International Journal of Pharmaceutics, 103 (1994) 179–186

IJP 3381

Effect of d-limonene on the transdermal permeation of nifedipine and domperidone

A.C. Calpena, J. Lauroba, M. Suriol, R. Obach and J. Domenech

Pharmacokinetics and Biopharmaceutics Laboratory, School of Pharmacy, University of Barcelona, Barcelona (Spain)

(Received 27 April 1993)
(Modified version received 29 July 1993)
(Accepted 3 August 1993)

Key words: Domperidone; Nifedipine; Transdermal penetration; Rat skin; Human skin; Enhancer; d-Limonene

Summary

The aim of this research is to study the profile of transdermal permeation of nifedipine and domperidone. Permeation studies were carried out using the skin of hairless rat and human skin as a membrane mounted in a permeation system in vitro. The influence of the type of skin used and the presence of d-limonene on the transdermal penetration of these drugs were studied. The drugs studied permeate approx. 3 times faster through rat skin than through human skin. The type of skin has no influence on the lag time. d-Limonene increases the values of the permeability constant (K_p), flux (J) and lag time (T_l) for both drugs, but not the lag time for domperidone when human skin is used. The effect of the enhancer on the enhancement index (EI) of domperidone varies with the type of skin used (EI = 15.7 (rat skin) and EI = 7.7 (human skin)). When human skin is used, although penetration is increased by d-limonene, the concentration values predicted at steady state are below therapeutic levels for both studied drugs.

Introduction

Various studies have demonstrated that the transdermal pathway may be a suitable alternative to the oral route in the administration of drugs with systemic activity. The advantages provided for the dosification of drugs in transdermal therapeutic systems (TTS) (Berba and Benakar, 1990; Thomas and Pfister, 1991; Wester and Maibach, 1992) are well known. Thus, drugs with a high intrinsic pharmacological activity that are used in pathologies that require relatively long-term therapy are studied in order to determine whether they are suitable for formulation in a TTS.

In the field of antihypertensive drugs, and also in antiemetics, it has been shown that certain drugs can be formulated as TTS, for example, clonidine (Weber and Drayer, 1984) in the former case and scopolamine (Shaw et al., 1987) in the latter. Further, calcium antagonists and orthopramides, owing to their pharmacological, pharmacokinetic and physico-chemical properties, may also be suitable candidates for formulation as TTS for the treatment of hypertension (Lowenthal

Correspondence to: J. Domenech Berrozpe, Departament de Farmàcia, Subunitat de Biofarmàcia i Farmacocinética, Facultat de Farmàcia, Universitat de Barcelona, Avgda Joan XXIII, s/n 08028 Barcelona, Spain.

SSDI 0378-5173(93)E0245-L

et al., 1986) and vomiting (Schmitt and Shaw, 1986).

Studies carried out with this aim indicated that both nifedipine, among a series of calcium antagonists (Diez et al., 1991), and domperidone, among a series of antiemetics (Blanes et al., 1990), have a poor profile of cutaneous permeation.

The aim of this study was to determine whether the use of the enhancer d-limonene, which has been shown to possess suitable activity as a enhancer of cutaneous permeation (Takayama and Nagai, 1991; Takayama et al., 1991; Williams and Barry, 1991a), produces substantial improvements in the permeation profile of nifedipine and domperidone. Differences between the transdermal penetration of these two drugs were also studied using rat or human skin as permeation membrane.

Materials and Methods

All experiments were performed under subdued lighting conditions in order to avoid the effects of light on the drugs.

Materials

Nifedipine was supplied by Chemo Ibérica (Spain) and domperidone was from Janssen Farmacéutica S.A. (Belgium). d-Limonene was from Fluka S.A. (Germany). HPLC-grade methanol and acetonitrile and analytical-grade ethanol and monosodium phosphate were from E. Merck (Germany).

Analytical method

The content of the samples of nifedipine and domperidone were analysed by HPLC using a Kontron chromatograph (HPLC system 400, Kontron Instruments, Zurich, Switzerland), equipped with two pumps (model 420), with a 432 variable UV detector, 460 autosampler and 450 data system. Working conditions were as follows:

Nifedipine: column, Merck Lichrocart 125–4 LiChrospher 100 RP-18 (5 μm); mobile phase, phosphate (KH_2PO_4 1.36 g/l, pH 4.5)-acetonitrile solution (30:70 v/v); flow rate, 1 ml/min; wavelength, 237 nm. The technique was exact and precise, with a percentage coefficient of variation (CV%) between 0.251 and 4.09, and a relative error (%E) between −2.44 and 2.85 for a range of concentrations between 0.195 and 50 μg/ml.

