medication.

RESULTS

No laboratory test abnormalities were noted and no patients experienced any adverse event.

Distribution of terbinafine

The distribution of terbinafine is shown in Fig. 1 and Table I. By far the highest concentrations were achieved in sebum, with a concentration of 56.1 µg/g after 14 days and 34.8 µg/g after 28 days of medication. The concentration was 35 days after stop of medication 1.6 µg/g and 55 days after the last dose the concentration of terbinafine was still 0.4 µg/g sebum. The second highest concentration of terbinafine, 14.4 µg/g tissue, was found in stratum corneum 1 day after the last day of medication and the concentration was still 2.1 µg/g 44 days after the last day of medication and 0.7 µg/g 55 days after the last dose (Fig. 1). The concentration of terbinafine in hair and nails was lower but the curve was more or less parallel to the curve for stratum corneum. The maximum concentration, 2.4 µg/g tissue, in hair was achieved at the last day of medication and the concentration was 55 days after the last dose 0.2 µg/g. In nails the concentration was lower with a maximum of 0.4 µg/g tissue 1 day after the last dose but still 0.1 µg/g tissue 55 days after the last day of medication. The highest plasma concentration (peak) was 1.39 µg/ml achieved at the last day of medication. Only 1 day later, the concentration was as low as 0.2 µg/ml and 55 days after the last dose the concentration in plasma was only 0.03 µg/ml.

After cessation of drug administration, all curves suggest a biphasic elimination being initially more rapid and for several tissues the half-lives could be estimated to be around 20 days. The concentration of terbinafine was still increasing for both hair and nails at day 28, but for all other tissue samples, including plasma, a steady state concentration was achieved before the last day of medication.

DISCUSSION

Terbinafine is an antifungal drug of the allylamine class with a high activity in vitro against especially dermatophytes with MICs of 0.002–0.008 µg/ml. It has been shown to be effective in treating patients with various dermatomycoses (7), especially tinea ungium (8).

In an earlier investigation we studied the distribution of terbinafine in various compartments of the skin after oral administration of 250 mg daily for 12 days (6). Very high concentrations (45.4 µg/g) were found in sebum. High concentrations (9.1 µg/g) were also found in stratum corneum, but in this compartment a plateau was never reached. Terbinafine was found to be delivered to the stratum corneum primarily with sebum and to a lesser extent through incorporation into the basal keratinocytes and diffusion through dermis–epidermis.

In the present study, the highest levels of terbinafine were found in sebum (56.1 µg/g). High concentrations were also seen in stratum corneum (14.4 µg/g). With the exception of nails, terbinafine was found in all other compartments in higher concentrations than in plasma. However, the concentration in nails was already measurable after 7 days (0.1 µg/g) and continued to increase until 1 day after stop of medication (0.4 µg/g). In our earlier terbinafine pharmacokinetic study (6), nails were not included and hairs were pulled with a forceps. However, although there was a difference in the sampling technique for hair, in the 2 studies the initial curves were identical, indicating that no sebum was contaminating hair in our first study. A steady-state concentration for terbinafine was achieved within 14 days for plasma, sebum, stratum corneum and dermis-epidermis (without stratum corneum). For both hair and nails the levels continued to increase until 1 day after the last day of medication. After cessation of medication all tissue concentration curves suggest a biphasic elimination being initially more rapid. Thirteen days after stop of medication the concentration of terbinafine in sebum, stratum corneum and hair was 6.0, 3, 2 and 1.0 µg/g respectively, and 45 days after cessation of medication the concentration in sebum, stratum corneum and hair was still 0.4, 0.7 and 0.2 µg/g respectively. These concentrations are more than 10 times as high as the MICs for most dermatophytes. For nails the elimination was slower than for the other compartments, and 45 days after stop of treatment the concentration was still 0.1 µg/g compared to 0.3 µg/g at the last day of medication. However, only 13 days after stop of medication the concentration of terbinafine in nails (0.3 µg/g) exceeded that in plasma (0.1 µg/ml), again indicating that plasma concentrations cannot be used as a parameter for the tissue concentration of terbinafine in the target organ.

