Sonophoresis. I. The Use of High-Frequency Ultrasound to Enhance Transdermal Drug Delivery

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Previous attempts to use ultrasound (≤1-MHz frequency and 1 to 3-W/cm^2 intensity) to enhance transdermal drug delivery (so-called sonophoresis) have produced inconsistent results. Theoretical analysis of ultrasound propagation in tissue predicts that higher-frequency ultrasound (>1 MHz) will increase the concentration of energy deposition in the stratum corneum (SC) (typically, the rate-limiting barrier to percutaneous penetration). This hypothesis was tested by comparing the passive transdermal delivery of salicylic acid with that under the influence of ultrasound at 2-, 10-, and 16-MHz frequency; measurements were performed *in vivo* in hairless guinea pigs. Total drug absorbed was quantified by determining the amount of salicylic acid (1) present in SC tape strips and (2) eliminated in urine. Sonophoresis for 20 min at 2 MHz caused no significant increase in salicylic acid delivery over passive diffusion; treatment with ultrasound at 10 and 16 MHz, on the other hand, significantly elevated salicylic acid transport, by 4-fold and 2.5-fold, respectively. Kinetic analysis of the sonophoretic data at 10 and 16 MHz also revealed that the diffusion lag time associated with transdermal drug delivery (TDD) was reduced. A shorter period (5 min) of sonophoresis again resulted in enhanced TDD (relative to the corresponding control) at the higher frequencies; the delivered dose, and the level of enhancement, however, were lower than those after the 20-min treatment. In a separate series of experiments, it was shown that (a) ultrasound did not alter the release kinetics of salicylic acid from the gel formulation used and (b) pretreatment of the skin with ultrasound at 10 and 16 MHz lowered skin barrier function such that the subsequent delivery of salicylic acid was enhanced compared to passive transport without sonophoresis pretreatment. It follows that the enhancing effect of sonophoresis is due to a direct effect of ultrasound on (presumably) the stratum corneum.

KEY WORDS: sonophoresis; ultrasound; transdermal delivery; salicylic acid; stratum corneum.

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INTRODUCTION

The need for enhancement methods to increase transdermal drug delivery (TDD) is well established (1) and approaches such as iontophoresis (2–5) and the use of chemicals (6,7) have been investigated. While appreciable increases in drug flux have been achieved, undesirable "side effects," such as irritation (8–10), have been observed. Hence, there remains a need for an effective enhancement method, which has minimal "toxicological" drawbacks.

Ultrasound has been used extensively over the last three decades for medical diagnostics and physical therapy. For such applications, the technique has been deemed safe, with no long- or short-term side effects (11). Thus, ultrasound satisfies a major criterion of an ideal approach to enhance TDD. The first published report on the use of ultrasound for increasing drug flux across the skin, so-called sonophoresis, appeared in 1954 (12). Since then, there have been numerous further studies, primarily *in vivo* (13,14). However, there is no consensus on the efficacy of ultrasound for increasing drug flux across the skin. While some studies report the successful employment of sonophoresis (15–19), others have obtained negative results (20–22). Although these different investigations have involved compounds (such as hydrocortisone, cortisol, salicylic acid, lidocaine, etc.) of various physicochemical characteristics, incorporated in a range of formulations (i.e., gels, creams and lotions), no consistent pattern of behavior has evolved. Common to these previous efforts were that ultrasound frequencies in the range of 1–3 MHz and applied intensity levels between 1 and 3 W/cm^2 were used because of the availability of commercial equipment.

