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Comparison of Thermal Effect with Ultrasound in Rat Calf Muscles after the Application of Five Non-Steroidal Anti-Inflammatory Drugs

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Background and Purposes: Phonophoresis has been defined as the migration of drugs through the skin under influence of ultrasound (US). The phonophoresis of nonsteroidal anti-inflammatory drugs (NSAIDs) was studied in vivo through hairless rat skin to determine the temperature changes in superficial and deep tissues in response to NSAID phonophoresis.

Study Design and Objectives: To measure and compare the temperature changes in tissues in response to five NSAIDs under phonophoresis.

Methods: Male Wistar rats weighing 250 to 350 g had one of five drugs; i.e., piroxicam (Feldene), indomethacin (Indocin), etofenamate (Rheumon), methylsalicylate (Salomethyl), or diclofenac (Voren) applied to one hindlimb followed by phonophoresis. The other hindlimb served as the sham-treated control. US intensities of 1.0W/cm^2 at a fixed frequency of 1 MHz were applied in continuous or pulsed waves with 50% and 25% duty cycles for 5, 10, or 20 minutes. Tissue temperatures were assessed by a digital recorder with hypodermic needle microprobes before and after phonophoresis.

Results: Significant temperature rises were produced in skin and muscle after five NSAID phonophoretic and sham treatments. But these temperature rises showed significant differences among five NSAIDs in the deep muscle of phonophoresis-treated limbs ($P< 0.05$). Especially after piroxicam and diclofenac phonophoresis, the deep temperatures were significantly higher in pulsed-wave US treated limbs than those treated with sham ($P< 0.05$).

Conclusion: The use of piroxicam or diclofenac enhances the thermal effect of deep tissue during US phonophoresis, which may potentially further increase the percutaneous absorption of these drugs.
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Key words: ultrasound, phonophoresis, nonsteroidal anti-inflammatory drugs, tissue temperature, rat

INTRODUCTION

Phonophoresis is the skin penetration-enhancing methods of a drug across the viable epidermis into the underlying tissues by the application of therapeutic ultrasound (US).[1] Although phonophoresis has been applied in physiotherapy clinics for almost 40 years, its

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therapeutic efficacy is still under question, and the biophysical mechanisms involved are not fully understood.[2] Percutaneous administration of drugs assisted by phonophoresis offers many advantages, but only a few drugs are currently available for transdermal application due to low permeability of the skin to many molecules.

According to Byl,[3] both the thermal and the nonthermal characteristics of US enhance the diffusion of topically administered drugs. Heat from US increases the kinetic energy of drug molecules and the cell membrane and enhances permeability of cutaneous entry points, such as hair follicles and sweat glands. It also increases local perfusion to the area, improving the likelihood that the drug will diffuse through the stratum corneum and be collected by the capillary network in the dermis. Therefore, US is frequently adopted for its thermal effects with local tissue temperature elevation.[3] The thermal effect of US seems to be the main factor which enhances percutaneous administration under the conditions used in physical medicine.[4] The exact mechanism of phonophoresis may be a result of the increased fluidity of the barrier domains and kinetic energy of the permeant molecules due to conversion of wave energy to mechanical energy, and heat generation within the stratum corneum. Therefore, the use of phonophoresis may be damaging to the skin or subcutaneous structures due to hyperthermia if frequency and intensity of application are extensive. In addition, the ability of a drug to penetrate the skin is closely related to its molecular weight and its affinity for the stratum corneum. It was concluded that the effect of phonophoresis depends on the nature of the drug, the formulation base, and conditions of US application.

Phonophoresis is often chosen when inflammation occurs in deep rather than superficial tissues.[5] Physiotherapists have particularly focused on hydrocorticone and nonsteroidal anti-inflammatory drugs phonophoresis (NSAID) in order to treat various inflammatory conditions.[6] Unfortunately, most of these treatments have been conducted on a rather subjective and non-quantitative basis.[7] Studies have been performed with various drugs[3,8] and using different US devices and conditions (intensity, duration, frequency, continuous or pulsed mode).[8-11] In this study, we investigated the thermal effect of continuous and pulsed US output using a therapeutic frequency (1 MHz) with on/off ratios of 1:1 and 1:3 on the phonophoretic delivery of five popular NSAID ointments in rats. The purpose of the present investigation is to quantify the temperature changes in superficial and deep tissues during the each NSAID phonophoresis at mode and duration of US commonly used in clinic.

MATERIALS AND METHODS

Preparation of Animals

Male Wistar rats (N= 75) weighing 250 to 350g were used and randomly divided into five groups for treatment with five different NSAIDs. The experiments were performed in a temperature-controlled room (25 °C). The animal experimental design and care in this study were approved by FooYin University Institutional Animal Care and Use Committee. The day before the experiment, the hair of the hindlimb was carefully removed with an electric clipper and razor without breaking the skin. The rats were fixed and anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg), and the resting body temperature was kept between 36.5 °C and 37.0 °C throughout the experiments. Circular skin sites about 2.0 cm in diameter on left and right hindlimbs were selected for drug application. The area around the application site was covered with Saran Wrap film (Asahi-Dow, Tokyo).

