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/cm² 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/cm² 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 supersistilation. 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 Medisomics precision power meter [Medisomics (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/cm². 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 mM 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 Aqualine coupling gel (2g) was placed on the treatment site on the right forearm and ultrasound treatment (3.0 MHz and 1.0 W/cm² continuous output or 0 W/cm², 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 mM 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/cm²) 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.