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Absorption of Amorolfine through Human Nail

Key Words
Amorolfine
Nail
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Pharmacokinetics

Abstract
The percutaneous absorption of a new antifungal agent, amorolfine, has been measured through both skin and nail using an in vitro technique. Application of a 5% concentration in either an ethanol or methylene chloride lacquer resulted in permeation rates through nail in the range of 20–100 ng/cm^2/h and somewhat higher through skin. Absorption was greater from the methylene chloride lacquer than the ethanol lacquer.

Introduction

Topical therapy for fungal infections of the human nail has been largely ineffective, and this failure may in part be due to poor penetration of the therapeutic agent into the nail plate. The thickness of the nail and its relatively compact construction make it a formidable barrier to the entry of topically applied agents. In this study the penetration of amorolfine [4-{3-[*p*-(1,1-dimethylpropyl)phenyl]-2-methyl-propyl}-2,6-*cis*-dimethylmorpholine hydrochloride] into human nail has been examined using an in vitro permeation model.

Materials and Methods

Two formulations of amorolfine were examined in this study. Both were supplied by Hoffmann-La Roche (Nutley, N.J.) and consisted of 5% drug in either a methylene chloride or ethanol lacquer. To each was added a tracer amount of ^{3}H-amorolfine (specific activity 100.7 μCi/mg), giving a final activity of approximately 100 μCi/ml.

Human nails were obtained at autopsy from local hospitals and stored in screw-cap glass vials at –20 °C. Prior to use the nails were cleansed of adherent blood or tissue using a small curette and/or tap water. They were then mounted in special glass diffusion chambers fitted with a plastic top having the same curvature as that of the nail. When using the great toenail or thumbnail, it was first split in half so that each test formulation could be run in duplicate on pieces of nail having the same thickness and obtained from the same donor. The receptor portion of the diffusion chambers was filled with phosphate-buffered isotonic saline (pH 6.0) and maintained at 37 °C by circulating temperature-controlled water through an outer jacket. Stirring of the receptor phase was achieved by a teflon covered magnet activated by a 600-rpm motor placed beneath the chamber. The outer portion of the nail was left open to ambient laboratory conditions, approximately 22–23 °C and 35–55% relative humidity.

Percutaneous absorption was measured using a modification of the method of Franz [1]. Following a 24-hour equilibration period, each formulation was applied to the nail by microsyringe at a dose of 0.01 ml/cm^2. The rate and extent of absorption was measured by removing the receptor solution at intervals over the next few days and assaying a

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1-ml aliquot for radioactive content by liquid scintillation spectroscopy. In some experiments, additional doses of amorolfine were applied after first removing the prior dose with acetone-soaked cotton swabs.

For comparative purposes, several experiments were conducted in which amorolfine absorption through human skin was also measured. Human trunk skin was obtained at autopsy, sectioned with a dermatome to a thickness of approximately 250 μm and stored at –20 °C. Just prior to use it was thawed by placing in 37 °C for 5 min, cut into small sections and mounted on 9-mm Franz cells. All other aspects of the experiments were similar to those described for the measurement of nail permeation.

The uptake of amorolfine at equilibrium by small pieces of nail plate was also determined. Nails from 2 donors were cut into multiple 20- to 40-mg sections and placed in small screw-cap vials to which 1 ml of either the ethanol or methylene chloride lacquer was added. After 48 h at room temperature, the sections were removed and placed for 1 min in 100 ml of ethanol, which was then vigorously stirred. Following this the sections were removed, blotted dry, and dissolved in 3 ml Soluene (Packard Instrument Company). An 0.1-ml aliquot was counted to determine radioactive content.

Results

The rate of absorption of amorolfine through human thumbnail following a single application of the ethanol and methylene chloride lacquer is shown in figure 1. The flux reaches a peak between 5 and 25 h, then appears to slowly decline. Following an acetone wash of the surface at 68 h, there is a further decline in the flux. Amorolfine absorption from the methylene chloride lacquer is somewhat greater than that from the ethanol lacquer over the entire time course of the experiment. At the peak, the flux approximates 100 ng/cm^2/h from the methylene chloride vehicle.

Reapplication of the lacquers to the same nail sections shown in figure 1 were made 3 times over the subsequent 8 days. The results are shown in figure 2. With each reapplication the rate of absorption profile was found to be similar to that seen with the first application. Following a peak reached after 10–20 h the rate slowly declined until the time of the next acetone wash and reapplication. The variable nature of the amorolfine peak on each reapplication of the ethanol lacquer is puzzling and its significance is not known at this time.

For comparison, amorolfine absorption through trunk skin is shown in figure 3. As with nail an initial peak is seen, at approximately 2 h, followed by a steady state over most of the subsequent 48 h. The flux is greater from both lacquers through skin than through nail, ranging from 100 to 200 ng/cm^2/h, but is again seen to be higher from the methylene chloride vehicle.

