A Method to Measure the Amount of Drug Penetrated across the Nail Plate

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INTRODUCTION

One of the major difficulties in development of products for the treatment of nail diseases is the lack of proper *in vitro* methods to measure the extent of drug penetration across the nail plate. A few albeit limited nail penetration models are available currently (1,2). One of the drawbacks of these models is that they may not provide an intimate contact between the receptor compartment and the nail surface. Moreover, the nail plate can be easily overhydrated beyond normal level.

To develop an *in vitro* nail penetration model, it is important to understand the physiochemical characteristics of the nail and the nature of nail diseases. The physiochemical properties of the nail, as evidenced in various experimental studies, indicate that it behaves more like a hydrophilic gel membrane as opposed to a lipophilic membrane, such as the stratum corneum (2–5). The degree of hydration of the nail is regarded as the most important factor influencing the physical properties of the nail. Another important factor to be considered is that fungi involved in onychomycosis could grow within different areas of the nail, i.e., in the nail plate, the nail bed, and the nail matrix (6,7).

The main objectives of this study were twofold: (1) to develop an experimental setup that simulates the human nail plate and the nail bed and (2) to measure the amount of drug penetrating across, as well as the amount within, the nail plate. Porcine hoof membrane and poloxamer gel were used to simulate the nail matrix and the nail bed, respectively, and ciclopirox was chosen as a model drug.

MATERIALS AND METHODS

Materials

Ciclopirox was a gift from Hana Pharm. Co. (Seoul, South Korea). All other chemicals were reagent grade or above and were used without further purification.

Assay Method

To block the *N*-hydroxyl group of ciclopirox, which interacts strongly with the silica-gel based stationary phase, the methylation of *N*-hydroxyl group was accomplished with dimethyl sulfate using a method reported previously with some modifications (8). The methyl derivative was analyzed by high-performance liquid chromatography (Shimadzu Scientific Instruments Inc., Columbia, MD).

Development of an *In Vitro* Penetration Model

Preparation of Porcine Hoof Membranes

Porcine horn hooves were acquired fresh and cleaned with distilled water. Clean porcine hoof membranes were placed in a vacuum oven at 40°C for about a day until they became free of removable water. The weight (A) and thickness of dry porcine hoof membranes were measured. The hoof membranes were kept for 24 h on a dampened gauze at 32°C, which allowed them to recover water lost during the drying process, and then they were weighed (B). The water content was measured again at the end of the penetration study (C). The "nail swelling" was calculated using the following equation:

$$\text{Nail swelling} = \frac{\frac{(B-A)}{A} + \frac{(C-A)}{A}}{2}$$

Penetration Study Procedure

Commercial ciclopirox lacquer (8%) was loaded on the dorsal side of porcine hooves. Each porcine hoof was placed carefully on a poloxamer gel prepared in a plastic chamber. During the penetration study, the chamber was sealed to protect the system from water evaporation, and the temperature was maintained at 32°C. At scheduled sampling points, the nail in the plastic penetration chamber was separated from poloxamer gel, and the lacquer was removed from the nail. The remaining poloxamer gel on the nail was removed by briefly vortexing the nail with 1 ml of distilled water in a scintillation vial. Then, the nail was wiped using clean tissues and weighed (C). It was cut into small pieces and extracted with 10 ml of 5% methanol aqueous solution at 60°C for 1 day. The same solvent extraction process was repeated twice. Two milliliters of extract was transferred into a screw-capped test tube for subsequent derivatization as described in the assay section.

The remaining poloxamer gel in the penetration chamber was added with 2 ml of distilled water, 1 ml of distilled water that was used to remove poloxamer gel on the nail, and 5 ml of ethylacetate. After centrifugation, 3.5 ml of the organic phase was taken into screw-capped test tube and left to evaporate under nitrogen stream. The residue was dissolved with 2 ml of 5% methanol aqueous solution for subsequent derivatization as described in the assay section.

Stability of Ciclopirox Methyl Derivatives

The stability of ciclopirox methyl derivative was evaluated at the following experimental conditions: –18°C/light protected, –1°C/light protected, ambient temperature/light protected, ambient temperature/under ambient light, and 40°C/light protected.