The physics of ultrasound propagation in tissue and the barrier properties of skin define the ultrasound parameters which should improve the efficacy of sonophoresis. The stratum corneum (SC), the outermost 15–25 μm of the skin, is the principal rate-limiting barrier for percutaneous absorption (23,24). Therefore, enhancement of percutaneous absorption must target primarily this barrier. Similar to audible sound, ultrasound waves can undergo reflection, refraction, and/or absorption when they encounter another medium with dissimilar properties (25). If the properties of the encountered medium are significantly different from those of the transmitting medium (for example, the second medium is more absorbing), the acoustic energy of the ultrasound beam is attenuated by the above process(es). The attenuation of ultrasound in tissue, which limits its depth of penetration, is a function of the frequency and of the properties of the absorbing tissue (25). For most biological tissues, the empirical relationship between frequency and energy loss due to attenuation predicts that higher frequency waves undergo greater energy loss (energy loss = 1 dB/cm/MHz) (11,25,26) and lead to an increased energy concentration in the tissue. It follows that higher-frequency ultrasound will increase energy deposition within the SC (which is rather thin) and that the use of high-frequency ultrasound may render the membrane more permeable. The objective of this work was to test this hypothesis. We have examined the skin penetration enhancement of salicylic acid induced by ultrasound at 2-, 10-, and 16-MHz frequencies *in vivo* using hairless guinea

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pigs. We have observed that the higher frequencies (i) increased drug flux across the skin and (ii) reduced the lag time typically associated with TDD.

MATERIALS AND METHODS

High-frequency continuous-wave ultrasound was generated using a setup consisting of a signal generator (Model 8116A, Hewlett Packard, Palo Alto, CA), a power amplifier (325LA, ENI Co., Santa Clara, CA), and a set of commercially available ultrasound transducers (Precision Acoustic Devices, Fremont, CA, and Panametrics, Waltham, MA). The resonant frequencies of the transducers were confirmed using a network analyzer, and the transducers were driven at their fundamental resonant frequencies in all the experiments. The electrical energy content of the amplified high-frequency electrical signals was measured using a power meter (Model 4421-101, Bird Electronics, Ojai, CA). The transducers converted these electrical signals into ultrasound waves. To evaluate the acoustic efficiency of the transducers [efficiency of converting the electrical energy to mechanical (ultrasound) energy], radiation force measurements (i.e., the acoustic power output of the ultrasound waves was measured by the weight recorded on a sensitive balance) were made. These are described in detail elsewhere (27,28).

Hairless guinea pigs (8–16 weeks old) were obtained from Charles River Laboratories (Boston, MA). Salicylic acid was obtained from Aldrich Chemicals (St. Louis, MO). Carbopol was a gift from B. F. Goodrich & Co (Akron, OH). The gel formulation, which acts as both coupling agent and drug reservoir, was prepared as follows: a saturated solution of salicylic acid (0.5 g in 97 ml of water), including trace amounts of ^{14}C-labeled salicylic acid (New England Nuclear, DE) was mixed with 0.5 g of Carbopol (polyacrylic acid polymer). The formulation was left to stand overnight. After 24 hr, 0.5 g of NaOH was added so that the polymer network would extend, due to neutralization of the resin, causing the gel to swell. The addition of NaOH increased the formulation pH to 5.5. Since the pK_a of salicylic acid is 2.97, the drug was completely ionized and dissolved in the gel.

At least three animals were used for each experiment, and to reduce errors due to interanimal variability, each animal served as its own control. Treatment and control procedures followed identical protocols except that the transducer was not activated during the control procedure.

(a) Tape-Stripping and Urinary Excretion. Sufficient gel was spread on the face of the transducer to cover it completely with a thin film. Based on the amount of drug which was ultimately found to have penetrated, the dose of drug applied was effectively infinite. The transducer was placed in contact with the skin on one flank of the animal and was activated for 5 or 20 min. The intensity of the input energy was set to be 0.2 W/cm^2. At the end of the treatment period, the skin surface was cleaned of all residual gel by wiping with wet cotton swabs, followed by a water wash. The control experiments were carried out in an identical fashion on the contralateral flank of the animal. The efficacy of the ultrasound treatment was determined by comparing the amount of drug penetrated into the animal with and without ultrasound. The control and ultrasound treatment procedures were separated by at least 2 weeks and were conducted in a random order. The amount of drug that had penetrated into the animal was quantified in two ways: (i) sequential tape strips of the SC were removed using Scotch tape (3M Co., St. Paul, MN) and the amount of radioactivity, which was recovered in each strip, was determined; and (ii) the animals were housed in metabolic cages, and urine was collected periodically and monitored for radioactivity for 1–3 days. To 0.5 ml of urine, 0.5 ml of 1 *N* HCl and 4.5 ml of scintillation fluid (Readygel, Beckman, Irvine, CA) were added. The vials were kept in the dark for at least 12 hr so that the radioactivity measured by the scintillation counter was the result of true radioactive disintegration, rather than artifactual chemiluminescence. Subsequently, the amount of radioactivity was measured using a liquid scintillation counter (Beckman LS4000, Irvine, CA). All radioactivity data were corrected for background radioactivity and, in the case of urine, quenching due to coloration.

