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A comparative in vitro study of percutaneous penetration of β-blockers in human skin☆

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Abstract

In vitro diffusion experiments with propranolol, oxprenolol, metoprolol and atenolol were carried out using excised human abdominal skin. The main permeation parameters (permeability coefficient, flow and lag time) were calculated and compared as measurement of intrinsic permeability across human skin. A long lag time and a low steady-state flow were found for all drugs assayed. Skin permeability predicted at steady state did not reach therapeutic concentrations, which indicated the need for appropriate chemical penetration enhancers or vehicles to overcome limiting factors. The results, including those of celiprolol and bisoprolol reported previously, correlated with physicochemical properties, especially with lipophilicity, one of the main factors in drug permeability prediction through human skin.

Keywords: Percutaneous permeation; Propranolol; Oxprenolol; Atenolol; Metoprolol; Human skin; In vitro

1. Introduction

The utilisation of transdermal route for systemic action of drugs has brought out an important number of new clinical applications which use transdermal drug delivery (TDD) with patches (Brouwers, 1996). This route shows certain biopharmaceutical benefits such as increasing bioavailability and therapeutic efficacy bypassing hepatic first-pass metabolism. This may be accompanied by the longer dosing frequency required for a chronic treatment and, thus, improved adherence.

In recent years, owing to advantages offered by transdermal administration and the extensive use of β-blockers in the treatment of various cardiovascular disorders, several authors have studied percutaneous permeation and TDD with patches of β-blockers across artificial (Green and Hadgraft, 1987), animal (Ogiso et al., 1988; Corbo et al., 1990; Ghosh et al., 1993; Demou et al., 1994; Ghosh et al., 1995) or human skin (Mey et al., 1989; Spieker et al., 1989; Krishna and Pandit,

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1996) membrane models. However, few of them compare the permeation characteristics of these drugs through human skin.

In the present study, as a continuation of a previous one in which two β-blockers celiprolol and bisoprolol were reported (Modamio et al., 1998), transdermal permeation properties across human skin in vitro of propranolol, oxprenolol, metoprolol and atenolol were compared (including also both celiprolol and bisoprolol), and the potential of each individual drug studied assessed. In addition, correlations between physicochemical parameters of β-blockers chosen, were established, especially lipophilicity expressed as intrinsic partition coefficient, in order to determine whether optimal permeability, and so the predictive potential of these physicochemical parameters can be established.

2. Material and methods

2.1. Materials

Both, propranolol chlorhydrate and atenolol base were provided by ICI-Farma (Madrid, Spain), oxprenolol chlorhydrate and metoprolol tartrate by Novartis Farmacéutica (Barcelona, Spain). The different chemical forms employed for drug assayed were in accordance with pharmaceutical dosage forms available in the market. Acetonitrile and methanol, which were HPLC grade, were purchased from Scharlau (Germany). Triethylamine was purchased from Fluka Chemika-Biochemika (Alcobendas, Spain), 85% ortophosphoric acid from Probus (Badalona, Spain), potassium phosphate mono-basic and disodium hydrogen phosphate anhydrous from Panreac Química (Montcada i Reixach, Spain) and *n*-octanol from Farmaitalia Carlo Erba (Milan, Italy). All of them were analytical grade chemicals and used without further purification.

2.2. Sample analysis

The HPLC system consisted of a Kontron (model 420, Kontron Instruments, Barcelona, Spain) equipped with an automatic sampling system with a variable volume injector (model 465), two pumps (model 420), a mixer (model 491), a capilar UV-visible detector with variable-wavelength (model 433) and a computerised integration system data output (model MT-450). Liquid chromatographic analyses were performed on a C18 Nucleosil column (5-μm particle diameter, 12.5 cm × 4 mm i.d.) (Teknokroma, Barcelona, Spain) at room temperature. The mobile phase was composed of acetonitrile/67 mM Sorensen's phosphate buffer (pH 5.0) with 0.2% (w/v) of triethylamine and the pH adjusted to 3 with 85% ortophosphoric acid (40/60, 30/70, 25/75 and 10/90 (v/v) for propranolol, oxprenolol, metoprolol and atenolol, respectively). The flow rate was delivered at 0.8 ml min^{-1}. Standard solutions of each drug calibration curve range (25–0.78 μg ml^{-1}) were obtained by suitable dilution from stock solutions prepared at 0.25 mg ml^{-1} in 67 mM Sorensen's phosphate buffer (pH 7.4). Intra- and interassay validation of analytical methods indicated were exact and precise. Accuracy, expressed as a percentage of the mean recovery, ranged from 100.96 to 102.32%. Precision, expressed as a relative standard deviation (S.D.), ranged from 0.01 to 3.68%. Accelerated studies of stability in solution were previously carried out with β-blockers assayed (Modamio et al., 1994, 1996).