Domperidone: column, Novapack C-18 ref. 5NVC 184, particle size 4 (μm) with internal diameter of 5 mm; mobile phase, phosphate (KH_2PO_4 1.36 g/l, pH 4.5)-acetonitrile solution (60:40 v/v); flow rate, 1 ml/min; wavelength, 274 nm. The technique was exact and precise, with a percentage coefficient of variation (CV%) between 0.98 and 9.98, and a relative error (%E) between −6.85 and 3.70 for a range of concentrations between 0.39 and 25 μg/ml.

Solubility determination

The solubility of nifedipine was determined in an ethanol/water solution of 50% (v/v), and that of domperidone in an ethanol/water solution of 70% (v/v), by addition, in both cases, of an excess of the drug to the ethanol. The mixture was then completed with water and stirred for 24 h at 37°C. After centrifugation (2000 × g) for 5 min, an aliquot of the supernatant was filtered (nylon, 0.22 μm), and used for determination of the concentration of each substance by HPLC. The study was performed for each drug, in the presence and absence of enhancer. Solubility values were measured in triplicate.

Permeation membrane

Dorsal full-thickness skin of male hairless rats (Charles River, Italy), aged 9–15 weeks and weighing 190–250 g, was used as a permeation membrane. Animals were housed under standard conditions (20 ± 2°C, 50% relative humidity and 12 h light/dark cycle), and provided with food and water ad libitum until the day of the experiment.

Human skin, provided by a plastic surgery clinic (Clínica Planas, Barcelona), was also used as a permeation membrane. The skin was abdominal, obtained from healthy women aged between 20 and 50 years. Sections of 400 μm thickness were prepared with a dermatome (Aesculap, Germany).

Skin permeation procedure

A system of nine amber glass improved Franz diffusion cells, with a diffusional area of 3.14 cm^2 (FDC 400, Crown Glass Co., Somerville, NY) was used for permeation studies. Rats were killed by cervical dislocation and a circular section, of approx. 25 mm diameter, of full-thickness skin was excised from the dorsal site, and then carefully freed of subcutaneous fat. After thawing, the human skin was cut into slices of 400 μm with a dermatome, from which sections about 25 mm in diameter were obtained. Rat and human skin sections were hydrated at 4°C in normal saline solution for 24 h, and then mounted between the donor and receptor compartments of the cells and clamped with the dermal side in direct contact with the receptor medium. The working conditions were as follows:

Nifedipine: The receptor fluid, with a volume of approx. 15 ml, which was maintained in the lower part of the cell at 37 ± 0.5°C by means of a circulating-water jacket, was a 50% ethanol/water solution (v/v). In the donor compartment, 1 ml of a saturated solution of nifedipine (in the presence of solid) in ethanol/water (50% v/v) was applied on the stratum corneum side in the absence and presence of enhancer.

The donor compartment was covered with parafilm to prevent evaporation. Samples (500 μl) were withdrawn periodically from the receptor liquid and replaced by the same volume of the receptor medium, kept at 37°C. The samples were analyzed for drug content by the HPLC procedure mentioned above.

Domperidone: The receptor fluid for domperidone was a 70% ethanol/water solution (v/v). In the donor compartment, 1 ml of a solution of domperidone at a concentration of 1 mg/ml in ethanol/water (70% v/v) was applied on the stratum corneum side in the absence and presence of enhancer. After each sample was removed, the totality of the donor liquid was replaced in order to maintain a constant concentration of the drug in the compartment throughout the experiment, and the donor compartment was covered with parafilm to prevent evaporation. The sampling technique was as described for nifedipine.

Data analysis

As described by Okamoto et al. (1986), the partition parameter P_1 and the diffusion parameter P_2 are defined by Eqns 1 and 2, respectively:

$$P_1 = K \cdot L \tag{1}$$

$$P_2 = D / L^2 \tag{2}$$

where D is the diffusion constant, L denotes the thickness of the membrane and K is the partition coefficient of the drug between the membrane and the donor solution.

