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Erbium:YAG laser resurfacing increases skin permeability and the risk of excessive absorption of antibiotics and sunscreens: The influence of skin recovery on drug absorption

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ABSTRACT

While laser skin resurfacing is expected to result in reduced barrier function and increased risk of drug absorption, the extent of the increment has not yet been systematically investigated. We aimed to establish the skin permeation profiles of tetracycline and sunscreens after exposure to the erbium:yttrium-aluminum-garnet (Er:YAG) laser during postoperative periods. Physiological and histopathological examinations were carried out for 5 days after laser treatment on nude mice. Percutaneous absorption of the permeants was determined by an in vitro Franz cell. Ablation depths varied in reaching the stratum corneum (10 μm, 2.5 J/cm^2) to approach the epidermis (25 μm, 6.25 J/cm^2) and upper dermis (40 μm, 10 J/cm^2). Reepithelialization evaluated by transepidermal water loss was complete within 2–4 days and depended on the ablation depth. Epidermal hyperplasia was observed in the 40-μm-treated group. The laser was sufficient to disrupt the skin barrier and allow the transport of the permeants into and across the skin. The laser fluence was found to play an important role in modulating skin absorption. A 25-μm ablation depth increased tetracycline flux 84-fold. A much smaller enhancement (3.3-fold) was detected for tetracycline accumulation within the skin. The laser with different fluences produced enhancement of oxybenzone skin deposition of 3.4–6.4-fold relative to the untreated group. No penetration across the skin was shown regardless of whether titanium dioxide was applied to intact or laser-treated skin. However, laser resurfacing increased the skin deposition of titanium dioxide from 46 to 109–188 ng/g. Tetracycline absorption had recovered to the level of intact skin after 5 days, while more time was required for oxybenzone absorption. The in vivo skin accumulation and plasma concentration revealed that the laser could increase tetracycline absorption 2–3-fold. The experimental results indicated that clinicians should be cautious when determining the dose for postoperative treatment.

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1. Introduction

Laser skin resurfacing is an effective surgery for treating wrinkles, scarring and cutaneous photodamage (Tanzi and Alster, 2003). One of the commonly used tools for surgical resurfacing is the erbium:yttrium-aluminum-garnet (Er:YAG) laser. It can precisely ablate the skin with minimal thermal damage (Lee et al., 2009). This device is suitable for treating epidermal processes in clinical situations (Regan et al., 2010). The Er:YAG laser is excellent for treating acne and burn scars, melasma, rhytides and skin hyperpigmentation (Fang et al., 2004; Karsai et al., 2010).

The use of resurfacing lasers has raised concerns about side effects, potential hazards, collateral damage and other safety issues (Doukas and Kollias, 2004). Safety issues are based on toxicity characteristics and on the potential promotion of the extent of skin permeability. Because the laser ablates the skin, there are concerns that the associated cutaneous injury may result in rapid absorption of allergens and topically applied drugs in large amounts. Some drugs, such as antibiotics, anti-inflammatory agents, and sunscreens are generally used for postoperative therapy/prevention with laser resurfacing (Pan et al., 2010). The stratum corneum (SC) and epidermis are the primary barriers and rate-limiting

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steps in the percutaneous absorption of drugs. Systemic absorption through the skin occurs as the barrier function has been reduced (Kasichayanula et al., 2007). One important issue for laser resurfacing is the risk of excessive drug absorption after treatment. Currently, no systematic evaluation of this risk is available. Moreover, there is no standard for the optimal dose of topically applied drugs after laser irradiation. The present work aimed to detect skin absorption of drugs and sunscreens by resurfacing laser application with clinically relevant depth. The permeants used herein were tetracycline, oxybenzone and titanium dioxide. Antibiotics such as tetracycline are always used to prevent infection after laser resurfacing. In addition to antibiotics, sunscreens are also always applied to the skin following laser resurfacing. Many cosmetics and hair products contain sunscreens, which often result in the daily application of a sunscreen without the user making a conscious decision to use one (Calafat et al., 2008). The action site for these chemicals should be restricted to the skin surface or uppermost SC layers (Cross et al., 2001). Therefore, the percutaneous absorption of antibiotics and sunscreens is essential to the accurate risk assessment of the patients.

The nude mouse was used in this work as the animal model. The dorsal skin was irradiated with the laser to depths of 10, 25 and 40 μm, which correspond to the nearly complete ablation of the SC, epidermis and upper dermis, respectively. The skin structure can recover within a few days following irradiation. The percutaneous permeation of these drugs was also examined by their ability to penetrate across laser-treated skin with different recovery periods. The present work utilized Franz cells to determine percutaneous drug absorption into and across the skin. The disruption and recovery of skin by laser treatment were examined by histopathological studies. The postoperative skin recovery was examined by measuring the transepidermal water loss (TEWL), skin erythema and surface pH level. The in vivo skin absorption and plasma concentration of tetracycline were also examined after laser treatment and the subsequent topical administration.

2. Materials and methods

2.1. Materials

Tetracycline hydrochloride and oxybenzone were purchased from Sigma–Aldrich Chemical (St. Louis, MO, USA). Titanium dioxide was supplied by Alfa Aesar Chemical (Ward Hill, MA, USA). Titanium dioxide-loaded lotion (2.53%, Menturm UV Shield S2®) was obtained from Omi Brotherhood (Shiga, Japan).

2.2. Laser assembly

The laser device (Contour, Sciton Laser, Palo Alto, CA, USA) possesses a wavelength of 2940 nm and pulse duration of 100 μs. An articulated arm was used to deliver the laser beam onto the nude mouse skin. A 1.5-cm × 1.5-cm ablation area was created by a square scanning handpiece. The laser irradiation was performed after being calibrated to ablation levels of 10 (2.5 J/cm^2), 25 (6.25 J/cm^2) and 40 μm (10 J/cm^2), with 10% overlap and zero coagulation.

