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Phonophoresis of hydrocortisone with enhancers: an acoustically defined model

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Abstract

The aim of the study was to define an acoustic model to determine the effect of ultrasound on the penetration of hydrocortisone through whole rat skin. Methods: ultrasound dosimetry measurements were used to define an ultrasound source used to measure the phonophoretic enhancement of hydrocortisone transport through rat skin in vitro. The effect of conductive heating was also studied. Results: acoustic dosimetry measurements indicated that the skin barrier was exposed to ultrasound standing waves and this focused heat generation within the tissue. While sonication alone did not significantly enhance hydrocortisone permeation, a significant synergistic effect was observed with Azone but not with oleic acid. The ultrasound-Azone effect could be duplicated with conductive heating. Conclusion: synergism between phonophoresis and Azone treatment was observed in the enhancement of hydrocortisone percutaneous transport. It is probable that the thermal effects of ultrasound were responsible for accelerated Azone diffusion through the stratum corneum.

Keywords: Azone; Dosimetry; Enhancement; Synergism; Transdermal; Ultrasound

1. Introduction

Strategies to facilitate transdermal drug delivery, by increasing the normally the low permeability of the stratum corneum, include molecular modification to optimise the physico-chemical properties of the drug (Chan and Li Wan Po, 1989), diverse pharmaceutical formulations with a range of vehicles (Barry, 1983; Cooper, 1985), the use of chemical enhancers (Barry, 1991), occlusion (Bucks et al., 1989) and iontophoresis (Tyle and Kari, 1988). A further approach involves the application of ultrasound to increase the penetration of drugs into tissues—a phenomenon termed phonophoresis (Tyle and Agrawala, 1989). Although phonophoresis has been applied in physio-

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therapy clinics for almost 40 years, the therapeutic efficacy of the technique is still under question while the biophysical mechanisms involved are not fully understood (Meidan et al., 1995). Physiotherapists have particularly focused on hydrocortisone phonophoresis in order to treat various inflammatory conditions (Newman et al., 1992). Unfortunately, most of this treatment has been conducted on a rather subjective and non-quantitative basis (Williams, 1983). For example, in one trial involving 102 arthritic patients (Griffin et al., 1967), hydrocortisone with 1 MHz ultrasound was compared to 1 MHz ultrasound alone. Treatment efficacy was evaluated by pain and range of motion criteria that assessed limb movement. It was shown that the combination of ultrasound plus steroid was more effective than ultrasound alone ($P = 0.001$, t-test). In a retrospective study of 285 patients treated for several different types of inflammatory conditions (Kleinkort and Wood, 1975), the authors compared 1 MHz ultrasound plus 10% hydrocortisone versus 1 MHz ultrasound plus 1% hydrocortisone. It was found that phonophoresis of 10% steroid was superior to phonophoresis of 1% steroid. However, in both studies, there were no non-sonicated control groups and the beam intensity was varied according to individual patient tolerance.

Hydrocortisone phonophoresis has also been evaluated in in vivo animal models with mixed results. One research team (Davick et al., 1988) applied cream containing 5 and 10% tritiated hydrocortisone to the medial aspect of the knees of mongrel dogs. Continuous wave 0.5 W cm^{-2} ultrasound was administered at 0.87 MHz for 8 min. A non-sonicated drug application acted as a placebo. Autoradiography of the excised skin indicated that phonophoresis of the 10% hydrocortisone cream resulted in significantly more drug penetration into the viable epidermis than cream alone. In contrast, Muir and co-workers (Muir et al., 1990) determined that 2.75 W cm^{-2} ultrasound at 1 MHz did not significantly enhance hydrocortisone penetration through the surface of the joints of greyhounds. Similarly conflicting results have been obtained in in vitro studies. Although one group obtained significant flux enhancement through hairless mouse skin using 1 MHz ultrasound (Machluf and Kost, 1993), another group reported no enhancement through whole human skin with 1 MHz ultrasound (Machet et al., 1997).

