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Ultrasound-enhanced diffusion into coupling gel during phonophoresis of 5-fluorouracil

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Abstract

Purpose: To investigate the competitive transport across skin and back-diffusion of 5-fluorouracil into coupling gel under the influence of ultrasound, heat-alone and Azone enhancement. *Methods:* The ultrasound effect on 5-fluorouracil penetration through whole rat skin was investigated in modified diffusion cells using a commercial ultrasound generator which was calibrated with a bilaminar membrane hydrophone. *Results:* Ultrasonic dosimetry measurements demonstrated that the skin membrane was subjected to a complex and unpredictable standing wave field which induced physiologically acceptable heating of the tissue. Surprisingly, ultrasonication produced a decrease in percutaneous drug penetration. Quantification studies indicated that this effect was due to the diffusive loss of the hydrophilic substance 5-fluorouracil from the skin surface into the overlying volume of coupling gel. This phenomenon could be duplicated by the application of conductive heating, indicating that the thermal effects of ultrasound were probably responsible for accelerated 5-fluorouracil diffusion through the gel. *Conclusion:* This study acutely demonstrates how formulation design of the donor vehicle/coupling gel may radically affect therapeutic efficacy in phonophoretic systems. Published by Elsevier Science B.V.

Keywords: Azone; Coupling gel; Enhancement; 5-Fluorouracil; Percutaneous absorption; Phonophoresis

1. Introduction

5-Fluorouracil is a cytotoxic agent used topically on the skin for the treatment of actinic keratosis and various epithelial neoplasmas. As the drug is highly polar (Log $P_{\text{oct/water}} = -0.95$), it permeates relatively slowly through the lipoidal stratum corneum. However, the requirement for improved therapy has brought into use various ways towards increasing 5-fluorouracil absorption. Diverse strategies have included the development of prodrugs (Mollgaard et al., 1982), occlusion (Martindale, 1982), the application of chemical enhancers (Touitou and Abed, 1985; Philips and Michniak, 1995) and ion-

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tophoresis (Welch et al., 1997). Yet another potential approach involves the use of ultrasound to enhance percutaneous absorption—a procedure known as phonophoresis (Singh and Singh, 1990). Although phonophoresis has been used in physiotherapy clinics for almost 40 years, its mode of action and therapeutic effectiveness are still under discussion (Meidan et al., 1995). A comprehensive theory of phonophoresis has not yet emerged, partly because different research teams have employed different ultrasonic parameters, i.e. frequency, intensity, duration and mode; as well as different skin types, drugs, and vehicles. A further problem is that the physiotherapeutic ultrasound generators which are used for phonophoresis studies are often poorly calibrated and the control dial values on the device may not correlate to the actual ultrasound intensities emitted. (Meidan et al., 1995). Confusion has also arisen from the fact that ultrasound can reflect back on itself at an interface between two media exhibiting a large mismatch in acoustic impedance, e.g. a vessel wall–solution interface in vitro. This produces a standing wave which 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. Moreover, during ultrasound propagation, the beam volume begins to expand at a critical distance from the source. The value of this critical distance is related to the ultrasonic wavelength and transducer radius (Williams, 1983).

The objective of this study was to investigate the effect of ultrasound on 5-fluorouracil permeation through full-thickness rat skin in vitro. Significantly, unlike most other phonophoretic studies, the output of the ultrasound source was independently validated and a hydrophone technique was employed to define the ultrasonic field in the vicinity of the skin membrane. Since the primary effect of sonication is heat generation, a heat-only application was devised and its effect on 5-fluorouracil penetration was also investigated. In addition, the effect of the combination treatment of ultrasound with Azone was also examined.