NSAID Ointments and Reagents

Five ointments commonly used in rehabilitation clinics; i.e., Feldene (piroxicam; N=15), Indocin (indomethacin; N=15), Rheumon (etofenamate; N= 15), Salomethyl (60% methylsalicylate, 8% eugenol and 32% menthol; N=15) and Voren-G (diclofenac sodium; N=15), were purchased from Pfizer Inc. (Germany), German Organic Pharm. Co. (Taiwan), Bayer AG (Germany), Tanable Seiyaku Co. (Taiwan), and Yung-Shin Pharm. Co. (Taiwan), respectively. The components of the US gel (Aquasonic 100; Parked Laboratories, Orange, NJ) are water, ethylenediamine tetra-acetate, methylisothiazolinone, methylchloroisothialzolinone, pro- pylene glycol, imidazolidinyl urea sodium hydrate, and Pattern Blue V.

US and Phonophoretic Techniques

An NSAID ointment (0.4 g) was rubbed into the skin of the left hindlimb, and a standard coupling medium (1.5

g) was applied over the medication. Simultaneously, the right hindlimb in the same rat was used for the sham treatment; i.e., only US application without NSAID delivery. One-MHz continuous or pulsed US with on/off ratios of 1:1 or 1:3 was then applied for 5, 10, or 20 minutes with an averaged intensity of 1.0 W/cm^2, i.e., the spatial average - temporal average intensity (I_{SATA}) of 1.0, 0.5 or 0.25 W/cm^2, respectively. When the on/off ratios were 1:1 and 1:3, the times for sonication and non-sonication were 2 ms/2 ms and 2 ms/6 ms, respectively. Sonication was produced using a commercially available device (US-3; ITO Co., Japan) with a treatment head of 1.7 cm diameter and an effective radiating area of 0.75 cm^2. The US head was moved over a treated area using small, continuous, circular movements. The US unit was less than 1 year old and was calibrated via a US power meter before the study.

Temperature Measurements in vivo

Two electronic digital thermometers (Physitemp Thermalet Model TH-8; Physithemp Instruments, Clifton, NJ) which display the temperature in degrees Celsius were simultaneously used for recoding the temperatures of NSAID-treated and sham-treated sides in the same animal. The temperature measurements of rats were performed with the experimenter blind to which group was treated with the drug phonophoresis. Each thermometer had two thermistors (hypodermic needle microprobes) that were used for superficial and deep measurements (Physitek MT29/5; Physithemp Instruments). The thermistor was sterilized the evening before each data collection by immersing it in glutaraldehyde solution (Cidex) for eight hours. This procedure was repeated for 15 min between each pair of animals. One of the thermistors was inserted (slight slanting to the skin) at a 45° angle into the belly. The distance from the muscle through the sagittal plane to the tip of the needle was about 1 cm. This represented the 1 cm tissue thickness that US waves needed to penetrate to cause the thermistor needle to react to any change in temperature. The other thermistor needle was placed on the skin overlying the muscle belly. The thermistors were then connected to the monitor, and the temperature was recorded. Temperatures before and after phonophoresis were measured in the skin and muscle. The thermistors were not removed until the phonophoresis treatments were finished. The same technique of temperature measurement was practiced throughout the experiments. During the experimental period, rectal temperature was also monitored to control the stable animal's core temperature.

Data Analysis

Means and standard deviations (SD) of the temperatures were calculated for the different US modes. The differences in the temperatures (Δt) before and after phonophoresis treatments were also recorded and statistically analyzed by a paired *t*-test of variance. General linear model (GLM) procedures with Tukey HSD post hoc tests were used to analyze the differences and to test for statistical significances among the five NSAID phonophoresis and three modes of US output. A *P* value of less than 0.05 was considered significant. All data were analyzed using the Statistical Package for the Social Sciences Version 10.0 for Windows (SPSS Inc., Illinois).

RESULTS

Effects of NSAIDs with Phonophoretic Delivery under Continuous-output US

The effect of continued US on the temperature rise was studied by supplied energy for 5 minutes at an intensity of 1.0W/cm^2. The mean increase in the skin temperature ranged from 0.48 °C to 1.07 °C in five NSAID-treated limbs and from 0.52 °C to 0.81 °C in sham-treated limbs, while the temperature elevations in the muscle were from 0.47 °C to 2.17 °C and from 0.99 °C to 1.79 °C, respectively. When the baseline temperature (temperature at time 0, pre-treatment) was selected as the reference temperature for pair-wise comparisons, changes in skin and muscle temperatures were statistically significant after treatment with NSAID phonophoresis-treated limbs and sham-treated limbs (paired *t*-test, $P < 0.05$). There were no significant differences temperature rise (Δt) of the skin and muscle between the NSAID and the sham treatments for each drug (paired *t*-test, $P > 0.05$). There were significant differences in the temperature rise (Δt) measured in the muscle among the five NSAIDs (GLM, $P < 0.05$), especially between piroxicam and indomethacin (Tukey HSD, $P < 0.05$). The rectal

temperatures did not change significantly (paired *t*-test, $P > 0.05$) at any point during the experiments (Table 1).