The values of P_1 and P_2 were estimated by fitting Eqn 3, which is derived according to Fick's second law of diffusion, to the experimental results (accumulated permeated amount/time), as follows:

$$Q_t = (AP_1C_0)\left[P_2 t - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2} \times \exp(-P_2 n^2 \pi^2 t)\right] \tag{3}$$

where Q_t is the total amount of drug appearing in the receptor fluid in time t, A represents the area of application and C_0 is the constant concentration of the drug in the donor solution. Since it is difficult to determine the real diffusion barrier thickness correctly, two directly related parameters, P_1 and P_2, were used. They were calculated using a nonlinear least-squares regression programme (MULTI) (Yamaoka et al., 1981). The permeability constant (K_p), the lag time (T_l) and the flux (J) were calculated from the following equations:

$$K_p = P_1 \cdot P_2 \tag{4}$$

$$T_l = 1/(6P_2) \tag{5}$$

$$J = C_0 \cdot K_p \tag{6}$$

The amounts permeated at 24 h (Q_{24}) were calculated from Eqn 3, where t = 24 h.

TABLE 1

Solubility of nifedipine in a 50% ethanol / water solution (v / v), and of domperidone in a 70% ethanol / water solution (v / v) in the absence and presence of the enhancer (n = 3) (mean ± SD)

Drug	Enhancer	Solubility (mg/ml)
Nifedipine	–	6.51 ± 0.10
	d-limonene 5%	7.82 ± 0.06
Domperidone	–	6.84 ± 0.32
	d-limonene 5%	14.54 ± 0.74

Assuming that extrapolation of the data obtained in vitro to what would happen in vivo is speculative, the present study measured the transdermal dose (D_t), the amount permeated at 24 h (X_{24}), the dose permeated daily (D_{ss}) and the plasmatic concentration at steady state (C_{ss}), supposing that a 16 cm^2 patch would be a reasonable size for treatment. The following equations were used:

$$D_T = D_0 \cdot [1 - E] \tag{7}$$

where D_0 is the daily oral dose and E denotes the extraction ratio,

$$X_{24} = Q_{24} \cdot TTS_{area}/A \tag{8}$$

where TTS_{area} is the theoretical surface area of the patch (16 cm^2) and A denotes the area of permeation of the cell (in our case 3.14 cm^2).

$$D_{ss} = J \cdot TTS_{area} \cdot t \tag{9}$$

where J is the flux obtained when the donor compartment of the Franz cell contains a supersaturated solution of the drug, and t denotes the time; (since D_{ss} is the dose permeated daily, t is equal to 24 h).

$$C_{ss} = J \cdot TTS_{area}/Cl \tag{10}$$

where Cl is the plasma clearance. In order to evaluate the effect of the permeation promoter assayed (d-limonene at 5%) on the lag time, the flux and the permeability constant (Ito et al., 1988; Williams and Barry, 1991b), the following equations were used:

$$X_r = [P_1](\text{with enhancer}) / [P_1](\text{without enhancer}) \tag{11}$$

where X_r is the coefficient of relative activity and P_1 is the partition parameter of Eqn 3.

$$C_r = [C_v](\text{with enhancer}) / [C_v](\text{without enhancer}) \tag{12}$$

where C_r is the relative solubility of the drug in the vehicle in the presence and absence of the enhancer.

$$D_r = [T_l](\text{without enhancer}) / [T_l](\text{with enhancer}) \tag{13}$$

TABLE 2

Values of the constant of permeability (K_p), flux (J) and lag time (T_l) for nifedipine and domperidone in rat and human skin, in the presence or absence of d-limonene, together with the enhancer index (EI)

Drug	Membrane	Enhancer	K_p ($cm\ h^{-1}$) ($\times 10^3$) (mean ± SD)	T_l (h) (mean ± SD)	J ($mg\ h^{\times 1}\ cm^{-2}$) ($\times 10^2$) (mean ± SD)	EI
Nifedipine	rat skin	–	1.46 ± 0.40	14.5 ± 3.2	0.95 ± 0.26	
		d-limonene 5%	4.29 ± 0.69	5.4 ± 4.6	3.34 ± 0.54	2.9
	human skin	–	0.54 ± 0.19	12.6 ± 2.3	0.34 ± 0.12	
		d-limonene 5%	1.66 ± 0.28	4.0 ± 2.5	1.29 ± 0.22	3.1
Domperidone	rat skin	–	0.75 ± 0.19	27.4 ± 3.5	0.075 ± 0.019	
		d-limonene 5%	11.77 ± 1.40	3.6 ± 1.0	1.18 ± 0.14	15.7
	human skin	–	0.25 ± 0.13	19.3 ± 7.8	0.025 ± 0.013	
		d-limonene 5%	1.88 ± 0.62	20.3 ± 6.4	0.18 ± 0.062	7.7

