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ORIGINAL ARTICLE

Skin pretreatment with an Er:YAG laser promotes the transdermal delivery of three narcotic analgesics

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Abstract Because of their low oral bioavailabilities and short half-lives, it may be more feasible to administer narcotic analgesics via the skin. However, this delivery method is limited by the low permeability of the stratum corneum (SC). The aim of this study was to enhance the transdermal delivery of three narcotic drugs, including morphine, nalbuphine, and buprenorphine, with an erbium:yttrium–aluminum–garnet (Er:YAG) laser pretreatment. In an in vitro pig skin permeation experiment, Er:YAG laser pretreatment of the skin produced a 10~35-fold enhancement in drug permeation that was dependent on the laser fluence and the narcotic analgesic used. The permeation of morphine and nalbuphine showed higher enhancement with Er:YAG laser treatment as compared to that of buprenorphine. This may have been due to the higher lipophilicity and molecular mass of buprenorphine than the other two narcotic drugs. A photomechanical wave was generated by filtering laser radiation through a polystyrene target. The experimental results showed that a single photomechanical wave was sufficient to enhance morphine permeation by sevenfold. This enhancement was significantly lower than that produced by direct laser irradiation, indicating the predominant mechanism of SC ablation by the Er:YAG laser for transdermal drug delivery.

Keywords Narcotic analgesics · Er:YAG laser · Transdermal delivery · Skin

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Introduction

Opioid analgesics such as morphine, nalbuphine, and buprenorphine (Fig. 1) are used clinically for the relief of postsurgical pain, cancer pain, and other pain. Opioids can be differentiated according to the categories of μ and κ receptors, which they preferentially occupy and activate [1]. Morphine, nalbuphine, and buprenorphine are respectively the agonist, mixed agonist–antagonist, and partial agonist of opioid receptors. Because of their low oral bioavailabilities (15~30%) and short elimination half-lives (1.4~3.4 h) [2–4], frequent injections are needed with these analgesics to achieve adequate pain control. Transdermal drug delivery offers further improvement in their administration and enables the continuous systemic application through the skin, producing constant plasma concentrations

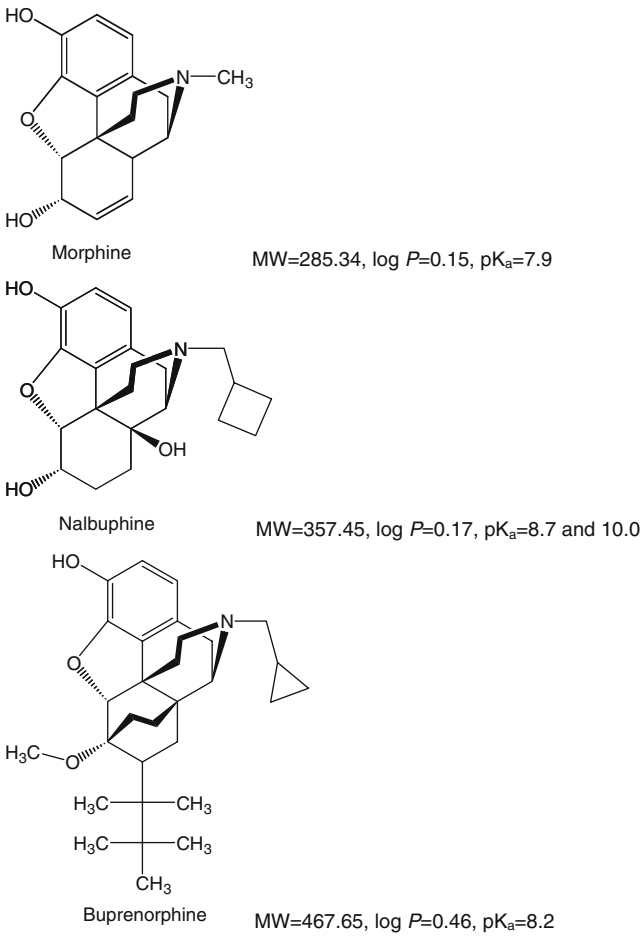

Fig. 1 Chemical structures, molecular weights (*MW*), octanol/water partitioning coefficients (log *P*), and dissociation coefficients (pK_a) of morphine, nalbuphine, and buprenorphine

[5]. Due to the low permeability of opioid analgesics across the skin, several approaches have been utilized to drive opioids through the skin barrier, including chemical enhancers, iontophoresis, and ultrasound [6, 7].

