

Terbinafine Levels in Serum, Stratum Corneum, Dermis-Epidermis (without Stratum Corneum), Hair, Sebum and Eccrine Sweat

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We determined terbinafine levels in serum, stratum corneum, dermis-epidermis (without stratum corneum), hair, sebum and eccrine sweat before, during and after 250 mg doses orally to volunteers once daily. Terbinafine is concentrated rapidly in stratum corneum (up to 9.1 µg/g of tissue) primarily by diffusion from the vascular system through the dermis-epidermis. It also reaches high concentration in sebum (up to 45.1 µg/ml) after several days and continue to concentrate in sebum for up to two days after discontinuation of drug. Hair concentration reach levels of 2.6 µg/g of tissue indicating high drug levels in and around the hair follicle. It is not found in sweat. Plasma levels range between 0.1 and 1.0 µg/ml. There is a tenfold accumulation of drug in stratum corneum by day 2. Elimination of drug from tissue occurs with a half-life of 4 to 5 days and with the potential for drug levels above fungicidal concentrations for dermatophytes for more than 3 weeks. The tissue pharmacokinetic profile of terbinafine is similar to that of another lipophilic drug, itraconazole, but is very different from ketoconazole and griseofulvin. Higher levels of terbinafine are achieved than of either of the imidazoles and remain longer than griseofulvin. **Key words:** *Terbinafine; Pharmacokinetic.*

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Terbinafine (Lamisil[®], Sandoz code SF 86-327) is a synthetic antifungal agent of the allylamine class and

is the most effective member of this new chemical class of antimycotic compounds so far developed (1). The compound has proved to be highly active against dermatophytes such as *Trichophyton spp.*, *Microsporum spp.* and strains of *Epidermophyton floccosum*, with minimum inhibitory concentrations (MIC) ranging from 0.0015 to 0.01 µg/ml in vitro (2). Terbinafine is also active against molds and dimorphic fungi, and against many pathogenic yeasts of the genera *Pityrosporum*, *Candida* and *Rhodotorula* (3). The dose for patients with dermatomycosis is 125 mg twice daily or 250 mg once daily.

The antimycotic activity of terbinafine is due to its interference with ergosterol biosynthesis, specifically its inhibition of fungal squalene epoxidase. Thus the formation of lanosterol, the first complete sterol (2 steps after squalene epoxydation) is inhibited (4, 5).

In animals the maximal concentrations were found after 6 h in the liver, kidney, fat tissue, skin and blood. These results are consistent with the known high level of drug concentration in lipophilic tissue such as fat and skin. The pharmacokinetics of terbinafine in Man has been elucidated in healthy volunteers using either radiolabelled or unlabelled drugs. 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 was calculated to be 0.8-1.2 h. The total plasma clearance is approximately 1250 ml/min if a bioavailability of 80% is assumed.

To evaluate the tissue distribution of terbinafine to its primary sites of action, this study of drug levels in eccrine sweat, sebum, hair and skin has been carried out. It was considered of particular importance to determine the drug levels in target tissue, whether it is accumulating there, and the duration of tissue

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levels after drug discontinuation. For purpose of comparison, the available information on the distribution of the other antifungal drugs griseofulvin, ketoconazole, and itraconazole is also reviewed.

MATERIAL AND METHODS

Volunteers

Twelve healthy male volunteers were enrolled in the study after providing informed consent. Institutional review board approval was obtained from the University of California. Two volunteers did not complete for reasons unrelated to the study, so that ten subjects were fully evaluable.

Dosage and duration of therapy

The volunteers were given two tablets of 125 mg terbinafine once daily for 12 days. Day 0 was the first day of medication, and day 11 was the last day. Samples were taken on day 0, before the start of medication, and then at days 2, 6, 12, 13, and 16.

Collection of samples

Serum. Blood was drawn from all volunteers at days 0, 2, 6, 12, 13, and 16. On days 2 and 6 blood was taken immediately before the tablets were given and then 2 h after. The blood was allowed to clot, and serum was kept frozen at -20°C .

Stratum corneum collection. The back of the subject was divided into 6 sections; each section was studied only once. 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 stratum corneum determined by subtracting the tare from the total weight. The dish was sealed and stored at -20°C .

Dermis-epidermis (without stratum corneum) collection. In the same quadrant a punch biopsy, 4 mm in diameter, 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 -20°C .

