International Journal of Pharmaceutics 395 (2010) 84–90

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Low-frequency ultrasound sonophoresis to increase the efficiency of topical steroids: A pilot randomized study of humans

Annabel Maruani[a,b,*], Alain Boucaud[c], Elodie Perrodeau[d], David Gendre[d], Bruno Giraudeau[a,d], Laurent Machet[a,b]

[a] Université François Rabelais Tours, France
[b] INSERM, U930 Tours, CHRU de Tours, Service de Dermatologie, France
[c] Transderma Systems, Tours, France
[d] INSERM, CIC 202, CHRU Tours, France

ARTICLE INFO

Article history:
Received 31 January 2010
Received in revised form 3 May 2010
Accepted 8 May 2010
Available online 22 May 2010

Keywords:
Low-frequency ultrasound
Sonophoresis
Clinical study
Topical steroids

ABSTRACT

Topical steroids are efficient in vasoconstriction potential, which is linked to their anti-inflammatory activity. Low-frequency ultrasound (US) applied on the skin (sonophoresis) may enhance the transdermal transport of various steroids. We aimed to assess, in a simple, blinded, randomized controlled pilot study, the clinical efficiency of sonophoresis in increasing vasoconstriction by enhancing the transdermal penetration of topical steroids in human skin.

The study took place in the Clinical Investigation Center of the University Hospital of Tours and involved healthy volunteers. Three circular zones were delimited on each of the subjects' forearms: zone 1 (right and left) received topical steroids with 1-h occlusion, zone 2 with 2-h occlusion, and zone 3 with massage. Forearms were randomized to first undergo US, using a 36 kHz probe, delivered in a pulsed mode (2 s on/5 s off), during 5 min, with a US intensity of 2.72 W/cm^2, or no US. We used betamethasone 17-valerate in cream form as the topical steroid. The primary outcome was difference between forearms in skin color (increased whiteness reflecting the intensity of vasoconstriction) measured by 2 scores: values obtained with a chromameter (the higher the value, the whiter the skin) and a clinical visual score. The measurements were taken by a dermatologist by blinded assessment.

Fifteen subjects were included. Vasoconstriction was significantly higher with the topical steroid applied after US, especially in zone 2, than without US. Vasoconstriction was increased at 1, 2, 3, 4, and 6 h (e.g., chromameter score 63.4 *versus* 65.2, $p = 0.017$ at 4 h) and disappeared at 24 h. Moreover, 2-h occlusion gave higher vasoconstriction scores than did 1-h occlusion or massage alone, whether US was applied or not.

The use of low-frequency US coupled with 2-h occlusion is a synergistic way to increase the efficiency of topical steroids by enhancing skin permeability.

1. Introduction

The anti-inflammatory activity of topical steroids is linked to their vasoconstriction potential, according to which they are classified into 4 classes of activity (Mc Kenzie and Stoughton, 1962; Täuber, 1994). The vasoconstriction effect of steroids depends on the molecules' effects and their ability to penetrate skin. Thus, use of topical steroids with transdermal enhancers increases the vasoconstriction. Low-frequency ultrasound (US) applied to the skin, called sonophoresis, has been investigated for enhancing the transdermal transport of various drugs with a low and even high weight, such as insulin (Byl, 1995; Liu et al., 2006; Mitragotri and Kost, 2001; Smith et al., 2003). US has been investigated *in vitro* on excised skin, *in vivo* in animals and in only a few studies in humans (Becker et al., 2005; Katz et al., 2004; Santoianni et al., 2004). Sonophoresis is a noninvasive method and is well tolerated with use of defined parameters of US delivery (duration of delivery, intensity, pulsed/continuous mode) (Machet and Boucaud, 2002). The most common adverse effects are moderate erythema, pain and tinnitus, which are all reversible (Maruani et al., 2010). Increased transdermal permeability after US is attributed to thermal effects but mainly to cavitation phenomenon—the generation of gas bubbles, which oscillate and may implode at the skin surface, thus provoking disorganization and/or the creation of an aqueous pathway through

* Corresponding author at: Service de Dermatologie, CHRU de Tours, Avenue de la République, 37044 Tours Cedex 9, France. Tel.: +33 2 47 36 62 56; fax: +33 2 47 36 62 56.
E-mail address: annabel-maruani@wanadoo.fr (A. Maruani).

0378-5173/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2010.05.010

the stratum corneum (Simonin, 1995; Tang et al., 2002; Tezel et al., 2002).

We aimed to investigate in a simple, blinded, randomized controlled pilot study of healthy subjects the clinical efficiency of sonophoresis in enhancing transdermal penetration of topical steroids in human skin, as measured by vasoconstriction scores.

2. Patients and methods

2.1. Setting and participants

The study was carried out from May to July 2008 in the Clinical Investigation Center of the University Hospital of Tours, France. Healthy volunteers were included if they were ≥18 years old, were not pregnant or breastfeeding, did not use topical therapy, and had neither dermatological nor neurological disease. Each volunteer signed an informed consent form.