Many types of lasers have been developed and are used in dermatology. The erbium:yttrium–aluminum–garnet (Er:YAG) laser can ablate the stratum corneum (SC) with minimal residual thermal damage to the skin; it is currently used for the resurfacing of rhytides, scars, and photodamage [8]. We recently suggested that the Er:YAG laser can effectively enhance and precisely control drug delivery via the skin, including 5-fluorouracil, ascorbic acid, and dextran [9–11]. The aim of this study was to evaluate the feasibility of using an Er:YAG laser to control and promote the transdermal delivery of opioid analgesics. Depending on the molecular size and polarity of the various analgesics, there may be differences of several orders of magnitude in the rates of permeation across the skin. This present study utilized in vitro Franz cells to examine the transdermal delivery of analgesics with laser treatment. Histological changes in the skin structure due to interactions with the Er:YAG laser were also evaluated. To obtain pure photomechanical waves generated by the laser, a polystyrene lens was used to filter the laser light for comparison. A porcine animal model was used in this study because it is the most relevant animal model for human skin [12].

Materials and methods

Materials

Morphine HCl was supplied by the National Bureau of Controlled Drugs (Taipei, Taiwan). Nalbuphine HCl was purchased from Du Pont Merck (North Billerica, MA, USA). Buprenorphine HCl was purchased from Macfarlan Smith (Edinburgh, UK). All other chemicals and solvents were of analytical grade and were used as received.

Er:YAG laser assembly

The Er:YAG laser (Continuum Biomedical, Santa Clara, CA, USA) used here has a wavelength of 2,940 nm and a pulse duration of 250 μs. An articulated arm was used to deliver the laser beam onto the skin. Output energies of 0.35~0.65 J with a beam spot 7 mm in diameter could achieve fluences of 1.4~2.6 J/cm^2. The energy of the laser pulse was monitored with an energy meter (Nova Display, Ophir, Israel) before and after treatment.

Skin samples

Pigs (a hybrid of the genuses of Landrace and Duroc at ~1 week old) were supplied by the laboratory of Dr. Chi-Feng Hung, Fu Jen Catholic University (Hsinchuang, Taipei County, Taiwan). Full-thickness skin was excised from the dorsal region. The hair of skin samples was carefully removed with curved surgical scissors. The subcutaneous adipose tissue was also dissected away. The animal experiment protocol was received and approved by the Institutional Animal Care and Use Committee of Chang Gung University.

In vitro transdermal delivery

For the in vitro study, we used a Franz vertical diffusion assembly. A piece of excised pig skin was mounted on the receptor compartment with the SC side facing upward into the donor compartment. The laser handpiece was located approximately 3.7 cm from the skin surface. After pretreatment with the laser, the skin surface was wiped with a cotton wool swab several times. A black polystyrene target material was positioned on the skin for laser irradiation if necessary. A double-distilled water was used

to the acoustic coupling medium between the target material and the skin. To obtain SC-stripped skin, adhesive tape was applied to intact pig skin with uniform pressure and then removed. This procedure was repeated 30 times per sample. The receptor compartment (5.5-ml) was filled with a pH-7.4 citrate-phosphate buffer. The donor compartment (0.5-ml) contained morphine, nalbuphine, or buprenorphine (4-mM) in pH-5 buffer and was occluded with paraffin film. The available area of the Franz cell was 0.79 cm^2. The receptor was maintained at 37°C and stirred by a magnetic bar at 600 rpm. At appropriate intervals, a 200-μl aliquot of receptor medium was withdrawn and immediately replaced by an equal volume of fresh medium. The length of the sampling period was 36 h. The amounts of drugs in the receptor medium were determined by high-performance liquid chromatography (HPLC).