Amorolfine absorption through nail following pretreatment with the penetration enhancer dimethyl sulfoxide

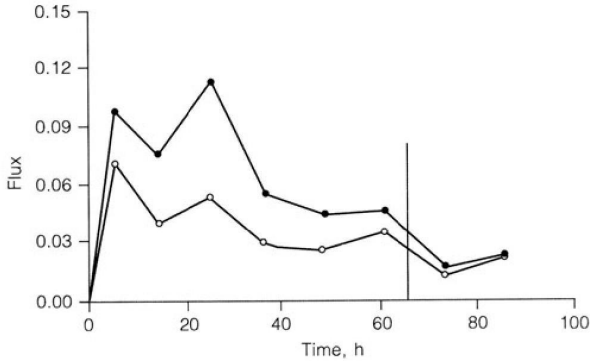

Fig. 1. Rate of absorption (μg/cm^2/h) following a single application of amorolfine to duplicate halves of both thumbnails from a single donor. Data were averaged for each vehicle. Vertical line indicates surface wash. ● = Methylene chloride; ○ = ethanol.

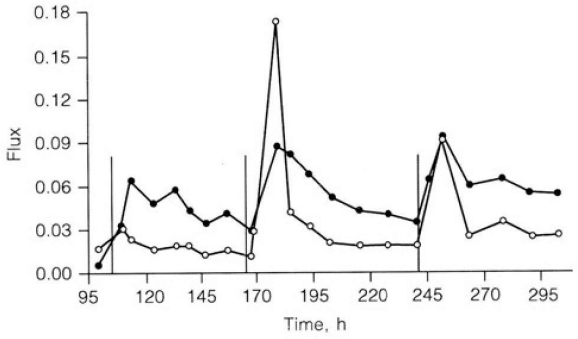

Fig. 2. Rate of absorption (μg/cm^2/h) following reapplication (vertical lines) of amorolfine to the same nail sections shown in figure 1. Data were averaged for both vehicles. ● = Methylene chloride; ○ = ethanol.

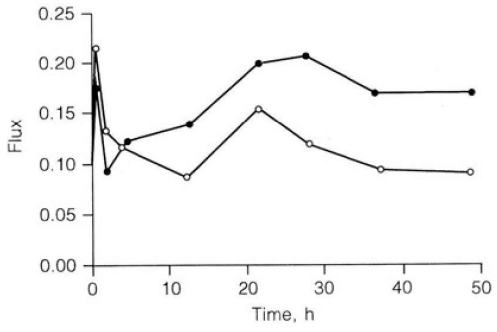

Fig. 3. Rate of amorolfine absorption (μg/cm^2/h) through human trunk skin. Each vehicle was applied to triplicate sections from the same donor and the data averaged. ● = Methylene chloride; ○ = ethanol.

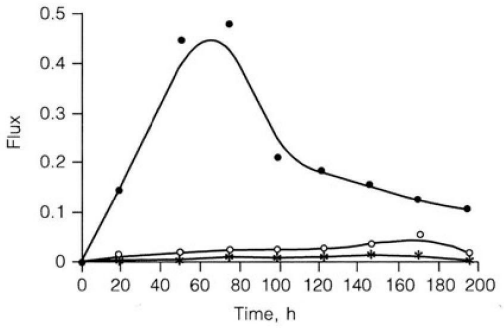

Fig. 4. Rate of amorolfine absorption (% of dose) following application from toluene. DMSO was applied to one of three nail sections from the same donor 5 min prior to drug application. ● = DMSO; ○ = no DMSO; * = no DMSO.

(DMSO) is shown in figure 4. DMSO was applied to one section of a single donor's nail for 5 min, then removed and the surface carefully dried. A tracer amount of ^{3}H-amorolfine in toluene was then applied and the rate of absorption measured. For comparison, the rate of absorption through two nail sections from the same donor not pretreated with DMSO is also shown. The results are similar to those seen following use of DMSO on skin [2]. A large increase in drug penetration is noted in the pretreated as opposed to untreated nail sections, and this increase persists over the entire 8 days of the experiment.

The uptake of amorolfine into the nail plate from both of the lacquer formulations following a 48-hour equilibration is given in table 1. Uptake is greater from the methylene chloride lacquer than the ethanol lacquer (2.9 vs. 1.2 μg drug/mg nail, respectively), and this in keeping with the flux data.

Table 1. Nail uptake of amorolfine following 48-hour soak

Vehicle	Drug, μg/mg nail	n
Methylene chloride	2.9 ± 0.6	4
Ethanol	1.2 ± 0.4	4

Values are mean ± SD.

Conclusions

The absorption of amorolfine through human nail and trunk skin has been measured using an in vitro technique. When applied from either a methylene chloride or ethanol lacquer the drug is found to penetrate both nail and skin. In nail the rate of absorption profile is characterized by a peak followed by a slow decline. Rates of permeation through nail are in the range 20–100 ng/cm^{2}/h, and somewhat higher through skin. Absorption was found to be consistently higher from the methylene chloride lacquer than the ethanol lacquer. Pretreatment of nail with the penetration enhancer DMSO resulted in a large increase in amorolfine penetration.

References

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