Low-Frequency Sonophoresis: A Noninvasive Method of Drug Delivery and Diagnostics

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Transdermal drug delivery offers an attractive alternative to injections and oral medications. However, applications of transdermal drug delivery are limited to only a few drugs as a result of low skin permeability. Application of low-frequency ultrasound enhances skin permeability, a phenomenon referred to as low-frequency sonophoresis. In this method, a short application of ultrasound is used to permeabilize skin for a prolonged period of time. During this period, ultrasonically permeabilized skin may be utilized for drug delivery. In addition, a sample of interstitial fluid or its components may be extracted through permeabilized skin for diagnostic applications. In this paper, we report our in vivo studies that demonstrate the principles of both of these concepts. Detailed studies on drug delivery are performed using inulin and mannitol as model drugs. Studies on diagnostics are performed using glucose as a model analyte. Applications of this technology to drug delivery and diagnostics are discussed.

Introduction

Transdermal drug delivery offers several advantages over traditional drug delivery systems such as oral delivery and injections, including elimination of first pass metabolism, minimization of pain, and possible sustained release of drugs (1). The transdermal route of administration can be especially beneficial in the delivery of chronic injectable medications where patient compliance is low. However, transdermal transport of molecules is slow as a result of the low permeability of the stratum corneum, the outermost layer of the skin. Therefore, it is difficult to deliver drugs across the skin at a therapeutically relevant rate. This, in fact, is the main reason only a handful of low-molecular weight drugs are administered by this route today. A possible solution to this problem is to increase skin permeability using physicochemical forces, referred to as penetration enhancers, for example, ultrasound (2–8), chemical enhancers (9, 10), and electric fields (11, 12). Enhanced skin permeability induced by enhancers may be used for delivering drugs or for extraction of clinically relevant analytes such as glucose.

Ultrasound has been used for enhancing transdermal transport under a variety of conditions (2–5, 7, 13). This phenomenon is referred to as sonophoresis. The enhancement of transdermal transport induced by ultrasound varies from a few percent to several orders of magnitude depending on the condition. Among several ultrasound conditions that have been used for sonophoresis, low-frequency conditions (f < 100 kHz) have been found to be most effective in enhancing transdermal transport. In

Materials and Methods

Ultrasound-Induced Skin Permeabilization. All animal procedures were performed using institutionally approved protocols. Rats (Sprague Dawley, either sex) were anesthetized with a mixture of ketamine (60 mg/kg) and xylazine (10 mg/kg) injected ip or im. After anesthesia was confirmed, a flanged glass cylinder (Crown Glass Co., diameter 15 mm, height 2 cm) was glued on the rat's shaved lateral flank using a minimal amount of cyanoacrylate adhesive (Permabond International or Vet Bond) on the outer edge of the flange. The chamber was filled with 2 mL of PBS and the skin was hydrated for 1 h. At the end of hydration, ultrasound was applied using methods described below. The coupling medium (the liquid present between the ultrasound horn and the skin) was PBS. An Ag/AgCl electrode was inserted subcutaneously to measure skin electrical conductivity according to methods described in section C.

Ultrasound Application. Ultrasound was applied only once to each animal for typically less than 2 min using a sonicator (VCX 400, Sonics and Materials) operating at a frequency of 20 kHz. Before each new experiment, the sonicators were “tuned” according to a procedure specified by the manufacturer to ensure that the applied signal optimally matched the resonance frequency of the piezoelectric crystal. The horn was positioned 1 cm above the skin inside the donor chamber. The sonicators were operated in a pulsed mode (5 s on, 5 s off). This choice of pulsing parameters was made to minimize thermal effects and was otherwise arbitrary. Measurement of ultrasound intensity was performed using a calorimetric method and is described elsewhere (14). Note that calorimetry measures intensity based on
the increase in temperature of a known amount of liquid in which the horn is immersed. Hence, the heating of the horn itself may lead to artificially high-intensity values. To assess the importance of horn heating in intensity measurements, we measured the temperature of the horn itself during the measurements. No significant increase in horn temperature was noticed. This result indicates that the effect of horn heating on intensity measurements is minimal, although additional studies are required to confirm this conclusion. The intensity of ultrasound used for pretreatment was about 7 W/cm². This intensity corresponds to spatial average pulse average ($I_{\text{sAPA}}$) values, that is, it corresponds to the spatial average temporal average intensity during the ON time of ultrasound.