The conflicting data has resulted from the fact that different research groups have employed different ultrasonic parameters i.e. frequency, intensity, duration and mode, as well as different skin membranes and vehicles. However, more fundamentally, many of the physiotherapeutic ultrasound generators which are used for phonophoretic research often suffer from poor calibration and the amount of sound energy emitted may not truly be reflected by the control dial on the machine (Walmsley and Squier, 1991). Another variable is that ultrasound can reflect back on itself at a tissue-bone interface in vivo or at a vessel wall-solution interface in vitro to produce a standing wave. Such a standing wave is characterised by a regular repeating pattern of acoustic nodes where the ultrasonic intensity is zero and acoustic antinodes where the intensity is double that of the incident beam. Yet another problem is that as an ultrasound beam propagates away from its source, the beam area begins to expand after a certain critical distance that depends mathematically upon the ultrasonic wavelength and transducer radius-an effect associated with constructive and destructive wave interference (Williams, 1983).

The aim of this study was to investigate the ultrasound effect on hydrocortisone penetration through whole rat skin in vitro. Crucially, unlike previous such studies, the output of the ultrasound source was independently validated and a hydrophone technique was used to define the ultrasonic field in the region of the skin barrier. Since the main effect of sonication is heat generation, a heat-alone application was also developed and its effect on hydrocortisone permeation was also investigated. Additionally, the interactions between ultrasound and the chemical enhancers Azone and oleic acid was also examined.

2. Materials and methods

2.1. Diffusion studies

Sections of intact whole skin, of side length 2–3 cm, were excised from the backs of 4–5 month old male Wistar rats (250–300 g). These sections were either used immediately, or stored in the frozen state at −20°C between sheets of aluminium foil for a period of up to 1 month. Each skin sample was transferred to a Franz diffusion cell (Franz, 1975), placed upon the steel mesh in the recess, and secured as a barrier between the donor and receptor halves. The receptor solution consisted of 5% aqueous ethanol. This solution had been initially degassed by heating to 30°C and then sonicating in an ultrasound bath (Kerry, Pulsatron 125) for 3 min. The fluid in the receptor compartment was maintained at 28°C by a thermostatic water pump (Churchill-Matrix, Churchill Instrument Company, Perivale) which circulated water through the jacket surrounding the main chamber. The donor solution consisted of a small volume (100 μl) of ethanolic solution containing 5% v/v of [^{3}H]-hydrocortisone (63.36 pmol, 185 kBq, Amersham). For the chemical enhancer studies, the skin, whilst mounted in the Franz cell, was pretreated with ethanolic solutions of either 1% v/v Azone or 1% v/v oleic acid (Merck, Poole). The enhancers were allowed to soak into the skin over a period of 1 h before the subsequent addition of the donor solution at time zero.

One hour after the application of the donor solution, the ethanol had largely evaporated from the skin surface and the Franz cell was inverted to remove any bubbles from the skin underside. The donor compartment was then filled with ultrasonic coupling gel (Henleys Medical Supplies, Welwyn Garden City). Care was taken to minimise the production of air bubbles within the gel. For the next 4 h, the skin was subjected to either: control conditions in which the donor compartment was merely sealed with parafilm; ultrasound-exposure; or heat-alone. Throughout the 5 h experimental period, 1.1 ml aliquots of receptor solution were withdrawn at 30 min intervals and replaced with an equal volume of receptor medium. Each 1.1 ml aliquot sampled was vortexed with 10 ml of scintillation fluid (Optiphase Hisafe 3 Fisher Chemicals, Loughborough) and then placed in a liquid scintillation counter (Packard 1900T.R. Packard, Pangbourne). The emitted activity value of each aliquot was converted to a drug concentration value according to the activity/mole ratio of the hydrocortisone. From these results, taking into account the cumulative dilution of the receptor solution (Meidan et al., 1998), it was possible to determine the flux, permeability coefficient and lag time values for the data.

2.2. Ultrasound application

An ultrasound generator (Therasonic 1032, model no. 50, EMS Greenham, Wantage) and its associated transducer (frontal surface area of 2.0 cm^2) were employed as the ultrasound source. The machine can generate continuous ultrasonic waves at a frequency of either 1.1 or 3.3 MHz throughout an indicated intensity range of 0 to 2.5 W cm^{-2}. The effective radiating area of the transducer was 0.7 cm^2 at 1.1 MHz or 0.6 cm^2 at 3.3 MHz. In addition to producing continuous wave ultrasound, the device could also generate 2 ms pulses of ultrasound at a 1:2 on/off ratio. Initially, both the 1.1 and 3.3 MHz outputs of the device were validated, across their entire intensity range, by applying a non-compensating radiation force technique (Davidson, 1991). For this purpose, Newell's original radiation force meter was used (Newell, 1963).

