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International Journal of Pharmaceutics 237 (2002) 209–214

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Effect of different terpene-containing essential oils on permeation of estradiol through hairless mouse skin

D. Monti [a,*], P. Chetoni [a], S. Burgalassi [a], M. Najarro [a], M. Fabrizio Saettone [a], E. Boldrini [b]

[a] *Department of Bioorganic Chemistry and Biopharmaceutics, University of Pisa, Via Bonanno 33, I-56126 Pisa, Italy*
[b] *Farmigea SpA Industria Chimico-Farmaceutica, Pisa, Italy*

Received 26 October 2001; received in revised form 11 January 2002; accepted 25 January 2002

Abstract

Purpose of the present investigation was to evaluate six terpene-containing essential oils for their capacity to promote permeation of estradiol (ES) through hairless mouse skin in vitro. Tests on cajuput, cardamom, melissa, myrtle, niaouli and orange oil, all used at the 10% w/w concentration in propylene glycol (PG), evidenced niaouli oil (NIA) as the best permeation promoter for ES. Tests on the main terpene components of NIA (1,8 cineole, α-pinene, α-terpineol and D-limonene), evaluated neat (10% w/w in PG) or in admixture, confirmed the better promoting activity of whole NIA. The present data point to the validity of complex terpene mixtures, such as that composing NIA, as transdermal penetration enhancers for moderately lipophilic drugs like ES.

Keywords: Estradiol; Penetration enhancers; Hairless mouse skin; Terpenes; Essential oils; Niaouli essential oil

1. Introduction

The undesirable side-effects of estradiol (ES) administered orally can be offset by using the transdermal route, which avoids hepatic first-pass metabolism and attenuates the fluctuating hormone levels resulting from oral therapy (Balfour and McTavish, 1992; Corson, 1993). Success of the transdermal route depends on the ability of drugs to permeate the skin at a rate and in amounts sufficient to attain effective plasma concentrations, and it is well known that for sex hormones, including ES, this goal cannot be attained without an absorption promoter (Guy, 1996). Although ethanol appears to be the agent of choice for commercial applications, a variety of promoters, often in combination with skin occlusion, have been investigated and proposed for ES. Terpenes, neat or in combination with propylene glycol (PG) or ethanol have been extensively investigated as skin permeation enhancers for this hormone and for other drugs (Cf. e.g. Williams and Barry, 1991; Obata et al., 1991; Okabe et al., 1992; Barry and Williams, 1993; Kobayashi et al.,

* Corresponding author. Tel.: +39-50-24000; fax: +39-50-21002.
E-mail address: montid@farm.unipi.it (D. Monti).

0378-5173/02/$ - see front matter
PII: S0378-5173(02)00032-7

1994; Cornwell and Barry, 1995; Moghimi et al., 1996a,b,c).

Purpose of the present investigation was to evaluate six terpene-containing essential oils (cajuput, cardamom, melissa, myrtle, niaouli and orange) for their capacity to promote transdermal permeation of ES. Although these oils are traditionally used in medicine for their balsamic and antiseptic properties, information on their promotion of skin permeation is not present in the literature. This preliminary investigation was carried out in vitro using hairless mouse skin, while keeping in mind the limitations of this model (Bond and Barry, 1988).

2. Materials and methods

2.1. Materials

The following products were used as received: β-estradiol (ES) and α-terpineol (Sigma Chemical Co., St. Louis, MO); melissa and cardamom essential oils (Alban Muller International, Montreuil, France); sweet orange, myrtle and cajuput essential oils (Curt Georgi Imes SpA, Milano, Italy); niaouli essential oil (NIA) and 1,8-cineole (ACEF SpA, Piacenza, Italy); α-pinene (Fluka Chemie AG, Buchs, Switzerland); PG and sodium azide (Carlo Erba, Milano, Italy). All other chemicals used were of pharmaceutical or analytical grade.

2.2. In vitro skin permeation experiments

Permeation tests through excised hairless mouse skin were carried out as previously described (Monti et al., 1995) using horizontal cells (Chien and Valia, 1984). Each half-cell had a volume of 8.5 ml, and the skin surface available for permeation was 2.0 cm^2. Male hairless mice (Strain MF1-hr/hr/Ola, Nossan S.r.l., Correzzana, Milano) aged 7–10 weeks were used in all cases. The donor phase consisted of PG containing 1.0% w/w ES and 10.0% w/w enhancer. This enhancer concentration was chosen on the basis of results obtained in a previous investigation, also dealing with essential oils (Monti and Saettone, 1997). The receiving phase was isotonic, 66.7 mM, pH 7.4 phosphate buffer containing 0.003% w/v sodium azide to prevent bacterial growth. At predetermined time intervals, 5.0 ml samples of the receiving phase were withdrawn for analysis, and replaced with an equal volume of fresh receptor phase. Each permeation test was replicated at least four times.

