Transdermal Drug Delivery Using Low-Frequency Sonophoresis

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Purpose. Application of therapeutic ultrasound (frequency: 1–3 MHz and intensity: 0–2 W/cm^2) enhances transdermal drug transport, although typically by a factor of less than 10. In this paper, we show that application of ultrasound at 20 KHz induces transdermal transport enhancements of up to 1000 times higher than those induced by therapeutic ultrasound.

Methods. *In vitro* (human cadaver epidermis) as well as *in vivo* (hairless rat skin) permeation experiments were performed to assess the effect of low-frequency ultrasound on transdermal transport.

Results. Application of low-frequency ultrasound (20 KHz, 125 mW/cm^2, 100 msec pulses applied every second) enhanced transdermal transport of several permeants, including estradiol, salicylic acid, corticosterone, sucrose, aldosterone, water, and butanol, across human cadaver skin by a factor in the range of 3 to 3000 and that of salicylic acid across hairless rat skin in vivo by a factor of up to 300. Low-frequency ultrasound did not induce a long-term loss of the barrier properties of the skin *(in vitro)* or damage to living skin of hairless rats. At a mechanistic level, it is hypothesized that application of low-frequency ultrasound enhances transdermal transport through aqueous channels in the SC generated by cavitation-induced bilayer disordering. Support for this hypothesis is provided using experimental and theoretical analyses of low-frequency sonophoresis.

Conclusions. Low-frequency ultrasound enhances transdermal transport of drugs more effectively than that induced by therapeutic ultrasound.

KEY WORDS: transdermal drug delivery; ultrasound; sonophoresis; cavitation.

INTRODUCTION

Transdermal drug delivery offers an alternative to oral delivery and injections. However, its application has been restricted to only a few drugs because of the skin's low permeability (1). The enormous barrier properties of the skin are attributed to the stratum corneum (SC), the outermost layer of the skin. The SC consists of keratinocytes, which are dead cells filled with cross-linked keratin fibers that are surrounded by lipid bilayers. The transdermal transport of drugs occurs through the intercellular lipid bilayers whose *ordered* structure confers a low permeability to the skin (2). A variety of approaches have been suggested to enhance transdermal drug transport. These include: i) use of chemicals to either modify the skin structure or to increase the drug concentration in the transdermal drug patch (3,4), ii) application of electric fields to create transient transport pathways (electroporation) (5,6) or to increase the mobility of charged drugs through the skin (iontophoresis) (7), and iii) application of ultrasound (sonophoresis) (8).

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Sonophoresis has been shown to enhance transdermal transport of various drugs (9–11). Although a variety of ultrasound conditions have been used for sonophoresis, the most commonly used condition corresponds to therapeutic ultrasound (frequency in the range of 1–3 MHz, and intensity in the range of 0–2 W/cm^2). It is commonly observed that the typical enhancement induced by therapeutic ultrasound is less than 10-fold, and in many cases, no enhancement of transdermal drug transport has been observed upon application of therapeutic ultrasound (9–11). Due to this uncertainty and inefficiency, therapeutic sonophoresis has found limited clinical application. Accordingly, a better selection of ultrasound parameters is needed to induce a higher enhancement of transdermal drug transport by sonophoresis. In a previous study, we have found that ultrasound-induced cavitation is the dominant mechanism responsible for sonophoresis (8). Since cavitational effects vary inversely with ultrasound frequency (12), we hypothesize that ultrasound at frequencies lower than that corresponding to therapeutic ultrasound should be more effective in enhancing transdermal drug transport.

In this paper, we present an investigation of low-frequency sonophoresis undertaken to assess the validity of the above mentioned hypothesis. This investigation includes: (i) *in vitro* and *in vivo* experiments performed to assess the sonophoretic transdermal transport enhancement induced by low-frequency ultrasound, (ii) a preliminary safety analysis to assess the recovery of the skin barrier properties after sonophoresis (*in vitro*) and the effect of ultrasound on living skin cells (*in vivo*), (iii) a mechanistic analysis of low-frequency sonophoresis, and (iv) a quantitative analysis of low-frequency sonophoresis.

MATERIALS AND METHODS

A. Transdermal Transport Measurements

Transdermal transport of a variety of permeants, including estradiol, salicylic acid, corticosterone, sucrose, aldosterone, water, and butanol (either ^{3}H or ^{14}C labelled obtained from New England Nuclear), was studied in the presence as well as in the absence of low-frequency ultrasound. These permeants cover a broad range of molecular characteristics, including molecular weight and lipophilicity, thus allowing us to study the dependence of sonophoretic enhancement on these characteristics. The permeability experiments were performed *in vitro* using human cadaver skin (obtained from local hospitals and the National Disease Research Institute). The skin was heat stripped by keeping the full-thickness skin in water at 60°C for two minutes followed by removal of the epidermis. The epidermis was then stored at 4°C in a humidified chamber for up to 2 weeks. A piece of the epidermis was taken out from the chamber prior to the experiments and was mounted on a Franz diffusion cell (Crown Glass Co., FDC 200). The Franz diffusion cell consists of two compartments, the donor and the receiver compartments, with the stratum corneum facing the donor compartment. The epidermis was supported by a nylon mesh (Tetko, Inc.) to avoid any damage due to possible mechanical oscillations upon ultrasound application. The donor and receiver compartments were then clamped. The receiver compartment was filled with Phosphate Buffer Saline (PBS, phos-

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phate concentration = 0.01 M, NaCl concentration = 0.137M) (Sigma Chemicals Co.), and the donor solution was filled with ~1 μCi/ml solution of the radiolabelled permeant in PBS. The concentration of the permeant in the receiver compartment was measured every hour using a scintillation counter (model 2000 CA, Packard). The epidermis permeability was calculated from the transdermal flux using the equation, $P = V\Delta C/(AC_d\tau)$, where V is the volume of the receiver compartment (15.8 ml), A is the skin area (3.14 cm^2), ΔC is the measured increase in the permeant concentration of the solution in the receiver compartment over a time period τ, and C_d is the permeant concentration of the solution in the donor compartment.

B. Ultrasound Application

Ultrasound was applied using a sonicator (VCX 400, Sonics and Materials) operating at a frequency of 20 KHz. The ultrasound intensity was measured using a hydrophone (Model 8106, Bruel and Kjaer). In order to avoid any thermal effects, a pulsed application of ultrasound (100 msec pulses applied every second) was chosen, although the choice of pulse rate was arbitrary. The temperatures of the solutions in the donor and the receiver compartments were measured using a thermocouple (Digithermo, VWR Scientific). No significant increase in either temperature (<2°C) was observed upon ultrasound exposure.

