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Report

The Effect of Ultrasound on the *in Vitro* Penetration of Ibuprofen Through Human Epidermis

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The objective of this study was to develop an *in vitro* method to investigate the effect of ultrasound on the *in vitro* absorption of ibuprofen from a propylene glycol/water vehicle through human epidermis. A diffusion cell was modified so ultrasound could be applied to the vehicle and skin. Since ultrasound can increase the temperature underneath the area of application, control representing temperature effects ran concurrently to the ultrasound experiment. The results demonstrate that ultrasound can increase the penetration of ibuprofen through human skin. This increase in diffusion was greater than for controls where an equivalent increase in temperature was utilized. The results also indicate that evaporation of vehicle components may alter the skin/vehicle partition coefficient, decreasing the effects of ultrasound on the penetration of ibuprofen through the skin.

KEY WORDS: ultrasound; phonophoresis; temperature; ibuprofen; human skin; *in vitro* percutaneous absorption.

INTRODUCTION

Ultrasonic energy is a form of mechanical energy generated by using a piezoelectric crystal which is made to vibrate by passing an alternating current through the material (1). This form of energy has been used in physical medicine for the treatment of a variety of localized inflammatory conditions of nerves, muscle, ligaments, and skin. Both thermal and nonthermal effects are considered to be responsible for the clinical benefit of ultrasound (2).

One reported property of ultrasonic energy is its ability to increase the percutaneous penetration of drug molecules through the skin; this effect is known as phonophoresis (3). Phonophoresis with topical antiinflammatories or local anesthetics is currently utilized by physical therapists as part of their treatment plans. Early experiments in pigs have concluded that ultrasonic energy is capable of driving hydrocortisone into underlying tissues (4–8). Recently, however when phonophoresis was administered with a variety of drugs in double blind crossover clinical trials using human volunteers, a significant increase in the absorption of the drug was reported (fluocinolone acetonide gel, lidocaine/prilocaine cream) (9,10). The effect of ultrasound on the skin penetration of mannitol, inulin, and physostigmine in rats and guinea pigs was studied. These results indicated that ultrasound eliminated the lag time for transdermal penetration of these drugs and significantly increased the amount absorbed (11). In other clinical trials there was not a significant increase in percutaneous absorption (lidocaine cream, benzydamine gel) (12,13).

Clinically the ultrasonic dose can range from 0.001 to 2 W/cm^2 and is chosen on the basis of what gives the patient a sensation of warmth during treatment which is tolerable (2). The transducer can be applied either in a stationary position or with a moving technique; either method requires a medium to transmit sound waves which is called a coupling medium. In clinical practice the moving technique is more frequently used. The physical therapist moves the transducer in a gentle motion in direct contact with the areas to be treated. The pattern of movement and the dose of ultrasound are determined by the physical therapist depending on the condition, area of treatment, type of patient, and type of machine output used. Intensities up to 2 W/cm^2 can be achieved with the moving technique but very few individuals can tolerate up to 0.2 W/cm^2 for more than 2 min using the stationary technique (2).

Another consideration is the length of ultrasound treatment. From the literature cited in this article the most common treatment time is 5 min, up to about 20 min.

Ultrasound treatments may also be administered either on a continuous or on a pulsed mode. As the name implies, with the pulsed mode there is a time interval between ultrasonic outputs. When conducting a study using pulsed output, the pulse period and pulse duration should be recorded. With pulsed output it is possible to use higher intensities of ultrasound with a lesser chance of tissue damage (2).

The main objectives of this study were to develop an *in vitro* method and to investigate the effect ultrasound has on

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the *in vitro* percutaneous penetration of ibuprofen through human epidermis from a propylene glycol/water vehicle. Another objective was to differentiate the effect of ultrasound from the effect of an equivalent temperature increase, on the penetration of ibuprofen from the same vehicle.

MATERIALS AND METHODS

Materials and Equipment

Materials and equipment were as follows: glass scintillation vials, Kimble Glass Company (Toledo, Ohio); nine-cell Franz diffusion apparatus (1.5-cm diameter), Crown Glass Company (Somerville, N.J.); constant temperature circulator, Exacal EX-100B, Neslab Instrument Inc. (Newington, N.H.); ultrasound generator Dynasound 601 and ultrasound transducer (15-mm diameter), lead zirconate titanate, Dynawave Corporation (Geneva, Ill.); digital thermocouple thermometer, Digi-Sense, and type K thermocouples (0.029-in. diameter) with miniconnectors Kapton-insulated, Cole Parmer (Chicago); Fisher pump Model A-1, Fisher Scientific Company (Itasca, Ill.); constant-temperature circulator, Thermomix II, B. Braun (Melsungen, West Germany); and liquid scintillation counter, Packard Tri-Carb 4640, Packard Instrument Company (Downers Grove, Ill.).