Linear regression analysis of pseudo steady-state diffusion data allowed calculation of J, the steady-state flux (given by Q/At, where Q is the amount of permeant diffusing across the area A in time t). The permeation lag times (indicating the time taken by the drug to saturate the skin and to reach the receiving compartment) were calculated from the x-axis intercept values of the regression lines. Enhancement factors (EF), expressing the relative activity of each promoter, were calculated from the ratio J_b/J_a, where J_b and J_a are the average fluxes in presence and in absence of promoter, respectively.

2.3. Analytical methods

The concentration of the ES in the samples was determined by HPLC (liquid chromatograph with LC 6A pump and 20 μl Rheodyne injector, SPDM6A detector and computer integrating system, Shimadzu Corp., Kyoto, Japan). The column (30 cm × 3.9 mm) was packed with μ-Bondapack C18 (pore size 10 μm, Waters, USA-Milford, MA). The mobile phase consisted of 45% v/v methanol, 45% v/v acetonitrile and 10% v/v water (flow rate 0.8 ml/min). The retention time and the detection wavelength were 6.4 min and 260 nm, respectively.

The oils composition was determined by GC (HP 5890 gas-chromatograph equipped with capillary HP-WAX and HP-5 30 × 0.25 mm columns). The GC operating conditions were: oven temperature 60 °C (10 min), 60–220 °C at 5 °C/min, final temperature held for 20 min; injector and detector temperatures: 250 °C; dual FID detector; carrier gas nitrogen flow: 5 ml/min; split ratio: 1/30; injected volume: 0.5 μl.

Identification of components was made on the basis of retention times of the corresponding

Table 1
Main terpene components of the essential oils tested in the study, as determined by GC analysis

Essential oil	Main terpene components (%, v/v)
Melissa (*Melissa officinalis*)	Geranial (37.8); neral (29.0)
Cajuput (*Melaleuca leucadendron*)	1,8-Cineole (56.3); α-terpineol (8.7); D-limonene (6.5); α-pinene (5.1)
Cardamom (*Elettaria cardamomum*)	1,8-Cineole (29.8); α-terpinyl acetate (39.2); α-terpineol (4.7)
Myrtle (*Myrtus communis*)	1,8-Cineole (34.6); α-pinene (21.3); myrtenyl acetate (20.4)
Niaouli (*Melaleuca viridiflora*)	1,8-Cineole (50.04); α-pinene (16.3); α-terpineol (8.3); D-limonene (6.0)
Orange (*Citrus aurantium*, var. *Dulcis*)	D-Limonene (98)

pure compounds; the relative amounts of individual components were calculated from the peak areas.

2.4. Statistical evaluation of data

Statistical differences between means were assessed by ANOVA (StatView software, Abacus Concepts inc., Berkeley, CA). The evaluation included calculation of means and standard errors, and group comparisons using the Fisher PLSD test. Differences were considered statistically significant at $P < 0.05$.

3. Results

The main terpene components of the six tested essential oils (cajuput, cardamom, melissa, myrtle, niaouli and orange) are listed in Table 1. None of the oils contained ethanol, or other alcohols as diluents. Six vehicles containing 1.0% ES and 10% essential oil in PG, (no. 2–7, Table 2) were tested against vehicle no. 1, containing 1.0% ES in PG alone, for their effect on permeation of ES through hairless mouse skin in vitro. The results of the tests (flux, lag time, percent permeated ES after 5 h, EF) are reported in Table 2 for each vehicle.

In the absence of added oil (vehicle 1), the transdermal flux of ES was 0.2 ± 0.02 μg/cm^2 h. The presence of melissa or cardamom oil (vehicles 2 and 4, respectively) failed to improve the drug permeation rate. The other oils (vehicles 3, 5, 6 and 7) produced a 16- to 52-fold flux increase over vehicle 1. The permeation lag times ranged from 1.0 to 1.8 h, without statistically significant differences among the individual vehicles. Vehicle 6, containing niaouli (NIA) essential oil proved the best enhancer for ES (EF = 52.1, significantly different from those of all other tested vehicles).

