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RESEARCH ARTICLE

The Effect of Bubble Surface Charge on Phonophoresis: Implication in Transdermal Piroxicam Delivery

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ABSTRACT

It is over several decades that ultrasound is used to enhance the transdermal drug delivery (phonphoresis). The mechanism of the enhancement is not fully understood and the ability of ultrasound on the enhancement for some drugs is unclear. The effect of continuous wave 870 KHz ultrasound at intensity of 1 W/cm^2 for 15 minutes on transdermal absorption of piroxicam from solution and gel formulations in hairless rat skin was studied. Exposure to ultrasound increased the rate of diffusion from gel and solution of piroxicam to 10 and 3 times higher than that in skins not exposed to ultrasound. We strongly believe that the lower diffusion of piroxicam from the solution is caused by extra-bubbles generated by ultrasound. It can be suggested that cavitation activity and its negative surface charges play a dominant rule in phonophoresis.

Keywords: *Piroxicam, Phonophoresis, Cavitation, Micro-streaming*

Transdermal drug delivery is designed to transfer drugs form the surface of the skin and through its various layers into the systemic circulation [1]. In normal condition, the diffusion of drug into the skin and beyond that is limited. This limitation is attributed to stratum corneum of the skin. In order to increase diffusion of drugs through the skin, physical & chemical approaches have been adopted to change stratum corneum properties. Phonophoresis is a physical method to disturb the stratum corneum and is defined as the enhancement and movement of drugs through intact skin and into soft tissue under influence of ultrasound perturbation [2]. Although significant attention has been devoted to the investigation of phonophoresis, its mechanism of action has not been clearly understood.

There are several parameters, which may affect the skin upon exposure of ultrasound. These parameters as summarised by Mitragotri are cavitation, thermal effect, induction/convection processes and mechanical effects. The role of cavitation in diffusion processes is well documented. Mitrogotri stated that enhancement of drug by phonophoresis is due to disordering of lipid bilayer of stratum corneum which is resulted from cavitation effects [3]. In another report, Mitragotri concluded that ultrasound has no effect on increasing diffusion of some drugs [4]. Some other researchers believed that the enhancement effect of phonophoresis was more pronounced for polar compounds compared to non-polar compounds [5]. Although the importance of cavitation has been realised by other investigators, its effect on diffusion of drugs has not been fully described.

Bubbles are usually produced in the sound field from small inactive bubble nuclei which are normally present in a medium activated by the pressure fluctuation of the sound field. This causes volume pulsations of the nuclei, growth by rectified diffusion to resonant size, and concentration of acoustic energy in their vicinity [6,7]. The nuclei will grow by a process of rectified diffusion [7-9]. Research by Watmough on cavitations revealed that the mapping of ultrasound field on paper is strongly dependent on the charge of the ions of the dyes and presence of micro-bubbles [10,11]. Shiran has shown that there would be no pattern of ultrasound field in the absence of micro-bubble [12].

In this study, the effects of micro-bubbles and their electrical surface charges on the rate of drug diffusion in phonophoresis have been assessed.

