Influence of Ultrasound on the Percutaneous Absorption of Nicotinate Esters[1]

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The influence of ultrasound on the percutaneous absorption of three nicotinate esters was investigated in 10 healthy volunteers in a double-blind placebo controlled crossover clinical trial. Using a specially designed experimental protocol, the effect of continuous output ultrasound (at frequency 3.0 MHz and intensity 1.0 W/cm^2 for 5 min) on the percutaneous absorption of methyl, ethyl, and hexyl nicotinates, from gel bases, was investigated. A placebo control, involving massage with each of the gels, without ultrasound for 5 min, was also incorporated. The pharmacodynamic parameter of vasodilation caused by the nicotinates was used to monitor the percutaneous absorption of the drugs. Laser Doppler velocimetry, a noninvasive optical technique, was used to measure vasodilation of the cutaneous vessels within the treatment site. Ultrasound treatment led to enhanced vasodilator response to the nicotinates, therefore indicating an enhancement of their percutaneous absorption. These agents may prove to be useful compounds in examination of the mechanism of action of phonophoresis.

KEY WORDS: phonophoresis; percutaneous absorption; ultrasound; nicotinate esters; laser Doppler velocimetry.

INTRODUCTION

Ultrasound therapy is routinely used by physiotherapists for the treatment of a wide range of conditions and, in particular, in the management of soft tissue injuries. Topical pharmaceuticals containing antiinflammatories are also useful in the treatment of soft tissue injuries. It would, therefore, seem rational to combine these two effective treatment modalities in the hope of obtaining a synergistic effect. Two important aspects exist concerning the concomitant use of topical drug application together with ultrasound in the treatment of inflammatory conditions. First, drug application and ultrasound therapy can each act independently to control symptoms and aid recovery, and second, ultrasound energy may enhance the percutaneous absorption of the applied drug. It is the enhancement of percutaneous absorption by ultrasound, a technique termed phonophoresis, which is examined in this paper. Phonophoresis involves placing the topical formulation on the skin and massaging the area with the ultrasonic source.

Previous investigations have reported successful phonophoretic administration of a wide range of drugs, for example, corticosteroids, local anesthetics, and nonsteroidal antiinflammatories. Enhanced local anesthesia has been reported following treatment with phonophoretically administered local anesthetic drugs. Novak (1), for example, showed that the concentration of lidocaine in rabbit muscle tissue was much greater when the tissues had been subjected to ultrasound during application of lidocaine (180% increase in drug concentration in tissue excised 30 min after treatment). Griffin and co-workers (2) investigated the clinical effects of ultrasonically administered hydrocortisone, as compared to ultrasonically administered placebo in 102 arthritic patients. They reported that 68% of those patients receiving hydrocortisone in conjunction with ultrasound exhibited a marked decrease in pain and significant increase in range of motion, while only 28% of those receiving placebo plus ultrasound showed similar improvement. They did not, however, examine the effects of application of hydrocortisone alone. Kleinkort and Wood (3) conducted a retrospective study of 85 patients treated with ultrasonically driven hydrocortisone which was applied as a 10% (w/w) ointment or a 1% (w/w) cream. They concluded that phonoretically treated 10% hydrocortisone ointment provided a "painless alternative to steroid injections." Many of the clinical trials on phonophoresis which have been carried out to date have, however, been poorly controlled, inadequate information on ultrasound conditions has been provided, and measurement systems used have often not been sufficiently accurate. This led Skauen and Zentner (4), who reviewed extensive literature on phonophoresis in 1984, to conclude that although a number of investigators have reported ultrasonically mediated enhancement of percutaneous absorption, further well-controlled and well-designed studies were required to substantiate these reports.

McElnay and co-workers (5) recently reported that ultrasound treatment led to enhanced percutaneous absorption of fluocinolone acetonide in healthy volunteer subjects in a double-blind placebo controlled clinical trial. This was in contrast to similarly conducted studies which indicated that therapeutic ultrasound did not significantly increase the percutaneous absorption of lidocaine (6) or benzydamine (7). Ultrasound has, however, been shown by these workers to enhance the local anesthetic effect of a lidocaine/prilocaine eutectic mixture applied topically as Emla cream (8).

Ultrasound energy is rapidly attenuated in air; therefore in order to be effective, it must be transferred efficiently from the ultrasound transducer into the skin. The transmission characteristics of a number of topical proprietary preparations containing drugs suitable for use with ultrasound have recently been investigated (9). Gel formulations were found to be the most suitable coupling agents.