In the phonophoretic system selected, the transducer was positioned inside the donor cell, facing downwards so that its radiating surface was 2 cm distance from the skin surface. A specially designed Teflon collar which slotted over the top rim of the donor cell, fixed the transducer in position, perpendicular to the skin surface. Continuous wave 1.1 MHz ultrasound was applied at 2.25 W cm^{-2}. Continuous wave 3.3 MHz ultrasound was applied across a successive range of intensities (0, 0.75, 1.0, 1.5 and 2.25 W cm^{-2}). Pulsed 3.3 MHz ultrasound was also administered at 1:2 pulsed mode exhibiting a SATA intensity of 0.75 W cm^{-2}. In all cases, ultrasound transmission between the transducer and skin sample was mediated by the coupling gel which filled most of

the donor compartment. A schematic illustration of the equipment is presented in Fig. 1.

Since the ultrasound generator was not designed to be continuously in operation for 4 h periods, the machine was switched off for 10 min intervals following each 50 min period of sonication. An in-built feature of the generator was that the transducer face must be free of coupling gel when the ultrasound is being switched on. This permits the transducer to re-calibrate itself. Therefore, at the end of each 10 min rest interlude, the transducer was removed from the Franz cell, wiped clean of residues of coupling gel, recalibrated, re-inserted into the Franz cell, and switched on. The sonication experiments were always performed using the same individual diffusion cell so that the beam acoustics within the apparatus remained as reproducible as possible.

2.3. Heat-alone application

A heat-alone application was developed which would simulate the thermal effects of ultrasound without generating any additional effects. This application was mediated by a heating probe that was electrically connected to a variable ac supply module (Variac). The probe, which exhibited a frontal surface area of 2.0 cm^2 (identical to the transducer), was fixed by a Teflon collar, through the central axis of an individual donor cell. The probe was thus aligned normal to the skin sample and its front surface was a distance of 2 cm from the skin surface. By adjusting the module control dial to a specific applied voltage, it was possible to warm the skin surface to a particular temperature.

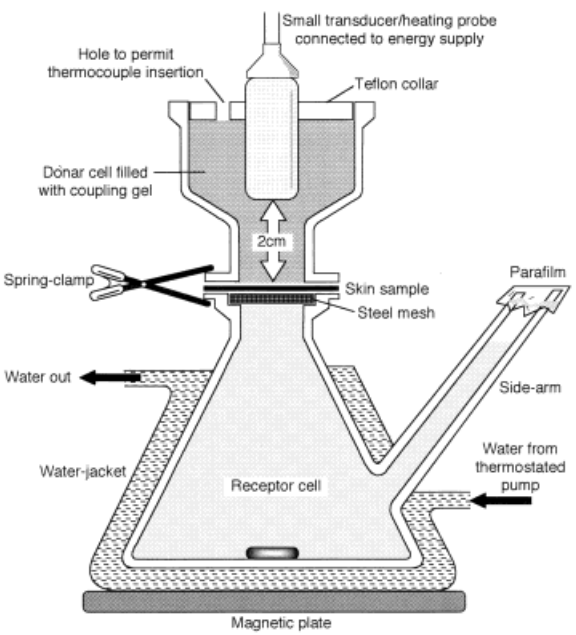

Fig. 1. Schematic diagram showing the ultrasound dosimetry arrangement for the modified Franz-cell model.

2.4. Ultrasound dosimetry measurements

A bilaminar membrane hydrophone (Marconi Y-337611), which had been pre-calibrated at the National Physical Laboratory, was employed to make the dosimetry measurements. The instrument consists of a central, 1 mm wide, piezoelectric element, embedded within an annular holder, 10 cm in diameter. The sensor was connected via a single-ended amplifier to an oscilloscope (Hameg 60 MHz, HM605). The oscilloscope timebase was set to 1 s cm^{-1} and the amplitude was set at 10 mV cm^{-1}. Ideally, the measurements should have been conducted with the hydrophone inserted horizontally through the diffusion interface of the Franz cell. Unfortunately, the protruding side-arm prevented the insertion of the large annular holder through the Franz cell junction. Fortunately, in each phonophoresis experiment, the skin was supported by a steel mesh lying within the receptor cell recess. Since steel exhibits a high ultrasonic reflectivity (Chivers, 1991), much of the sound energy would be reflected upwards at this interface and this effect would dominate the acoustic field at the skin sample. Consequently, it was possible to omit the Franz cell altogether and measure the effect of the steel mesh on the ultrasound beam emitted by the transducer. Fig. 2 displays a diagram of the arrangement used. The transducer was clamped into position at a distance of 2 cm directly above the hydrophone. The hydrophone was placed on an inclined stand making a 5° angle with the horizontal. This prevented the partial reflection of any energy back towards the transducer. The ultrasound was then switched on (1.1 MHz, 2.25 W cm^{-2}). Ultrasound intensity

