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In vitro phonophoresis of mannitol, oestradiol and hydrocortisone across human and hairless mouse skin

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Abstract

Ultrasound is frequently used in physical medicine. In a preliminary study, we demonstrated that the thermal effect of ultrasound was the principal explanation for the increase in the diffusion rate of digoxin. The aim of this study was to investigate further the phonophoresis of three drugs with a cooling thermostat, thus suppressing the thermal effect of ultrasound. Sonication was carried out at 1.5 W/cm^2, 1.1 MHz for 20 min. We used modified Franz diffusion cells adapted for sonication. During sonication the temperature in the donor compartment was continuously monitored and maintained at 31°C by the cooling coil. Diffusion of the tritiated drugs (hydrocortisone, mannitol, oestradiol) across hairless mouse and whole human skin was determined by liquid scintillation counting for up to 24 h, and the steady-state flux was determined. No enhancement in steady-state diffusion rates was observed for the three drugs in comparison with controls. The thermal effect of ultrasound seems to be the main factor which enhances percutaneous administration under the conditions used in physical medicine.

Keywords: Percutaneous administration; Ultrasound; Human skin; Hairless mouse skin; Phonophoresis; Electron microscopy

1. Introduction

The skin is the target for topical treatment of dermatosis and is an increasingly used route of administration of systemic drugs. Percutaneous administration offers some major advantages:

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zero-order kinetics, avoidance of the gastrointestinal tract responsible for alteration of polypeptides, avoidance of hepatic first-pass effect and better compliance. The limitation of topical and, to a greater degree, systemic delivery is that the skin constitutes a very efficient barrier against the environment and particularly against the penetration of chemicals. The ability of a drug to penetrate the skin is closely related to its molecular weight and its affinity for the stratum corneum. In order to enhance percutaneous administration of drugs, the use of chemical enhancers, iontophoresis and phonophoresis has been proposed.

Phonophoresis of drugs has recently been emphasized (Byl, 1995) using various ultrasound conditions. Very high frequency (15 MHz) (Bommannan et al., 1992), high frequency (1–3 MHz) (Mitragori et al., 1995), low frequency (150 kHz) (Ueda et al., 1996) and very low frequency conditions (20 kHz) (Mitragori et al., 1996) have been used. We have previously investigated the phonophoresis of azidothymidine and digoxin using a therapeutic frequency (1.5 MHz and 3.3 MHz, respectively) and we demonstrated that the suppression of thermal effect with a cooling coil resulted in absence of percutaneous enhancement of azidothymidine (Pelucio-Lopes et al., 1993) and that the thermal effect of ultrasound was the main explanation for enhancement of percutaneous absorption of digoxin (Machet et al., 1996). In this study, we used a cooling coil to refrigerate the donor compartment in order to demonstrate the non-thermal effect of ultrasound at an intensity, frequency, mode and duration commonly used in medicine.

2. Materials and methods

2.1. Drugs

Tritiated mannitol (spec. act. 30 Ci/mmol; purity 99%), tritiated hydrocortisone (spec. act. 49.1 Ci/mmol; purity 99%) and tritiated oestradiol (spec. act. 49 Ci/mmol; purity 99%) were purchased from NEN FRANCE. We performed high pressure liquid chromatography for tritiated oestradiol and hydrocortisone and paper chromatography for mannitol after exposure of all three drugs to ultrasound (3 W/cm^2) for 1 h to check the absence of ultrasound-induced damage.

2.2. Membranes

Female hairless mice (aged 6–7 weeks) were killed by cervical dislocation. The skin from the back was removed and the fat trimmed off. Human skin samples were obtained from freshly excised surgical specimens from abdominal areas and the breast. The subcutaneous fat was trimmed off and whole skin (thickness 1.5–2 mm) was used for the diffusion experiments. When skin was not used immediately, it was stored at -20°C and used in less than three months (Bronaugh et al., 1986). Skin membranes were mounted in the receiving compartment and allowed to reach equilibrium for 10 h.

