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ORIGINAL ARTICLE

Therapeutic effects of a novel DA5505 formulation on a guinea pig model of tinea pedis

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ABSTRACT

Background/Objective: Tinea pedis, a superficial mycosal infection affecting the soles and toe webs of feet, is generally treated with terbinafine. However, the main problem with available marketed formulations is their inability to deliver terbinafine to the viable skin layers. Hence, the main aim of this study was to prepare a novel DA5505 formulation capable of penetrating and retaining terbinafine in deeper skin layers.
Methods: Skin permeation and retention of terbinafine were analyzed following administration of different formulations (DA5505, Terbinew® gel, Lamisil Cream®, and Lamisil Once®) to mouse and porcine skin *in vitro*. *In vivo* efficacy of DA5505 in the guinea pig model was also evaluated.
Results: Compared to marketed gel, cream, and solution formulations, DA5505 exhibited significantly higher skin permeation and retention of terbinafine in both mouse and porcine skin ($p < 0.05$). *In vivo* skin permeation studies further showed higher terbinafine content with improved pharmacokinetics for DA5505, following a single application similar to Lamisil Once®, compared with those for commercial solution and gel formulations. In addition, *in vivo* experiments revealed that DA5505 exhibited the highest antifungal effect among the formulations tested, by significantly reducing the number of culture-positive feet and intensity of infection ($p < 0.001$) in the guinea pig model.
Conclusion: Hence, DA5505 exhibits potential for short-course (single application) effective treatment of tinea pedis.

Introduction

Superficial mycoses have an increasing incidence rate, with 20–25% of the world population being affected.[1] The main reasons attributed to the rise in these infection rates include migration, tourism, and socioeconomic conditions.[2] These infections are mainly caused by dermatophytes (pathogenic keratin-digesting fungi) belonging to the genera *Epidermophyton*, *Microsporum*, and *Trichophyton*. Commonly, dermatophytes are limited to superficial skin, hair, and nails, causing disfiguring and discomfort.[3]

The soles and toe webs of feet are the most frequently affected sites in tinea pedis infection. Common clinical manifestations include intertriginous dermatitis presented with maceration, peeling, and fissuring in toe interspaces. *Trichophyton mentagrophytes* causes an acute inflammatory condition characterized by the formation of pustules, vesicles, and sometimes bullae.[4] Tinea pedis is generally treated with topical application of different antifungal agents in the form of creams, gels, sprays, liquids, and powders.[5] Topical terbinafine has been widely applied for the treatment of tinea pedis. Terbinafine is a fungicidal allylamine compound with an excellent safety profile. It inhibits the synthesis of ergosterol, an important fungal cell membrane and cell wall component.[6,7]

Although different topical formulations of terbinafine are available on the market, effective drug delivery to the target site has still not been achieved. The main problem with conventional topical formulations is the easy removal of formulation from the site of application, leading to insufficient amounts of drug reaching

Conflicts of interest: S.D. Han, G.H. Lee, and J.H. Jun are employees of Dong-A Pharmaceutical Co., Ltd., while all the other authors have no conflicts of interest to declare.

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the site of action. Different strategies can be adopted to enhance skin permeation, including preparation of bioadhesive formulations or increasing lipid bilayer permeability by hydration techniques. In this study, film-forming gel containing terbinafine was prepared to enhance terbinafine permeation by affecting lipid bilayer permeability through hydration. Chitosan is a natural, cationic, hydrophilic biopolymer that interacts with the negatively charged skin surface, conferring enhanced bioadhesiveness and percutaneous penetration.[8,9] In this study, a film-forming gel of terbinafine (DA5505) was prepared using chitosan for effective delivery of terbinafine in a guinea pig model of tinea pedis.

Methods

Materials

Terbinafine was purchased from Dong-Woo Syntech (Eumsung, South Korea). Chitosan was purchased from Koyo Chemical Co. (Sakai-minato, Japan). DA5505 was provided by Dong-A Pharm Co. (Seoul, South Korea), and Lamisil Once (Novartis Consumer Health UK Ltd., Brentford, UK), Lamisil Cream (Novartis Consumer Health) and Terbinew gel (Dong-A Pharm) were purchased from the market. All other chemicals used were of analytical grade. Each gram of chitosan-based film-forming gel (DA5505) contains 1% w/v terbinafine. Other ingredients of the gel are chitosan and ethanol. The pH of DA5505 formulation was 4.0 ± 0.1 and terbinafine drug content was 99.8 ± 0.1.

Animals

For this study, 5–7-week-old Balb/c nude mice, male Hartley guinea pigs, and Sprague–Dawley rats were purchased from Central Lab Animal Inc. (Seoul, South Korea). The excised skin of Balb/C nude mice was used for *in vitro* studies. In addition, fresh skin of 6–8-week-old swine of ~40 kg body weight was obtained from a slaughterhouse for *in vitro* experiments. Rats and guinea pigs were housed under pathogen-free conditions, with food and water *ad libitum*. All animal care and experimental protocols were approved by the Institutional Animal Ethical Committee, Yeungnam University, South Korea.