The concentrations of narcotic analgesics within the skin were determined after the 36-h experiment was completed. The skin tissue was rinsed with double-distilled water and blotted with a paper towel to remove any drug adhering to the skin surface. The tissue was weighed and minced with scissors, positioned in a glass homogenizer containing 1 ml of 0.1-N HCl, and ground for 5 min with an electric stirrer. The resulting solution was centrifuged for 10 min at 9,300×g and then filtered through a polyvinylidene difluoride (PVDF) membrane with a pore size of 0.45 μm. The drug amount in the supernatant was determined by HPLC.

HPLC analytical methods

Morphine, nalbuphine, and buprenorphine were quantified using an HPLC system. A 25-cm-long, 4-mm inner diameter stainless C18 column (LiChrospher®, Merck, Darmstadt, Germany) was used for morphine, while a Kanto Mightysil® C18 column (Tokyo, Japan) was used for nalbuphine and buprenorphine. The mobile phases consisted of acetonitrile: 20 mM phosphate aqueous solution (32:68); methanol, pH-3.5 citrate-phosphate buffer (15:85); and acetonitrile, pH-5 citrate-phosphate buffer (55:45) for morphine, nalbuphine, and buprenorphine, respectively. The flow rate was set at 1 ml/min, and the wavelength of the UV detector was set at 210 nm for all three analgesics.

Histological examination of pig skin

The pig skin was irradiated by an Er:YAG laser with or without a black polystyrene filter. Immediately after treatment, a specimen of the exposed area was taken for histological examination. The adjacent untreated skin was also assessed as a control. Each specimen was fixed in a 10% buffered formaldehyde solution at pH 7.4 for at least 48 h. Each section was dehydrated using ethanol, embedded in paraffin wax, and stained with hematoxylin and eosin (H&E). Three different sites for each sample were examined under an Eclipse 4000 light microscope (Nikon, Tokyo, Japan). Photomicrographs of skin samples were taken with a Coolpix 4500 digital camera (Nikon).

Statistical analysis

The statistical analysis of differences between different treatments was performed using unpaired Student's t test. A 0.05 level of probability was taken as the level of significance.

Results

Transdermal delivery of morphine across laser-treated skin

To evaluate the effect of the Er:YAG laser on the transdermal delivery of narcotic analgesics, morphine was first permeated across the skin. In the in vitro permeation study, the total amount of morphine permeating across a unit diffusion surface and into the receptor was calculated and plotted as a function of time. The drug flux was calculated by the slope of the linear portion of the cumulative amount–time plots and was expressed as the mass of drug passing across 1-cm^2 of skin over time, as shown in Fig. 2. Morphine showed very low permeability across the skin without laser pretreatment. The Er:YAG laser was effective in enhancing the skin permeation of morphine for all fluences tested. Laser ablation of 49% of the SC surface area produced 3~22-fold increases in the flux of morphine. An increase in the laser intensity led to a further promotion of morphine permeation from 1.4 to 2.1 J/cm^2. Nevertheless, the use of the highest fluence (2.6 J/cm^2) did not further increase the morphine permeation as compared to the fluence of 2.1 J/cm^2 ($p>0.05$). As shown in Fig. 2, no statistically significant difference ($p>0.05$) was observed in the flux of morphine across SC-stripped and 2.1 J/cm^2-treated skin.

Comparison of the transdermal delivery of three narcotic analgesics

The skin permeation of morphine, nalbuphine, and buprenorphine was evaluated with or without treatment with the Er:YAG laser for comparison. As shown in Table 1, there was no difference ($p>0.05$) among the fluxes of analgesics across intact skin. Fluences of 1.7 and 2.6 J/cm^2 both significantly accelerated ($p<0.05$) the fluxes of all three narcotic analgesics. Exposure of a limited area of the skin surface (49%) to the Er:YAG laser increased drug permeation to different enhancement levels; morphine and

