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The influence of ultrasound on the percutaneous absorption of fluocinolone acetonide *

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Summary

Phonophoresis (improved percutaneous absorption by the application of ultrasound) has received increased attention in recent years; however, there is a lack of controlled clinical data using the technique. In the present study phonophoresis was examined using the model drug, fluocinolone acetonide. Twelve healthy volunteers took part in a double-blind cross-over trial of the effects of ultrasound on the percutaneous absorption of fluocinolone acetonide (as measured by the skin blanching test). Using two different experimental protocols, blanching on two study periods was examined for up to 12 h and up to 35 h post-drug application both under control (massage only for 5 min) and test (ultrasound massage; 2.0 $W \cdot cm^{-2}$ for 5 min) conditions. The area under the blanching curve, which was used to monitor the percutaneous absorption of the fluocinolone acetonide from the gel base, increased when ultrasound was used to a statistically significant extent ($P < 0.05$; paired t-test) for both treatment regimens. Mean $\pm$ S.E. values for day 1 were 10.4 ± 1.5 and 7.9 ± 1.4 and for day 2 were 45.6 ± 5.7 and 38.5 ± 5.2 for ultrasound and no ultrasound treatment respectively (all units are in blanching scale units $\times$ hours). We conclude that ultrasound treatment led to an enhanced percutaneous absorption of fluocinolone acetonide from the gel base (Synalar gel). The change in absorption was small and unlikely to give significant differences in clinical effect. Comparing these data with our recent negative findings with lignocaine and benzydamine it appears that the physicochemical properties of both the drug and the formulation base may be important for phonophoresis to take place and that the magnitude of the phonophoretic effect is small.

Introduction

The technique of using ultrasound to enhance the percutaneous absorption of drugs (phonophoresis) was first reported by Fellinger and Schmid in 1954. The technique involves placing the topical preparation on the skin over the area to be treated and massaging the area with an ultrasound source. The ultrasonic energy is said to enhance the percutaneous absorption and tissue penetration of the active ingredients from the topical product. The technique is used primarily in physiotherapy and in sports medicine; however, it may find use in other localised inflammatory conditions in the future since Quillen (1980) has stated that phonophoresis offers a safe and painless alternative to injection for inflammatory conditions. A major problem with the therapy, however, is the lack of information on treatment protocols to-

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gether with a paucity of definite experimental proof that the technique is capable of enhancing the percutaneous absorption of drugs across human skin. Although there is no shortage of reports on the use of phonophoresis in volunteer subjects and patients, e.g. for lignocaine (Novak, 1964), carbocaine (Cameroy, 1966) and hydrocortisone (Griffin, 1966; Griffin et al., 1967; Kleinkort and Wood, 1975; Wing, 1982), the lack of adequate controls together with poor study design have invalidated many of the conclusions reached (Skauen and Zentner, 1984). A particular problem in interpreting the efficacy of phonophoresis in the treatment of inflammatory conditions is that ultrasound alone (i.e. without concomitant use of an anti-inflammatory topical product) has anti-inflammatory activity (e.g. Aldes et al., 1954; Middlemast and Chatterjee, 1978).

The aim of the present study was to evaluate whether ultrasound enhances percutaneous absorption using the model drug fluocinolone acetonide. This drug was chosen for two reasons: (a) much of the current literature on phonophoresis involves corticosteroids; and (b) the percutaneous absorption of corticosteroids can be easily monitored in volunteer subjects using the skin blanching test (Barry and Woodford, 1974).

Materials and Methods

The study, which was carried out on healthy volunteer subjects, was approved by the University Ethics Committee. Initial experimentation was carried out using two healthy volunteers to allow an adequate protocol to be drawn up for use with the corticosteroid skin blanching test (Barry and Woodford, 1974). To facilitate data collection it was desired that the maximum blanching response should appear within 6–8 h and last for up to 35 h after application of the gel. The preliminary experimentation was used to standardise the following variables:

(a) the length of the initial pre-soak period, i.e. the time period that gel remained in contact with the skin before the application of ultrasound;
(b) total length of contact time of the topical steroid gel with the skin, i.e. occlusion time after ultrasound treatment; and
(c) the weight of gel applied.