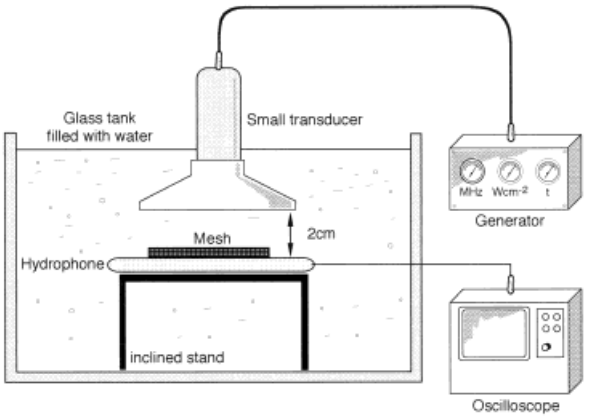

Fig. 2. Schematic illustration of the apparatus used to investigate phonophoresis.

measurements were then made both with and without the steel mesh lying over the hydrophone.

2.5. Temperature measurement techniques

Experiments were initiated to determine the skin surface temperature profile associated with each specific ultrasound and heat-alone regimen. To this end, a hole was drilled through each Teflon collar, so as to permit the insertion of a thermocouple probe (Digitron instruments, 3202 type K) right through the donor cell, so as to make contact with the skin surface. Consequently, it was possible to record the temperature at points on the skin surface, approximately midway between the centre and edge of the skin sample. The repeated insertion of the thermocouple probe through the donor cell could potentially compromise the skin barrier, create air bubbles in the coupling gel or possibly remove drug from the skin surface. Consequently, the temperature profile associated with each ultrasound/heat-alone regimen was determined from separate studies in which the skin samples were drug-free. In each experiment, successive temperature measurements were made at 30 min intervals throughout the 5 h period. In the ultrasound-exposure experiments, the beam was momentarily switched off during the actual measurement as otherwise, the thermocouple wire would itself heat up as it attenuated the energy (Williams, 1983).

2.6. Histological techniques

The Azone-only and Azone-plus-ultrasound treatments were conducted by mounting whole skin sections in modified Franz cells and pipetting 100 μl of 1% v/v Azone on to each skin surface. After 1 h, 100 μl of ethanol was deposited and after a further 1 h, each donor cell was filled with coupling gel. Each skin sample was then exposed for 4 h to either ultrasound (1.1 MHz, 2.25 W cm^{-2}) or control conditions. In either case, the skin sections were removed from the diffusion cells after 4 h and residues of coupling gel were wiped off with lens tissue. Control samples of whole rat skin were obtained by direct excision from freshly sacrificed animals. Each experimental treatment was conducted in triplicate.

In all cases, three 1 mm wide, 20 mm long strips were excised from the centrally exposed area of each skin section. These skin strips were fixed by overnight immersion in 4% neutrally buffered formalin and subsequently dehydrated and cleared in an automatic tissue processor (Shandon Hypercenter 2, Basingstoke). The skin strips were then wax-impregnated with paramat wax (Merck) under vacuum. The wax-impregnated strips were blocked out by immersion in fresh molten paraffin wax delivered by an automated wax dispensing machine (Boova 39 Professional Embedding Centre, Klanestaff). The wax was allowed to cool down to form a solid block encasing the tissue strip. The blocks were placed on ice prior to cutting with a base sledge microtome (IS300 Anglia Scientific, Basingstoke). Transverse strips (5 μm wide) were cut out from the block in the cassette and 'floated out' on a beaker of water at room temperature and then in a beaker of water at 48°C. The folds and wrinkles in the wax were carefully removed by gentle stretching with tweezers. The sections were placed on slides and dried for 10 min (Life Sciences International, Basingstoke). The sections were then placed in an enclosed automatic stainer (Shandon Linistain GLX) which removed the wax with sequential washes of xylene, IMS and water and then stained the tissue with Gill's haematoxylin and eosin dye. Cover slips were placed on the slides using xam (adherent in 60% acetone, BDH) as the mountant.