C. Electrical Resistance Measurements

Two Ag/AgCl electrodes (E242, Invivo Metrics) were introduced in the donor and the receiver compartments to measure the electrical resistance of the epidermis. These measurements were taken every hour, before, during, and after ultrasound exposure. In order to measure the electrical resistance of the epidermis, an AC electric field, typically having a voltage of 100 mV and a frequency of 10 Hz, was applied across the electrodes for a short time (typically 5 seconds) using a signal generator (model HP 4116 A). The electric current through the skin was measured using an ammeter (Micronta, Tandy Corporation). The electrical resistance was then calculated from Ohm's law. The saline resistance was measured separately using the same assembly but without mounting the epidermis. Since the measured epidermis resistance is the sum of the actual epidermis resistance and the saline resistance, the latter was subtracted from the measured epidermis resistance to obtain the actual epidermis resistance. The epidermis resistivity was calculated by multiplying the epidermal electrical resistance by the skin area (3.14 cm^2).

D. *In Vivo* Sonophoresis Experiments

In vivo experiments were performed to assess the efficacy of sonophoresis across living skin. Hairless rats (IFFA, Charles River, about 16 weeks old, either sex) were used as an animal model for these studies, since it has been shown that the transport properties of hairless rat skin are comparable to those of human skin (13). The hairless rats were anesthetized with a mixture of ketamine (60 mg/kg) and xylazine (10 mg/kg). After about an hour into anesthesia, a flanged glass cylinder (Crown Glass Company, diameter 20 mm, height 2 cm) was glued on the rat's back using a minimal amount of superglue (Permabond International) or vacuum grease (Dow Chemicals) on the outer edge of the flange. The center of the cylinder was located about 3 cm from the rear end of the rat. This particular site was chosen to avoid application of ultrasound directly on a sharp bone close to the body surface, which otherwise might have caused damage to the blood capillaries near the edge of the bone. The cylinder was filled with a solution of radiolabelled salicylic acid (about 2 μCi/ml). Ultrasound (20 KHz, 125 mW/cm^2, 100 msec pulses applied every second) was applied by immersing the transducer in the donor solution. The concentration of salicylic acid in the rat urine was measured using a scintillation counter (model 2000 CA, Packard).

E. Histological Studies

Histological studies of the hairless rat skin exposed to ultrasound were performed to assess the safety of low-frequency sonophoresis as a penetration enhancer. These studies were performed at Deborah Heart and Lung Institute, New Jersey. The hairless rat skin exposed to ultrasound (20 KHz, 125 mW/cm^2, 100 msec pulses applied every second for 1 hour) was removed within 5 minutes after sacrificing the rat using pentobarbital. These samples were stored in a formalin solution for up to 5 days. The samples were then stained with hematoxylin and eosin (Deborah Heart and Lung Institute, New Jersey). The stained skin samples were later observed under a light microscope (40-fold magnification) to assess possible structural damage to the skin.

RESULTS AND DISCUSSION

A. Low-Frequency Ultrasound Enhances Transdermal Transport of Drugs

Application of low-frequency ultrasound (20 KHz, 125 mW/cm^2, 100 msec pulses applied every second) enhances transdermal transport across human cadaver epidermis *in vitro* for all the permeants examined, including estradiol, salicylic acid, corticosterone, aldosterone, sucrose, water, and butanol, by a factor in the range of 3 to 5000. As an example, Figure 1 shows the effect of ultrasound on the amount of salicylic acid transported (represented as the percent of the amount present in the donor compartment) across the skin (filled circles). The amount of salicylic acid transported by passive permeation, though detectable, is very small and is also shown (empty circles). The transdermal salicylic acid flux (proportional to the slope of the curves) increases with time and reaches a nearly steady-state value after 3 hours (indicated by the near constant slope of the curve shown in Figure 1). The sonophoretic permeability of the epidermis to salicylic acid was calculated based on the transdermal salicylic acid flux during the 4th and 5th hour, and was found to be 0.04 cm/hr. This sonophoretic permeability is about 400 times higher than the passive permeability of salicylic acid (1×10^{-4} cm/hr, measured in a different set of experiments). Application of ultrasound also enhances permeability of the epidermis to a variety of other permeants as shown in Table 1. It is noteworthy that the sonophoretic permeabilities to all the permeants listed in Table 1 are nearly the same, that is, in the range of 0.01 cm/hr to 0.07 cm/hr, although their physico-chemical properties, including molecular weight and lipophilicity (represented in terms of the octanol-water partition coefficient, $K_{o/w}$) are quite different. This behavior is different from that exhibited by the passive permeability

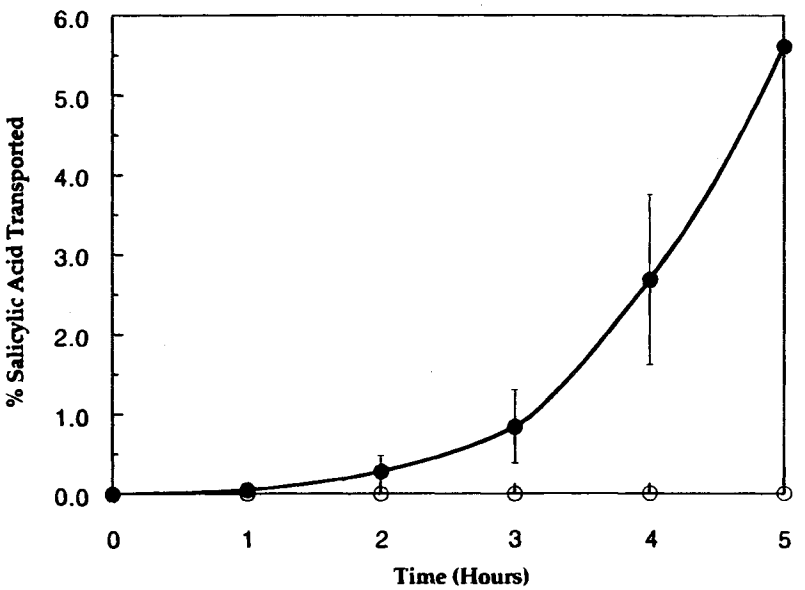

Fig. 1. Percent salicylic acid transported across the skin in the presence of ultrasound (●)(20 KHz, 125 mW/cm^2, 100 msec pulses applied every second) as a function of time. Controls are shown by (○). Each number indicates mean ± SD (error bars) of 3 experiments.

which decreases dramatically with increasing permeant molecular weight and decreasing lipophilicity, thus allowing only a few drugs to permeate the skin at a therapeutically significant rate (14). The least hydrophobic drug that is currently delivered transdermally for clinical applications is nicotine ($K_{o/w}$ = 25), and the largest drug that is delivered transdermally is fentanyl (MW = 336).

