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The retention of isoconazole in the skin after once or twice daily application of 1% isoconazole nitrate cream (Travogen®) over a 14-day period

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Summary

The concentration of the antimycotic agent isoconazole nitrate was measured in the horny layer and in skin tissues after once or twice daily topical applications of 1% isoconazole nitrate cream to the volar aspect of both arms in 12 volunteers. No difference in drug levels either within the horny layer or whole skin were found between the treatments. From this evidence it is anticipated that a once daily treatment regimen would be as clinically effective as a twice daily treatment regimen. High levels of the drug were found in the horny layer 10 days after the end of both treatments. This large and long lasting reservoir of the drug in the horny layer may provide protection of the skin against new infection for some time after treatment.

A twice daily application of topical antimycotics is usually recommended for the treatment of superficial dermatomycoses. With bifonazole it has been shown that a once daily application is clinically equally as effective as a twice daily application (Stettendorf, 1983; 1984). Other new antimycotics have also been successfully used when applied once daily (Meinicke, Striegel & Weidinger, 1984; Baumgartner & Graber, 1984).

All evidence for this new, simplified, treatment scheme comes from clinical trials. It is difficult, however, if not even impossible, to be sure whether patients have really applied their treatments according to the intended treatment scheme.

The present study is concerned with the pharmacokinetics of isoconazole nitrate—a broad spectrum antimycotic which is highly effective in dermatomycoses when applied twice daily as cream, solution or spray (Herms & Wendt, 1980). The objectives of this study, which was performed in healthy volunteer subjects, were:

1. To determine how long isoconazole is retained in the different layers of the skin following 14 days' daily application.

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2. To establish any differences in the concentration of isoconazole in the skin after once daily and twice daily topical administration over 14 days.

Materials and methods

Study design

Twelve normal, male volunteer subjects, without a history of skin disease, participated in the study after they had given their written, witnessed informed consent.

Isoconazole cream (Travogen®—Schering AG, Berlin) containing 1% ^{3}H-isoconazole nitrate (specific radioactivity 0·925 MBq/mg) was applied to 2 × 2 cm areas of the volar aspect of the arm and the application sites were occluded using Finn chambers and Scanpor tape. The total dosage per area was 20 mg cream (=0·2 mg isoconazole nitrate=0·185 MBq ^{3}H) which corresponds to a dose of 5 mg/cm^2 cream (=4·6 LBq ^{3}H). Each of the 12 subjects received 20 mg cream to a site on one arm twice daily and to a site on the other arm once daily for a total of 14 days. This was sufficient to leave the surface visibly covered by the topical application and is slightly in excess of the amount usually applied in treatment. The choice of left or right arms for the two treatments was randomized.

Before renewal of the cream the skin surface was cleaned with cotton pads. These pads were collected, together with the Finn chambers, in a glass vessel to estimate the total recovered radioactivity.

The arms were washed once per day with tap water before the morning application.

The 12 subjects were divided into three subgroups (A, B, C) for skin sampling, which was performed on the 15th day after the start of treatment for Group (A), on the 20th day for Group (B), and on the 25th day for Group (C). For sampling the horny layer skin surface biopsies (Marks & Dawber, 1971) were taken, and analysed as described previously (Finlay & Marks, 1985). For sampling viable epidermis and dermis, punch biopsies were taken.

Table 1. Recovery of ^{3}H activity on the skin after once or twice daily application of 1% ^{3}H isoconazole nitrate cream in a dose of 5 mg cream/cm^2 skin to the volar aspect of the arm of volunteers over 14 days. Values are expressed as percentage of the total dose administered ± standard deviation (n=4)

	Treatment	
Group	1 × daily	2 × daily
A	71·1 ± 9·0	83·0 ± 5·2
B	81·2 ± 4·4	81·1 ± 7·0
C	74·0 ± 7·2	85·0 ± 4·5
Mean total recovery	75·4 ± 13·6	83·1 ± 10·3

^{3}H radioactivity was measured by scintillation counting of extracts from skin surface swabs, skin surface biopsies and punch biopsies. Data that was collected on once or twice daily application was compared by the paired *t*-test.

Results

Recovery of the skin surface

The total ^{3}H recovery on the skin surface (removed by the pads during the 14 days' treatment) amounted to approximately 80% of the total dose (Table 1). Differences between both treatment schemes or between subgroups could not be detected.