The pharmacological response to an applied drug can be utilized to measure percutaneous absorption of compounds that elicit measurable responses, such as local anesthesia, inflammatory responses, and allergic reactions. The vasoconstrictor assay, which involves comparing the blanching

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effect of vasoactive compounds, such as corticosteroids, has been widely used (e.g., Refs. 10–12). The properties of vasodilative compounds, such as nicotinates, can be measured with the aid of photopulse plethysmography (PPG) and laser Doppler velocimetry (LDV), techniques which measure changes in skin blood volume and flow, respectively (13–15).

The aim of the present study was to investigate the effect of ultrasound on the percutaneous absorption of three model compounds, namely, methyl, ethyl, and hexyl nicotinate, from a gel formulation in healthy human volunteers. Methyl (and ethyl) nicotinate possess hydrophilic properties, while hexyl nicotinate is lipophilic in character [the measured partition coefficients (isopropyl myristate–water at 25°C) are 2.5, 7.1, and 1155, respectively (16)]. It was envisaged that the different physicochemical properties of the drugs could provide some clues with regard to the mechanism of action of phonophoresis.

MATERIALS AND METHODS

The nicotinate gels used in the present study were prepared using Carbopol 940 (BF Goodrich Co. Chemical Group, Cleveland, Ohio), a synthetic polymer composed of polyacrylic acid linked to sucrose (17), which is widely used in the cosmetic and pharmaceutical industries (18). The methyl and ethyl nicotinate gels were prepared by first adding the drug to HPLC-grade water (produced by filtration through a Milli-Q reagent water system, Millipore Ltd., Middlesex, U.K.) Carbopol 940 (0.5%, w/v) was slowly added and stirred for 2 min with a vortex mixer, and sodium hydroxide solution (0.02%, w/v; BDH Chemicals Ltd., Poole, U.K.) was then stirred in using a magnetic stirrer. Hexyl nicotinate is immiscible with water, therefore the hexyl nicotinate gel was prepared by vortex mixing of the drug into the preferred gel base. The resulting gels were left under vacuum overnight to remove incorporated air. Three gels were prepared using these methods, i.e., 0.01% (w/v) methyl nicotinate, 0.0125% (w/v) ethyl nicotinate, and 0.015% (w/v) hexyl nicotinate (methyl and ethyl nicotinates were obtained from Sigma Chemical Co., Dorset, U.K.; hexyl nicotinate was a gift from Zimmerman Hobbs Ltd., U.K.). Initial experimentation in three volunteers indicated that these three formulations gave rise to approximately equipotent submaximal responses (see experimental protocol).

The transmission of ultrasound energy (at 3.0 MHz) through the gel preparations was measured using a Medisonics precision power meter [Medisonics (U.K.) Ltd., Surrey, U.K.] using a previously reported technique (9). Percentage transmission relative to deionized degassed water recorded for each of the gels (mean ± SE) was 122 ± 0.4% (n = 5; 0.01%, w/v, methyl nicotinate), 120 ± 0.5% (n = 5; 0.0125%, w/v, ethyl nicotinate), and 123 ± 0.5% (n = 5; 0.015%, w/v, hexyl nicotinate). These data indicate that the gels possessed good ultrasound coupling characteristics.

The study was carried out in 10 healthy volunteers (1 male and 9 females aged 20 to 28 years) who gave their written informed consent to take part in the trial. The study was approved by the University Ethics Committee.

Experimental Protocol

Initial experimentation was carried out to develop a suitable experimental protocol, in particular, to standardize the concentration of each drug to be applied, the duration of application of ultrasound, and the contact time of the vasodilator gels with the skin. In preliminary studies, in three volunteers, the concentration of each of the nicotinates which gave rise to readily measurable submaximal vasodilation responses when applied to the skin for 10 min were established by examination of the vasodilation obtained from each of the esters over a range of concentration. The chosen concentrations were then used in the main study, namely, 0.01% (w/v) methyl nicotinate, 0.0125% (w/v) ethyl nicotinate, and 0.015% (w/v) hexyl nicotinate. In addition, ultrasound treatment was applied with placebo gel to establish the influence of ultrasound alone on cutaneous blood flow. No increase in blood flow was recorded.

