ELSEVIER

European Journal of Pharmaceutical Sciences, 5 (1997) 119–127

EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES

Nail penetration of the antifungal agent oxiconazole after repeated topical application in healthy volunteers, and the effect of acetylcysteine

E.J. van Hoogdalem*, W.E. van den Hoven, I.J. Terpstra, J. van Zijtveld, J.S.C. Verschoor, J.N. Visser

Yamanouchi Europe B.V., P.O. Box 108, 2350 AC Leiderdorp, Netherlands

Received 21 December 1995; accepted 19 November 1996

Abstract

Poor response of fungal nail infections to topical treatment with antimycotics is probably related to poor drug penetration into the infected nail. The aim of the present study was to evaluate the in vivo nail penetration of the antimycotic oxiconazole from a 1% w/v lotion, and to evaluate the potential penetration enhancing properties of co-delivered acetylcysteine. Six healthy volunteers were treated with 1% w/v oxiconazole lotions with or without acetylcysteine (15% w/v), according to a left–right study design. Treatment was performed twice daily for 6 weeks. Nail clippings were collected every 2 weeks over an 8-week period, until 2 weeks after the treatment stopped. Oxiconazole levels at various depths in sectioned nail clippings were measured by gas chromatographic analysis of ethanolic nail extracts. Topical treatment with oxiconazole nitrate without acetylcysteine resulted in maximum drug levels in the upper 0–50-μm layers varying between 120 and 1420 ng mg^{-1}; levels decreased downwards. Total uptake into the nail was less than 0.2% of the topical dose, indicating a substantial barrier function of the nail. Co-delivery with acetylcysteine statistically significantly prolonged the mean residence time of oxiconazole in the upper 51–100-μm ring finger nail layers from 3.7–4.9 weeks to 4.1–6.4 weeks, implying increased retention of the drug in the nail. Mean (±S.D.) peak drug levels increased from 790±420 ng mg^{-1} to 1570±820 ng ml^{-1} in the upper 0–50-μm layer, which suggested a variably enhancing effect of acetylcysteine on the extent of oxiconazole nail penetration in the upper nail layers. The effect of acetylcysteine was speculated to be related to increased binding of oxiconazole to nail constituents.

Keywords: Oxiconazole; Nail penetration; Acetylcysteine; Volunteers

1. Introduction

Onychomycosis, infection of the nail by fungi, represents a significant therapeutic problem in dermatology because of failure in establishing and maintaining effective drug levels at the infection site (Zaias, 1990). Recent advances in this therapeutic area were made by the development of the antimycotic agents terbinafine and itraconazole, both diffusing into the nail up to efficacious levels on repeated oral dosing (Roseeuw and De Doncker, 1993a; Schäfer-Korting, 1993). With respect to topical drug application, amorolfine nail lacquer has become available, showing rapid intraungual diffusion in vitro and reaching effective drug levels at the target site (Reinel and Clarke, 1992; Polak, 1993). The present study addresses the nail uptake of the imidazole antifungal agent oxiconazole after its topical application, and it explores the feasibility of nail penetration enhancement.

Oxiconazole is applied topically as nitrate in cream, solution or powder in the treatment of fungal infections of the skin. It is active against various dermatomycetic and non-dermatomycetic fungi and yeasts, comparable to econazole and miconazole (Jegasothy and Pakes, 1991). A once-daily applica-

*Corresponding author. Tel.: +31 71 455515; Fax: +31 71 455560.

0928-0987/97/$32.00
PII S0928-0987(97)00270-4

tion frequency maintained for 2–7 weeks is generally adequate in the treatment of cutaneous infections with oxiconazole.

