Phonophoresis of Methyl Nicotinate: A Preliminary Study to Elucidate the Mechanism of Action

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The skin penetration enhancement effect of ultrasound (phonophoresis) on methyl nicotinate was investigated in 10 healthy volunteers in a double-blind, placebo-controlled, crossover clinical trial. Each treatment consisted of the application of ultrasound massage (3.0 MHz, 1.0 W/cm^2 continuous output) or placebo massage (0 MHz) for 5 min to the forearms of the volunteers, followed by a standardized application of methyl nicotinate at intervals of 15 sec, 1 min, and 2 min postmassage. Percutaneous absorption of methyl nicotinate was monitored using laser Doppler velocimetry. Ultrasound treatment applied prior to methyl nicotinate led to enhanced percutaneous absorption of the drug, for example, ultrasound treatment data versus control data at 2 min showed significant increases ($P < 0.05$; analysis of variance) in the peak blood flow (125.8 ± 12.0 vs 75.3 ± 10.4% flux) and in the area under the curve for blood flow (2630.3 ± 387.5 vs 1567.6 ± 183.5% flux · min). The results of this study suggest that ultrasound affects the skin structure to provide skin penetration enhancement. This finding is consistent with the proposed hypothesis that phonophoresis acts by disordering the structured lipids in the stratum corneum.

KEY WORDS: phonophoresis; percutaneous absorption; ultrasound; methyl nicotinate; penetration enhancement; laser Doppler velocimetry.

INTRODUCTION

Phonophoresis is the use of ultrasound energy to enhance percutaneous penetration of topically applied drugs (1). Ultrasound therapy is widely used by physiotherapists in the management of a range of conditions, in particular, musculoskeletal conditions and soft tissue injuries. Ultrasound energy is not transmitted in air, therefore the standard treatment procedure involves the application of an unmedicated coupling agent (usually gel) to transmit the ultrasound energy from the ultrasound transducer to the treatment site.

The use of a medicated coupling agent with ultrasound therapy has been investigated by several groups. Griffin *et al.* (2), for example, examined the clinical effects of administration of ultrasound with either hydrocortisone or placebo ointment to 102 arthritic patients. Of those patients receiving hydrocortisone/ultrasound treatment, 68% exhibited a marked decrease in pain and a significant increase in range of movement, while only 28% of those receiving placebo with ultrasound showed a similar improvement. The effect of hydrocortisone application alone was not investigated. A number of other authors have examined the concomitant use of antiinflammatory topical products with ultrasound (e.g., Refs. 3–5). Enhanced clinical effectiveness in the case of inflammatory conditions may result from increased percutaneous absorption of the applied drug due to ultrasound (i.e., phonophoresis) or a synergistic combination resulting from concomitant use of topical drug application and ultrasound treatment, both of which are effective in the treatment of inflammation.

The present paper continues our investigations of the influence of ultrasound on the percutaneous absorption of drugs. In our earlier studies the ability of ultrasound to enhance percutaneous absorption of certain drugs has been clearly established (6–8). We have shown, for example, that ultrasound treatment (3.0 MHz, 1.0 W/cm^2 continuous output, 5 min) increases the percutaneous penetration of a range of nicotinate esters as measured by the extent of vasodilator response using laser Doppler velocimetry (LDV) (8). The increase in percutaneous absorption of nicotinate esters following ultrasound treatment was of the order of 59% for methyl nicotinate, 79% for ethyl nicotinate, and 21% for hexyl nicotinate compared with control data.

The mechanism by which ultrasound acts as a penetration enhancer is, however, unclear. One possibility is that ultrasound may alter the structure of stratum corneum lipids since ultrasound energy is known to cause a mechanical disturbance in an absorbing medium (9). It is also possible that ultrasound improves the rate of solution of the drug into the stratum corneum lipids, perhaps even permitting supersaturation. This would provide a greater thermodynamic driving force across the stratum corneum.

The aim of the present study was to investigate whether ultrasound affects the structure of the skin, by monitoring the vasodilator response to methyl nicotinate applied at intervals *after* ultrasound treatment of the skin in healthy volunteer subjects.

MATERIALS AND METHODS

Materials and Equipment

Methyl nicotinate was obtained from Sigma Chemical Co., Dorset, UK. It was applied to the skin using 1-cm filter disks (Millipore 100 prefilters type AP10, B.N. 05855, Millipore Ltd., Middlesex, UK).