Photomicrographs of the skin samples were obtained at $\times 100$ magnification (Olympus BH microscope; Olympus PM6 camera; Fujicolor 35 mm Super G Film, Olympus Optical Company, London).

3. Results and discussion

3.1. Validation of the ultrasound source

Radiation-force measurements indicated that the control dial values on the ultrasound generator correlated well with the actual ultrasonic intensities emitted by the transducer. The performance of the generator was well within the limits set by the British Standards Institute for ultrasonic therapy devices (pamphlet no. 5724: section 2.5: 1985).

3.2. Ultrasound dosimetry within the Franz cell

When the steel mesh was placed between the transducer and hydrophone, there was a 75% reduction in the ultrasonic intensity detected at the hydrophone. This indicated that the remainder of the 1.1 MHz beam was reflected back at the steel mesh-water interface. It was found that if the angle the mesh makes with the horizontal was varied by merely a few degrees, the proportion of energy reflected increased. Since the mesh fitted loosely within its recess and the Franz cell was inverted during the course of the diffusion experiment, the precise mesh orientation would have varied within and between individual phonophoresis experiments. Thus, the steel mesh acts as an efficient, though unpredictable ultrasound reflector, reflecting at least three-quarters and probably more of the 1.1 MHz beam back upwards to produce a complex and unpredictable standing wave within the region of the skin sample and coupling gel. A basic understanding of ultrasound propagation indicates that the same process also occurred during phonophoresis at 3.3 MHz (Williams, 1983). The quantity of energy contained in an ultrasonic wave as it passes through any given point is usually expressed in terms of the intensity at that site. Intensity is defined as the power developed through an imaginary plane orientated at right angles to the direction of wave propagation. i.e. $I = P/A$ where I is the intensity (W cm^{-2}), P is the beam power (W) and A is the area of the imaginary plane (cm^2). Since $P = I \times A$ for any given intensity, the total power conveyed by the 1.1 MHz beam (radiating surface area of 0.7 cm^2) would be greater than that of the 3.3 MHz beam (radiating surface area of 0.6 cm^2).

As an ultrasound beam propagates away from the transducer, it can be described as consisting of two regions (Meidan et al., 1995). These are the near-field or Fresnel zone and the far-field or Fraunhofer zone. The near field is a cylindrical beam of spatially fluctuating acoustic intensity caused by the constructive and destructive interference of ultrasonic waves. The far field is a diverging beam exhibiting a central acoustic intensity peak in the centre of the beam which smoothly falls off at either side. The boundary between these two zones occurs at a distance d from the transducer which may be determined from the equation $d = r^2/\lambda$ where r is the radius of the radiating surface of the transducer and λ is the wavelength. As long as $r > 5$, which usually holds true, the beam diverges in the far zone, with divergence angle θ (in degrees) according to the equation $\theta = 35\lambda/r$. Beam divergence, calculated to begin at 1.65 and 4.24 cm from the transducer surface with the 1.1 and 3.3 MHz outputs respectively, resulted in the standing wave field covering much of the active skin surface of the Franz cell (3.3 cm^2). During phonophoresis, this energy was continually attenuated into heat within the skin tissue. Skin surface temperature measurements provide an indication of this process.

3.3. Skin surface thermal data

Fig. 3A presents the temperature-time profiles associated with control conditions, 2.25 W cm^{-2} ultrasound at 1.1 MHz, and 2.25 W cm^{-2} ultrasound at 3.3 MHz. It can be seen that under control conditions, the skin surface temperature was initially just above room temperature. However, at 60 min there was a marked increase in temperature which reached a plateau at 90 min.