Isoconazole concentration in horny layer

Table 2 shows the percentage of the dose in successive skin surface biopsies of the stratum corneum after both treatments 1, 5 and 10 days after the last treatment. The ^{3}H-radioactivity

Table 2. Percentage of dose in different portions of the horny layer: 1 (Group A), 5 (Group B) and 10 days (Group C) after the end of a 14-day treatment of the volar aspects of the arm with 1% ^{3}H isoconazole nitrate cream. Comparison between a once and a twice daily treatment

		Treatment			
		1 × daily		2 × daily	
Group	Horny layer depth	Per cent of single dose	Concentration* (μg/ml)	Per cent of single dose	Concentration* (μg/ml)
A	I	9·57 ± 4·09		7·36 ± 3·37	
	II	3·77 ± 0·71		5·20 ± 3·58	
	III	3·74 ± 1·44		1·86 ± 0·54	
	IV	2·07 ± 0·90		2·29 ± 1·28	
	Total	19·1	9574	16·71	8351
B	I	0·13 ± 0·03		0·15 ± 0·05	
	II	0·12 ± 0·01		0·07 ± 0·01	
	III	0·08 ± 0·01		0·04 ± 0·01	
	IV	0·01 ± 0·00		0·03 ± 0·00	
	Total	0·34	195	0·29	144
C	I	0·09 ± 0·03		0·09 ± 0·05	
	II	0·05 ± 0·01		0·03 ± 0·01	
	III	0·02 ± 0·00		0·02 ± 0·00	
	IV	0·01 ± 0·00		0·01 ± 0·00	
	Total	0·17	85	0·15	81

* Calculation is based on a mean thickness of the horny layer of 10 μm.
I = uppermost distal layer; IV = inner proximal layer.

Table 3. Mean concentration of isoconazole in living skin: 1 (Group A), 5 (Group B) and 10 (Group C) days after the end of a 14-day treatment of the volar aspects of the arm with 1% isoconazole nitrate cream. Comparison between a once daily and a twice daily treatment

Group	dpm	Concentration* (ng/ml)	dpm	Concentration* (ng/ml)
A	3724 ± 948	680 ± 170	4575 ± 1856	830 ± 340
B	518 ± 41	94 ± 75	607 ± 145	110 ± 26
C	353 ± 68	64 ± 12	306 ± 23	56 ± 4

* Calculation is based on a mean thickness of the skin of 1 mm.

within the horny layer declined from distal to proximal by a factor of 5, independent of the time point investigated. At one day after the end of both 14 days' treatment regimes, 15–20% of a single dose was found in the horny layer. This corresponds to mean drug levels of 8,000–10,000 μg/ml, assuming a horny layer depth of 10 μm. Five days later 0·3% of a single dose was detected in the horny layer, which corresponds to an isoconazole concentration of approximately 150 μg/ml. Ten days after the end of the treatment, 0·16% (=80 μg/ml) was found in the horny layer. There were no differences between the two treatment regimes.

Isoconazole concentration in the living skin

Distinctly lower drug levels were found in the living skin (epidermis and dermis) (Table 3). One day, after the end of the treatment period, the mean concentration of radioactivity in skin amounted to 700–800 ng isoconazole equiv./ml. After 5 days, levels of 100 ng/ml, and after 10 days, levels of 60 ng/ml were found. No differences between the two treatments could be detected.

Discussion

A rapid release of the drug from the chosen formulation and its ready availability in the superficial layers of the skin, in concentrations above the minimal inhibitory or the minimal biocidal concentration for adequate periods of time, are necessary prerequisites for the clinical efficacy of a topical antimycotic (Täuber, 1980; Täuber & Rzadkiewicz, 1979). After a single application of isoconazole preparation (Travogen® cream), a rapid penetration of isoconazole into the horny layer and the living epidermis has been shown (Täuber & Rzadiewicz, 1979). In clinical trials, excellent healing rates of more than 90% have been reported in dermatomycosis when applied in the recommended twice daily treatment scheme. In the present study the levels of the antimycotic were determined in the stratum corneum and skin biopsies at different time points after a once or twice daily treatment of volunteers with ^{3}H-labelled isoconazole preparation (Travogen® cream) over 14 days. No differences in the concentration of the

^{3}H-activity in the stratum corneum and skin biopsies could be found between the treatment schemes. Since isoconazole is not metabolized in human skin in significant amounts (Täuber & Rzadkiewicz, 1979) the radioactivity measured in the horny layer represents essentially unchanged drug. It is obvious that a twice daily application of 1% isoconazole nitrate cream does not further increase the levels of the drug, either in the horny layer or in the living skin, as compared to a once daily application.