Hair collection. At each sampling time, 10 strands of scalp hair were removed by a quick pull with a forceps. The hair specimens were collected, transferred to a tared tube, and stored at -20°C .

Collection of eccrine sweat. Eccrine sweat was collected as thermogenic sweat after the subjects has been for approximately 15–20 min in a sauna bath operating at 55–65°C. Sweat was collected in a plastic bag placed around the arm and fastened with tape around the shoulder. It was divided into two portions, i.e. one that was immediately stored at -20°C and one that was separated by centrifugation into a clear supernatant phase and a turbid sediment phase. The volume of each was determined and the fractions stored at -20°C .

Collection of sebum. Sebum was collected indirectly by collecting from the forehead area skin surface lipids primarily of sebaceous origin. One piece of cigarette paper measuring 5 cm² 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 hours. The cigarette paper was transferred to a tared glass tube. The lipids were extracted by ethyl acetate, weighed and stored at -20°C .

Assay for terbinafine

Serum. Serum was extracted with ethyl acetate. 0.1 ml internal standard (IS) and 2 ml of 50 mM phosphate buffer pH 6.0 were added to 0.5 ml of serum. The resulting solution (pH 6.2) was extracted with 3 ml of ethyl acetate by shaking the test tube in a horizontal position for 1 h. After 10 min of centrifugation (2500 r.p.m.), an aliquot of 2 ml was evaporated to apparent dryness. 300 µl of 85% 0.5 NH₂SO₄ and 15% 2-propanol (v/v) were added to the residue, and 200 µl of this solution were 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 5N NaOH and heating as described before. After neutralization (addition of 0.8 ml H₃PO₄ 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 ethylacetate 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 pre-weighed skin punch biopsies were transferred into thick-walled stoppered glass tubes and 0.5 ml of 5N NaOH was added to each tube. Complete hydrolysis was performed as described under the procedure for hair. The hydrolysate was neutralized by the addition of 0.37 ml H₃PO₄ 85% to each tube. IS (50 µl = 50 ng IS/sample) was added before the hydrolysates were extracted with water-saturated ethyl acetate as described above. After centrifugation, an aliquot of 1.2 ml of the organic phase was evaporated to apparent dryness, and the residue was redissolved in 350 µl of ethyl acetate. The solution was transferred into glass vials and an aliquot of 175 µl injected for HPLC analysis.

Hair. Hair strands of each sample were first cut into short (2–5 mm) pieces and transferred into thick-walled stoppered glass tubes. 5N NaOH (1.5 ml) was added to each tube, and the stoppered tubes were heated for 1.5 hours in a water bath at 90°C to hydrolyze the hair pieces. Immediately after cooling to room temperature, neutralization of each hydrolysate to pH 6–7 was achieved by the addition of H₃PO₄ 85% (260 µl). IS (50 ng/sample) was added, and the extraction of terbinafine and IS was achieved by shaking the hydrolysates horizontally for 10 min with 1.5 ml of water-saturated ethyl acetate on a LAB-shaker (250 rpm). Thereafter, the tubes were centrifuged for 10 min (2,500 rpm) in order to improve phase separation. An aliquot of 1.2 ml of each organic phase was evaporated to apparent dryness (evacuated at 30°C in the Vortex-Evaporator), and the remaining residue redissolved

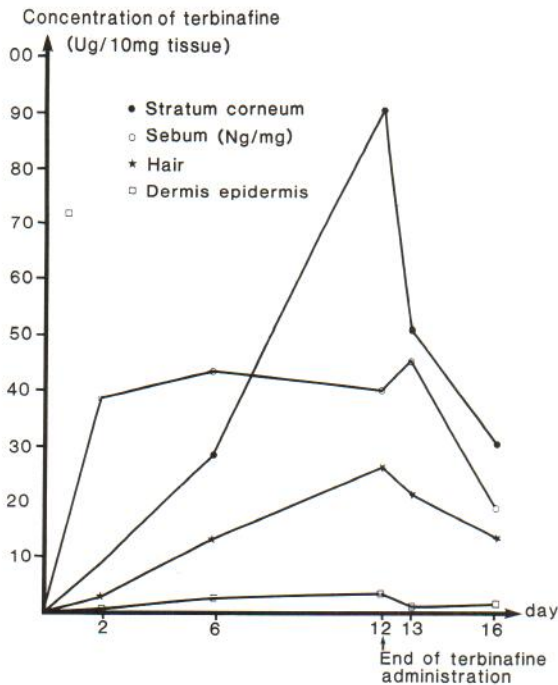


Fig. 1. Distribution of terbinafine in various skin compartments after 12 days of daily administration of 250 mg/day.

in 300 μ l of ethyl acetate. After transfer into vials, aliquots of 175 μ l were injected for HPLC analysis.

