Effect of Pulsed Output Ultrasound on the Transdermal Absorption of Indomethacin from an Ointment in Rats

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The effect of pulsed output ultrasound (1 MHz) with on/off ratios of 1 : 2, 1 : 4 and 1 : 9 on transdermal absorption of indomethacin from an ointment was studied in rats. Ultrasound energy was supplied for between 10 and 19 min at a range of intensities ($1.0\text{--}2.5\text{ W}\cdot\text{cm}^{-2}$), energy levels commonly used for therapeutic purposes. The on/off pulsed ratio, intensity and the time of application were found to play an important role in the transdermal phonophoretic delivery system of indomethacin; 1 : 2 pulsed output ultrasound appeared to be the most effective in improving the transdermal absorption. The highest penetration was observed at an intensity of $1.0\text{ W}\cdot\text{cm}^{-2}$ and application time of 15 min. With pulsed output it was possible to use higher intensities of ultrasound without increasing skin temperature or damaging skin.

Key words phonophoresis; pulsed output ultrasound; continuous output ultrasound; transdermal absorption; indomethacin; skin temperature; skin damage

Phonophoresis is the enhancement of transdermal absorption of a drug by the application of ultrasound. There are several reviews of transdermal drug delivery by phonophoresis.¹⁻⁴⁾ Studies have been performed with various drugs^{2,4)} using different devices and different ultrasound conditions in terms of intensity, duration, frequency,⁵⁻⁷⁾ and continuous or discontinuous, *i.e.* pulsed mode. Although transdermal phonophoresis is normally carried out with a continuous output, the use of a pulsed output has been suggested as a method to obtain higher drug flux and to reduce skin damage.³⁾ Experimental studies with lignocaine and prilocaine⁸⁾ or inulin and physostigmine⁹⁾ appear to support this suggestion. However, there is limited quantitative information on the effect of pulsed ultrasound irradiation on the delivery of a transdermally administered drug.

In previous papers^{10,11)} it was demonstrated that therapeutic continuous ultrasound (1 MHz) could enhance the transdermal absorption of indomethacin from an ointment in rats. In subsequent work,¹²⁾ the effect of a pulsed output with an on/off ratio of 1 : 3 was examined at different intensities and durations. Pulsed ultrasound was found to be less effective as an enhancer than the same energy delivered in continuous mode. The on/off pulsed ratio, intensity and the time of application were suggested to play an important role in the transdermal phonophoretic delivery system. However, a 1 : 3 pulsed output ultrasound was used in these studies since a variable-on/off ratio generator was not available. In the present study, we investigated the effect of pulsed output ultrasound with on/off ratios of 1 : 2, 1 : 4 and 1 : 9 in the phonophoretic delivery of the model drug from an ointment in rats.

MATERIALS AND METHODS

Materials Indomethacin was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Gel ointment used was the commercially available Inteban ointment from Sumitomo Pharmaceutical Co. (Osaka, Japan), which contains 1% indomethacin. Other reagents used were of analytical grade.

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In Vivo Study Male Wistar rats weighing 250—350 g were used. The experiments were performed in a temperature-controlled room (21—3 °C). The day before the experiment the hair of the abdominal parts was carefully removed with an electric clipper and a razor without breaking the skin. On each study day the rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg), and 0.4 g of 1% indomethacin ointment was applied to a circular site (1.7 cm in diameter) on their abdominal skin. The area around the application site was covered with Saran Wrap® film (Asahi-Dow, Tokyo), followed by 1.5 g of ultrasonic gel (Echo Jelly, Aloka Co., Osaka). One MHz pulsed ultrasound with on/off ratios of 1 : 2, 1 : 4 and 1 : 9 or continuous ultrasound was then applied at the treated area for between 10—19 min with a range of intensities ($0.5\text{--}2.5\text{ W}\cdot\text{cm}^{-2}$). When the on/off ratios were 1 : 2, 1 : 4 and 1 : 9, the time proportion for sonication and nonsonication were 2 ms/4 ms, 2 ms/8 ms and 2 ms/18 ms, respectively. Ultrasonication was produced by a commercially available ultrasound device (Therasonic 1032, Electro-Medical Supplies, England) with a small treatment head which has a 1.7 cm diameter and effective radiating-area of 0.75 cm^2 . The ultrasound transducer was placed in a clamp attached to a stand, which could be easily raised or lowered. Control animals were treated by the same procedure, except that the ultrasonic transducer was not applied.