It has to be assumed that the levels of the drug in the horny layer and in the living skin 1 day after 14 days' treatment corresponds to the steady-state levels in these skin layers during therapy. The drug levels in the horny layer in the present study proved to be two to three times higher than those found in previous *in vitro* investigations with the thigh skin after a single application of the cream (Täuber & Rzadkiewicz, 1979). The higher levels in the horny layer *in vivo* could be explained by regional differences in penetration since different anatomical sites have been used. They could be explained, likewise, as an effect of the occlusion or could be caused by an accumulation in the skin during the multi-application experiment.

After a single application in the *in vitro* experiment, high levels were found in epidermis and dermis as compared to the *in vivo* situation. This may be due to an accumulation of substance in the skin which does not occur *in vivo* where the antimycotic is removed via the blood circulation.

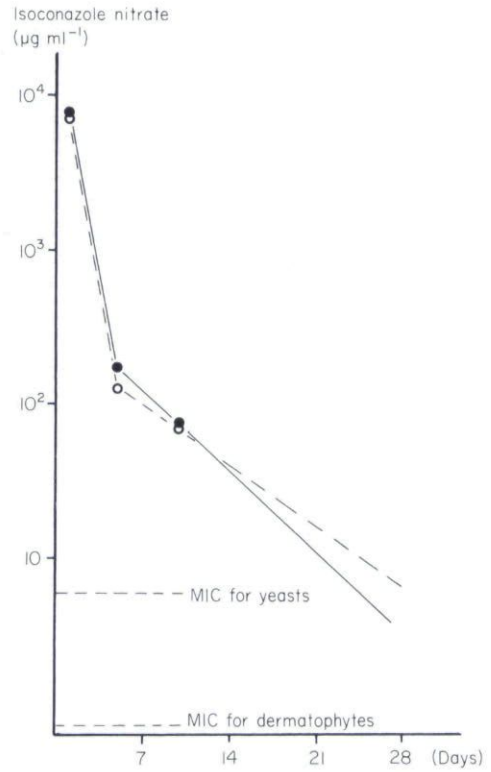

Figure 1. Mean isoconazole concentration in the horny layer 1, 5 and 10 days after a 14-day treatment with 1% isoconazole nitrate cream. Comparison between once daily (●–) and twice daily (○– –) applications.

The mean concentration of isoconazole in the horny layer decays bi-phasically with half-lives of approximately $t_{\frac{1}{2}\alpha} = 12$ h and $t_{\frac{1}{2}\beta} = 4$–5 days (Fig. 1). Again, no differences between the two treatment regimens could be seen. Even 10 days after the end of both treatments the mean drug levels in the horny layer were more than 10-fold above the MIC-values for yeasts and yeast-like fungi (median of 24 strains 6 μg/ml) and approximately 100-fold above the MIC-values for dermatophytes (median of 18 strains 0·8 μg/ml) (Kessler, 1980; Täuber, 1980).

Extrapolation of the decay curves of Fig. 1 for a longer period of time indicates that drug levels in the horny layers would be above MIC-values for yeasts and dermatophytes for between approximately 4 and 6 weeks after the end of therapy. This is substantially longer than the normal turnover time for the stratum corneum and would suggest that there is a diffusion of the drug downwards into the newly formed stratum corneum.

Since no differences were found between the two treatment regimens in steady-state levels of isoconazole nitrate, it has to be assumed that a once daily treatment of dermatomycoses with the isoconazole preparation used is as equally effective when compared with a twice daily treatment. Dermatomycoses should be treated with topical antimycotics for 2–4 weeks until the skin and horny layer are completely normal. Due to the long-lasting drug reservoir in the horny layer, active protection against re-infection may be available for several weeks after the end of treatment.

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