2.3. Skin recovery evaluated by physiological parameters

The dorsal region of each nude mouse (ICR-Foxn1nu strain, 8 weeks old) was irradiated by laser. Transepidermal water loss (TEWL), colorimetric parameters and skin pH were measured for 5 days post-application. A Tewameter® (TM300, Courage and Khazaka, Köln, Germany) was utilized to measure TEWL. The recording was taken at a stable level 30 s post-application of the TEWL probe to the skin. A spectrocolorimeter (CD100, Yokogawa Electrical, Tokyo, Japan) was used to determine the skin erythema (a^*). The skin surface pH was determined by Skin-pH-Meter® pH 905 (Courage and Khazaka). The baseline standard was detected by an adjacent untreated site. The temperature and relative humidity in the laboratory were kept at 27 °C and 50%, respectively. The sample number for each test was ten ($n = 10$).

2.4. Histopathological examination by light microscopy

The animals were sacrificed at 0, 1, 3 and 5 days after laser treatment. The skin specimens were excised for histological examination. Each specimen was fixed in a 10% pH 7.4-buffered formaldehyde solution for at least 72 h. Each slice was then dehydrated using ethanol, embedded in paraffin wax and stained with hematoxylin and eosin (H&E). For each skin sample, three different sites were examined and evaluated under light microscopy (Eclipse 4000, Nikon, Tokyo, Japan) with a digital camera (DX71, Olympus, Tokyo, Japan).

2.5. In vitro percutaneous absorption

Full-thickness skin was excised from the dorsal region after sacrifice. The skin was mounted between the donor and receptor compartments of a Franz cell with the SC facing upward into the donor. A pH 7.4 buffer (5.5 ml) and 30% ethanol in pH 7.4 buffer was used as the receptor medium for tetracycline and sunscreens, respectively. The donor was pipetted with 0.5 ml of double-distilled water containing tetracycline at a dose of 0.5% (w/v). The respective donor concentrations for oxybenzone and titanium dioxide were 0.3% and 0.5%, respectively, in 30% ethanol/water. The permeated area between the compartments was 0.785 cm^2. The stirring rate and temperature were kept at 600 rpm and 37 °C. At the determined intervals, 300-μl aliquots of the medium from the receptor were withdrawn and immediately replaced with an equal volume of fresh medium.

The skin was removed from the cells and washed briefly in water at the end of the in vitro experiment. Subsequently, the skin was weighed, cut with scissors and positioned in a glass homogenizer containing 1 ml of 0.1 N HCl (for tetracycline) or methanol (for oxybenzone and titanium dioxide) for 5 min. The homogenized mixture was then centrifuged for 10 min at 10,000 rpm, and the supernatant was collected. Each sample obtained from the in vitro study was analyzed by atomic absorption spectrophotometry (AAS) or high-performance liquid chromatography (HPLC).

2.6. Analytical methods for the permeants

The HPLC system included a Hitachi L-7110 pump, a Hitachi L-7200 sample processor and a Hitachi L-7400 UV detector (Hitachi, Tokyo, Japan). A 25-cm-long, 4-mm inner diameter stainless steel C18 column (Merck, Darmstadt, Germany) was used. The mobile phase consisted of methanol:0.05 M potassium phosphate in water (40:60) for tetracycline and methanol:water (80:20) at pH 3 adjusted with phosphoric acid for oxybenzone. The flow rate was 1 ml/min. The UV detector was set to the wavelengths of 270 and 288 nm for tetracycline and oxybenzone, respectively. Titanium dioxide was analyzed by measuring the absorbance using graphite furnace (flameless) AAS (Z-5000, Hitachi).

2.7. In vivo percutaneous absorption

The nude mouse was used as the animal model in the in vivo experiment. All animals were starved overnight prior to the experiment. A glass cylinder with an available area of 0.785 cm^2 was placed on the dorsal skin with glue (Instant Super Glue®, Kokuyo, Japan). An aliquot of 0.2 ml of double-distilled water with tetracycline (0.5%) was added to the cylinder. The application time was 6 h. The application region of the skin was excised at the end of the experiment. The procedures for washing and extraction of the compound from the skin were the same as for the in vitro experiment.

An aliquot of a 200-μl blood sample was withdrawn from a heart puncture into a heparin-rinsed vial at 6 h after dosing. Each blood sample was centrifuged at 3000 × g for 10 min. The resulting plasma sample (100 μl) was vortex-mixed with a 150-μl acetonitrile. The denatured protein precipitate was separated by centrifugation at 8000 × g for 10 min. An aliquot (20 μl) of the supernatant was directly injected into HPLC for analysis.

2.8. Statistical analysis

The Kruskal–Wallis test was used to analyze statistical differences between different treatments. A post hoc Dunn's test was used to examine individual differences between treatments. A 0.05 level of probability ($p < 0.05$) was selected as the level of significance. Data entry and analysis were completed using the SPSS version of the 11.5 statistical package program (SPSS, Chicago, IL, USA).

3. Results

3.1. Skin recovery evaluated by physiological parameters

Since the Er:YAG laser with 1 J/cm^2 projects an ablation depth of 4 μm, 2.5, 6.25, and 10 J/cm^2 exhibited respective penetration depths of 10, 25 and 40 μm. According to a previous study (Lee et al., 2001), the thicknesses of the SC and the epidermis of the nude mouse dorsal skin are 11 and 13 μm, respectively. It can be expected that low-energy application (2.5 J/cm^2) would result in selective removal of the SC, intermediate energy (6.25 J/cm^2) would produce an ablation depth that penetrated the viable epidermis and

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