Concerning penetration into nails and skin, it has been shown that oxiconazole penetration behaviour is dependent on the formulation; tinctures gave better in vitro nail penetration than ointments, but in abdomen skin the reverse was observed (Stüttgen and Bauer, 1982). In vitro data demonstrate an outspoken barrier function of the nail for oxiconazole from a 1% tincture; amounts penetrating into the ventral nail plate proved to be a 30-fold lower than those into the dorsal side (Stüttgen and Bauer, 1982). Stüttgen suggested that co-delivery of 10% of urea increased uptake of oxiconazole into the nail, possibly by influencing keratin structure in the nail (Stüttgen, 1989). Following this suggestion, the mucolytic agent *N*-acetylcysteine (acetylcysteine) was speculated to exert a penetration promoting action by disrupting keratin disulfide bonds.

The aim of the present study was to evaluate the penetration of oxiconazole into the human nail after repeated administration and to assess the potential influence of acetylcysteine on this process.

2. Experimental procedures

2.1. Chemicals

Oxiconazole nitrate (lot no. 711017) was obtained from Hoffman–La Roche (Basle, Switzerland). Econazole nitrate was purchased from Sigma (St. Louis, USA). Ethanol (99.8%; p.a.) was supplied by Merck (Darmstadt, Germany). Helium (pure; E-70-H) and nitrogen gas (very pure; S-71-H) were purchased from Hoek Loos (Amsterdam, Netherlands). Aqueous solutions were prepared using deionized water supplied by a Milli-Q water purification system (Millipore–Waters, Etten-Leur, Netherlands). Other reagents were of analytical grade, and these were used as received.

2.2. Clinical experiment

The clinical study protocol was reviewed by an independent Medical Ethical Committee and the study was started after obtaining their approval. The study was conducted in accordance with the Principles of the Declaration of Helsinki on biomedical research involving human subjects, updated in Hong Kong in 1989.

Healthy volunteers, 2 males and 4 females, were randomly selected from a volunteer panel. During a treatment period of 6 weeks, volunteers applied the oxiconazole lotion without penetration enhancer on nails of one hand, and the lotion with acetylcysteine on nails of the other hand. Application was done twice daily, after getting up and before going to bed, on the nails of middle finger and ring finger. A 10-μl volume of solution was applied per finger nail, using a fixed volume pipette (Socorex Micropipette; Omnilabo Nederland, Breda, Netherlands). Volunteers were instructed to wash the nails with tap water prior to each application, and to avoid all exposure to water within a 1-h period of application. In addition they were instructed to avoid circumstances giving strong nail hydration, such as swimming, sauna visits and dish washing with ungloved hands, during the entire 8-weeks study period. To avoid in vivo oxiconazole degradation, subjects were requested not to expose themselves to excessive UV-light.

Volunteers returned to the laboratory on weekly intervals, for nail inspection, photography of the nails, collection of nail clippings and to check protocol adherence. Prior to application, during and after the 6-week treatment period, viz. after 2, 4 and 6 weeks' treatment and after week 8, nail cuttings were collected. Cutting was done prior to the subsequent application, after thoroughly brushing the nails under tap water.

Nail clippings were collected individually in polyethylene vials (Packard Instrument, Groningen, Netherlands), and they were stored at room temperature until analysis.

2.3. Sample analysis

Clippings were fixed, ventral side downwards, on a specimen holder using cyanoacrylate glue (Permacol 302 FS contact cement; Permacol-Ede, Ede, Netherlands), thus enabling nail sectioning over its entire surface. Subsequently, the nail clippings were cut parallel to the surface in sections of 20-μm thickness (Microtome 5030; Bright Instrument, Huntingdon, UK). Of the middle finger nails, three series of five subsequent sections were collected in separate vials, representing nail layers at depths of 0–100, 101–200 and 201–300 μm, respectively. On interim analysis

of these results it was decided to collect the first 100-μm layer of ring finger cuttings in two separate fractions. Consequently, three series of subsequent 25-μm sections of ring finger nails were collected, representing the 0–50, 51–100 and 101–200-μm layers. All sectioning was done at room temperature. Assuming an uniform density throughout the nail, weights of the sections thus collected as one sample were calculated as:

weight sectioned layer in sample
= (number of sections/sample)
× (section thickness)
× (total weight clipping)/
(total thickness clipping)