Effects of NSAIDs with Phonophoretic Delivery under Pulsed-output US

With an on/off ratio of 1:1, there was a mean increase in the skin temperature in the range of 0.43 °C to 0.98 °C in five NSAID-treated limbs, and 0.74 °C to 0.85 °C in the sham-treated limbs. The mean temperature elevations in the muscle were from 0.65 °C to 2.62 °C in five NSAID-treated limbs and from 0.65 °C to 1.37 °C in sham-treated limbs. Comparison of the baseline and post-treatment temperatures showed statistically significant changes in both the skin and the muscle with NSAID phonophoresis-treated limbs and sham-treated limbs (paired *t*-test, $P < 0.05$). A 50% pulsed-output US exposure produced temperature rise (Δt) in both skin and muscle with all treatments. A significant difference of Δt between NSAID and sham treatment was shown in the muscle during application of diclofenac (paired *t*-test, $P < 0.001$). Significant differences in the temperature rise in the muscle were seen among the five NSAIDs (GLM, $P<0.05$), particularly between diclofenac and the other four drugs (Tukey HSD, $P < 0.05$). The rectal temperatures did not change significantly (Table 2).

With an on/off ratio of 1:3, the mean increase in the skin temperature was in the range of 0.22 °C to 1.10 °C in five NSAID-treated limbs, and 0.39 °C to 0.97 °C in sham-treated limbs. The temperature elevations in the muscle were from 0.91°C to 2.25°C with NSAID treatments and from 0.78°C to 1.44°C with sham treatments. The difference between the baseline and post-treatment temperatures in the skin and muscle were statistically significant with all of five NSAID-phonophoresis treated limbs and sham-treated limbs (paired *t*-test, $P < 0.05$). A 25% pulsed output of US produced temperature rise (Δt) both at the skin and in the muscle with both NSAID and sham treatments. A significant difference was observed in the muscle temperature between sham treatment and the application of piroxicam (paired *t*-test, $P < 0.05$) and diclofenac (paired *t*-test, $P < 0.05$). The temperature rise (Δt) in the skin and muscle were significant different among the five NSAIDs (GLM, $P < 0.05$). Differences were observed between indomethacin and etofenamate in skin temperature and in diclofenac compared with the others for muscle temperature (Tukey HSD, $P < 0.05$). The rectal temperatures did not change significantly (Table 3).

Comparison of Pulsed- and Continuous-output US

The temperature increases induced by continuous US output for 5 minutes and 1:1 pulsed output for 10 minutes and 1:3 pulsed output for 20 minutes were compared (Figure 1). The piroxicam and diclofenac phonophoretic delivery at 1.0 W/cm^2 produced a significant temperature rise in the muscle among continuous, 50%, and 25% pulsed US output modes (GLM, $P < 0.05$). Significant differences were shown between continuous and 50%, and between 25% and 50% pulsed US outputs with piroxicam phonophoresis (Tukey HSD, $P < 0.05$) and between continuous and two pulsed modes with diclofenac phonophoresis (Tukey HSD, $P < 0.05$). With these two drugs, the muscle temperatures were higher than with the sham treatments.

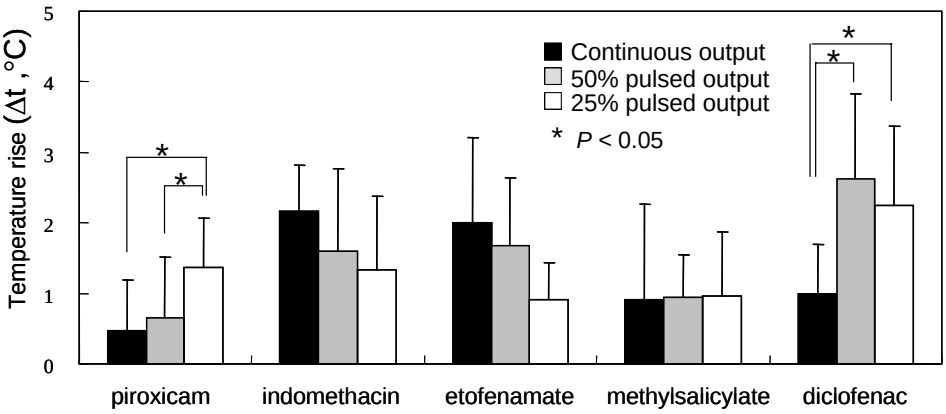

Figure 1. Temperature rise in muscle with continuous and 50% and 25% pulsed-output US with piroxicam, indomethacin, etofenamate, methylsalicylate, and diclofenac phonophoresis.