(b) Urinary Excretion Without Tape-Stripping. Excluding the tape-stripping step, a protocol identical to that described above, in Section a, was followed.

(c) Pretreatment Experiments. The selected site was pretreated with ultrasound for 10 min using water as the coupling medium. Thereafter, the skin was exposed to the drug-loaded gel for 5 min. Subsequently, tape-stripping and urinary analysis were performed as described in Section a. The control procedure involved an identical protocol on the contralateral flank without the transducer being activated.

(d) In Vitro *Release Experiments.* The gel was applied as a thin film onto the transducer surface, which was then immersed in 20 ml of well-stirred water (receiver solution). Postimmersion, 0.2-ml samples were withdrawn periodically and assayed. Quadruplicate measurements were made in both treatment and control experiments.

(e) Skin Temperature Measurements. Skin temperature was monitored using a digital thermometer with an ultrasound transparent probe head (BAT-12, Sensortek, Clifton, NJ). The thermocouple probe head (0.6-mm diameter) was positioned between the transducer and the skin, and the local tissue temperature was recorded at periodic intervals.

(f) Data Analysis. From the amount of radioactivity initially present in the gel, the recovered radioactivity (from tape-stripping the SC and from the urine) was converted to micrograms of drug. The latter quantity equaled the amount of drug that had penetrated into the animals. The transducers used had different cross-sectional areas. In order to compare the results obtained using different transducers, the amount (μg) of drug recovered was divided by the cross-sectional area of the transducer employed. To quantify the effect of ultrasound on the drug flux, we defined the percentage enhancement (E) as

$$E = \frac{\text{radiolabel (drug) recovered postsonophoresis}}{\text{radiolabel (drug) recovered after passive diffusion}} \times 100$$

Enhancement values are expressed as mean ± SD (n = 3).

RESULTS

Figure 1 compares the amount of salicylic acid, which penetrated hairless guinea pig skin in 20 min, (a) by passive diffusion and (b) with sonophoresis using 2-, 10-, and 16-MHz frequencies at a 0.2-W/cm^2 intensity. The hatched bars represent drug present in the SC, which was recovered by

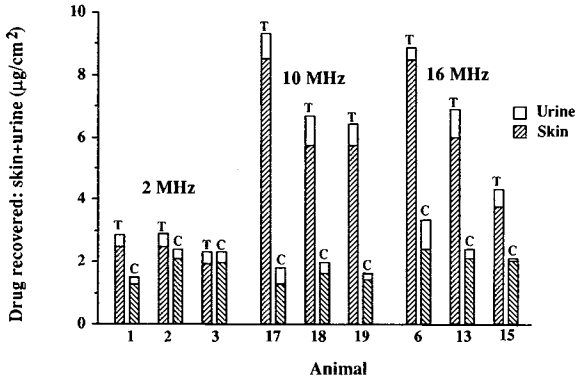

Fig. 1. TDD of salicylic acid following 20 min of sonophoresis (T), using 2-, 10-, and 16-MHz frequencies, and passive diffusion (C). A paired t test reveals that there is no significant difference between T and C when 2 MHz is used. However, there is a significant difference between T and C after sonophoresis using 10 MHz ($P \leq 0.03$) and 16 MHz ($P \leq 0.05$).