2.3. Solubility determination

Drug solubility (S) was measured in 67 mM Sorensen's phosphate buffer (pH 7.4). The solubility assay was carried out in small volumes owing to both the limited quantity of drug available and the very soluble or soluble descriptive terms in water described in the literature for drugs assayed, except for atenolol (Luch, 1983; Caplar et al., 1984; Clarke, 1986; USP XXIII, 1995; Martindale, 1996; The Merck Index, 1996; British Pharmacopoeia, 1998). An excess of drug was added to 0.4 ml of vehicle in a small glass vial. The whole mixture was incubated in a shaking water bath maintained at 32 ± 1°C for 24 h. After centrifugation at 3000 × g, the supernatant was passed through a membrane filter (nylon, 0.22 μm) and diluted with Sorensen's phosphate buffer

(pH 7.4) prior to HPLC assay. The solubility was measured in triplicate.

2.4. Determination of n-octanol-Sorensen's phosphate buffer (pH 7.4) partition coefficient (P)

The *n*-octanol-Sorensen's phosphate buffer (pH 7.4) partition coefficient serves as a parameter of lipophilicity. *n*-Octanol and 67 mM Sorensen's phosphate buffer (pH 7.4) were presaturated with each other for at least 24 h before the experiment. For each drug, a solution of 20 μg ml^{-1} was prepared in Sorensen's phosphate buffer (pH 7.4). Then, 3 ml of this was transferred to a 30-ml tube containing a constant volume of 1 ml of organic phase. The tubes were stoppered and mixed for 24 h in a shaking thermostated bath at 32 ± 1°C. After separating the phases by centrifugation at $3000 \times g$ for 10 min, the aqueous phase was analysed for drug content by HPLC. The concentration in *n*-octanol was calculated as the difference between this and the total concentration, also previously analysed by HPLC in the buffer phase. The *P* values were measured in replicates of six.

In the case of compounds ionising at physiological pH, such as β-blockers, the intrinsic partition coefficient (P') may be a more appropriate parameter to measure the partition coefficient of the non-ionised compound. This is in agreement with the pH-partition hypothesis, according to which the permeability of the non-ionised form should be higher since it is more lipophilic and hence more permeable across skin. So, P' was calculated as follows, according to the Henderson–Hasselbalch equation, using the p*K* values previously determined experimentally and considering a pH of 7.4 (Clarke, 1984; Betageri and Rogers, 1987; Clarke and Cahoon, 1987; Barbato et al., 1990):

$$P' = P[\text{antilog}(\text{p}K - \text{pH}) + 1] \quad (1)$$

2.5. pK determination

The p*K* values were determined experimentally by potentiometry. For each drug, a solution of 100 μg ml^{-1} was prepared in 67 mM Sorensen's phosphate buffer (pH 7.4). Five ml of this was transferred to a battery of tubes (between 8 and 12) containing increasing volumes of 0.001 M HCl or 0.001 M NaOH standard solution depending on whether the pH of drug solution was basic (atenolol) or acid (the rest of drugs), respectively. The tubes were shaken for 1 min, and pH was then measured by potentiometric titration, using a Crison MicropH 2001 pH-meter. The sudden change of pH near the equivalence point was calculated graphically from the plot of the increment of pH divided by the volume ($\Delta\text{pH}/\Delta V$) versus the volume of HCl or NaOH initially added. The p*K* value was obtained from the same plot by the inclusion of the pH values as another *y*-axis. It was measured in replicates of four.

2.6. Permeation studies

The in vitro permeation was studied across abdominal human skin removed from four healthy women during plastic surgery. Following the same methodology already used by us in a previously reported work (Modamio et al., 1998), after freezing (-20°C), the skin was cut with a dermatome (model GA 630, Aesculap, Germany) into 1.2 mm thick layers and was hydrated in normal saline solution at 4 ± 1°C for 24 h. The permeation study was conducted with Franz diffusion cells (Franz, 1975) in the static mode (Crown Glass, Somerville, NJ) with a diffusional area of 3.14 cm^2. The capacity of receptor compartments was 15 ml, and temperature was maintained at 32 ± 1°C by means of a surrounding jacket. Drug concentration in donor compartments was 5 mg ml^{-1} prepared in 67 mM Sorensen's phosphate buffer (pH 7.4). Receptor solution was Sorensen's phosphate buffer (pH 7.4), which was continuously stirred at 600 rpm with a teflon-coated bar magnet placed inside the cell.

