Low Intensity Ultrasound as a Probe to Elucidate the Relative Follicular Contribution to Total Transdermal Absorption

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Received June 17, 1997; accepted October 6, 1997

Purpose. To investigate the effect of ultrasound on the histological integrity and permeability properties of whole rat skin in vitro.

Methods. A defined, field-free source of ultrasound was used to irradiate excised rat skin prior to in vitro transport studies in Franz-type cells using sucrose, mannitol, hydrocortisone, 5-fluorouracil and aminopyrine.

Results. High intensity ultrasound irradiation (1 to 2 W cm⁻²) irreversibly damaged cutaneous structures and increased the percutaneous transport rate of permeants. In contrast, skin integrity was largely maintained with low intensity ultrasound (0.1 to 1 W cm⁻²) which merely discharged sebum from the sebaceous glands so as to fill much of the hair follicle shafts. This effect caused the transfollicular absorption pathway to be blocked for hydrophilic molecules that penetrate via this route and reduced the transport rate significantly.

Conclusions. This phenomenon may be used as a probe to elucidate the relative follicular contribution to total penetration for hydrophilic permeants. It was demonstrated that the shunt pathway was responsible for virtually all mannitol and sucrose penetration, perhaps half of hydrocortisone transport but negligible aminopyrine and 5-fluorouracil penetration.

KEY WORDS: Follicular transport; mechanism; percutaneous absorption; phonophoresis.

INTRODUCTION

Phonophoresis (sonophoresis) is the use of ultrasound to enhance percutaneous drug delivery. Ultrasound may potentially accelerate drug absorption via several mechanisms such as heating, radiation pressure, acoustic microstreaming or cavitation. However, there have been mixed reports on the effectiveness of this procedure; some workers have reported that ultrasound can significantly enhance percutaneous drug absorption whilst other groups have found ultrasound to have no effect whatsoever (1). Much of the literature on phonophoresis suffers from the defect of inadequate dosimetry of the ultrasound source which is often due to complex and unpredictable standing wave patterns occurring within the experimental assembly (2). This major limitation has added to the confusion in this area.

The aim of this study was to determine the effect of ultrasound on the structural morphology and barrier properties of whole rat skin in vitro. An exposure system was specially developed so that the skin was exposed to a definable ultrasonic field with no standing waves. This is termed an ultrasound free-field. It was convenient to classify effects into those produced by low intensity ultrasound (<1 W cm⁻²) and high intensity ultrasound (1 to 2 W cm⁻²) respectively. In order to elucidate the nature of any ultrasound-induced perturbations, a heat-alone control application was devised which simulated the ultrasonic heating effect without generating mechanical forces. In addition to histological analysis, control and sonicated rat skin samples were also used as barriers for percutaneous absorption experiments involving sucrose, mannitol, 5-fluorouracil, aminopyrine, and hydrocortisone. These compounds encompass a variety of octanol-water partition coefficients ranging from log k = –2.6 to log k = 1.55 and may be expected to show a variation in percutaneous penetration ranging from transfollicular (hydrophilic compounds) to transcellular (more hydrophobic compounds).

METHODS

Development of the Ultrasonic Free-Field Exposure System

In order to subject skin samples to an ultrasonic free-field, a suitable exposure system had to be initially designed. The simplest such system involves placing the skin section flat on a sheet of clingfilm, stretched across a water-filled beaker. If the beaker is composed or lined with sound-absorbing material then this attenuates the incoming energy and prevents standing wave development. Initially, a plastic beaker (d = 12 cm; h = 12 cm) was investigated for its sound-absorbing properties. To this end, a 3.5 MHz reversible transducer (595522B, 19 mm, Long I. F. Series L, A71110Hr, Picker International, Cleveland, Ohio) was used in conjunction with an A-mode scan (Clinical Diagnostic, Series 4100MG, Kretz-Technik AG, Zippf, Austria). This equipment essentially acts as a reversible piezoelectric transducer, emitting a brief pulse of ultrasound and then recording the energy of any returning ultrasonic echo (3). The plastic beaker was filled with water and the transducer of the A-scan was then placed just below the water surface, with its radiating surface pointing downwards toward the base of the container. Since ultrasound undergoes almost total reflection at a stainless steel-water interface, the acoustic reflectivity of a stainless steel beaker was similarly measured in order to obtain a reference reading representing virtually 100% reflection. It was determined that the plastic-water interface reflected back approximately 10% of the energy. In order to develop a more sound-absorptive system, the echo tests were repeated with the beaker entirely lined on all its inside surfaces with sections of thick-pile carpet. Common household carpet has been used for many years as an inexpensive, readily available, ultrasound-absorbing material (4). The carpet lining had to be left submerged in water for 10 min so as to permit entrapped air to escape to the water surface. It was determined that the carpet lining absorbed at least 99% of the incoming acoustic beam and that the reflected energy component was therefore negligible. Consequently, it was decided to employ the carpet-lining for the free-field exposure system. Further tests with the A-scan
demonstrated that tiny variations in the angle made by the transducer with the water did not greatly affect the magnitude of the reflections, thus indicating that holding the transducer by hand would be acceptable. A sheet of cling film was stretched taught over the surface of the water and secured with rubber bands. This provided a physical base to support the skin samples to be sonicated. Coupling gel (Henleys Medical Supplies, Welwyn Garden City, UK), exhibiting a low attenuation coefficient, was applied to make contact between the transducer, skin and cling film. Figure 1 depicts the ultrasound free-field exposure system developed for these studies.