This effect is due to the deposition of coupling gel which insulates the skin from the cooler air above and thus allows heat from the receptor compartment (jacketed at 28°C) to be more efficiently retained within the skin. The application of the 1.1 MHz beam at 60 min caused the skin temperature to rise, rapidly at first and then progressively less so until an equilibrium temperature of 37°C was attained at 120 min. During this phase, the energy output of the transducer was equal to

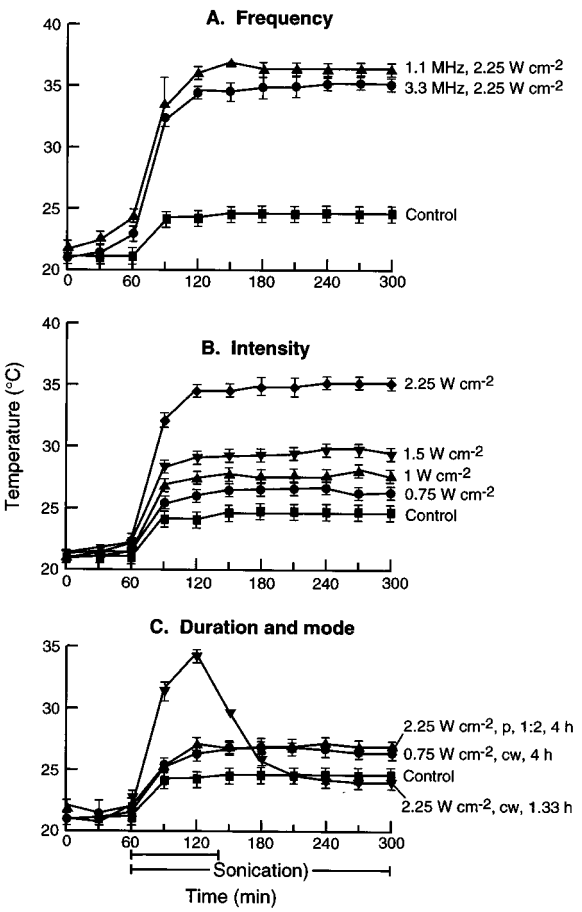

Fig. 3. Skin surface temperature profile during sonication. A, the effect of frequency (▲, 1.1 MHz, 2.25 W cm^{-2}; ●, 3.3 MHz, 2.25 W cm^{-2}; ■, control). B, the effect of intensity at 3.3 MHz frequency (◆, 2.25 W cm^{-2}; ▼, 1.5 W cm^{-2}; ▲, 1 W cm^{-2}; ●, 0.75 W cm^{-2}; ■, control). C, the effect of duration and mode (▼, 2.25 W cm^{-2}, p, 1:2, 4 h; ▲, 2.25 W cm^{-2}, cw, 1.33 h; ●, 0.75 W cm^{-2}, cw, 4 h; ■, control). (Sonication was applied from 60–300 min and included 4 × 10 min off periods; *p*, pulsed; cw, continuous wave).

the energy lost from the apparatus via conduction. It can be seen that the temperature-time profile of the 3.3 MHz beam was similar to the profile of the 1.1 MHz beam except that a slightly lower equilibrium temperature of approximately 35°C was attained at 3.3 MHz. Switching off the ultrasound for 10 min intervals did not produce noticeable deviations in the thermal profiles and this is probably due to the relatively high specific heat capacity exhibited by the coupling gel. Sonication at either frequency did not produce temperature increases that should cause irreversible damage to the skin structure. Some workers have suggested 45°C as a cut-off point with temperatures in excess of this value being potentially destructive to skin (Bronaugh et al., 1982). Even taking into account ultrasound-induced deep heating, it is unlikely that such temperatures are being generated within the rat skin samples in this model. Fig. 3B shows the temperature versus time data for various intensities of 3.3 MHz ultrasound. Again, the same general profile was obtained and it is clear that the equilibrium temperature attained is dependent upon the intensity and ultimately the power conveyed by the ultrasonic beam.

The skin samples in the diffusion cells were exposed to three different regimens of 3.3 MHz ultrasound, each of which conveyed the same total energy. The three regimens were a 0.75 W cm^{-2} beam delivered for 4 h, a 2.25 W cm^{-2} beam delivered in 1:2 (on/off) pulsed mode for 4 h and a 2.25 W cm^{-2} beam delivered for 1.33 h. The thermal profile of each of these beams is presented in Fig. 3C. It can be seen that both of the 4 h applications resulted in similar plateau temperatures being attained. This is due to the fact that both beams conveyed the same power output. The 1.33 h application induced much greater temperature increase initially but the skin temperature then decreased, reaching control levels from 210 min onwards. It was determined from pilot studies that by adjusting the potential difference at the a.c. supply module to 12 V, it was possible to simulate the surface temperature profile generated by a 2.25 W cm^{-2} beam at 3.3 MHz. By employing a 25 V supply, it was possible to create a thermal profile that formed a plateau