An area of skin equidistant from the elbow and wrist on the flexor surface of the forearm was used during all experiments as inconsistencies of blanching have been observed in sites close to the elbow and wrist (Burdick, 1974; Barry and Woodford, 1978). The area around the application site was covered with Blenderm tape to protect it from spread of the gel during ultrasonic massage. Initially the application site consisted of 5 holes (10 mm diameter) punched out of the Blenderm tape. To increase the contact area this was later increased to a rectangular area 2.5×2.0 cm. As previously demonstrated by Kirsch et al. (1982), no differences were found in the blanching response between the right and left forearms. Based on the initial results in two volunteers, a 5-min pre-soak period, 5-min ultrasound treatment followed by a 3-h occlusion period (Blenderm tape) prior to assessment of blanching was chosen as the most suitable study protocol. If the gel was left in contact with the skin for 4 h post-application, blanching was already established on removal of the occlusive tape. Control data utilising KY Jelly and KY Jelly plus ultrasound indicated that the blanching effect seen was indeed due to the steroid and not due to occlusion of the site or to the ultrasound.

The weight of gel required was the minimum weight that could produce an easily measurable response within the given time period. As too much steroid could mask any effects of the ultrasound, initial experimentation with different gel weights was also carried out. A weight of 1.5 g was found to be ideal but on ultrasonic massage a substantial amount was lost due to adhesion to the ultrasound head. This latter problem was overcome by applying 3 g for the initial pre-soak period, removing this totally after the ultrasonic massage and reapplying a weighed amount of fresh gel for the remainder of the 3-h contact period. This meant that a specific weight of gel was in contact with the application site at all times.

As some individuals do not blanch at all even

on application of large amounts of the most potent types of corticosteroid formulation (Haigh and Kanfer, 1984), preliminary screening of blanching activity in 24 volunteers was undertaken. Twelve "respondors" were selected for entry into the finalised protocol.

Treatment periods

The finalised protocol involved two treatment periods, two weeks apart. A typical treatment protocol for one volunteer is given below.

(a) Day 1. Synalar Gel (3 g; fluocinolone acetonide 0.025%) was placed on a 2.5×2.0 cm isolated site (Blenderm tape) on the flexor surface of the left forearm. The gel was left for 5 min (in an attempt to saturate the stratum corneum) and then massaged using an ultrasound head for 5 min ($2.0\ W \cdot cm^{-2}$; test). The treatment was then repeated for the right arm ($0\ W \cdot cm^{-2}$; control). Treatment with the ultrasound was fully randomised and blinded so that neither the subject nor the ultrasound administrator knew whether the instrument (Sonostat Model 633, Siemens) had been turned on or off. The instrument gave a pulsed output at a frequency of 870 kHz. The head used for the ultrasonic massage had a nominal radiating area of 4 cm^2 and a maximum power output of 12 W.

After ultrasound treatment the excess gel was totally removed and replaced by a second weighed amount of gel (1 g). The site was then occluded with Blenderm tape and left for 3 h. The subjects kept both sleeves rolled down during the occlusion period to minimise fluctuations in skin temperature. After occlusion the gel and tape were removed; the adhesive tape being removed slowly to reduce erythema which would interfere with the blanching assay. Blanching was assessed on a scale of 0–4 (Haigh and Kanfer, 1984) for up to 12 h after removal of the occlusive dressing. The observations were carried out in a double-blind fashion by a trained observer from 3 to 8 h and thereafter by self-assessment by the volunteers (final year pharmacy students who were familiar with the assay procedure).

(b) Day 2. The experimental procedure was as for day 1 except a 2 g aliquot of gel was reapplied to each arm after ultrasound treatment and the blanhing was monitored over 35 h. On day 2 the arms exposed to the ultrasound treatments ($2.0\ W \cdot cm^{-2}$ and $0\ W \cdot cm^{-2}$) were also crossed over.

A period of two weeks was allowed between treatments because of the steroid reservoir effect (Barry and Woodford, 1974). Cross-over of the treatments would overcome inconsistencies caused by any small residual reservoir effects which may have persisted for more than 14 days. Since the area under the blanching curve is a measure of the percutaneous absorption of the steroid (Barry et al., 1984), this parameter was used to assess the percutaneous absorption of fluocinolone acetonide. The *AUC* values were measured using the trapezoidal rule and values obtained for no ultrasound treatment ($0\ W \cdot cm^{-2}$) and ultrasound treatment ($2.0\ W \cdot cm^{-2}$) were compared for both study periods using the paired *t*-test. The paired *t*-test was used since each subject acted as his/her own control and since the data clearly indicated that there was a large variation in blanching activity between subjects but that blanching was reproducible for each individual subject.