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Diffusivity of Ciclopirox in Poloxamer Gel

Empty plastic syringes (25 ml) with the top removed were filled with approximately 10 ml of 20% poloxamer solution from the open end with the plungers in the pulled-out position. The syringes were kept in the open-end up position at room temperature to form a semisolid gel (9). Two-hundred microliters of hexane solution containing 5 mg/ml of ciclopirox was applied on the gel surface and left for the evaporation of hexane by purging nitrogen gas. After incubating the syringe sets for 3 days in an oven at 32°C, the gel in the syringe was slowly expelled out using the plunger at an approximate rate of 1 ml of the gel per push. After discarding the first slice, a total of six slices with a uniform volume were sequentially cut and weighed. The concentration of ciclopirox in each gel slice was quantitated.

Development of an Extraction Method

Extraction of Ciclopirox from Hoof Membranes

To find an optimum temperature, solvent composition, and duration of time required for extracting ciclopirox from the hoof membranes, the following experiments were performed. The hoof membranes in pieces were transferred into 20-ml vials, and ciclopirox was extracted from the hoof using 10 ml of aqueous solution containing various concentrations of methanol (2.5, 5, 10, and 20 w/w %) at ambient temperature, 37°C, and 60°C for 4 days. Then, the extract was transferred into a vial for quantification. The same solvent extraction process was repeated twice.

The extraction period required for optimum extraction process, in which 5% methanol aqueous solution was used as an extraction solution, was evaluated at 60°C. The extraction process was repeated until the ciclopirox in the extract was not detectable.

Extraction of Ciclopirox from the Poloxamer Gel

To find an optimum solvent for extraction of ciclopirox from 20% poloxamer gel, the extraction efficiency of various solvents was compared. Five milliliter of the poloxamer solution containing ciclopirox was transferred into a screw-capped test tube, and each test solvent was added. The mixture was shaken for 20 minutes. Two phases were separated by centrifugation. Three milliliter of each extraction solvent was transferred into another screw-capped test tube and left to evaporate under nitrogen stream. The residue was dissolved by 2 ml of 5% methanol aqueous solution. The control group consisted of 5% methanol solution containing ciclopirox.

RESULTS AND DISCUSSION

Stability of Ciclopirox Methyl Derivatives

The degradation rate of ciclopirox methyl derivative was accelerated by light, but it was not affected by the temperature up to 50 days. The degradation rate of ciclopirox methyl derivative followed pseudo first-order kinetics. The half-life of ciclopirox methyl derivatives under the ambient and lighted condition was approximately 130 h, with the first order rate constant of 0.0053 h^{-1}. When ciclopirox methyl derivative was protected from light, it was stable at temperature ranging from −18°C to 40°C for at least 50 days. Based on these results, all analysis were performed under light-protected conditions.

Diffusivity in Poloxamer Gel

Poloxamer was chosen as the receptor phase because it undergoes sol-gel transformation. Because it is a gel under the conditions described for the penetration study, the nail plate can be placed on the poloxamer gel, achieving intimate contact with each other. It is easy to analyze the drug in the poloxamer gel because of its solution status at low temperatures.

The diffusivity of ciclopirox within poloxamer gel was investigated to find whether or not it is suitable as a receptor base. The diffusion profile of ciclopirox in poloxamer gel at 32°C is shown in Fig. 1. The diffusivity and the solubility of ciclopirox in poloxamer gel were high enough to be used as a receptor phase, considering typical concentration of the drug and the duration of experiment used in this study.

Development of the Extraction Method

Extraction of Ciclopirox from the Hoof Membranes

To optimize the extraction condition of ciclopirox from the hoof membranes, the effects of the temperature and solvent composition on the extraction ratio were investigated. Although water is a good permeant through the nail (3,4), it is not a good solvent for ciclopirox. Therefore, methanol was added into water to improve solubility of ciclopirox in the extraction solvent. Figure 2 shows the extraction ratio in various compositions of water/methanol mixture. The highest extraction ratio of ciclopirox was achieved by 5% methanol aqueous solution at 60°C after the 4-day extraction.

To find an adequate period of extraction, ciclopirox was extracted from the hoof membranes using 5% methanol aqueous solution at 60°C. The extraction efficiency of ciclopirox from the hoof membrane increased until the duration of extraction reached 24 h. Therefore, the extraction period was set at 24 h and the process was repeated three times.