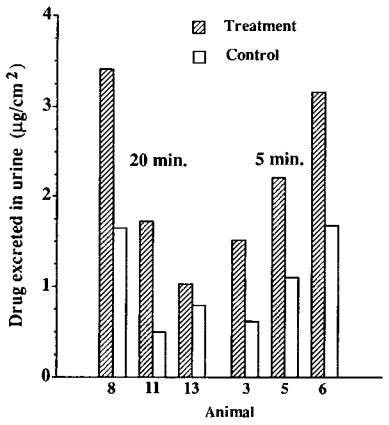

Fig. 3. The total amount of salicylic acid absorbed transdermally (calculated from the radioactivity eliminated in the urine) following 20 and 5 min of sonophoresis at 16 MHz. A paired t test reveals that there is a significant difference between treatment and control ($P \leq 0.08$).

tape-stripping. The unfilled segments above the hatched rectangles indicate drug recovered in the urine. The results obtained for each animal after sonophoresis (T) and passive diffusion (C) are presented side by side to facilitate direct comparison. Sonophoresis at 2 MHz did not significantly increase the total drug flux compared to passive diffusion ($E_{2MHz} = 133 \pm 63\%$, $P \geq 0.25$). However, sonophoresis using 10- and 16-MHz frequencies resulted in a significantly enhanced TDD ($E_{10MHz} = 415 \pm 89\%$, $P \leq 0.03$; $E_{16MHz} = 253 \pm 42\%$, $P \leq 0.05$).

In another series of experiments, to assess the effect of shorter ultrasound exposure, sonophoresis was carried out for only 5 min. The results of these experiments are shown in Fig. 2. Similar to the results obtained following 20 min of sonophoresis, the shorter exposure at 10 and 16 MHz enhanced the flux of salicylic acid through the skin for all the animals used ($E_{10MHz} = 160 \pm 24\%$, $P \leq 0.02$; $E_{16MHz} = 177 \pm 21\%$, $P \leq 0.04$). As expected, due to the reduced contact time between the formulation and the skin, TDD overall was lower.

Figure 3 shows the results of the experiments (following treatment at 16 MHz), in which drug penetration was assessed from urine analysis alone, with no tape-stripping

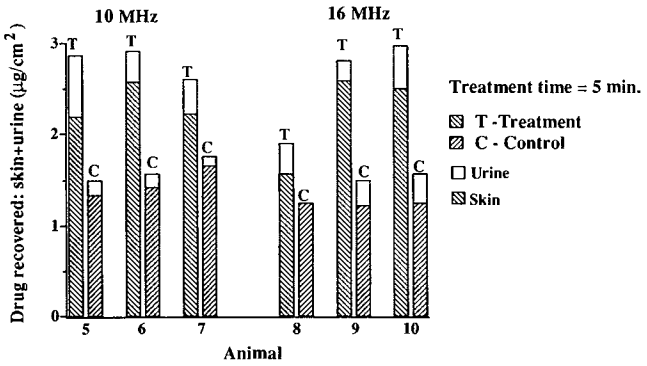

Fig. 2. TDD of salicylic acid following (a) sonophoresis (at 10 and 16 MHz) for 5 minutes (T) and (b) passive diffusion (C). There is a significant difference between T and C for both the frequencies used (10 MHz, $P \leq 0.02$, and 16 MHz, $P \leq 0.04$). The effect of sonophoresis exposure time can be assessed by comparison with Fig. 1.

(Section b in Materials and Methods). Sonophoresis for 5 and 20 min increased the amount of radioactivity eliminated in the urine of all animals tested, indicating again that more drug entered the systemic circulation under the influence of ultrasound ($E_{5min} = 226 \pm 106\%$; $E_{20min} = 211 \pm 29\%$; $P \leq 0.08$ for both). To assess the effect of ultrasound on diffusional lag-time, temporal evolution of the data in Fig. 3 are plotted in Figs. 4a and 4b. Two important features are as follows: (i) the area under the treatment curve (indicating the total amount of radioactivity eliminated) is significantly larger than the corresponding control; and (ii) radioactivity is detected in the urine much sooner following sonophoresis, indicating that the lag time for diffusion is reduced. Ultrasound did not appear to influence the volume of urine excreted: there was no significant difference between the volume of urine excreted with, and that excreted without, ultrasound.