The four main terpene components of NIA, 1,8 cineole (50.04%), α-pinene (16.3%), α-terpineol (8.3%) and D-limonene (6.0%) are known skin penetration enhancers (cf. e.g., Barry and Williams, 1993), and were also present in some of the other (less active) oils. It was thus sought to verify which terpene, or association, was responsi-

Table 2
Skin permeation data of ES from liquid vehicles containing different essential oils

Vehicle[a] no.	Essential oil	$J \pm$ S.E. (μg/cm^2/h)	Lag time ± S.E. (h)	ES permeated $\times 10^2$ (% w/w)	EF[b] ± S.E.
1	None	0.20 ± 0.02	–	0.5 ± 0.04	1.00
2	Melissa	–	–	–	–
3	Cajuput	3.35 ± 0.1	1.6 ± 0.1	2.6 ± 0.6	16.7 ± 2.5
4	Cardamom	–	–	–	–
5	Myrtle	7.72 ± 0.7	1.7 ± 0.1	4.5 ± 0.9	38.6 ± 3.5
6	Niaouli, NIA	10.43 ± 0.8	1.8 ± 0.2	7.3 ± 0.4	57.8 ± 4.8[c]
7	Orange	5.43 ± 0.2	1.0 ± 0.4	5.2 ± 1.0	27.1 ± 2.8

[a] Each vehicle consisted of PG containing 1.0% w/w ES. Essential oils were present at the 10.0% w/w concentration.
[b] Enhancement factor.
[c] Significantly different from all other vehicles ($P < 0.05$).

Table 3
Composition and in vitro skin permeation data of ES from liquid vehicles containing different terpenes

Vehicle[a]	Enhancer	$J \pm$ S.E. (μg/cm^2/h)	Lag time $\pm$ S.E. (h)	ES permeated $\times 10^2$ (% w/w)	EF[b] $\pm$ S.E.
1	None	0.20 ± 0.02	–	0.5 ± 0.04	1.00
6	Niaouli, NIA	10.43 ± 0.8	1.8 ± 0.4	7.3 ± 0.4	52.1 ± 4.0
8	1,8-Cineole	6.68 ± 0.5	2.1 ± 0.1	5.3 ± 0.1	33.0 ± 2.4^c
9	α-Pinene	1.66 ± 0.1	0.9 ± 0.2	1.7 ± 0.1	8.1 ± 0.6^c
10	α-Terpineol	0.43 ± 0.1	–	0.8 ± 0.3	$2.0 \pm 0.6^{c,d}$
11	D-Limonene	1.87 ± 0.3	0.56 ± 0.1	1.8 ± 0.2	9.4 ± 0.8^c
12	MIX-1	9.25 ± 0.7	1.2 ± 0.2	6.4 ± 0.4	46.3 ± 3.3^c
13	MIX-2	8.9 ± 0.6	1.4 ± 0.1	6.2 ± 0.2	44.8 ± 3.0^c

[a] Each vehicle consisted of PG containing 1.0% w/w ES. Enhancers (neat, NIA or NIA-like mixture) were present at the 10.0% w/w concentration.
[b] Enhancement factor.
[c] Significantly different from vehicle 6 ($P < 0.05$).
[d] Not significantly different from vehicle 1.

ble for the particular promoting activity of NIA. The four terpene components were tested individually at the 10% concentration in PG (vehicles 8–11, Table 3): 1,8-cineole proved the best promoter for ES (EF = 33.0). This EF value, however, was significantly lower than that corresponding to NIA (52.1). A mixture of four terpenes ('MIX-1'; vehicle 12) containing 62% 1,8-cineole, 20% α-pinene, 10% α-terpineol and 7.4% D-limonene (all v/v, same proportions as those present in NIA), tested at the 10% concentration in PG (vehicle 12), showed a time lag in the same range as that of NIA and a relatively high EF value (46.3), which, however, was significantly lower than that produced by the whole essential oil. Due to the poor enhancing activity of α-terpineol, verified with vehicle 10, this was omitted in the composition of a second combination of terpenes ('MIX-2', vehicle 13), containing 69.2% 1,8-cineole, 22.5% α-pinene, and 8.3% D-limonene (all v/v). The results obtained with this ternary mixture, tested at the 10% w/w concentration in PG, were similar to those reported for the quaternary mixture, MIX-1.