Table 1. Temperature changes during phonophoretic delivery of five NSAIDs with continuous-output 1 MHz US at 1.0 W/cm^2 for 5 minutes

Temperature (°C)		Piroxicam (n=15)		Indomethacin (n=15)		Etofenamate (n=15)		Methylsalicylate (n=15)		Diclofenac (n=15)		Differences among drugs*	
		Exp.	Sham	Exp.	Sham	Exp.	Sham	Exp.	Sham	Exp.	Sham	Exp.	Sham
Skin	Pre	35.62±0.51	35.53±0.28	35.35±0.47	35.43±0.37	35.57±0.44	35.23±0.34	35.40±0.34	35.35±0.29	35.31±0.50	35.40±0.46		
	Post	36.10±0.73†	36.05±0.45†	36.05±0.66†	36.06±0.55†	36.21±0.53†	36.05±0.67†	36.31±0.59†	36.13±0.58†	36.38±0.62†	36.11±0.41†		
	Δt	0.48±0.59	0.52±0.52	0.70±0.54	0.63±0.55	0.63±0.57	0.81±0.52	0.91±0.62	0.79±0.51	1.07±0.66	0.71±0.58	NS	NS
P, exp. vs. sham (Δt)‡		0.8453		0.7155		0.3733		0.5459		0.1234			
Muscle	Pre	36.65±0.70	36.51±0.32	36.11±0.42	36.25±0.38	36.05±0.53	36.17±0.43	36.14±0.39	36.31±0.42	36.65±0.32	36.38±0.68		
	Post	37.12±0.69†	37.50±0.83†	38.29±2.71†	37.91±1.02†	38.05±1.16†	37.96±1.14†	37.06±1.02†	37.49±0.90†	37.65±0.61†	37.91±1.29†		
	Δt	0.47±0.72	0.99±0.85	2.17±2.65	1.67±0.98	2.00±1.21	1.79±1.16	0.92±1.35	1.18±0.86	0.99±0.71	1.53±1.26	*P*< 0.05	NS
P, exp. vs. sham (Δt)‡		0.0859		0.4929		0.6269		0.5352		0.1651			
Rectal	Δt	0.01±0.34		0.03±0.29		0.02±0.23		0.05±0.29		0.08±0.22			
	p§	0.3413		0.4739		0.4023		0.5857		0.7618			

* GLM = general linear model

† P <0.05 (paired *t*-test) comparing difference between baseline (Pre) and post-treatment (Post) temperature.

‡ P <0.05 (paired *t*-test) comparing difference between temperature rise (Δt) of experimental (exp.) and sham hind limbs.

§ Rectal temperature changes in pre- vs. post-treatment tested by paired *t*-test.

NS: not significant.

Table 2. Temperature changes during phonophoretic delivery of five NSAIDs with 50% pulsed-output 1 MHz US at 1.0W/cm^2 for 10 minutes

Temperature (°C)		Piroxicam (n=15)		Indomethacin (n=15)		Etofenamate (n=15)		Methylsalicylate (n=15)		Diclofenac (n=15)		Differences among drugs*	
		Exp.	Sham	Exp.	Sham	Exp.	Sham	Exp.	Sham	Exp.	Sham	Exp.	Sham
Skin	Pre	35.41±0.37	35.34±0.40	35.53±0.24	35.33±0.35	35.48±0.47	35.28±0.39	35.34±0.77	35.40±0.31	35.37±0.62	35.43±0.54		
	Post	35.84±0.55†	36.08±0.42†	36.39±0.63†	36.17±0.51†	36.09±0.68†	36.08±0.67†	36.27±0.81†	36.15±0.63†	36.35±0.50†	36.17±0.52†		
	Δt	0.43±0.77	0.74±0.56	0.86±0.64	0.85±0.39	0.61±0.73	0.80±0.57	0.93±0.70	0.75±0.60	0.98±0.63	0.75±0.52	NS	NS
P, exp. vs. sham (Δt)‡		0.2250		0.9455		0.4260		0.4716		0.2780			
Muscle	Pre	36.30±0.59	36.46±0.33	36.21±0.51	36.13±0.49	36.05±0.91	36.16±0.48	36.29±0.27	36.30±0.59	36.34±0.53	36.36±0.29		
	Post	36.95±0.76†	37.11±0.61†	37.81±1.28†	37.50±0.77†	37.71±1.42†	37.78±0.98†	37.23±0.63†	37.70±1.00†	38.96±1.28†	37.56±0.75†		
	Δt	0.65±0.86	0.65±0.59	1.60±1.17	1.37±0.74	1.67±0.97	1.79±1.16	0.94±0.60	1.40±1.18	2.62±1.21	1.20±0.82	*P*< 0.05	NS
P, exp. vs. sham (Δt)‡		1.0000		0.5186		0.8947		0.1899		**0.0008**‡			
Rectal	Δt	-0.03±0.29		0.06±0.25		0.07±0.31		0.01±0.25		0.01±0.36			
	p§	0.7728		0.8237		0.1702		0.6037		0.7121			

* GLM = general linear model

† P <0.05 (paired *t*-test) comparing difference between baseline (Pre) and post-treatment (Post) temperature.