One hundred μl of internal standard solution (econazole nitrate; 860 ng ml^{-1}) and 900 μl of ethanol were added to each sample of sections. Subsequently, the samples were incubated for 72 h at 50°C in a water bath (GFL, Hannover-Vinnhorst, Germany). One μl of the resulting extracts was injected on a gas chromatograph with electron capture detection (HP 5880A, Hewlett–Packard, Avondale, USA), using a HP 7673A automatic sampler. The temperature of injection port, column and detector were maintained at 240°C, 250°C and 300°C, respectively. Helium (25 ml min^{-1}) and nitrogen (40 ml min^{-1}) were used as carrier gas and make-up gas, respectively. In order to accommodate for interference by glue components, the lower nail sections were chromatographed at flows of 18 ml min^{-1} and 150 ml min^{-1}, respectively. Split flow and septum flow were kept at 220 ml min^{-1} and 5 ml min^{-1}, respectively.

Data processing was performed on a HP 5880A GC terminal. Oxiconazole contents in nail samples were calculated by relating their detector responses to a calibration curve, using unweighted linear regression. The calibration curve spanned the range of 5–650 ng/sample. Subsequently, nail concentrations, expressed as oxiconazole nitrate levels (ng g^{-1}), were calculated as:

nail concentration
= (amount oxiconazole nitrate/sample)/
(weight sectioned layer in sample)

2.4. Data analysis

Pharmacokinetic data analysis was performed using Siphar 4.0 b (SIMED, Créteil, France). For each nail layer, the maximum concentration reached during the 8-week study period (C_{max}), the time at which the maximum level was reached (t_{max}), and the residual nail concentration after 8 weeks ($C_{week\ 8}$) were determined. The areas under the nail concentration versus time curves from 0–8 weeks (AUC_{0-8}) were calculated using the linear–logarithmic trapezoidal rule (Chiou, 1978). The mean residence time (MRT) was calculated as $AUMC_{0-8}/AUC_{0-8}$, AUMC referring to the area under the moment versus time curve (Rowland and Tozer, 1989). The MRT reflects the mean time period during which a compound is present in the sampled compartment.

The effects of the presence of acetylcysteine were analysed statistically by comparing within-subject results with and without enhancer, using the Wilcoxon rank sign test and maintaining a comparison-wise error of $\alpha = 0.05$.

3. Results

3.1. Bioanalytical method

In an attempt to spike blank nail material with oxiconazole, nail sections were pre-incubated in 50 and 100 μg ml^{-1} solutions of oxiconazole nitrate in ethanol. Extraction of these spiked sections with ethanol during 1–72 h was found to reach a plateau after a 48 h incubation period (data not shown). After 72 h extraction at 50°C, the recovery (±S.D.) from sections pre-incubated in 50 and 100 μg ml^{-1} of oxiconazole amounted to 91±15% ($n = 10$) and 114±31% ($n = 10$), respectively. Recoveries were expressed relative to the amount of drug which penetrated into the nail during incubation, and they were assessed in parallel experiments with radiolabelled oxiconazole. These results indicate complete extraction of oxiconazole from nail sections in the currently used method.

Under these circumstances retention times of econazole and oxiconazole amounted to 3.2 and 5.5 min, respectively (Fig. 1). The detection limit, defined as (2× noise level)/(slope calibration curve), amounted to 1.9 ng/sample. In the analysis of the lower layers, the modified flows of helium and nitrogen increased the detection limit to 2.4 ng/sample.