Based on maximal effects achieved with ultrasound in previous investigations (e.g., Ref. 8), an ultrasound frequency of 3.0 MHz at an intensity of 1.0 W/cm^2 was chosen for the study. The main study, which consisted of three treatment sessions, was carried out on three separate occasions, 7 days apart, in a double-blind, placebo controlled, crossover fashion, with each person acting as his/her own control. Neither the subject nor the person applying the ultrasound knew the gel type being used or the frequency/intensity of ultrasound being used. One operator applied the ultrasound throughout the study.

Application to Subjects

The three treatment periods were as follows.

Session 1. Each subject washed his/her forearms with soap and water and rinsed them thoroughly to remove any trace of soap. The arms were then dried before marking out a 3.5-cm-diameter treatment site (size of LDV probe holder) on each forearm using a ballpoint pen. The LDV probe [Periflux PF2 laser Doppler flowmeter, standard probe PF108 with thermostated probe holder (32°C); Perimed, Stockholm, Sweden] was then held manually in place on the treatment site to obtain a control blood flow measurement (see measurement of cutaneous blood flow). A portion of gel (1.5 g) was then applied to the treatment site on the right forearm and left for a 5-min contact period. This 5-min contact time, prior to the application of ultrasound, was used to help allow the drug concentration to equilibrate between the gel and the stratum corneum. Gel application was randomized, using one of the three nicotinate formulations. The ultrasound head (Sonacel Multiphon Mk II ultrasonic generator, SCI Instruments Ltd., Hertfordshire, U.K.) was then used to massage the area in a standardized circular motion for a 5-min period (6), the nicotinate gel acting as the coupling medium. Ultrasound treatment was randomized, the ultrasound generator being set to a frequency of 0 MHz (control, placebo) or 3.0 MHz continuous output ultrasound at an intensity of 1.0 W/cm^2. [Prior to the study the ultrasound generator was calibrated using a coplanar PVDF (polyvinylidene fluoride) membrane hydrophone (GEC-Marconi Electronics Ltd., Essex, U.K.) and a tethered-float radiometer (National Physical Laboratory, Middlesex, U.K.)] Following ultrasound treatment the gel was removed from the forearm and blood flow monitoring started.

The complete procedure was repeated with the left forearm using a different ultrasound/gel combination.

Session 2. The procedure was exactly as for Session 1, using different ultrasound/gel combinations.

Session 3. The procedure was exactly as for Sessions 1 and 2 using different ultrasound/gel combinations.

The ultrasound/gel combinations used were such that each subject received treatment with each of the three drug formulations, i.e., 0.01% (w/v) methyl nicotinate, 0.0125% (w/v) ethyl nicotinate, and 0.015% (w/v) hexyl nicotinate, in both the absence (0 MHz) and the presence (3.0 MHz, 1.0 W/cm^2) of ultrasound treatment.

Measurement of Cutaneous Blood Flow

The esters of nicotinic acid are potent vasodilators which produce erythema on application to human skin (19–22). The time taken for them to produce erythema is indicative of the speed at which they penetrate the skin (22,23), and for this reason, nicotinic acid esters are widely used as model drugs in the investigation of percutaneous absorption. The increase in cutaneous blood flow due to the vasodilator nicotinates can be utilized to monitor the rate and extent of percutaneous absorption of the drugs using, for example, laser Doppler velocimetry (LDV). The laser Doppler technique has been described in detail by a number of authors (e.g., Refs. 14, 24–26). In this technique, monochromatic light at 632.8 nm from a 2-mW helium–neon laser is directed into the skin. The magnitude and frequency distribution of the Doppler shifted light can be related to the number of red blood cells reflecting the laser light and their velocity. The Doppler signal is processed to provide a measure of tissue perfusion quantified as the percentage flux value: % flux = (or proportional to) number of red blood cells moving in the measured volume × mean cell velocity. The percentage flux values are instrument-generated arbitrary values which are proportional to cutaneous blood flow. The point at which the optical fibres interface with the skin is called the probe. The probe is housed within a probe holder which can be attached to the skin with a double-sided adhesive disk. A thermostated probe holder can be used to record skin perfusion at specified and stable temperatures.

The LDV probe held within the thermostated probe holder (at 32°C) was attached to the treatment site with an adhesive ring. Cutaneous blood flow (% flux) was recorded immediately and at 1-min intervals thereafter. Monitoring was continued until blood flow returned to the control blood flow measurement recorded prior to treatment.