**Development of the Heat-Alone Exposure Protocol**

Initially, an ultrasonic free-field of intensity 1.5 W cm\(^{-2}\) was beamed across samples of whole rat skin by using the beaker assembly shown in Figure 1. After the 5 min application period, the skin surface temperature at the centre of the sonicated region was measured by thermocouple and was found to be 42°C. An aluminium and perspex cylinder (d = 3.7 cm; l = 6 cm; approximately the same surface area as the large transducer) was subsequently employed to simulate the heating effect of the 1.5 W cm\(^{-2}\) ultrasonic output. The hollow interior of the probe, was connected by rubber tubing to a thermostatically controlled water pump (Churchill-Matrix, Churchill Instrument Company Ltd., Perivale, UK). When the pump was switched on, the circulating hot water heated the aluminium probe towards an equilibrium temperature. When the thermostat indicated that the desired temperature had been attained, the cylinder was employed to heat skin samples on the carpet-lined beaker using the same technique as described for the ultrasound transducer. Due to inefficiencies in heat conduction within the system, the actual temperature at the skin surface, in the centre of the probe-exposed area, was slightly lower than that indicated by the thermostat dial. Therefore, the heating probe was pre-calibrated with a differential thermocouple (3202 type K, d = 1 mm; Digitron Instruments Ltd., Hertford, UK) and it was determined that the skin temperature was approximately 10% lower than that indicated by the thermostat. Therefore, the thermostat dial was set to 46°C in order to produce a final skin surface temperature of 42°C. It should be noted that temperature readings at specific points can only give an indication of total heat generated. The temperature distribution throughout the skin is inhomogeneous due, for example, to spatial fluctuations in the output intensity and partial reflections at the interfaces between the skin strata.

**Transport Studies**

Whole skin was obtained from male Wistar rats, 4 to 5 months old and weighing 250 to 300 g. The animals were sacrificed by cervical dislocation and the dorsal region of each rat was shaved with electric clippers (Model no. 50, Sunbeam-Oster, Oxfordshire, UK). A pair of scissors was used to excise a sheet of whole-thickness intact skin from the back of each animal. Adhering fat and other visceral debris were removed from the undersurface of this skin. The isolated skin was cut into 6 rectangular sections of tissue (side length, 2 to 3 cm). These skin samples were either used immediately as barrier membranes, or alternatively stored at -20°C between sheets of aluminium foil for a period of up to 1 month (5). Each skin section was coated with 0.5 g of ultrasound coupling gel (Henley's Medical Supplies Ltd., Welwyn Garden City, UK) on the inner surface and 1 g of gel on the outer surface. The skin sample was placed flat upon the sheet of cling film comprising one of the two exposure systems described above (i.e. ultrasound or heat-alone). For the ultrasound-exposure regime, the large transducer of an ultrasound generator (Therasonic 1032, model no. 50, EMS Greenham Ltd., Wantage, Oxfordshire) was placed on top of the sample so that the skin was compressed flat between the transducer and the cling film. A 1.1 MHz frequency was selected and the generator was switched on at a set intensity (0, 0.1, 1, 1.5, 1.75 or 2 W cm\(^{-2}\)). The transducer was hand-held perpendicular to the skin surface for 5 min whilst the skin was sonicated. The area of skin exposed to the ultrasound was 4.4 cm\(^2\) while the active area of the penetration chamber varied from 2.2 to 3.3 cm\(^2\). For the heat-alone studies, the aluminium heating probe was similarly used to heat the skin samples to a central surface temperature of 42°C over 5 min (mimicking the thermal effect of 1.5 W cm\(^{-2}\) ultrasound). Following sonication or direct heating, the skin sections were repeatedly washed with distilled water and dried with a paper towel in order to remove residues of coupling gel. Subsequently, the sections were mounted on Franz diffusion cells jacketed at 37°C (6). The receptor volumes varied from 24 to 33 ml; and sample aliquots were 1.1 ml. In these permeation experiments, the donor phase consisted of 100 ml of ethanol solution containing 5% v/v of the appropriate radiolabelled drug. The drugs were \(^{14}C\)-sucrose (1.819 pmol, 0.0370 MBq, Amersham International Plc, Amersham, UK); \(^{14}C\)-mannitol (17.874 pmol, 0.0370 MBq, Amersham International Plc); \(^{3}H\)-hydrocortisone (63.36 pmol, 0.1850 MBq, Amersham International Plc); \(^{3}H\)-5-fluorouracil (333.3 pmol, 0.1628 MBq, NEN Life Science Products, Hounslow, UK); and \(^{3}H\)-aminopyrine (2313 pmol, 0.0093 MBq, Amersham International Plc). The composition of the receptor phase depended upon the nature of the penetrant. For sucrose, mannitol, 5-fluorouracil and aminopyrine, the receptor solution was distilled water but for hydrocortisone, 5% v/v aqueous ethanol was employed. In either case, the solutions were partially degassed by heating to 38°C and then sonicated in an ultrasound bath (Kerry, Pulsatron 125) for 3 min.

The permeation experiment was allowed to proceed for 5 h during which 1.1 ml aliquots of receiver solution were withdrawn at 30 min intervals and replaced by an identical volume of drug-free solution. Each 1.1 ml sample was vortexed which 10 ml of scintillation fluid (Optiphase Hisafe 3, Fisher Chemicals, Loughborough, UK) and the emitted activity was counted.