Calibration curves proved to be linear in the concentration range used. Correlation coefficients

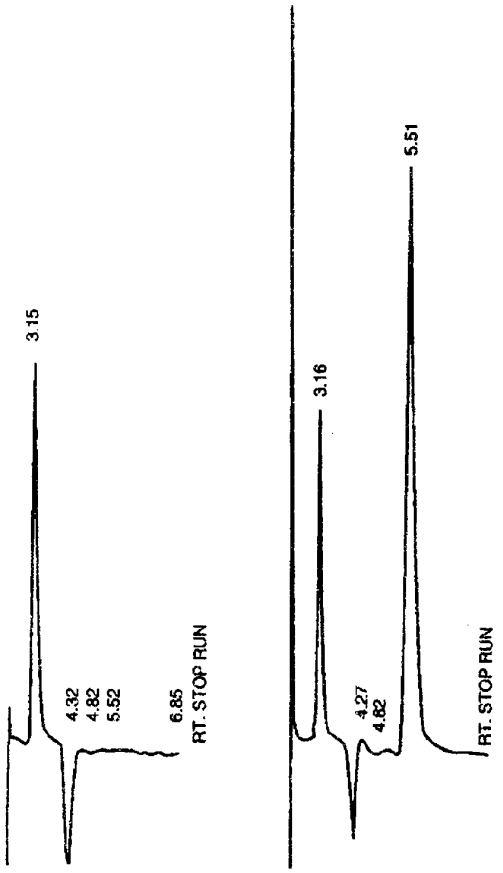

Fig. 1. Chromatograms of untreated nail (left panel), and of a sample of 5 pooled sections (0–100 μm) of a nail treated for 2 weeks with an oxoconazole 1% w/v lotion, the sample containing 261 ng mg^{-1} oxiconazole nitrate (right panel); peaks at 3.2 and 5.5 min represent econazole and oxiconazole, respectively.

amounted to 0.9991 or higher. Reproducibility of injection, expressed as coefficient of variation ($n=9$), amounted to 1.9–5.1% in the concentration range of 5–700 ng/sample. Performance of the method was not affected by presence of acetylcysteine (data not shown).

3.2. Penetration kinetics

Repeated application of oxiconazole nitrate on middle finger nails resulted in maximum drug levels in the upper 100-μm layer varying between 70 and 1200 ng mg^{-1}, and lower profiles were present in the 101–200-μm layer underneath (data not shown). Since the major trends in acetylcysteine effects appeared to be observed in the upper 100-μm layer of middle finger nails, it was decided to make further discrimination between the 0–50-μm and 51–100-μm layer using ring finger nails.

In ring finger nails, the upper 50-μm layer showed a tendency of increased oxiconazole contents on delivery with acetylcysteine (Fig. 2a). In the lower layers, effects with and without acetylcysteine were comparable, apart from a statistically significant increase in MRT and $C_{week\ 8}$ (Table 1).

Generally, the effect of acetylcysteine on C_{max} and AUC_{0-8} of oxiconazole nitrate was not reproducible in all subjects. In terms of individual AUC_{0-8}-values, the effect of acetylcysteine on upper layer drug contents varied between a 7-fold decrease and a 16-fold increase as compared with application without acetylcysteine. For the upper layers of ring finger nails, this implied a two-fold increase in mean uptake (Table 1), as also observed for the upper 100-μm layer of middle finger nails (data not shown). Effects on the parameters C_{max} and AUC_{0-8}, however, did not reach statistical significance (Table 1).

The maximum extent of oxiconazole nail uptake was estimated from the data set showing highest C_{max} and AUC_{0-8} values, viz. the right ring finger of one subject, treated with lotion containing acetylcysteine. From the last nail clipping, collected after week 8, 1159 ng of oxiconazole nitrate was recovered. Assuming that this clipping represented 1/7 of the total nail plate, and assuming homogenous distribution of drug over the nail, the total amount of oxiconazole nitrate present in the nail after week 8 amounted to 8 μg. From previously collected clippings, 3221 ng were collected, adding up to 11.3 μg of drug which penetrated into the nail over the entire treatment period. In this period, comprising twice daily application of 10 μl of 1% w/v oxiconazole for 6 weeks, a total dose of 9.7 mg of oxiconazole nitrate had been applied. Consequently, the extent of oxiconazole uptake into the nail may be estimated at 0.12% at the highest.