**Electrical Resistance Measurements.** The skin conductivity was measured between the subcutaneous electrode and the metal sonicator horn. To measure the electrical resistivity of the skin, a 100 mV AC electric field (10 Hz) was applied across the skin for a short time using a signal generator (model HP 4116 A). Current measurements were made with an ammeter (Micronta, Tandy Corporation). The electrical resistivity was then calculated from Ohm’s law. Since the measured resistance is the sum of the actual skin resistance and the saline resistance, the latter was subtracted from the measured resistivity. Since the measured resistance is obtained by multiplying the skin electrical resistance (measured experimentally) by the skin area ($1.7 \text{ cm}^2$). Skin conductivity was calculated by taking the reciprocal of the skin resistivity.

**Transdermal Extraction.** Extraction was performed using glucose ($\text{MW} = 180$). At the end of sonication, the chamber contents were removed and replaced (after rinsing) with $0.5–2 \text{ mL}$ of fresh buffer (PBS). Radiolabeled glucose was injected through a catheter placed in the jugular vein. Extraction of analytes was performed for 15 min by diffusion or by application of additional ultrasound ($1.6 \text{ W/cm}^2$, 5 s ON 5 s OFF, 20 kHz). At the end of 15 min of extraction, the chamber content was analyzed for glucose using a scintillation counter (Packard 2400 CA). Skin permeability was calculated (assuming steady state) on the basis of the equation $P = \frac{V\Delta C}{A\Delta t} / (A_{\text{CM}})$, where $V$ is the volume of the receiver compartment (1 mL), $A$ is the skin area ($1.7 \text{ cm}^2$), $\Delta C/ \Delta t$ is the measured increase in analyte concentration in the solution in the collection chamber over a period of time $\Delta t$ (typically 15 min), and $A_{\text{CM}}$ is the analyte concentration in serum (assuming that glucose concentration in the interstitial fluid is comparable to that in serum).

**Transdermal Drug Delivery.** Delivery was measured using two molecules, mannitol ($\text{MW} = 180$) and inulin ($\text{MW} = 5000$). Skin was pretreated with ultrasound using methods described above. At the end of sonication, PBS was removed, and the chamber was rinsed with PBS. The chamber was then filled with a solution of radiolabeled mannitol or inulin ($10 \mu\text{Ci/mL}$). A catheter was placed in the rat’s bladder to collect urine samples. Concentration of inulin or mannitol in the urine was measured every 30 min for up to 5 h. Skin permeability was calculated (assuming steady state) on the basis of the equation $P = \frac{V\Delta C}{A\Delta t} / (A_{\text{CM}})$, where $V$ is the collected urine volume, $A$ is the skin area ($1.7 \text{ cm}^2$), $\Delta C/ \Delta t$ is the measured increase in the inulin or mannitol concentration in urine over a period of time $\Delta t$, and $A_{\text{CM}}$ is the inulin or mannitol concentration in the donor compartment.

![Figure 1](Image 332x586 to 543x744)

**Figure 1.** Transdermal delivery of mannitol through rat skin. Closed circles show transport across ultrasound-pretreated skin. Open circles show transport through untreated skin. The amount of drug transported is measured in units of dpm (disintegrations per minute).

**Results and Discussion**

**Ultrasound-Induced Skin Permeabilization.** Ultrasound was applied only once to permeabilize the skin. Enhanced skin permeability was monitored using skin conductivity. Skin conductivity is an excellent indicator of its permeability (14). This occurs because the lipid bilayers of the stratum corneum (SC), which offers electrical resistance to the skin, also retard transdermal transport of molecules. The relationship between skin permeability and skin conductivity can be mathematically explained on the basis of the mechanism of low-frequency sonophoresis (15). Specifically, we hypothesize that application of low-frequency ultrasound produces cavitation, which in turn disorders lipid bilayers of the skin. This leads to the formation of aqueous channels in the skin (4). Current-carrying ions as well as drugs can permeate through these channels. Therefore the transport pathways for the drugs during LFS are the same as those for the current-carrying ions. Hence, the correlation between skin conductivity and skin permeability is fundamentally understandable. Quantitative aspects of this correlation are discussed elsewhere (15).