2.2. Randomization of US and blinding

A randomization sequence was computer generated. For each subject, the scientific engineer (A.B.) randomized each subject's forearm, right or left, to undergo US or not. The dermatologist, who assessed primary and secondary outcomes, was blinded to the US treatment.

2.3. Interventions

2.3.1. Ultrasound device

The US device (Transderma Systems, Tours, France) was a Langevin-type transducer commonly used in sonophoresis studies. Its working frequency was 36 kHz. The tip of the device included a cylindrical ultrasonic horn (titanium), 1.8 cm in diameter, housed in a tube made of Delrin. The device also includes a chamber where cavitation occurs, called a "cavitation chamber," consisting of an O-ring with a 7-cm^2 inner surface placed between the skin surface and the ultrasonic horn. It contained 10 ml of saline ([NaCl] 9‰) used as a coupling medium. The tip vibration was parallel to the axis of the acoustic system. During the experiments, the tip of the ultrasonic horn was always positioned 5 mm from the skin. The power unit of the device allowed for adjusting various parameters, including intensity, continuous or pulsed mode, and application time. Before each application, the equipment was calibrated to ensure that the electrical signal matched the resonance frequency of the US probe. US intensity was determined by a calorimetric method, which is usually used to measure low-frequency US intensity (Weimann and Wu, 2002; Zderic et al., 2002).

2.3.2. Topical steroid

We used a topical steroid with class 2 activity, betamethasone 17-valerate, in cream form (Betneval®, GlaxoSmithKline). A quantity of 0.2 ml corresponding to about 0.2 g (i.e., 0.1 mg of active principle) was used for application.

2.4. Protocol

Each subject sat in a medical chair. For each forearm, the research engineer (in room 1) delimited 3 circular zones (zones 1, 2 and 3 each on the right and left forearms) 12 mm in diameter (Fig. 1). Each forearm was randomized to receive treatment without US on the 3 zones or US delivered in a pulsed mode (2 s on/5 s off) during 5 min, with a US intensity of 2.72 W/cm^2. If toxicity occurred (major pain, necrosis), US was immediately discontinued. Immediately after US application, the engineer applied betamethasone 17-valerate on zones 1 and 2 (right and left forearm) with occlusion (i.e., retained on the skin by use of a polyurethanne dressing, Tegaderm® (3 M Santé, France). For zone 1 (right and left forearm), the dressing was taken off after 1 h and for zone 2, after 2 h. For zone 3, betamethasone 17-valerate was massaged on the skin for 30 s. The dermatologist (A.M.) (in room 2) took all measurements (see Section 2.5) before the application of betamethasone 17-valerate, at 30 min (zone 3 only), 1 h (zones 1 and 3), then 2, 3, 4, 6 and 24 h (all zones).

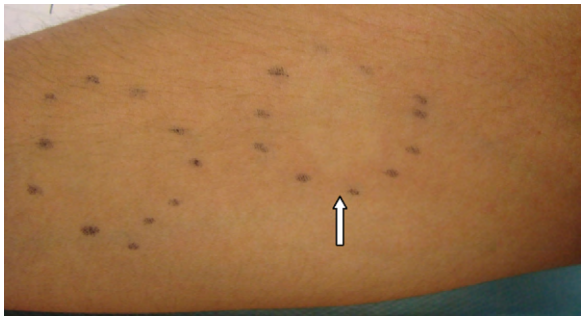


Fig. 1. Definition of 6 zones on the anterior part of forearms (R, right; L, left) before low-frequency ultrasound and treatment with topical steroid betamethasone 17-valerate.

2.5. Outcomes

2.5.1. Primary outcome

The main objective was to evaluate skin penetration of betamethasone 17-valerate after low-frequency US. We assessed the difference in skin color (increased whitening reflecting the intensity of vasoconstriction) between the forearm with US and betamethasone 17-valerate and the forearm with betamethasone 17-valerate alone. Color was evaluated by use of a chromameter and clinical vasoconstriction evaluation at 30 min and 1, 2, 3, 4, 6 and 24 h. Forearm color was assessed before the application of betamethasone 17-valerate – immediately after US – to take into account the variation in skin color specifically linked to US.

A chromameter (CR300, Minolta, Japan) is used to objectively assess change in skin color (Van den Kerckhove et al., 2001); the probe of the chromameter is placed in contact with the cutaneous zones. The chromameter provides 2 parameters reflecting the whiteness and the redness (linked to pigmentation and vascularisation) of the zone. We analyzed whiteness in this study because it reflects vasoconstriction; the higher the value (given with arbitrary units), the whiter the zone; the lower the value, the redder the zone.

The dermatologist clinically evaluated skin color using a visual scale from 0 to 10, 0 corresponding to maximal whiteness (maximal vasoconstriction) and 10 maximal redness (maximal pigmentation and vasodilatation); 5 represents normal skin (measured before the application of US/betamethasone 17-valerate).