The observed independence of the sonophoretic permeability on permeant characteristics suggests that low-frequency sonophoresis may potentially provide a method for the delivery of a wide variety of compounds, irrespective of their physicochemical properties. The observed sonophoretic drug permeabilities in the range of 0.01 cm/hr to 0.07 cm/hr may be sufficiently high to deliver therapeutic doses of many drugs, since the passive skin permeability of all seven drugs that are currently administered transdermally in clinical applications by passive transport is less than 0.02 cm/hr (14). In addition, the sonophoretic permeability may be further increased by optimizing the ultrasound parameters, including intensity and pulse length.

In order to assess the efficacy of low-frequency ultrasound in enhancing transdermal transport across living skin, we performed *in vivo* experiments using hairless rats as an animal model. We used salicylic acid as the model molecule for these studies since salicylates are one of the most important nonsteroidal anti-inflammatory agents used for the treatment of joint disorders, such as arthritis. Transdermal delivery of salicylates provides an advantageous alternative to oral delivery or injections, since the former allows local delivery of salicylate, thus avoiding its high systemic concentrations which could be toxic to the body. Figure 2 shows the variation of the amount of salicylic acid excreted in urine (represented as the percent of the total applied dose) with time upon 1 hour of sonophoresis performed according to protocols described in section II D.

Table 1. List of Permeant Characteristics and the Experimentally Measured Sonophoretic Permeabilities. The Typical Error in the Reported Sonophoretic Enhancements Is About 40%

Compound	Molecular Weight (Da)	Octanol-Water Partition Coefficient $K_{o/w}$	Passive Skin Permeability P^p (cm/hr)[a]	Sonophoretic Permeability P^{us} (cm/hr)	Enhancement Ratio $E = P^{us}/P^p$
Aldosterone	360	12[c]	5.0×10^{-5}	0.070	1400
Butanol	74	7.5[c]	2.2×10^{-3}	0.064	29
Corticosterone	346	87[b]	3.0×10^{-4}	0.024	80
Estradiol	272	7000[b]	3.0×10^{-3}	0.010	3
Salicylic Acid	138	0.1[c]	1.0×10^{-4}	0.040	400
Sucrose	342	0.0046[c]	5.2×10^{-6}	0.026	5000
Water	18	0.042[c]	3.0×10^{-4}	0.034	113

[a] Measured by the authors.
[b] Johnson, M. E., unpublished data.
[c] Hanch, C., Leo, A., In *Substituent Constants for Correlation Analysis*, Wiley: New York, 1979.

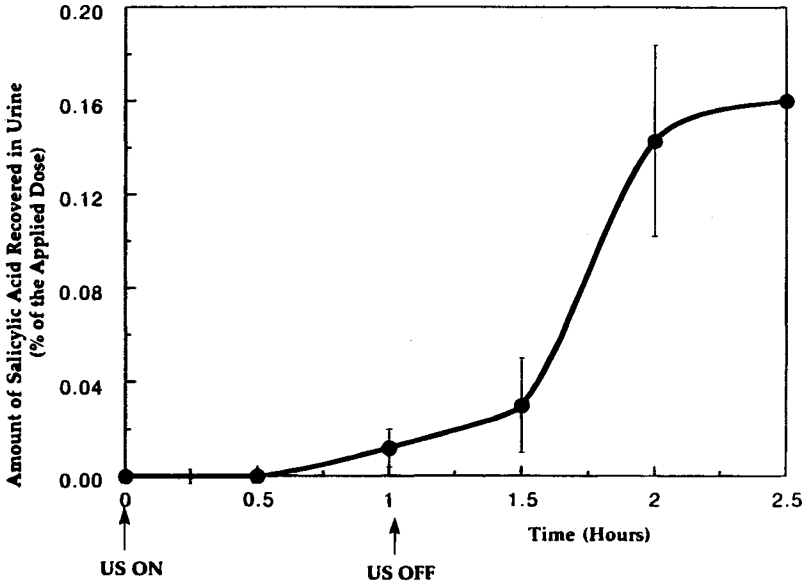

Fig. 2. Amount of salicylic acid measured in the urine of hairless rats (in dpm) as a function of time. Each number indicates mean ± SD (error bars) of 3 experiments. Ultrasound (20 KHz, 125 mW/cm^2, 100 msec pulses every applied second) was turned ON at t = 0 and turned OFF at t = 1 hr.

About 0.15% of the applied dose was found in the urine of rats exposed to ultrasound at the end of 2 hours. The amount in the urine of control rats was below our detection limit (0.0005% of the applied dose). This result suggests that application of low-frequency ultrasound enhances transdermal salicylic acid transport *in vivo* by at least 300-fold, an enhancement comparable to the 400-fold enhancement measured *in vitro* across human cadaver skin.

B. Safety Analysis of Low-Frequency Sonophoresis

Safety of a transdermal transport enhancer is crucial for its applicability. The safety of low-frequency sonophoresis involves two main issues: i) the reversibility of the epidermal barrier properties after turning ultrasound OFF, and ii) the effect of low-frequency ultrasound on the living parts of the skin and underlying tissues. Below, we examine each issue.

Recovery of the Skin Barrier Properties After Sonophoresis. The sonophoretic flux of permeants exhibited significant recovery after turning ultrasound OFF. For example, the permeability to salicylic acid during the fifth hour of sonophoresis was about 0.04 cm/hr (400-fold higher than the passive permeability). When ultrasound was turned OFF after 5 hours, the transdermal salicylic acid flux over the next two hours was below the detection limit (permeability detection limit of about 1×10^{-3} cm/hr).

