Optimisation of low-frequency ultrasound parameters for transcutaneous protein delivery

A. Dahlan, H. O. Alpar and S. Murdan
Department of Pharmaceutics, School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, UK. Email: afendi.dahlan@ulsop.ac.uk

Transdermal delivery using low-frequency ultrasound has been largely focused on small drug molecules and knowledge on macromolecular delivery is scant. As we are interested in the application of ultrasound waves for vaccine delivery to the skin, the aim of this study was to establish optimum ultrasound setting for parameters such as type of wave (continuous or pulse), application time, duty cycle, pulse length and possible synergistic action with chemical enhancer (1% SLS aqueous solution) for the transcutaneous delivery of a model antigen (BSA). Permeation studies using Franz cells were conducted with full-thickness rat skin and PBS as the receptor medium. The donor compartment was filled with 2mL of coupling medium (1% SLS aqueous solution or water) and ultrasound (19% amplitude for SLS and 50% for water; either continuous or pulsatile – 10% or 50% duty cycle) was applied with the transducer being 10mm from the skin surface. Following ultrasound application, the coupling medium was removed from the donor compartment, the skin was rinsed and blotted dry, and 50_L of Iodine-125 labelled BSA was applied onto the skin. Twenty-four hours later, the levels of radioactivity in the receptor compartment and in the skin were measured using a gamma counter. Gel electrophoresis on the receptor phase confirmed the presence of BSA. It was found that application of ultrasound, inclusion of SLS, a pulse wave, 50%duty cycle (0.5 s on 0.5 s off) and longer application time (3 min) compared with absence of ultrasound and of SLS, a continuous wave, 10%duty cycle (0.1 s on, 0.9 s off) and shorter application time (1 min), respectively (Table 1), resulted in greater permeation and deposition of BSA in the skin as expected and as reported previously for small molecular weight drugs (Mitragotri et al 1996). However, the use of longer pulse length (5 s on, 5 s off) resulted in lower protein permeation through the skin compared with a shorter pulse length (0.5 s on, 0.5 s off) even though the total time was the same. This is due to depletion of gas in the coupling medium during the initial long pulses. A shortage of gaseous molecule inhibits effective formation and collapse of bubbles (or microjets), which is believed to cause skin disruption and permeation of drugs. From these preliminary studies we can conclude that ultrasound does influence permeation of the largemolecule BSAinto and through the skin, total pre-treatment timematters and duty cycle and pulse length are important parameters. Finally pulse mode is also better than continuous mode even though less sound waves generated.

Table 1 Amount of labelled BSA that permeated skin at 24 h

Ultrasound potocol	Amount of BSA, cpm (n = 4)
Continuous modeþwater	54_2.4
0.5 s on pulse waveþ1% SLS (3 min)	220_46.5
0.1 s on pulse waveþ1% SLS (3 min)	112.5_2.1
0.5 s on pulse waveþwater (1 min)	55_3.6
5.0 s on pulse waveþwater (3 min)	107_2
0.5 s on pulse waveþwater (3 min)	154_19.5
Control	30_7.2

Mitragotri, S. et al (1996) Pharm. Res. 13: 411–420