Extraction of Ciclopirox from Poloxamer Gel

A proper extraction solvent should have a high solvent capacity for ciclopirox and be immiscible with 20% pol-

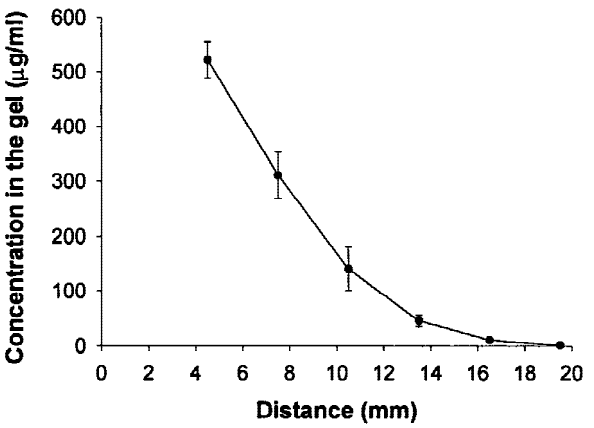

Fig. 1. Concentration profile of ciclopirox in poloxamer gel at 32°C after 3 days. Data are shown as mean ± S.D. ($n = 3$).

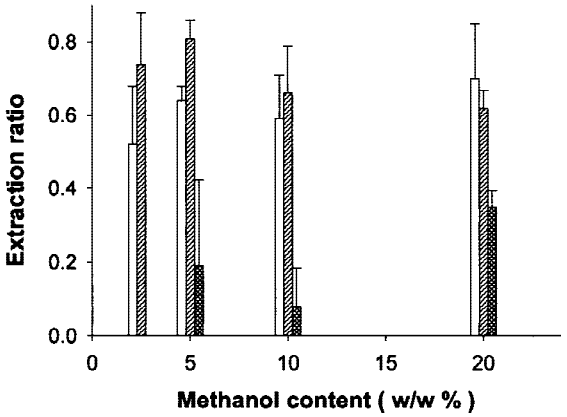

Fig. 2. Effect of temperature and the composition of extraction solvent on the extraction ratio of ciclopirox from the nail plate. Data are the average of three measurements and shown as mean ± SD. (□: 32°C; ▨: 60°C; ▩: ambient temperature).

oxamer gel. The extraction solvent should also have a low boiling point so it can be easily dried. Because of these stipulations, diethyl ether and ethylacetate were chosen as candidate solvents. The extraction ratios of ethylacetate and diethyl ether were 85 ± 3% and 98 ± 7% (mean ± SD), respectively. Even though ethylacetate had a lower extraction ratio than diethyl ether, the former was chosen as an optimum extraction solvent for poloxamer gel because of its better separation efficiency. When ethylacetate was used to extract the ciclopirox in the poloxamer gel, a linear regression coefficient (>0.99) of the calibration curve with the concentration range from 6–50 μg/ml was demonstrated.

In Vitro Penetration Model

Effect of Water Content and Thickness of Nail

The thickness and the degree of hydration of porcine hoof membrane could affect the amount of drug within and across the hoof membrane. To investigate the effect of both parameters in the penetration study, the effects of the water content and the thickness of porcine hoof membrane on the amount of drug across or within the hoof membrane were investigated (Fig. 3). When relationships among parameters were evaluated by a multiple regression analysis, no statistically significant relationship was observed except for the relationship between water content and the amount of drug in poloxamer gel ($P < 0.05$). The fact that the water content had a statistically significant effect on the amount of the drug penetrated across the nail plate emphasized the importance of controlling water content.