High-performance liquid chromatography for terbinafine

Quantitation of terbinafine was performed using an Agilent (Agilent Technologies, Santa Clara, CA, USA) high-performance liquid chromatography (HPLC) system equipped with Agilent 1200 series G1314C variable wavelength detector, a G1312B pump, a G1329B ALS SL autosampler, and a reversed phase C_{18} column (4.6 mm × 150 mm) preceded by a guard column (4 mm × 4 mm). LC solution software was used for HPLC analysis. The mobile phase used was a mixture of acetonitrile/tetrahydrofuran/10mM phosphate buffer (pH 7.8; 650:150:250), and a flow rate of 2 mL/min was applied. Analyses were performed at a detection wavelength of 280 nm and column temperature of 30 °C.

Liquid chromatography–tandem mass spectrometry for terbinafine

Quantitation of terbinafine was performed using an Agilent liquid chromatography–tandem mass spectrometry LC-MS/MS system equipped with Unison UK-C_{18} (2 mm internal diameter × 50 mm length, 3 μm) column maintained at 35°C. The MS system was maintained with gas temperature of 350°C, gas flow of 12 L/min, a nebulizer with 380 kPa, and capillary with 4000 V. Clotrimazole was used as an internal standard.

Determination of skin permeation of terbinafine from different formulations

Skin was obtained from Balb/C nude mice and washed with phosphate buffered saline (PBS). Receptor compartment of the diffusion cell was filled with 5 mL of 50% ethanol. The temperature of the cell was maintained at 37°C with stirring at 300 rpm. Mouse skin (3 cm × 3 cm) was attached between the donor and the receptor compartment. Different marketed formulations, along with DA5505 (300 mg terbinafine equivalent), were added to the receptor compartment. Aliquots (200 μL) from the receptor compartment were sampled at predetermined time intervals and replaced with the same amount of fresh receptor fluid. The samples were centrifuged at 15,700 *g* for 10 minutes and 100 μL of the supernatant was collected and analyzed by HPLC.

Determination of terbinafine retention in porcine skin

The receptor compartment was filled with 5 mL of 50% ethanol. Porcine skin (3 cm × 3 cm), prehydrated for 30 minutes, was mounted on the diffusion cell. Different formulations (DA5505, Terbinew gel, Lamisil Cream, and Lamisil Once) were added to the donor compartment and left for 24 hours to allow terbinafine skin penetration. Upon completion of the experiment, diffusion cells were dismantled and receptor phases were removed. The skin surface was washed in distilled water to remove any residual formulation or drug. Skin surface exposed to the donor compartment was punched out. The punched skin samples were then milled and extracted at ambient temperature for 12 hours with continuous shaking. Afterwards, samples were filtered through 0.45-μm membrane filters and terbinafine concentration was analyzed by HPLC, as described previously.

In vivo skin retention and pharmacokinetics evaluation of terbinafine

In vivo pharmacokinetics of terbinafine was evaluated in rats that were divided into three groups, with 10 rats/group. Backs of the rats were shaved to remove hair. A different formulation (DA5505, Lamisil Once, or Terbinew gel) was applied to each group. Formulations equivalent to 500 $\mu g/cm^2$ of terbinafine were applied to 4 cm × 4-cm areas on the shaved backs of the rats. After 24 hours, the formulations were wiped off and the skin was washed with water and tape-stripped to remove excess drug. At appropriate time points (1 day, 2 days, 3 days, 6 days, 7 days, 14 days, 21 days, and 28 days) following the application of different formulations, the skin was harvested to determine the amount of drug retained, by HPLC and LC-MS/MS methods as described previously. The amount of terbinafine permeated at Day 1 was analyzed by HPLC. Similarly, the amount of retained terbinafine in the skin from Day 2 to Day 28 was evaluated by LC-MS/MS. Different pharmacokinetic parameters were evaluated for comparison between formulations.

Development of the guinea pig model of tinea pedis

T. mentagrophytes (ATCC 24953) was used to infect the guinea pigs. Conidia were harvested from cultures grown on K agar slants (0.2% Bacto-peptone, 0.1% glucose, 0.1% KH_2PO_4, 0.1% $MgSO_4$ $7H_2O$, and 2% agar) at 27°C for 3 weeks. The conidial fluid was adjusted to 1×10^8 conidia/mL, for use as the inoculum, by suspending it in sterile saline supplemented with 0.05% Tween 80. The procedure for the development of infection in guinea pigs was similar to that reported previously.[10,11] The sites for inoculation of the fungus were the plantar parts of the hind feet. Cotton swab dipped with 80% v/v ethanol was used to clean the locus. Sterile gauze was dampened

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