

Levels of Fluconazole in Normal and Diseased Nails during and after Treatment of Onychomycoses in Toe-nails with Fluconazole 150 mg Once Weekly

JAN FAERGEMANN¹, and HEINRICH LAUFEN²

¹Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden, and ²Research and Development Laboratories Pfizer Mack, Illertissen, Germany

Thirty-six patients with onychomycoses of their toe-nails were included in a double-blind, parallel-group comparative study of fluconazole 150 mg once weekly and griseofulvin 1,000 mg once daily for 12 months, or earlier if cured. Every month during treatment and in cured patients 3 and 6 months after stop of treatment one toe-nail was clipped and serum samples were taken. In patients treated with fluconazole the concentration of fluconazole was measured in serum and nails. We found a very high concentration of fluconazole in nails (peak 8.54 µg/g) and the nail concentration was statistically significantly higher than serum concentrations ($p < 0.001$). In cured patients fluconazole was still present in high concentrations 3 (1.7 µg/g) and 6 (1.4 µg/g) months after stop of treatment. These results indicate that fluconazole should be effective in the treatment of onychomycosis in a dose of 150 mg once weekly. The results also indicate that the treatment period could be shortened because fluconazole is still present in high concentrations 6 months after stop of therapy. The concentration of fluconazole found in nails is much higher than that found in the case of terbinafine and itraconazole, indicating that fluconazole should be at least as effective as these drugs in the treatment of tinea unguium.

(Accepted November 24, 1995.)

Acta Derm Venereol (Stockh) 1996; 76: 219–221.

J. Faergemann, Department of Dermatology, Sahlgrenska University Hospital, S-413 45 Gothenburg, Sweden.

Fluconazole is a synthetic bis-triazole derivative which inhibits the synthesis of ergosterol (1, 2).

Fluconazole has been found in high concentrations in the skin (3–5). We have earlier studied the distribution of fluconazole in serum, various sections of the skin and eccrine sweat during and after a 50-mg daily dose of fluconazole for 12 days and 150 mg fluconazole once a week for 2 weeks (5).

As a part of a European multicenter double-blind, parallel-group comparative study of fluconazole 150 mg once weekly and griseofulvin 1,000 mg once daily in the treatment of tinea unguium of the toe-nails, we sampled toe-nails for analysis of fluconazole levels. In this pharmacokinetic study peripheral nail clippings were sampled during and after treatment with fluconazole 150 mg once weekly.

MATERIAL AND METHODS

Patients

Thirty-six patients with onychomycoses of their toe-nails were included in a double-blind, parallel-group comparative study of fluconazole 150 mg once weekly and griseofulvin 1,000 mg once daily for 12 months, or earlier if cured. This study was part of a European multicenter study, and not all centers have fulfilled the trial at the time of writing this paper.

Collection of samples

Patients returned for control every month during treatment, and cured patients returned for control 3 and 6 months after stop of treatment. At each visit one diseased and one normal toe-nail were clipped at the free border, from all patients included in the study, transferred to a tared tube, and stored at -70°C . The same nail (target) was followed during the whole study. Serum samples were also taken at each visit.

Assay for fluconazole

Before analysis the investigator responsible for the assay (H. Laufen) broke the code, and he only analysed nails and sera from patients treated with fluconazole. The code was not known to the investigator responsible for treatment and control of the patients (J. Faergemann).

Assay principle

Fluconazole was extracted from nail and serum samples with ethyl acetate, and the extracts were chemically derivatized through a silylation reaction and subsequently analysed by gas chromatography with electron capture detection. The separation of fluconazole from coextracted components of the biological matrix was carried out on a fused silica capillary column of 30 m length (DB 5.625 bonded phase), after temperature-controlled vaporization of the extracts.

Quantification was performed on the basis of chromatographic peak ratios of fluconazole and an internal standard. The extraction procedure of fluconazole from plasma was the same as described earlier (5), with an extraction yield being greater than 90% (6). The nails were solubilized before the extraction by being treated with 1 M sodium hydroxide at 60°C for about 90 min. There was no loss of fluconazole through this treatment.