‡ P <0.05 (paired *t*-test) comparing difference between temperature rise (Δt) of experimental (exp.) and sham hind limbs.

§ Rectal temperature changes in pre- vs. post-treatment tested by paired *t*-test.

NS: not significant.

Table 3. Temperature changes during phonophoretic delivery of five NSAID with 25% pulsed-output 1 MHz US at 1.0W/cm^2 for 20 minutes

Temperature (°C)		Piroxicam (n=15)		Indomethacin (n=15)		Etofenamate (n=15)		Methylsalicylate (n=15)		Diclofenac (n=15)		Differences among drugs*	
		Exp.	Sham	Exp.	Sham	Exp.	Sham	Exp.	Sham	Exp.	Sham	Exp.	Sham
Skin	Pre	35.48±0.43	35.31±0.38	35.35±0.42	35.38±0.29	35.47±0.41	35.67±0.49	35.31±0.22	35.41±0.52	35.24±0.38	35.21±0.34		
	Post	36.34±0.78†	35.85±0.40†	36.45±0.82†	36.25±0.47†	35.69±0.50†	36.05±0.85†	35.75±0.63†	36.39±0.84†	36.15±0.53†	35.98±0.54†		
	Δt	0.86±0.74	0.55±0.58	1.10±0.95	0.87±0.56	0.22±0.39	0.39±0.79	0.44±0.66	0.97±0.89	0.91±0.51	0.77±0.49	$P<0.05$	NS
P, exp. vs. sham (Δt)‡		0.2098		0.4182		0.4722		0.0739		0.4289			
Muscle	Pre	36.29±0.44	36.15±0.25	36.09±0.46	36.41±0.33	36.00±1.53	36.17±0.59	36.35±0.32	36.46±0.35	36.20±0.41	36.39±0.40		
	Post	37.65±0.81†	36.93±0.55†	37.43±1.16†	37.42±0.58†	36.91±1.67†	37.11±0.73†	37.31±0.90†	37.97±0.72†	38.45±1.30†	37.83±0.83†		
	Δt	1.36±0.71	0.78±0.58	1.33±1.04	1.01±0.70	0.91±0.53	0.94±0.92	0.96±0.91	1.51±0.70	2.25±1.12	1.44±0.73	$P<0.05$	NS
P, exp. vs. sham (Δt)‡		**0.0212‡**		0.3316		0.9039		0.0765		**0.0261‡**			
Rectal	Δt	0.00±0.36		0.01±0.36		-0.01±0.28		0.02±0.26		0.07±0.29			
	p§	0.3362		0.8306		0.5038		0.7186		0.9515			

* GLM = general linear model

† $P<0.05$ (paired *t*-test) comparing difference between baseline (Pre) and post-treatment (Post) temperature.

‡ $P<0.05$ (paired *t*-test) comparing difference between temperature rise (Δt) of experimental (exp.) and sham hind limbs.

§ Rectal temperature changes in pre- vs. post-treatment tested by paired *t*-test.

NS: not significant.

DISCUSSION

The present study demonstrated that therapeutic continuous and pulsed US as physical enhancers of transdermal delivery of five NSAIDs could enhance the temperature rise. Especially after piroxicam and diclofenac phonophoresis, the muscle temperatures were significantly higher in pulsed-wave US treated limb than those treated with sham. Several phenomena may explain the increase in temperature at the skin surface and within skin when exposed to US. The extent of the temperature increase during NSAID phonophoretic application depends on the US field parameters, the US absorption, thermal conduction, drug actions and blood perfusion of the tissue.

It has been generalized without preliminary studies of phonophoresis to show real increase in transdermal transport and in vitro studies carried out till the 1980's have demonstrated increased percutaneous absorption of various drugs. The limitation of topical delivery is that the skin constitutes a very efficient barrier against the penetration of exogenous compounds. Skin permeability is increased by increase in temperature.[12,13] The energy of US should be high enough to increase skin permeability and obtain the desired absorption enhancement but low enough not to cause any significant rise in skin temperature or any skin damage. However, conflicting findings have been published showing only slight effects or even the absence of effect on skin permeability. The ability of a drug to penetrate the skin is also related to its molecular weight. More recently, the possibility of rendering the skin permeable to large molecules such as insulin or low molecular weight heparin has been confirmed with in the use of US to permeate the skin.[14,15] A rise in temperature is one of major factors which significant increased in percutaneous diffusion rates with various molecules within molecular weights varying from 138 to 781, including hydrocortisone and salicylic acid.[4,16] In the present study, the molecular weight of five NSAIDs with molecular weights within 380 belonged to low- molecular-weight compounds, so temperature rise was a major factor that influenced percutaneous absorption.