3.3. Adverse events

Topical application of the lotions was well tolerated. At post-study physical examination, no abnormalities were reported. Concerning the influence of topical treatment with acetylcysteine on nail growth, no outspoken effects of any additive on nail thickness

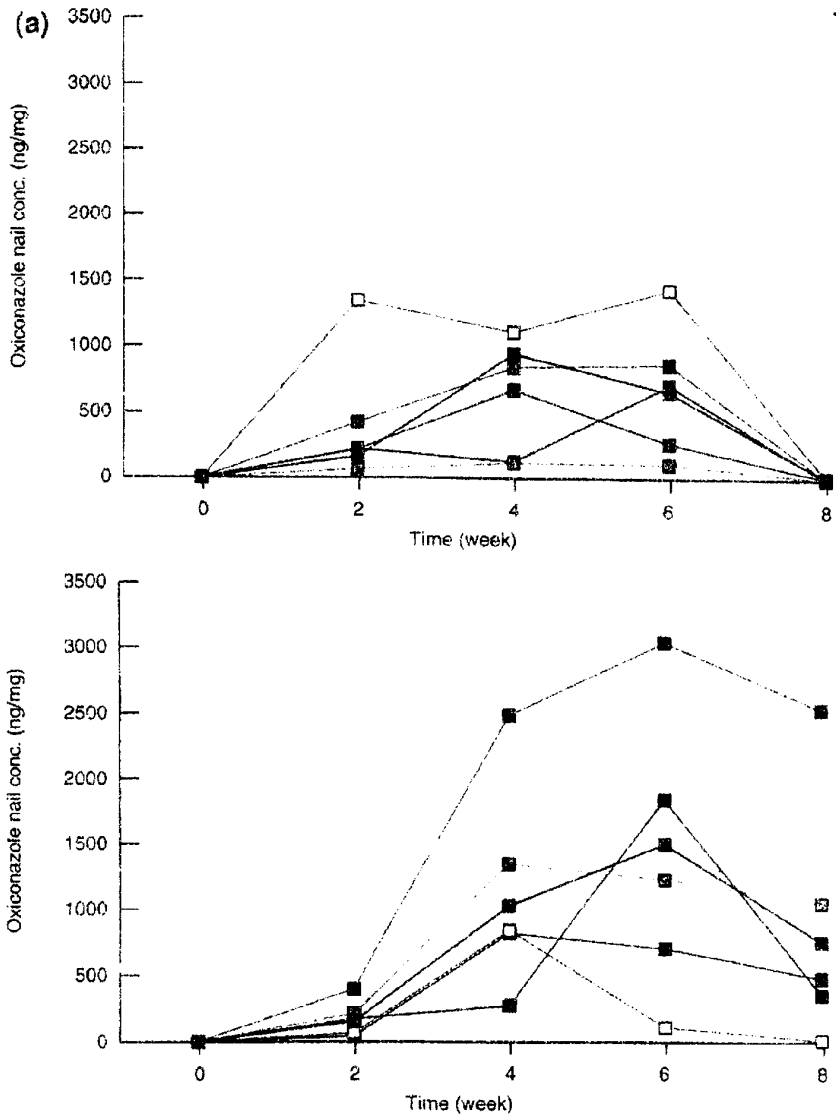

Fig. 2. Individual oxiconazole ring finger nail levels during and after 6 weeks' treatment with 1% w/v oxiconazole lotion without (upper panels) and with (lower panels) acetylcysteine 15% w/v in 6 healthy subjects; profiles as observed at three subsequent nail layers are shown, viz. (a) the upper 50-μm, (b) 51–100-μm and (c) 101–200-μm.

or nail clipping weight were observed (data not shown).

4. Discussion

Repeated application of oxiconazole lotions to the nail resulted in measurable drug levels in the upper nail layer; the concentrations decreased downwards (Fig. 2; Table 1). These profiles, and the low extent of uptake in the nail, viz. less than 0.2%, indicate an important barrier function of the upper nail layer. This low permeating potential is in line with the previously reported poor systemic absorption of topical oxiconazole, only 0.3% of the topical dose being recovered from urine and faeces (Jegasothy and

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.