2. Materials and Methods

2.1. Thin-layer Chromatography

Thin-layer chromatography was conducted in order to assess the purity of the [^{3}H]-labelled 5-fluorouracil (NEN) used in this study; a sample of unlabelled 5-fluorouracil (Sigma) was employed for reference purposes. The stationary phase consisted of a 30 mm-wide and 80 mm-long, pre-coated plastic sheet (Merck, 0.2 mm layer thickness), pencil marked into 8 mm-wide divisions. This pre-coated sheet was placed inside a development chamber (Panglass Shandon Chromatatank) filled with a mixture consisting of 60 ml *n*-butanol (BDH), 15 ml 1% v/v aqueous acetic acid (BDH) and 15 ml distilled water. The base of the stationary phase was immersed in the solvent to a depth of 8 mm. A 1 ml volume of either: radiolabelled 5-fluorouracil (66.66 pmol, 0.0326 MBq, NEN); or 'cold' 5-fluorouracil (0.05% w/v); was deposited at a central origin, located 16 mm above the initial level of the solvent. The chamber was covered and the system left for 3 h until the solvent front had diffused to a distance of 64 mm above the origin. The plate was removed from the chamber and dried in a current of warm air. The strip was then cut into 8 mm wide sections and each of these was transferred to a labelled vial, filled with scintillation fluid (10 ml, Optiphase Hisafe 3). The vials were vortexed and their emitted activities were analysed in a scintillation counter (Packard 1900 T.R.). Qualitative analysis of the unlabelled drug was performed by exposing the stationary phase to 254 nm light generated by a UV lamp (Allen 425 LCF 750Q). The region of 5-fluorouracil deposition could be observed by the emission of blue light.

2.2. 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, placed upon the steel mesh in the recess, and secured as a barrier between the two cell compartments. The receptor phase consisted of degassed, distilled water. Degassing was achieved by heating to 30°C and then sonicating for 3 min in an ultrasound bath (Kerry, Pulsatron 125). The receptor cell fluid was maintained at 28°C by a thermostatic water pump (Churchill-Matrix) which circulated water through the jacket surrounding the inner chamber. At time zero, 100 ml of an ethanolic solution containing 5% v/v [^{3}H]5-fluorouracil (333.3 pmol, 0.1628 MBq, NEN) was deposited on the skin surface. For the Azone studies, the skin, whilst mounted in the diffusion cell, was pretreated with 100 ml of 1% v/v Azone in ethanol. This solution was allowed to soak into the skin for a period of 1 h before the subsequent addition of the 5-fluorouracil solution.

One hour after the application of the donor solution, the ethanol had mostly evaporated from the skin surface and the Franz cell was inverted, thus facilitating the removal of any air bubbles from the lower surface of the skin. The donor compartment was then filled with ultrasonic coupling gel (Henleys Medical). Care was taken to minimise the development 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; sonication; or heat-only treatment. Throughout the 5-h experimental period, 1.1 ml aliquots of receptor phase were withdrawn at 30 min intervals and replaced with an equal volume of blank receptor medium. Each 1.1 ml aliquot sampled was vortexed with 10 ml of scintillation fluid (Optiphase Hisafe 3) and then placed in a liquid scintillation counter (Packard 1900 T.R.). The emitted activity value of each aliquot was converted to a 5-fluorouracil concentration value according to the activity/mole ratio of the 5-fluorouracil. By taking into account the cumulative dilution of the receptor phase (Franz, 1975 Meidan et al., 1998a,b), it was possible to calculate the flux, permeability coefficient and lag time values for all the derived data. 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 $J = (dM/dt)/A = DKC_d/h$. 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 $k_p = DK/h = J/C_d$. The lag-time is the time taken for steady-state transport to be reached and depends upon diffusion coefficient and membrane thickness such that $t_L = h^2/6D$.