Results

The procedures were well tolerated by all volunteers and informal questioning indicated that the volunteers and the physician were indeed unaware as to whether the ultrasound instrument had been switched on or off during treatment periods.

The results obtained for day 1 are shown in Fig. 1. It is clear that at all assessment times the mean blanching score was higher in the case of ultrasound treatment ($2.0\ W \cdot cm^{-2}$). The mean *AUC* value ($\pm$S.E.) was also significantly ($P < 0.05$) higher (10.4 ± 1.5 versus 7.9 ± 1.4; Table 1) when ultrasound data were compared with massage only ($0\ W \cdot cm^{-2}$). The blanching response in both cases followed a similar curve with increased mean blanching scores occurring for up to 11 h in both cases.

A similar pattern was seen on day 2 of the study. The addition of twice the amount of gel post-ultrasound therapy, however, led to a marked increase in the blanching scores achieved, e.g. at

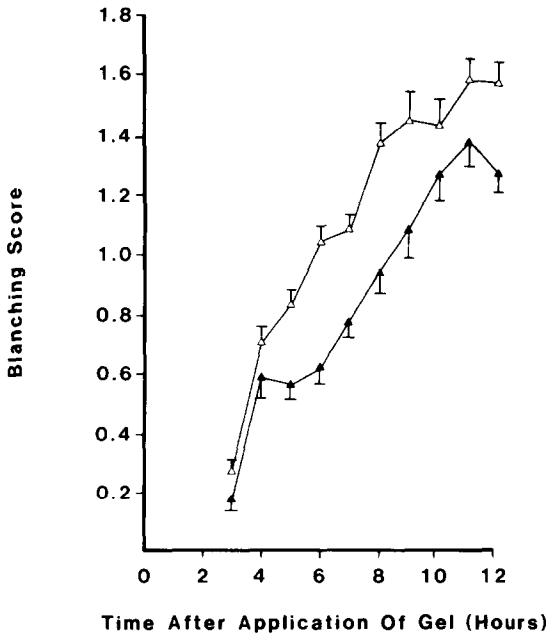

Fig. 1. Blanching score (mean ± S.E.) versus time profile in 12 volunteer subjects on experimental day 1 after application of ultrasound at an intensity of 0 $W \cdot cm^{-2}$ (▲; control) and 2.0 $W \cdot cm^{-2}$ (△; test).

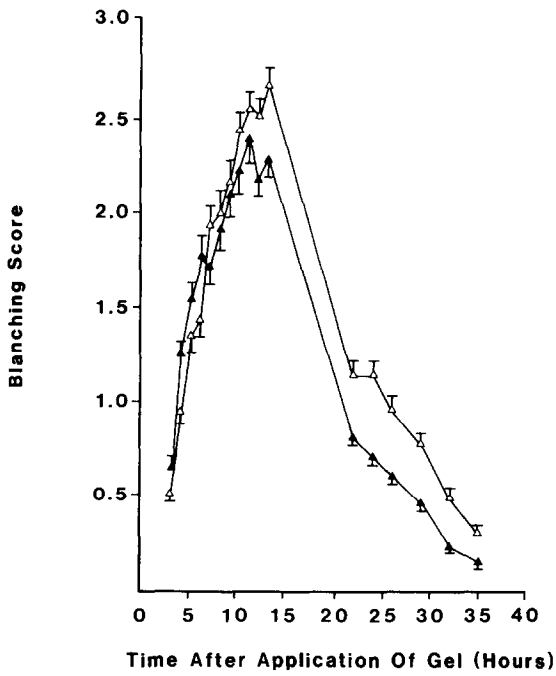

Fig. 2. Blanching score (mean ± S.E.) versus time profile in 12 volunteer subjects on experimental day 2 after application of ultrasound at an intensity of 0 $W \cdot cm^{-2}$ (▲; control) and 2.0 $W \cdot cm^{-2}$ (△; test).

