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Mini review

Phonophoresis: efficiency, mechanisms and skin tolerance

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Abstract

Phonophoresis or sonophoresis is the use of ultrasound to increase percutaneous absorption of a drug. The technique has been widely used in sports medicine since the sixties. Controlled studies in humans in vivo have demonstrated absence or mild effects of the technique with the parameters currently used (frequency 1–3 MHz, intensity 1–2 W/cm^2, duration 5–10 mins, continuous or pulse mode). However, it was demonstrated in 1995 that administration of macromolecules with conserved biological activity was feasible in animals in vivo using low frequency ultrasound. This led to new research into this method of transdermal administration. The aim of this review is to present the main findings published with low frequency and high frequency ultrasound over the last ten years, and to discuss the respective roles of thermal, cavitational and non-cavitational effects on the reduction of the skin barrier. Particular attention is paid to the biological effects on living skin which might be of importance for tolerance and practical use in humans.

Keywords: Administration; Cutaneous; Biological transport; Permeability; Pharmacology; Ultrasonics

1. Introduction

The skin provides an essential barrier function, effectively limiting exchanges with the external environment and penetration of exogenous compounds. Nevertheless, the skin is also a general route administration of drugs whose target is extra-cutaneous (oestradiol, nitroglycerin, fentanyl, etc). Many other drugs could be administrated by this route because percutaneous administration offers many advantages including 1) suppression of first hepatic pass effect 2) stable plasma levels, and 3) absence of degradation by the digestive tract (e.g. polypeptides such as insulin). Making a drug of high molecular weight penetrate is quite impossible with conventional chemical enhancers. The creation of wide pathways through the epidermis is therefore the aim of certain techniques such as electroporation (Lombry et al., 2000), if possible without permanent damage to the skin (Vanbever et al., 1998).

The use of ultrasound as a physical enhancer since the 1950's in sports medicine, a method referred to as sonophoresis or phonophoresis, has been generalized without preliminary studies to show real increase in transdermal transport (Byl,

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1995). In vitro studies carried out since the 1980's have demonstrated increased percutaneous absorption of various drugs. However conflicting findings have been published showing slight effects or even the absence of effect on skin permeability. This was reviewed under the provocative title: Phonophoresis— is it a reality? (Meidan et al., 1995). More recently, the possibility of rendering the skin permeable to large molecules such as insulin (Tachibana and Tachibana, 1991; Mitragotri et al., 1995a; Boucaud et al., 2002a) or low molecular weight heparin (Mitragotri and Kost, 2001) has raised interest in the use of ultrasound to permeate the skin.

The aim of this review is to present the main pharmacokinetic findings and the possible mechanisms responsible for increasing skin permeability and to draw attention to the remaining problems in terms of skin tolerance and applicability in humans.

2. Physical characteristics of ultrasound

Ultrasound is produced by a transducer composed of a piezoelectric crystal which converts electric energy into mechanical energy in the form of oscillations which generate acoustic waves. These waves are partially reflected by the medium in which they are propagated, the other part penetrates and propagates into the medium. During its propagation, a wave is partially scattered and absorbed by the medium, resulting in attenuation of the emitted wave; the lost energy is converted into heat.

2.1. The frequency

The frequency F of an emitted wave depends on the size of the crystal. It is by definition higher than 20 kHz. Attenuation of an acoustic wave is inversely proportional to its frequency, and thus the more the frequency increases, the less deeply the ultrasound penetrates into and under the skin. High frequencies (1–3 MHz) were first investigated as physical enhancers for transdermal delivery of drugs (Pottenger and Karalfa, 1989; Byl, 1995). These high frequencies are still used in current treatments. Because the outer layer of epidermis, the stratum corneum, is the main barrier to percutaneous penetration of drugs it initially seemed logical to concentrate the ultrasonic energy on this skin layer using very high frequency (10–20 MHz) (Bommannan et al., 1992a,b). The use of low frequency ultrasound (20–150 kHz) was recently shown to be more effective in enhancing transdermal transport (Tachibana and Tachibana 1991; Tachibana, 1992; Mitragotri et al., 1995a; Ueda et al., 1995).

2.2. Mode

Ultrasound waves can be emitted continuously (continuous mode) or in a sequential mode, e.g. 0.1 s applied every second (discontinuous or pulsed mode). The rise in temperature is faster and more intense with the continuous mode

2.3. Intensity

The intensity I is directly dependent on the acoustic energy (E) emitted and the speed of sound (c) in the medium: $I = cE$. Energy E is itself dependent on the density of the propagation medium R, on the total pressure p and on the speed of sound (equal to the sum of the atmospheric pressure and the pressure created by the ultrasound wave): $E = p^2/rc^2$ The intensities usually employed lie between 0.5 and 2 W/cm^2. The increase in pressure is approximately 0.2 bar with 1 W/cm^2 in water (Simonin, 1995).

3. Does ultrasound enhance percutaneous absorption?

This question was previously addressed in a general review (Meidan et al., 1995). The conditions of sonication vary between the published studies, thus explaining the disparity of the results. The conclusion was that phonophoresis does exist, but a need for further research in order to clarify the mechanisms and optimize the process was emphasized. It has been clear since 1995 that frequency is a major parameter in phonophoresis since interesting results were re-

ported with 20 kHz ultrasound (Mitragotri et al., 1995a). It was also demonstrated that greater enhancement ratios were obtained in vitro with various molecules using separated epidermis. Moreover significant in vivo transdermal permeation was confirmed across hairless rat skin for large molecules such as insulin (Mitragotri et al., 1995a) and low molecular weight heparin (Mitragotri, 2001). Results from in vivo studies appear in Tables 1 and 2 and from in vitro studies in Table 3.

3.1. High frequency phonophoresis (1–3 MHz)

The first studies involved corticosteroids (Griffin and Touchstone, 1963) and anaesthetics (Novak, 1964), and the technique became very popular in the United States. An investigation carried out in 52 military hospitals showed that 45 hospitals used phonophoresis (Pottenger and Karalfa, 1989). Ultrasound was used in sports medicine at frequencies ranging from 0.7 to 1.1 MHz, with intensities from 1 to 2 W/cm^2, in continuous or pulse mode (Byl, 1995). The principal criterion was the subjective reduction of pain, but controlled studies did not show any difference from controls (McElnay et al., 1985). Studies in the last decade have been performed in vitro to quantify the increase in cutaneous permeability induced by ultrasound. Differences between studies were related to ultrasound conditions (continuous or pulse mode, duration, intensity), the presence or absence of a cooling system, and the processing of the membranes used (epidermis alone, controlled skin thickness using a dermatome, or full thickness skin). It was been concluded that the increase in percutaneous flux remains moderate (enhanced-ratio generally between 1 and 5) when ultrasound is applied for a short time (5 to 15 mins) (Meidan et al., 1995; Meidan et al., 1998a,b). Increasing the exposure time from 10 to 60 mins results in a 2- to 5-fold increase in transdermal diffusion of prednisolone in vitro with 1 MHz ultrasound, 4.3 W/ cm^2, and continuous mode (Hikima et al., 1998). Interestingly in a later study, no ultrasound-induced transdermal transport was demonstrated after removing the stratum corneum. With longer exposure of skin to ultrasound (up to 20 h) providing periodic replacement of donor and receptor compartment solution to ensure sufficient dissolved gas concentration and using isolated epidermal sheets, permeability may be increased from 1 to 13 (Mitragotri et al., 1995b; Johnson et al., 1996). This is due to increased diffusitivity of the drug within stratum corneum rather than an increase in partition coefficient (Mitragotri, 2001).