2.3. Sonication Procedure

An ultrasonic generator (Therasonic 1032, model no. 50, EMS Greenham) and its complementary transducer (frontal surface area of 2.0 cm^2, effective radiating area of 0.7 cm^2) were employed as the ultrasound source for these studies. The device produces continuous 1.1 MHz ultrasonic waves throughout an indicated intensity range 0–2.54 W cm^{-2}. Initially, the acoustic output of the device was validated, across its entire intensity range. This was achieved by employing a non-compensating radiation force technique (Davidson, 1991), utilising Newell's original radiation force meter (Newell, 1963). In the devised sonication system, the transducer probe was placed inside the donor compartment, facing downwards so that its radiating surface was 2 cm above the skin surface. A specially constructed 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}. Ultrasound transmission between the transducer and skin sample was mediated by the coupling gel which filled most of the donor compartment (Meidan et al., 1998b). Since the ultrasound generator was not designed to be continuously operated for 4-h periods, the machine was switched off for a 10 min interval following each 50 min sonication period. The generator is designed so that the transducer face must be free of gel when the ultrasound is being switched on. This allows the transducer to re-calibrate itself. Consequently, at the end of each 10 min rest interlude, the transducer was removed from the diffusion cell, wiped clean of coupling gel residues, recalibrated, re-inserted into the diffusion cell, and switched on.

The sonication experiments were always conducted using the same individual Franz cell so that the beam acoustics within the apparatus remained as reproducible as possible.

2.4. Heat-only application

A heat-only application was devised which would mimic the thermal effects of the 1.1 MHz, 2.25 W cm^{-2} beam without generating any additional effects. This application was mediated by a heating probe that was electrically connected to a variable a.c. supply module (Variac). The probe, which exhibited a frontal surface area of 2.0 cm^2—identical to that of 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 at a distance of 2 cm above the skin surface. By adjusting the voltage dial on the module, it was possible to control the heating of the skin surface. In another variant of the heat-only application, the probe was placed directly on the skin surface with no intervening coupling gel present.

2.5. Ultrasound dosimetry measurements

A bilaminar membrane hydrophone (Marconi Y-337611), which had been pre-calibrated at the National Physical Laboratory, was used for the dosimetry measurements. The hydrophone consists of a central, 1 mm-wide, piezoelectric element, embedded within an annular ring 10 cm in diameter. The sensor was connected via a single-ended amplifier to an oscilloscope (Hameg 60 MHZ, HM605). The oscilloscope time-base was fixed at 1 ms cm^{-1} and the amplitude was set to 10 mV cm^{-1}. Initially, it was planned that measurements would be performed with the hydrophone inserted horizontally through the diffusion interface of the Franz cell. Unfortunately, the protrusion of the Franz cell side-arm prevented such a configuration. However, in each phonophoresis experiment, the skin sample was supported by an underlying steel mesh. As steel exhibits a high ultrasonic reflectivity (Chivers, 1991), much of the incident ultrasound undergoes reflection at this interface and this effect would dominate the ultrasonic field at the skin sample. It was therefore possible to omit the Franz cell altogether and measure the effect of the steel mesh on ultrasound in a tank of water. The transducer was clamped into position at a height of 2 cm directly above the hydrophone. The hydrophone was placed on a stand, inclined at 5° from the horizontal to prevent the partial reflection of any ultrasound back towards the transducer. Sonication was then initiated and intensity measurements were then made both with and without the steel mesh lying over the hydrophone (Meidan et al., 1998b)

2.6. Temperature measurement method

Experiments were performed in order to determine the skin surface thermal profile induced by the sonication and the heat-only applications. For this purpose, a hole was drilled through each Teflon collar, so as to allow the insertion of a thermocouple probe (Digitron Instruments, 3202 type K) right through the donor compartment, thus making contact with the skin surface. This facilitated the taking of temperatures at points on the skin surface approximately midway between the centre and edge of the skin sample. The repeated thrusting of the thermocouple through the donor compartment could possibly compromise the skin barrier or induce the mixing of 5-fluorouracil into the coupling gel. In order to avoid this, the temperature profile produced by each application was determined from separate studies in which the skin membranes were drug-free. In each study the skin surface temperatures were recorded at successive 30 min intervals throughout the 5-h period. In the sonication experiments, the beam was momentarily switched off for the actual recording as otherwise, the thermocouple would heat up as it directly attenuated the ultrasound (Williams, 1983).