Chemicals

Chemicals were as follows: ibuprofen USP (98% purity), Upjohn Company (Kalamazoo, Mich.); ^{14}C-ibuprofen (20 μCi/mg, 98% purity), The Boots Company (England); propylene glycol USP, American Drug Industries (Chicago); sodium phosphate monobasic and sodium phosphate dibasic anhydrous, Mallinckrodt Chemical Works (St. Louis, Mo.); sodium chloride, Aldrich Chemical Company Inc. (Milwaukee, Wis.); and scintillation cocktail, Ready-Solv MP, Beckman Instruments Inc. (Fullerton, Calif.).

Diffusion Cell

The diffusion-cell apparatus used in these experiments is shown in Fig. 1. Instead of the glass caps provided by the

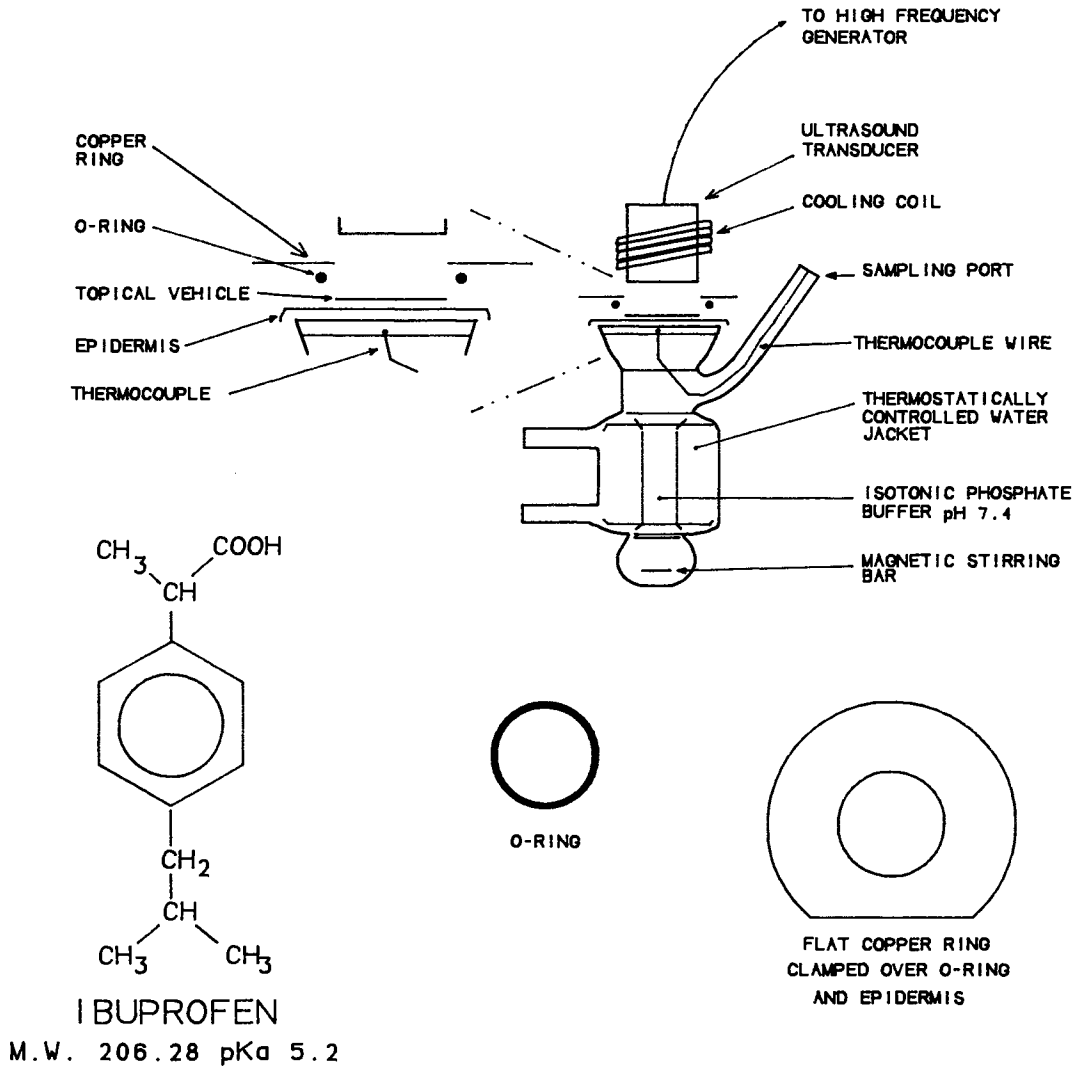

Fig. 1. Illustration of the diffusion chamber, similar to the one described by Franz (14), modified for the application of ultrasound.