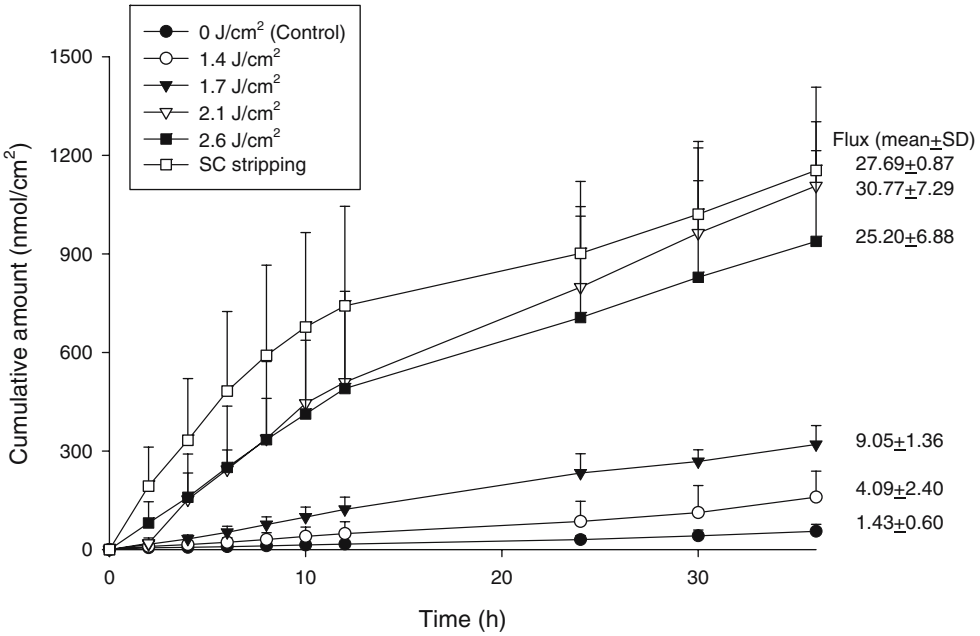

Fig. 2 In vitro cumulative amount–time profiles of the topical permeation of morphine after Er:YAG laser pretreatment of porcine skin at various fluences from 1.4 to 2.6 J/cm^2. Each value represents the mean±SD (n=4~8)

nalbuphine showed a more-significant enhancement. Extrapolating the original flux data of the laser-pretreated area to an area of 100% exposure (normalized flux) resulted in 10~35-fold increases of enhancement ratios. The fluxes of morphine and nalbuphine across the SC-stripped skin were significantly lower (p<0.05) than those across 2.6 J/cm^2-treated skin. However, buprenorphine showed an opposite result as compared to the other analgesics.

After each in vitro permeation experiment, drug was extracted from the skin to determine the drug accumulation in the skin. As shown in Table 2, no significant increase (p>0.05) in the skin deposition after laser treatment was found for morphine or nalbuphine. Tape stripping even decreased (p<0.05) the skin accumulation of morphine. A similar result was observed for buprenorphine. The 2.6-J/cm^2 fluence and tape-stripping technique both greatly reduced buprenorphine deposition into the skin.

The effect of a photomechanical wave on the transdermal delivery of morphine

To elucidate the mechanisms of the Er:YAG laser on transdermal drug delivery, a photomechanical wave was generated by laser ablation of a polystyrene target and then launched into the skin. In this experimental arrangement, the laser radiation was totally absorbed by the target so that only the photomechanical wave reached the skin [13]. As shown in Fig. 3, it is clear that a single photomechanical wave from the Er:YAG laser was sufficient to significantly enhance (p<0.05) the transport of morphine through pig skin

Table 1 Flux and enhancement ratio of narcotic drugs across pig skin with treatment using Er:YAG laser