3.2. Low frequency sonophoresis: (20–100 kHz)

This range of frequency has been studied more recently in vitro and in some species in vivo.

In vitro low frequency sonophoresis using a 20 kHz probe showed increased transdermal diffusion of various molecules in vitro. Diffusion of low molecular weight molecules was increased by 2–5000 across isolated epidermis in vitro (Mitragotri et al., 1996). A synergistic action of chemical enhancers has been shown with low molecular weight molecules (Mitragotri et al., 2000a). Although statistically significant, the enhancement ratio remains relatively low in some in vitro studies performed on mice skin (Fang et al., 1999), especially for hydrophilic drugs (Monti et al., 2001). Using 350 mm thickness human dermatomed skin including the epidermis and upper dermis, a significant but moderate increase was also demonstrated for caffeine and fentanyl, the enhancement ratio being four and 34 during sonication, respectively (Boucaud et al., 2001a).

In vivo Tachibana was the first to demonstrate transdermal diffusion of insulin using hairless mice exposed to 48 kHz ultrasound applied for 5 mins in vivo, resulting in a marked decrease ($\sim$ 80%) in glycaemia (Tachibana and Tachibana, 1991). In hairless mice and in humans, reverse skin permeability of glucose measured after 20 kHz ultrasound exposure was 100-fold enhanced compared to controls (Mitragotri et al., 2000b; Kost et al., 2000). The increase was even more spectacular with large molecules such as insulin (Mitragotri et al., 1995a) and low-molecular weight heparin (Mitragotri and Kost, 2001) and, due to the effect of ultrasound on mice skin permeability, demonstrated biological activity of the applied drug on skin in vivo, i.e a decrease in

Table 1
In vivo studies in animals

Author, year	F (kHz)	I (W/cm^2)	Mode	Duration (min)	Molecule	Animal	Effect
Griffin and Touchstone, 1963	1000	1–3	C	5	Cortisol	Swine	Intramuscular concentration $\times 3$
Levy et al., 1989	1000 1000	1.5 3	C P	3 5	D-mannitol	Rats	Increased diffusion $\times$ 5-20
Bommannan et al., 1992a	2000 10 000 16 000	0.2	C	20	Salicylic acid	Guinea pigs	Urinary excretion increased at 10 MHz ($\times$ 4) and 16 MHz ($\times$ 2.5) but not at 2
Tachibana and Tachibana, 1993	48	0.17	C	5	Lidocaïne	Mice	Increased pain threshold
Vyas et al., 1995	20 000	3	P	15	Diclofénac	Rats	Reduction in provoked paw oedema
Mitragotri et al., 1995a	20	0.225	P	60	Insuline	Rats	Marked decrease in blood glucose levels
Mitragotri et al., 1996	20	0.125	P	300	Salicylic acid	rats	Flux $\times$ 300
Asano et al., 1997	1000	1–2.5	P C	10–19 10	Indomethacin	rats	Mild increase in blood concentrations
Mitragotri and Kost, 2000	20	7	P	2	Mannitol Inulin Glucose	Rats	$\times$ 33 enhancement $\times$ 20 enhancement $\times$ 65 enhancement
Mitragotri and Kost, 2001	20	7	P	2	Dalteparin	rats	Anti-Xa activity
Boucaud et al., 2002a	20	2.5	P	15	Insulin	rats	Marked decrease in blood glucose levels

Table 2
In vivo studies in humans

Author, year	F (kHz)	I (W/cm^2)	Mode	Duration (min)	Molecule	Number of patients	Effect
Griffin et al., 1967	1000	0–3	C	5	Hydrocortisone	102	Reduced pain (68% vs 28%)
McElnay et al., 1985	870	2	P	5	lignocaine	10	Non-significant
McElnay et al., 1986	870	2	P	5	Fluocinolone acetonide	12	Non-significant
Benson et al., 1988	750–3000	1–1.5	C P	5	Prilocaine Lignocaine	11	Significant increase in duration of anaesthesia
Benson et al., 1989	750–3000	1.5	C	5	Benzydamine	10	Non-significant
Williams, 1990	1100	0.25	C	5	Anaesthetic drugs	6	Non-significant
Benson et al., 1991	3000	1	C	5	Nicotinates	10	Non-significant
Ciccone et al., 1991	1000	1.5	P	5	Salicylate	40	Non-significant
McElnay et al., 1993	3000	1	C	5	Nicotinate	10	Vasodilatation × 1.7
Kost et al., 2000	20	10	P	2	Glucose	7	Increased reverse transport of glucose

Table 3
In vitro studies of phonophoresis according to frequency across rat, mouse or human skin.

Author, year	F (kHz)	I (W/cm^2)	Mode	Duration (min)	Molecule	Membrane	Effect
Vyas et al., 1995	20 000	3	P	15	Diclofénac	Mice skin	× 2–30 enhancement
Machet et al., 1996	3300	3	C	10	Digoxin	Hairless mice skin	Flux × 3 but heating resulted in the same effect
Meidan et al., 1998b	3300 1100	2.25	C	240	Hydrocortisone	Rat skin	× 2.2 enhancement × 1.8
Meidan et al., 1998a	1100	2	C	5	Sucrose Mannitol Hydrocortisone	Rat skin	× 4.5 enhancement × 4.1 × 7.7
Pelucio-Lopes et al., 1993	1100	1.5	C	20	Azydothymidine	Human skin	Flux × 1 with a cooling coil
Hikima et al., 1998	1100	4.3	C	10–30	Prednisolone	Hairless mouse skin	× 2–5
Machet et al., 1998	1100	1.5	C	20	Mannitol Oestradiol Hydrocortisone	Hairless mouse skin Human skin	Flux × 1 with a cooling coil Flux × 1 with a cooling coil
Brucks et al., 1989	1000	1	C	240	Ibuprofene	Human epidermis	× 3 enhancement
Mitragotri et al., 1995b	1000	2	C	300	7 molecules	Human epidermis	Flux × 13 estradiol, × 5 testosterone, × 4 cortisol, × 1.5 butanol, × 1.2 caffeine.
Johnson et al., 1996	1000	1.4	C	1440	4 molecules with chemical enhancer	Human epidermis	Flux x1 à × 75
Mitragotri, 2001	1000	2	C	300	5 molecules	Human stratum corneum	× 2– 15 enhancement
Ueda et al., 1995	150	0.111	C	60	9 molecules	Hairless rat skin	Flux × 2 –15
Ueda et al., 1996	150	0.111	C	60	Benzoate sodium Deuterium oxyde	Hairless rat skin	× 7 enhancement × 4 enhancement
Monti et al., 2001	40	0.44	C	240	Caffeine Morphine	Hairless mouse skin	× 4 enhancement × 10 enhancement
Mitragotri et al., 1996	20	0.125	P	300	7 molecules	Human epidermis	Flux × 3 (oestradiol), × 80 (cortisol), × 113 water), × 400 salicylic acid), × 5000 (sucrose)
Zhang et al., 1996	20	0.2	P	120	Vasopressine	Human epidermis	Kp = 1 10^{-5} cm/h with ultrasound Kp = 0 without ultrasound
Fang et al., 1999	20	0.1–0.3	P C	240 240	Clobetasol 17-propionate	Hairless mice	× 1.9–4 enhancement × 1.5–5.9 enhancement
Mitragotri et al., 2000c	20	1.6–14	P	90	Mannitol	Pig skin	× 10 enhancement
Mitragotri and Kost, 2001	20	7	P	10	Low molecular weight Heparin	Pig skin	× 21 enhancement
Boucaud et al., 2001a	20	2.5	P	60	Caffeine Fentanyl	Human skin	× 4 enhancement × 34
	20	2.5	C	10	Caffeine Fentanyl		× 1 × 4