manufacturer, a flat copper ring was devised and clamped over the O-ring. As a result, the ultrasound probe could be positioned over the skin surface, without interference from the glass caps. The diffusion cells were taped to the diffusion-cell assembly to prevent any movement when the transducer was positioned on the cell. The surface area of the diffusion cell is 1.77 cm^2. The receptor volume was approximately 7 ml, and the volume was calibrated for each cell prior to use. The contents of the cell were stirred with a small magnetic stirring bar operated at 600 rpm. Temperature control was provided by a constant-temperature bath with an external circulator, which when circulated through the diffusion cells, kept the skin surface temperature at approximately 32 ± 1°C under ambient conditions. Isotonic sodium phosphate buffer, pH 7.4, prepared with Milli-Q water, was used as the receptor fluid. Sink conditions were maintained since ibuprofen is soluble at this pH (5.2 mg/ml). Prior to use, the receptor fluid was filtered through a 0.22-μm membrane filter and degassed for 30 min using both an ultrasonic bath and a vacuum simultaneously.

Human Skin

Human skin samples were obtained from cosmetic breast reduction cases (female, Caucasian, 18–20 years old). The epidermis was removed by a heat separation technique (15). Upon receipt, the subcutaneous fat was trimmed and the full-thickness human skin was immersed in distilled water at 60°C for 2 min. The epidermis was gently teased off with a blunt spatula, allowed to dry in a desiccator, and refrigerated until further use. The skin tissue was rehydrated by immersing the sample in distilled water for 1 hr prior to use.

Vehicle Preparation and Assay

^{14}C-Labeled ibuprofen dissolved in ethanol was added to a scintillation vial and allowed to evaporate. A solution of propylene glycol/water (65/35, v/v) containing unlabeled ibuprofen was added to make a final concentration of 1.1% (w/v) of ibuprofen. The formulation was sonicated for 10 min and allowed to stand overnight. The final formulation contained 5.76 μCi per 0.3-ml aliquot. On each diffusion cell a 0.3-ml aliquot was applied to the stratum corneum side of human epidermis. This aliquot of drug was not occluded and was exposed to ambient laboratory conditions. The receptor fluid was sampled at appropriate time intervals. Each sample of 100 μl was replaced by an equal volume of isotonic phosphate buffer. The analysis of each subsequent sample was corrected for all the previous samples that had been removed. Scintillation cocktail was added to each sample, vortexed for 2 min, and left overnight to equilibrate before assaying for ibuprofen concentration.

Ultrasound and Heat Application

Since the application of ultrasound also increases skin temperature, the ultrasound effect was compared with two controls: one with no treatment (except addition of drug formulation to the skin) and a second control where heat was applied after application of the formulation to the skin (to differentiate the effect of temperature from that of ultrasound). For these experiments, the vehicle itself served as the coupling medium. Ultrasound was applied for 30 min at the beginning and at 6 hr under the following conditions.

Intensity:	1 W/cm^2
Mode:	Continuous
Application:	Stationary
Area:	1.77 cm^2
Frequency:	1 MHz

The ultrasound equipment used for these experiments is typical for clinical practice. The ultrasound transducer was placed in a clamp attached to an adjustable ring stand, which could be easily raised or lowered. A copper cooling coil was placed around the transducer to counteract any deleterious temperature rise which may occur during the operation of the ultrasound device. Water at 5°C was circulated through this copper coil when the transducer was in use.

A control representing temperature effects ran concurrent to the ultrasound procedure. Changes in the temperature during the heat application were adjusted manually to a magnitude similar to that manifested with the ultrasound experiments (see Fig. 2). A thermocouple probe was positioned through the sample arm of the diffusion cell so that the thermocouple was underneath the center of the skin. The wire was then taped into position. The temperature readings were recorded every 10 min using a digital thermometer, when the ultrasound dose was administered. A brass weight covered in aluminum foil, which had the same surface area as the ultrasound probe, served as the temperature probe. Heated water, circulated through a copper coil surrounding this probe, provided the heat source.