Drug	Fluence (J/cm^2)	0	1.7	2.6	Stripping
Morphine	Original flux (nmol cm^{-2} h^{-1})[a]	1.43±0.60	9.05±1.36	25.20±6.88	31.69±0.87
	Normalized flux (nmol cm^{-2} h^{-1})[b]	–	16.97±2.56	49.93±13.64	31.69±0.87
	Enhancement ratio[c]	–	11.87	34.92	22.16
Nalbuphine	Original flux (nmol cm^{-2} h^{-1})	1.55±0.70	18.80±4.20	25.28±3.43	36.81±3.90
	Normalized flux (nmol cm^{-2} h^{-1})	–	36.75±8.21	49.99±6.78	36.81±3.90
	Enhancement ratio	–	23.71	32.25	23.75
Buprenorphine	Original flux (nmol cm^{-2} h^{-1})	1.41±0.83	7.87±3.44	10.92±1.41	26.27±3.50
	Normalized flux (nmol cm^{-2} h^{-1})	–	14.60±6.39	20.82±2.68	26.27±3.50
	Enhancement ratio	–	10.35	14.77	18.63

[a] The original flux was directly calculated from the flux across partially laser-treated skin area (49%).
[b] The normalized flux was calculated from the flux across the fully laser-treated skin area (100%) that was calibrated by the flux of the control group.
[c] The enhancement ratio (ER) was the normalized flux of laser treatment group/flux of the control group.
Each value represents the mean±SD (n=4–8).

Table 2 Narcotic drug amount deposited in the pig skin (nmol/mg) with treatment using Er:YAG laser

Fluence (J/cm^2)	0	1.7	2.6	Stripping
Morphine	1.07±0.15	1.60±0.38	1.16±0.31	0.56±0.14
Nalbuphine	1.53±0.51	1.77±0.51	2.25±0.39	1.69±0.54
Buprenorphine	18.59±9.58	16.72±5.73	6.58±7.00	3.48±12.99

Each value represents the mean±SD (n=4–8).

in vitro. However, this effect was much lower ($p<0.05$) than that generated by direct laser radiation.

Pig skin was exposed to the laser with or without the polystyrene target to determine the effects on the integrity of the skin structure. Light microscopy indicated that the structure of the whole skin remained intact in the untreated pig skin (Fig. 4a). Histologic observations demonstrated a thinning of the SC after treatment with 2.6 J/cm^2 of laser irradiation (Fig. 4b). The Er:YAG laser also flattened the SC layer above the viable skin. No gross changes in the viable epidermis or dermis were noted when compared with the control. When the Er:YAG laser radiation was filtered through the polystyrene target, no SC thinning was observed in the skin histology (Fig. 4c). Some focal compaction and homogenization of the SC were detected in the skin treated by a single photomechanical wave. No alteration was noted in the morphology or thickness of viable skin.

Discussion

The permeation of morphine across SC-stripped skin was much higher than that across intact skin, showing the SC to be the principal barrier to its penetration. The Er:YAG laser is an ablative tool for the SC with the capability of precise control. A single pulse of 1.4 J/cm^2 was sufficient to enhance morphine permeation across the pig skin. As demonstrated in our previous study [14], the etched thickness of the SC layer after laser ablation appeared to be proportional to the treated fluence. The rate-limiting step for hydrophilic morphine molecule penetration lies at the level of the SC. Partial ablation of the SC by the Er:YAG laser reduced the inherent barrier properties of the skin and, thus, enhanced morphine permeation.

The more-significant reduction in the SC thickness by the higher laser fluences may lead to greater enhancement of drug permeation. However, morphine flux with a fluence of 2.6 J/cm^2 did not exceed that with 2.1 J/cm^2. This may indicate that forces other than SC ablation predominated in increasing morphine permeation after laser exposure. The permeant is first partitioned into the SC, after which it progresses through the skin to deeper layers. Although removal of a part of the SC can reduce the inherent barrier property of the skin and increase drug permeation, the partitioning of the drug into the SC should be decreased after partial removal, which results in an offset effect. This phenomenon was proven for other drugs permeated across laser-pretreated skin, such as 5-fluorouracil and ascorbic acid [9, 10].