glycaemia with insulin and anti-Xa activity with low-molecular weight heparin. Transdermal delivery of insulin was confirmed in vivo with a short (15 min) application time of 20 kHz ultrasound in hairless rats (Boucaud et al., 2002a) and, interestingly, in a preliminary study in pigs in vivo (Boucaud et al., 2002b).

4. How does it work?

The propagation of an ultrasonic wave within the skin has two main physical consequences: heating and cavitation, and these mechanisms may be linked as cavitation may cause heating (Holt and Roy, 2001). The overall consequence is increased skin permeability due to increased fluidity of intercellular lipids by heating or mechanical stress and/or by enlarging intercellular space, or by creating permanent or transient holes through corneocytes and keratinocytes as a consequence of cavitation and/or by driving the drug and the vehicle through the permeabilized skin by convection. This increase in skin permeability to drugs may not persist after the end of sonication (Boucaud et al., 2002b).

4.1. Heating

Several phenomena may explain the increase in temperature at the skin surface and within skin exposed to ultrasound. When an ultrasonic wave crosses the skin or another structure, the wave gradually decreases as it propagates. This attenuation phenomenon is explained by three mechanisms, i.e. absorption, reflection and dispersion, and it depends on the frequency of the ultrasonic wave and on the density and heterogeneity of the structure. Attenuation of the skin is four times higher than that of soft tissues (Goss et al., 1978, 1979), and this is due to the heterogeneity of the skin. The use of an aqueous gel decreases the reflection and the rise in temperature. To minimize the increase in temperature an ultrasound-pulsed mode, or a less focused ultrasound wave can be used.

4.2. Heating the skin surface

Several authors have measured in vitro the rise in skin temperature at the surface and have reported a slight rise of about a few degrees Celsius which cannot explain the increase in percutaneous absorption (Ueda et al., 1995; Mitragotri et al., 1995a). However other studies have reported greater rises in temperature. Miyazaki et al. (1991) showed a rise of 6 °C with 1 MHz for a fairly low intensity of 0.25 W/cm ^{2} and 12 °C for an intensity of 0.75 W/cm^2. Despite the use of a cooling coil, an increase in 11 °C was observed in the donor compartment (Brucks et al., 1989). In our group, we reported an increase of 15 to 30 °C for intensities ranging from 1 to 3 W/cm^2 (Machet et al., 1996). Moreover, we obtained an equivalent increase in percutaneous flow while heating with an electric resistance. Rise in temperature is thus one of the major factors which can explain the increase in percutaneous absorption in the frequency range 1–3 MHz and in continuous mode. Moreover our findings obtained with a cooling system did not showed any significant increased in percutaneous diffusion rates with various molecules whithin molecular weights varying from 138 to 781 (azidothymidine, digoxin, hydrocortisone, mannitol, oestradiol, salicylic acid) (Pelucio-Lopes et al., 1993; Machet et al., 1998), but with vasopressin V-2 antagonist (molecular weight 1014) a 3-fold increase was observed. With lower frequencies using synthetic cellulose membranes and monitoring temperature, the flux of hydrocortisone was multiplied four-fold with ultrasound (20 kHz, 10–30 W/cm^2) and the temperature increased from 25 to 75 °C (Julian and Zentner, 1990). The diffusion flux measured was close to that of controls at a similar temperature.

4.3. Heating within the skin

It is known that the in-depth penetration of ultrasound is inversely proportional to its frequency: at 90 kHz 50% of energy penetrates up to 10 cm in depth, whereas at 1 MHz the same amount of energy only penetrates to 2 cm. This in-depth transmission suggests vasodilatation and collection of the drug by the dermal capillaries.

Using a thermal probe under the skin of ultrasound exposed rats (1 MHz, 1 to 2 W/cm^2, continuous mode) Asano et al. showed that the temperature increased from 6 °C with 1 W/cm^2 to 11 °C with 2 W/cm^2 and this was accompanied by skin damage (Asano et al., 1997). Using a 20 kHz probe, the temperature measured in vivo just beneath the sonicated skin increased by a few degrees Celsius during sonication (Singer et al., 1998; Boucaud et al., 2001b) while in the donor compartment a dose relationship between intensity and heating was demonstrated. However, heating remains moderate with the intensity reported to be effective in low frequency sonophoresis, suggesting that other mechanisms are involved.

4.4. Cavitation

Cavitation can easily be observed in liquid media, especially under high intensity and low frequency conditions: the local variations in acoustic pressure induced by the ultrasound wave generate small gas bubbles (Suslick, 1988). During the negative phase, bubbles grow around their equilibrium radius. On the other hand the pressure increases during the positive phase, and induces a reduction (sometimes violent) in the bubble radius. When implosion of the bubble occurs the local rise in temperature can reach few thousands degrees Celsius. The phenomenon of bubble growth and decrease and the lifespan of these bubbles are dependent on the ultrasonic parameters (amplitude of the acoustic pressure, environmental pressure, frequency, characteristics of the liquid, existence of dissolved gas in the liquid). Two types of cavitation occur in liquid media: stable and unstable. Stable cavitation corresponds to a bubble that oscillates many times around its equilibrium radius (resonance radius Rr expressed in μm). Rr is roughly related to frequency (F in kHz) by the following equation: $FxRr = 3000$. Thus the resonance radius is 3 μm with 1 MHz and 150 μm with 20 kHz. The size of bubbles in living tissue may be greater (Ter Haar and Daniels, 1981). Unstable cavitation exists for a very short length of time, during which the gas cavity grows very fast and then implodes, creating a shock wave and by creating many bubbles of smaller size. The occurrence of cavitation in water is facilitated by the presence of dissolved gas and needs very high intensity above the frequency of 3 MHz since more ultrasound intensity is needed (Fig. 1) (Esche, 1952; Simonin, 1995). Nuclei for cavitation is possible in the skin as microclefts exist between corneocytes (Menon and Elias, 1997).