Blood samples (0.6 ml) were taken from the jugular vein at hourly intervals after drug administration. The assay of indomethacin was performed using HPLC as described previously.¹³⁾ The area under the plasma concentration curve (AUC) up to 4 h post-administration was calculated according to the trapezoidal rule.¹⁴⁾ The Student's *t*-test was utilized to estimate the significant differences between each ultrasound treatment group and the control group.

Skin hypodermic temperature was recorded on a thermometer (D613, Takara Kogyo Co., Tokyo) by means of a small incision in the rat abdominal skin and insertion of the temperature coupler (Takara SZL-64) into the hypodermis of the treated skin.

Histological Examination of the Skin Tissue The effect

of ultrasound on the skin tissue was checked by microscopic observation. The tissue samples excised after ultrasound treatment were fixed in formalin by the standard procedure, stained with hematoxylin-eosin, and observed with a microscope (model PM-10ADS, Olympus, Tokyo).

RESULTS

Effect of Pulsed Output Ultrasound on the Transdermal Absorption The effect of pulsed ultrasound on the drug absorption was studied by setting an ultrasound generator to an on/off ratio of 1:2, 1:4 or 1:9 pulsed output; this was supplied for between 10 and 19 min at an intensity range of $1.0\text{--}2.5\text{ W}\cdot\text{cm}^{-2}$, energy levels commonly used for therapeutic purposes.

Figure 1 shows the results of the experiment in which sonication was carried out with an on/off ratio of 1:2 pulsed mode at a range of intensities (1.0, 1.5 and $2.0\text{ W}\cdot\text{cm}^{-2}$) for 10 min. Table 1, summarizes the *AUC* value up to 4 h post-administration. The enhancement effect of 1:2 pulsed output ultrasound on the transdermal absorption of indomethacin was observed at the three ultrasound energy levels studied. As shown in Table 1, the mean *AUC* values after irradiation at these intensities were 1.5–1.7 times greater than the control value.

Figure 2 shows the change in hypodermic temperature during exposure to 1:2 pulsed ultrasound for 10 min at the different intensities. As the intensity of the ultrasound was increased, hypodermic temperature rose. Hypodermic temperatures were 35.3 , 38.7 and 40.2°C when ultrasound energy was applied for 10 min to rats at 1.0, 1.5 and $2.0\text{ W}\cdot\text{cm}^{-2}$, respectively.

To examine the effect of ultrasound on the skin, histological comparisons were made between rat skin tissue with and without the ultrasound. Figure 3 shows photomicrographs of rat skin at 10 min after application

of the 1:2 pulsed ultrasound. There was no change in skin tissue (epidermis, dermis and hypodermis) during exposure to the pulsed ultrasound of $1.0\text{ W}\cdot\text{cm}^{-2}$ (Fig. 3A) as compared with the control (not shown in figure). Rat skin treated with 1.5 and $2.0\text{ W}\cdot\text{cm}^{-2}$ (Fig. 3B and 3C) ultrasound, on the other hand, showed atrophy (arrow) and necrosis (arrows) in the epidermis, respectively.

The use of pulsed ultrasound with an on/off ratio of 1:4 at a range of intensities (1.5 , 2.0 and $2.5\text{ W}\cdot\text{cm}^{-2}$) for 10 min has also been shown to promote the transdermal absorption of indomethacin (Table 1). The effect was proportional to the intensity of the ultrasound; the higher the energy applied, the greater the drug penetration through the skin. The hypodermic temperature induced by a 1:4 pulsed ultrasound at 2.0 and $2.5\text{ W}\cdot\text{cm}^{-2}$ for 10 min was approximately 38°C in each case; however, atrophy in the epidermis was noted as the intensity was increased.