2.5.2. Secondary outcome

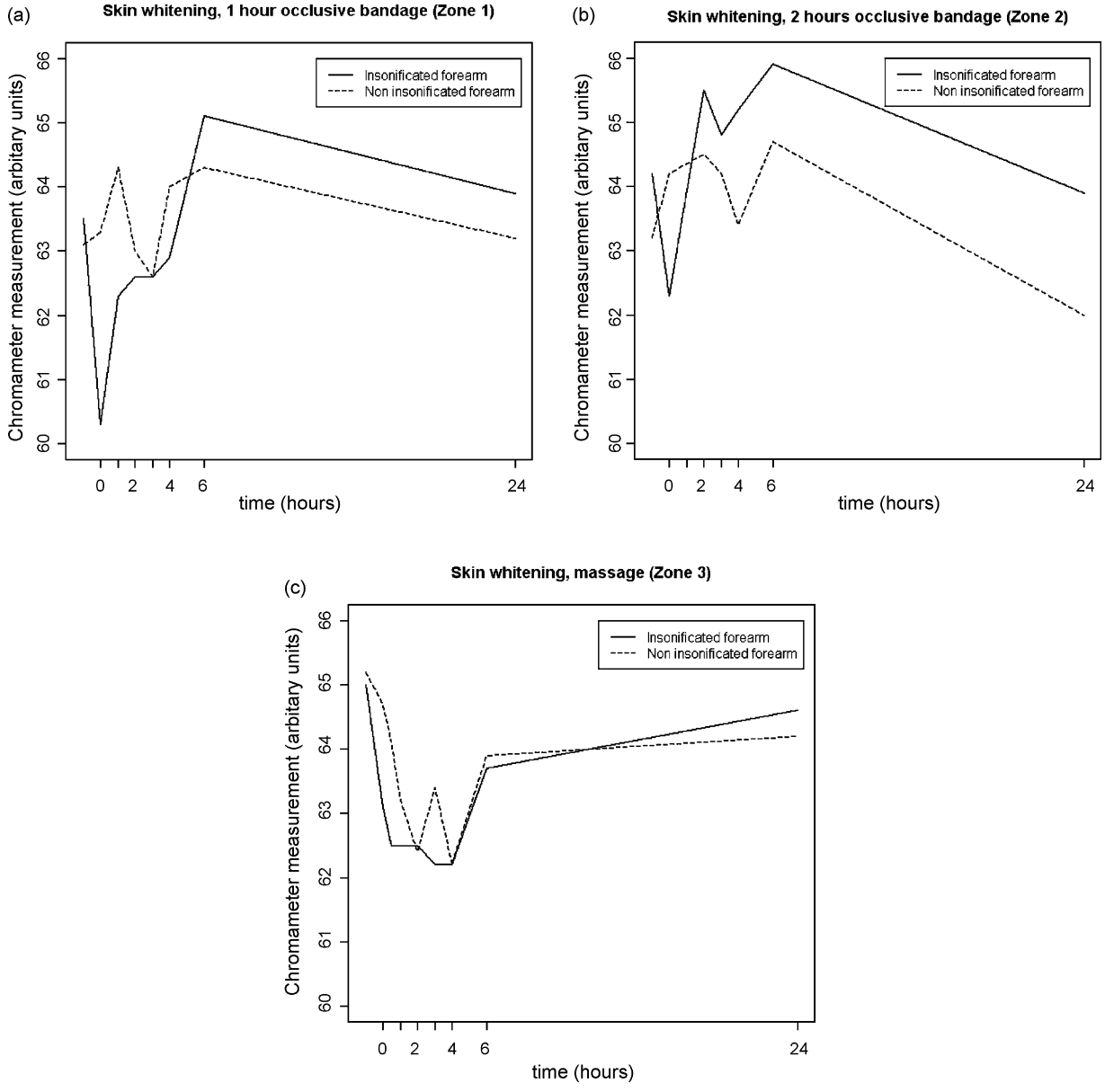
The secondary outcome was whether application of betamethasone 17-valerate with 1- or 2-h occlusion after low-frequency US was more efficient than usual treatment (application of topical cream by massage alone). This outcome was assessed by the kinetics of cutaneous whitening (vasoconstriction) in terms of delay and duration of action as measured by chromametric and visual vasoconstriction scores.

Adverse events were systematically evaluated by the research engineer and dermatologist. Pain was self-reported by the subject on a visual analog scale, from 0 (no pain) to 100 (maximal pain). Pain > 40/100 was considered important and led to the discontinuation of US.

Table 1a
Whitening of zones of forearms assessed by a chromameter.