2.7. Quantification of 5-fluorouracil in the skin and gel

Initially, it was necessary to determine whether the contact time between the skin sample and the

5-fluorouracil formulation is, in itself, a parameter which affects the scintillation measurements. To this end, nine sections of whole rat skin were placed on plastic supports with the epidermis uppermost. A 100 μl volume of ethanolic solution containing 5% v/v 5-fluorouracil was deposited onto each skin sample. The sections were left undisturbed for a specific time period (15, 60 or 240 min) with each time-span experiment being conducted in triplicate. Each skin sample was then transferred to a labelled scintillation vial and 10 ml of scintillation fluid (Optiphase Hisafe 3) was dispensed into each vial. The vials were vortexed. and the emitted activities were counted in a scintillation counter. For the quantification studies, 5-fluorouracil absorption experiments were carried out in triplicate under sonicated, heat-only and control conditions; samples were not taken during the diffusion period. After 5 h, the Franz cells were disassembled and each skin sample was transferred to a labelled scintillation vial. In addition, the coupling gel was scraped out of each donor cell and these contents were also transferred to a separate set of scintillation vials. After the addition of scintillation fluid, all the vials were vortexed and their emitted activities were counted.

3. Results and discussion

3.1. Acoustic dosimetry within the Franz cell

Radiation force measurements indicated that the control dial values on the ultrasound generator correlated well with the actual ultrasonic intensities emitted by the transducer. Both the general performance and efficiency of the machine were well within the limits set by the British Standards Institute for ultrasonic therapy devices (Pamphlet No. 5724, Section 2.5, 1985). As in previous studies (Meidan et al., 1998a,b), the presence of the steel mesh between the transducer and hydrophone resulted in a large (75%) decrease in the ultrasonic intensity detected at the hydrophone, the remainder of the beam undergoing reflection at the steel mesh–water interface. Importantly, it was also found that if the angle the mesh makes with the horizontal was varied by only a few degrees, the proportion of energy reflected increased. Since the mesh was loosely fitted within its recess and the diffusion cell was inverted during the course of the experiment, the precise mesh orientation would have varied within and between individual phonophoresis experiments. Thus, the steel mesh acts as an unpredictable ultrasound reflector, reflecting at least 75% and probably more of the beam back upwards to produce a complex and unpredictable standing wave field within the region of the skin barrier and coupling gel. Beam divergence, calculated to begin at 1.65 cm from the transducer 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 readings provide an indication of this process.

3.2. Skin surface temperature profiles

Fig. 1 shows the various temperature versus time profiles associated with control conditions, sonication, heat-only with coupling gel (simulating sonication), and heat-only without coupling gel. It can be seen that under control conditions, the skin surface temperature was initially just

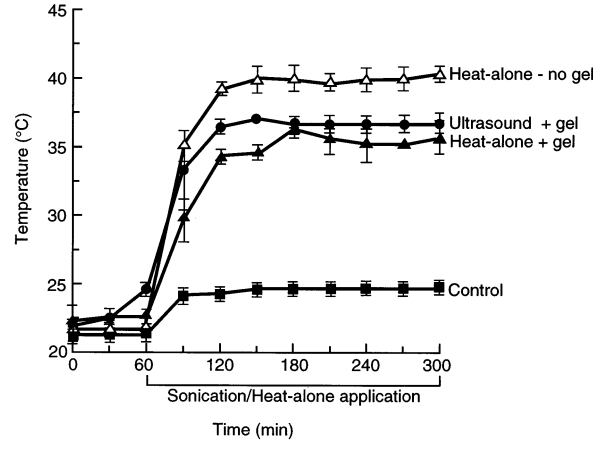

Fig. 1. Skin surface temperature profiles produced by each treatment regimen (■, control, with gel; ●, ultrasonication, with gel; ▲, heat-alone, with gel; △, heat-alone, without gel). Error bars represent S.D., $n = 3$.