Data Analysis

The amount of ibuprofen penetrated through epidermis at each time point was compared. One-way analysis of variance followed by the Tukey–Kramer method was used to compare the means ($P < 0.05$). If the sample variances were not homogeneous as demonstrated by the F max test, comparison of means was performed by using the Games and Howell method (16). Statistical tables by Rohlf and Sokal (17) were used along with this analysis. Calculations were performed with a Hewlett–Packard HP-41C calculator using

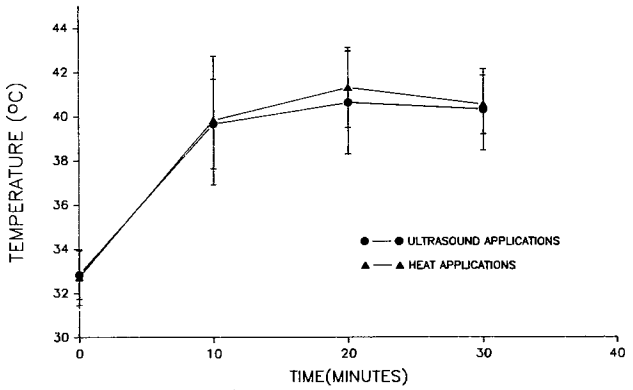

Fig. 2. Temperature profile beneath the surface of the skin during ultrasound and heat applications (mean ± SD; N = 11 or 12 determinations).

the Stat-Pac statistics application software for ANOVA calculations.

Initial fluxes and lag times were calculated from the slope of the best-fit regression line at the beginning of the experiment. Steady-state fluxes were calculated from the slope of the best-fit regression line between the treatments. Final fluxes were also calculated after the 360-min time point for 30 min.

RESULTS AND DISCUSSION

The dose of ultrasound used in this study was chosen to allow any increase in diffusion to occur within a possible clinical time limit. A stationary technique was used because it was not possible to move the transducer in a reproducible fashion on the diffusion cell without causing damage to the epidermal membrane. A 1-MHz frequency was used since a variable-frequency generator was not available. Different frequencies may alter the amount of drug diffused through the skin (7,10).

The transducer can also cause a temperature increase in the area of tissue beneath it. The temperature versus time data are graphed in Fig. 2. The temperature at a certain intensity tends to rise to a maximum and stay constant within a certain standard deviation until the ultrasound application is stopped. The thermocouple was positioned as accurately as possible in the center of the diffusion cell and as close to the epidermis as possible. Since the surface of this transducer, and all transducers on the market, does not have a uniform intensity emitted throughout the surface of contact, the temperature can vary depending on the position of the thermocouple. The maximum temperature during these experiments (N = 11 or 12) was 43.9°C from an initial average reading of 32.8°C. This rise in temperature did not visually damage the skin membrane. Also, the temperature did not increase to above 45°C, which may be potentially destructive depending on the length of exposure and type of cell or tissue (18).

The results for the diffusion experiments are graphed in Fig. 3. Table I contains the lag times and the calculated fluxes for the various treatments. The total amount, in micrograms, of ibuprofen permeated through the epidermis at each time point was compared to the control experiments. Treatment with ultrasound resulted in an 11.3-fold increase at the 30-min time point in *in vitro* penetration of total amount of ibuprofen compared to the untreated control. As anticipated, changes of a lesser magnitude were observed when the effect of temperature was isolated. This demonstrates the individual effect of ultrasound on the penetration of ibuprofen. The initial flux due to ultrasound is approximately nine times the control. The initial flux due to heat is five times the control.

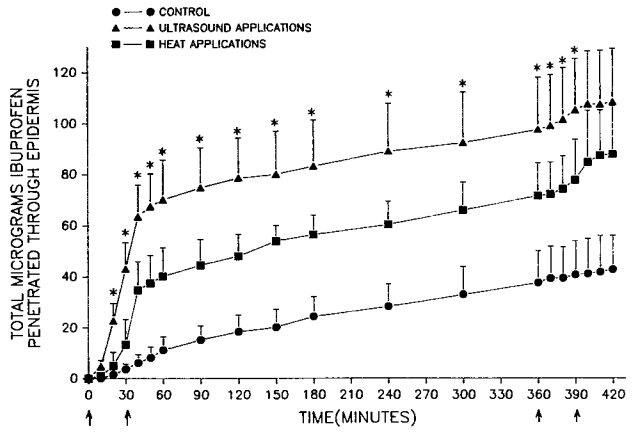

Fig. 3. Total micrograms (mean ± SD) of ibuprofen penetrated versus time. Ultrasound and heat applied from time 0 to 30 min and from 360 to 390 min as indicated by the arrows. Asterisks denote where ultrasound is significantly different ($P < 0.05$) from heat application and control.

