Erbium–Yttrium–Aluminum–Garnet Laser Irradiation Ameliorates Skin Permeation and Follicular Delivery of Antialopecia Drugs

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ABSTRACT: Alopecia usually cannot be cured because of the available drug therapy being unsatisfactory. To improve the efficiency of treatment, erbium–yttrium–aluminum–garnet (Er–YAG) laser treatment was conducted to facilitate skin permeation of antialopecia drugs such as minoxidil (MXD), diphencyprone (DPCP), and peptide. *In vitro* and *in vivo* percutaneous absorption experiments were carried out by using nude mouse skin and porcine skin as permeation barriers. Fluorescence and confocal microscopies were used to visualize distribution of permeants within the skin. Laser ablation at a depth of 6 and 10 μm enhanced MXD skin accumulation twofold to ninefold depending on the skin barriers selected. DPCP absorption showed less enhancement by laser irradiation as compared with MXD. An ablation depth of 10 μm could increase the peptide flux from zero to 4.99 and 0.33 $\mu g\ cm^{-2}\ h^{-1}$ for nude mouse skin and porcine skin, respectively. The laser treatment also promoted drug uptake in the hair follicles, with DPCP demonstrating the greatest enhancement (sixfold compared with the control). The imaging of skin examined by microscopies provided evidence of follicular and intercellular delivery assisted by the Er–YAG laser. Besides the ablative effect of removing the *stratum corneum*, the laser may interact with sebum to break up the barrier function, increasing the skin delivery of antialopecia drugs. The minimally invasive, well-controlled approach of laser-mediated drug permeation offers a potential way to treat alopecia. This study's findings provide the basis for the first report on laser-assisted delivery of antialopecia drugs. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:3542–3552, 2014
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INTRODUCTION

Alopecia is known as hair loss on the scalp because of an androgenetic process (androgenetic alopecia) or an inflammatory process (alopecia areata). The prevalence of androgenetic alopecia in Caucasian men is 96%.[1] On the contrary, alopecia areata affects 2.1% of the population.[2] Minoxidil (MXD) is developed for the treatment of both alopecia types. This drug at a 5% dose is the first-line topical therapy for androgenetic alopecia.[3] It can reduce baldness by the mechanisms of vasodilation, enhanced proliferation, and angiogenesis. Diphencyprone (DPCP) is a contact allergen for topical immunotherapy of alopecia areata. Alopecia treatment by drugs is always inefficient. The effect of MXD is not permanent, and the treatment cessation contributes to hair loss in 4–6 months.[4] Moreover, contact dermatitis occurs in 6% of patients receiving 5% MXD.[5] A lag time of 3 months from initiation of treatment to initial hair growth is observed for DPCP with a success rate of only 50%. The adverse effects of DPCP occur in 24% of patients.[6] Recent advances in biotechnology have developed macromolecules and nanoparticles for alopecia therapy. These include growth factors, proteins, genes, and fullerenes.[7–9] Abundant investigations document the use of peptides to counter the effect of alopecia. These include soymetide-4,[10] formyl–methyonyl–leucyl–phenylalanine,[11] prolactin,[12] and calcitonin gene-related peptide.[13] These biologics, however, show unsatisfactory results in treating alopecia because of their instability and molecular size, which is too large to penetrate the skin.

Efficient topical drug delivery is challenging because of the formidable barrier function of the *stratum corneum* (*SC*). *SC* removal by tape-stripping, mechanical abrasion, and laser treatment has shown to be effective for promoting drug permeation via the skin.[14] The approaches of tape-stripping and abrasion are limited and lack reproducibility because of the poor ability to control the *SC* ablation level. Laser treatment can precisely and selectively remove *SC* in a controlled and noncontact manner.[15] The duration of laser irradiation is in the range of nano- to microseconds, indicating a quick operation for enhancing drug absorption.[16] It has been shown that the ablative lasers can increase topical delivery of small molecule drugs, macromolecules, and nanoparticles.[17,18] The improvement of antialopecia drug delivery for achieving efficacious therapy is urgent. We sought to evaluate the impact of ablative laser treatment on cutaneous delivery of antialopecia drugs, including MXD, DPCP, and peptide. Hair follicles are the main target for these drugs in treating hair loss. The follicular openings of

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the scalp occupy 10% of the total scalp area.[19] Whether laser treatment could facilitate drug delivery into the follicles was examined in this report.

Using the erbium–yttrium–aluminum–garnet (Er–YAG) laser as the ablative tool, we measured the *in vitro* and *in vivo* skin permeation of antialopecia drugs via nude mouse and porcine skins. The role of the follicles on laser-assisted drug delivery was evaluated using the cyanoacrylate casting technique. The biodistribution of permeants in skin tissues was imaged by fluorescence microscopy and confocal laser scanning microscopy (CLSM) for vertical and horizontal observation, respectively. In our study, we demonstrated for the first time the utilization of the Er–YAG laser for topical delivery of antialopecia actives.

