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In vitro phonophoresis of digoxin across hairless mice and human skin: thermal effect of ultrasound

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Abstract

The phonophoresis of digoxin was studied in vitro through human and hairless mouse skin. Sonication was carried out with continuous mode at an intensity of 1 and 3 W/cm^2 and a frequency of 3.3 MHz for 10 min. Sonication at 3W/cm^2 significantly increased the absorption of digoxin through mouse skin. Percutaneous penetration was not increased using an intensity of 1W/cm^2 under the same experimental conditions. Enhanced digoxin penetration at 3W/cm^2 can be explained by the mechanical and/or thermal action of ultrasound waves. Thermal simulation from electrical resistance increased digoxin flux in comparable amounts to those obtained by sonication at 3 W/cm^2. There was no enhancement of digoxin absorption across human skin by ultrasound, probably due to dermal retention of this lipophilic drug. Further studies will be necessary to determine the relative importance of the thermal effects of ultrasound on percutaneous administration of drugs.

Keywords: Percutaneous administration; Digoxin; Ultrasound; Human skin; hairless mouse skin; Phonophoresis

1. Introduction

Though percutaneous administration of drugs offers many advantages (zero-order plasma kinetics, avoidance of first-pass effect, better compliance), only a few drugs are currently available for transdermal application. This is due to the low permeability of the skin to many molecules. Enhancing the delivery rate of molecules across skin in order to attain significant therapeutic level is therefore a great challenge. Several approaches have been used: chemical enhancers, iontophoresis and ultrasound.

Ultrasound has been widely used in physical medicine, alone or associated with various anti-inflammatory agents, but controlled studies are lacking. The ultrasound parameters currently used are a range of 0.5 to 5 MHz for frequency and between 0.1 to 3 W/cm^2 for intensity (McElnay et al., 1993).

Phonophoresis (or sonophoresis) has been defined as the migration of drugs through the skin under the influence of ultrasound. Studies have

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been performed with various drugs (Tyle and Agrawala, 1989), using different devices and different ultrasound conditions in terms of intensity, frequency (Bommannan et al., 1992a; Mitragotri et al., 1995), duration and continuous or discontinuous mode.

In a previous work (Machet et al., 1991), we investigated the percutaneous administration of digoxin without ultrasound. The percutaneous fluxes were not sufficient, despite the use of a chemical enhancer, dipropylene glycol. The aim of this study was to quantify the ultrasound effects on the diffusion rate of tritiated digoxin in vitro through excised human skin and hairless mouse skin.

2. Materials and methods

2.1. Molecule

[12α-^{3}H (N)] Digoxin (spec. act. 10 Ci/mmol; purity 99%) was purchased from NEN FRANCE. In order to check that the molecules of digoxin and the digoxin-tritium link had not been damaged by ultrasound, we performed high pressure liquid chromatography of a donor solution which had been treated for 1 h at an intensity of 5 W/cm^2 and a frequency of 3.3 MHz. This control test indicated no ultrasound-induced ^{3}H-digoxin degradation.

2.2. Membranes

Female hairless mice, (age: 6–7 weeks) were killed by cervical dislocation. The skin from the back was removed and fat removed. 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 was used for the diffusion experiments. When skin was not used immediately, it was stored at -20°C and used in less than 3 months. Skin membranes were mounted in the receiving compartment and alowed to reach equilibrium for 10 h.

2.3. Diffusion cells

Permeation experiments were performed with specially modified Franz diffusion cells which allowed the introduction of an ultrasound probe into the donor compartment. The length of donor compartment was 2.4 cm with an inside diameter of 2.5 cm and an outside diameter of 4 cm. The total membrane area available for diffusion was 2 cm^2. The donor compartment was filled with normal saline (0.9%) containing 5 μCi of tritiated digoxin per ml. The receiving medium (15 ml) was composed of 60% saline solution, 20% polyethyleneglycol 400 (PEG 400) and 20% ethanol. The content of the receptor was degassed before each experiment. It was 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 constant temperature (37°C) in order to warm the receptor solution.

