A Double-blind Comparison of Levels of Terbinafine and Itraconazole in Plasma, Skin, Sebum, Hair and Nails During and After Oral Medication

Sir,
Both itraconazole and terbinafine are lipophilic and both drugs are new potent orally active antifungal drugs belonging to two different chemical classes (1–7). However, comparative data from controlled trials are not available for the distribution of these drugs in various skin compartments and nails.

In the present double-blind comparative study levels of both itraconazole and terbinafine were studied in plasma, stratum corneum, dermis-epidermis (without stratum corneum), sebum, clipped hair and nails during and after 200 mg itraconazole or 250 mg terbinafine orally once daily for 28 days.

MATERIAL AND METHODS

Volunteers and medication procedure
In a double-blind, double-dummy randomized comparative study, 12 healthy male volunteers (mean age 29 years; range 21–47) received itraconazole 200 mg once daily for 28 days and another 12 healthy male volunteers (mean age 28 years; range 21–33) 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.

Collection of samples
Samples were taken on days 0, 7, 14 and 28 during medication as well as on days 1, 6, 12, 24, 36, 48, 54, 90 (nails only) and 180 (nails only) after cessation of drug intake. Samples were always taken 2 h after intake of medicine. Plasma, skin, nail and hair were sampled according to the procedure earlier described (1, 2).

Analytical methods
Terbinafine and itraconazole were both determined in plasma, sebum and the other tissues by specific reversed-phase high-performance liquid chromatography (RP-HPLC) methods with UV detection. Only in plasma, itraconazole was quantified by its own fluorescence following excitation at 260 nm and detection at 355 nm emission wavelength. For the other determinations of both itraconazole and terbinafine, UV absorption at 261 nm and 224 nm, respectively, was used. The methods have been described in detail earlier (1–4).

Fig. 1. Levels of itraconazole and terbinafine in plasma, sebum and nails during and after 28 days of oral medication.

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Fig. 2. Levels of itraconazole and terbinafine in dermis and epidermis (without stratum corneum) and hair during and after 28 days of oral medication.

Blood analysis
Before start of medication and on the last day of medication (day 28), blood analysis for liver enzymes, serum creatinine, cholesterol and triglycerides were made to detect side-effects.

Statistics
Linear regression was used to calculate the concentration in each sample.

RESULTS
The levels of terbinafine and itraconazole in plasma, nails and sebum are shown in Fig. 1 and the levels in dermis-epidermis (without stratum corneum) and hair in Fig. 2. Unfortunately, analysis of itraconazole in stratum corneum was impaired by a baseline interference that was not observed in the other tissues. Hence, no validated data for itraconazole were available in this tissue. The concentration of itraconazole in nails was lower than that earlier reported (peak 0.006 µg/g) and no itraconazole was observed before 6 days after stop of medication (34 days after start of medication).

No side-effects were seen in the terbinafine group, but in the itraconazole group one volunteer developed liver changes with a 10-fold increase in liver enzymes (GOT and GPT) and a biopsy revealed signs of necrosis in the liver. Blood analyses for hepatitis, hemoglobin, sedimentation rate, white and red blood cell count and platelets were normal. Liver enzymes were normalised after 20 days and the volunteer did not experience any signs of disease.

DISCUSSION
The concentrations of terbinafine in skin and nails is from 10 to 1,000 times higher than MICs against dermatophytes, and this together with the persistence of terbinafine especially in stratum corneum, hair and nails and its fungicidal effect on dermatophytes is a good explanation for the efficacy of terbinafine in the treatment of dermatophyloses. Compared to earlier studies the concentration of terbinafine was around 10 times lower in stratum corneum, sebum and hair (1, 2). The reason for this is difficult to explain. The sampling procedure as well as the analytic methods were similar to earlier procedures.

Earlier studies have shown that itraconazole also persists in nails in concentrations above MICs for most dermatophytes for up to 6 months after stop of treatment (0.67 µg/g) (6, 7). The concentrations found in this study were much lower (0.006 µg/g) and itraconazole was first measurable at day 34 or 6 days after the stop of 28 days of medication.

The most striking differences between the two drugs were observed in their concentrations in hair and nails, where much higher terbinafine concentrations could be measured in these tissues. No side-effects were seen in the terbinafine group, but in the itraconazole group one volunteer developed liver changes, with increase in liver enzymes and necrotic changes in the liver.

Information of skin distribution of oral antifungal drugs rather than the traditional use of blood levels should be the basis for a more rational, pharmacodynamically oriented approach to antifungal therapy.

REFERENCES
1. Faergemann J, Zehender H, Denouil, Millieroux L. 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. Acta Derm Venereol (Stockh) 1993; 73: 305–309.
Small Proline-rich Proteins in Hair Follicles

Sir,

Transglutaminase 1 (TGase1, TGK, TGM) of about 92 KDa is membrane-associated keratinocyte transglutaminase, first discovered in keratinocytes, but is now known to be widely expressed in epithelial and non-epithelial tissues. TGase 1 is thought to be a critically important enzyme involved in the formation and assembly of the cornified cell envelope (CE) of terminally differentiating epidermis.

Recently we have raised a new anti-human TGase 1 antibody in goats against a purified recombinant protein expressed in bacteria (1). This antibody reacted with high specificity with only TGase 1 in the epidermis and cultured keratinocytes by Western blotting and immunoprecipitation. It reacted with all epidermal layers, with some potentiation of the granular layer in normal human epidermis. However, these stainings are very different from those of a widely used TGase 1 monoclonal antibody (B.C1), which labels upper spinous and granular layers of normal epidermis. By Western blotting, B.C1 antibody recognized a group of bands of 15-20 KDa. Amino acid analysis and amino acid sequencing revealed that these bands represented the small proline-rich (SRP) 1 and 2 proteins. Also with a series of blocking experiments with TGase 1 proteins and synthetic peptides, it is now considered that the main epitope of the B.C1 antibody resides on the amino-terminus of these two SRP proteins.

In a recent report in this journal, Tamada et al. (2) reported the expression of TGase 1 in human anagen hair follicles, by using B.C1 antibody. B.C1 antibody decorated the hair cuticle and the three layers of the inner root sheath in the bulb and suprabulbar portion. Subsequently, the translocation of the B.C1 epitope occurred to the inner site of the outer root sheath in the middle part of the hair follicle. In the distal portion of the isthmus and the infundibulum, the epitope was seen in the internal part of the outer root sheath and upper spinous and granular layers of epidermis.

There are several reports (3-5) of immunocytochemistry of hair follicles using antibodies of SPR1 and/or SPR2 (see Fig. 8 in (3), Fig. 3 in (4) and Fig. 6 in (5)). The distribution of SPR1 and/or SPR2 proteins is similar to the B.C1 epitope of hair follicles observed by Tamada et al. (2). We observed TGase 1 expression at hair follicles, our antibody stained outer root sheath and inner root sheath cells, with potentiation of the cuticle of the inner root sheath and hair in the bulbar and suprabulbar portion (6). The work conducted by Tamada et al. is thorough and may be a valuable report of SPR proteins, which are expressed and served as CE precursor proteins at hair follicles.

REFERENCES