Plots of flux values (%) against time (min) were drawn from the original data. A number of parameters were recorded, namely, peak blood flow (% flux), time to peak blood flow (min), area under the curve of blood flow against time (AUC: % · min), and modified AUC (obtained by subtracting the baseline blood flow data from the AUC: % · min). The time to peak blood flow data yielded information on the rate of drug absorption, while peak blood flow and AUC data yielded information on the extent of drug absorption.

Statistical Analysis

The parameters obtained for control (no ultrasound) and test (ultrasound) data were compared using the paired *t* test for each of the three gels. Significance was not tested for across treatments since the different agents were used at arbitrary concentrations, albeit concentrations which led to approximately equivalent responses.

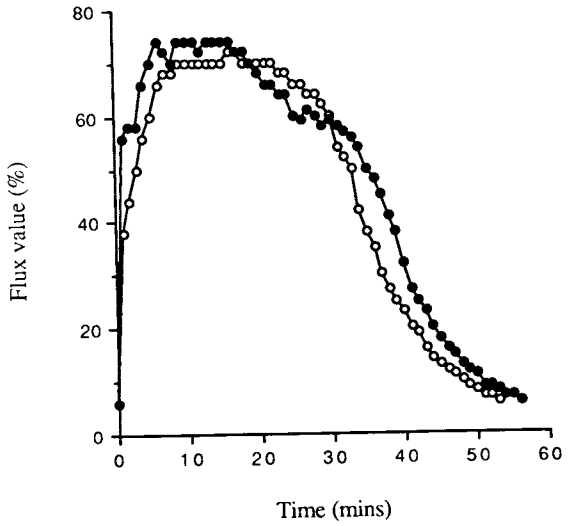

Fig. 1. The influence of ultrasound (3.0 MHz; 1.0 W/cm^2) on the percutaneous absorption of methyl nicotinate as measured by LDV; Volunteer 3. (—○—) Control; (—●—) ultrasound.

RESULTS

Examples of curves of flux values against time, obtained following treatments with methyl nicotinate, in the absence and presence of ultrasound, are shown in Figs. 1 and 2. These two figures serve to illustrate the large interindividual variations which were noted between some volunteers.

Peak blood flow (±SE), actual times to peak blood flow (±SE), AUC (±SE), and modified AUC (±SE) are recorded for methyl, ethyl, and hexyl nicotinate in the absence and presence of ultrasound treatment [0 MHz (control) or 3.0 MHz; 1.0 W/cm^2] in Tables I, II, and III, respectively.

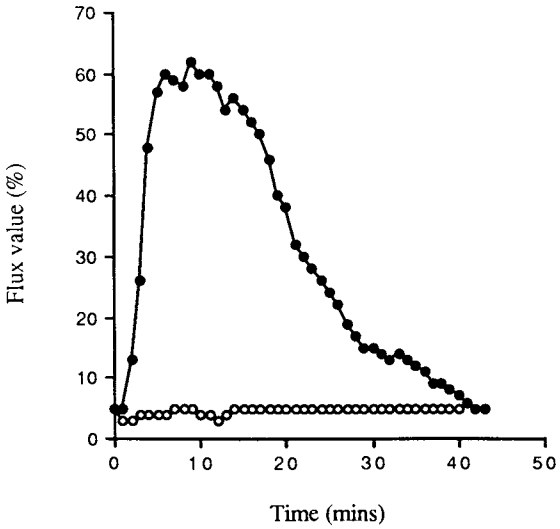

Fig. 2. The influence of ultrasound (3.0 MHz; 1.0 W/cm^2) on the percutaneous absorption of methyl nicotinate as measured by LDV; Volunteer 9. (—○—) Control; (—●—) ultrasound.