Machet et al. reported an increase of 15 °C to 30 °C

for intensities ranging from 1 to 3 W/cm^2 of 3.3 MHz with continuous mode for 10 minutes, and significantly increased absorption of digoxin through mouse skin.[7] Miyazaki et al. 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.[17] Moreover, Julian and Zentner reported the diffusion flux of hydrocortisone was multiplied four-fold with low frequency phonophoresis (20 kHz, 10-30 W/cm^2), and the temperature increased from 25 °C to 75 °C.[18] The increase in temperature primarily depends on the acoustic frequency, intensity, duration of US and thermal characteristics of the medium.[7] However, a rise in skin temperature has been proved to be one of the major factors which can explain the increase in percutaneous absorption in the high and low frequency and in continuous mode of US.[7,12] Greater absorption, with the potential for undesirable US-induced temperature increase, is found in skin, tendon, and spinal cord.[19] In the present study, piroxicam, indomethacin, etofenamate, methylsalicylate, and diclofenac delivery by US resulted in temperature increases from 0.5 °C to 2.5 °C in skin surface and muscle tissue with high frequency US (1 MHz) for 5 to 20 minutes. Although it cannot directly explain how US could increase percutaneous absorption, it does suggest that US could potentially enhance transdermal absorption of these drugs due to its thermal effect. Most authors have focused on measuring the rise in skin temperature at the surface in vitro. Paradoxically, temperature within the skin and deep tissues has rarely been measured in the literature. In the present study, piroxicam and diclofenac produced the greatest effect in temperature rise at deep muscle tissue. In general, biological tissues and cells may be considered to be at greater risk of hyperthermic destruction with these drugs when US is applied for a long time. Future in vivo studies are needed to investigate tolerance and transdermal transport in human.

Phonophoresis treatment may be administered in either a continuous or a pulsed mode of US. When the same total energy (5 W/min/cm^2) was compared, the pulsed mode with an on/off ratio of 1:2 was found to be better for phonophoretic transport of the indomethacin from topical formulations.[20] The 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.[1] In a comparison of pulsed-output US with on/off ratios of 1:2, 1:4, and 1:9, 1:2 appeared to be the most effective in encouraging the transdermal absorption of indomethacin.[20] The 1:1 pulsed mode is more effective in inducing transdermal absorption of benzydamine hydrochloride in healthy subjects.[20] The previous results suggested that pulsed US with larger on/off ratios is more effective as an enhancer. The hyperthermia-induced skin lesion was less marked with pulsed mode for long-term application.[21] The present study demonstrated that deep tissue temperature could be enhanced by all of five NSAID phonophoretic treatments, with both regardless continuous and pulsed output of US. But the on/off ratio and the time of application had important effects on the transdermal phonophoretic delivery of piroxicam and diclofenac. Clearly, the thermal effects caused by US output varied in drug transdermal transport and absorption, and probably depend on the individual characteristics and composition of drugs.

In the present study, the data reflects the augmentation of the positive synergistic thermal action between diclofenac, piroxicam and US at the deep tissue level. The mechanism by which diclofenac and piroxicam phonophoresis enhances the thermal effects of pulsed US is not entirely clear. A recent study demonstrated that therapeutic US (1 MHz US, I_{SATA}= 0.5 W/cm^2) enhanced the percutaneous penetration of the topical diclofenac gel and plasma diclofenac mass.[22] Therefore, the muscle microvascular permeability was probably affected by penetration of diclofenac and piroxicam. Diclofenac and piroxicam, the cyclooxygenase inhibitors, inhibited the vascular permeability response and decreased blood flow in muscular layer.[23,24] This means that further US energy is deposited as heat due to the slower heat dissipation caused by diclofenac or piroxicam-induced reduction of thermal diffusion and local blood flow. In addition, the total application time of US regarded as the sum of times with and without US application may be a factor which aggravates the thermal effect of diclofenac and piroxicam phonophoresis; i.e., the longer application time corresponds to higher thermal enhancement effect caused by repetitively stroke of a US head (2.27 cm^2) over a small skin area (3.14 cm^2). In the present study, with a 20-minute exposure of pulsed mode of US, the rise in temperature of muscle was higher significantly for

phonophoretic transport of the diclofenac or piroxicam from topical formulations. This temperature rise due to the longer clinical use of phonophoresis of these drugs over a limited area is noteworthy.