Ultrasound energy was applied using a Sonacel Multiphone Mk II ultrasound generator (SCI Instruments Ltd., Hertfordshire, UK) with Aquasonic 100 ultrasound transmission gel as a coupling agent (Parker Laboratories Inc, Orange, NJ). The transmission of ultrasound energy (at 3.0 MHz) through Aquasonic gel was measured using a Medisonics precision power meter [Medisonics (U.K.) Ltd., Surrey, UK] as reported previously (10). Percentage transmission relative to deionised degassed water recorded for Aquasonic gel (mean ± SE) was 98.14 ± 0.32, indicating that the

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gel promotes efficient transmission of ultrasound energy from the ultrasound generator to the skin. Prior to the study the ultrasound generator was calibrated using a coplanar PVDF (polyvinylidene fluoride) membrane hydrophone (GEC-Marconi Electronics Ltd., Essex, UK) and a tethered-float radiometer (National Physical Laboratory, Middlesex, UK) (11).

Cutaneous blood flow was measured using a Periflux Pf 2 laser Doppler flow meter using a standard Pf 108 probe and probe holder (Perimed, Stockholm, Sweden) (8).

Protocol Development

Based on our previous findings (6–8), the ultrasound treatment selected involved a 5-min massage with ultrasound of a frequency of 3 MHz and an intensity of 1.0 W/cm^2. Initial experimentation was carried out in three volunteers to help optimize two further experimental variables, namely, the amount of methyl nicotinate to be applied to the skin (and its mode of application) and the time period between ultrasound treatment and application of the methyl nicotinate.

Application of the methyl nicotinate as an aqueous solution on filter disks was chosen as a convenient method of applying the drug to the skin. This method of application has been used previously (12). The method adopted involved saturating the filter disk in an aqueous methyl nicotinate solution and placing it in contact with the skin for 15 sec. It was shown in preliminary experiments that, using this method of application, different volunteers responded submaximally to differing methyl nicotinate concentrations. It was therefore decided that each volunteer would undergo a preliminary screen to select a suitable concentration from 5, 2.5, or 1 m*M* methyl nicotinate. Several time intervals between treatment with ultrasound and application of the methyl nicotinate were examined. The time intervals chosen for the main study protocol were as follows: 15 sec, 1 min, and 2 min.

Application to Subjects

The study was conducted on 10 healthy volunteers who gave their written informed consent before participating in the trial. The study was approved by the University Ethics Committee.

The study, which consisted of three treatment sessions, was carried out on three separate occasions, 7 days apart, in a double-blind, randomized, placebo-controlled crossover fashion, with each person acting as his/her own control. Neither the subject nor the person applying the ultrasound knew the ultrasound parameters being used, and one operator applied the ultrasound throughout to ensure uniformity of application. The study was conducted in a temperature-controlled room at 18.5–20°C.

All subjects underwent initial screening to determine the concentration of methyl nicotinate required to produce a measurable submaximal response. The three experimental sessions for each volunteer were as follows.

Session 1. A 3.5-cm-diameter treatment site (size of LDV probe holder) was marked on the flexor aspect of each forearm using a ballpoint pen. The LDV probe (Periflux Pf2 laser Doppler flow meter, standard probe Pf108 with probe holder; Perimed) was held in place manually on the treatment site to obtain a control blood flow measurement (see measurement of cutaneous blood flow). A quantity of Aquasonic coupling gel (2g) was placed on the treatment site on the right forearm and ultrasound treatment (3.0 MHz and 1.0 W/cm^2 continuous output or 0 W/cm^2, i.e., massage only) was applied. The ultrasound head was used to massage the area for a 5-min period using a standardized circular motion. Following treatment the gel was removed from the forearm using paper tissue.

After a time period (15 sec, 1 min, or 2 min) a 1-cm-diameter filter disk fully saturated with the appropriate strength of methyl nicotinate solution (5, 2.5, or 1 m*M* as determined previously to provide a submaximal response in the volunteer) was placed in the center of the circle for a contact period of 15 sec. The disk was then removed, the skin wiped with tissue paper, and blood flow monitoring started.

The complete procedure was repeated with the left forearm using a different ultrasound treatment/nicotinate application delay period combination.

Session 2. The procedure was as for Session 1 using different ultrasound/nicotinate application delay period combinations.