TABLE 3

Effect of d-limonene (5%) on the permeation parameters of nifedipine and domperidone using rat and human skin expressed as relative values of flux (J_r), constant of permeability (K_{pr}), constant of activity (X_r), coefficient of diffusion (D_r) and solubility (C_r)

Drug	Membrane	J_r	K_{p_r}	X_r	D_r	C_r
Nifedipine	rat skin	3.5	2.9	1.1	2.7	1.2
	human skin	3.8	3.1	0.98	3.2	
Domperidone	rat skin	15.3	15.7	2.1	7.5	2.1
	human skin	7.2	7.7	8.1	0.95	

where D_r is the relative coefficient of diffusion and T_l denotes the lag time.

$$J_r = X_r \cdot D_r \cdot C_r \quad (14)$$

$$K_{p_r} = X_r \cdot D_r \quad (15)$$

where J_r and K_{p_r} are the fluxes and the constants of relative permeability in the presence and absence of enhancer.

Statistical methods

Comparisons of mean values of the parameters of permeation obtained in the presence and absence of enhancer, using both rat and human skin, were performed following the method of Williams et al. (1992), by the application of a non-parametric statistical test, in our case the U test of Mann Whitney. Values were considered to be significant at $p < 0.05$.

Results and Discussion

The solubilities of the drugs in the vehicle in the absence and presence of the enhancer are listed in Table 1. The permeation parameters obtained for nifedipine and domperidone in the absence and presence of enhancer, using rat and human skin as permeation membrane are given in Table 2. Table 2 also shows the values of the enhancement index (EI = K_p(with enhancer) /K_p(without enhancer). The effects of the enhancer on the permeation parameters of nifedipine and domperidone according to Eqns 11–15 are shown in Table 3. The values of the parameters of prediction according to Eqns 7–10, in the absence and presence of enhancer, using human skin as permeation membrane, are given in Table 4. The permeation profiles of the mean amounts permeated in the absence and presence of enhancer, using rat and human skin as permeation membrane, are represented in Figs 1 and 2 for nifedipine and domperidone, respectively.

Nifedipine

Influence of skin type The results of the experiments carried out indicate that, for nifedipine, there are statistically significant differences ($p < 0.05$) between the mean values of the permeability constant (K_p), the value obtained with rat skin being approx. 3-fold that found for human skin (Table 2). As a consequence, significant differences ($p < 0.05$) were also found between mean flux values, which were also approx. 3-fold higher when using rat skin than in human skin

TABLE 4

Relevant parameters for nifedipine and domperidone used to formulate a therapeutic transdermal system with d-limonene 5%

Drug	D_0 [a] (mg)	D_t [b] (mg)	X_{24} [c] (mg) (mean ± SD)	D_{ss} [d] (mg) (mean ± SD)	C_{ss} [e] (ng/ml) (mean ± SD)	C_t [f] (ng/ml)	Cl [g] (l/h)
Nifedipine	30	13	4.15 ± 0.96	4.98 ± 0.85	2.5 ± 0.4	6–51	83.0
Domperidone	30	11	0.22 ± 0.08	8.33 ± 2.73	8.3 ± 2.7	18	42.1

[a] Oral daily dose. [b] Theoretical daily transdermal doses. [c] Predicted of permeated amount during the first 24 h. [d] Predicted permeated amounts under steady-state conditions. [e] Predicted plasma levels at steady state. [f] Therapeutic plasma concentrations. [g]Clearance.