Calibration and quality control

All calibration and quality control samples required for the complete analysis of the clinical samples were prepared together, prior to the start of the analysis. Calibration curves were set up, covering the concentration range of 0–100 ng/sample for nail and 0–25 µg/ml for serum. The coefficient of correlation of the calibration curves (polynomial of 2nd degree) was greater than 0.998 for nails and 0.994 for serum. With serum the within-day precision of the assay at a concentration of 0.10 µg/ml was 2.6%, at 0.51 µg/ml it was 3.4%, and at 1.52 µg/ml it was 2.0% (each $n = 6$ quality control samples). Day-to-day precision, as determined from the assay of duplicate quality control samples, was 2.8% at 0.10 µg/ml, 4.9% at 0.51 µg/ml, and 1.9% at 1.52 µg/ml. For nails, the within-day precision was 2.6% at 2.53 ng/sample, 1.1% at 10.12 ng/sample, and 2.2% at 50.60 ng/sample. The corresponding day-to-day precision amounted to 7.3%, 0.8% and 3.2%, respectively.

Limit of quantification

The limit of quantification for fluconazole was taken as 0.25 ng/sample in nail and $0.04 \mu\text{g ml}^{-1}$ in serum, which was also the lowest calibration point. At that concentration, the relative standard deviation for the back-calculated concentrations for nails was 10.49%, with a mean relative error of 0.00% ($n = 6$), and for serum 6.45%, with a mean error of +2.77%.

Statistics

The observed differences in fluconazole concentrations between serum, infected nail and healthy nail were tested for significance. An ANOVA with patients, visits and type of sample as defined sources of variance was performed with all measured fluconazole concentrations. The F-test was applied and multiple comparisons were made using the Scheffé test.

RESULTS

The concentration of fluconazole in diseased and healthy nails during and after treatment is shown in Fig. 1. Sampling took place once monthly during treatment and, in cured patients, 3 and 6 months after stop of treatment. An increase in fluconazole concentration in the nails was seen during the first 6 months of treatment. Then the concentration was more or less stable until treatment was stopped. The concentration of fluconazole in distal nail clipping was after only 1 month higher than in serum (Fig. 1). During treatment there was no statistically significant difference between healthy and diseased nails. However, the concentration both in diseased and healthy nails was significantly higher than in serum ($p < 0.001$). The mean concentration in healthy nails was $3.09 \mu\text{g/g}$ after only 1 month, and it increased to $8.54 \mu\text{g/g}$ after 6 months of treatment. The serum concentration was more or less stable during treatment at a concentration of 0.45 to $1.36 \mu\text{g/ml}$.

The concentration of fluconazole remained high 3 months after stop of treatment, and the mean concentration was then $1.7 \mu\text{g/g}$ in healthy toe-nails and $2.08 \mu\text{g/g}$ in diseased toe-nails ($n = 6$). Six months after stop of treatment the concentration was still $1.4 \mu\text{g/g}$ in healthy toe-nails and $1.9 \mu\text{g/g}$ in

diseased toe-nails ($n = 3$). The concentration of fluconazole obtained in toe-nails during and 6 months after treatment with 150 mg once weekly is very high, and after only 1 month the concentration is far above the MICs (minimal inhibitory concentration) of fluconazole against both dermatophytes and yeasts. Since after visit 5 the number of subjects from which samples were obtained decreased, until there were only 3 subjects (after visit 9), the mean values of visits 10 to 12 may be less representative.

DISCUSSION

In an earlier study we have showed that fluconazole reached the stratum corneum very fast through sweat and direct diffusion from the dermal capillaries through dermis and epidermis (5). The peak concentration obtained in the stratum corneum was $73.0 \mu\text{g/g}$ after 12 days of 50 mg fluconazole once daily for 12 days and $23.4 \mu\text{g/g}$ 4 h after a second dose of 150 mg fluconazole once weekly (5). In that study sweat and skin sampling was continued for 1 week after stop of medication, and fluconazole was still found in concentrations far above MICs for dermatophytes and yeasts.