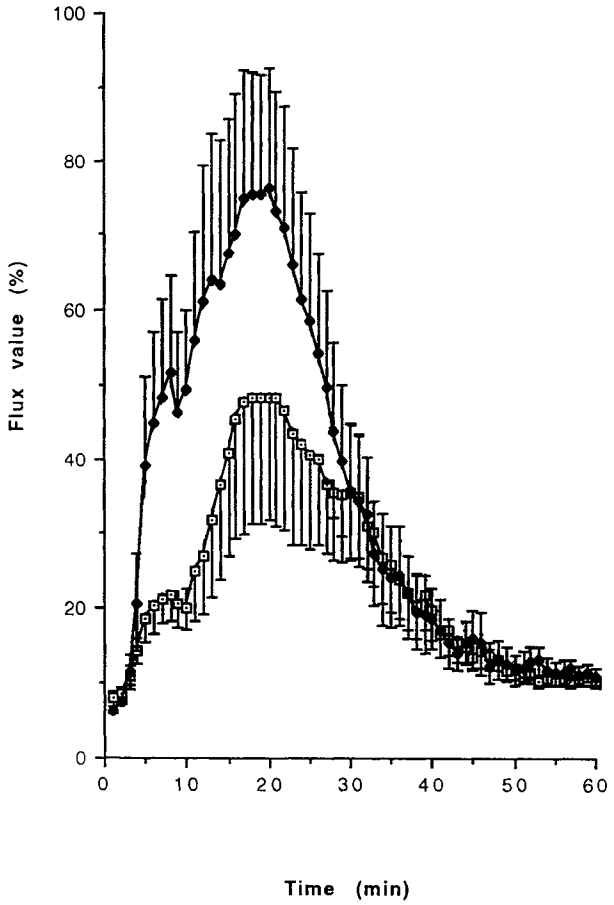

Fig. 1. The influence of ultrasound (3.0 MHz, 1.0 W/cm^2) on the percutaneous absorption of methyl nicotinate as measured by LDV: ultrasound applied 15 sec prior to methyl nicotinate. Mean percentage flux ± SE. (——⊡——) Control; (——◆——) ultrasound.

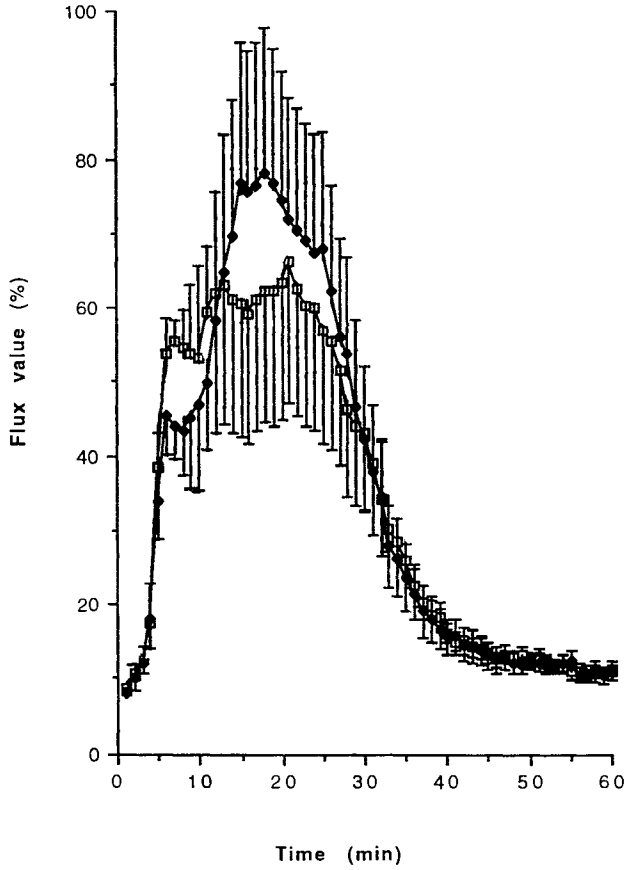

Fig. 2. The influence of ultrasound (3.0 MHz, 1.0 W/cm^2) on the percutaneous absorption of methyl nicotinate as measured by LDV: ultrasound applied 1 min prior to methyl nicotinate. Mean percentage flux ± SE. (——⊡——) Control; (——♦——) ultrasound.

Session 3. The procedure was as for Sessions 1 and 2 using different ultrasound/nicotinate application delay period combinations.

The ultrasound treatment/nicotinate application delay period combinations used were such that all volunteers received randomized applications of their appropriate methyl nicotinate solution at each of the three delay periods (i.e., 15 sec, 1 min, 2 min) following massage with the ultrasound head, in both the absence (0 W/cm^2) and the presence (3.0 MHz, 1.0 W/cm^2) of ultrasound energy.