The role of cavitation in increasing percutaneous permeability due to ultrasound is supported by a series of in vitro experiments: 1) the importance of keeping dissolved gas in the medium to form nuclei of cavitation (Mitragotri et al., 1995b), 2) the possibility of permeating cell membranes in vitro (Greenleaf et al., 1998) is enhanced in the presence of artificial cavitation nuclei, 3) demonstration of possible pores created by ultrasound on the skin surface (Machet et al., 1998), and within the stratum corneum (Singer et al., 1998; Wu et al., 1998), 4) demonstration of multiple pits induced by bubble implosion on aluminium foil exposed to ultrasound and its correlation with intensity and skin conductivity (Terahara et al., 2002). The possible occurrence and the consequences of cavitation in cells or tissues (Carstensen et al., 2000; Poliachik et al., 2001) and possible applications in therapy for

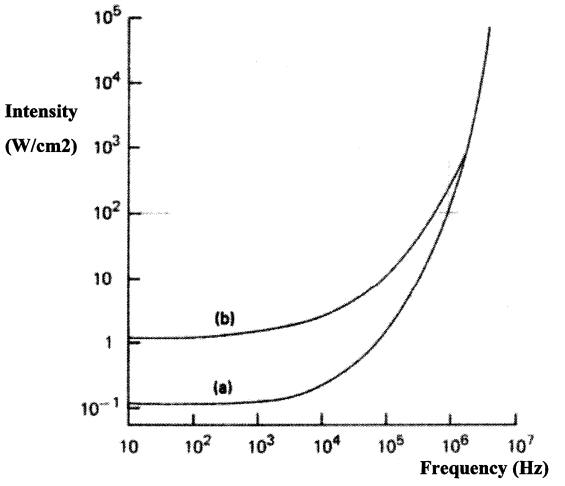

Fig. 1. Occurrence of cavitation in liquid medium according to the intensity and presence or absence of dissolved gas (Esche, 1952).

destruction of cancers (Huber and Debus, 2001) have been studied. Despite a very low increase in temperature, bovine red blood cells in suspension exposed to ultrasound (24 kHz) display a dose-dependent change in membrane permeability assessed by measuring the quantity of extracellular release of haemoglobin (Liu et al., 1998). This study demonstrated the existence of a total exposure time threshold of 100 ms to permeate the erythrocyte membrane while pulse length and duty cycle were found to be less important. A similar trend was demonstrated in vitro in prostate cancer cells (Cochran and Prausnitz, 2001) and with pig skin, a dose-dependent relationship was found between intensity, duration of exposure and cavitation (Mitragotri et al., 2000c). Indirect proof of cavitation was demonstrated in isolated epidermis in vitro, after incubating epidermis with fluorescein, resulting in bleaching of fluorescence probably secondary to production of hydroxyl radicals induced by cavitation (Mitragotri et al., 1995b). The existence of dissolved gas deep in living tissue can allow the development of cavitation bubbles, but it is much more difficult to highlight it (Ter Haar and Daniels, 1981). Small cavities the size of a few microns, which could correspond to 'the print' of cavitation bubbles on the surface of the stratum corneum, were shown in vitro using electronic scan microscopy (Machet et al., 1998). Scanning electron microscopy showed 1–3 mm holes on the surface of the stratum corneum after exposure to ultrasound (1.1 MHz, 1.5 W/cm^2). Such crater-like images of 5–15 mm were also reported in hairless mouse skin exposed to ultrasound in vitro (1 MHz, 4.3 W/cm^2) (Hikima et al., 1998). Such 'prints' have been shown when experimentally exposing aluminum foil to 20 kHz ultrasound (Mitragotri et al., 2000c; Terahara et al., 2002) and the quantity of pits increased with intensity and reduction of the distance between the skin and the probe. After having exposed rats to ultrasound in vivo, scanning electron microscopy examination demonstrated lesions of 100–150 μm diameter on the surface of the stratum corneum (Yamashita et al., 1997), corresponding to the size of the bubbles of stable cavitation at 20 kHz (Simonin, 1995). In the same study, slight changes were noticed around the base of the hairs of human skin in vitro. Cellular membrane disruption and inter and intracytoplasmic vacuolae were demonstrated in fish, without a significant increase in temperature within the skin (Frenkel et al., 1999). No lesions were demonstrated after sonication using degassed water, indicating that cavitation was the causative mechanism.

However this does not imply that cavitation occurs in vivo inside the human stratum corneum. Theoretically, cavitation is able to occur because of the presence of oxygen and nitrogen dissolved in the stratum corneum and the existence of lacunae between corneocytes (Menon and Elias, 1997). Cavitation could also occur in vivo in the 5 μm diameter sweat and sebaceous gland ducts. Explosion of cavitation bubbles and the currents of convection created in the sweat ducts might shorten the latency time. However this has not yet been demonstrated and histological findings do not reveal changes in sweat glands after ultrasound.

4.5. Removal or modification of stratum corneum lipids

In a model of lipid bilayers using an atomic force microscope, defects were demonstrated with diameters between ten and hundred nm, and diameters were three times greater at 168 kHz compared to 707 kHz (Malghnani et al., 1998). Intercellular lipid content was measured in hairless rat skin in vitro after ultrasound exposure in a solution containing Tween 20. Release of lipids from the skin was demonstrated which increased with length of time of exposure to ultrasound. The lipid release was correlated with decreased skin resistance and enhanced transport of polar molecules (Ueda et al., 1996). These findings are probably associated with the sebaceous gland involvement demonstrated on histological section (Meidan et al., 1998a). Changes in intercellular lipid, whatever the cause are, are evidenced by widening of intercellular spaces, and intercellular migration of a tracer has been demonstrated (see section 5.1).