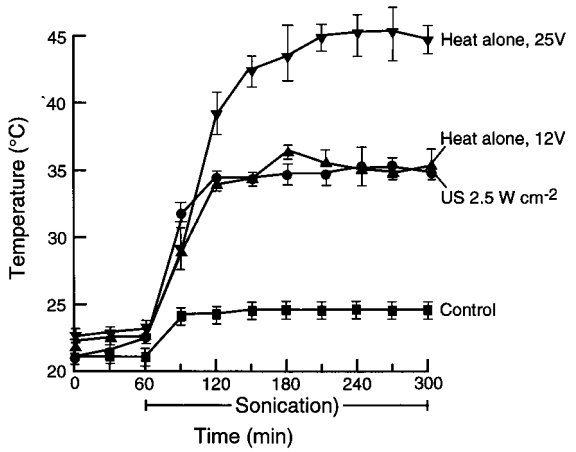

Fig. 4. Skin surface temperature associated with 3.3 MHz ultrasound and heat-alone (▼, heat alone, 25 V; ▲, heat alone, 12 V; ●, ultrasound, 2.25 W cm^{-2}; ■, control).

at 45°C. All these temperature-time profiles are presented in Fig. 4. It must be noted that while sonication caused heating throughout the depth of tissue, the heat-alone applications mediated a more surface-confined effect.

3.4. Permeation data

Table 1 displays the flux values, permeability coefficients and lag times derived from linear regression analysis of the resulting transport curves. It should be noted that lag time comparisons may not be an ideal way to evaluate these data since sonication was initiated 1 h after hydrocortisone application. Fig. 5A presents the permeation profiles for the treatments; control, 1.1 MHz ultrasound only; oleic acid only; and oleic acid with 1.1 MHz. Under control conditions, hydrocortisone penetration was characterised by a mean permeability coefficient of 0.12 $\mu m\ h^{-1}$. It can be seen that sonication from 60 min onwards resulted in an increase in hydrocortisone flux. Although the increase was significant at the 90% confidence level, it was not significant at the 95% confidence level ($P = 0.052$). The pre-application of oleic acid resulted in an approximately 7-fold enhancement in hydrocortisone penetration. However, sonication of oleic acid-pretreated skin did not produce a significant further enhancement ($P = 0.324$, t-test). It must be remembered that in the stratum corneum, oleic acid undergoes phase separation from the endogenous solid lipids. The existence of oleic acid in a liquid state results in high permeability being exhibited at the boundary between the solid and liquid domains and this effect is primarily responsible for the enhancement action of this agent (Naik et al., 1995). This effect normally occurs throughout the entire depth of the horny layer and it appears that ultrasonic heating does not affect this process.

The permeation profiles of the treatments; control, 1.1 MHz ultrasound only, Azone only and Azone with 1.1 MHz ultrasound are shown in Fig. 5B. It can be seen that Azone pretreatment enhanced hydrocortisone permeability almost 6-fold. Crucially, sonication of Azone-pretreated skin enhanced hydrocortisone permeation by a further 2.5-fold, compared to Azone pretreatment alone. This represents a significant synergistic phenomenon. Fig. 6 illustrates the effect of Azone followed by heat-alone on hydrocortisone permeation. Here, heating to a surface temperature of 35°C increased the steady-state rate and that more intense heating to 45°C enhanced permeation further. These results strongly suggest that the synergistic phenomenon is mediated by the thermal effects of ultrasound rather than other mechanical effects such as radiation pressure, acoustic microstreaming or cavitation (Williams, 1983; Meidan et al., 1995). Current thinking is that Azone acts as an enhancer by fluidising intercellular lipids of the stratum corneum and that this effect occurs initially only within the uppermost layers of keratinocytes (Hadgraft et al., 1995). It seems likely that ultrasonic heating accelerates Azone penetration to a greater depth within the cornified layer, thus permeabilising it to hydrocortisone diffusion. Photomicrography of haematoxylin and eosin-stained skin samples indicated that following both Azone-only treatment and Azone plus ultrasonication (1.1 MHz, 2.25 W cm^{-2}), the stratum corneum and other cutaneous structures remained intact, and unaltered in comparison to control skin. Thus, ultrasound—Azone synergism was not associated with any detrimental changes to the skin barrier. From Table 1, it can be seen that sonicating at 3.3 MHz had the same general