The effect of pulsed ultrasound with an on/off ratio of 1:9 on transdermal absorption of indomethacin was examined at an intensity of $2.5\text{ W}\cdot\text{cm}^{-2}$. There was no significant difference between ultrasound-treated and

Table 1. Comparison of *AUC* Values with and without Ultrasound

Treatment	Pulse ratio	Intensity ($\text{W}\cdot\text{cm}^{-2}$)	Time (min)	<i>AUC</i> ^{a)} ($\mu\text{g}\cdot\text{h}\cdot\text{ml}^{-1}$)	Enhancement factor ^{b)}
Control	—	—	—	4.19 ± 0.56	1.0
Pulsed ultrasound	1:2	1.0	10	6.17 ± 1.73	1.5
		1.0	15	9.65 ± 1.32^c	2.3
		1.0	19	7.05 ± 1.21	1.7
		1.5	10	7.24 ± 1.70	1.7
		2.0	10	7.04 ± 0.64^e	1.7
	1:4	1.5	10	5.53 ± 0.37	1.3
		2.0	10	6.94 ± 1.74	1.7
		2.5	10	7.88 ± 0.74^c	1.9
	1:9	2.5	10	4.83 ± 0.94	1.2
		2.5	10	7.62 ± 0.43^d	1.8
Continuous ultrasound	—	0.5	10	9.44 ± 1.31^c	2.3
	—	1.0	10	6.45 ± 0.80	1.5

a) Each value represents the mean $\pm$ S.E. ($n=4$). b) Enhancement factor relative to *AUC* from the control. c) $p<0.01$, d) $p<0.02$, e) $p<0.05$ vs. controls, by Student's *t*-test.

Fig. 1. Effect of Intensity of 1:2 Pulsed Ultrasound on the Plasma Levels of Indomethacin from an Ointment in Rats

Each rat was irradiated with the pulsed ultrasound for 10 min at the intensity levels of 0 ($\circ$), 1.0 ($\bullet$), 1.5 ($\blacktriangle$), and $2.0\text{ W}\cdot\text{cm}^{-2}$ ($\blacksquare$). Each value is the mean $\pm$ S.E. of 4 experiments.

Fig. 2. Effect of Intensity of 1:2 Pulsed Ultrasound on the Hypodermic Temperature of Rats

Each rat was irradiated with the pulsed ultrasound for 10 min at the intensity levels of 1.0 ($\circ$), 1.5 (Δ), and $2.0\text{ W}\cdot\text{cm}^{-2}$ ($\square$).

Fig. 3. Photomicrographs of Rat Skin after 1:2 Pulsed Ultrasound Irradiation for 10 min at the Intensity Levels of 1.0 (A), 1.5 (B), and 2.0 (C) $\text{W} \cdot \text{cm}^{-2}$

control skin.

The effect of the duration of treatment on the transdermal absorption of indomethacin was also studied. In this study, 1:2 pulsed output ultrasound was applied at a fixed intensity of $1.0 \text{ W} \cdot \text{cm}^{-2}$ while the duration of ultrasound application was varied: 10, 15, and 19 min. Figure 4 shows the mean plasma level profile of indomethacin and the effect of the duration of phonophoresis treatment on the AUC values is summarized in Table 1.

Fig. 4. Effect of the Time of Application of 1:2 Pulsed Ultrasound on the Plasma Levels of Indomethacin from an Ointment in Rats

Each rat was irradiated with the pulsed ultrasound at $1.0 \text{ W} \cdot \text{cm}^{-2}$ for 0 (○), 10 (●), 15 (▲), and 19 (■) min. Each value is the mean $\pm$ S.E. of 4 experiments.

The time of ultrasound application was also found to be important in the transdermal phonophoretic delivery of indomethacin. The magnitude of enhancement in the transdermal absorption of the drug following phonophoresis treatment was not proportional to the time of application. AUC values increased up to 15 min and then declined as exposure time lengthened (19 min) at an intensity of $1.0 \text{ W} \cdot \text{cm}^{-2}$. The increase in hypodermic temperature during exposure to ultrasound for 15 min at $1.0 \text{ W} \cdot \text{cm}^{-2}$ was similar to that induced by 10 min irradiation at this intensity (Fig. 2). This rise in the temperature did not damage the skin membrane, so far as could be determined microscopically.