		T0	T1	T2	T3	T4	T6	T24
Zone 1	IF	63.5 [60.1; 65.9]	62.3 [59.9; 67.0]	62.6 [60.3; 67.7]	62.6 [60.2; 67.0]	62.9 [60.5; 66.9]	65.1 [60.4; 67.0]	63.9 [59.6; 67.6]
	NIF	63.1 [60.4; 67.7]	64.3 [61.7; 67.1]	63.0 [60.5; 67.9]	62.6 [60.9; 67.5]	64.0 [60.9; 68.0]	64.3 [60.9; 67.5]	63.2 [60.5; 68.5]
	p	0.198	**0.026**	1	0.776	0.397	0.187	0.599
Zone 2	IF	64.2 [60.1; 67.8]	–	65.5 [62.1; 69.7]	64.8 [61.5; 68.9]	65.2 [61.4; 68.4]	65.9 [61.4; 68.3]	63.9 [60.6; 68.4]
	NIF	63.2 [60.7; 67.2]	–	64.5 [61.2; 68.7]	64.2 [59.4; 67.4]	63.4 [60.8; 67.4]	64.7 [60.3; 67.7]	62.0 [60.6; 68.5]
	p	0.330	–	0.083	**0.031**	**0.017**	0.112	0.167
Zone 3	IF	65.0 [61.1; 68.1]	62.5 [59.1; 68.5]	62.5 [58.9; 67.8]	62.2 [60.0; 68.3]	62.2 [60.1; 68.0]	63.7 [59.1; 66.4]	64.6 [59.7; 68.9]
	NIF	65.2 [60.7; 67.2]	63.2 [59.6; 67.9]	62.4 [60.1; 67.3]	63.4 [60.3; 67.8]	62.2 [59.8; 67.2]	63.9 [60.6; 66.8]	64.2 [61.0; 68.1]
	p	0.181	0.950	0.776	0.362	0.328	0.798	0.706

Data are medians [interquartile range].

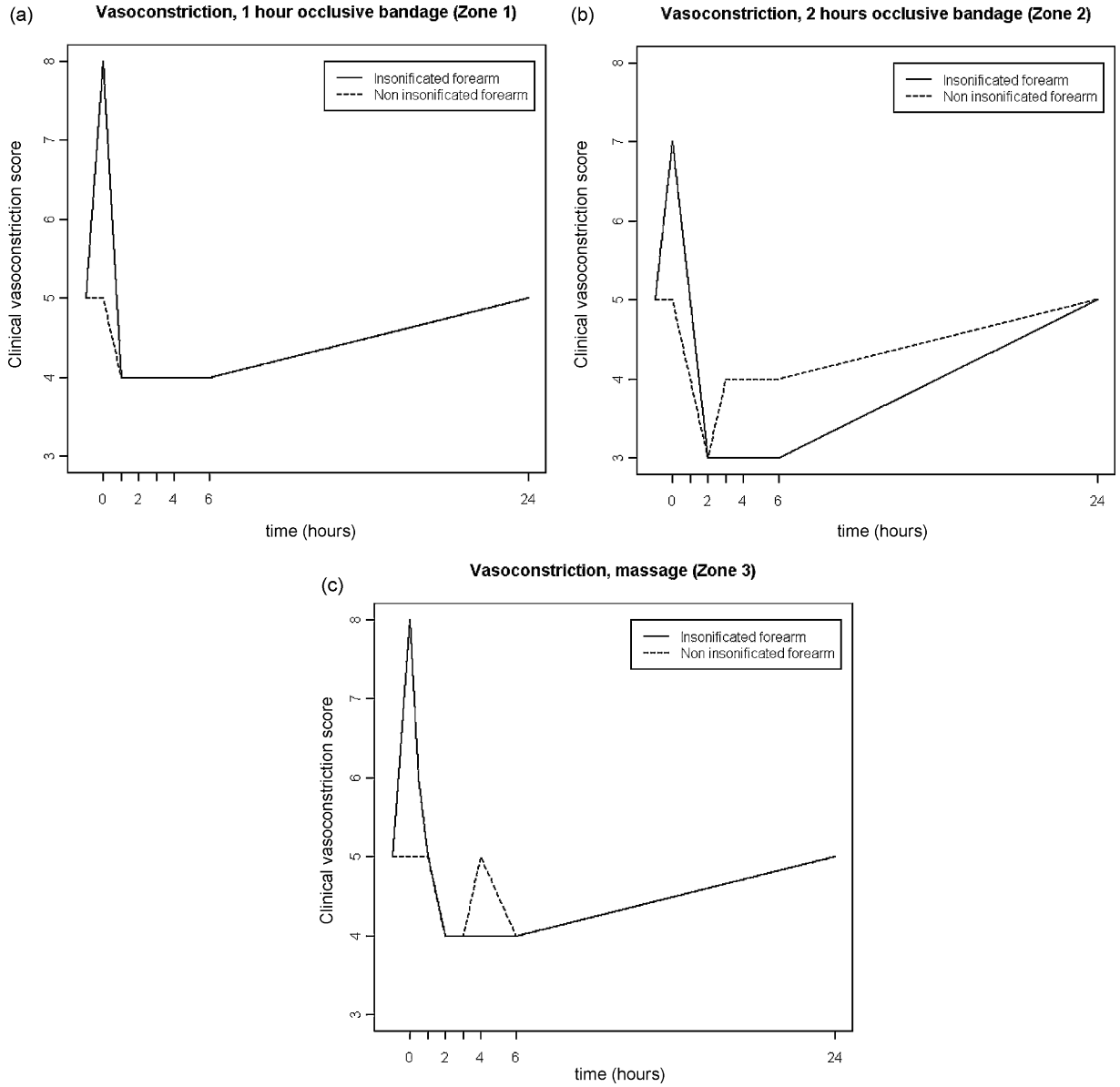


Graph 1. Median skin whitening measured by chromameter (arbitrary values which reflect whiteness: the higher the value, the whiter the skin) at each time for each zone.