Later open clinical trials have shown that fluconazole 150 mg once weekly is effective in the treatment of tinea corporis, tinea cruris, tinea pedis and tinea unguium (7–9). In a multicenter, double-blind, controlled study in Sweden and Norway of 239 patients with tinea corporis or tinea cruris, we recently showed that fluconazole 150 mg once weekly for 4 to 6 weeks (4 to 6 dosages) tended to be more effective (74%

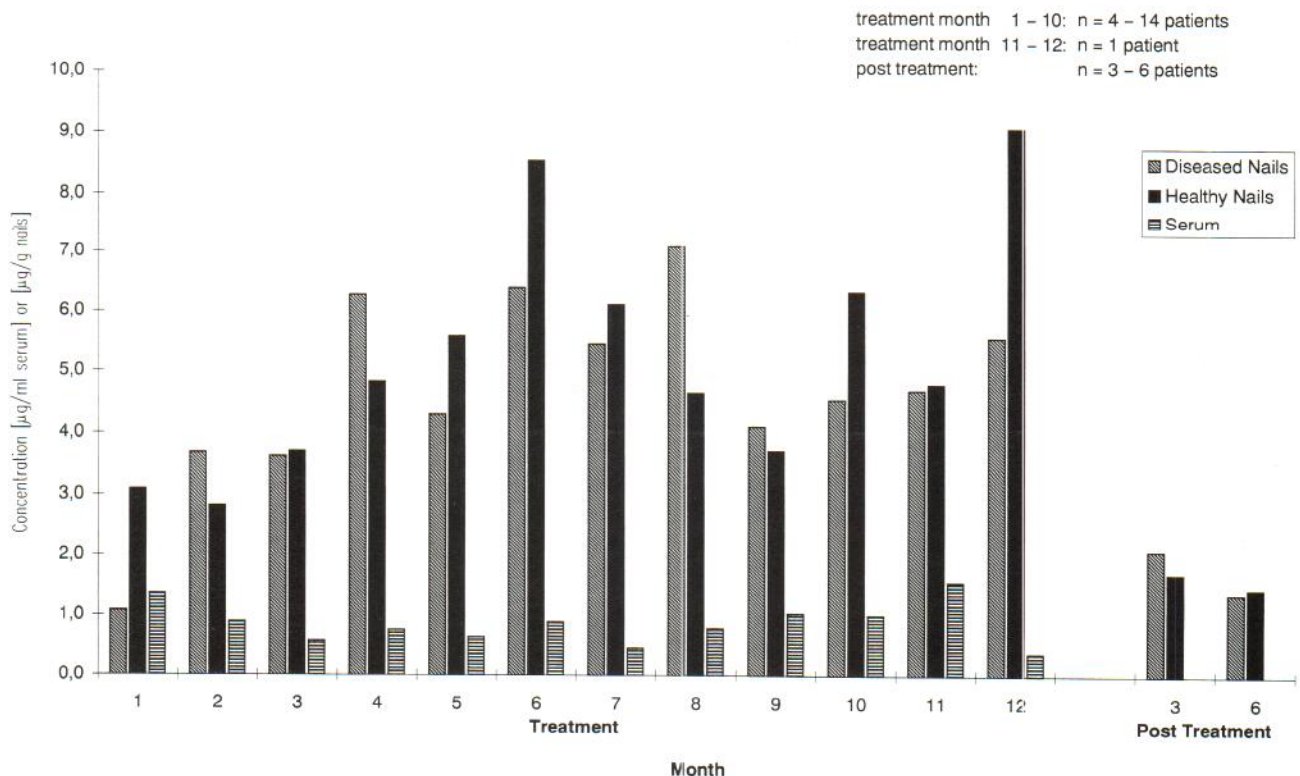


Fig 1. Levels of fluconazole in serum, diseased and normal nails during and after treatment of onychomycosis with fluconazole 150 mg orally once weekly.

cured) than griseofulvin 500 mg once daily for 4 to 6 weeks (62% cured) (unpublished data).