4.6. Miscellaneous

Non-thermal and non-cavitational effects are less well reported. Emission of the ultrasonic wave involves a rise in the pressure of the liquid medium in the donor compartment. The first hypothesis is to imagine that this pressure mechanically pushes the active ingredient (but not its vehicle) through the skin, as through a sieve. This hypothesis is not tenable: the active ingredient is a small molecule in solution and pressure is exerted both on the active ingredient and on the vehicle (aqueous solution or gel). The pressure induced by the ultrasonic wave can be estimated by taking speed of sound in intercellular lipids at 1000 m/s (Simonin, 1995), i.e. about 5×10^{-6} bar with 1 W/cm^2 and 5×10^{-4} bar with 100 W/cm^2 The relative contribution of this flow induced by the pressure can be calculated for the diffusion of urea through a synthetic dialysis membrane and represents 0.02% with 1 W/cm^2 and 2% with 100 W/cm^2. The rise in the pressure thus does not have a significant contribution to the increased percutaneous flow induced by ultrasound (Simonin, 1995). Phonophoresis of mannitol across pig skin in vitro was recently reported to be the same when ultrasound was applied before application of mannitol or at the same time, suggesting the absence of significant effect of convection in ultrasound-induced transdermal transport (Mitragotri et al., 2000c). However, with a larger molecule such as insulin, pretreatment with ultrasound was ineffective in increasing skin transport, though effective transdermal transport of insulin was achieved during sonication, (Boucaud et al., 2002a).

Under experimental conditions where the temperature is controlled, the transport of small molecules through synthetic membranes is increased. This is probably to the acoustic streaming induced by diffusion of the ultrasound wave. In vitro, the receiver compartment is subjected to permanent agitation by a magnetic bar. The layer of water immediately adjacent to the membrane is less well mixed and constitutes an additional resistance to diffusion through the skin. However the reduction in resistance is weak (about 10%) (Simonin, 1995).

Finally, a recent study in conditions where rise in temperature was negligible and cavitation was absent showed evidence of intercellular widening secondary to mechanical stress induced by transverse (shear) waves (Frenkel et al., 2000, see Section 5.1).

5. Imaging of biological effects of ultrasound: disorganization of the stratum corneum and skin tolerance

Because the stratum corneum constitutes the principal resistance to molecule diffusion, a modification by ultrasound of the corneocytes and/or the intercellular spaces should be demonstrated. The progression of the ultrasonic wave in the tortuous channel of intercorneocyte spaces and the reflection of the wave by the corneocytes could induce mechanical and thermal disturbance within the intercellular lipid bilayers. However, it is possible that modifications of the intercellular space can be transitory under the effect of the ultrasound, without visible changes using optic or electron microscopy.

5.1. Creation of a pore pathway through the stratum corneum

Human stratum corneum exposed to 168 kHz at 1.2 W/cm^2 for 15 min was examined by Wu et al. using epifluorescence microscopy and the attenuation coefficient was measured. Cavities measuring 20 mm were revealed and considered to be the consequence of cavitation. Attenuation of the stratum corneum was increased because of the multiplication of interfaces between cells, intercellular lipids and entrapped air pockets (Wu et al., 1998). After in vitro exposure of human skin to ultrasound (1.1 MHz, 1.5 W/cm^2), scanning electron microscopy showed holes of 1–3 mm in the surface of the stratum corneum (Machet et al., 1998). Similar images of 5–15 mm were also reported in hairless mouse skin exposed to ultrasound in vitro (1 MHz, 4.3 W/cm^2) (Hikima et al., 1998). A tracer (lanthanum) was shown to migrate between intercorneocyte space after exposure to high frequency ultrasound (10–16 MHz)

and the tracer reached the dermis (Bommannan et al., 1992b). Widening of intercellular space and some desmosomal changes were demonstrated (Menon and Elias, 1997). These modifications were transient. The assumption that intercellular cavities were secondary to cavitation due to entrapped gas has been questioned since at this frequency and at the intensity used the occurrence of cavitation is doubtful (Simonin, 1995). It is therefore more likely that widening of intercellular space was secondary to mechanical stress causing disruption of the lipid bilayers. This was supported by a further study carried out on fish at 3 MHz and 2 W/cm^2, below the cavitional threshold intensity dose-dependent intercellular widening was shown, while cavitation was not detectable (Frenkel et al., 2000). Maximum disorganization affecting the outer layers of the epidermis occurred with a 45° angle of incident radiation, suggesting a role of transverse waves in the occurrence of cell to cell disruption. However, operating in cavitational conditions (1 MHz, 0.75 W/cm^2), the same authors demonstrated rupture of membrane cells responsible for cell to cell disruption (Frenkel et al., 1999).

5.2. Trans-adnexa pathway

The transfollicular route may be significant in mice and in humans. The relative surface of these channels is weak (1.10^{-3}) but their resistance to diffusion is much weaker, particularly for polar molecules and electrolytes, creating a shunt of diffusion through the stratum corneum. Debulking of sebaceous gland contents from the stratum corneum was demonstrated by histological study (Meidan et al., 1998a,b). This occurred with 1.1 MHz ultrasound at very low intensity 0.1 W/cm^2. This was not thought to be due to a heating effect because of the low intensity, nor to acoustic pressure, but was more probably the consequence of cavitation occurring within sebaceous units. However this was found to be responsible for negative effects on percutaneous absorption of polar molecules such as mannitol and sucrose, while hydrocortisone transport was unaffected (Meidan et al., 1998b).

5.3. Skin tolerance to ultrasound

Macroscopic alterations can be seen after in vitro sonication of human skin with high frequency ultrasound (1–3 MHz) at intensities ranging from 2 to 3 W/cm^2 (Machet et al., 1996). Histological studies showed multiple necrosis of keratinocytes with epidermal detachment, oedema and degeneration of collagen fibers in the upper part of the dermis while treatment with heat alone did not produce such damages (Machet et al., 1996; Asano et al., 1997; Meidan et al., 1998a). Transmission electron microscopy revealed changes in intracytoplasmic organites and in collagen fibers (Machet et al., 1996) and holes were seen in the skin surface (cf section 5-1) (Hikima et al., 1998).

In vitro low frequency ultrasound experiments with hairless mouse skin exposed to 20 kHz ultrasound for 4 hours showed epidermal and dermal lesions with relatively low intensity 0.2 W/cm^2 but with continuous mode. The lesions were less marked with pulsed mode (Fang et al., 1999). Using human skin in vitro we showed an absence of lesions below 2.5 W/cm^2, and a dose-dependent severity of skin lesions beyond 4 W/cm^2. Severity was also more obvious with continuous ultrasound. A dose-dependent increase in temperature also occurred in the donor compartment, the temperature measured in the donor compartment varying from 33 to 65 °C (Boucaud et al., 2001b). Similar findings were reported in dogs (Singer et al., 1998). Experiments were also carried out in vivo with hairless rat skin exposed to 2.5 W/cm^2. Macroscopic and microscopic appearance was normal immediately after sonication. However, overt macro and microscopic lesions appeared 24 hours later, thus demonstrating the delayed constitution of ultrasound-induced lesions (Fig. 2). These lesions were not due to heating of the donor compartment since the increase was moderate, and the same heating with an electrical resistance produced no lesions. Additionally, lesions were not due to diffusion of heated water into skin since the same ultrasound protocol applied with a plastic film between the ultrasound probe and the skin resulted in the same epidermal and dermal lesions. Finally, the increase in tempera-