Application of ultrasound after 6 hr did not produce an increase in flux to the same degree seen with the ultrasound application at the beginning. A reason for this may be due to the experimental procedure. If the vehicle is changing its composition with time to an extent that it changes the activity of the drug within that vehicle, the rate of penetration through the skin would be altered. This may account for the observed changes seen when ultrasound was applied after 6 hr. The drug/vehicle was left open to the ambient conditions of the laboratory environment, which simulates *in vivo* use conditions. This procedure allows the vehicle to evaporate and essentially change its composition. The ibuprofen was saturated when first applied. The solubility is 7.5 mg/ml in the propylene glycol/water vehicle versus a solubility of 207 mg/ml in the propylene glycol alone. With time the aqueous component of the vehicle will evaporate and hence the solubility of the drug in the vehicle increases but the amount of drug present remains constant. When the solubility of the drug in the vehicle increases, the partition coefficient (skin/vehicle) falls; therefore the drug tends to stay in the vehicle, and not partition into the skin. The composition of the vehicle can change to the point where further increases in the diffusion of ibuprofen which could be gained by the application of ultrasound may be offset by the high degree of interaction between the drug and the vehicle.

The lag time was not eliminated in either the ultrasound or the heat application experiments. This is the opposite of what was reported by Kost *et al.* (11); they concluded that the lag time was eliminated with an ultrasound application when studied *in vivo* with rats and guinea pigs. A possible explanation is that rats (18) and perhaps guinea pigs have a greater number of hair follicles as compared to human breast skin and ultrasound may also promote the flux of a compound through these appendages to a greater extent then the bulk stratum corneum.

The observed increase in the flux of ibuprofen occurred at the beginning of the experiment only when ultrasound or heat was applied and remained for 10 min after the energy source was turned off. After this time period the fluxes of ibuprofen for the two treatments and control were essentially the same. This suggests that there is no permanent damage to the barrier properties of the membrane.

Ultrasound can produce both thermal and nonthermal effects. In addition to a temperature rise, nonthermal effects which are produced are cavitation, radiation pressure, and acoustic microstreaming (19). These mechanisms may effect the vehicle, diffusion coefficient, or membrane itself. The amount of the drug in solution in a suspension-type vehicle may increase and therefore elevate the flux of the drug

Table I. Calculated Parameters (mean ± SD) for the Various Treatments

	Lag time (min)[a]	Flux (μg/cm² · min)		
		Initial[a]	Steady state[b]	Final[c]
Control (N = 5)	10.9 ± 0.83	0.12 ± 0.06	0.19 ± 0.32	0.054 ± 0.02
Ultrasound (N = 5)	8.0 ± 2.1	1.1 ± 0.25	0.12 ± 0.15	0.14 ± 0.07
Heat (N = 6)	12.4 ± 3.9	0.62 ± 0.21	0.13 ± 0.18	0.12 ± 0.06

[a] Initial fluxes and lag times calculated from the slope of the best-fit regression line at the beginning of the experiment.
[b] Steady-state flux calculated from the slope of the best-fit regression line after the initial treatments to the 360-min time point.
[c] Final fluxes calculated from the slope of the regression line from the 360- to the 390-min time points.

through the skin. Ultrasound may affect the stratum corneum itself by altering the lipids within the stratum corneum as suggested by McElnay *et al.* (9), which in turn may increase the diffusion of certain compounds. Finally, ultrasound may simply increase the diffusion coefficient solely by decreasing the activation energy required for diffusion to occur as described by Julian (20) for the ultrasonic enhancement of diffusion through polymeric membranes.

CONCLUSIONS

(1) The use of ultrasound to enhance the penetration of compounds through skin can be studied *in vitro* using a modified version of a commercially available diffusion cell and an ultrasound transducer which is a miniature version of the type used in clinical practice.

(2) Ultrasound can increase the *in vitro* penetration of ibuprofen through human skin to a greater extent than what is manifested with a temperature increase of a similar magnitude.

(3) Under certain conditions, such as a low skin/vehicle partition coefficient, ultrasound may not significantly increase the flux of drug through the skin.

(4) This study suggests that there is no permanent damage of the barrier properties of the skin after ultrasound was applied under the specified conditions.

(5) Mechanisms were discussed which outline various reasons for the enhancement of diffusion through human skin noted with an ultrasound application.

(6) Further studies are needed in order to elucidate a mechanism for the enhanced diffusion noted with ultrasound. Also, *in vivo* experiments should be undertaken in order to characterize the depth of penetration into local subcutaneous tissue that can be achieved using phonophoresis.

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