In the present study we found a very high concentration of fluconazole in nails, and no statistically significant difference was seen between healthy and diseased nails, but the nail concentrations were statistically significantly higher than serum concentrations ($p < 0.001$). Fluconazole was in cured patients still present in high concentrations 3 and 6 months after stop of treatment.

These results indicate that fluconazole should be effective in the treatment of onychomycosis due to dermatophytes in a dose of 150 mg once weekly. The results also indicate that the treatment period could be shortened, since fluconazole is still present in high concentrations 6 months after stop of therapy. It was very interesting that the concentration of fluconazole found in nails was much higher than that found in the case of terbinafine (10) and itraconazole (11) indicating that fluconazole should be at least as effective as these drugs in the treatment of tinea unguium.

The inter-individual variations were rather high in both serum and nail concentrations. In controlled studies with a short treatment time, such as pharmacokinetic studies in healthy subjects, the variations in the serum concentrations of fluconazole are usually as low as 20–30%. As a reason for the observed large variations in fluconazole serum concentrations of the present study, heterogeneity of patient groups, intra- and inter-subject variations in daily dosage time or variable compliance to daily drug intake are factors that may be considered. The observed variability in nail concentrations may reflect variations in the distribution kinetics of fluconazole into the nails but may as well have been influenced by similar factors as the variation in serum concentrations.

In conclusion, this pharmacokinetic study indicates that fluconazole 150 mg once weekly should be effective in the treatment of onychomycosis, and with weekly dosing the risk of side effects is minimized. As we have shown earlier, skin

pharmacokinetic studies should be the background for more relevant clinically oriented trials.

REFERENCES

1. Grant SM, Clissold SP. Fluconazole. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in superficial and systemic mycoses. *Drugs* 1990; 39: 877–916.
2. Bennett JE. Fluconazole: a novel advance in therapy for systemic fungal infections. *Rev Infect Dis* 1990; 12(Suppl 3): 263–390.
3. Haneke E. Pharmacokinetic evaluation of fluconazole in plasma, epidermis and blister fluid. *Int J Dermatol* 1992; 31(Suppl 2): 3–5.
4. Hay RJ. Pharmacokinetic evaluation of fluconazole in skin and nails. *Int J Dermatol* 1992; 31(Suppl 2): 6–7.
5. Faergemann J, Laufen H. Levels of fluconazole in serum, stratum corneum, epidermis-dermis (without stratum corneum) and eccrine sweat. *Clin Exp Dermatol* 1993; 18: 102–106.
6. Wood PR, Tarbit MH. Gas chromatographic method for the determination of fluconazole, a novel antifungal agent in human plasma and urine. *J Chromatogr* 1986; 383: 179.
7. Kuokkanen K, Alava S. Fluconazole in the treatment of onychomycosis caused by dermatophytes. *J Dermatol Treatm* 1992; 3: 115–117.
8. Suchil P, Gei FM, Robles M, Perera-Ramirez A, Welsh O, Male O. Once-weekly oral doses of fluconazole 150 mg in the treatment of tinea corporis/cruris and cutaneous candidiasis. *Clin Exp Dermatol* 1992; 17: 397–401.
9. Del Aguila R, Gei FM, Robles M, Perera-Ramirez A, Male O. Once-weekly oral doses of fluconazole 150 mg in the treatment of tinea pedis. *Clin Exp Dermatol* 1992; 17:402-406.
10. Faergemann J, Zehender H, Denouël J, Millerioux 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.
11. Willemsen M, De Doncker P, Willems J, Woestenborghs R, Van de Velde V, Heykants J, et al. Posttreatment itraconazole levels in the nail. New implications for treatment in onychomycosis. *J Am Acad Dermatol* 1992; 26: 731–735.