Measurement of Cutaneous Blood Flow

Methyl nicotinate is a potent vasodilator, producing erythema on application to human skin (12). Although early workers depended on the development of erythema as a measure of the rate of percutaneous absorption of nicotinates (13), the development of laser Doppler velocimetry (LDV) allows the measurement of cutaneous blood flow and therefore a more accurate determination of the rate and extent of nicotinate percutaneous absorption. The laser Doppler technique has been described in detail by a number of authors (e.g., Refs. 8, 12, 14, and 15).

In the present investigation, after removing the nicotinate filter disk and wiping the skin with tissue paper, the LDV probe held within a probe holder was attached to the treatment site using an adhesive disk. Cutaneous blood flow (% flux) was recorded immediately and at 1-min intervals thereafter. Monitoring was continued for 1 hr or until blood flow returned to pretreatment values.

Plots of flux values (%) against time (min) were drawn from the original data. A number of parameters were recorded, i.e., peak blood flow (% flux), time to peak blood flow (min), area under the curve of blood flow against time (AUC; % · min) and modified AUC (mAUC; obtained by subtracting the baseline blood flow data from the AUC, % · min).

Statistical Analysis

The parameters (as outlined above) obtained for control (no ultrasound) and test (ultrasound) data at the three time intervals combined were compared using analysis of variance. In addition, Scheffe's test was applied to the data for each of the three ultrasound treatment/nicotinate application delay periods to determine at which time intervals a significant difference existed between control and ultrasound treatment.

RESULTS

Curves of mean flux values (±SE) against time, ob-

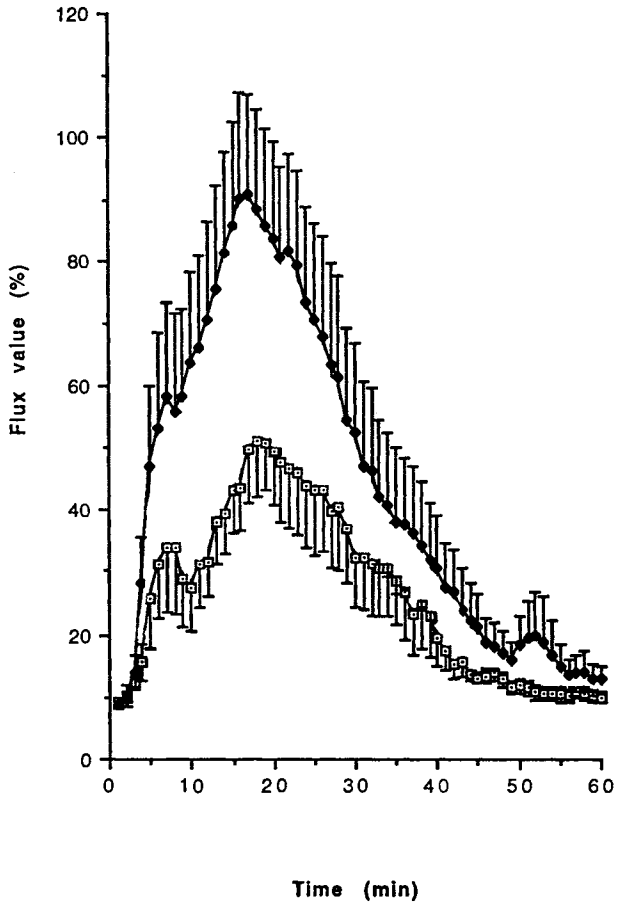

Fig. 3. The influence of ultrasound (3.0 MHz, 1.0 W/cm^2) on the percutaneous absorption of methyl nicotinate as measured by LDV: ultrasound applied 2 min prior to methyl nicotinate. Mean percentage flux ± SE. (——⊡——) Control; (——♦——) ultrasound.

Table I. Peak Blood Flow (% Flux) vs Time (min) Following Percutaneous Absorption of Methyl Nicotinate Applied 15 sec, 1 min, and 2 min After Ultrasound Treatment (3.0 MHz and 1.0 W/cm^2 or 0 W/cm^2 Control)

	15 sec		1 min		2 min	
Volunteer	Control	Ultrasound	Control	Ultrasound	Control	Ultrasound
1	25	105	80	135	74	108
2	38	56	81	37	30	41
3	20	153	210	198	79	159
4	26	65	78	50	114	153
5	40	129	48	45	20	83
6	38	99	120	132	84	150
7	57	117	83	114	76	144
8	59	64	37	68	60	135
9	162	126	147	150	126	135
10	150	210	86	59	90	150
Mean	61.5	112.4	97.0	98.8	75.3	125.8
±SE	16.3	14.8	16.0	17.2	10.4	12.0
Significance[a]	$P < 0.05$		$P > 0.05$ (ns)		$P < 0.05$	

[a] Scheffe's test.

tained following application of methyl nicotinate post ultrasound treatment (3.0 MHz and 1 W/cm^2 or 0 W/cm^2 control) for each of the three ultrasound/nicotinate application delay periods (15 sec, 1 min, 2 min), are shown in Figs. 1–3.