Skin samples were mounted between donor and receptor compartments of the cells and clamped with the dermal side in contact with the receptor medium. Then, at time zero, 1 ml of the drug solution was placed in the donor compartment and the cell was covered with parafilm to avoid solvent evaporation. Samples of 1 ml were taken

from receptor compartments and immediately replaced with 1 ml of the receptor solution, at the same temperature. At the same time, donor cell contents were entirely replaced by the same volume of new solution, in an attempt to maintain drug concentration constant throughout the experiment (72 h). Initial experiments confirmed the maintenance of sink conditions by this procedure. The amounts of drug permeated from receptor solutions at predetermined times were analysed by HPLC. Seven parallel experiments were conducted with each drug, using all specimens skin in each drug. This same methodology was already used by the authors in a previously reported work (Modamio et al., 1998), what has made possible to establish different comparisons among drug assayed in both studies.

2.7. Data analysis

The penetration profiles were analysed on the basis of a diffusion model for the infinite dose system, which considers human skin to be a one-plane barrier membrane. Following Okamoto et al. (1986), the next parametrised equation, which is derived according to Fick's second law of diffusion, was used:

$$Q_t = (Ap_2C_0) \times \left[P_1 t - \frac{1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \exp(-P_1 n^2 \pi^2 t) \right] \quad (2)$$

Q_t is the total amount of drug appearing in the receptor solution at a given time t; A represents the diffusion area (3.14 cm^2) and C_0 is the constant concentration of the donor solution (5 μg ml^{-1}). Due to the difficulties in determining the real diffusion barrier thickness, the two parameters directly related, the diffusion parameter (P_1) and partition parameter (P_2), were estimated by fitting Eq. (2) to experimental data, by a non-linear least-squares computer program (Multi) (Yamaoka et al., 1981). P_1 and P_2, were defined by the Eqs. (3) and (4), respectively:

$$P_1 = \frac{D}{L^2} \quad (3)$$

$$P_2 = KL \quad (4)$$

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

Subsequently, permeation parameters, permeability coefficient (K_p), flow (J) and lag time (T_{lag}), were calculated from the following Eqs. (5)–(7):

$$K_p = P_1 P_2 \quad (5)$$

$$J = C_0 K_p \quad (6)$$

$$T_{lag} = \frac{1}{6P_1} \quad (7)$$

The mean values of estimated permeation parameters among drugs assayed were compared by Kruskall–Wallis one-way analysis of variance (ANOVA) (Williams et al., 1992).

On the basis of permeation parameters obtained and pharmacokinetic parameters for drugs studied, the daily transdermal dose (D_t), the theoretical amount permeated daily in steady-state conditions (D_{ss}) and the theoretical plasma steady-state concentrations (C_{ss}) were predicted with the following Eqs. (8)–(10), respectively:

$$D_t = D_0 \frac{[100 - E(\%)]}{100} \quad (8)$$

$$D_{ss} = J \mathrm{TTS}_{area} t \quad (9)$$

$$C_{ss} = \frac{J \mathrm{TTS}_{area}}{\mathrm{Cl}_p} \quad (10)$$

where D_0 is the oral daily dose, $E(\%)$ is the percentage extraction ratio, TTS_{area} is the surface area of a patch (in this case 16 cm^2) and Cl_p is the plasmatic clearance.

3. Results and discussion

The results of the current study were obtained following the same methodology as already used by us in a previously reported work (Modamio et al., 1998), in order to make easier comparisons among drug assayed in both studies.

The stability of all drugs at the temperature of this study (32°C) was confirmed before further studies, by extrapolation from the Arrhenius equation (Modamio et al., 1994, 1996).

Physicochemical properties of the penetrant (lipophilicity, pK and molecular weight) have a marked influence on drug permeation through biological membranes, including the stratum corneum (Karzel and Liedtke, 1989). The values of physicochemical parameters for the β-blockers assayed are listed in Table 1. Molecular weight of the free base (MW), melting point (mp) and solubility in water (S*) are taken from the literature (Luch, 1983; Caplar et al., 1984; Barbato et al., 1990; British Pharmacopoeia, 1998), while solubility in Sorensen's phosphate buffer (pH 7.4) (S), pK and partition coefficient (P) and the intrinsic partition coefficient (P') are obtained experimentally.

