Topical Drug Delivery in Humans with a Single Photomechanical Wave

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Purpose. Assess the feasibility of *in vivo* topical drug delivery in humans with a single photomechanical wave.
Methods. Photomechanical waves were generated with a 23 nsec Q-switched ruby laser. *In vivo* fluorescence spectroscopy was used as an elegant non-invasive assay of transport of 5-aminolevulinic acid into the skin following the application of a single photomechanical wave.
Results. The barrier function of the human stratum corneum *in vivo* may be modulated by a single (110 nsec) photomechanical compression wave without adversely affecting the viability and structure of the epidermis and dermis. Furthermore, the stratum corneum barrier always recovers within minutes following a photomechanical wave. The application of the photomechanical wave did not cause any pain. The dose delivered across the stratum corneum depends on the peak pressure and has a threshold at ~350 bar. A 30% increase in peak pressure, produced a 680% increase in the amount delivered.
Conclusions. Photomechanical waves may have important implications for transcutaneous drug delivery.

KEY WORDS: 5-aminolevulinic acid; ruby laser; photoacoustics; shock waves; stress waves; transdermal drug delivery.

INTRODUCTION

In topical drug application, the drug is delivered where it is needed while minimizing it at places where it is not needed or wanted. All else being equal, lower drug doses should be needed for topical application as compared with oral application, since losses of the drug (e.g., systemic deactivation or degradation) are reduced. Additionally, localization of the drug coupled to lower administered doses minimizes potential side effects. For topical application to be effective, the drug has to pass through the stratum corneum (SC). However, the SC of the skin is an effective barrier to molecular transport. Depending on the size and charge of the drug, there may be several orders of magnitude difference in the rate of penetration through the SC. Furthermore, even the most rapidly penetrating drugs actually diffuse very slowly (1). This has limited drugs for topical application to those molecules that can penetrate in sufficient quantities to produce a therapeutic effect. Therefore, a number of methods have been investigated for transdermal drug delivery.

In addition to vehicle formulations and chemical enhancers, physical methods such as the application of electrical current (iontophoresis and electroporation) and ultrasound (phonophoresis) have been investigated for drug delivery. In 1747, Veratti suggested the application of electrical current for transcutaneous drug delivery (2). The pathway for iontophoresis appears to be mainly transappendageal. It has also been suggested that electroporation of the SC lipids can occur at high voltages (>50 V) (3). Some of the disadvantages associated with iontophoresis are that drugs must be ionized and that the current used can cause iontophoretic burns (4).

The application of phonophoresis has been studied since 1954 with a variety of topical drugs such as counterirritants (e.g., menthol), anti-inflammatories (e.g., salicylates, hydrocortisone, dexamethasone), and anesthetics (e.g., lidocaine). Reports on the enhancement of transdermal delivery with phonophoresis have been mixed (5,6). More recently, it was reported that low frequency ultrasound (20 kHz, 100 ms pulses applied every second, 12.5 to 225 mW/cm^2 for 4 hr) induced transport of proteins (ranging from insulin [~6 kDa] to erythropoeitin [~48 kDa]) *in vitro* across human cadaver epidermis (7). In the same report, it was shown that insulin could be delivered in a rat model *in vivo* using low frequency ultrasound. No physical damage to the rat's skin was observed under a light microscope (40-fold magnification of samples stained with hematoxylin and eosin) (7). However, tissue damage may occur in phonophoresis (8). Electron micrographs of tissue after ultrasound exposure show significant cytotoxic effects which were attributed to cavitation (9). It has also been suggested that cavitation may be the primary mechanism of phonophoresis (10). Cavitation has been attributed to the presence of a tensile cycle (negative pressure), which is always present in ultrasound (11).

Photomechanical stress waves are broadband compressive waves (Fig. 1) (12). While the action of ultrasound is mostly mediated by heat or cavitation (13), photomechanical waves appear to interact directly with cells and tissue by mechanical forces (14). It has been shown that photomechanical waves can induce or enhance delivery of molecules across the plasma membrane of cells *in vitro* without loss of viability (15–17). These observations suggest that photomechanical waves may be provide an approach to enhance the delivery of molecules across the SC.

Experiments in a rat model *in vivo* showed that large molecules penetrated through the SC following the application of a single photomechanical wave (18). Both 40 kDa dextran molecules and latex particles 20 nm in diameter were delivered into the viable layers of the skin by a single photomechanical wave. Histology indicated that 40 kDa dextran molecules penetrated to a depth of approximately 40 μm beyond the SC. The rat SC is approximately 7 μm thick and the viable epidermis is ~13 μm (19). In contrast, human SC is ~10 μm thick and the viable epidermis is ~100 μm. Therefore, it is important to assess the feasibility of *in vivo* photomechanical topical delivery in humans.