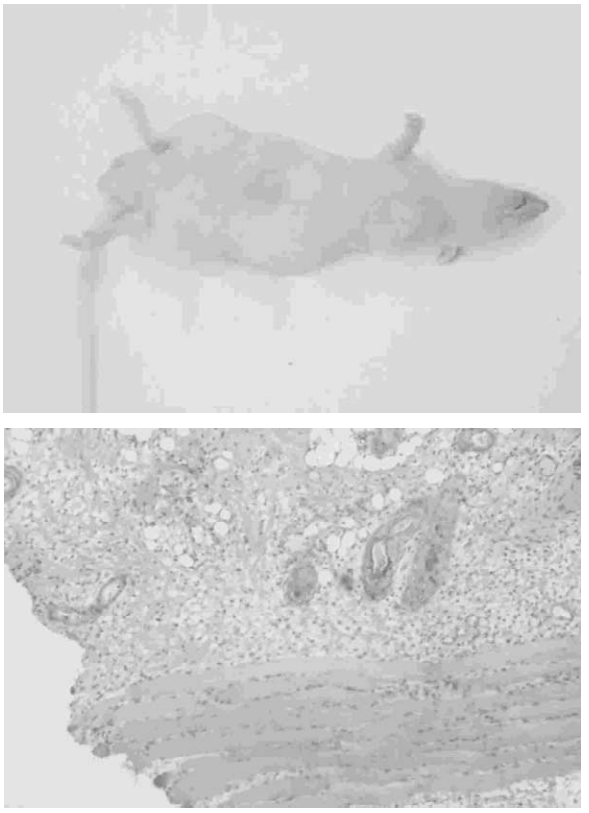

Fig. 2. Immediate macroscopic erythema and histologic lesion at 48 h in hairless mice exposed to 20 kHz ultrasound at 2.5 W/cm^2.

ture measured was moderate (+3 °C) and the lesions severe, with vessel and muscle damage suggesting a possible in depth cavitational effect (Boucaud et al., 2001a). Hairless mouse skin, and particularly the stratum corneum, is thinner than human skin and this could explain why the threshold tolerance of human skin to ultrasound is higher in vitro (see above) and probably also in vivo, since preliminary findings in 2 human volunteers showed the absence of immediate or delayed cutaneous lesions (Fig. 3) after sonication (5 W/cm^2, pulse duration 1.6 s, duty cycle 1:4, 15 minutes) (Boucaud et al., 2002b).

6. Stability of sonicated drugs

Eventual degradation of drugs to ultrasound was studied in vitro and showed absence of degradation for oligodeoxynucleotides (Meidan et al., 1995), insulin (Boucaud et al., 2002a), fentanyl and caffeine (Boucaud et al., 2001a). The persistence of biological activity of insulin and low-molecular weight heparin in vivo is also in accordance with the absence of degradation in the conditions used (Mitragotri et al., 1995a; Mitragotri and Kost, 2001).

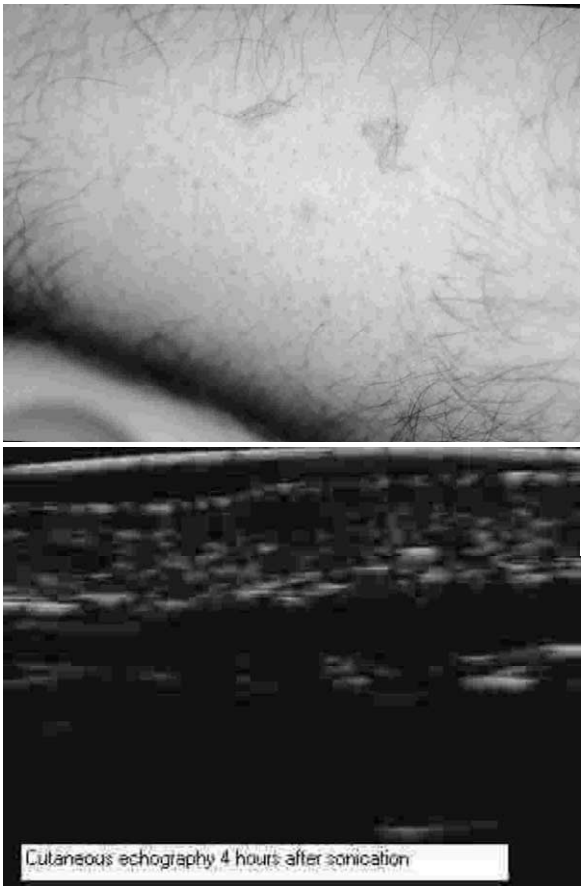

Fig. 3. Normal macroscopic appearance of human skin 4 hours after ultrasound (5 W/cm^2, pulse duration 1.6 s, duty cycle 1:4, 15 mins). Echography shows slight dermal oedema.

7. Coupling agent

To ensure the transmission of ultrasound a coupling medium (generally a gel or water) is interposed between the transducer and the skin. The viscosity of the coupling agent and the quantity of gas dissolved in the medium may significantly affect transdermal transport (Mitragotri et al. 1995b). A decrease in sonophoretic transport of insulin, vasopressin and estradiol was reported in vitro when molecules were administered in a gel (Zhang et al., 1996; Mitragotri et al. 1995b). Similar findings were reported with lidocaine in vivo in hairless mice (Tachibana and Tachibana, 1993).

8. The future

There is no doubt that ultrasound can markedly increase percutaneous absorption. Current published findings are encouraging, especially for diabetes. It is possible to decrease glucose blood levels with a non-invasive device in vivo in animals and moreover, measurement of blood glucose levels could be achieved in humans (Kost et al., 2000). However, the daily dose required to treat an adult diabetic patient is usually about 30 to 60 IU insulin, and the quantity delivered is about 0.5 to 1 IU for a short period, thus continuous administration throughout the day would theoretically allow the administration of a daily dose, though no human in vivo study has yet been published.

Recent studies have demonstrated both cavitational and non-cavitational effects leading to reduced skin barrier efficiency. There is a dose–response relationship between the intensity of ultrasound and increased transdermal transport and also with the severity of skin lesions. Thus future in vivo studies are needed to investigate tolerance and transdermal transport in humans.

References

Asano, J., Suisha, F., Takada, M., Kawasaki, N., Myasaki, S., 1997. Effect of pulsed output ultrasound on the transdermal absorption of indomethacin from an ointment in rats. Biol. Pharm. Bull. 20, 288–291.

Benson, H.A.E., McElnay, J.C., Harland, R., 1988. Phonophoresis of lignocaine and prilocaine from Emla cream. Int. J. Pharm. 44, 65–69.

Benson, H.A.E., McElnay, J.C., Harland, R., 1989. Use of ultrasound to enhance percutaneous absorption of benzydamine. Phys. Ther. 69, 113–118.

Benson, H.A.E., McElnay, J.C., Harland, R., Hargraft, J., 1991. Influence of ultrasound on the percutaneous absorption of nicotinates esters. Pharm. Res. 8, 204–209.