In order to further assess the recovery of the barrier properties of the epidermis after short (1 hour) as well as long (5 hours) ultrasound exposures, we performed the following experiments. We exposed epidermis pieces to ultrasound (20 KHz, 125 mW/cm^2, 100 msec pulses applied every second) for 1 hour in the diffusion cell with the donor and receiver compartments filled with PBS. We then added ^{3}H labeled water in the donor compartment, and measured the transdermal flux of water for up to 12 hours after ultrasound exposure. These experiments were repeated for a 5-hour ultrasound exposure using different epidermis pieces. In the case of a 1-hour long exposure, the epidermal permeability to water measured within 2 hours post-exposure was comparable to the passive epidermal permeability to water. In the case of a 5-hour long exposure, the epidermal permeability 2 hours post-exposure was about 6 times higher than the passive permeability to water. However, this value continued to decrease, and was within a factor of 2 of the passive water permeability 12 hours post-exposure. Hence, it appears that application of low-frequency ultrasound does not cause a long-term change in the epidermal barrier properties measured in terms of water permeability. We also measured recovery of the epidermal electrical resistance after sonophoresis. After a 1 hour ultrasound exposure (20 KHz, 125 mW/cm^2, 100 msec pulses applied every second) the electrical resistance of the epidermis decreased by 60% (see Figure 5). Two hours after turning ultrasound OFF, the resistance increased by a factor of 1.2 compared to the value immediately after turning ultrasound OFF (final value = 70% of the skin resistance before sonophoresis). After a 5-hour-long ultrasound exposure, the epidermal electrical resistance decreased by about 25-fold (see Figure 5). Two hours after tuning ultrasound OFF, the epidermal resistance increased by about 2-fold compared to that at the end of ultrasound exposure. The relevance of these results to the safety of low-frequency sonophoresis needs to be further investigated.

Biological Effects of Low-Frequency Ultrasound. Low-frequency ultrasound (frequency in the range of 20–85 KHz) is currently used by dentists for tooth cleaning (15). In view of this, significant efforts have been devoted to investigate probable biological effects of low-frequency ultrasound (16,17). However, no conclusions have been reached regarding the limiting ultrasound conditions required to ensure safe exposure.

In order to assess the effect of low-frequency ultrasound on living skin cells, we performed initial histological studies using hairless rats as an animal model. Our histological studies

indicated no physical damage in the skin exposed to ultrasound at all the intensities used in the experiments described above (see Figures 3A and 3B). Furthermore, the regions of hairless rat epidermis exposed to ultrasound were intact and showed no signs of abnormality. Although further research focusing on safety issues is required before arriving at any final conclusions regarding the safety of low-frequency ultrasound, our preliminary studies indicate that low-frequency ultrasound does not induce damage to the skin and underlying tissues. Note that, in view of the deeper penetration of low-frequency ultrasound into the body, evaluation of the effects of low-frequency ultrasound on tissues deep into the body is important for the eventual evaluation of the safety of low-frequency ultrasound.

C. Comparison of Low-Frequency and Therapeutic Ultrasound

Transdermal transport enhancement induced by low-frequency ultrasound under conditions reported in this paper is much more significant than that induced by therapeutic ultra-

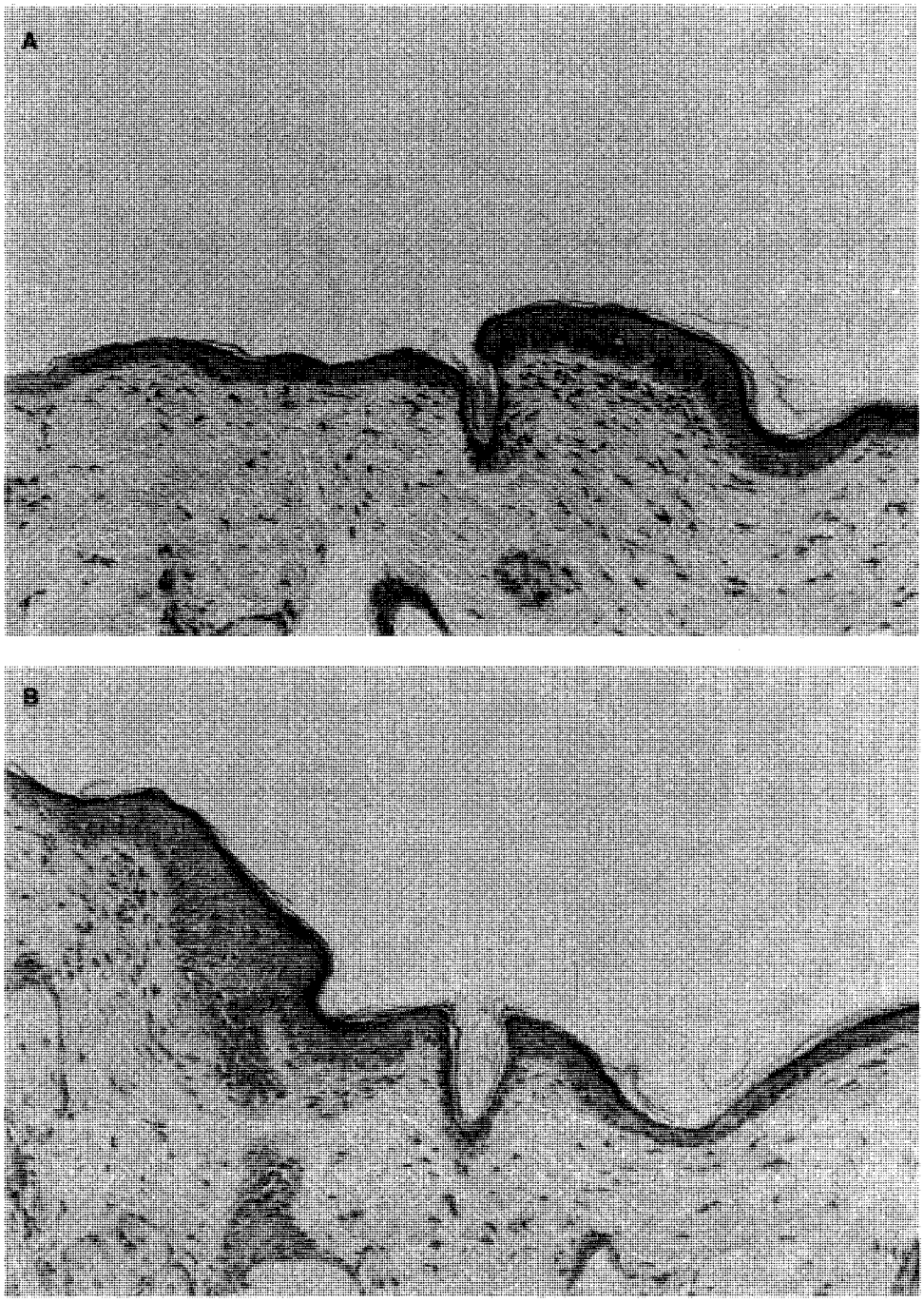

Fig. 3. Histological results of the hairless rat skin exposed to ultrasound. Figure 3A corresponds to a control (skin not exposed to ultrasound), while Figure 3B indicates skin exposed to ultrasound (20 KHz, 125 mW/cm^2, 100 msec pulses applied every second).