2.4. Ultrasound conditions

Ultrasound (US) was generated by a 15-mm diameter ceramic transducer (lead titanate zirconate ceramic P-189 Quartz and Silice CO.) with an output of 90% and area of 1.77 cm^2. Continous mode ultrasound was applied for 10 min at an intensity of 1, 2 or 3 W/cm^2 and a frequency of 3.3MHz (Hewlett Packard 3314 A). The power was controlled by a Rhode and Schwarz wattmeter.

The temperature was recorded continuously during sonication using a fine rigid wire thermocouple probe and a digital thermometer; the measurement was taken in the donor solution-surface membrane interfacial region.

2.5. Diffusion protocol

Each diffusion experiment was carried out over 48 h. 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

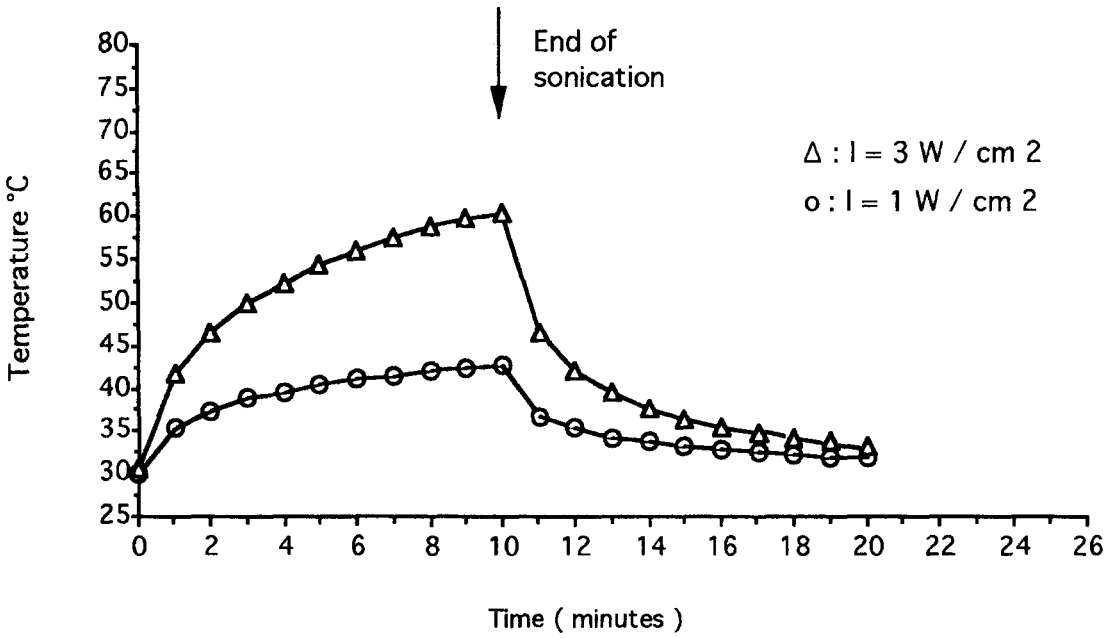

Fig. 1. Increase in temperature in donor cell compartment during sonication.