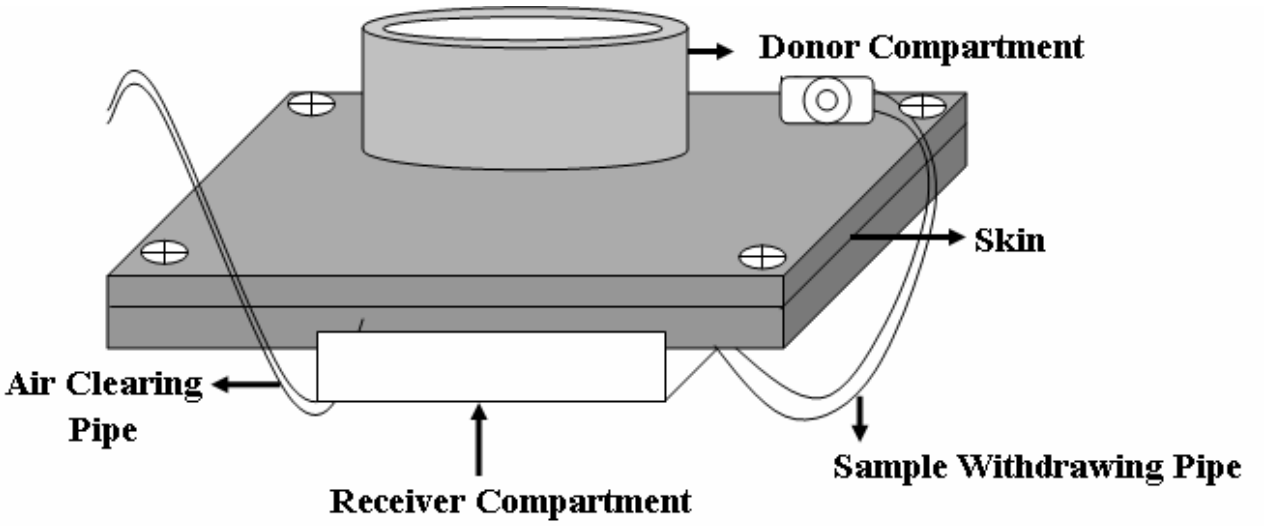

Fig. 1. Two compartments chamber used for mounting the isolated skin

MATERIALS AND METHOD

A costume-made chamber with two compartments was constructed as shown in Fig 1. The receiving compartment was covered with rubber (1 mm thickness) as an absorber to prevent standing wave formation and multiple reflections during sonification. The temperature of the skin inside receiving compartment was measured by a digital thermometer (LT Luton TM 905). Two plastic tubes (1 mm diameter) were fitted into the receiver compartment to take sample from, and to clear the air under the skin. A magnetic stirrer with Perspex cover and speed of 100 rpm placed in a ban to keep temperature constant at 33°C throughout the experiments. Male Wistar rats (200-300 g) were sacrificed by cervical dislocation and the abdominal hair was carefully removed with electrical clips.

The stratum corneum side of the prepared abdominal skin was mounted on ring-shaped donor compartment with 2.5 cm diameter; large enough to allow the ultrasound to pass through without any disturbances. The donor and receiver compartments were screwed together and 55 ml of normal saline (9 g/L) was injected into the receiver compartment and left for 15 hours in refrigerator for the skin to achieve steady state diffusion rate. Prior to the experiment, the skin was washed with 55 ml fresh normal saline. Pre-experimental solution was then replaced by fresh normal saline at 33°C.

A plane circular 870 KHz transducer, 4 cm in diameter powered by sonostat 633 generator, was placed vertically in the donor compartment. The fixed skin in the chamber was covered either with 10 g of piroxicam gel (0.5%) or piroxicam solution (5×10 ml; 20 mg/ml) and exposed to ultrasound in continuous wave mode at intensity of 1W/cm^2 for 15 minutes. The equipment was calibrated by radiation force and dye/paper method before use. Each experiment was repeated four times and the mean of the values were obtained.

Aliquots of 5 ml were withdrawn from the receiver chamber immediately after the end of sonification and then at 2-hour intervals up to 6 hours. After withdrawal of each sample, the same volume of normal saline was added to keep the receiver chamber volume constant during experiment. The drug concentration was determined using HPTLC system.

RESULTS

Fig. 2 shows the results of continuous wave ultrasound applied to the skin covered with the piroxicam gel. The rate of piroxicam diffusion through the skin, using gel formulation, was over 10 fold higher compared to its control for 6 hours of sampling, with sharp increase in piroxicam concentration two hours after the termination of sonification.

The rate of diffusion of piroxicam from solution in

Table 1. The mean value of the amount of piroxicam from gel formulation in the receiver chamber with and without ultrasound (US) radiation

Time (minute)	Amount of Piroxicam (μg) (Mean ± SE)		t-test	Ratio of the amount of Piroxicam (with US/without US)
	Without (US)	With (US)		
15	0	0	-	00.0
120	3.71±0.5	40.3±4.5	S*	10.9
240	5.98±1.2	58.64±2.3	S	10.0
360	6.55±1.2	76.41±3.9	S	11.6
480	8.74±2.3	91.83±6.9	S	10.5

*S=significant ($p<0.05$)

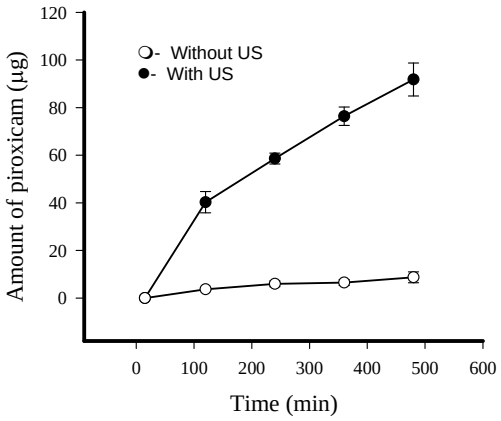

Fig. 2. The time-concentration curve of piroxicam gel upon 15 minutes ultrasound treatment (data are means ± standard error)