US has been used in sports medicine, alone or associated with various anti-inflammatory agents, to treat strains, sprains, tendonitis, bursitis, and epicondylitis at frequencies ranging from 0.7 to 1.1 MHz, with intensities from 1 to 2 W/cm^2, in continuous or pulsed mode.[3,5,25] Clinical reports have documented successful phonophoresis of iodide solution for treating osteoarthritis associated with gout attacks,[26] trolamine salicylate cream for muscle soreness,[27] and interferon for herpetic keratitis.[28] Anti-inflammatory agents, such as corticosteroids and salicylates, have been a frequent focus of interest as the topical drug for phonophoresis studies. It has been assumed that the US energy was assumed to have distributed the drug through the inflamed tissue and enhanced its absorption. However, on the basis of the research reviewed, the benefit of enhancement of benzydamine, indomethacin, and salicylate absorption is questionable.[3,20,29] The US of 1 MHz of therapeutic US could enhance the transdermal absorption of indomethacin from an ointment in rats.[20,30] Diclofenac is a well-established NSAID and analgesic, with a high therapeutic index for the management of acute painful conditions. A previous study suggested that the use of Voltaren Emulgel®, a topical percutaneous formulation of diclofenac, as a coupling medium during US therapy is an effective alternative to the currently used gel.[31] Because of the deep penetration of US (up to 5 cm below the skin), however, periosteal burns and tissue necrosis may result if treatment time or intensity is excessive.[32] But according to our results, piroxicam and diclofenac phonophoresis should be carefully used in the treatment of acute inflammation because of their hyperthermia effect in deep tissues.

CONCLUSION

One of the possible mechanisms of ultrasounically enhanced transdermal drug delivery is temperature increase which potentially related to percutaneous absorption of drugs. The rise in temperature depends on the US frequency, intensity, duration of US and thermal characteristics of the medium. For continued effective use of phonophoresis, researchers must carefully identify the important physiochemical properties of various drug molecules that could be influenced by US. Treatment effectiveness will improve when health care providers are able to more carefully match the topical agent with the specific patient condition. Aggressive research is also needed on variables of NSAID delivery, appropriate length of treatment sessions, and overall duration of phonophoretic therapy. However, there is a small risk of unwanted deep temperature increases during long-term application of diclofenac or piroxicam phonophoretic deliveries. The findings of this study should be of significance to clinicians who regularly use NSAID phonophoresis.

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REFERENCES

1. Tyle P, Agrawala P. Drug delivery by phonophoresis. Pharm Res 1989;6:355-61.
2. Meidan VM, Walmsley AD, Irwin WJ. Phonophoresis - is it a reality? Int J Pharm 1995;118:129-49.
3. Byl NN. The use of ultrasound as an enhancer for transcutaneous drug delivery: phonophoresis. Phys Ther 1995;75:539-53.
4. Machet L, Cochelin N, Patat F, et al. In vitro phonophoresis of mannitol, oestradiol and hydrocortisone across human and hairless mouse skin. Int J Pharm 1998;165:169-74.
5. Kassan DG, Lynch AM, Stiller MJ. Physical enhancement of dermatologic drug delivery: iontophoresis and phonophoresis. J Am Acad Dermatol 1996;34:657-66.
6. Newman JT, Nellermoe MD, Carnett JL. Hydrocortisone phonophoresis. A literature review. J Am Podiatr Med Assoc 1992;82:432-5.
7. Machet L, Pinton J, Patat F, et al. In vitro phonophoresis of digoxin across hairless mice and human skin: thermal effect of ultrasound. Int J Pharm 1996;133:39-45.
8. Meidan VM, Walmsley AD, Docker MF, et al. Ultrasound-enhanced diffusion into coupling gel during phonophoresis of 5-fluorouracil. Int J Pharm 1999:185:

205-13.