12 h the mean blanching score on day 1 with ultrasound was 1.6 while on day 2 the corresponding value was 2.6. The two curves on day 2 were, however, very similar up to 12 h (Fig. 2) and indeed the *AUC* values obtained for this period were not significantly different between the treatments ($P > 0.05$; Table 1). Thereafter, the *AUC* values were significantly higher ($P < 0.05$) in those cases where ultrasound had been administered (Table 1). It should be stressed that the differences

TABLE 1

Summary of results obtained for area under the blanching versus time curves for the 2 ultrasound treatments on the 2 experimental days

	Mean *AUC* value (blanching scale value × h ± S.E.)		Calculated *t*-value	Statistical significance *
	Control (0 $W \cdot cm^{-2}$)	Test (2 $W \cdot cm^{-2}$)		
Treatment day 1, 3–12 h *AUC* values	7.9 ± 1.4	10.4 ± 1.5	2.225	$P < 0.05$ S
Treatment day 2, 3–12 h *AUC* values	16.6 ± 2.6	16.1 ± 2.5	−0.318	$P > 0.05$ NS
Treatment day 2, 3–22 h *AUC* values	32.6 ± 4.5	36.4 ± 4.4	2.685	$P < 0.05$ S
Treatment day 2, 3–35 h *AUC* values	38.5 ± 5.2	45.6 ± 5.7	3.750	$P < 0.05$ S

* Paired *t*-test. NS = not significant; S = significant.

obtained were small and if compared using an unpaired t-test were statistically insignificant. The paired t-test is, however, more appropriate for use with the present results since each subject acted as his/her own control and since control and test data in each volunteer was monitored simultaneously.

Discussion

Percutaneous absorption of drugs from products designed for local effects is particularly difficult to monitor in vivo in human subjects since the rate of drug absorption is often slow and the extent of drug absorption is often too low to allow quantification of the drug in serum without the use of radiolabelled compounds. The blanching test for corticosteroids (Barry and Woodford, 1974) overcomes this difficulty since the degree of blanching has been shown to correlate well with the amount of a steroid absorbed across the skin barrier (Christie and Moore-Robinson, 1970). It is also convenient that the kinetics of this pharmacodynamic effect can be easily monitored. Since corticosteroids are one group of drugs commonly used with phonophoresis (Wing, 1982), the blanching test was an obvious choice of experimental technique to assess whether phonophoresis is effective.

The experimental protocols were devised specifically for this investigation. The aim of the 5-min contact time of the gel prior to application of the ultrasound was to help saturate the outer layers of the skin (stratum corneum) prior to application of the ultrasonic driving force. This 5-min period was a compromise since in clinical practice a presoak period is not generally used. A gel formulation of fluocinolone acetonide was chosen since gels are more likely than creams or ointments to couple the ultrasonic head with the skin and so allow transmission of the ultrasonic energy from the apparatus to the volunteer. (Ultrasonic energy does not travel in air.)

The blanching response depends on 3 main factors (Wallace et al., 1979):

(a) the ability of the steroid to penetrate the skin barrier after release from the applied vehicle;
(b) the intrinsic activity of the steroid at the receptor site; and
(c) the clearance of the drug from the active site.

Each subject acted as his/her own control and therefore the intrinsic activity remained unchanged. Clearance of drug from the active site, if changed, would be increased during ultrasound administration due to the increased blood flow caused by localised heat production (Coakley, 1978). Since the area under the blanching curve was increased after ultrasound treatment, the present results clearly illustrate that ultrasound enhanced the percutaneous absorption of fluocinolone. The fact that no difference was observed between the two treatments on day 2 up to 12 h (Table 1) can be attributed to the larger mass of gel (2 g) left in contact with the skin; the increased blanching, due to doubling of gel weight, initially masked the effects of the ultrasound. It should be stressed, however, that the increases were small and likely to be clinically insignificant.

Studies with hydrocortisone have indicated that the rate-limiting step in the percutaneous absorption of steroids is passage across the stratum corneum (Feldman and Maibach, 1965). A proposed mechanism for enhanced percutaneous absorption of drugs, e.g. by decylmethyl sulphoxide, is via increased lipid fluidity of the stratum corneum (Goodman and Barry, 1986). This is also a possible mechanism for enhanced percutaneous absorption when the skin is treated with ultrasonic energy. Differential scanning calorimetric studies are currently underway to find out if ultrasound increases lipid fluidity of the stratum corneum. Some localised heat is also produced by ultrasonic massage. This may also have played a role in enhanced drug absorption noted in the present study.

In conclusion ultrasound increased the percutaneous absorption of fluocinolone acetonide to a small but statistically significant extent from Synalar Gel. This is in contrast to our previously reported studies of a lack of a statistically significant phonophoretic effect with lignocaine cream (McElnay et al., 1985) and benzydamine gel (Benson et al., 1986).

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