Skin Laser Treatments Enhancing Transdermal Delivery of ALA

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ABSTRACT: Drug delivery across skin has been limited due to barrier properties of the skin, especially those of the stratum corneum (SC). Use of the laser radiation has been suggested for the controlled removal of the SC. The purpose of this study was to study *in vitro* the influence of infrared radiation from the erbium:yttrium–aluminum–garnet (Er:YAG) laser ($\lambda = 2940$ nm), and visible from the 2nd harmonic of a neodymium:yttrium–aluminum–garnet (Nd:YAG) laser ($\lambda = 532$ nm) on transdermal delivery of 5-aminolevulinic acid (ALA). Pinna skin of the inner side of rabbit ear was used for skin permeation. The light sources were an Er:YAG laser (Key III Plus KaVo) and a Q-switched Nd:YAG laser (Lotis TII SL-2132). Permeation study, morphological and structural skin examination by histology and differential scanning calorimetry (DSC) were carried out. Permeation profiles and histological observations obtained after irradiation with infrared and visible laser radiation differed due to different biophysical effects on irradiated skin. Wavelength of 2940 nm required lower energy contribution to produce the same level of permeation than visible radiation at 532 nm. Structural analysis by DSC shows a selective impact on the lipidic structure. Laser pretreatment enhanced the delivery of ALA trough the skin by SC ablation. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:223–231, 2011

Keywords: transdermal drug delivery; 5-ALA; Er:YAG laser; laser ablation; second harmonic of Nd:YAG laser; stratum corneum

INTRODUCTION

Photodynamic therapy (PDT) involves a combination of a photosensitizer, light, and oxygen to cause destruction of selected cells, mainly through generation of highly cytotoxic singlet oxygen.[1] Common photosensitizers, such as porphyrins, cannot be administered by topical application to neoplastic skin lesions, because their high molecular weights preclude successful penetration of the stratum corneum (SC). When administered intravenously, these agents accumulate in normal skin and lead to prolonged cutaneous photosensitivity. In contrast, the protoporphyrin IX (PpIX) precursor 5-aminolevulinic acid (ALA, MW 167.6 Da), which is not associated with cutaneous photosensitivity, can be applied topically.[2] However, due to the limited ALA permeation through intact SC the best results by topical application have been reported in the case of superficial lesions with a damaged barrier function, such as basal cell carcinoma, Bowen's disease and actinic keratosis.[3–5]

ALA is a hydrophilic molecule with a SC/water partition coefficient of 0.1 and thus, as expected from skin permeability theory, ALA permeates poorly across intact skin.[6] Various strategies have been employed in order to enhance topical penetration of ALA, including curettage/debulking of nodular lesions, use of more lipophilic derivatives, use of chemical penetration enhancers and iontophoresis.[7–9]

Although SC-stripping technique may also produce high ALA permeation in clinical situation, use of laser radiation may offer a more-precise and quicker method to control the enhancement process. Lasers are physical devices that have been used for medical diagnosis and therapeutic purposes. The erbium:yttrium–aluminum–garnet (Er:YAG) laser ($\lambda = 2940$ nm) is very popular nowadays, and has the capability to cause ablation of the SC with minimal residual thermal damage to the skin.[10,11] It is currently used for the resurfacing of rhyrides, scars, and photodamage.[12] Recently, some studies

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have suggested that the Er:YAG laser could effectively enhance and control *in vitro* drug delivery via skin, including: nalbuphine, indomethacin, 5-fluorouracil, vitamin C, lidocaine.[13–16] Although Er:YAG laser-mediated enhancement of drug delivery has been demonstrated for a number of *in vitro* studies, the effect of the second harmonic of the neodymium:yttrium–aluminum–garnet (Nd:YAG) laser ($\lambda = 532$ nm) has only been demonstrated for 5-fluorouracil.[17] The aim of the present study was to assess the feasibility of the Er:YAG laser and the second harmonic of a Nd:YAG laser for enhancing the topical delivery of ALA in a clinical safety way.