Narcotic analgesics are weakly basic drugs with pK_a values of >7.9 (Fig. 1). These analgesics were almost completely ionized in the donor solution (pH 5). The ionized form of a drug always shows a lower permeation compared to the un-ionized form because of the lipophilic characteristics of the SC. This may contribute to the low permeability of these analgesics. Although opioid analgesics have different molecular masses and lipophilicities, no

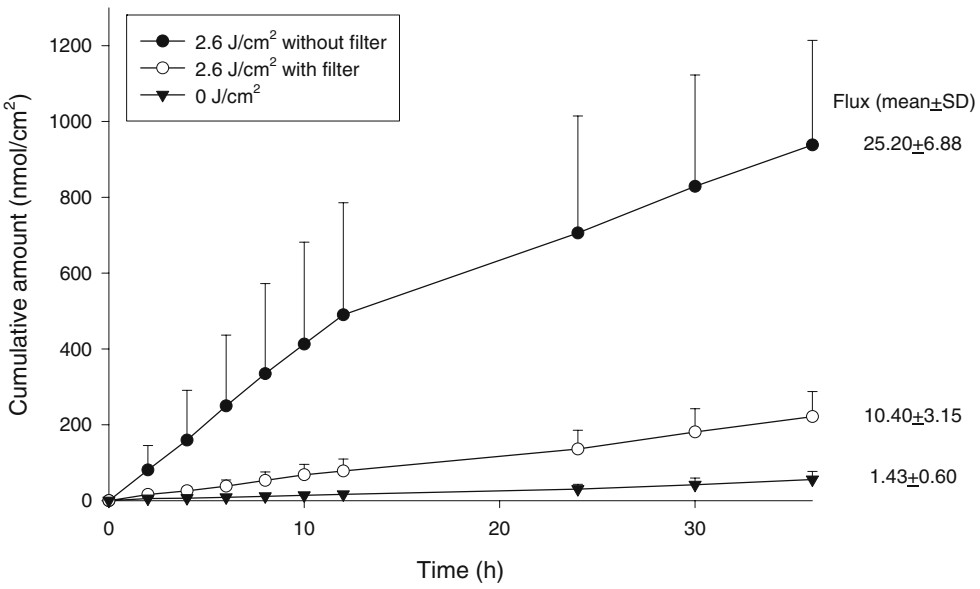

Fig. 3 In vitro cumulative amount–time profiles of the topical permeation of morphine after Er:YAG laser pretreatment of porcine skin at a 2.6 J/cm^2 fluence with or without polystyrene target filtering. Each value represents the mean±SD (n=6~8)

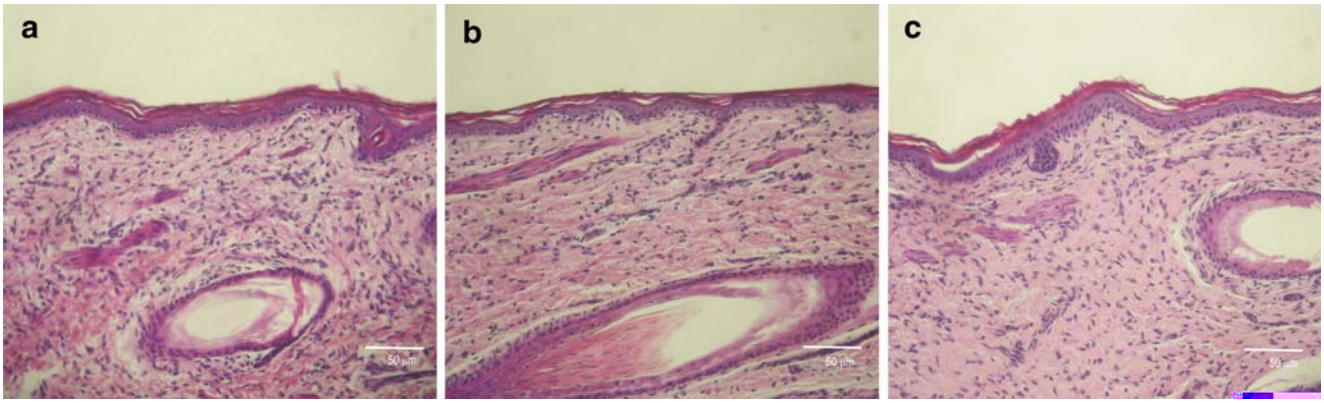

Fig. 4 Histological examination of pig skin with nontreatment (control; **a**); Er:YAG laser treatment at 2.6 J/cm^2 (**b**); and Er:YAG laser treatment at 2.6 J/cm^2 with polystyrene target filtering (photomechanical wave; magnification ×200)