Bommannan, D., Okuyama, H., Stauffer, P., Guy, R.H., 1992a. Sonophoresis. I. The use of high-frequency ultrasound to enhance transdermal drug delivery. Pharm. Res. 9, 559–564.

Bommannan, D., Menon, G.K., Okuyama, H., Elias, P.M., Guy, R.H., 1992b. Sonophoresis. II. Examination of the mechanism(s) of ultrasound-enhanced transdermal delivery. Pharm. Res. 9, 1043–1047.

Boucaud, A., Machet, L., Arbeille, B., Machet, M.C., Sournac, M., Mavon, A., Patat, F., Vaillant, L., 2001a. In vitro study of low-frequency ultrasound-enhanced transdermal transport of fentanyl and caffeine across human and hairless rat skin. Int. J. Pharm. 228, 69–77.

Boucaud, A., Montharu, J., Machet, L., Arbeille, B., Machet, M.C., Patat, F., Vaillant, L., 2001b. Clinical, histologic, and electron microscopy study of skin exposed to low-frequency ultrasound. Anat. Rec. 264, 114–119.

Boucaud, A., Garrigue, M.A., Machet, L., Vaillant, L., Patat, F., 2002a. Effect of sonication parameters on transdermal delivery of insulin to hairless rats. J. Control. Release 81, 113–119.

Boucaud, A., Machet, L., Garrigue, M.A., Vaillant, L., Patat, F., 2002b. A practical use of low frequency ultrasound for rapid and reproducible transdermal delivery of insulin, Proc. IEEE Ultrasonics Symp, Atlanta, 2001.

Brucks, R., Nanavaty, M., Jung, D., Siegel, F., 1989. The effect of ultrasound on ibuprofen through human epidermis. Pharm. Res. 6, 697–701.

Byl, N.B., 1995. The use of ultrasound as an enhancer for transcutaneous drug delivery: phonophoresis. Phys. Ther. 75, 539–553.

Carstensen, E.L., Gracewski, S., Dalecki, D., 2000. The search for cavitation in vivo. Ultrasound Med. Biol. 26, 1377–1385.

Cochran, S.A., Prausnitz, M.R., 2001. Sonoluminescence as an indicator of cell membrane disruption by acoustic cavitation. Ultrasound Med. Biol. 27, 841–851.

Ciccone, C.D., Leggin, B.G., Callamaro, J.J., 1991. Effects of ultrasound and trolamine salicylate phonophoresis on delayed-onset muscle soreness. Phys. Ther. 71, 666–678.

Esche, R., 1952. Untersuchungen der Schwingungskavitation in Flüssigkeiten. Acustica 2, 208–218.

Fang, J.Y., Fang, C.L., Sung, K.C., Chen, H.Y., 1999. Effect of low frequency ultrasound on the in vitro percutaneous absorption of clobetasol 17-propionate. Int. J. Pharm. 191, 33–42.

Frenkel, V., Kimmel, E., Iger, Y., 1999. Ultrasound-cavitation damage to external epithelia of fish skin. Ultrasound Med. Biol. 25, 1295–1303.

Frenkel, V., Kimmel, E., Iger, Y., 2000. Ultrasound-induced intercellular space widening in fish epidermis. Ultrasound Med. Biol. 26, 473–480.

Goss, S.A., Fritzell, L., Dunn, F., 1978. Comprehensive compilation of empirical ultrasonic properties of mammalian tissues. J. Acoust. Soc. Am. 64, 423–457.

Goss, S.A., Johnson, R.L., Dunn, F., 1979. Ultrasonic absorption and attenuation of high frequency sound in mammalian tissue. Ultrasound Med. Biol. 5, 181–186.

Greenleaf, W.J., Bolander, M.E., Sarkar, G., Goldring, M.B., Greeleaf, J.F., 1998. Artificial cavitation nuclei significantly enhance acoustically induced cell transfection. Ultrasound Med. Biol. 24, 587–595.

Griffin, J.E., Touchstone, J.C., 1963. Ultrasonic movement of cortisol into pig tissue. I -Movement into skeletal muscle. Am. J. Phys. Med. 42, 77–85.

Griffin, J.E., Echternach, J.L., Price, R.E., Touchstone, J.C., 1967. Patients treated with ultrasonic driven hydrocortisone and with ultrasound alone. Phys. Ther. 47, 594–601.

Hikima, T., Hirai, Y., Tojo, K., 1998. Effect of ultrasound application on skin metabolism of prednisolone 21-acetate. Pharm. Res. 15, 1680–1683.

Holt, R.G., Roy, R.A., 2001. Measurements of bubble-enhanced heating from focused MHz-frequency ultrasound in a tissue-mimicking material. Ultrasound Med. Biol. 27, 1399–1412.

Huber, P.E., Debus, J., 2001. Tumor toxicity in vivo and radical formation in vitro depend on the shock wave-induced cavitation dose. Radiat. Res. 156, 301–309.

Johnson, M., Mitragotri, S., Patel, A., Blankschtein, D., Langer, R., 1996. Synergistic effects of chemical enhancers and therapeutic ultrasound on transdermal drug delivery. J. Pharm. Sci. 85, 670–679.

Julian, T.N., Zentner, G.M., 1990. Mechanism for ultrasonically enhanced transmembrane solute permeation. J. Control. Release 12, 77–85.

Kost, J., Mitragotri, S., Gabbay, R., Pisko, M., Langer, R., 2000. Transdermal monitoring of glucose and other analytes using ultrasound. Nature. Med. 6, 347–350.

Levy, L., Kost, J., Meshulam, Y., Langer, R., 1989. Effect of ultrasound on transdermal drug delivery to rats and guinea pigs. J. Clin. Invest. 83, 2074–2078.

Liu, J., Lewis, T.N., Prausnitz, M.R., 1998. Non-invasive assessement and control of ultrasound-mediated membrane permeabilization. Pharm. Res. 15, 918–924.

Lombry, C., Dujardin, N., Préat, V., 2000. Transdermal delivery of macromolecules using skin electroporation? Pharm. Res. 17, 32–37.

Machet, L., Pinton, J., Patat, F., Arbeille, B., Pourcelot, L., Vaillant, L., 1996. In vitro phonophoresis of digoxin across hairless mice and human skin: thermal effect of ultrasound. Int. J. Pharm. 133, 39–45.

Machet, L., Cochelin, N., Patat, F., Arbeille, B., Machet, M.C., Lorette, G., Vaillant, L., 1998. In vitro phonophoresis of mannitol, oestradiol and hydrocortisone across hairless mouse and human skin. Int. J. Pharm. 165, 169–174.

McElnay, J.C., Matthews, M.P., Harland, R., McCafferty, D.F., 1985. The effect of ultrasound on the percutaneous absorption of lignocaine. Br J Clin Pharmacol 20, 421–424.