It should be pointed out that analogous results (to be reported elsewhere) were obtained with norethindrone, a synthetic progestin. Also in this case, the promoting effect of NIA was greater with respect to that of other essential oils, and significantly higher than that produced by both terpene combinations.

4. Discussion

As mentioned in the introduction, terpenes as transdermal penetration promoters for ES have been investigated in depth by Barry and his associates (cf. e.g., Williams and Barry, 1991; Moghimi et al., 1996a,b,c). These authors found, in agreement with the present data, that some hydrocarbon and cyclic ether terpenes (e.g., α-pinene, D-limonene, 1,8-cineole) induced an increase of ES permeability coefficient in human epidermis, while other terpenes (such as α-terpineol) provided no enhancement. The same authors suggested that for this moderately lipophilic drug (log octanol/water partition coefficient = 2.3) hydrocarbon terpenes may act by increasing the diffusivity in the stratum corneum, while cyclic ether terpenes may increase partitioning. The latter mechanism has been proposed also for PG (Yamane et al., 1995).

While the enhancing effect of individual terpenes has been extensively investigated, few data seem to exist on their combinations, either natural (essential oils) or artificial. Levison et al. (1994) have reported on a computer optimisation technique, applied to development of a hydrogel formulation containing indomethacin. The result of their study indicated a combination of D-limonene, L-menthol and 1,8-cineole (in ethanol and PG as solvents) as optimum penetration enhancer. Occasional reports on essential oils as

skin penetration enhancers can be found in the literature (Williams and Barry, 1989; Namba et al., 1992; Abdullah et al., 1996).

Niaouli oil (NIA) is employed in medicine since long time for its balsamic, antibacterial, hyperemising and wound-healing properties. NIA is commonly obtained by distillation of the leaves of *Melaleuca viridiflora*, a myrtacea from New Caledonia (Fenaroli, 1963), although other sources denominate niaouli the distillate obtained from the leaves of another myrtacea: *Melaleuca quinquenervia*, a large tree from eastern Australia (Lawrence, 1997). The two plants may originate oils with different quali/quantitative composition. GC analysis showed the present NIA sample (from *Melaleuca viridiflora*) to be composed of the terpenes listed in Table 1, plus β-pinene (5.0%), *p*-cymene (4.0%), linalool (2.0%), and minor amounts of other components. Different compositions have been reported for niaouli oils from *Melaleuca quinquenervia* plants grown in different countries (Lawrence, 1997; Christoph et al., 2000).

Although some relatively ancient reports (Quevauviller and Huyen, 1976; Quevauviller and Panouse-Perrin, 1952) mention the capacity of NIA to 'penetrate though the skin and mucosae', whole NIA has apparently never been tested as skin penetration enhancer.

The present data, even if needing validation with other skin models, point to the activity of complex terpene mixtures present in whole essential oils, such as the tested NIA sample, and (albeit to a lesser extent) of terpene combinations mimicking their composition, as transdermal penetration enhancers for ES. Further experiments with NIA from other sources, and with other hydrophilic and lipophilic drugs are under way. Another point to be further clarified is the significantly different promoting activity of vehicles of similar composition, such as 7 (orange essential oil) and 11 (D-limonene), or 3 (cajuput) and 6 (niaouli). It cannot be excluded that undefined components, present in the oils at low concentration, may significantly influence the enhancing activity.

References

Abdullah, D., Ping, Q.N., Liu, G.J., 1996. Enhancing effect of essential oils on the penetration of 5-fluorouracil through rat skin. Yao Xue Xue Bao 31, 214–221.

Balfour, J.A., McTavish, D., 1992. Transdermal estradiol. A review of its pharmacological profile, and therapeutic potential in the prevention of postmenopausal osteoporosis. Drugs and Aging 2, 487–507.

Barry, B.W., Williams, A.C., 1993. Terpenes as skin penetration enhancers. In: Walters, K.A., Hadgraft, J. (Eds.), Pharmaceutical Skin Permeation Enhancement. Marcel Dekker, New York, pp. 95–111.

Bond, J.R., Barry, B.W., 1988. Hairless mouse skin is limited as a model for assessing the effects of penetration enhancers in human skin. J. Invest. Dermatol. 90, 810–813.

Chien, Y.W., Valia, K.H., 1984. Development of a dynamic skin permeation system for long-term permeation studies. Drug Dev. Ind. Pharm. 10, 575–599.