significant difference was found in their permeation rates across intact pig skin. One may expect lower permeation to be associated with higher-molecular-weight drugs. Nevertheless, this is not the case with narcotic analgesics. It is well established that compounds with higher partition coefficients (log *P*), such as buprenorphine, are likely to be the better permeants across the skin. When the SC was totally stripped, however, significant differences in the fluxes among these analgesics were detected. Morphine and nalbuphine permeation showed comparable enhancements after the SC was stripped, which were higher than that of buprenorphine. Buprenorphine is a very lipophilic drug. Its hydrochloride salt still remains lipophilic despite the addition of a small amount of a hydrophilic substance (HCl) that is not able to shield the bulky hydrophobic moiety [15]. Under general in vitro conditions, a drug must pass through the thicker and hydrophilic viable epidermis/dermis, and the permeation resistance decreases the permeation of lipophilic drugs. Thus, it appears that the SC is not the sole significant contributor to resistance for buprenorphine. Viable skin appeared to be responsible for some bulk of the resistance. Of course, it cannot be neglected that the SC still contributed an important barrier for buprenorphine permeation. This can be confirmed by a proportional relationship between laser fluence and buprenorphine flux as depicted in Table 1. The Er:YAG laser could accelerate morphine and nalbuphine permeation to a greater degree than buprenorphine, especially at a fluence of 2.6 J/cm^2. This finding confirms that viable skin acts as a permeation barrier for buprenorphine. Because partitioning from the vehicle to the SC is important for lipophilic drugs, the loss of the SC by laser exposure of the skin may limit buprenorphine partitioning into the skin. Another possible reason is that the larger molecular mass of buprenorphine may have impeded its permeation across the laser-pretreated skin, although a part of the SC had been ablated.

Information on the skin reservoir of drugs may supplement the understanding of the complete process of permeation across the skin. The skin deposition of narcotic analgesics was not increased by laser irradiation. A fluence of 2.6 J/cm^2 actually reduced buprenorphine deposition. This may have been due to the lack of an SC drug reservoir within the skin, resulting in insufficient space for the deposition of drugs. This effect was especially important for buprenorphine because of its lipophilicity for partitioning into the SC. The higher buprenorphine contents in the skin among the three analgesics confirm the importance of SC partitioning for buprenorphine permeation. However, it was noted that the extraction rates of analgesics from the skin may differ. Hence, comparisons of the skin deposition data among various analgesics should be done with caution.

As depicted in Table 1, the Er:YAG laser increased the transdermal transport of morphine and nalbuphine to greater levels than did the SC-stripping technique. Because the Er:YAG laser only partly ablated the SC layer, there may be other mechanisms involved in the laser treatment. Three mechanisms, including direct ablation, optical breakdown (by the photomechanical wave), and a photothermal effect, are involved in laser–tissue interactions [16]. The Er:YAG laser emits light with a 2,940-nm wavelength that ablates the SC with a minimal residual thermal effect because this wavelength corresponds to the main peak of water absorption [17]. The photomechanical wave is a broadband, unipolar, compressive wave. Previous studies suggested that the photomechanical wave induces expansion of the lacunar spaces within the highly tortuous intercellular domains, leading to the formation of transient channels within the SC [16, 18]. Corneocytes may also be affected by the photomechanical wave [18]. Local propagation of the photomechanical wave also causes cracking of the upper layers of the epidermis [19]. This cracking of the epidermis can lead to greater drug permeation across the skin. Previous investigations showed that a pure photomechanical wave promotes transdermal drug delivery [20, 21]. A single photomechanical wave accelerated morphine flux by sevenfold. No significant alteration in the skin morphol-

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