Table 1b
Whitening of zones of forearms assessed by clinical vasoconstriction score from 0 (maximal vasoconstriction) to 10 (maximal vasodilatation).

		T0	T1	T2	T3	T4	T6	T24
Zone 1	IF	5.0 [5.0; 5.0]	4.0 [4.0; 5.8]	4.0 [3.0; 4.0]	4.0 [3.2; 4.0]	4.0 [3.9; 5.0]	4.0 [3.0; 4.8]	5.0 [4.0; 5.0]
	NIF	5.0 [5.0; 5.0]	4.0 [4.0; 4.0]	4.0 [4.0; 4.0]	4.0 [4.0; 4.8]	4.0 [4.0; 5.0]	4.0 [3.2; 4.8]	5.0 [4.0; 5.0]
	p	–	0.105	0.665	0.588	0.890	0.766	0.572
Zone 2	IF	5.0 [5.0; 5.0]	–	3.0 [2.0; 3.0]	3.0 [2.0; 3.0]	3.0 [3.0; 4.0]	3.0 [2.2; 4.0]	5.0 [4.0; 5.0]
	NIF	5.0 [5.0; 5.0]	–	3.0 [3.0; 4.0]	4.0 [4.0; 4.0]	4.0 [4.0; 4.0]	4.0 [3.0; 4.0]	5.0 [4.2; 5.0]
	p	–	–	**0.015**	**0.007**	**0.008**	**0.006**	0.345
Zone 3	IF	5.0 [5.0; 5.0]	5.0 [4.0; 6.0]	4.0 [4.0; 5.0]	4.0 [3.2; 4.0]	4.0 [4.0; 4.1]	4.0 [4.0; 4.8]	5.0 [4.0; 5.0]
	NIF	5.0 [5.0; 5.0]	5.0 [4.0; 5.0]	4.0 [4.0; 5.0]	4.0 [4.0; 4.8]	5.0 [4.0; 5.0]	4.0 [4.0; 4.8]	5.0 [5.0; 5.0]
	p	–	0.098	1	**0.008**	**0.015**	0.773	0.766

Zone 1: topical steroid with 1-h occlusion; zone 2: topical steroid with 2-h occlusion; zone 3: topical steroid applied by massage alone.
T0: before treatment; T1: 1 h after treatment; T2: 2 h after treatment; T3: 3 h after treatment; T4: 4 h after treatment; T6: 6 h after treatment; T24: 24 h after treatment.
IF: forearm treated with ultrasound; NIF: forearm not treated with ultrasound.



Graph 2. Median skin whitening measured with clinical vasoconstriction score at each time for each zone.

2.6. Statistical analysis

We arbitrarily planned to include 15 subjects in this pilot study. Quantitative data are presented as medians [interquartile range] and qualitative data as frequencies. Statistical analysis involved use of R Project for Statistical Computing v2.8.1. A Wilcoxon signed rank for paired data (significance = 5%) was used to compare skin whiteness on both forearms (US and no US), the primary outcome, and to compare the variation in skin whiteness in all zones (by massage and 1- and 2-h occlusion) on each forearm and at each time, the secondary outcome. A descriptive analysis was performed for tolerance data.

2.7. Ethical approval

The protocol was approved by the ethics committee of the University Hospital of Tours, France (registration no. 2005-05).

3. Results

We enrolled 15 healthy subjects in the study (12 females; median age 24 years (range 19–60 years).