2.3. Diffusion cells

Permeation experiments were performed with modified Franz diffusion cells which allowed the introduction of an ultrasound probe into the donor compartment and thermoregulation in the donor compartment (Pelucio-Lopes et al., 1993). The donor compartment (6 ml volume) was filled with 3 ml of normal saline (0.9%) containing 6 μCi/ml hydrocortisone and 3 μCi/ml oestradiol to test hydrocortisone and oestradiol diffusion and 5 μCi/ml tritiated mannitol was added to a saturated solution of unlabelled mannitol to test mannitol diffusion. The receiving medium (volume 15 ml) was composed of 60% saline solution, 20% polyethyleneglycol 400 (PEG 400) and 20% ethanol for hydrocortisone and oestradiol and normal saline for mannitol. The contents of the receptor were degassed before each experiment and then mixed with a magnetic stirring bar which was driven by an external magnetic stirrer at a controlled speed (300 rpm). Water was pumped through the jacketed receiving compartment at a constant temperature (32°C) in order to warm the receptor solution.

2.4. Ultrasound conditions

Ultrasound (US) was generated by a ceramic transducer (15 mm diameter plane disk, lead titanate zirconate ceramic P-189 Quartz and Silice CO.) with a power output of 90% and area of 1.77 cm^2. The transducer was introduced to the donor compartment solution at a distance of about 3 mm from the skin. Continuous mode ultrasound was applied for the first 20 min of the diffusion assay at an intensity of 1.5 W/cm^2 and a frequency of 1 MHz (Hewlett Packard 3314 A). The power was controlled by a Rhode and Schwarz wattmeter. The temperature was recorded continuously in the donor solution-surface membrane interface region during sonication using a fine rigid wire thermocouple probe linked to a digital thermometer. The temperature in the receptor compartment was not recorded.

2.5. Diffusion protocol

Each diffusion experiment was performed for up to 48 h with human skin and 25 h with hairless mouse skin. At regular intervals of time 500-μl samples were taken from the sampling port in the receiving compartment. The same volume was replaced with initial receiving solution to maintain a constant volume and the replacement dilution effects were corrected for in the assay calculations. Diffusion rates or flux (J) were determined from the slope of diffusion curves and expressed as the amount of drug passing a square centimeter of skin surface per hour ($pg/cm^2/h$). The permeability coefficient (K_p) was determined by the equation $K_p = J/C_o$, where C_o was the initial concentration in the donor compartment.

2.6. Counting method

Each sample was placed in scintillation vials filled with 7 ml Ophti-phase Hifa scintillation liquid. Radioactivity was determined in a RACK B LKB 1217 liquid scintillation counter. Quenching phenomenon was evaluated using an internal standard and efficiency was determined for each drug (38.5%, 38.2%, and 39.3% for hydrocortisone, mannitol and oestradiol, respectively).

2.7. Histology and electron microscopy of skin

The effects of ultrasound on skin were analyzed by light and electron microscopy. In order to eliminate the artifacts caused by freezing and duration of the experiment, a specimen was taken immediately after trimming off the fat and was fixed in Bouin's solution for light microscopy and in a paraformaldehyde/glutaraldehyde solution for electron microscopy. Another skin sample of the same origin was mounted on a diffusion cell and was sonicated at 1.5 W/cm^2 for 20 min. A further sample was taken after sonication and then fixed as previously described.

3. Results

3.1. Thermal variations

Without a cooling coil the temperature gradually increased to 42°C in 20 min and the temperature was maintained between 29 and 31°C during phonophoresis experiments.

3.2. Diffusion kinetics

No significant increase in mean diffusion flux was observed with any of the three drugs. In vitro diffusion fluxes and permeability coefficients (K_p) of mannitol, hydrocortisone and oestradiol are expressed in Tables 1 and 2.