Figure 5 shows the effect of pretreating the skin with ultrasound followed by application of the drug-containing gel *without* ultrasound (Section c in Materials and Methods). As observed above, salicylic acid absorption through ultrasound-pretreated skin is greater than that through the corresponding control. These data suggest that pretreating the skin with ultrasound makes the SC more permeable.

Figure 6 shows drug release profiles from the gel with and without 16-MHz ultrasound. There is no statistically significant difference between the release profiles, showing that ultrasound is not facilitating drug release from the gel.

The radiation force measurements are shown in Fig. 7. The 2- and 16-MHz transducers (received from the same manufacturer) had the same acoustic efficiency. The 10-MHz transducer (obtained from a different manufacturer) had a relatively higher efficiency.

DISCUSSION

Previous attempts to enhance percutaneous absorption using ultrasound have achieved, at best, only modest success, despite the use of high intensities (≥ 1 W/cm^2). We hypothesized that the relatively small effects observed were due to the use of low ultrasound frequencies (1–3 MHz),

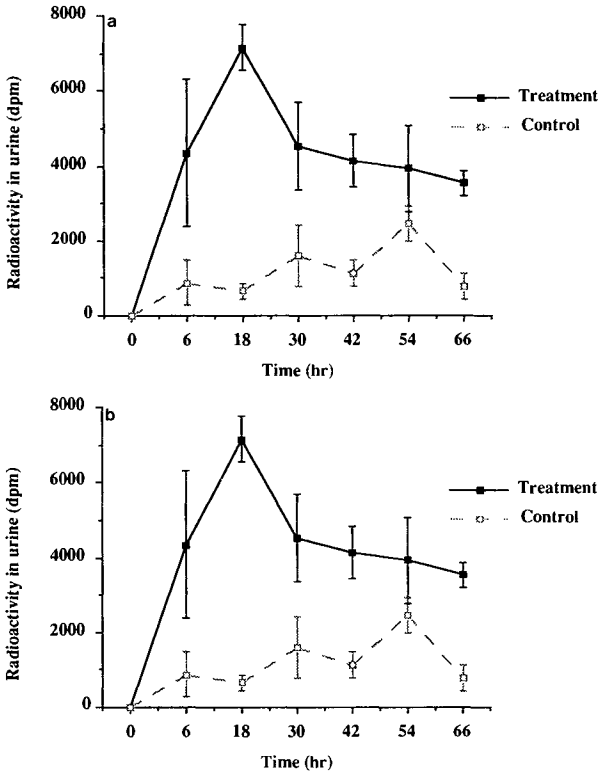

Fig. 4. Radioactivity (mean ± SE; $n = 3$) eliminated in the urine, as a function of time, after sonophoresis at 16 MHz for 20 min (a) and 5 min (b). Different amounts of radiolabeled tracer were administered in the two experiments making direct, quantitative comparison inappropriate. However, in both cases, the total amount of radioactivity eliminated postsonophoresis is significantly greater ($P \leq 0.02$ for 5 min, $P \leq 0.05$ for 20 min) than the control. Furthermore, the value at each time point is significantly different from the corresponding time point after passive diffusion ($P \leq 0.01$ for 5 min, $P \leq 0.2$ for 20 min).

which would penetrate deep into tissue and produce very little energy dissipation within the SC. Therefore, in this study, we have used frequencies of 2, 10, and 16 MHz. The availability of transducers dictated the choice of these frequencies. The intensity of 0.2 W/cm^2, a value much lower than that previously used, falls in the range used for diagnostic applications of ultrasound; the higher levels employed before are characteristic of those considered beneficial in physical therapy. Our preference for avoiding high intensity was based on the fact that continuous and/or repeated application of high-intensity ultrasound, as may be needed for TDD enhancement, can lead to significant, and often intolerable, heating. To minimize heating, physical therapists either use pulsed waves and/or move the transducer around the treatment area when water is used as the coupling medium, while minimizing direct contact with the skin. Obviously, appreciable local heating may be expected to lead to poor patient compliance. Under our experimental conditions, little increase in skin temperature (i.e., less than 1°C) was observed, presumably because of the low intensities employed. We can, therefore, discount heating of the skin as a mechanism by which the observed enhancement is induced. Parenthetically, we note that, for water, a 10°C increase in temperature is necessary for a doubling of skin permeability (29).