An idea of the reliability of S calculation can be obtained by comparison with the solubility in water (found in the literature, S*) and the theoretical prediction from the Yalkowsky equation (Yalkowsky and Valvani, 1980). This equation, shown at the foot of Table 1, permits calculation of the aqueous solubility of compounds expressed in moles per liter by means of partition coefficient and mp data. As can be seen, the magnitude of experimental solubility data keeps the same order as bibliographic values in all drugs assayed. This happens also between experimental and theoretical Yalkowsky solubilities except for atenolol. Some of the differences found can be explained because, first, phosphate buffer (pH 7.4) was used as aqueous phase instead of water in the calculation of both experimental solubility and partition coefficient and, second, the study was performed at 32 instead of 25°C. Atenolol's lack of correlation with the theoretical solubility found may be due to its low partition coefficient, which makes it be the only hydrophilic β-blocker of the four assayed.

Results for partition coefficients determined in n-octanol/Sorensen's phosphate buffer (pH 7.4) system at 32°C, were very similar to those measured by Barbato et al. (1990) for these β-blockers (Table 2). In this case, the different temperatures used in the determination (20°C) may explain some of the differences found. So, the order of lipophilicity of the β-blockers evaluated was confirmed.

Since the intrinsic partition coefficient is important in predicting the ability of drugs to cross lipophilic membranes, this parameter was also compared (Table 2). The results corroborated those of other authors, although some of the differences can be attributed to both the temperature and the pK employed. In this study pK values were determined experimentally in phosphate buffer instead of being taken from the literature and measured in water as previous authors did. The biggest variation was found in the case of atenolol.

Mean permeation profiles of each drug, including celiprolol and bisoprolol, which were reported previously (Modamio et al., 1998), i.e. the time

Table 1
Physicochemical parameters corresponding to β-blockers assayed (mean ± S.D.)[a]

β-blocker	MW (g mol^{-1})	mp (°C)	S* ($\times 10^4$) (μg ml^{-1})	S ($\times 10^4$) (μg ml^{-1})	$S_{Yalkowsky}$ ($\times 10^4$) (μg ml^{-1})	pK	P	P'
Propranolol	259.3	166	3.3–10.0	16.8 ± 0.43	4.4	9.50 ± 0.15	14.57 ± 3.67	1849.00 ± 465.70
Oxprenolol	265.4	110	100.0	63.5 ± 4.04	19.7	9.30 ± 0.20	1.20 ± 0.04	96.73 ± 2.88
Metoprolol	267.4	124	100.0	80.2 ± 29.1	55.5	9.53 ± 0.09	0.31 ± 0.01	42.22 ± 0.89
Atenolol	266.3	155	1.0–3.3	1.9 ± 0.02	210.6	8.64 ± 0.05	0.04 ± 0.03	0.73 ± 0.45

[a] MW, molecular weight; mp, melting point; S*, water solubility from literature; S, experimental solubility in the conditions of our study; $S_{Yalkowsky}$, obtained from equation $\log S(M) = -0.01\,(mp-25) - \log P + 0.8$; P and P', experimental and intrinsic values of the partition coefficient.

Table 2
Comparison of partition coefficient and intrinsic partition coefficient corresponding to β-blockers assayed[a]

β-blocker	log P	log P(a)	log P'	log P'(b)	log P'(c)	log P'(d)
Propranolol	1.16	1.07	3.27	3.37	3.37	3.39
Oxprenolol	0.08	0.13	1.99	2.62	2.18	2.68
Metoprolol	−0.51	−0.26	1.63	2.28	1.80	2.04
Atenolol	−1.40	−1.61	−0.13	1.95	0.16	0.27

[a] log P, logarithm of experimental partition coefficient; log P(a), obtained from Barbato et al. (1990) at 20°C, log P', logarithm of experimental intrinsic partition coefficient; log P'(b)), obtained from Betageri and Rogers (1987) at 30°C; log P'(c),obtained from Burgot et al. (1990) at 25°C; log P'(d), obtained from González et al. (1995) at 25°C.

course of accumulated amounts permeated through human skin are shown in Fig. 1. Experiments finished after 3 days (72 h). In order to characterise their permeability, after the calculation of P_1 and P_2, K_p, J, and T_{lag} parameters were calculated for each drug by the method described above. Table 3 summarises mean and S.D. values of these permeation parameters in the conditions of the study. Significant differences ($P < 0.05$) among drugs for K_p, J and T_{lag} parameters were found. Mean K_p and J values were very low for all drugs, but the highest and lowest values were for propranolol and atenolol, respectively. Drugs that have the potential to be formulated in transdermal therapeutic systems (TTS) should reach a suitable J, which is directly related to drug solubility in the vehicle. Very low J values were found despite the appropriate solubility for all drugs.