above room temperature. However, at 60 min there was a pronounced increase in temperature which reached a plateau at 90 min. This is caused by the deposition of coupling gel which insulates the skin from the cooler air above and thus permits heat from the receptor compartment (jacketed at 28°C) to be more effectively retained within the skin. Ultrasound application at 60 minutes caused the skin temperature to increase, rapidly at first and then gradually less so until an equilibrium value of 37°C was reached at 120 min. From this point onwards, the energy delivered by the transducer was equal to the energy lost from the apparatus by conduction. As a result of the relatively high specific heat capacity exhibited by the coupling gel, switching off the sound for 10 min intervals did not produce erratic behaviour in the temperature plot. Importantly, ultrasound exposure did not elevate skin temperatures to 45°C—a critical point at which the skin barrier becomes irreversibly damaged (Bronaugh et al., 1982).

Fig. 1 also shows the thermal profile obtained by applying a 12 V potential difference to the heating probe. It can be seen that the derived plot resembles the one produced by sonication. However, it must be remembered that while attenuation of ultrasound-generated heat throughout the skin depth, the heat-only regimen induced a more surface-confined effect. A second heat-only application was devised in which the heating probe was placed directly on the skin surface, in the absence of coupling gel. By applying a potential difference of 10 V, it was possible to elevate the skin surface temperature to an equilibrium value of about 40°C.

3.3. Permeation data

Radiometric analysis of the TLC plate indicated that 98.7% of the emitted activity of the chromatoplate was located within a band 40–56 mm from the origin (length of run, 64 mm). A similar activity distribution was found in a repeat run of the experiment. Empirical observations of the distribution pattern of pure 5-fluorouracil showed that this sample was also concentrated in this region of the chromatoplate. These results

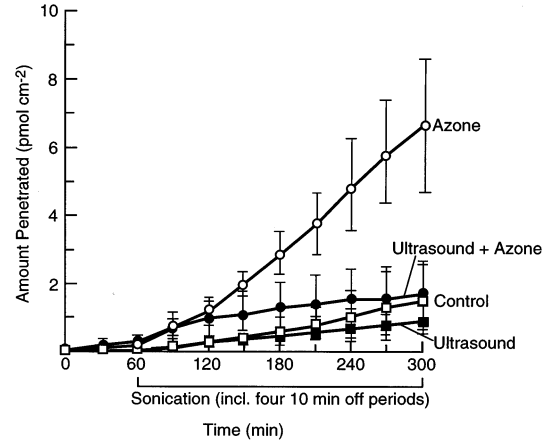

Fig. 2. The effects of ultrasound and Azone on 5-fluorouracil penetration (□, control, $n = 5$; ■, ultrasound; ○, Azone (1%); ●, Azone (1%) plus ultrasound). Error bars represent S.D., $n = 3$ except where stated.

indicate that the available samples of [^{3}H]5-fluorouracil had not degraded. A series of permeation studies were conducted in order to evaluate the effects of ultrasound, heat-alone, and Azone on the percutaneous absorption of 5-fluorouracil. Fig. 2 illustrates the permeation profiles of the treatments: control, ultrasound only, Azone only, and Azone with ultrasound. Under control conditions, 5-fluorouracil absorption can be described in terms of two distinct phases. There is an initial mean lag time of almost 90 min. This is followed by a mean steady-state flux of 0.42 pmol cm^{-2} h^{-1} which is equivalent to a mean permeability coefficient (k_p) of 1.27×10^{-4} cm h^{-1}.