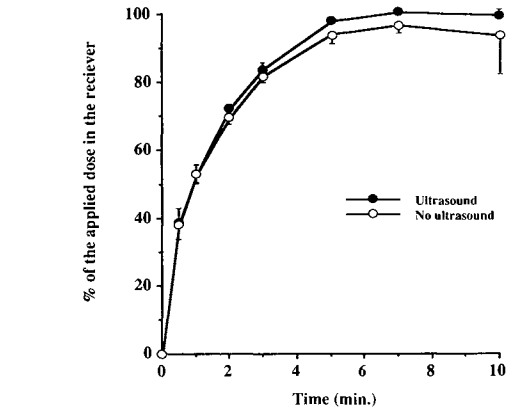

Fig. 6. *In vitro* release of salicylic acid from the formulation by passive diffusion and under the influence of ultrasound. Ultrasound does not significantly affect the release rate of salicylic acid from the gel formation.

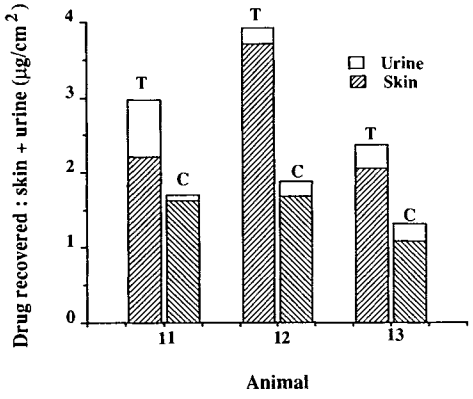

Fig. 5. TDD of salicylic acid following (a) pretreatment of the skin with ultrasound (16 MHz, 10 min) and then a 5-min application of drug-containing gel (T) and (b) a 5-min application of the same gel without ultrasound pretreatment (C). Significantly more drug was delivered when the skin was pretreated with ultrasound ($P \leq 0.04$).

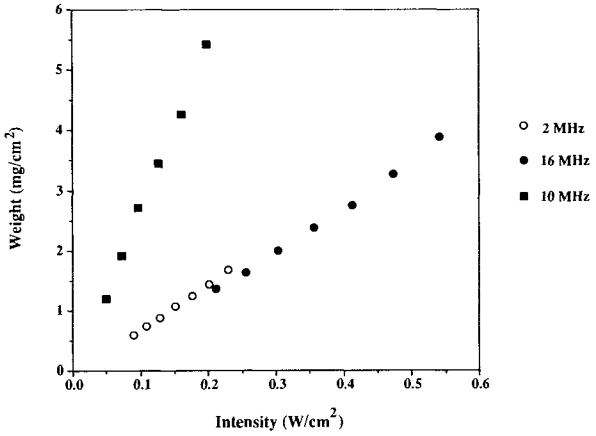

Fig. 7. Radiation force measurements to determine the acoustic efficiency of the different transducers. The weight registered on a sensitive balance for a given electrical energy is plotted as a function of the input.

Although the biological effects of ultrasound, at the relatively high intensities and low frequencies commonly used in diagnostic procedures, have been well documented, the same cannot be said of high-frequency ultrasound (25). From a safety standpoint, therefore, if one proposes to test the hypothesis that high-frequency ultrasound will be more effective in sonophoresis, it makes sense to begin the investigation at low intensities. Because the attenuation of ultrasound is directly proportional to the frequency, the depth of penetration of the acoustic energy varies inversely with frequency. The combination of high frequency and low intensity, therefore, should concentrate the effect of the ultrasound beam in the superficial skin layers, and minimize any negative effects of ultrasound on deeper tissues. In a subsequent paper, this issue is addressed by examining the impact of ultrasound on epidermal tissue morphology.