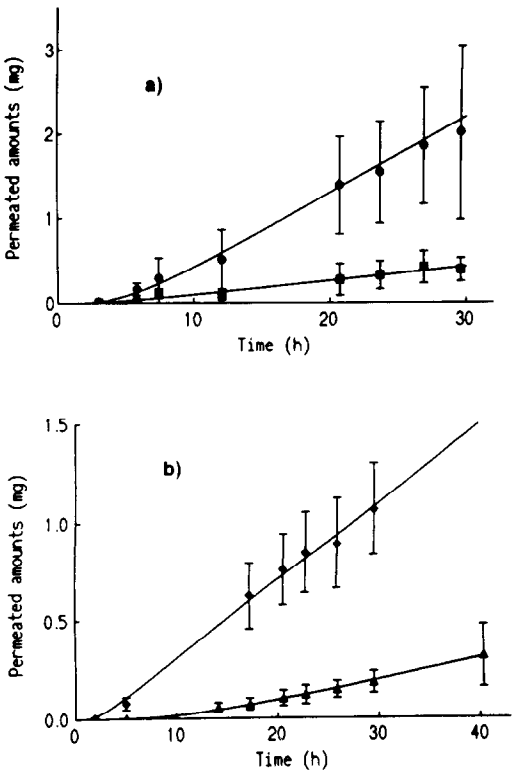

Fig. 1. Time course of the mean permeated amounts of nifedipine and corresponding theoretical fitting curves. (a) (●) Nifedipine in rat skin with enhancer, (■) nifedipine in rat skin without enhancer; (b) (♦) nifedipine in human skin with enhancer, (▲) nifedipine in human skin without enhancer.

(Table 2). However, no significant difference ($p < 0.05$) was found between the mean values of lag time (T_l). For this parameter, the value obtained was practically the same in rat skin (T_l = 14.5 h) and in human skin (T_l = 12.6 h) (Table 2). This value is slightly higher than 10% of a reasonable mean application time of 3 days.

Influence of enhancer For nifedipine the presence of the enhancer (d-limonene 5%) in the formulations assayed led to significant differences ($p < 0.05$) between mean values of permeation (K_p, T_l and J) when rat skin was used. In the presence of enhancer, the permeability constant and the flux increased almost 3-fold (Table 2). d-Limonene reduced the lag time (T_l) of nifedipine by about 50% (Table 2). According to Eqns 14–15, the increases in K_p and J and the decrease in T_l are due to the fact that the penetration promotor enhances the diffusion of the drug ($D_r = 2.7$), since the coefficient of relative activity ($X_r = 1.1$) and the relative solubility in the vehicle ($C_r = 1.2$) remain practically constant (Table 3).

When human skin was used, the presence of d-limonene in the nifedipine formulations also led to statistically significant ($p < 0.05$) differences between the mean values of the permeation parameters. The values of both K_p and J increase about 3-fold. In this case, the lag time (T_l) in the presence of enhancer was approximately one third of that obtained in the absence of enhancer. In human skin the reduction in the lag time of nifedipine in the presence of d-limonene and the increases in the values of K_p and J are

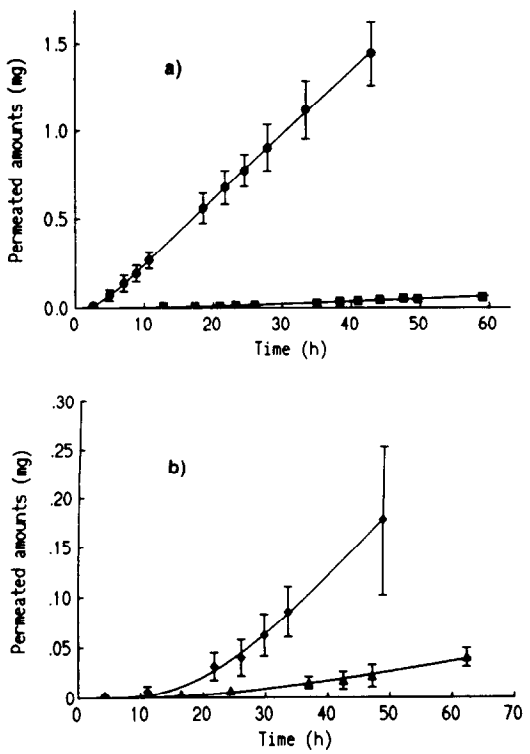

Fig. 2. Time course of the mean permeated amounts of domperidone and corresponding theoretical fitting curves. (a) (●) Domperidone in rat skin with enhancer, (■) domperidone in rat skin without enhancer; (b) (♦) domperidone in human skin with enhancer, (▲) domperidone in human skin without enhancer.

also due to the fact that the penetration promotor enhances the relative diffusion of the drug ($D_r = 3.2$), since the relative coefficients of activity ($X_r = 0.98$) and solubility ($C_r = 1.2$) remained practically constant, according to Eqns 14 and 15 (Table 3).