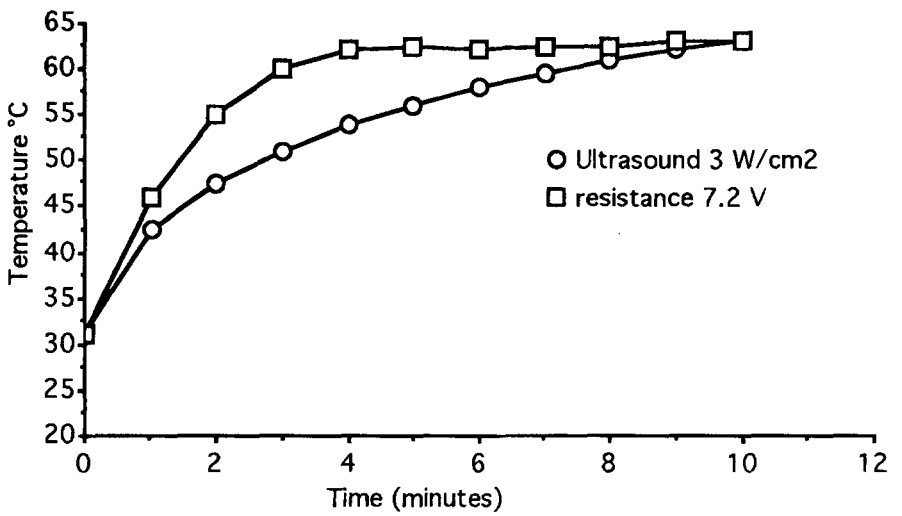

Fig. 2. Increase in temperature with ultrasound and electrical resistance.

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 (Kp) was determined by the equation Kp = J/Co where Co is the initial concentration in the donor compartment.

2.6. Counting method

Each sample was placed in scintillation vials filled with 7 ml of Ophti-phase Hifa scintillation liquid. Radioactivity was determined in a RACK B LKB 1217 liquid scintillation counter (35% efficiency). We sought a quenching phenomenon using an internal standard. We found that the

efficiency was 92%, showing that the quenching phenomenon in the receiving solution is very limited. Knowing that the counter efficiency for tritium is of 35%, we obtained the total efficiency of 32%.

2.7. Histology and electron microscopy of skin

The effects of ultrasound on skin were analyzed by electron and light microscopy. In order to eliminate the artefacts caused by freezing and duration of the experiment, we adopted the following protocol: a biopsy was immediately performed after trimming off the fat and 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 W/cm^2 or 3 W/cm^2 for 10 min. After sonication, a biopsy was performed and then fixed as previously described. Hairless mice skin was also analysed by the same procedure. Histological control of heated skins with electrical resistance was also carried out.

2.8. Thermal modelisation

In order to determine whether the thermal action of ultrasound was solely responsible for the increased digoxin flux, we heated the donor solution using an electrical resistance of 10 Ohms. The resistance was placed in the solution of digoxin for 10 min and adjusted in order to obtain a thermal variation which was comparable to that recorded during sonication.

2.9. Statistical analysis

Values of flux and permeability coefficients were expressed as mean $\pm$ SD. A Student's t-test was used for statistical analysis.

3. Results

3.1. Thermal variations

The increase in temperature induced by US is shown (Fig. 1). The increase in temperature induced by electrical resistance was comparable (Fig. 2).

3.2. Diffusion kinetics

3.2.1. Hairless mouse skin

Table 1 summarizes the mean values of diffusion flux and permeability coefficients for each experimental condition. No significant difference was found between control and experimental diffusion cells treated at 1 W/cm^2 or 2 W/cm^2. However, treatment at 3 W/cm^2 significantly increases the absorption of digoxin across mouse skin ($P < 0.01$). However, treatment by an electrical resistance in similar thermal conditions increased the percutaneous flux of digoxin in similar proportions (Fig. 3).

3.2.2. Human skin

Mean diffusion kinetics across human skin are given in Fig. 4 for each experimental condition. Table 2 summarizes the mean values of diffusion flux and permeability coefficients across human skin. There was no significant difference between ultrasound-treated and control skin.

3.3. Histology and electron microscopy of skin

Both hairless mice and human skin showed epidermal and dermal alterations in comparison with controls. Light microscopy study revealed considerable keratinocyte necrosis, in some cases dermal epidermal bullae and densification of dermal structure. Ultrastructural alteration consisted of intracytoplasmic changes especially for mitochondria, nuclear damage, and changes in collagen fibres in the dermis.