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 μ l were injected for HPLC analysis.

Eccrine sweat. Solid NaCl was added to aliquots of 2 ml (or 1 ml, or 0.5 ml depending on the total sample volume) of eccrine sweat to improve phase separation after the extraction with 0.5–2 ml of water-saturated ethyl acetate as described for hair. After centrifugation, 1.4 ml of the organic phase was evaporated to apparent dryness. The remaining residue was redissolved in 400 μ l of ethyl acetate, vortexed and centrifuged for 3 min. From this solution, an aliquot of 175 μ l was injected for HPLC analysis. Determination of terbinafine utilized an reversed phase HPLC method.

Calibration and calculations. Calibration and quality control samples were spaced throughout every batch 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 by mean of RS/1-SABAC procedures (6). Calibration lines were obtained from extended least square linear regression (ELSFIT) (7). All samples within a batch were evaluated with the same calibration parameters.

Haematology and blood biochemistry. Blood samples were taken for analysis of hemoglobin, erythrocytes, white blood cells, platelets, liver function test (SGOT, SGPT,

alkaline, phosphatase, bilirubin), renal function test (urea and creatinine), cholesterol, triglycerides, and blood glucose. The tests were done before the start of medication and after 12 days of treatment.

RESULTS

A. Data set and demographic data. The patients were all male, mean age 33 years (range 18–52 years), and all were Caucasians. All were healthy except one (patient 2), who had seborrhoeic dermatitis and a history of a gastric ulcer operated in 1972.

B. Adverse events, tolerability, laboratory data. No adverse events were recorded, and overall tolerability was rated as very good by all subjects. One volunteer had a low hemoglobin at baseline, and the same level at day 12 (13.3–13.4 mg/dl). Three volunteers with elevated cholesterol levels at baseline (one also with elevated glucose and one with elevated triglyceride) had persistently elevated levels at day 12. One volunteer had an increase in triglyceride from the normal to the abnormal range (169 mg/dl to 240 mg/dl). This was not considered medically relevant or significant since no control diet was chosen during the study.

C. Distribution of terbinafine in serum and tissues (Fig 1). No terbinafine was measurable in eccrine sweat. Low concentrations were found in dermis-epidermis (around or below the limit of detection) on the second day of study examination, but by day 6 these concentration exceeded plasma trough levels. During the study period increasing amounts of terbinafine were identified in stratum corneum (stratum corneum: plasma ratios measured from 13 to 73 times from 2 to day 12).

Terbinafine rapidly accumulated in sebum where it reached the highest concentration of all specimens analysed. In sebum, the concentration remained almost unchanged until day 13. In hair, dermis-epidermis and in stratum corneum, the concentration of terbinafine increased with time until day 12. In these tissues, the elimination of terbinafine started upon cessation of the administration of terbinafine, whereas in sebum the elimination apparently was delayed for several days. Inter-subject variability was identified in all tissue sampled.

Terbinafine rapidly accumulated in the serum within two h after administration. The elimination of the drug from blood after day 12 had a half-life of 4 days.

Table I. Comparison of the distribution of 4 orally active antifungal agents.

Level in: ($\mu\text{g/g}$ or $\mu\text{g/ml}$)	Griseofulvin (500 mg \times 2 for 14 days)	Ketoconazole (200 mg for 14 days)	Itraconazole (200 mg for 7 days)	Terbinafine (250 mg for 12 days)
Serum (peak)	2.0	7.9	0.5	1.0
Sebum	not done	0	4.64	45.1
Sweat	200-300	0.084	0.072	0
Stratum corneum	20.6	5.18	0.79	9.1

DISCUSSION

The mechanisms by which a drug may arrive at the surface of the skin from the vascular system include: (1) diffusion through the dermis-epidermis, (2) incorporation into the basal cells of the epidermis and migration during the normal turnover of keratinocytes, (3) concentration in and secretion in sebum, and (4) concentration and secretion in sweat. Each mechanism may be associated with a different effect on diseases of the skin. For example, diseases of hair and hair follicles should respond best to drugs concentrated in sebum, whereas superficial stratum corneum infections would likely respond well to drugs secreted in sweat. Very chronic conditions leading to thickened epidermis should respond best to drugs that diffuse through the skin rather than those secreted on the surface of the skin.