The baseline conductivity of rat skin is about 0.01 (kΩ cm²)⁻¹. Skin conductivity increased with application of ultrasound. The increase in skin conductivity varies with the total energy, $E$, of ultrasound ($E = Ir$, where $I$ is ultrasound intensity (W/cm²) and $r$ is the net exposure time (s)) delivered to the skin). There exists a threshold of ultrasound energy, $E_{\text{threshold}}$, below which no significant change in the electrical conductivity of skin is observed. For rat skin (in vivo), this threshold is about 10 J /cm² (16). After application of an ultrasound dose of 1000 J/cm², the skin conductivity typically reaches a value of 0.6 (kΩ cm²)⁻¹, an enhancement of 60-fold over the skin conductivity of untreated skin. Skin remained in a state of elevated conductivity for at least 5 h (the duration of the experiment). Thus, a short application of ultrasound increased skin permeability for a prolonged period of time. Note that the skin permeability would eventually recover to its baseline value. For example, we have utilized ultrasound under similar conditions on diabetic human volunteer (17). These studies showed that skin remained permeable for about 15 h after ultrasound application and decreased to its baseline permeability after 24 h.

**Transdermal Drug Delivery.** Figure 1 shows the amount of mannitol delivered transdermally through
ultrasonically permeabilized skin (closed circles) as well as through untreated skin (open circles). In these experiments mannitol was placed on the skin after ultrasound pretreatment of the skin. Hence, the only mechanism of enhancement of mannitol transport is enhanced diffusion through structurally altered skin. Mannitol transport through untreated skin is very slow, corresponding to a permeability of $1.2 \times 10^{-5}$ cm/h. In contrast, mannitol permeability through ultrasonically permeabilized skin is $4 \times 10^{-4}$ cm/h, an enhancement of about 33-fold. Figure 2 shows the results of similar experiments performed using inulin. The results are very similar to those for mannitol. Specifically, the passive permeability of inulin is very low ($7.4 \times 10^{-6}$ cm/h). However, inulin permeability through ultrasound-pretreated skin is relatively high ($1.5 \times 10^{-4}$ cm/h), corresponding to an enhancement of 20-fold. A comparison of data in Figures 1 and 2 shows that the enhancement of skin permeability for mannitol and inulin is comparable (20-fold for inulin and 33-fold for mannitol), although their molecular weights differ significantly (180 for mannitol and 5000 for inulin). The significance of these results is discussed in detail in a later section.

The delivery rate of drugs can be further enhanced by providing an additional driving force that may induce convection through ultrasound-pretreated skin. To assess this possibility, we performed additional in vivo experiments using inulin. In these experiments, skin was pretreated using ultrasound as before. After that, 1 mL solution of radiolabeled inulin ($10 \mu$Ci/ml) was placed on the skin. Additional ultrasound was applied at a much lower intensity ($1.6$ W/cm², 20 kHz, 5 s ON 5 s OFF). Inulin transport across the skin was measured as before. Figure 3 shows the results of these studies. Closed circles show transdermal inulin transport in the presence of ultrasound driving force, while open circles show inulin transport due to pretreatment alone. Transdermal transport during additional ultrasound application was about 21-fold higher than that due to pretreatment alone. The enhancement induced by additional ultrasound decreased immediately after turning ultrasound OFF. These data are shown in Figure 4. Ultrasound pretreatment was performed at time zero, and ultrasound driving force was applied immediately after that. When ultrasound was turned OFF at 90 min, the amount of inulin delivered transdermally began decreasing and became close to baseline (that is, pretreatment alone) within 3 h. This lag time between turning ultrasound OFF and returning of the flux to baseline is likely to originate from the lag time associated with the clearance of inulin from skin.

Application of ultrasound induced no visible damage to the skin. We performed histological studies on rat skin exposed to ultrasound (4). No gross damage to the stratum corneum or underlying tissues was observed, although further studies are necessary to confirm the safety of low-frequency sonophoresis. Thus, the data presented here show that sonophoresis offers a viable method for drug delivery. Applications of this method are discussed in a later section.