MATERIALS AND METHODS

Chemicals

ALA (5-aminolevulinic acid, hydrochloride salt) was obtained from Sigma–Aldrich, Madrid, Spain. Trypsin (trypsin from bovine pancreas 13,000 U/mg) was obtained from Sigma–Aldrich. All other chemicals were of analytical reagent grade.

Skin Samples

Pinna skin of the inner side of rabbit ear (New Zealand rabbits of 2.9–3.1 kg from Granja Conicular San Bernardo, Navarra, Spain) was used for skin permeation studies. The animals were sacrificed and the ears were removed. Pinna skin was peeled away from the underlying cartilage, then it was washed with saline solution (NaCl 0.9%) and frozen at −80°C.

Laser Treatment

The Light Source for Laser Treatment was:

(a) A Q-switched Nd:YAG laser (Lotis TII SL-2132), which produces pulses at 532 nm (up to 90 mJ/pulse, ~11 ns, 1–15 Hz). The laser beam was Gaussian-shaped and passed through a BK7 spherical lens of focal length $f = 250$ mm. The sample was mounted on a X–Y (New Focus, San Jose, CA, USA) translation stage and placed normal to the incident focused laser beam. Output energies ranged between 50 and 55 mJ with a beam spot size focused to 1 mm in diameter, rendering fluences of 6.6–7 J/cm^2.

(b) An Er:YAG laser (KEY 3+; KaVo, Biberach, Germany) at 2940 nm and a pulse duration of 250 μs. The laser beam was delivered onto the skin through a KEY Laser Handpiece 2060 (universal) displaced perpendicular to the skin surface. Output energy of 40 mJ with a beam spot diameter of 1 mm and repetition rates of 1–2 Hz was applied to the samples through microscope slides, in order to reduce the amount of energy incident to the sample down to 8 mJ. Therefore, the fluences applied ranged between 1 and 1.6 J/cm^2. The sample was located on a glass support to make easier its irradiation.

Energy measurements of the laser pulse incident on the sample were performed by using a pyroelectric detector Gentec-DE 500+ (in combination with Gentec-EO SOLO console). The fluence (energy/surface) of the radiation incident on the sample surface was controlled by varying the pump energy.

In Vitro Topical Delivery of 5-ALA

The diffusion cell used was a Franz vertical diffusion assembly. The skin was mounted on the receptor compartment with the SC side facing upwards into the donor compartment. The receptor compartment (20 mL) was filled with potassium phosphate buffer, pH 5. The donor vehicle was filled with 10 mL of 0.57% (w/v) ALA in potassium phosphate buffer, pH 5. The available area of the Franz cell was 3.8 cm^2. The diffusion cells were placed in a double walled vessel (7 cm × 5.5 cm i.d.), connected to a water recirculating thermostat to maintain a constant temperature of 37°C, and the receptor fluid was stirred by a magnetic bar at 500 rpm (Velt multiposition electromagnetic stirrer, Italy). At appropriate intervals, 100 μL aliquots of receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution.

Spectral Scanning Multimode Reader Analysis for 5-ALA

This method employs a prederivatization of ALA samples with acetyl acetone and formaldehyde and fluorescence detection. In brief, acetil acetone reagent was prepared mixing 15 mL acetyl acetone, 10 mL ethanol, and 75 mL distilled water. Derivatization was based on a modification of the Hantzsch reaction, in which amine compounds react with acetyl acetone and formaldehyde. To a vial, 3.5 mL acetyl acetone reagent, 50 μL of sample, and 0.45 mL 10% (w/w) formaldehyde solution were added and mixed on a vortex mixer for ~5 s. This mixture was heated for 20 min at 100°C. The vial was then cooled in an ice bath.

The solutions containing ALA-acetyl acetone/formaldehyde reagent derivative, were analyzed with a Thermo Scientific Spectral Scanning Multimode Reader (Varioskan Flash), through the reading of the fluorescence signal at 460 nm of the ALA derivatized samples placed in microplates and excited at 370 nm.

Data Analysis

In the *in vitro* permeation study, the total amount of drug permeating across the unit diffusion surface and into the receptor was calculated and plotted as a