4. Discussion

We demonstrated in this study that ultrasound significantly enhanced the diffusion rate of digoxin across hairless mice skin in vitro. The

Table 1
In vitro percutaneous diffusion of digoxin across hairless mice skin: mean fluxes and permeability coefficents

		Mean flux ± SEM (pg/cm²/h)	Mean K_p ± SEM (10^{-3} cm/h)
Control (n = 8)		233 ± 40	0.60 ± 0.10
Ultrasound	1. W/cm² (n = 6)	255 ± 21	0.66 ± 0.05
	2. W/cm²(n = 6)	269 ± 33	0.69 ± 0.09
	3. W/cm² (n = 8)	556 ± 84	1.43 ± 0.22
Heating (n = 7)	7.2 V	601 ± 111	1.54 ± 0.28

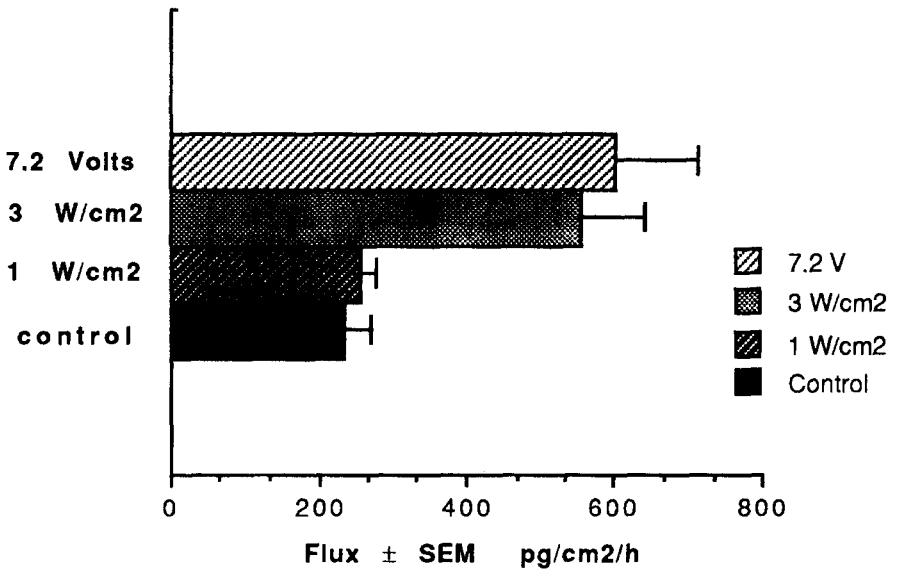

Fig. 3. Diffusion of digoxin in vitro across hairless mice skin.

increase in diffusion rate was observed across hairless mice skin and not across human skin, despite the presence of morphological and histological changes both in human and mice skins. The permeability coefficient of digoxin across hairless mice skin was 36 times greater than that measured across human skin (Machet et al., 1991). We think that the dermal retention of digoxin in vitro could explain the discrepancies in percutaneous flux across human skin, and could mask enhancement of percutaneous transport due to ultrasound.

The possible mechanisms of ultrasonically enhanced transdermal drug delivery are numerous (Bommannan et al., 1992b). Ultrasound causes mechanical disturbance in the absorbing medium. As ultrasound propagates through a medium, some of its energy is absorbed and converted into heat. The increase in temperature depends on the acoustic frequency, intensity, duration of sonication and thermal characteristics of the medium. Paradoxically, temperature in the donor compartment has rarely been controlled in the literature. Brucks et al. (1989) demonstated that ultrasound applied for 30 min with an intensity of 1W/cm² and frequency of 1MHz increased temperature by 11°C, despite the use of a cooling coil. The diffusion rate was increased during sonication. In many other studies, there was no measurment of temperature, nor use of cooling system in the donor compartment. In the present study, we have shown that ultrasound increases the temperature in donor compartment.