Table I. Peak Blood Flow (±SE), Time to Peak Blood Flow (±SE), Area Under Curve (AUC; ±SE), and Modified AUC (±SE) Following Percutaneous Absorption of Methyl Nicotinate in the Absence (0 W/cm^2) and Presence (3.0 MHz; 1.0 W/cm^2) of Ultrasound Treatment[a]

	Peak blood flow (%)		Time to peak blood flow (min)		AUC (% · min)		Modified AUC (% · min)	
Volunteer	Control	Ultrasound	Control	Ultrasound	Control	Ultrasound	Control	Ultrasound
1	38	62	23	19	1055.0	2787.5	864.3	2549.1
2	4	40	1	15	160.0	858.0	0.0	598.3
3	72	74	16	6	2181.6	2722.5	1643.5	2386.5
4	68	69	16	7	2448.0	2096.0	2155.8	1851.0
5	58	62	8	3	1896.5	2022.5	1571.5	1622.5
6	52	59	20	6	1840.0	1292.0	1646.6	1082.0
7	29	74	7	6	644.0	1456.0	325.0	1038.0
8	62	78	16	18	807.0	1346.0	527.0	1079.7
9	5	62	0	9	180.0	1308.0	0.0	1121.7
10	6	52	12	25	155.5	787.5	12.5	601.5
Mean	39.4	63.2	11.9	11.4	1136.8	1667.6	874.6	1393.0
±SE	±8.5	±3.6	±2.4	±2.3	±280.3	±224.2	±258.4	±217.0
Significance[b]	Sig. ($P < 0.01$)		Not sig. ($P > 0.05$)		Sig. ($P < 0.025$)		Sig. ($P < 0.025$)	

[a] (%) in all cases refers to percentage flux value obtained using LDV.
[b] Paired t test.

Time to peak blood flow following percutaneous absorption of methyl nicotinate was reduced by ultrasound treatment, though not significantly ($P > 0.05$). Ultrasound treatment significantly enhanced the peak blood flow ($P < 0.01$), AUC ($P < 0.025$), and modified AUC ($P < 0.025$) for methyl nicotinate (Table I). The increase in flux following percutaneous absorption of methyl nicotinate treatment with ultrasound as compared to control data was of the order of 59% (modified AUC data).

Results (Table II) showed that ultrasound treatment significantly enhanced the percutaneous absorption of ethyl nicotinate as determined by all the parameters measured [peak blood flow ($P < 0.05$); time to peak blood flow ($P < 0.025$); AUC ($P < 0.01$); modified AUC ($P < 0.01$)]. Ultrasound treatment caused an 79% (modified AUC data) increase in the percutaneous absorption of ethyl nicotinate.

Ultrasound treatment enhanced the percutaneous absorption of hexyl nicotinate as determined by all the parameters measured, although the differences were not statistically significant ($P > 0.05$; Table III). The measured increase in percutaneous absorption of hexyl nicotinate due to ultrasound treatment was considerably lower, i.e., 21% (modified AUC data).

DISCUSSION

The experimental protocol was developed specifically for this investigation based on previous experience with the

Table II. Peak Blood Flow (±SE), Time to Peak Blood Flow (±SE), Area Under Curve (AUC; ±SE), and Modified AUC (±SE) Following Percutaneous Absorption of Ethyl Nicotinate in the Absence (0 W/cm^2) and Presence (3.0 MHz; 1.0 W/cm^2) of Ultrasound Treatment[a]

	Peak blood flow (%)		Time to peak blood flow (min)		AUC (% · min)		Modified AUC (% · min)	
Volunteer	Control	Ultrasound	Control	Ultrasound	Control	Ultrasound	Control	Ultrasound
1	52	60	2	1	1948.5	2634.5	1668.5	2298.7
2	14	45	15	7	180.5	1117.0	50.5	865.0
3	82	84	16	7	2697.5	1963.5	2414.1	1718.5
4	62	60	11	9	1087.0	1561.5	896.6	1326.5
5	62	56	4	3	1787.5	2102.5	1499.5	1754.5
6	53	52	13	4	1263.0	1814.0	1040.8	1645.7
7	40	60	5	2	1030.7	1929.0	633.5	1652.0
8	10	68	28	13	261.0	2046.0	1.3	1710.0
9	23	36	4	10	345.0	759.5	170.0	544.2
10	42	68	22	13	584.0	2506.0	432.0	2254.8
Mean	44.0	58.9	12.0	6.9	1118.5	1843.4	880.7	1577.0
±SE	±7.3	±4.2	±2.7	±1.4	±261.6	±181.9	±249.7	±173.1
Significance[b]	Sig. ($P < 0.05$)		Sig. ($P < 0.025$)		Sig. ($P < 0.01$)		Sig. ($P < 0.01$)	

[a] (%) in all cases refers to percentage flux value obtained using LDV.
[b] Paired t test.