3.3. Histologic and electron microscopy

Histologic examination showed no difference between control and sonicated skin. Scanning electron microscopy revealed holes at the surface of corneocytes of sonicated skin (Fig. 1). Transmission electron microscopy showed no cellular abnormalities and well-preserved intercellular spaces without signs of intercellular disruption (Fig. 2).

Table 1
In vitro percutaneous diffusion across hairless mice skin[a]

	Mean flux ± S.E.M. (pg/cm^2/h)		K_p ± S.E.M. (10^{-3} cm/h)	
	Control	Ultrasound	Control	Ultrasound
Hydrocortisone	40.4 ± 7.2 ($n = 5$)	46.8 ± 4.6 ($n = 5$)	0.9 ± 0.2	1.1 ± 0.1
Mannitol	27.1 ± 5.5 ($n = 9$)	44.0 ± 10.6 ($n = 5$)	0.9 ± 0.2	1.4 ± 0.3
Oestradiol	1206 ± 179 ($n = 9$)	1156 ± 144 ($n = 10$)	75 ± 11	72 ± 9

[a] Mean steady state flux was calculated over the first 12 h with oestradiol, and over 24 h with mannitol and hydrocortisone.

4. Discussion

Ultrasound has been used widely in physical medicine for many years either alone or associated with various anaesthetic or anti-inflammatory agents (Byl, 1995). The possible mechanisms of ultrasonically enhanced transdermal drug delivery are numerous: temperature increase, reduction in the boundary layer thickness, radiation pressure, decrease in the donor solution-membrane interface potential energy barrier and cavitation (Simonin, 1995). The cavitation phenomenon results from the creation of bubbles which can collapse violently (Simonin, 1995). The cavitation threshold increases with ultrasound frequency, and this is easily demonstrated in cells in suspension within fluids. Some experiments have supported the possible occurrence of cavitation within the stratum corneum (Mitragori et al., 1995) but this phenomenon has never been demonstrated. One other study of phonophoresis in electron microscopy has been reported (Menon et al., 1994). They observed large clefts within intercellular spaces of the stratum corneum, but ultrasound conditions (15 MHz, 0.1 W/cm^2) were below the cavitation threshold. In this study, scanning electron microscopy showed holes at the surface of the stratum corneum. Though the interpretation of such holes remains uncertain, they could be the consequence of cavitation occurring from microbubbles at the interface area with the stratum corneum. This does not imply that cavitation occurred within the stratum corneum or deeper in the epidermis and no such images were seen with transmission electron microscopy.

Another important parameter in phonophoresis is heating. The increase in temperature depends on the acoustic frequency, intensity, duration of sonication, and the thermal characteristics of the medium. Brucks et al. (1989) demonstrated that ultrasound applied for 30 min at an intensity of 1 W/cm^2 and a frequency of 1 MHz increased temperature by 11°C, despite the use of a cooling coil. The diffusion rate was increased during sonication. Mitragori et al. (1996) reported a 7°C increase in the donor compartment during the 30 first min of exposure to ultrasound (continuous mode, 2 W/cm^2, 1 MHz). In many other studies there was no measurement of temperature, nor use of a cooling system in the donor compartment. In a previous study, we demonstrated that ultrasound (continuous mode, 3 W/cm^2, 3.3 MHz, 10 min) increased temperature in the donor compartment (maximum 60°C) and increased percutaneous absorption of digoxin in a similar way to heating with electric resistance (Machet et al., 1996). In this study, a preliminary assay without a cooling coil demonstrated that the temperature increased to 42°C, but this increase was subsequently well controlled using a cooling coil during diffusion assays. No significant enhancement of percutaneous absorption of mannitol, hydrocortisone and estradiol occurred using high frequency ultrasound in these conditions (1.5 MHz for 20 min). We have previously studied phonophoresis of azidothymidine (1.1 MHz, 1.5 W/cm^2, 20 min) with a cooling coil and we failed to show any difference from controls (Pelucio-Lopes et al., 1993). Thus, our in vitro studies did not demon-