McElnay, J.C., Kennedy, T.A., Harland, R., 1986. Enhancement of the percutaneous absorption of fluocinolone using ultrasound. Br. J. Clin. Pharmac. 21, 609–610.

McElnay, J.C., Benson, H.A.E., Harland, R., Hadgraft, J., 1993. Phonophoresis of methyl nicotinate : a preliminary study to elucidate the mechanism of action. Pharm. Res. 10, 1726–1731.

Malghnani, M.S., Yang, J., Wu, J., 1998. Generation and growth of bilayer defects induced by ultrasound. J. Acoust. Soc. Am. 103, 1682–1685.

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

Meidan, V.M., Docker, M.F., 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, 85–92.

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.

Menon, G.K., Elias, P.M., 1997. Morphologic basis for a pore-pathway in mammalian stratum corneum. Skin Pharmacol. 10, 235–246.

Mitragotri, S., Blankschtein, D., Langer, R., 1995a. Ultrasound-mediated transdermal protein delivery. Sciences 269, 850–853.

Mitragotri, S., Blankschtein, D., Langer, R., 1995b. A mechanistic study of ultrasonically-enhanced transdermal drug delivery. J. Pharm. Sci. 84, 697–706.

Mitragotri, S., Blankschtein, D., Langer, R., 1996. Transdermal drug delivery using low-frequency sonophoresis. Pharm. Res. 13, 411–420.

Mitragotri, S., Ray, D., Farell, H., Tang, H., Yu, B., Kost, J., Blankstein, D., Langer, R., 2000a. Synergistic effect of ultrasound and sodium lauryl sulfate on transdermal drug delivery. J. Pharm. Sci. 89, 892–900.

Mitragotri, S., Coleman, M., Kost, J., Langer, R., 2000b. Transdermal extraction of analytes using low-frequency ultrasound. Pharm. Res. 17, 466–470.

Mitragotri, S., Kost, J., 2000. Low-frequency sonophoresis: an non invasive method of drug delivery and diagnostics. Biothechnol. Prog. 16, 488–492.

Mitragotri, S., Farell, J., Tang, H., Terahara, T., Kost, J., Langer, R., 2000c. Determination of threshold energy dose for ultrasound-induced transdermal transport. J. Control. Release 63, 41–52.

Mitragotri, S., Kost, J., 2001. Transdermal delivery of heparin and low-molecular weight heparin using low frequency ultrasound. Pharm. Res. 18, 1151–1156.

Mitragotri, S., 2001. Effect of therapeutic ultrasound on partition and diffusion coefficient in human stratum corneum. J. Control. Release 71, 23–29.

Miyasaki, S., Mizuoka, H., Oda, M., Takada, M., 1991. External control of drug release and penetration: enhancement of the transdermal absorption of indomethacin by ultrasound irradiation. J. Pharm. Pharmacol. 43, 115–116.

Monti, D., Gianelli, R., Chetoni, P., Burgalassi, S., 2001. Comparison of the effect of ultrasound and of chemical enhancers on transdermal permeation of caffeine and morphine through hairless mouse skin in vitro. Int. J. Pharm. 229, 131–137.

Novak, J., 1964. Experimental transmission of lidocaine through intact skin by ultrasound. Arch. Phys. Med. 45, 231–232.

Pelucio-Lopes, C., Machet, L., Vaillant, L., Patat, F., Letiecq, M., Furet, Y., Lorette, G., 1993. Phonophoresis of azidothymidine (AZT) ex vivo across human and hairless mice skin. Int. J. Pharm. 96, 249–252.

Poliachik, S.L., Chandler, W.L., Mourad, P.D., Ollos, R.J., Crum, L.A., 2001. Activation, aggregation and adhesion of platelets exposed to high-intensity focused ultrasound. Ultrasound Med. Biol. 27, 1567–1576.

Pottenger, F.J., Karalfa, B.L., 1989. Utilization of hydrocortisone phonophoresis in United States army physical therapy clinics. Military Medicine 154, 355–358.

Simonin, J.P., 1995. On the mechanisms of in vitro and in vivo phonophoresis. J. Control. Release 33, 125–141.

Singer, A.J., Homan, C.S., Church, A.L., McClain, S.A., 1998. Low-frequency sonophoresis: pathologic and thermal effects in dogs. Acad. Emerg. Med. 5, 35–40.

Suslick, KS. Ultrasound – its chemical, physical and biological effects. VCH Publishers, New York, 1988, 336p.

Tachibana, K., Tachibana, S., 1991. Transdermal delivery of insulin by ultrasonic vibration. J. Pharm. Pharmacol. 43, 270–271.

Tachibana, K., 1992. Transdermal delivery of insulin to alloxan-diabetic rabbits by ultrasound exposure. Pharm. Res. 9, 952–954.

Tachibana, K., Tachibana, S., 1993. Use of ultrasound to enhance the local anesthetic effect of topically applied lidocaine. Anesthesiology 78, 1091–1096.

Ter Haar, G.R., Daniels, S., 1981. Evidence for ultrasonically induced cavitation in vivo. Phys. Med. Biol. 26, 1145–1149.

Terahara, T., Mitragotri, S., Kost, J., Langer, R., 2002. Dependance of low-frequency sonophoresis on ultrasound parameters, distance of the horn and intensity. Int. J. Pharm. 235, 35–42.

Ueda, H., Sugibayashi, K., Morimoto, Y., 1995. Skin penetration-enhancing effect of drugs by phonophoresis. J. Control. Release 37, 291–297.

Ueda, H., Ogihara, M., Sugibayashi, K., Morimoto, Y., 1996. Change in the electrochemical properties of skin and the lipid packing in stratum corneum by ultrasonic irradiation. Int. J. Pharm. 137, 217–224.

Vanbever, R., Fouchard, D., Jadoul, A., De Morre, N., Preat, V., Marty, J.P., 1998. In vivo noninvasive evaluation of hairless rat skin after high-voltage pulse exposure. Skin Pharmacol. Appl. Skin Physiol. 11, 23–34.

Vyas, S.P., Singh, R., Asati, R.K., 1995. Liposomally encapsulated diclofenac for sonophoresis induced systemic delivery. J. Microencapsulation 12, 149–154.

Williams, A.R., 1990. Phonophoresis: an in vivo evaluation using three topical anaesthetic preparations. Ultrasonics 28, 137–141.

Wu, J., Chappelow, J., Yang, J., Weimann, L., 1998. Defects generated in human stratum corneum specimens by ultrasound. Ultrasound Med. Biol. 24, 705–710.

Yamashita, N., Tachibana, K., Ogawa, K., Tsujita, N., Tmita, A., 1997. Scanning electron microscopic evaluation of the skin surface after ultrasound exposure. Anat. Rec. 247, 455–461.

Zhang, I., Shung, K.K., Edwards, D.A., 1996. Hydrogels with enhanced mass transfer for transdermal drug delivery. J. Pharm. Sci. 85, 1312–1316.