MATERIALS AND METHODS

Experimental Arrangement

Photomechanical waves were generated by ablation of the target material with a Q-switched ruby laser and launched into

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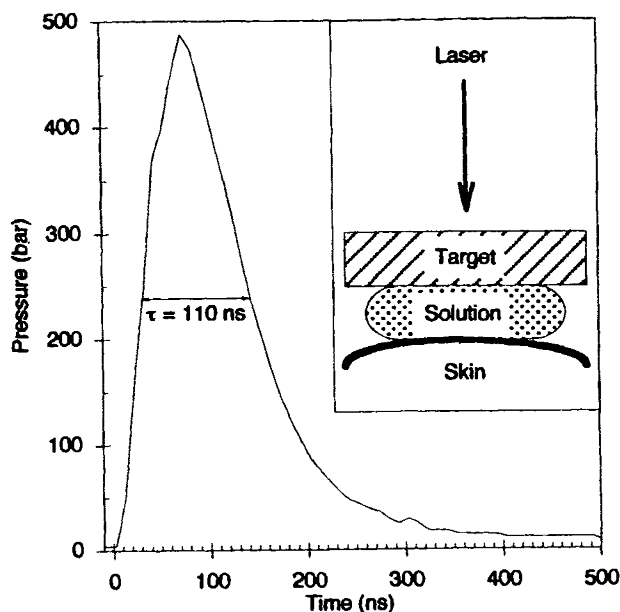

Fig. 1. Waveform of a photomechanical wave generated in a black polystyrene target by ablation with a Q-switched ruby laser. The laser system was equipped with an articulated arm which allows the laser pulse to be easily directed to the target while confining the path of the laser for safety. Insert: Photomechanical transdermal delivery.

the reservoir containing the molecular probe solution. An articulated arm was used to deliver the beam onto the target. The beam size at the target was ~6 mm in diameter to achieve fluence of ~7 J/cm^2. The laser pulse was completely absorbed by the target so that only the photomechanical wave propagated through the probe solution and impinged onto the skin. The temporal profile of the photomechanical wave used in these experiments which was measured with a calibrated transducer (under identical conditions of laser parameters, target material, and propagation distance through the coupling medium) is shown in Fig. 1.

The experimental arrangement used for drug delivery through the SC (18) is shown in the insert in Fig. 1. A flexible washer (~7 mm I.D., ~19 mm O.D., ~1.6 mm thick) placed onto the skin formed a reservoir for the solution to be delivered through the SC. The (black polystyrene) target material was positioned on top of the washer in contact with the solution which acted as the acoustic coupling medium.

Experimental Procedure

The research followed the tenets of the Declaration of Helsinki promulgated in 1964 and was approved by the Massachusetts General Hospital Subcommittee on Human Studies. Informed consent was obtained from the 7 human volunteers. The solution to be delivered through the SC was localized on the skin by a flexible plastic washer placed onto the skin (volar forearm of volunteers). The center part of the washer was filled with the solution and covered with the target. The laser radiation was completely absorbed by the target so that the SC was exposed only to the photomechanical wave. The solution serves as the coupling medium for the photomechanical wave to propagate into the SC. A single laser pulse was delivered to the target material which generated a single photomechanical wave. The solution was allowed to remain in contact with the skin for five minutes after the application of the photomechanical wave. Subsequently, the solution was removed and the surface of the skin cleaned with water. Control sites, adjacent to the sites exposed to photomechanical waves, were treated with the donor solution in an identical manner except that they were not exposed to photomechanical waves. *In vivo* fluorescence spectroscopy was used as an elegant non-invasive assay of transport of ALA into the skin following the application of a single photomechanical wave.