Table III. Peak Blood Flow (±SE), Time to Peak Blood Flow (±SE), Area Under Curve (AUC; ±SE), and Modified AUC (±SE) Following Percutaneous Absorption of Hexyl Nicotinate in the Absence (0 W/cm^2) and Presence (3.0 MHz; 1.0 W/cm^2) of Ultrasound Treatment[a]

	Peak blood flow (%)		Time to peak blood flow (min)		AUC (% · min)		Modified AUC (% · min)	
Volunteer	Control	Ultrasound	Control	Ultrasound	Control	Ultrasound	Control	Ultrasound
1	72	78	9	4	2933.5	3410.0	2587.1	3037.6
2	8	66	14	19	163.0	2236.0	60.3	1911.0
3	80	80	46	2	5075.0	5690.5	4752.6	5283.2
4	72	74	37	9	4611.5	4042.0	4026.5	3536.7
5	51	67	43	42	3559.5	4212.5	2965.5	3492.5
6	52	54	6	3	2639.5	2724.0	2164.5	2329.0
7	60	78	5	8	1344.0	2910.0	1123.0	2490.0
8	70	80	32	32	2725.5	3970.0	2335.5	3488.3
9	68	66	23	29	2783.5	3138.5	2307.5	2688.8
10	74	60	15	10	3430.0	2787.0	3110.0	2467.0
Mean	60.7	70.3	23.0	15.8	2926.5	3512.1	2543.3	3072.4
±SE	±6.6	±2.9	±4.9	±4.4	±452.1	±316.5	±421.7	±302.0
Significance[b]	Not sig. ($P > 0.05$)		Not sig. ($P > 0.05$)		Not sig. ($P > 0.05$)		Not sig. ($P > 0.05$)	

[a] (%) in all cases refers to percentage flux value obtained using LDV.
[b] Paired t test.

technique and initial experimentation. LDV was found to be a suitable method for the measurement of cutaneous blood flow under the present experimental conditions.

Results showed that ultrasound treatment led to significant enhancement of the percutaneous absorption of methyl and ethyl nicotinates. The rate and extent of percutaneous absorption of hexyl nicotinate were enhanced by ultrasound treatment, though not statistically significantly ($P > 0.05$, paired t test; Table III).

A possible mechanism of improved percutaneous absorption by ultrasound is that ultrasound may interact with the structured lipids located in the intercellular channels of the stratum corneum. In the same way that some penetration enhancers act by disordering these lipids (27,30), the ultrasound energy may act to facilitate diffusion through the lipid domains. If this is the case, it would be expected that the more polar the molecule, the greater the degree of enhancement. It can be proposed that the absorption of methyl and ethyl nicotinates was enhanced because diffusion through the intercellular lipid channels is the rate limiting process. For hexyl nicotinate, the rate-limiting step in absorption is partitioning from the lipid-rich stratum corneum environment and would therefore not be influenced by lipid fluidisation.

Although preliminary experimentation in three volunteers indicated that the concentration of nicotinates chosen gave rise to approximately equivalent submaximal responses, a retrospective evaluation of the results indicated that the mean control values obtained for the 10 trial subjects for hexyl nicotinate were higher (60.7) than the control values for the other two esters (39.4 and 44.0) and indeed approximated to the ultrasound values for the other two esters. Careful consideration of the data presented in Tables I to III indicates that the augmentation by ultrasound was greatest when control values were lowest and was likely related to maximal response values of approximately 75–85%. This effect is highlighted by examining the responses to methyl nicotinate in both the absence and the presence of ultrasound in volunteers 3 and 9 (Figs. 1 and 2).

In conclusion, the present results indicate that ultrasound augments the vasodilator response of nicotinate esters and therefore that ultrasound increases their percutaneous absorption. Although it is tempting to suggest, since augmentation of response was greatest in the case of the methyl and ethyl nicotinates, that the phonophoretic effect was dependent on lipophilicity (and in turn on lipid fluidisation), due to the complication of differences in control values, the present data do not provide clear information on the mechanism of action of ultrasound. Further experiments are under way using a range of concentrations of nicotinate esters in combination with ultrasound and on the response to nicotinate esters after the application of ultrasound to the skin (using a drug-free coupling gel). Preliminary investigations of the latter effect have shown that ultrasound treatment prior to the application of methyl nicotinate leads to an enhancement of percutaneous absorption of this ester (31). The latter observation suggests that ultrasound energy affects the structure of the skin, so supporting the lipid fluidisation hypothesis.

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