**Transdermal Glucose Extraction.** Figure 5 shows extraction of glucose through ultrasonically permeabilized skin (closed circles), as well as through untreated skin (open circles). The passive permeability of untreated skin to glucose is $4 \times 10^{-4}$ cm/h. In contrast, the permeability of skin treated with a single application of ultrasound is $3.4 \times 10^{-3}$ cm/h (an enhancement of about 9-fold). This enhancement may be further enhanced by application of additional driving forces such as ultrasound to ultrasonically pretreated skin. We performed additional experiments to test this hypothesis. Specifically, we pretreated rat skin according to procedures described earlier. We then filled the chamber with PBS...
and applied additional ultrasound at low intensity (1.6 W/cm², 20 kHz, 5 s ON 5 s OFF). Figure 6 shows the results of these experiments. Closed circles show glucose extraction in the presence of ultrasound driving force. Open circles show glucose extraction by pretreatment alone. Glucose permeability during ultrasound-based extraction is $2.6 \pm 1.1 \times 10^{-2}$ cm²/h (an enhancement of about 65-fold over diffusion across untreated skin). This permeability is about 7-fold higher than that induced by pretreatment alone. These data clearly show that additional application of ultrasound (after pretreatment) significantly enhanced transdermal glucose flux. These data are similar to those obtained in the case of drug delivery, although the additional enhancement induced by low-intensity ultrasound is different for insulin delivery and glucose extraction. The origin of this difference requires further investigations. With a permeability of $2.6 \times 10^{-2}$ cm²/h, an extraction flux of about $26 \mu$g/cm²/h of glucose could be achieved when the rat’s blood glucose level is 100 mg/dL (flux = permeability $\times$ glucose concentration). We collected the extracted glucose into a collection chamber containing 1 mL of PBS. The concentration of glucose in the collection chamber at the end of 15-min extraction was about 1 mg/dL. This concentration can be measured using glucose sensors and can be used to predict blood glucose concentrations after a one-point calibration (17). A detailed description of the correlation between blood glucose levels and transdermally extracted fluxes is provided in the next section. Thus, the data presented here show that application of ultrasound results in significant enhancements of transdermal glucose extraction. Next, we discuss applications of this method.

**Applications of Sonophoresis in Drug Delivery and Diagnostics.** Enhanced skin permeability by ultrasound application may be used for delivery of various therapeutic agents. We have previously shown that proteins such as insulin, γ-interferon, and erythropoetin can be delivered through permeabilized skin (3). With the permeability reported in Figure 2, an insulin dose of about 1 U/h can be delivered through a patch having an area of 10 cm² and containing insulin solution at a concentration of 500 U/mL. This dose is comparable to a typical baseline insulin dose for a Type I diabetic patient (3). Note that this dose can be further increased by providing additional application of low-intensity ultrasound as shown in Figure 3. Sonophoretic drug delivery method can also be used for several other drugs including low-molecular weight heparin and leutinizing hormone releasing hormone (LHRH). Further work in this area should focus on optimization of ultrasound parameters for increasing the delivery rates, performing detailed safety studies, and tests on human volunteers.

Low-frequency sonophoresis also has several applications in diagnostics. Specifically, the method proposed here has been tested in human volunteers for glucose extraction (17). Specifically, a 2-min ultrasound application was used to permeabilize skin of Type I diabetic volunteers. Skin remained permeable for about 15 h. Glucose was extracted through sonicated site using vacuum (10 in. Hg). The first extraction flux was used for calibration, and subsequent fluxes were used for prediction of blood glucose levels. Details of calibration are discussed in ref 17. Briefly, the first transdermal flux, $F_1$ (nmol/cm²/h) was used to calculate the calibration factor, $K_C$ ($K_C = B_i F_1$) for each individual, where $B_i$ is the blood glucose value (mg/dL) measured 30 min before measuring flux $F_i$. Blood glucose level of diabetic volunteers was changed using a standard meal, sustacal. These blood glucose values, $B_i$, were predicted on the basis of the calibration factor and the measured glucose fluxes, $F_i$, as $B_i = K_C F_i$. The relationship between reference glucose levels and sonophoretically predicted glucose levels was excellent (mean relative error of 17%) (17). Similar strategies may be used to noninvasively measure other analytes including electrolytes and blood gas. Initial safety studies indicated that ultrasound did not induce adverse effects on the skin. Specifically, no damage or irritation was observed by visual inspections. Further studies in this area should focus on conducting detailed safety studies and the development of a glucose sensor.

Note that several other strategies including the use of chemicals (9) and electric fields (11, 12) have been suggested for noninvasive transdermal drug delivery and/or diagnostics. Sonophoresis offers an advantageous method compared to these methods in that the enhancements are high and applicable to a variety of molecules (hydrophilic/lipophilic, charged/uncharged, high/low molecular weight). At the same time, this method requires a more sophisticated device compared to other methods. A detailed study of advantages/disadvantages of sonophoresis should be performed in the future.

Thus, the data presented here shows that low-frequency sonophoresis offers a noninvasive method of drug delivery and diagnostics. Both of these methods have several clinical applications. In addition, these two
methods may be combined to develop a self-regulated patch for delivery of drugs such as insulin and heparin (or low-molecular weight heparin) that will require minimum attention from the patient.

References and Notes

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