In Table II the distribution of terbinafine (present study and

Table II. *Comparison of 5 orally active antifungal agents*

	Serum/plasma (μg/ml)	Sebum (μg/g)	Sweat (μg/ml)	Epidermis–dermis (μg/g)	Stratum corneum (μg/g)
Griseofulvin 500 mg for 2 weeks (10)[a]	2.0	ND	200–300	ND	20.6
Fluconazole 50 mg for 12 days (9)	1.81	ND	4.89	2.93	66.4
Fluconazole 150 mg once a week for 2 weeks (9)	2.12	ND	4.69	4.62	23.4
Itraconazole 200 mg for 7 days (11)	0.5	4.64	0.072	ND	0.79
Ketoconazole 200 mg for 14 days (12)	7.9	0.0	0.084	ND	5.18
Terbinafine 250 mg for 12 days (6)	1.0	45.1	0.0	0.3	9.1
Terbinafine 250 mg for 28 days	1.39	56.07	ND	1.03	14.40

ND: not determined.
[a] reference.

ref 6), fluconazole (9), griseofulvin (10), itraconazole (11) and ketoconazole (12) is shown. Both the concentration and the route of delivery of the drug to stratum corneum are different for several of these antimycotics and this is of importance both for their efficacy and their indications. We have earlier shown that the concentration of terbinafine in eccrine sweat is nil but the concentration both in sebum and stratum corneum is high (6). This is of importance in the treatment of dermatomycoses localized to stratum corneum, hair and hair follicles. Fluconazole is found in high concentrations in stratum corneum, eccrine sweat and dermis–epidermis (without stratum corneum) (9), indicating that it might be effective not only in the treatment of dermatomycoses localized to stratum corneum but also in the treatment of fungal infections in the deeper part of epidermis and dermis. The level of griseofulvin was also high in stratum corneum and especially in sweat (10 and Table II) after a dose of 1 g/day for 14 days (10). However, griseofulvin disappeared rapidly from stratum corneum after cessation of therapy. Both itraconazole and ketoconazole reach stratum corneum by diffusion and sweat (11, 12). Itraconazole is also, just like terbinafine, excreted in sebum (11). However, the concentration of itraconazole is low in stratum corneum compared to terbinafine (Table II).

In an earlier study terbinafine was found in peripheral nail clippings from both normal nails and nails infected with dermatophytes in concentrations of 0.25 to 0.55 μg/g as early as 4 weeks after start of treatment (13). Clippings were taken every 4 weeks for 48 weeks but no clippings were taken between week 0 and 4 or after cessation of therapy. Even itraconazole has been studied in nails (14). It has been found in peripheral clippings in concentrations of 0.02 to 0.04 μg/g 4 weeks after start of treatment and these concentrations increased to 0.16 to 0.20 μg/g during treatment. Itraconazole persisted in nails for up to 7 months after cessation of treatment (14). In another nail kinetic study itraconazole 100 mg and 200 mg was given orally for 3 months to patients with onychomycosis, and nail concentrations were then followed for 6 months after stop of medication (15). Itraconazole has previously normally been given in 100 mg, and 200 mg is a high dose to give for several months. The levels of itraconazole in toenails were more or less constant during the 6-month follow-up, higher in the 200 mg group than in the 100 mg group. A decline in the concentration was seen in fingernails, most marked in the 200 mg group. The mean levels of itraconazole in toenails were 0.67 μg/g in the 200 mg group and 0.15 μg/g in the 100 mg group during the whole 6-month follow-up. In the present study we detected terbinafine in peripheral nail clippings as early as after 7 days in concentrations of 0.12 μg/g, increasing to 0.39 μg/g 1 day after the last day of treatment (28 days). Terbinafine was still detectable in peripheral nail clippings, in a concentration of 0.09 μg/g, as long as 55 days after the last day of treatment.

In conclusion the results from this study indicate that terbinafine is effective in the treatment of several dermatophytoses due to high concentrations of the drug in several tissue compartments. Several therapeutic studies indicate that therapy may be short-term (16, 17), and this is in agreement with our pharmacokinetic findings showing that terbinafine persists in stratum corneum, hair, nails and sebum for months after cessation of treatment. We suggest that details of skin distribution of an oral antifungal drug will provide the basis for a more rational, pharmacodynamic-oriented approach to antifungal therapy than the use of traditional blood levels only.

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