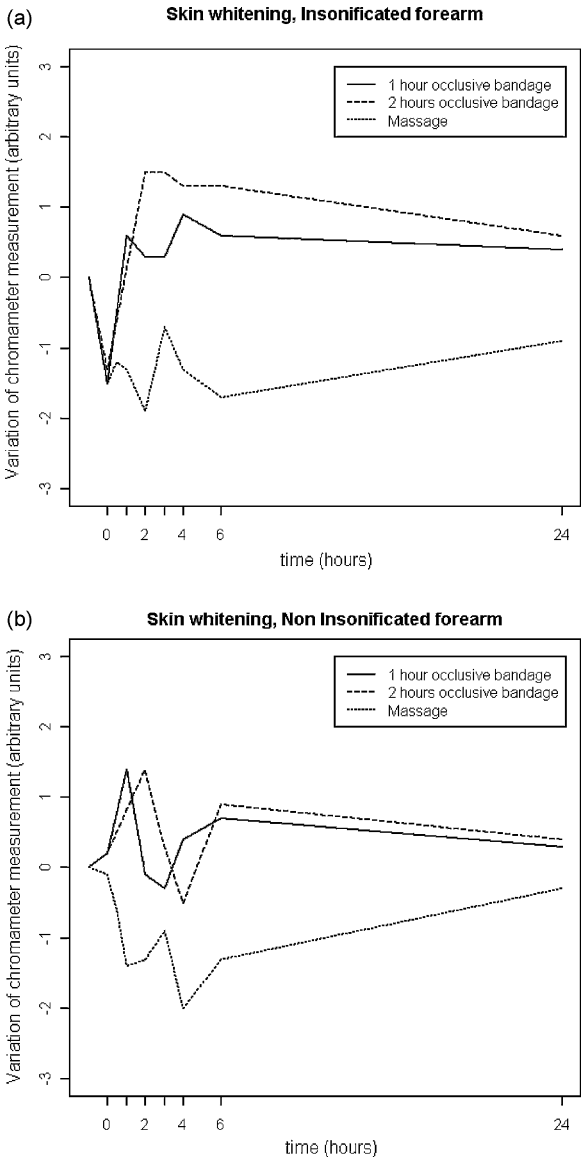
3.1. Primary outcome

Results of whitening of 3 zones, with and without US, investigated with use of a chromameter are in Table 1a. Graph 1a–c shows the chromameter measurements at time 0 and at 1, 2, 3, 4, 6 and 24 h. Immediately after US, whiteness decreased, subsequent to erythema induced by US, then whiteness increased slowly as the erythema disappeared and vasoconstriction occurred. The assessment of whitening of the 3 zones by clinical vasoconstriction score is in Table 1b and Graph 2a–c.

For zone 2 (topical steroid with 2-h occlusion), whitening (vasoconstriction) was greater on the forearm with US and topical steroid treatment than on the forearm with topical steroid alone, especially at T3 (chromameter score 64.2 *versus* 64.8, $p = 0.032$) and T4 (63.4 *versus* 65.2, $p = 0.017$) (Fig. 2).

3.2. Secondary outcome

Whitening (vasoconstriction) with application of the topical steroid assessed by chromameter, with and without US, comparing the 3 different zones, is in Table 2a and Graph 3a and b. Clinical vasoconstriction scores are in Table 2b. In forearms with US, whitening was significantly greater in zone 2 (2-h occlusion) than in zones 1



Graph 3. Variation of skin whitening measured by chromameter (arbitrary units: the higher the value, the whiter the zone) compared to the measurement before insonification.

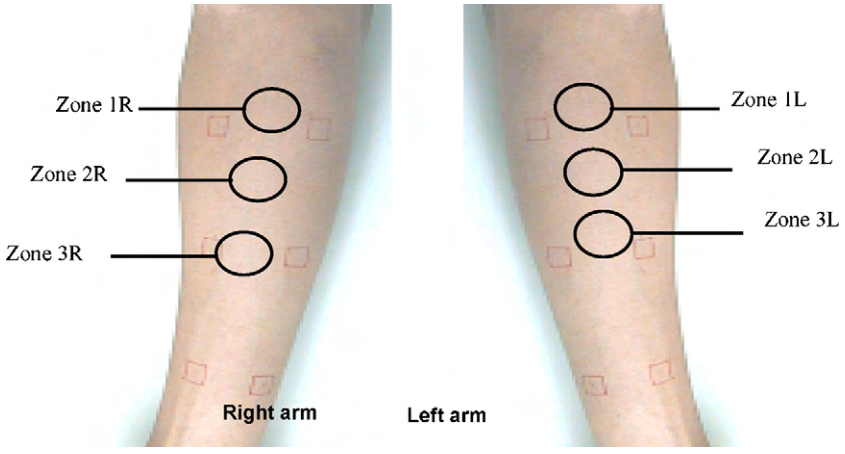


Fig. 2. Vasoconstriction (whitening) 4 h after the application of topical steroid, betamethasone 17-valerate, on a forearm that had undergone low-frequency ultrasound (sonophoresis).

Table 2a
Comparison of the variation of whitening measured with chromameter on the three zones.

	Difference in comparison with 1st measurement (median)			*p*-Value		
	Zone 1	Zone 2	Zone 3	Z1 *versus* Z2	Z1 *versus* Z3	Z2 *versus* Z3
Insonificated forearm						
T1	0.6	–	−1.3	–	**0.001**	–
T2	0.3	1.5	−1.9	**0.001**	**0.002**	**<0.001**
T3	0.3	1.5	−0.7	0.233	**0.011**	**0.001**
T4	0.9	1.3	−1.3	0.334	**0.011**	**0.001**
T6	0.6	1.3	−1.7	0.320	**0.001**	**<0.001**
T24	0.4	0.6	−0.9	0.887	0.121	0.083
Non-insonificated forearm						
T1	0.6	–	−1.3	–	**0.001**	–
T2	−0.1	1.4	−1.9	**0.003**	**0.031**	**<0.001**
T3	−0.3	0.3	−0.7	0.397	**0.035**	**0.018**
T4	0.4	0.5	−1.3	0.513	**0.005**	**0.002**
T6	0.7	0.9	−1.7	0.570	**0.008**	**0.020**
T24	0.3	0.4	−0.9	0.733	0.245	0.842

Table 2b
Comparison of the variation of whitening assessed with clinical vasoconstriction score on the three zones.