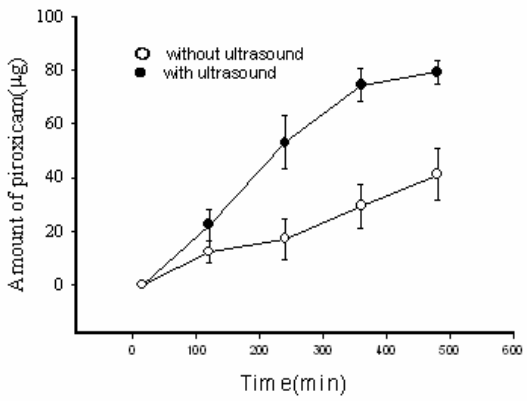

Fig 3. The time-concentration curve of piroxicam solution upon 15 minutes ultrasound treatment (data are means ± standard error)

Table 2. The mean value of the amount of piroxicam in solution form in the receiver chamber with and without ultrasound (US) radiation

Time (minute)	Amount of Piroxicam (µg) (Mean ± SE)		t-test	Ratio of the amount of Piroxicam (with US/without US)
	Without (US)	With (US)		
15	0	0	-	0.0
120	12.3±4.3	22.43±5.9	NS*	1.8
240	17.16±7.6	53.16±10	S**	3.1
360	29.52±8.2	74.47±6	S	2.5
480	41.24±9.8	79.45±4.5	S	2.0

*NS= no significant, **S=significant ($p<0.05$)

the presence and absence of ultrasound application is illustrated in Fig. 3. The graph shows an increase in piroxicam concentration in receiver chamber. The absolute increase in concentration is about 3 times higher than its control with very low diffusion rate two hours after termination of sonication.

The mean of the piroxicam concentrations found in receiver chamber with or without ultrasound radiation are shown in Tables 1 and 2 for gel and solution respectively. The solution of piroxicam in the absence of ultrasound also shows a more linear penetration of the drug compared to the gel formulation. A maximum 7°C temperature increase was recorded for both sets of experiments.

DISCUSSION

The results indicate that ultrasound has an enhancing effect on transdermal drug delivery. This enhancement is believed to be attributed to several factors such as thermal, cavitational and convective effects [3]. Ueda and co-workers found different diffusion rate for polar and non-polar drug, but they related this increase to thermal effect and diffusivity of the drug across the polar region in the stratum corneum [5]. The temperature rise in this study was about 7°C, which according to previous studies can not have a great effect on the enhancement of piroxicam diffusion through the skin [2,3].

The amount of piroxicam measured in receiver chamber from the gel formulation was almost 10 times higher than the control value, which was statistically significant. The concentration of piroxicam measured for the solution of piroxicam was almost three times higher compared to its control, even with higher concentration of drug applied to the skin. The increase of piroxicam permeation through the skin in both gel and solution forms upon ultrasound exposure signifies the involvement of other mechanisms rather than the change in the temperature alone. Since the increased permeation from gel is more pronounced than that from solution and also the possibility of bubble formation in solution is more than that in the gel, the bubble activities should be reviewed.

It seems that cavitation was an important factor which caused the rate of diffusion of piroxicam from solution to be less than that from the gel despite the higher concentration of drug in the solution. Usually, there are some bubble nucleus existing in the liquid medium and these nuclei will grow under rectified diffu-