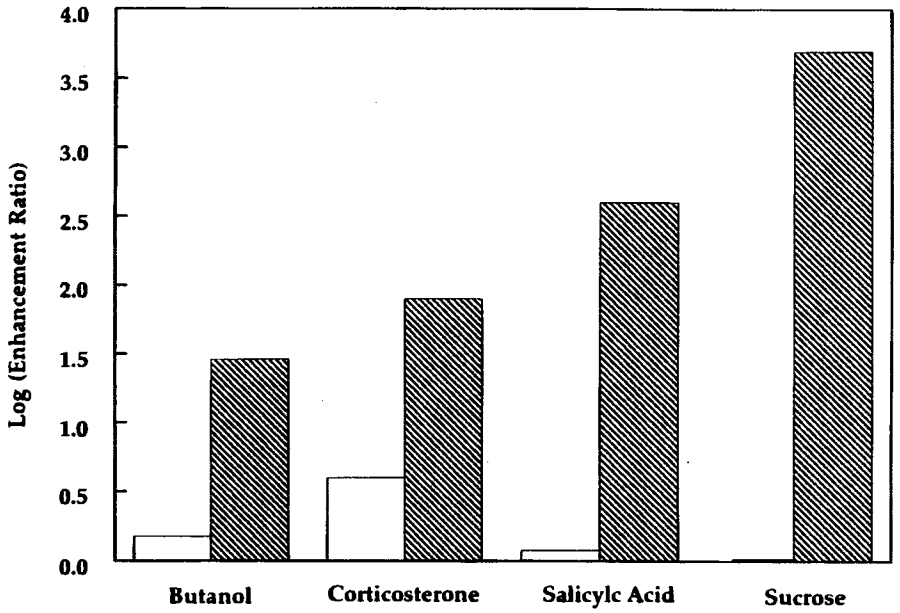

Fig. 4. Comparison of the sonophoretic enhancement ratio under therapeutic (white bars) and low-frequency (hatched bars) ultrasound conditions for butanol, corticosterone, salicylic acid, and sucrose.

sound. Figure 4 compares the enhancement ratio (ratio of the sonophoretic and passive permeabilities measured *in vitro* across human cadaver epidermis) induced by *therapeutic ultrasound* (1 MHz, 2 W/cm^2, continuous, indicated by empty bars) (8) and *low-frequency ultrasound* (20 KHz, 125 mW/cm^2, 100 msec pulses applied every second, indicated by hatched bars) (see also Table 1) in the case of four permeants, butanol, corticosterone, salicylic acid, and sucrose. The enhancement induced by low-frequency ultrasound is as much as 1000-fold higher than that induced by therapeutic ultrasound. Another advantage of low-frequency sonophoresis, as compared to therapeutic ultrasound, is that the former can induce transdermal transport of drugs which do not passively permeate across the epidermis, while in many cases, the latter cannot. This would be especially important in the case of hydrophilic drugs or large-molecular weight proteins which do not transport across the skin by passive transport. We have recently shown that application of low-frequency ultrasound, under conditions identical to those discussed in this paper, delivers therapeutic doses of high-molecular weight proteins, including insulin, γ-interferon, and erythropoeitin, across human epidermis *in vitro* as well as across hairless rat skin *in vivo* (18). A possible mechanistic explanation for this ability of low-frequency ultrasound to transport a wide variety of drugs across the skin is discussed in the next section.

MECHANISM OF LOW-FREQUENCY SONOPHORESIS

Various mechanisms have been put forward to rationalize the occurrence of sonophoresis. These include: (i) cavitation (generation and oscillation of gas bubbles), (ii) thermal effects (temperature increase), and (iii) induction of convective transport. Recently, we have shown (8) that cavitation plays a major role in sonophoresis performed using *therapeutic* ultrasound (frequency in the range of 1 MHz–3 MHz, and intensity in the range of 0–2 W/cm^2). Specifically, oscillations of cavitation bubbles induce disorder in the SC lipid bilayers, thereby enhancing the transport of drugs across the SC (8). Since it is known that cavitational effects in fluids vary inversely with ultrasound frequency (12), we anticipated that cavitational effects should play an even more important role in low-frequency sonophoresis.

Cavitation may occur inside as well as outside the skin upon ultrasound exposure. Cavitation at either location may enhance transdermal transport in two ways: (i) cavitation may induce convective transport across the skin, and (ii) oscillations of cavitation bubbles may disorganize the SC lipid bilayers. We performed experiments to assess the importance of (i) and (ii) in low-frequency sonophoresis.

(i) Role of Cavitation-Induced Convection in Sonophoresis. Oscillations of cavitation bubbles may induce convective velocities, a phenomenon referred to as microstreaming (19). If such velocities are generated inside or near the skin, they may induce convective transport across the skin. We hypothesized that the contribution of cavitation-induced convection could be isolated by measuring the effect of ultrasound on transport of water across delipidized SC. Since probably there are no lipid bilayers present in the delipidized SC, an observation of sonophoretic enhancement of water transport across the delipidized skin should indicate cavitation-induced convection rather than lipid bilayer disordering. This way, we could isolate the possible contribution of convective transport and bilayer disordering during sonophoresis. We chose water as a model permeant for these studies since water would be the primary fluid involved in convection across the SC.

We first separated the SC from the epidermis by soaking the heat-stripped epidermis in trypsin (20). The lipids in the SC were then removed by soaking it in a mixture of chloroform/

methanol (2:1) (21). After 3 days of soaking, the SC was washed with ethanol and water. We then measured the passive and sonophoretic water permeabilities of the delipidized SC. The passive permeability of the delipidized SC to water was found to be about 8 cm/hr (compared to 3×10^{-4} cm/hr in the case of normal epidermis). This observation shows that keratinocytes do not offer significant resistance to transdermal transport. Upon application of ultrasound (20 KHz, 125 mW/cm^2, 100 msec pulses applied every second), we did not observe any enhancement of water transport across the delipidized SC, suggesting that cavitation-induced convection does not play a major role in low-frequency sonophoresis.