	Difference in comparison with 1st measurement (median)			*p*-Value		
	Zone 1	Zone 2	Zone 3	Z1 *versus* Z2	Z1 *versus* Z3	Z2 *versus* Z3
Insonificated forearm						
T1	−1	–	0	–	0.210	–
T2	−1	−2	−1	**<0.001**	0.209	**0.001**
T3	−1	−2	−1	**0.003**	0.305	**0.009**
T4	−1	−2	−1	**0.002**	0.299	**0.002**
T6	−1	−2	−1	**0.006**	0.178	**0.005**
T24	0	0	0	0.850	0.530	0.233
Non-insonificated forearm						
T1	−1	–	0	–	0.052	–
T2	−1	−2	−1	**0.002**	**0.037**	**0.002**
T3	−1	−1	−1	0.110	0.407	**0.031**
T4	−1	−1	0	0.073	0.240	**0.008**
T6	−1	−1	−1	0.424	0.224	0.111
T24	0	0	0	0.203	0.120	1

Zone 1: topical steroid with 1-h occlusion; zone 2: topical steroid with 2-h occlusion; zone 3: topical steroid applied with massage.
T1: 1 h after treatment; T2: 2 h after treatment; T3: 3 h after treatment; T4: 4 h after treatment; T6: 6 h after treatment; T24: 24 h after treatment.

and 3 at all times (from 1 to 6 h), as assessed by both chromametric and clinical scores, with no significant difference in whitening between zones 1 and 3. In the forearms without US, whitening was significantly greater in only zone 2 than in zones 1 and 3, at 2 h only.

Zones of forearms showed no difference in whitening at 24 h, whether they underwent US or not.

3.3. Adverse events

During US delivery, subjects self-reported pain of moderate intensity (≤40 on a 100-point visual analog scale) for 38 of 45 zones and major pain (>40) for 7 zones, which led to the discontinuation of US. In all cases, pain stopped as soon as US was discontinued.

The dermatologist noted slight punctiform purpura on the site undergoing US in 3 cases: in 2 cases, purpura regressed at 24 h. One case of contact dermatitis occurred after occlusion, which resolved after 24 h.

4. Discussion

We aimed to assess, in a simple, blinded, randomized controlled pilot study, the clinical efficiency of sonophoresis in increasing vasoconstriction by enhancing the transdermal penetration of a topical steroid in human skin. Low-frequency US was efficient in enhancing the cutaneous penetration of the topical steroid *in vivo* on human skin. Indeed, whitening obtained with betamethasone 17-valerate treatment after US was greater than that without US in most cases, particularly when the topical steroid was applied under a bandage (occlusion as opposed to massage), especially with long-lasting occlusion (2 *versus* 1 h). The efficiency was greater in terms of intensity of whitening, as measured by chromametric and visual scores, and duration of whitening. Indeed, significant results were observed up to 6 h after US. However, US did not enhance the rapidity of whitening, probably because of the vasodilatation immediately induced by low-frequency US (Maruani et al., 2010).

The explanation of increased transdermal permeability due to low-frequency US is linked in part to the thermal effects of US and mainly attributed to cavitation (Simonin, 1995; Tang et al., 2002; Tezel et al., 2002). Cavitation increases with high-intensity low-frequency US, thus leading to a parallel increase in transdermal permeability. However, toxicity also increases: with high intensity or long duration, sonophoresis can induce necrosis of skin, fascia and muscles (Machet and Boucaud, 2002; Singer et al., 1998; Yamashita et al., 1997). The intensity we used was previously demonstrated to be well tolerated (Maruani et al., 2010). With US, we observed pain, erythema and slight punctiform purpura, which all rapidly resolved. Tinnitus is a commonly described side effect of high- and low-frequency US (Maruani et al., submitted for publication), but we did not observe tinnitus in our study.

Our secondary outcome was whether whitening with topical steroid applied with 1- and 2-h occlusion was more efficient than that applied by massage as measured by delay and duration of activity. Independent of US, topical steroid applied with 2-h occlusion induced more efficient vasoconstriction than did 1-h occlusion and

massage alone. The difference was still significant 6 h after US. The effect of occlusion as a known enhancer of skin absorption of drugs is linked to several mechanisms: an increase in stratum corneum hydration, an increase in skin temperature on the occluded site (and thus, a higher rate of vasodilatation), and a longer duration of the contact of the drug with the skin (Cevc et al., 2008).

Our pilot study suggests that the use of 2 different enhancers of skin permeability to topical steroids (low-frequency US and occlusion) is a synergistic way to increase the efficiency of topical steroids, considering both the intensity and duration of vasoconstriction. Indeed low-frequency US with 2-h occlusion induced greater vasoconstriction than did occlusion without US. The tolerance of the skin to this treatment was acceptable. Thus, the use of topical steroids with both sonophoresis and occlusion could be of interest for localized dermatologic lesions, which require treatment with high-intensity anti-inflammatory molecules. For instance, this combination could be used for lesions refractory to treatment because of thick skin, such as for cheloid scars or for topography of palms and soles.