The fact that the reduction in lag time was slightly more marked in human skin than in rat skin is attributable to the greater activity of the enhancer on the relative coefficient of diffusion of the drug in human skin ($D_r = 3.2$) than in rat skin ($D_r = 2.7$). Moreover, it could be considered that, according to the estimated enhancement index, (EI) (Table 2), the promotional activity of d-limonene on nifedipine is constant, irrespective of the type of skin used (EI = 2.9 in rat skin and EI = 3.1 in human skin).

Domperidone

Influence of type of skin used The type of skin influences the permeability constant (K_p) and flux (J) of domperidone, and statistically significant differences ($p < 0.05$) were found between the mean values of these parameters. The value of K_p in rat skin in this case was also 3-fold that found for human skin (Table 2), and obviously the flux value (J) was also 3 times greater in rat skin than in human skin (Table 2). Again, the type of skin did not influence the lag time for domperidone, since no significant difference ($p > 0.05$) was found between the mean values obtained for this parameter, which were greater than those determined for nifedipine ($T_l = 27.4$ h in rat skin, and $T_l = 19.3$ h in human skin) (Table 2).

Influence of enhancer In the case of domperidone, the presence of d-limonene in the formulation led to significant differences ($p < 0.05$) in the permeation parameters (K_p, J and T_l) in rat skin, with respect to those obtained without the enhancer. The values of K_p and J increased 15-fold (Table 2). Moreover, the lag time diminished approx. 7-fold in the presence of the enhancer (Table 2). According to Eqns 14 and 15, the increase in K_p and J was due, on the one hand, to the rise produced by d-limonene in the relative coefficient of activity of the drug ($X_r = 2.1$) and, on the other, the increase produced in the relative coefficient of diffusion ($D_r = 7.5$). Furthermore, the reduction in lag time may be attributed to the considerable increase in the relative coefficient of diffusion of the drug in the presence of d-limonene in the case of rat skin ($D_r = 7.5$) (Table 3).

When human skin was used, the presence of d-limonene led to a significant ($p < 0.05$) increase in both K_p and J of approx. 7-fold (Table 2). However, the lag time was not significantly ($p > 0.05$) affected by the presence of d-limonene (Table 2). As can be seen in Table 3, according to Eqns 14 and 15, the increases in the flux (J) and permeability constant (K_p) of domperidone produced by d-limonene are due to the fact that the enhancer increased the relative coefficient of activity of the drug ($X_r = 8.1$). The relative solubility in the vehicle ($C_r = 2.1$) has no practical effect, since the experiments with and without enhancer were performed at a constant concentration (1 mg/ml), the relative diffusion coefficient ($D_r = 0.95$) remaining practically constant. Thus, consistent with the observation that the constant for diffusion of the drug across human skin was unaffected by the presence of d-limonene, the lag time did not vary.

It is worth pointing out that, in contrast to what was found for nifedipine, in the case of domperidone the promotional action of d-limonene was greater in rat skin, as judged on the basis of the value of the enhancement index obtained (EI = 15.7 in rat skin and EI = 7.7 in human skin) (Table 2).

Notwithstanding the foregoing, it is worth commenting that, when human skin was used as a permeation membrane, although the enhancer assayed led to a marked improvement in the permeation profile of nifedipine and domperidone, increasing the values of flux (J) and permeability constant (K_p) while at the same time reducing the lag time in both cases, the presence of 5% d-limonene in the formulations assayed does not permit the prediction of therapeutic plasmatic concentrations under steady-state conditions (Table 4).

In conclusion, under our experimental conditions and for the drugs assayed, the type of skin has a significant influence on the permeability

constant (K_p) and the flux (J): permeation of these drugs through rat skin is approx. 3-fold greater than in human skin. This result is in agreement with the average obtained from a series of data published for various drugs (Cleary, 1984). However, the type of skin did not influence the lag time, which in both cases remained practically constant. On the other hand, our results indicate the difficulty in the prediction of the transdermal penetration of substances through rat and human skin, since the effect of the enhancer on the enhancement index (EI) may vary with the type of skin and as a function of the drug assayed. d-Limonene at 5%, under our experimental conditions, has proved to be a satisfactory enhancer, since it increased the value of K_p and J, and reduced T_l in the case of nifedipine, but not in the case of domperidone in human skin. This may be because the enhancer, in this case, does not modify the relative coefficient of diffusion of the drug. However, when human skin was used, the addition of 5% d-limonene to the formulations did not improve the penetrability of either drug to such values as would allow fluxes to be achieved that would permit the prediction of therapeutic plasmatic concentrations under steady-state conditions.