Terbinafine is an antifungal drug of the allylamine class with a high activity *in vitro* against dermatophytes and yeasts at MICs of 0.002–0.008 $\mu\text{g/ml}$. It has been shown to be effective in treating humans with various dermatomycosis (8). In the present study terbinafine levels up to 45 $\mu\text{g/ml}$ were found in sebum and levels up to 9 $\mu\text{g/ml}$ in stratum corneum. These values are far above the MICs of terbinafine against dermatophytes. Terbinafine is delivered to the stratum corneum by a passive diffusion from the blood stream through dermis-epidermis. It is also delivered to the stratum corneum and hair follicles by sebum. Its concentration continues to increase in the stratum corneum for the whole time of medication. Terbinafine may also be incorporated into the basal keratinocytes and transported to the stratum corneum during normal cell turnover.

A constant level of terbinafine was reached in sebum after 2 days, whereas it continued to increase in stratum corneum and hair. The onset of drug elimination was delayed by 1 day in sebum compared with stratum corneum and hair, but 5 days

after cessation of dosing the level in stratum corneum was higher than that in sebum. A level of terbinafine of 3 $\mu\text{g/g}$ in stratum corneum was still found 5 days after the end of terbinafine treatment. The high level in sebum is interesting and may be beneficial in the treatment of mycotic infections of the hair follicle. A lower level of terbinafine has been found in the stratum corneum by Lever and co-workers (9). However, they used a skin surface biopsy technique for obtaining stratum corneum and the terbinafine has been extracted by another method. They measured the concentration of terbinafine in ng/cm^2 . The highest concentration found in stratum corneum was 3.7 ng/cm^2 , which is lower than our results.

Table I shows the comparison of levels of drug identified in tissue in the present study with those of other drugs presented in the literature (10–15). Itraconazole is another antifungal drug delivered to the stratum corneum with sebum and, to a lesser extent, via eccrine sweat. The results reported here indicate that terbinafine is more like itraconazole than the other antifungal drugs since it strongly adheres to keratin, arrives at the stratum corneum via uptake through the dermis-epidermis (diffusion) and is extensively excreted in sebum but not found in sweat. These aspects of the kinetics of terbinafine may be responsible for the special efficacy of terbinafine in certain chronic dermatomycoses of skin, hair and nails. Terbinafine levels in sebum after 12 days of a therapeutic dose (250 mg/day) were 10 times higher than the level of itraconazole after approximate doses. The level of terbinafine in stratum corneum was 10 times higher than the level of itraconazole after 7 days of a higher dose of itraconazole (200 mg/day) (14). It should be noted that only 2 subjects participated in the itraconazole study, compared with 10 in the study of terbinafine reported here. The level of griseofulvin was also reported to be high in stratum corneum (11) after a dose of 1 g/day for 2

weeks. However, griseofulvin disappeared rapidly from stratum corneum after cessation of therapy. We suggest that details of skin distribution will provide the basis for a more rational, pharmacodynamic-oriented, approach to antifungal therapy than use only of traditional blood levels.

Our results with terbinafine enable the following statements to be made regarding the distribution of this drug in the skin:

(1) Terbinafine rapidly diffuses through dermis-epidermis and then binds to lipophilic keratinocytes in the stratum corneum.

(2) High concentrations of drug (well above the MIC for most dermatophytes) are reached within hours and gradually increase over several days to 9 µg/g of tissue. (In vitro *T. rubrum* is killed by 2–10 ng/ml.)

(3) Terbinafine is highly concentrated in sebum, reaching concentration of 40 µg/ml. This process occurs more slowly than diffusion, but steady state is reached within 2 days and persists for 2 days after the drug has been stopped.

(4) The elimination half-life from stratum corneum and sebum is 3–5 days. Therefore concentrations above the MIC for most dermatophytes may be present for 2–3 weeks after the oral therapy is discontinued.

(5) These distribution kinetics suggest that terbinafine should be highly effective in chronic diseases of the skin, hair, and nails, since it combines diffusion through skin to affected areas with high concentrations in the hair follicle, a common site for residual infections leading to relapse of the disease.

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