Conflict of interest

Dr. Alain Boucaud is a member of Transderma Systems, which supplied the ultrasound device.

Acknowledgements

The authors thank Jean-Marc Grégoire (UMR INSERM U930, CRNRS ERL 3106, Tours) for technical assistance and Aurélie Avarguès (CIC 202, CHRU de Tours) for support. The study was funded by a “Projet Hôpital Promoteur”, CHRU de Tours (HP) financing.

References

Becker, B.M., Helfrich, S., Baker, E., Lovgren, K., Minugh, A., Machan, J.T., 2005. Ultrasound with topical anesthetic rapidly decreases pain of intravenous cannulation. Acad. Emerg. Med. 12, 289–295.

Byl, N.N., 1995. The use of ultrasound as an enhancer for transcutaneous drug delivery: phonophoresis. Phys. Ther. 75, 539–553.

Cevc, G., Mazgareanu, S., Rother, M., Vierl, U., 2008. Occlusion effect on transcutaneous NSAID delivery from conventional and carrier-based formulations. Int. J. Pharm. 359, 190–197.

Katz, N.P., Shapiro, D.E., Herrmann, T.E., Kost, J., Custer, L.M., 2004. Rapid onset of cutaneous anesthesia with EMLA cream after pretreatment with a new ultrasound-emitting device. Anesth. Analg. 98, 371–376.

Liu, H., Li, S., Pan, W., Wang, Y., Han, F., Yao, H., 2006. Investigation into the potential of low-frequency ultrasound facilitated topical delivery of cyclosporin A. Int. J. Pharm. 326, 32–38.

Machet, L., Boucaud, A., 2002. Phonophoresis: efficiency, mechanisms and skin tolerance. Int. J. Pharm. 243, 1–15.

Maruani, A., Vierron, E., Machet, L., Giraudeau, B., Halimi, J.M., Boucaud, A. Tolerance of low-frequency ultrasound sonophoresis: a double-blind randomised study on humans. Skin Res. Technol., submitted for publication.

Maruani, A., Vierron, E., Machet, L., Giraudeau, B., Boucaud, A., 2010. Efficiency of low-frequency ultrasound sonophoresis in skin penetration of histamine: a randomized study in humans. Int. J. Pharm. 385, 37–41.

Mc Kenzie, A.W., Stoughton, R.B., 1962. Method for comparing percutaneous absorption of steroids. Arch. Dermatol. 86, 608–616.

Mitragotri, S., Kost, J., 2001. Transdermal delivery of heparin and low-molecular weight heparin using low-frequency ultrasound. Pharm. Res. 18, 1151–1156.

Santoianni, P., Nino, M., Calabro, G., 2004. Intradermal drug delivery by low frequency sonophoresis (25 KHz). Dermatol. Online J. 10, 24.

Simonin, J.P., 1995. On the mechanisms of in vitro and in vivo phonophoresis. J. Control Rel. 33, 125–141.

Singer, A.J., Homan, C.S., Church, A.L., McClain, S.A., 1998. Low-frequency sonophoresis: pathologic and thermal effects in dogs. Acad. Emerg. Med. 5, 35–40.

Smith, N.B., Lee, S., Shung, K.K., 2003. Ultrasound-mediated transdermal in vivo transport of insulin with low-profile cymbal arrays. Ultrasound Med. Biol. 29, 1205–1210.

Tang, H., Wang, C.C., Blankschtein, D., Langer, R., 2002. An investigation of the role of cavitation in low-frequency ultrasound-mediated transdermal drug transport. Pharm. Res. 19, 1160–1169.

Täuber, U., 1994. Dermocorticosteroids. Structure, activity, pharmacokinetics. Eur. J. Dermatol. 4, 419–429.

Tezel, A., Sens, A., Mitragotri, S., 2002. Investigations of the role of cavitation in low-frequency sonophoresis using acoustic spectroscopy. J. Pharm. Sci. 91, 444–453.

Van den Kerckhove, E., Staes, F., Flour, M., Stappaerts, K., Boeckx, W., 2001. Reproducibility of repeated measurements on healthy skin with Minolta Chromameter CR-300. Skin Res. Technol. 7, 56–59.

Weimann, L.J., Wu, J., 2002. Transdermal delivery of poly-l-lysine by sonomacroporation. Ultrasound Med. Biol. 28, 1173–1180.

Yamashita, N., Tachibana, K., Ogawa, K., Tsujita, N., Tomita, A., 1997. Scanning electron microscopic evaluation of the skin surface after ultrasound exposure. Anat. Record 247, 455–461.

Zderic, V., Vaezy, S., Martin, R.W., Clark, J., 2002. Ocular drug delivery using 20-kHz ultrasound. Ultrasound Med. Biol. 28, 823–829.