Correlation between the Nail Penetration Model and In Vivo Test

To evaluate the validity of the proposed nail penetration method for *in vivo* prediction, the *in vitro* penetration profile of ciclopirox from commercial lacquer formulation was compared with the *in vivo* profile reported in the literature (7) (Fig. 4). The amount of drug permeated *in vitro* method was standardized as drug weight per 1 mg of the nail because *in vivo* data were reported as such. A good correlation between the penetration profiles in porcine hoof membrane and the *in vivo* profile of human nail indicated that the proposed method composed of porcine hoof membrane and poloxamer gel can be an adequate model for *in vivo* prediction of ciclopirox peneteration through the nail. The fact that our

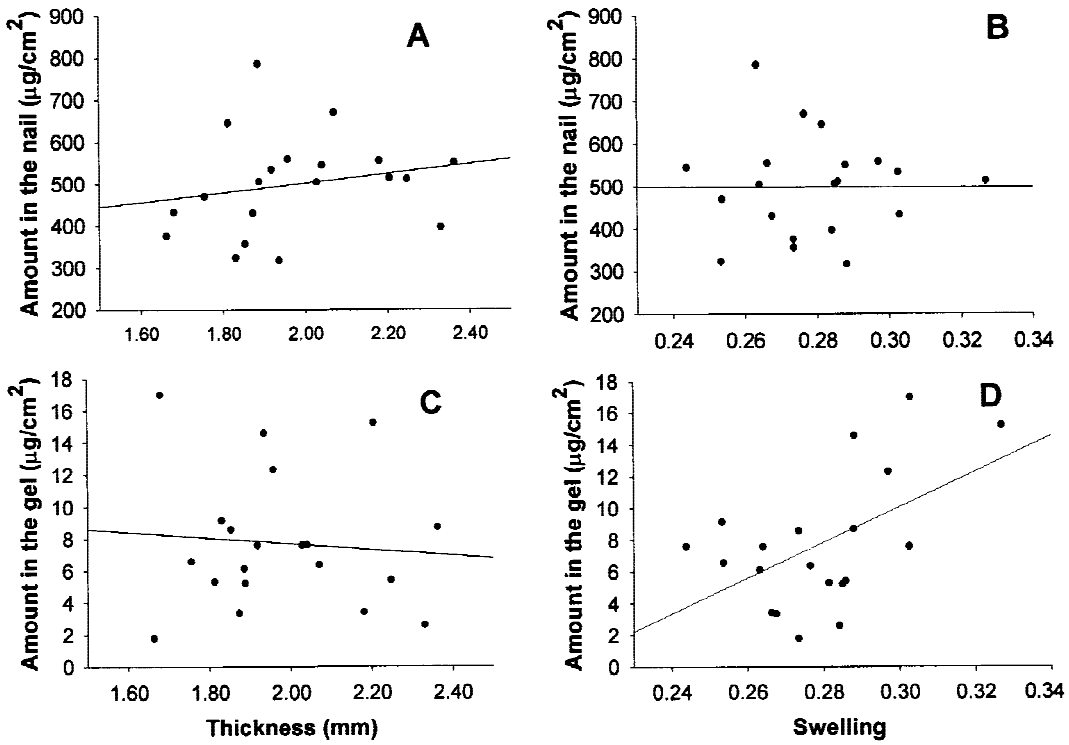

Fig. 3. Effect of the water content **(B, D)** and the thickness of the porcine hoof membrane **(A, C)** on the amounts of the drug across or within the hoof membrane. Each point represents a single experiment.

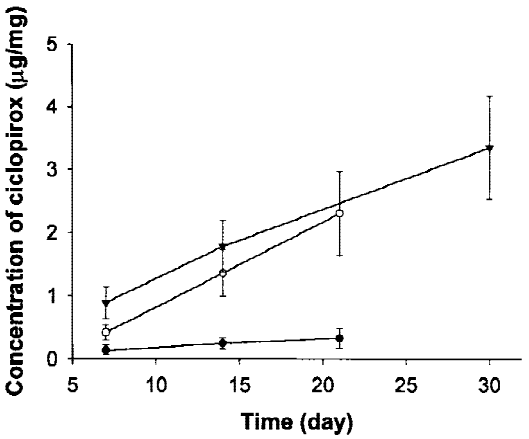

Fig. 4. The *in vitro* penetration profile of 8% ciclopirox lacquer from the *in vitro* model ($n = 8$) and *in vivo* profile reported (7). Data are shown as mean ± S.D. (●: amount in the gel; ○: amount in the porcine hoof membrane; ▼: amount in the human nail).

experimental results are similar to the *in vivo* data does not imply by itself that our model can simulate *in vivo* results. However, this is one of the many requirements necessary to develop a good methodology.

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