Sonication from 60 min onwards, produced a change in the permeation profile of 5-fluorouracil. The rate curve now exhibited three separate phases. Following the lag time, there followed a linear phase which was of a very similar magnitude to that observed in the control treatment. However, from 120 min onwards, the penetration rate slightly decreased with time. Since 333 pmol of 5-fluorouracil were initially deposited on the skin surface, this effect cannot be due to drug depletion on the donor side. One possibility is that ultrasound is accelerating drug diffusion from the skin surface, into the overlying volume of aqueous gel. The relatively large volume of

available gel ($\gg 6$ cm^3) and hydrophilicity of 5-fluorouracil (Log $P_{\text{octanol/water}} = -0.95$) would tend to facilitate such a process. Consequently, the concentration of 5-fluorouracil at the skin surface progressively declines during the period of ultrasound exposure. This results in the diminution of the thermodynamic driving force necessary for percutaneous absorption.

Following the application of Azone, 5-fluorouracil penetration was characterised by a mean lag-time of over 1 h. This was followed by a linear flux of mean value 1.84 pmol cm^{-2} h^{-1} which is equivalent to an average permeability coefficient (k_p) of 5.51×10^{-4} cm h^{-1}. This indicates that Azone pretreatment enhances 5-fluorouracil penetration by over 4-fold. This effect is mediated by the Azone molecules inserting themselves within the intercellular lipids of the stratum corneum, thus fluidising them. These perturbations are particularly effective at promoting the permeation of hydrophilic agents such as 5-fluorouracil (Sugibayashi et al., 1985). The combination of Azone pretreatment and ultrasound irradiation produced a complex 5-fluorouracil transport curve. Following the lag phase, there was a short period of steady-state flux which was comparable to the flux observed in the Azone alone experiments. However, from 90 min onwards, the permeation rate began to progressively decrease with time. By 240 min, the flux had become negligible. This curve-flattening effect is caused by the loss of permeant from the skin surface. Interestingly, the curve of the combination treatment is approximately parallel to the curve of the ultrasound-only treatment. This suggests that, in both cases, the same ultrasonic mechanism is accelerating 5-fluorouracil diffusion, from the skin surface into the coupling gel.

In order to determine whether ultrasonic heating was promoting the reverse diffusion effect, the effect of Azone with heat-only (to 35°C) on 5-fluorouracil permeation was investigated. It can be seen from Fig. 3 that under these conditions, a plateau effect also developed. A decrease in flux was first observed at 150 min and by 270 min, permeation was negligible. It can be concluded that the thermal effects of ultrasound are producing the back-diffusion effect. In a sequential experiment, Azone-pretreated skin was exposed to heat alone in the absence of coupling gel. From Fig. 3, it can be seen that following a short lag phase, 5-fluorouracil flux remained at a steady state throughout the experiment. Since the drug could not diffuse into an overlying gel layer, sink conditions were maintained for percutaneous absorption. Interestingly, the permeability coefficient in this experiment was lower than in any of the other Azone-exposed experiments. A possible explanation of this effect is that the coupling gel, which is approximately 90% water, is a powerful hydrating agent and will in itself act as an absorption enhancer. No such enhancement occurs in the absence of coupling gel.

Permeant quantification experiments were performed for both the skin and gel layer. Initially, the analytical technique was calibrated in order to ascertain whether the time of contact between the rat skin and 5-fluorouracil solution is a factor which affects the number of counts emitted from the sample vials. As can be seen from Table 1, the contact time between the drug formulation and skin did not affect the measured activity values. Following calibration, permeation experiments were conducted in which the skin samples were subjected to either control conditions, sonication, or heat-only. Fig. 4 presents the radioactivities detected from recovered volumes of coupling gel

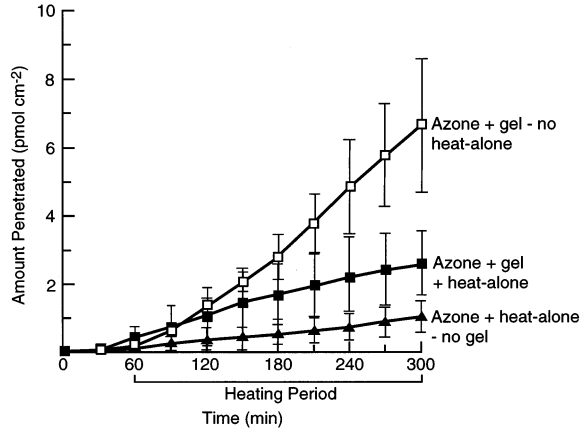

Fig. 3. The effects of Azone and heat-alone on 5-fluorouracil penetration (□, Azone (1%) without heat-alone, with gel; ■, Azone (1%) and heat-alone, with gel; ▲, Azone (1%) and heat-alone, without gel). Error bars represent S.D., $n = 3$.