The results presented here demonstrate that high-frequency ultrasound, at a diagnostic intensity (0.2 W/cm^2), can enhance the TDD of salicylic acid. Ultrasound requires a coupling medium for efficient energy transmission to the skin; an aqueous gel is a suitable coupling medium between the transducer and skin. Furthermore, the gel can also serve as a drug reservoir. Salicylic acid was selected as the model penetrant for this work: it has been previously used as an illustrative compound in other studies of TDD enhancement (e.g., Ref. 30), and it possesses appropriate solubility properties for the experiments described here. Although salicylic acid itself can alter epithelial membrane properties, exposure periods considerably longer (on the order of hours) than those used here are required for a measurable effect to be observed. Hence, we can be confident that altered flux is due to the ultrasound treatment, and not to the penetrant itself (a conclusion confirmed by the comparison between control and sonophoresis experiments). While it had been reported earlier that ultrasound can facilitate the release of molecules from polymeric matrices (31), we found that ultrasound did not alter the efflux of salicylic acid into an aqueous reservoir from the gel employed (see Fig. 6). The acoustic impedance mismatch between the transducer and the polymer matrix may explain the enhanced drug release previously observed (31). Conversely, the acoustic impedance values of water (gel) and skin do not differ significantly. Confirmation that ultrasound acts on the SC barrier (and not on the release of drug from the formulation) is provided by the pretreatment experiments. As no drug-containing gel is involved in the pretreatment of the skin with ultrasound, the enhanced drug delivery that is measured on subsequent application of the formulation must result from ultrasound-induced changes in the skin during the pretreatment process. The duration and reversibility of this effect, and the possible mechanism(s) of action, are discussed elsewhere.

Two major impediments to the more widespread use of TDD are the limited permeability of molecules through the skin and the unacceptably long lag times associated with the attainment of a therapeutically useful flux. The data presented here suggest that ultrasound can, to some extent, counter both of these problems. Figure 4a shows that the extent of TDD is increased using sonophoresis. Figure 4b indicates that the lag time can be reduced compared to that associated with passive diffusion. These advantages may be particularly significant, for example, in the delivery of local anesthetics.

A comment upon the characteristics of the transducers is warranted. High-frequency transducers (>2 MHz) are not commonly available. The transducers used in this work were supplied by two different manufacturers (2 and 16 MHz from one and 10 MHz from another), and they possessed different performance efficiencies (Fig. 7). For different input electrical energies, the 10-MHz transducer operated at a much higher efficiency, compared to the other two transducers, in converting the electrical signals to mechanical waves. The increased enhancements observed using the 10-MHz transducer (compared to the 16 MHz), which is contrary to our initial hypothesis are, we believe, due to its higher efficiency. To compensate for the lower acoustic efficiency of the 16-MHz transducer, we attempted to use higher intensities (~0.35 W/cm^2). Unfortunately, this caused a considerable (and unacceptable) increase in the temperature of the transducer (such that we could not distinguish between enhancement due to ultrasound and enhancement due to skin heating). Further experiments at higher intensities were therefore abandoned.

The exposure of the skin to ultrasound in the experiments reported here was of a much shorter duration than those previously described in the literature. In general, studies of the effects of different enhancers or enhancement methods have typically involved treating the skin for periods longer than 1 hr. The earlier work on sonophoresis is no exception to this rule. In contrast, the treatment times of 20 and 5 min, which have been used here, yielded significantly enhanced transport of the test molecule compared to its passive diffusion. Although the increase in TDD is modest (maximally about fourfold), the results support the hypothesis that higher-frequency ultrasound is more effective for sonophoresis. Furthermore, it is efficacious with relatively short treatment times, a potentially desirable feature from the standpoint of patient acceptability and compliance.

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