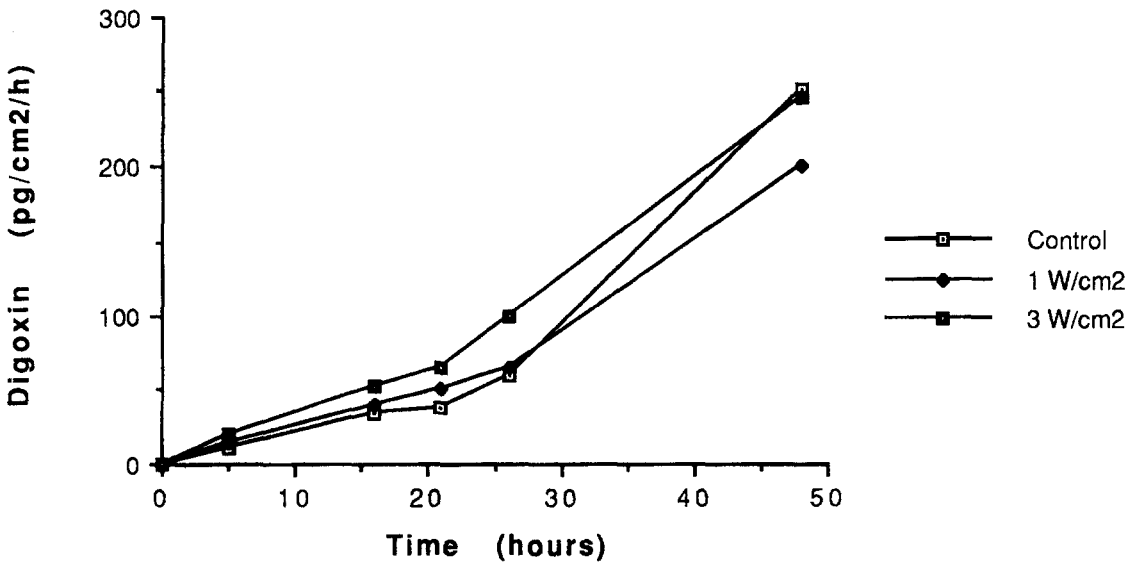

Fig. 4. Diffusion kinetics of digoxin in vitro across human skin.

Table 2
In vitro percutaneous diffusion of digoxin across whole human skin: mean fluxes and permeability coefficients

		Mean flux ± SEM (pg/cm²/h)	Mean K_p ± SEM (10^{-6} cm/h)
Control (n = 9)		6.2 ± 0.7	16 ± 2
Ultrasound	1 W/cm² (n = 6)	5.2 ± 0.6	13 ± 2
	3 W/cm² (n = 7)	5.2 ± 0.5	13 ± 1

In our study, overt histological and ultrastructural damage was observed in the epidermis and dermis. This damage could be due to the increase in temperature in the donor compartment, since histological changes after expsure to electrical resistance. Nevertheless, these changes were less intense and more superficial than those observed after exposure to ultrasound. The depth of penetration of ultrasound within the epidermis or demis could explain this difference. Another phenomenon called cavitation could explain these cellular lesions. Cavitation consists of creation and subsequent collapse of microbubbles from dissolved gas. It is a very energetic phenomenon which can create cellular damage to cells in suspension; this damage is thought to be reversible. Nevertheless it is not known whether cavitation could occur in biological tissues. However, using high frequency ultrasound (15 MHz) in vivo, Menon et al. (1994) showed evidence of lacunae in corneocytes possibly due to cavitation; these lacunae disappeared 48 h after sonication.

The stratum corneum comprises the main resistance to diffusion across the skin. Both exposure to ultrasound and electrical resistance result in a comparable increase in diffusion rate of digoxin. The thermal effect of ultrasound seems to be predominant in enhancing percutaneous diffusion of digoxin in vitro, by comparison with other effects of ultrasound. Because of the considerable increase in temperature and the major cellular changes that we observed in our study, it seems preferable to use a cooling system.

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