(ii) Role of Cavitation-Induced Bilayer Disordering in Sonophoresis. To assess the role of bilayer disordering in low-frequency sonophoresis, we performed electrical resistance measurements of the normal epidermis during sonophoresis.

The electrical resistance of the epidermis is a good, instantaneous indicator of the structural properties of the epidermis (22). Under normal conditions, ionic transport through the epidermis occurs partly through the follicles and partly through the intercellular lipid bilayers (23) Due to significant hindrance of the ionic transport through the lipid bilayers, the electrical resistance of the epidermis is very high. If application of ultrasound disorders the SC lipid bilayers, a decrease in the electrical resistance of the epidermis should be observed due to reduced hindrance. In addition, if significant bilayer disordering occurs, ions could transport across the lipid domains and keratinocytes, a phenomenon which would reduce the epidermal electrical resistance by more than 10-fold (23). Figure 5 shows the measured variation of the epidermal electrical resistance (normalized by the resistance in the absence of ultrasound) with time upon ultrasound application (20 KHz, 125 mW/cm^2, 100 msec pulses applied every second). The observed decrease in the epidermal electrical resistance suggests that low-frequency ultrasound induces disorder in the SC lipid bilayers. Moreover, the 25-fold decrease in the epidermal electrical resistance suggests that application of low-frequency ultrasound induces ionic transport across the lipid domains and keratinocytes. Note that a typical application of therapeutic ultrasound (1 MHz, 2 W/cm^2) reduces the epidermal electrical resistance by only about 30% (8). The observed difference in epidermal electrical resistance reduction induced by therapeutic ultrasound and low-frequency ultrasound supports the hypothesis that low-frequency ultrasound induces more bilayer disordering than that induced by therapeutic ultrasound. The occurrence of trans-keratinocyte pathways during low-frequency sonophoresis is also supported by the observed characteristic dependence of the sonophoretic permeability on the molecular characteristics of the permeants as explained below.

The passive transdermal transport of drugs occurs mostly through the lipid bilayers of the SC (2) (Figure 6A). Accordingly, the passive permeability is proportional to the lipid partition coefficient of the drug (23). On the other hand, the sonophoretic permeability under low-frequency ultrasound conditions is not proportional to the lipophilicity of drugs. The sonophoretic permeabilities of the seven permeants examined in this study, including water, sucrose, estradiol, corticosterone, aldosterone, salicylic acid, and butanol, are remarkably similar, although their lipophilicities (as reflected by their octanol-water partition coefficients, $K_{o/w}$) differ by five orders of magnitude (Table 1). The lack of correlation between the sonophoretic permeability and the permeant lipophilicity suggests that transdermal transport during low-frequency sonophoresis no longer occurs through the intercellular lipids. Instead, it occurs through aqueous pathways across the SC (the trans-keratinocyte route shown in Figure 6B). These aqueous pathways or channels may be generated due to the vigorous cavitation activity that occurs inside as well as outside the skin upon ultrasound application. Specifically, cavitation activity may disorganize the SC lipid bilayers followed by water penetration from the keratinocytes, leading to the formation of aqueous channels. Transdermal

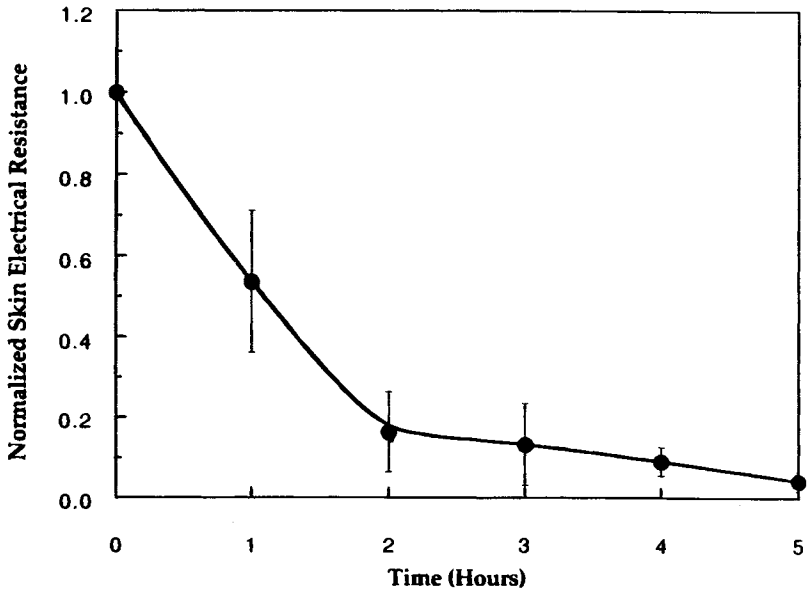

Fig. 5. Variation of the normalized skin electrical resistance in the presence of ultrasound (20 KHz, 125 mW/cm^2, 100 msec pulses applied every second). Each number indicates mean ± SD (error bars) of 3 experiments.

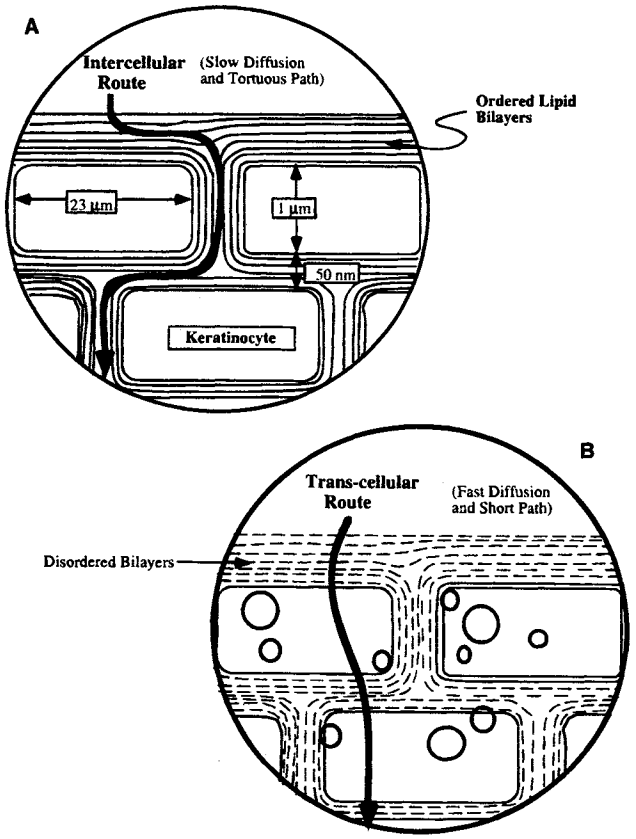

Fig. 6. Schematic illustration of the proposed mechanism of low-frequency sonophoresis. The figure illustrates transport pathways during passive transport (Figure 6A) and during low-frequency sonophoresis (Figure 6B).

transport of permeants may then occur through these channels (Figure 6B).