MATERIALS AND METHODS

Materials

Minoxidil, DPCP, rhodamine B, Nile red, polyethylene glycol (PEG) 400, and propylene glycol (PG) were purchased from Sigma–Aldrich (St. Louis, Missouri). Superglue (ethyl cyanoacrylate 7004T) was obtained from 3M (Taipei, Taiwan). Fluorescein isothiocyanate (FITC)–Pro–Arg–Leu–Leu–Tyr–Ser–Trp–His–Arg–Ser–His–Arg–Ser–His–COOH was synthesized by Tools Biotechnology (New Taipei City, Taiwan).

Animals

Female nude mice (ICR-Foxn1nu), 8-week-old, were supplied by National Laboratory Animal Center (Taipei, Taiwan). Specific pathogen-free pigs, 1-week-old, were provided by Animal Technology Institute Taiwan (Miaoli, Taiwan). The animal experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Chang Gung University. All animals used in this work were treated under the institutional guidelines.

Preparation of Skin

In the *in vitro* experiment, full-thickness skin from the dorsal area of the nude mice and pigs was excised after sacrifice. The sebum-removal skin was prepared by washing the *SC* side of the skin using cold hexane (4°C) five times.[20]

Er–YAG Laser

The Er–YAG laser (Contour; Sciton Laser, Palo Alto, California) irradiated a wavelength of 2940 nm with a pulse duration of 100 μs. A scanning hand piece was employed to ablate a skin area of 1.5 × 1.5 cm^2. The ablated depth of the skin by the laser was set to 6 and 10 μm by fluences of 1.5 and 2.5 J/cm^2, respectively.

In Vitro Skin Permeation

Skin penetration of antialopecia drugs was measured by a Franz diffusion cell. The nude mouse or porcine skin with or without laser exposure was mounted between the donor and receptor compartments. The donor vehicle was 0.5 mL 30% PEG400–water, 30% PG–water, and water for MXD, DPCP, and FITC–peptide, respectively. The dose of MXD, DPCP, and FITC–peptide was 0.2% (w/v), 0.08%, and 0.028%, respectively. The receptor medium consisted of 30% PG–pH 7.4 buffer, 30% ethanol–pH 7.4 buffer, and pH 7.4 buffer for these three permeants. The effective diffusion area between compartments was 0.785 cm^2. The stirring rate and temperature were kept at 600 rpm and 37°C, respectively. At appropriate intervals, 300 μL of the receptor medium was taken and immediately replaced by an equal volume of fresh medium. The samples of MXD and DPCP were analyzed by HPLC as described previously.[21] The samples of FITC–peptide were assayed by fluorescence spectrophotometry. The excitation and emission wavelengths were set to 490 and 520 nm, respectively. The accumulation of permeants within the skin was measured after a 24-h delivery. The skin was removed from the Franz cell, then rinsed with water and blotted with tissue paper. The skin sample was weighed and minced by scissors, positioned in a glass homogenizer with 1 mL methanol for samples treated by MXD and DPCP, and ground for 5 min with an electric stirrer. The homogenization medium for the peptide was 0.1 N HCl. The mixture was centrifuged at 9615 x*g* for 10 min. After filtration via the polyvinylidene fluoride (PVDF) membrane, the samples were detected by HPLC or fluorescence spectrophotometry.

Hair Follicle Uptake

Differential stripping and cyanoacrylate skin surface casting were used to detect the content of the permeants in the follicles.[22] Subsequent to stripping the *SC* of the skin removed from the Franz cell, a follicular cast was prepared. A drop of superglue was added on a glass slide, which was pressed onto the surface of the *SC*-stripped skin. The cyanoacrylate polymerized, and the slide was expelled with one quick movement after 5 min. The superglue remaining on the slide was scraped off and positioned in a tube with 2 mL methanol. The tube was shaken for 3 h. The final product was vacuumed to evaporate methanol. Mobile phase or water was added to dissolve the residuals for an HPLC or fluorescence spectrophotometry assay.

Vertical Observation of Skin by Fluorescence Microscopy

This experiment was performed in an *in vitro* Franz cell model by using porcine skin as a permeation barrier. Rhodamine B (0.3%, w/v, in 30% PEG400/water), Nile red (0.01%, w/v, in 30% PG/water), or FITC–peptide (0.028%, w/v, in water) was applied to the donor for a 6-h delivery. After the termination of skin permeation, the skin samples were sectioned in a cryostat microtome at a thickness of 20 μm, and then mounted by glycerin and gelatin. The slices were monitored with an inverted fluorescence microscope (IX81; Olympus, Tokyo, Japan) using a filter set at 450–490 and 515–565 nm for excitation and emission, respectively. Hematoxylin and eosin (H&E) stained the skin sections for observing the skin structure under bright-field imaging.

Horizontal Observation of Skin by CLSM

This experiment was carried out using an *in vivo* nude mouse model. A glass cylinder with a hollow area of 0.785 cm^2 was attached to the dorsal skin by superglue. An aliquot of 0.2 mL of vehicles, the same as used with the experiment of fluorescence microscopy, was pipetted into the cylinder. The application period was 3 h. The animal was then sacrificed, and the treated skin region was excised. The skin thickness was scanned at 5-μm increments via *Z*-axis of confocal microscopy (TCS SP2; Leica, Wetzlar, Germany). The excitation and emission wavelengths for rhodamine B and Nile red were set to 543 and 560–620 nm, respectively. The wavelengths of excitation and