Peak blood flow, time to peak blood flow, modified AUC, and AUC data (±SE) are recorded for methyl nicotinate applied at each of the three times intervals after ultrasound and control treatments in Tables I, II, III, and IV, respectively. Considerable intersubject variation in the response to methyl nicotinate existed. The control data obtained at 15-sec and 2-min intervals were very similar, however, control data recorded following the 1-min interval was unexpectedly higher.

Ultrasound treatment administered prior to application of methyl nicotinate (combined time interval data) significantly increased percutaneous absorption (peak blood flow $P = 0.006$, mAUC $P = 0.032$, AUC $P = 0.039$; analysis of variance). However, the rate of absorption as determined by the time to peak blood flow was not significantly enhanced ($P = 0.582$; analysis of variance).

Data obtained for the 2-min treatment interval showed that ultrasound significantly increased percutaneous absorption of methyl nicotinate (peak blood flow $P < 0.05$, mAUC $P < 0.05$, AUC $P < 0.05$; Scheffe's test). Although there was a trend of increased percutaneous absorption of methyl nicotinate at the 15-sec and 1-min time intervals (Figs. 1 and 2), with the exception of peak blood flow after the 15-sec delay ($P < 0.05$) this was not to a statistically significant degree (Scheffe's test; $P > 0.05$) for all the measured parameters.

The increase in flux following percutaneous absorption of methyl nicotinate with versus without ultrasound treatment was determined for each time interval from mean AUC data (mAUC data). Ultrasound treatment-induced increased flux was of the order of 41% (63%) at the 15-sec interval, 4%

Table II. Time to Peak Blood Flow (% Flux) vs Time (min) Following Percutaneous Absorption of Methyl Nicotinate Applied 15 sec, 1 min, and 2 min After Ultrasound Treatment (3.0 MHz and 1.0 W/cm^2 or 0 W/cm^2 Control)

	15 sec		1 min		2 min	
Volunteer	Control	Ultrasound	Control	Ultrasound	Control	Ultrasound
1	4	6	15	18	8	6
2	6	20	7	5	5	5
3	7	14	12	13	13	15
4	5	5	6	6	7	10
5	7	7	6	6	23	23
6	8	18	24	14	17	22
7	32	24	30	25	21	20
8	18	8	8	25	15	7
9	19	21	22	20	25	23
10	16	16	13	13	17	16
Mean	12.2	13.9	14.3	14.5	15.1	14.7
±SE	2.8	2.2	2.7	2.4	2.2	2.3
Significance[a]	$P > 0.05$ (ns)		$P > 0.05$ (ns)		$P > 0.05$	

[a] Scheffe's test.

Table III. Modified Area Under Curve of Blood Flow (% Flux) vs Time (min) Following Percutaneous Absorption of Methyl Nicotinate Applied 15 sec, 1 min, and 2 min After Ultrasound Treatment (3.0 MHz and 1.0 W/cm^2 or 0 W/cm^2 Control)

	15 sec		1 min		2 min	
Volunteer	Control	Ultrasound	Control	Ultrasound	Control	Ultrasound
1	70.5	1289.5	1471.0	2561.5	704.5	746.5
2	713.0	888.0	1071.0	300.0	225.0	608.5
3	371.5	1924.0	4289.5	3275.0	1093.0	1925.5
4	58.5	610.0	616.0	371.0	1133.5	2305.5
5	524.0	1472.0	283.0	419.0	84.5	660.0
6	385.5	1974.5	2505.5	3378.0	1062.5	3379.0
7	1208.5	2042.0	1120.0	1242.0	1125.0	2796.0
8	1512.0	903.0	556.0	1463.5	865.5	1734.5
9	2366.0	1281.0	2315.5	2554.5	2374.5	2918.5
10	3063.5	4410.0	830.5	693.0	1672.0	4000.0
Mean	1027.3	1679.4	1505.8	1625.8	1034.0	2107.4
±SE	320.7	341.0	385.7	385.3	208.7	376.2
Significance[a]	$P = 0.181$ (ns)		$P = 0.828$ (ns)		$P = 0.022$	

[a] Scheffe's test.