The occurrence of transdermal transport through aqueous channels across the disordered lipid regions may enhance transdermal transport as compared to passive transport because: i) the diffusion coefficients of permeants through water, which is likely to primarily occupy the channels generated by ultrasound, are up to a 1000-fold higher than those through the ordered lipid bilayers. Specifically, the diffusion coefficients of all the permeants listed in Table 1 in water are about 1×10^{-5} cm^2/s (24), while their passive diffusion coefficients through the SC vary between 1×10^{-6} cm^2/s and 1×10^{-9} cm^2/s (23), and ii) the transport path length of these aqueous channels may be much shorter (by a factor of up to 25 (23)) than that through the tortuous intercellular lipids in the case of passive transport. Note that under conditions where aqueous channels are formed through the disordered lipid regions, transdermal transport is unlikely to occur through the intercellular route since the intercellular lipids may lose continuity due to the formation of the aqueous channels.

Based on the discussion presented above, we summarize our proposed mechanism of low-frequency sonophoresis as follows. Application of low-frequency ultrasound may induce cavitation inside as well as outside the skin. Cavitation occurring at either location may cause disordering of the SC lipids. In addition, oscillations of cavitation bubbles may result in significant water penetration into the disordered lipid regions. This may cause the formation of aqueous channels through the intercellular lipids of the SC (Figure 6B). This allows permeants to transport across the disordered lipid domains. Once able to diffuse across the lipid domains, molecules may diffuse across keratinocytes and hence across the entire SC. This transport pathway may result in an enhanced transdermal transport as compared to passive transport.

QUANTITATIVE MODEL OF LOW-FREQUENCY SONOPHORESIS

As shown in Figure 6B, transdermal drug transport during low-frequency sonophoresis is assumed to occur through the aqueous channels across the disordered intercellular lipid regions. Since transport across keratinocytes is relatively fast owing to their relatively open structure compared to that of lipid bilayers, transport across the disordered intercellular lipid domains constitutes the rate limiting step in skin permeation during low-frequency sonophoresis. Support for this assumption is provided by our observation that water transport across delipidized SC is about 10000-fold higher than that across normal SC. Note that the SC consists of several layers of keratinocytes (denoted as N, with N typically = 15 (23)), suggesting that a permeant molecule has to cross about 15 intercellular lipid regions in order to transport across the SC. Consider a layer of intercellular lipid domain parallel to the skin surface (Figure 6B). Let ϕ be the average fractional area occupied by the aqueous channels generated by ultrasound application in the plane of the lipid domain across which drugs may diffuse. Since these channels are filled with saline, it may be assumed that small molecules (MW < 500) diffuse through these channels with a diffusivity, D_w, equal to that through water (typical value of 1×10^{-5} cm^2/s in the case of permeants having a molecular weight of less than 500 (24)). Large molecular weight permeants, such as proteins, may experience steric hindrance as they diffuse though these channels, and may therefore possess diffusion coefficients smaller than 1×10^{-5} cm^2/s. However, transdermal transport of proteins is not addressed here.

Based on the mechanistic explanation discussed above, the sonophoretic SC permeability, P^{us}, to small molecules (MW < 500) may be expressed as (23):

$$P^{us} = \frac{K\phi D_w}{Nl} \text{ (cm/h)} \qquad (1)$$

where K is the permeant partition coefficient in the aqueous channels (may be assumed to be unity since the donor compartment of the diffusion cell as well as the transport channels are likely to be filled with the same saline medium), N is the number of intercellular lipid regions that a molecule has to cross (typically 15 (23)), and l is the thickness of each lipid region (typically 50 nm (23)) (Figures 6A and 6B). The parameters ϕ, D_w, N, and l in Eq. (1) are all independent of the permeant characteristics, thus suggesting that the sonophoretic permeability should be independent of the permeant characteristics. This prediction is consistent with our observations that the sonophoretic permeabilities to all seven permeants studied are similar, although their physico-chemical characteristics are markedly different (Table 1).

Similarly, the electrical conductivity of the skin during sonophoresis, $\bar{\sigma}^{us}$ (related to the electrical resistivity, $\bar{\rho}^{us}$, by

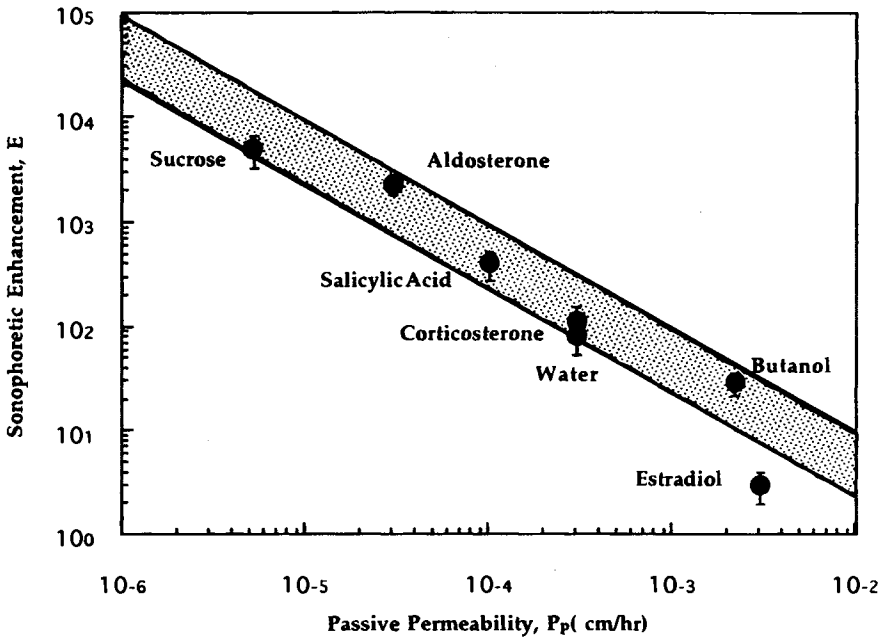

Fig. 7. Comparison of the sonophoretic enhancements, *E*, predicted using Eq. (4) with the experimentally observed sonophoretic enhancements (Table 1). The predicted E values are shown by two lines as the upper (corresponding to P^{us} = 0.09 cm/hr) and lower (corresponding to P^{us} = 0.023 cm/hr) limits on the predictions. The filled circles indicate experimentally measured E values. Each number indicates mean ± SD (error bars) of 3 experiments.