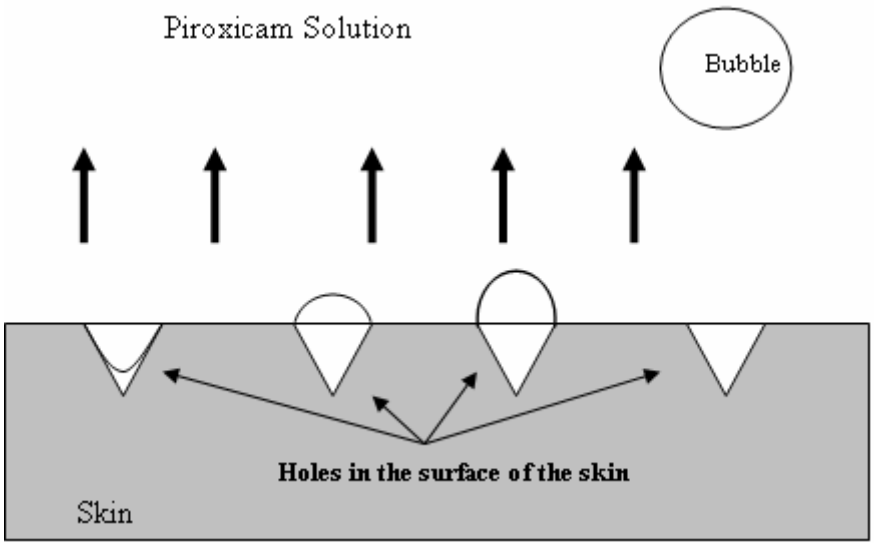

Fig. 4. Formation of bubble on the surface of the skin during sonication in solution

sion and form bubble in a sound field. There are three possible areas in which bubbles may form: in the liquid medium, on the surface of the skin and inside the epidermis. Close examination of the surface of the skin shows that the surface of the skin contains a large number of holes about 0.4mm in diameter [13]. These holes accommodate gas nuclei and when sonicated, the air will expand forming gas bubbles and because of the temperature rise in the skin, rectified diffusion may also contribute to their growth, as shown in Fig. 4. Mitragotri *et al.* and Wu *et al.* strongly believed that the growth of bubble by rectified diffusion does take place in the keratinocytes and lipid bilayer [3,14]. It was also believed that the presence of cavitation is the possible mechanism of the passage of high molecular weight drugs through the skin [14]. They realized that the cavitation influenced the penetration of drug through the skin, but the mechanism of action was not completely explained. It was reported by a number of researchers that the gas bubbles possessed a considerable negative electrical charges on the surface [10,15-20]. Margulis and Shiran experimentally measured the charge on bubble surface in distilled water to be 3×10^{-13} C and 10×10^{-13} C respectively [19,20]. Watmough *et al.* suggested that the charges on acoustically-excited bubbles are greater than gas/water interface in the absence of ultrasound [10]. They also stated that ultrasound is capable of maintaining the surface charges on the bubbles even in the presence of ions which would otherwise neutralize the charge.

The charged bubbles would thus be expected to attract the cationic drug ions and thus increase the local concentration of drug. Anionic drug ions, on the other hand, would be repelled by the electric field set up by micro-bubbles. Since piroxicam ions are negatively charged, local stirring caused by micro-streaming increases this depletion of piroxicam when resonant bubbles are excited by high local intensity [21]. Where the piroxicam concentration is maintained at its lowest level, less piroxicam enters the skin. There are greater numbers of induced gas bubble in piroxicam solution compared to gel formulation, which can repel the negatively-charged piroxicam ions. This explains why the amount of piroxicam measured from sonicated solution of piroxicam with five times higher concentration was lower than that from the gel. Such large difference has not been observed in the control groups. In fact, the penetration of drugs through the skin in control experiments with piroxicam solution was slightly higher. Bommannan *et al.* reported that penetration rate of drug through the skin for 2 MHz ultrasound was less than that for 16 MHz ultrasound [2]. They also stated that the treatment with ultrasound at 10 and 16 MHz significantly increased penetration of drug (almost 5 fold). They could not observe significant difference between the ultrasound-treated and controls at these frequencies. They concluded that "the enhancing effect of sonophoresis is due to direct effect of ultrasound on, presumably, stratum corneum". According to Hueter and Bolt "above 10 MHz frequency, a vapour type of cavitation is unlikely to occur" [22]. The presence of cavitation and presumably the negative charges of the drug ions suppressed the rate of penetration at 2 MHz frequency, while there were no bubbles at frequency of 10 and 16 MHz. The evidence provided here also suggests that it took almost 2 hours before the bubble (on the surface and possibly inside the keratinocytes and lipid bilayer) could dissolve and the passage for piroxicam diffusion be cleared to accelerate the drug passage.

In conclusion, this study has demonstrated that ultrasound significantly enhanced transdermal absorption of piroxicam from solution and gel formulations. It also suggests that the cavitation and its surface charge play a major rule in this enhancement. We also believe that the size of bubble over the surface of the skin, keratinocytes and lipid bilayer does affect the rate of permeation.

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