Fluorescence Spectroscopy

5-aminolevulinic acid (ALA) was chosen as a probe because it has been widely studied in our laboratory and provides a simple but elegant non-invasive assay of transport into the skin (20). ALA is a small charged molecule and therefore its rate of penetration is limited by normal human SC (21). ALA is a precursor of protoporphyrin IX (PpIX), an intermediate step in heme biosynthesis. In the presence of excess ALA, the conversion rate of ALA to PpIX is faster than the metabolism of PpIX, resulting in temporary accumulation of PpIX. The protocol was design to minimize or eliminate phototoxic damage due to this temporary accumulation of PpIX. ALA-induced PpIX accumulates predominantly in the viable epidermis and skin appendages (22). PpIX fluoresces at ~634 nm while ALA does not absorb or fluoresce at this wavelength. Thus, the transport of ALA can be followed non-invasively by monitoring the PpIX fluorescence. The intensity of fluorescence of PpIX is a measure of the amount of ALA transported through the SC by the photomechanical wave. The fluorescence was measured with a fiber coupled spectrofluorimeter (FluoroMax, Spex Industries, Edison, NJ). For (fair white) full thickness epidermis *in vitro*, the typical transmission of 400 nm (600–700 nm) light is ~0.5 (0.7 ~ 0.8) (23). Therefore, the fiber coupled spectrofluorimeter using 405 nm excitation and measuring the fluorescence emission around ~634 nm should be able to observe chromophores in the epidermis and at least the upper part of the dermis *in vivo*.

RESULTS

Following application of ALA for 5 minutes and a photomechanical pulse, the fluorescence signal of PpIX was observed to increase until it reached a maximum in a few hours. In Fig. 2, the fluorescence intensity at 634 nm as a function of time is shown for three different peak stresses. The permeation of the SC depended on the peak stress. The threshold permeation stress for the SC was observed at approximately 350 bar and increased dramatically at the highest stress. A 30% increase in peak pressure, produced a 680% increase in the amount delivered.

The fluorescence signal of PpIX was observed to increase (following application of a photomechanical pulse and ALA for 5 minutes) until it reached a maximum in 6~12 hours (see Fig. 3). In contrast, control sites (where only ALA was applied for 5 minutes) showed no fluorescence in the majority of the experiments, though occasionally, a weak fluorescence was observed. It is clear that a single photomechanical wave was sufficient to significantly enhance the transport of ALA through human SC *in vivo*.

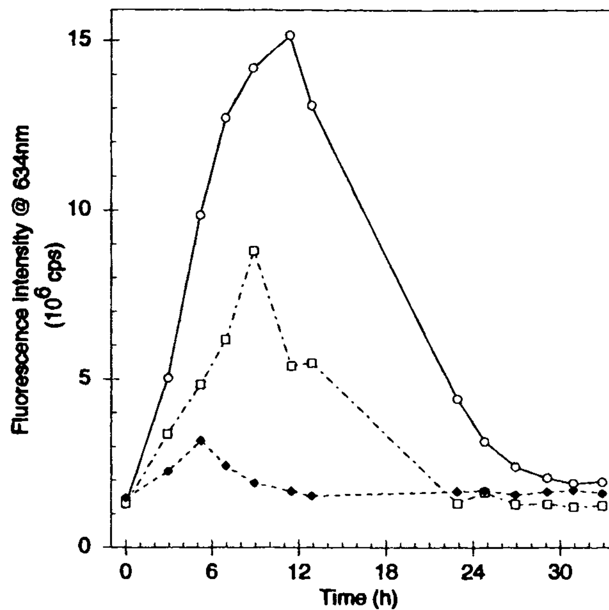

Fig. 2. Dependence of transdermal delivery on the peak stress of the photomechanical wave. The fluorescence intensity at 634 nm after application of a single photomechanical wave with different peak pressures of 388 bar [♦]; 442 bar [□]; and 503 bar [○]. The threshold permeation pressure for the SC was approximately 350 bar.

The barrier function of the SC following the application of a photomechanical wave always recovers. When ALA was applied immediately after a photomechanical wave using water as the coupling media, PpIX fluorescence was observed. If ALA was applied to a site 15–30 min after the application of a photomechanical wave, very little or no PpIX fluorescence was observed (Fig. 4) suggesting that the SC was no longer permeable to ALA. The recovery time of the barrier function of the SC probably depends on size as well as other characteristics (e.g., charge) of the probe molecules. In fact, in experiments on rats *in vivo*, the stratum corneum is no longer permeable to 40 kDa dextran molecules within a few minutes after the application of a single photomechanical wave (18).