9. Bommannan D, Menon GK, Okuyama H, et al. Sonophoresis II. Examination of the mechanism(s) of ultrasound-enhanced transdermal drug delivery. Pharm Res 1992;9:1043-7.
10. Bommannan D, Okuyama H, Stauffer P, et al. Sonophoresis I. The use of high-frequency ultrasound to enhance transdermal drug delivery. Pharm Res 1992;9: 559-64.
11. Mitragotri S, Blankschtein D, Langer R. Ultrasound-mediated transdermal protein delivery. Science 1995; 269:850-3.
12. Machet L, Boucaud A. Phonophoresis: efficiency, mechanisms and skin tolerance. Int J Pharm 2002;243:1-15.
13. Blank IH, Scheuplein RJ, MacFarlane DJ. Mechanism of percutaneous absorption. 3. The effect of temperature on the transport of non-electrolytes across the skin. J Invest Dermatol 1967;49:582-9.
14. Boucaud A, Machet L, Arbeille B, et al. In vitro study of low-frequency ultrasound-enhanced transdermal transport of fentanyl and caffeine across human and hairless rat skin. Int J Pharm 2001; 228:69-77.
15. Mitragotri S, Kost J. Transdermal delivery of heparin and low-molecular weight heparin using low-frequency ultrasound. Pharm Res 2001;18:1151-6.
16. Pelucio-Lopes C, Machet L, Vaillant L, et al. Phonophoresis of azidothymidine (AZT) ex vivo across human and hairless mice skin. Int J Pharm 1993;96:249-52.
17. Miyazaki S, Mizuoka H, Oda M, et al. External control of drug release and penetration: enhancement of the transdermal absorption of indomethacin by ultrasound irradiation. J Pharm Pharmacol 1991;43:115-6.
18. Julian TN, Zentner GM. Mechanism for ultrasounically enhanced transmembrane solute permeation. J Control Release 1990;12:77-85.
19. Lin WL, Liauh CT, Chen YY, et al. Theoretical study of temperature elevation at muscle/bone interface during ultrasound hyperthermia. Med Phys 2000;27:1131-40.
20. Asano J, Suisha F, Takada M, et al. Effect of pulsed output ultrasound on the transdermal absorption of indomethacin from an ointment in rats. Biol Pharm Bull 1997;20:288-91.
21. Fang J, Fang C, Sung KC, et al. Effect of low frequency ultrasound on the in vitro percutaneous absorption of clobetasol 17-propionate. Int J Pharm 1999; 191:33-42.
22. Duteil L, Queille C, Poncet M, et al. Objective assessment of topical corticosteroids and non-steroidal anti-inflammatory drugs in methyl-nicotinate-induced skin inflammation. Clin Exp Dermatol 1990;15:195-9.
23. Rosim GC, Barbieri CH, Lancas FM, et al. Diclofenac phonophoresis in human volunteers. Ultrasound Med Biol 2005;31:337-43.
24. Hirasawa N, Ohuchi K, Watanabe M, et al. Mechanism of the inhibitory action of cyclooxygenase inhibitors on leukocyte infiltration: involvement of endogenous histamine. Eur J Pharmacol 1987;144:267-75.
25. Roberts D. Transdermal drug delivery using iontophoresis and phonophoresis. Orthop Nurs 1999;18:50-4.
26. Kamenskaia NS, Fedorova NE. The therapeutic use of iodide-bromide-sodium chloride baths combined with hydrocortisone phonophoresis in patients with osteoarthrosis and gout. Vopr Kurortol Fizioter Lech Fiz Kult 1990 Nov-Dec;(6):47-50. (Abstract in English, full in Russian)
27. Ciccone CD, Leggin BG, Callamaro JJ. Effects of ultrasound and trolamine salicylate phonophoresis on delayed-onset muscle soreness. Phys Ther 1991;71: 666-75.
28. Benson HA, McElnay JC, Harland R. Use of ultrasound to enhance percutaneous absorption of benzydamine. Phys Ther 1989;69:113-8.
29. Benson HA, McElnay JC. High-performance liquid chromatography assay for the measurement of benzydamine hydrochloride in topical pharmaceutical preparations. J Chromatogr 1987;394:395-9.
30. Miyazaki S, Mizuoka H, Kohata Y, et al. External control of drug release and penetration. VI. Enhancing effect of ultrasound on the transdermal absorption of indomethacin from an ointment in rats. Chem Pharm Bull 1992;40:2826-30.
31. El-Hadidi T, El-Garf A. Double-blind study comparing the use of Voltaren Emulgel versus regular gel during ultrasonic sessions in the treatment of localized traumatic and rheumatic painful conditions. J Int Med Res 1991;19:219-27.
32. Singer AJ, Homan CS, Church AL, et al. Low-frequency sonophoresis: pathologic and thermal effects in dogs. Acad Emerg Med 1998;5:35-40.

比較大鼠後腿肌上超音波導入五種非類固醇抗炎藥物的熱效應

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研究背景：超音波導入療法是利用超音波將藥物推入皮膚下組織的一種方法。本篇研究主要探討在大鼠後腿無毛皮膚上以超音波導入非類固醇抗炎藥物後，在活體中淺層及深層組織溫度的變化。

實驗設計與目的：本篇研究主要探討在大鼠後腿皮膚上以超音波導入非類固醇抗炎藥物後，在活體中淺層及深層組織溫度的變化。

方法：在 250-300 克重的雄性 Wistar 大鼠單側後肢皮膚上，利用超音波隨機導入以下五種非類固醇抗炎藥物之其中一種：piroxicam (Feldene)、indomethacin (Indocin)、etofenamate (Rheumon)、methylsalicylate (Salomethyl)或 diclofenac (Voren)；另一側後肢則作爲不投藥之實驗對照組。以 1MHz，1W/cm^2 的持續性、50%或 20%間歇性輸出的超音波，分別給予 5、10 或 20 分鐘的處置。利用數位式針電極組織溫度測量儀記錄導入前、後的溫度變化。

結果：五種非類固醇抗炎藥物超音波導入實驗組及其對照組之處置後，淺層及深層組織溫度均明顯較處置前增加。但是，五種非類固醇抗炎藥物超音波導入實驗組的深層溫度增加程度比對照組顯著。其中，piroxicam 及 diclofenac 使用間歇性超音波輸出導入藥物後，深層溫度升高情形明顯比其他三種高。結論：使用超音波導入 piroxicam 或 diclofenac 兩種藥時，造成較高的深層組織熱效應，推測這兩種藥物的經皮吸收效果也較佳。（台灣復健醫誌 2006；34(1)：1 - 10）

關鍵詞：超音波(ultrasound)，超音波導入(phonophoresis)，非類固醇抗炎藥物(nonsteroidal anti-inflammatory drugs)，組織溫度(tissue temperature)，大鼠(rat)

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