Table 1
Permeation data for 4 h phonophoresis at 2.25 W cm^{-2}

Treatment	Flux (pmol cm^{-2} h^{-1})	k_p (μm h^{-1})	Lag time (h)
Control	0.0073 ± 0.0105	0.1161 ± 0.1661	2.27 ± 0.83
1.1 MHz	0.0133 ± 0.0016	0.2116 ± 0.0257	2.37 ± 0.33
3.3 MHz	0.0160 ± 0.0052	0.2543 ± 0.0824	2.19 ± 0.89
Oleic acid	0.0494 ± 0.0092	0.7859 ± 0.1349	1.79 ± 0.56
Oleic acid + 1.1 MHz	0.0583 ± 0.0029	0.9283 ± 0.0467	1.60 ± 0.16
Azone	0.0407 ± 0.0054	0.6478 ± 0.0858	2.01 ± 0.38
Azone + 1.1 MHz	0.1021 ± 0.0125	1.6254 ± 0.1981	1.89 ± 0.36
Azone + 3.3 MHz	0.0953 ± 0.0172	1.5170 ± 0.2737	2.08 ± 0.51
Azone + 35°C	0.0710 ± 0.0032	1.1295 ± 0.0513	2.12 ± 0.13
Azone + 45°C	0.1237 ± 0.0165	1.9682 ± 0.2622	2.19 ± 0.37

Values represent the mean ± S.E.M. derived from linear regression analysis between 150 and 300 min. The flux of the drug across the membrane (J) is the rate of transport (dM/dt) per unit area of membrane and is given by $J = (dM/dt)/A = D \cdot K \cdot C_d/h$ where, D denotes the diffusion coefficient of the drug in the membrane of thickness h, K is the partition coefficient between donor and membrane and C_d is the concentration of the drug in the donor solution. The permeability coefficient is $k_p = D \cdot K/h = J/C_d$. The lag-time is the time taken for steady-state transport to be reached and depends upon diffusion coefficient and membrane thickness such that $t_L = h^2/6D$.

effect as sonicating at 1.1 MHz although the ultrasound effects were slightly less pronounced, probably reflecting the slightly lower power output and therefore lesser heating conveyed by the 3.3 MHz beam.

Several permeation experiments were carried out in order to determine how the intensity parameter (at 3.3 MHz) affects hydrocortisone penetration through Azone-pretreated skin. Fig. 7 presents a plot of permeability coefficient as a function of intensity. It can be seen that the graph is approximately sigmoidal in profile. Compared to Azone alone, sonication at 0.75 W cm^{-2} did not increase the permeability coefficient. However, sonication at an intensity of 1 W cm^{-2} resulted in the mean permeability coefficient doubling in value while ultrasound exposure at 1.5 W cm^{-2} resulted in the permeability coefficient tripling in magnitude. However, augmenting beam intensity to 2.25 W cm^{-2} did not affect a further increase in permeability. This plateau effect may be due to the fact that at 1.5 W cm^{-2}, sufficient heating is generated for Azone to diffuse throughout the depth of the horny layer. Since Azone does not appreciably permeabilise the other strata of the skin (Sugibayashi et al., 1985), further ultrasonic heating exerts no effect on barrier permeability.

Other studies were conducted in order to ascertain the effect of duration and mode (at 3.3 MHz drive) on hydrocortisone penetration through Azone-pretreated skin. The relevant parameters are presented in Table 2. It can be seen that delivering the same amount of ultrasonic energy in different ways did not markedly affect the resultant flux or permeability coefficient.

4. Conclusions

A phonophoretic model was developed in which the skin barrier was exposed to a variable and complex ultrasound standing wave field. Implicit in this system was the fact that the acoustic energy, distributed over all of the active area of skin and overlying coupling gel, was progressively attenuated into heat while the skin surface temperatures remained well within the physiological range. Surprisingly, it was found that the 4 h application of 1.1 MHz, 2.25 W cm^{-2} ultrasound increased hydrocortisone permeation, although the significance of this effect was low ($P = 0.52$). However, we have shown recently that sonication of this type of rat skin in vitro causes the migration of lipids from the sebaceous glands into the hair follicle shafts (Meidan et al., 1998). This