the relation, $\bar{\sigma}^{us} = h/\bar{\rho}^{us}$, where h is the SC thickness) may be expressed as follows [23]:

$$\sigma^{us} = \frac{\phi\sigma_w}{N} \qquad (\Omega\text{cm})^{-1} \tag{2}$$

where σ_w is the electrical conductivity of saline (1.2×10^{-2} $(\Omega\text{cm})^{-1}$ (23)). Equations (1) and (2) relate the sonophoretic skin properties to fundamental transport properties and skin geometry. In these equations, ϕ is the only unknown parameter that needs to be determined experimentally. Note that ϕ depends on ultrasound parameters, including intensity, pulse length, and exposure time, and can be estimated from Eq. (2) by utilizing the experimentally measured values of $\bar{\rho}^{us}$. The epidermal electrical resistance, R^{us}, during the 4th and 5th hour of ultrasound exposure (20 KHz, 125 mW/cm^2, 100 msec pulses applied every second) was about 5100 ± 3100 Ω (about 4% of the electrical resistance in the absence of ultrasound (Figure 5)). This sonophoretic electrical resistance corresponds to $\bar{\rho}^{us}$ = 16000 ± 9600 Ω-cm^2 ($\bar{\rho}^{us} = AR^{us}$, where A is the epidermal area = 3.14 cm^2) which yields $\bar{\sigma}^{us} = 9 \times 10^{-8}$ (±60%) $(\Omega\text{cm})^{-1}$ ($\bar{\sigma}^{us} = h/\bar{\rho}^{us}$, $h = 15 \times 10^{-4}$ cm). Substituting this value of $\bar{\sigma}^{us}$ in Eq. (2) yields $\phi = 1.2 \times 10^{-4}$ (±60%). This suggests that about 0.012% of the SC area is available for sonophoretic transport under these ultrasound conditions. After substituting $\phi = 1.2 \times 10^{-4}$ in Eq. (1), with $D_w = 1 \times 10^{-5}$cm^2/s, N = 15, and $l = 50 \times 10^{-7}$ cm, and expressing P^{us} in units of cm/hr, one obtains:

$$P^{us} = \left[\frac{0.00012(1 \times 10^{-5})}{15(50 \times 10^{-7})}\right] \times 3600 = 0.057 \qquad \text{(cm/hr)} \tag{3}$$

The model predicts that the sonophoretic permeability of all low-molecular weight drugs (MW < 500) under the ultrasound conditions examined here (20 KHz, 125 mW/cm^2, 100 msec pulses applied every second) is about 0.057 (±60%) cm/hr, that is, in the range of 0.023 cm/hr to 0.09 cm/hr. This value is in reasonable agreement with the experimentally measured sonophoretic permeabilities of the permeants listed in Table 1 which are in the range of 0.01 cm/hr for estradiol to 0.07 cm/hr for aldosterone. The sonophoretic enhancement, *E*, defined as the ratio of the sonophoretic permeability, P^{us}, and the passive permeability, P^{p}, may then be expressed as follows:

$$E = \frac{0.057 \pm (60\%)}{P^{p}} \tag{4}$$

Figure 7 compares the range of sonophoretic enhancements predicted by Eq. (4) with the experimentally observed enhancements for seven permanents as reported in Table 1. The predicted enhancements are shown by two lines binding the shaded region. The lines correspond to the upper (P^{us} = 0.09 cm/hr) and lower (P^{us} = 0.023 cm/hr) limits of the predicted sonophoretic permeability. Figure 7 indicates that the predicted values of the sonophoretic enhancements are in reasonable agreement with the experimental values.

This analysis of low-frequency sonophoresis explains why low-frequency ultrasound can induce transdermal transport of drugs which exhibit very low passive transport. Drugs possessing low passive permeabilities are either: i) hydrophilic, which makes their partitioning into the SC lipid bilayers difficult, or ii) large in molecular size (for example, proteins), which reduces their diffusion coefficients in the SC. Low-frequency ultrasound overcomes both of these limitations by providing

aqueous transport channels across the SC. Since these channels are filled with saline, hydrophilic drugs can easily partition into the SC. In addition, diffusion of drugs through water is much faster than that through ordered lipid bilayer regions, thus allowing drugs to transport across the SC at a faster rate. Therefore, molecules such as hydrophilic drugs or proteins may permeate skin with relative ease in the presence of low-frequency ultrasound. On the other hand, transdermal transport of lipophilic drugs may not be enhanced significantly by low-frequency ultrasound. This is due to the fact that the partition coefficients of lipophilic drugs into the aqueous channels are likely to be lower than those into the lipid domains. As a result, the increase in the permeant diffusion coefficient in the SC due to channel formation may be partly offset by the reduction in the partition coefficient. Hence, the sonophoretic enhancement is likely to be less significant for hydrophobic drugs as compared to that for hydrophilic drugs. Indeed, we do observe that while low-frequency ultrasound enhances transdermal transport of aldosterone ($K_{o/w}$ = 17) by a factor of 1400, it enhances transdermal transport of estradiol ($K_{o/w}$ = 7000) only by a factor of 3, although both drugs possess similar molecular weights.

CONCLUSIONS

Low-frequency ultrasound enhances transdermal transport of a variety of drugs across human cadaver skin *in vitro* by a factor in the range of 3–5000. *In vivo* experiments performed using salicylic acid as a model drug show that low-frequency ultrasound enhances transdermal transport across living rat skin by a factor of at least 300. Application of low-frequency ultrasound does not appear to cause any long-term damage to the barrier properties of the epidermis. In addition, preliminary histological studies indicate no damage to the skin and underlying tissues of hairless rats exposed to low-frequency ultrasound, although additional studies are required before arriving at any final conclusions regarding the safety of low-frequency ultrasound.

A mechanistic explanation of low-frequency sonophoresis was proposed. It was suggested that application of low-frequency ultrasound generates aqueous channels across the SC, through which transdermal transport of drugs may occur. This hypothesis explains the observation that the sonophoretic permeability of various drugs is strikingly similar in spite of the fact that their physico-chemical characteristics are markedly different. A quantitative model was developed to predict the effect of low-frequency ultrasound on transdermal transport of drugs. The predictions of the model are in reasonable quantitative agreement with the experimental observations.

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