Table 1
[^{3}H]5-Fluorouracil radioactivity counts in skin as a function of formulation–skin contact time[a]

Absorption time (min)	DPM ± S.D. ($\times 10^{-3}$)
15	846.074 ± 195.278
60	724.311 ± 44.542
240	721.770 ± 166.338

[a] Values represent the mean disintegrations per second ± S.D. (DPM ± S.D.) of three replicates.

following their exposure to each of the three treatments. It can be seen that the heated gel exhibited the highest activity, the sonicated gel exhibited an intermediate level of radioactivity while the control gel exhibited the least activity. The sonication methodology involved the loss of small quantities of gel at the end of each hour, when the transducer was wiped clean and re-calibrated. No such loss of gel occurred in the heat-alone experiments. This probably explains the slightly lower activity in the sonicated gel in comparison to the heated gel. Fig. 5 shows the activities of the skin samples following their exposure to each of the three treatments. It can be seen that the sonicated and heated sections contained significantly less radioactive drug than control skin. Both these experiments show that heating within the donor cell, whether generated directly or via ultrasound attenuation, enhances the back-diffusion of 5-fluorouracil into the overlying gel. This

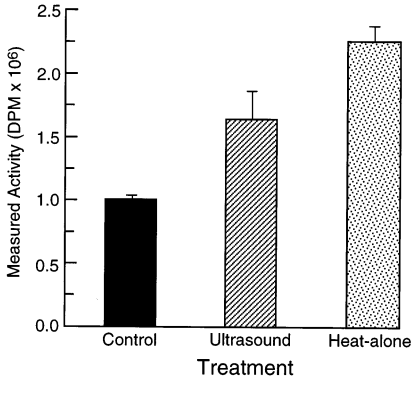

Fig. 4. Radioactivity of the recovered coupling gel following three different 5-fluorouracil exposure regimens. Error bars represent S.D., $n = 3$.

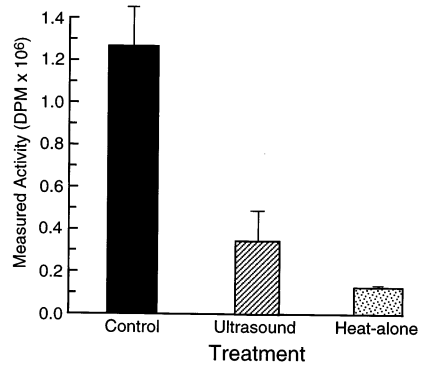

Fig. 5. Radioactivity of the recovered skin samples following three different 5-fluorouracil exposure regimens. Error bars represent S.D., $n = 3$.

back-diffusion process reduces the thermodynamic driving force for 5-fluorouracil permeation.

4. Conclusion

A modified diffusion cell system, in which the skin membrane was subjected to a complex ultrasound standing wave pattern, was devised. This exposure system distributed the sound energy over all of the active skin area as well as the overlying coupling gel. Progressive attenuation of the ultrasound heated the skin and gel although skin surface temperatures remained well within the physiological range. Surprisingly, sonication for 4 h with a 1.1 MHz, 2.25 W cm^{-2} beam produced a reduction in 5-fluorouracil penetration. This effect was caused by the accelerated back-diffusion of 5-fluorouracil from the skin surface into the overlying coupling gel, thus reducing 5-fluorouracil concentration at the skin surface. This phenomenon could be duplicated by the application of a simple heat source, indicating that the thermal effects of ultrasound were responsible for enhanced 5-fluorouracil diffusion into the gel. The coupling gel comprised a macromolecular network containing aqueous regions through which small hydrophilic agents could readily diffuse and heating has been shown to accelerate solute diffusion through such a system (Meidan, 1996). This back-diffusion effect has not been documented in the literature reports as in