Effect of Continuous Output Ultrasound on the Transdermal Absorption The effect of continuous ultrasound on the drug absorption was studied by supplying energy for 10 min at a range of intensities (0.5, 1.0 and $1.5 \text{ W} \cdot \text{cm}^{-2}$); $1.0 \text{ W} \cdot \text{cm}^{-2}$ was the intensity inducing the greatest absorption enhancement (Table 1). However, in our opinion, $0.5 \text{ W} \cdot \text{cm}^{-2}$ is the preferred intensity since its application for 10 min did not cause marked increase in skin temperature or any significant skin tissue damage. The mean AUC value after irradiation at this intensity was 1.8 times greater than the control value. Hypodermic temperatures after the ultrasound application at 0.5, 1.0 and $1.5 \text{ W} \cdot \text{cm}^{-2}$ for 10 min were 37.2 , 40.6 and 42.8°C , respectively. Higher ultrasound intensity and longer treatment sometimes cause skin burning.³⁾ Progressively more skin burning was noted in this study as the intensity was increased. Rat skin treated with $1 \text{ W} \cdot \text{cm}^{-2}$ ultrasound showed atrophy in the epidermis.

Comparison of Pulsed and Continuous Output Ultrasound The influence of 1:2 pulsed output at $1.5 \text{ W} \cdot \text{cm}^{-2}$ for 10 min and at $1.0 \text{ W} \cdot \text{cm}^{-2}$ for 15 min, 1:4 pulsed output at $2.5 \text{ W} \cdot \text{cm}^{-2}$ for 10 min, and continuous output at $0.5 \text{ W} \cdot \text{cm}^{-2}$ for 10 min with the same total energy ($5 \text{ W} \cdot \text{min} \cdot \text{cm}^{-2}$) were compared (Table 1). The results

indicated that the 1:2 pulsed output ultrasound at $1.0 \text{ W} \cdot \text{cm}^{-2}$ for 15 min was the most effective condition for the technique of phonophoresis with the topical formulation examined here. Its application did not cause marked skin temperature increase or any significant skin tissue damage. With this protocol, indomethacin delivery was enhanced 2.3 times relative to the control.

DISCUSSION

Ultrasound treatments may be administered either on a continuous or on a pulsed mode. As the term implies, with the pulsed mode there is a time interval between ultrasonic outputs. Pulsed mode, typically with 2–3 ms on and 10–20 ms off, allows a higher intensity to be used during the pulse with less chance of tissue damage.²⁾ The present study demonstrated that therapeutic pulsed ultrasound could enhance the transdermal absorption of indomethacin from an ointment to a statistically significant extent in rat. The on/off pulsed ratio, intensity and the time of application were found to have an important function in the transdermal phonophoretic delivery system of this drug; 1:2 pulsed output ultrasound appeared to be the most effective in improving the transdermal absorption. The highest penetration was observed at an intensity of $1.0 \text{ W} \cdot \text{cm}^{-2}$ and application period of 15 min (Fig. 4). The energy of ultrasound should be high enough to obtain the desired absorption enhancement but low enough not to cause any significant rise in skin temperature or skin damage. The maximum limit of exposure should be determined by measuring the skin temperature; $1.0 \text{ W} \cdot \text{cm}^{-2}$ of 1:2 pulsed ultrasound for 15 min did not cause notable skin temperature increase or any significant skin tissue damage. With this protocol, indomethacin delivery was enhanced 2.3 times relative to the control.

When the influence of pulsed and continuous ultrasound with the same total energy ($5 \text{ W} \cdot \text{min} \cdot \text{cm}^{-2}$) were compared, pulsed mode with an on/off ratio of 1:2 was found to be better than others for phonophoretic transport of the drug from the examined topical formulation. In previous work,¹²⁾ however, the continuous ultrasound was found to be more effective than 1:3 pulsed mode in improving the transdermal absorption of indomethacin. Benson *et al.*⁸⁾ investigated the influence of ultrasound on the transdermal absorption of lignocaine and prilocaine from Emla cream in healthy subjects, and they showed that 1:1 pulsed mode was better for phonophoretic transport of these drugs. The use of 1 MHz ultrasound at

$3 \text{ W} \cdot \text{cm}^{-2}$ pulsed 4:1 has also been shown to significantly promote the dorsal topical absorption in both rats and guinea pigs of inulin or physostigmine.⁹⁾ These results suggest that pulsed ultrasound with larger on/off ratios, such as 1:1 and 4:1, is more effective as an enhancer than the same energy delivered in the pulsed mode with smaller on/off ratios.

It would be appear from these results that the optimum value of on/off ratio varies with the intensity and the time of application applied. Therefore, it is essential to select optimum pulse mode parameters to attain the best facilitating effect of phonophoresis. The sensitivity and the tolerance of the skin in response to the irradiation conditions used should also be considered.

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