(8%) at the 1-min interval, and 68% (104%) at the 2-min interval.

DISCUSSION

The experimental protocol was developed for this investigation based on previous data (6–8) and initial experimentation. LDV was found to be a suitable method for the measurement of changes in cutaneous blood flow due to application of the vasodilator methyl nicotinate.

The data obtained in the study indicate that ultrasound energy applied prior to application of methyl nicotinate (combined time interval data) does lead to enhanced penetration of the drug across the skin (peak blood flow $P = 0.006$, mAUC $P = 0.032$, AUC $P = 0.039$; analysis of variance). Percutaneous absorption of methyl nicotinate applied 2 min after ultrasound treatment was significantly enhanced (peak blood flow $P = 0.005$, mAUC $P = 0.022$, AUC $P = 0.023$; Scheffe's test). Although not enhanced to a significant degree, there was also a trend of enhanced nicotinate absorption at the other two time intervals. Incidentally the increase in AUC data for the 2-min interval corresponds closely with values obtained for concomitant application of methyl nicotinate and ultrasound in a previous study (8).

The results show considerable variation in response between subjects, a phenomenon reported previously (8). Control data obtained for the 1-min interval between ultrasound treatment and application of methyl nicotinate were unexpectedly greater than data for the other time intervals [for example, AUC (±SE) 1 min, 1999.2 (±373.8); 15 sec, 1451.0 (±302.4); and 2 min, 1567.6 (±183.5)]. No obvious explanation can be offered for this finding, which resulted in the small increase in AUC values obtained for the 1-min interval data.

Table IV. Area Under Curve of Blood Flow (% Flux) vs Time (min) Following Percutaneous Absorption of Methyl Nicotinate Applied 15 sec, 1 min, and 2 min After Ultrasound Treatment (3.0 MHz and 1.0 W/cm^2 or 0 W/cm^2 Control)

	15 sec		1 min		2 min	
Volunteer	Control	Ultrasound	Control	Ultrasound	Control	Ultrasound
1	711.5	1695.5	2341.0	2967.5	1400.5	1152.5
2	1119.0	1294.0	1597.0	938.0	921.0	1130.5
3	661.5	2330.0	4695.5	3565.0	1557.0	2389.5
4	638.5	900.0	1370.0	951.0	1655.5	2885.5
5	988.0	1820.0	805.0	825.0	664.5	1124.0
6	733.5	2264.5	2911.5	3958.0	1584.5	3727.0
7	1672.5	2332.0	1526.0	1822.0	1589.0	3550.0
8	1918.0	1309.0	904.0	1869.5	1387.5	2439.5
9	2656.0	1745.0	2605.5	2902.5	2722.5	3208.5
10	3411.5	4816.0	1236.5	1041.0	2194.0	4696.0
Mean	1451.0	2050.6	1999.2	2084.0	1567.6	2630.3
±SE	302.4	342.7	373.8	373.3	183.5	387.5
Significance[a]	$P = 0.213$ (ns)		$P = 0.874$ (ns)		$P = 0.023$	

[a] Scheffe's test.

Collectively the data show that ultrasound energy is capable of causing an enhancement in percutaneous penetration of methyl nicotinate which exists for a period after the application of ultrasound has ceased. This in turn suggests that ultrasound energy affects the structure of the skin. Ultrasound energy is known to cause a mechanical disturbance in an absorbing medium (9), which may disorder the structured lipids located in the intercellular channels of the stratum corneum. As a result, diffusion of drug through the lipid regions of the stratum corneum would be facilitated [a similar mechanism to that implicated for some chemical penetration enhancers (16,17)]. Data obtained from a previous study (8) suggest that ultrasound exhibited a greater penetration enhancement effect with the more polar methyl and ethyl nicotinates than the more lipophilic hexyl nicotinate. This would tend to confirm the ultrasound-mediated lipid disordering hypothesis since the rate-limiting process in percutaneous absorption of polar molecules is diffusion through the intercellular lipid channels (18). Partitioning from the lipid-rich stratum corneum environment into the viable epidermis is the rate-limiting step in absorption of lipophilic molecules and would therefore not be influenced by lipid disordering. As mentioned in the Introduction, a further explanation is that the solubility of the drug in the stratum corneum is affected.

Further studies are under way to characterize the nature of the ultrasound-induced changes in skin structure and resistance to drug penetration.

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