most models employed previously, the permeant was homogeneously dispersed within the bulk of the donor vehicle. However, a protocol in which drug deposition is followed by the separate application of coupling gel—as reported here—has been applied by some researchers (Hofmann and Moll, 1993) and is more likely to be used clinically. This study acutely demonstrates how formulation design of the donor vehicle/coupling gel may radically affect therapeutic efficacy in phonophoretic systems.

References

Bronaugh, R., Stewart, R., Congdon, E., 1982. Methods for in vitro percutaneous absorption studies 2. Animal models for human skin. Toxicol. Appl. Pharmacol. 62, 481–488.

Chivers, R.C., 1991. Fundamentals of ultrasonic propagation. In: Preston, R.C. (Ed.), Output Measurements for Medical Ultrasound. Springer, London.

Davidson, F., 1991. Ultrasonic power balances. In: Preston, R.C. (Ed.), Output Measurements for Medical Ultrasound. Springer, London.

Franz, T.J., 1975. Percutaneous absorption. On the relevance of in vitro data. J. Invest. Dermatol. 64, 190–195.

Hofmann, D., Moll, F., 1993. The effect of ultrasound on in vivo penetration of benzyl nicotinate. J. Control. Release 27, 185–192.

Martindale, 1982. The Extra Pharmacopoeia, 28th ed. The Pharmaceutical Press, UK, pp. 209–211.

Meidan V.M., 1996. PhD Thesis, Aston University.

Meidan, V.M., Walmsley, A.D., Irwin, W.J., 1995. Phonophoresis—is it a reality? Int. J. Pharm. 118, 129–149.

Meidan, V.M., Walmsley, A.D., Irwin, W.J., 1998a. Low intensity ultrasound as a probe to elucidate the relative follicular contribution to total transdermal absorption. Pharm. Res. 15, 86–93.

Meidan, V.M., Docker, M.F., Walmsley, A.D., Irwin, W.J., 1998b. Phonophoresis of hydrocortisone with enhancers: an acoustically defined model. Int. J. Pharm. 170, 157–168.

Mollgaard, B., Hoelgaard, A., Bundgaard, H., 1982. Prodrugs as drug delivery systems. 23. Improved dermal delivery of 5-fluorouracil through human skin via n-acyloxymethyl pro-drug derivatives. Int. J. Pharm. 12, 153–162.

Newell, J.A., 1963. A radiation pressure balance for the absolute measurement of ultrasonic power. Phys. Med. Biol. 8, 215–221.

Philips, C.A., Michniak, B.B., 1995. Transdermal delivery of drugs with differing lipophilicities using Azone analogs as dermal penetration enhancers. J. Pharm. Sci. 84, 1427–1433.

Singh, S., Singh, J., 1990. Transdermal delivery of drugs by phonophoresis. A review. Drug Des. Del. 5, 259–265.

Sugibayashi, K., Hosoya, K.I., Morimoto, Y., Higuchi, W.I., 1985. Effect of the absorption enhancer Azone on the transport of 5-fluorouracil across hairless rat skin. J. Pharm. Pharmacol. 37, 578–580.

Touitou, E., Abed, L., 1985. Effect of propylene glycol, Azone and *n*-decylmethylsulphoxide on skin permeation kinetics of 5-fluorouracil. Int. J. Pharm. 27, 89–98.

Welch, M.L., Grabski, W.J., McCollough, M.L., et al., 1997. 5-Fluorouracil iontophoretic therapy for Bowen's disease. J. Am. Acad. Dermatol. 36, 956–958.

Williams, A.R. (Ed.), 1983. Ultrasound: Biological Effects and Potential Hazards. Academic Press, London.