Acknowledgments

This work is a part of project no. 1071 granted by 'Comisión Interministerial de Ciencia y Tecnología (CYCIT), Spain'. The authors thank Mr R. Rycroft for assistance in translation.

References

Berba, J. and Benakar, U., Clinical efficacy of currrent transdermal drug delivery systems: a retrospective evaluation. *Am. Pharm.*, 11 (1990) 33–41.

Blanes, C., Colom, H., Moreno, J., Obach, R., Peraire, C. and Domenech, J., Comparative study in vitro of transdermal absorption of a series of antiemetic drugs. *Eur. J. Drug Metab. Pharmacokinet.*, (Special Issue) (1990) 410–414.

Clearly, G.W., *Medical Applications of Controlled Release*, CRC Press, Boca Raton, FL, 1984, pp. 212–217.

Diez, I., Colom, H., Moreno, J., Obach, R., Peraire, C. and Domenech, J., A comparative in vitro study of transdermal absorption of a series of calcium channel antagonists. *J. Pharm. Sci.*, 80 (1991) 931–934.

Ito, Y., Ogiso, T. and Iwaki, M., Thermodynamic study on enhancement penetration of drugs by azone. *J. Pharmacobio-Dyn.*, 11 (1988) 749–757.

Lowental, D., Saris, S., Paran, E., Cristal, N., Sharif, K., Bies, C. and Fagan, T., Efficacy of clonidine as transdermal therapeutic system: the international clinical experience. *Am. Heart J.*, 112 (1986) 893–897.

Okamoto, H., Kamatsu, H., Hasida, M. and Sezaki, H., Effects of β-cyclodextrin and di-*O*-methyl-β-cyclodextrin on the percutaneous absorption of butylparaben, indomethacin and sulfanilic acid. *Int. J. Pharm.*, 30 (1986) 35–45.

Schmitt, L.G. and Shaw, J.E., Alleviation of induced vertigo. Therapy with transdermal scopolamine and oral meclizine. *Arch. Otolaryngol. Head Neck Surg.*, 112 (1986) 88–91.

Shaw, J.E., Prevo, M.E. and Amkraut, A.A., Testing of controlled release transdermal dosage form. *Arch. Dermatol.*, 123 (1987) 1548–1556.

Takayama, K., Kikuchi, K., Obata, Y., Okabe, H., Machida, Y. and Nagai, T., Terpenes as percutaneous absorption promotors. *STP Pharm. Sci.*, 1 (1991) 83–88.

Takayama, K. and Nagai, T., Simultaneous optimization for several characteristics concerning percutaneous absorption and skin damage of ketoprofen hydrogels containing d-limonene. *Int. J. Pharm.*, 74 (1991) 115–126.

Thomas, X. and Pfister, W.A., The emerging role of silicones used in transdermal drug delivery systems. *STP Pharm. Sci.*, 1 (1991) 38–46.

Weber, M.A. and Drayer J.M., Clinical experience with rate-controlled delivery of antihypertensive therapy by a transdermal system. *Am. Heart J.*, 108 (1984) 231–236.

Wester, R.C. and Maibach, H.I., Percutaneous absorption of drugs. *Clin. Pharmacokinet.*, 23 (1992) 235–266.

Williams, A.C. and Barry, B.W., 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 (1991a) 115–126.

Williams, A.C. and Barry, B.W., Terpenes and lipid-protein-partitioning theory of skin penetration enhancement. *Pharm. Res.*, 8 (1991b) 17–24.

Williams, A.C., Cornwell, P.A. and Barry, B.W., On the non-Gaussian distribution of human skin permeabilities. *Int. J. Pharm.*, 86 (1992) 69–77.

Yamaoka, K., Taniwara Y., Nakagawa T. and Uno T., Pharmacokinetic analysis program (MULTI) for microcomputer. *J. Pharmacobio-Dyn.*, 4 (1981) 879–885.