Christoph, F., Kaulfers, P.-M., Stahl-Biskup, E., 2000. A comparative study of the in vitro antimicrobial activity of tea tree oils s.l. with special reference to the activity of β-triketones. Planta Med. 66, 556–560.

Corson, S.L., 1993. A decade of experience with transdermal estrogen replacement therapy: overview of key pharmacologic and clinical findings. Int. J. Fertil. 38, 79–91.

Cornwell, P.A., Barry, B.W., 1995. Effects of penetration enhancer treatment on the statistical distribution of human skin permeabilities. Int. J. Pharm. 117, 101–112.

Fenaroli, G., 1963. Le sostanze aromatiche. In: Sostanze Aromatiche Naturali, vol. I. Ulrico Hoepli, Milano.

Guy, R.H., 1996. Current status and future prospects of transdermal drug delivery. Pharm. Res. 13, 1765–1769.

Kobayashi, D., Matsuzawa, T., Sugibayashi, K., Morimoto, Y., Kimura, M., 1994. Analysis of the combined effect of 1-menthol and ethanol as skin permeation enhancers based on a two-layer skin model. Pharm. Res. 11, 96–103.

Lawrence, B.M., 1997. Progress in essential oils. Perfumer and Flavorist 22, 49–58.

Levison, K.K., Takayama, K., Isowa, K., Okabe, K., Nagai, T., 1994. Formulation optimization of indomethacin gels containing a combination of three kinds of cyclic monoterpenes. J. Pharm. Sci. 83, 1367–1372.

Moghimi, H.R., Williams, A.C., Barry, B.W., 1996a. A lamellar matrix model for stratum corneum intercellular lipids II. Effects of geometry of the stratum corneum on permeation of model drugs 5-fluorouracyl and oestradiol. Int. J. Pharm. 131, 117–129.

Moghimi, H.R., Williams, A.C., Barry, B.W., 1996b. A lamellar matrix model for stratum corneum intercellular lipids III. Effects of terpene penetration enhancers on the release of 5-fluorouracyl and oestradiol from the matrix. Int. J. Pharm. 145, 37–47.

Moghimi, H.R., Williams, A.C., Barry, B.W., 1996c. A lamellar matrix model for stratum corneum intercellular lipids IV. Effects of terpene penetration enhancers on the permeation of 5-fluorouracyl and oestradiol through the matrix. Int. J. Pharm. 145, 49–59.

Monti, D., Saettone, M.F., Giannaccini, B., Galli-Angeli, D., 1995. Enhancement of transdermal penetration of dapiprazole through hairless mouse skin. J. Control. Rel. 33, 71–77.

Monti, D., Saettone, M.F., 1997. Development of a transdermal patch for delivery of propafenone. Preliminary studies in vitro. S.T.P. Pharma Sci. 7, 235–240.

Namba, T., Sekiya, K., Kadota, S., Hattori, M., Katayama, K., Koizumi, T., 1992. Studies on the baths with crude drug: the effects of Senkyu extract as skin penetration enhancer. Yakugaku Zasshi 112, 638–644.

Obata, Y., Takayama, K., Machida, Y., Nagai, T., 1991. Combined effect of cyclic monoterpenes and ethanol on percutaneous absorption of diclofenac sodium. Drug Des. Discov. 8, 137–144.

Okabe, H., Takayama, K., Nagai, T., 1992. Percutaneous absorption of ketoprofen from acrylic gel patches containing D-limonene and ethanol as absorption enhancers. Chem. Pharm. Bull. 40, 1906–1910.

Quevauviller, A., Huyen, V.N., 1976. Etude expérimentale des brûlures par l'acide fluorhydrique et de leur traitement. Thérapie (Paris) 31, 413–420.

Quevauviller, A., Panouse-Perrin, J., 1952. Exaltation du pouvoir anestésique local de la cocaïne par l'essence de niaouli purifiée. Anéstesie et Analgésie 9, 421–426.

Williams, A.C., Barry, B.W., 1989. Essential oils as novel human skin penetration enhancers. Int. J. Pharm. 57, R7–R9.

Williams, A.C., Barry, B.W., 1991. The enhancement index concept applied to terpene penetration enhancers for human skin and model lipophilic (oestradiol) and hydrophilic (5-fluorouracil) drugs. Int. J. Pharm. 74, 157–168.

Yamane, M.A., Williams, A.C., Barry, B.W., 1995. Terpene penetration enhancers in propylene glycol/water co-solvent systems: effectiveness and mechanism of action. J. Pharm. Pharmacol. 47, 978–989.