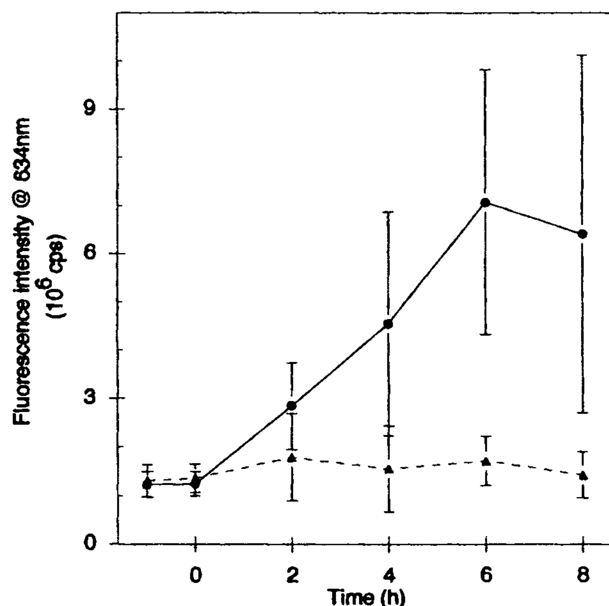

Fig. 3. Transdermal delivery of ALA. Fluorescence emission spectra of control sites exposed only to ALA [▲] and sites exposed to a single photomechanical wave in the presence of ALA [●]. Each point represents the average of the data from 7 human volunteers (except at 6 h [n = 5] and 8 h [n = 3]).

The observation of PpIX fluorescence from the skin indicates that the epidermis is still viable and converting ALA into PpIX. On the other hand, there are probably variations in the rate of PpIX biosynthesis among individuals (24). This may be one of the reasons for the differences in the intensity of the observed PpIX fluorescence signal from different volunteers (as seen in Fig. 3). The conversion of exogenous ALA into PpIX depends on both the rate of PpIX biosynthesis and the amount of ALA present. Consequently, the differences in the fluorescence intensity also depend on the quantity of ALA transported through the SC. At this time, the relative contributions to the observed biological variability cannot be determined. However, it is clear from these experiments that a single photomechanical wave can deliver ALA through the SC and does not adversely affect PpIX biosynthesis. It has been shown previously that the keratinocytes are a sensitive indicator of tissue damage due to photomechanical waves (25). Transmission electron microscopy indicated that there was no observable damage to the keratinocytes (see Fig. 5) from the photomechanical wave in the pressure range used in these experiments. In addition, all volunteers did not report any pain sensation and most did not report feeling any sensation at all (one reported feeling a slight non-painful sensation).

DISCUSSION

The mechanism by which a photomechanical wave enhances the transport of molecules through the SC is not known at this time. Three possible pathways (transappendageal, trans-cellular, and inter-cellular) have been suggested for penetration through the SC. The transappendageal (via cutaneous appendages) pathway is primarily via the follicles. However, the amount of transappendageal skin penetration in humans is

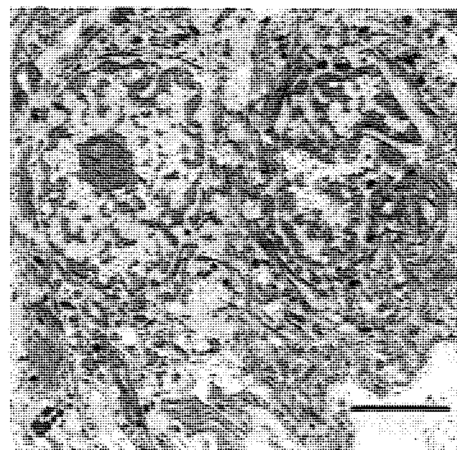

Fig. 4. Transmission electron micrograph of two basal keratinocytes from a biopsy obtained 24 hr following exposure to a photomechanical wave (500 bar) with water as the coupling solution. No significant damage is present in the subcellular organelles (scale bar = 3 μm).

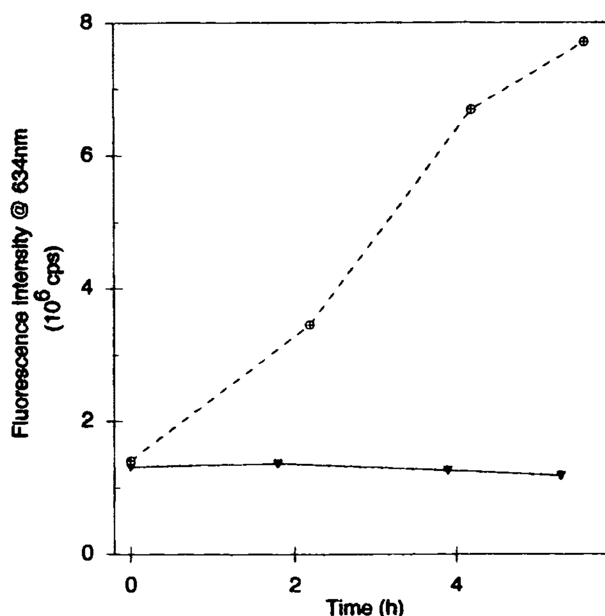

Fig. 5. The fluorescence intensity at 634 nm as a function of time of two adjacent sites. The first site was exposed to a single photomechanical wave in the presence of ALA [⊕]. The second site was exposed to a single photomechanical wave and the ALA was added 30 min later [▼]. There is no formation of PpIX indicating that the barrier function of SC had recovered.