Table 2
In vitro percutaneous diffusion across human skin[a]

	Mean flux ± S.E.M. (pg/cm^2/h)		K_p ± S.E.M. (10^{-3} cm/h)	
	Control	Ultrasound	Control	Ultrasound
Hydrocortisone	3.0 ± 0.6 ($n = 5$)	3.5 ± 1.3 ($n = 5$)	0.06 ± 0.01	0.08 ± 0.03
Mannitol	0.50 ± 0.15 ($n = 4$)	0.40 ± 0.15 ($n = 4$)	0.016 ± 0.005	0.013 ± 0.005
Oestradiol	128 ± 31 ($n = 5$)	114 ± 36 ($n = 5$)	8 ± 2	7 ± 2

[a] Mean steady state flux was calculated over the first 24 h with oestradiol, mannitol and hydrocortisone.

strate effectiveness of ultrasound when applied for a short time (10–20 min) for the enhancement of percutaneous diffusion of drugs when the temperature is controlled. This is reinforced by some controlled in vivo studies which failed to demonstrate any valuable effect of commonly used therapeutic ultrasound (1–3 MHz, 0.25–1 W/cm^2) (Benson et al., 1989; Williams, 1990).

However, this does not imply that phonophoresis is ineffective. First the optimal duration of exposure of the skin to ultrasound is not known. Mitragori et al. (1995) showed a 13-fold in vitro enhancement in the percutaneous diffusion of oestradiol when ultrasound (continuous mode, 1 MHz, 2 W/cm^2) was applied for 24 h. However, the increase was less intense with testosterone and corticosterone (5- and 4-fold, respectively) and was even absent with benzene, butanol, caffeine, and progesterone. Secondly, the use of low frequency ultrasound (20 KHz), which facilitates the occurrence of cavitation, seems to be very promising, since percutaneous flux enhancement varies from 3 to 5000 (Mitragori et al., 1996).

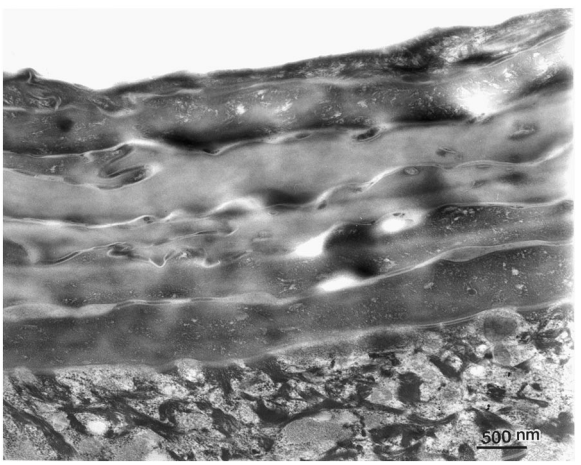

Fig. 2. Transmission electron microscopy: stratum corneum has a normal appearance.

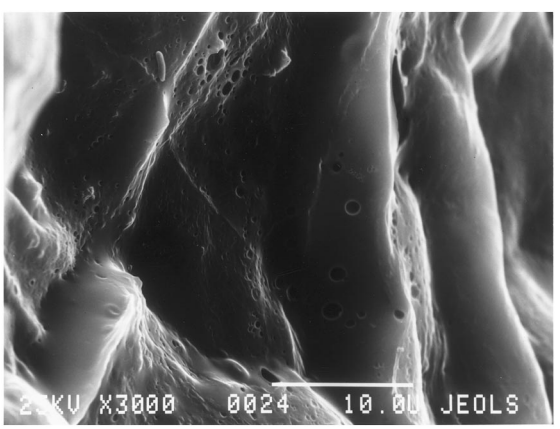

Fig. 1. Scanning electron microscopy: numerous holes (1–3 mm diameter) can be seen at the surface of sonicated skin.

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