limited by the small available surface area (the surface area of the cutaneous appendages is $\sim 10^{-2}$ to $\sim 10^{-5}$ of the surface area of the skin). The trans-cellular pathway requires the substrates to travel through the corneocytes. The inter-cellular pathway is via the extracellular lipids between the corneocytes. Experiments have suggested that the preferred pathway for percutaneous absorption is inter-cellular (26). For inter-cellular skin penetration, hydrophilic substrates are rate limited by the essentially lipid environment of the intercellular regions of the SC (27). Ionic substrates have very low partitioning into lipophilic environments on the basis of thermodynamics. It was shown that there was no observable skin absorption of the anion of salicylic acid or the cation of carbinoxamine (28). Therefore, percutaneous absorption along the intracellular pathway is expected to be low for ionic species such as ALA. As can be seen from Fig. 3, in the majority of the volunteers there is no observable PpIX fluorescence from sites where a photomechanical wave was not applied.

It has been suggested that cavitation is the primary mechanism for phonophoresis (10). Enhanced transdermal estradiol flux was observed for heat separated human skin *in vitro* following the application of ultrasound (13-fold enhancement for 1 MHz, 2 W/cm^2 for 30 min and 50% enhancement for 3 MHz, 2 W/cm^2 for 30 min). Confocal micrographs show significant bleaching of fluorescein loaded tissue after ultrasound exposure. This bleaching has been attributed to the oxidation of the fluorescein by cavitation generated peroxide radicals. In contrast, no bleaching was observed for 3 MHz (2 W/cm^2 for 30 min) ultrasound exposure. Histology suggests that the cavitational effect induced by sonophoresis (15 MHz, 0.1 W/cm^2 for 5 min) causes a disruption in the lamellar bilayers of the SC (29). Thus allowing molecular transport to proceed via inter-cellular pathways. However, cavitation may also be the explanation of some of the tissue damage that may occur in phonophoresis (8). Electron micrographs (9) of tissue after (16 MHz, 0.2 W/cm^2 for 20 min) ultrasound exposure show significant cytotoxic effects which were attributed to cavitation.

Cavitation has been attributed to the presence of a tensile cycle (negative pressure), which is always present in ultrasound (11). On the other hand, evidence indicates that photomechanical waves (650 bar peak pressure) do not generate cavitation (14). In addition, experiments in photomechanical-wave-induced membrane permeation in red blood cells indicate that cell loading is dependent on membrane integral proteins (functional aquaporins) (15). This suggests that the mechanisms of phonophoresis and photomechanical wave may be different. One possibility is that the photomechanical wave may affect plasma membranes and thus open up trans-cellular pathways for molecular transport through the SC. The cornified envelope of the corneocytes may not behave like typical plasma membranes. However, desmosomal domains may play a role similar to membrane integral proteins and be affected by photomechanical waves. Another interesting possibility is the formation of inter-cellular pathways. The photomechanical waves may induce a transient formation of continuous permeable lacunar system within the stratum corneum (30). Further work is needed to determine if the effect of photomechanical wave is to open up trans-cellular pathways, inter-cellular pathways, or a combination of both.

The ability of photomechanical waves to temporarily increase the permeability of plasma membranes of cells while the majority of the cells survive the procedure has been observed in experiments *in vitro* (16). This enhanced permeability has allowed the introduction of macromolecules into the cell cytoplasm. Photomechanical waves show promise for drug delivery at the cellular and organ level. An intriguing possibility is to adjust photomechanical wave parameters so that the photomechanical waves not only deliver molecules through the SC but to also load these molecules into cells *in vivo*.

In conclusion, a single 110 nsec photomechanical wave increases the permeability of human stratum corneum *in vivo*. The barrier function of the stratum corneum recovers within minutes. The dose delivered depends on the peak pressure and has a threshold at ~350 bar. A 30% increase in peak pressure, produced a 680% increase in the amount delivered. Photomechanical waves do not cause any pain nor do they adversely affect the viability and structure of the epidermis and dermis.

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