T_{lag} obtained according to the described methodology, showed that atenolol has the shortest T_{lag} and oxprenolol the longest one. However, no significant amounts were detected in receptor compartments during the first 20 h of diffusion experiments. Considering that a patch remains on the skin between 24 and 72 h, T_{lag} value is also a constraining factor in the formulation of a TTS for all drugs.

In an attempt to facilitate prediction of percutaneous permeation through human skin, a number of model in vitro experimental systems have been developed. These models try to quantify the permeation dependence of the penetrants studied on various physicochemical factors such as molecular weight and lipophilicity (Flynn, 1990). Along these lines, various correlations for the log permeability coefficient as a sum of log terms, including intrinsic partition coefficient, molecular weight, and/or degree of ionisation, have been determined.

MW directly affects the diffusion across simple or complex membranes, mainly in an inverse relationship, and is usually considered in the passage

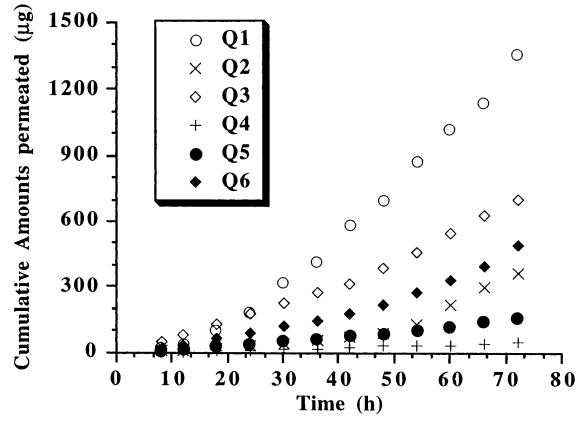

Fig. 1. Time course of cumulative amount permeated of propranolol (Q_1), oxprenolol (Q_2), metoprolol (Q_3), atenolol (Q_4), including bisoprolol (Q_5) and celiprolol (Q_6) ($n = 7$).

Table 3
Main permeation parameters for β-blockers assayed, shown as mean and standard deviation (S.D.) ($n = 7$)[a]

β-blocker	K_p ($\times 10^{-3}$) (cm h^{-1})	J (µg h^{-1} cm^{-2})	T_{lag} (h)
Propranolol	1.78 ± 0.63	8.90 ± 3.41	25.35 ± 8.22
Oxprenolol	1.54 ± 1.54	6.80 ± 6.52	58.84 ± 27.20
Metoprolol	0.83 ± 0.10	3.71 ± 0.32	13.03 ± 12.02
Atenolol	0.05 ± 0.02	0.24 ± 0.11	4.47 ± 2.90

[a] K_p, permeability rate constant; J, flow; T_{lag}, lag time.

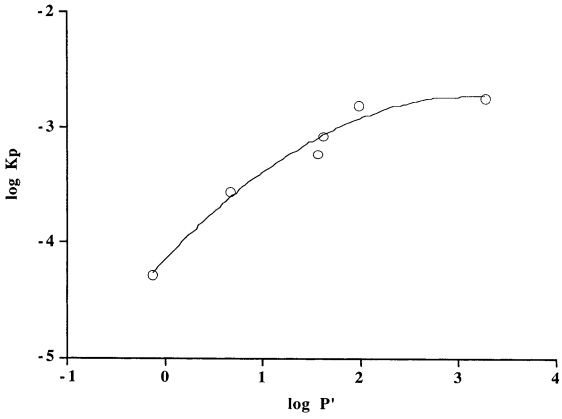

Fig. 2. Log–log plot of permeability coefficient through abdominal human skin membrane ($\log K_p$) and *n*-octanol/Sorensen's phosphate buffer (pH 7.4) intrinsic partition coefficient ($\log P'$) for all six β-blockers.

of drugs across skin. The differences in permeability of the β-blockers assayed seem not to be caused by molecular weight differences, since they are within a narrow range (Table 1).

Lipophilicity, measured as the intrinsic partition coefficient, is a determinant factor in the potential permeation of a drug across different biological membranes including skin, and could be a very useful parameter to identify optimal permeability. In 1983, Schoenwald and Huang compared permeability across excised rabbit corneas of 12 β-blockers grouped into very lipophilic, lipophilic and hydrophilic categories (drugs varied in octanol–water partition coefficient over a 4-fold logarithmic range). They found that the best fit was with a parabola where the plateau region corresponded with very lipophilic compounds like propranolol. Later, Le Brun et al. (1989) found a linear relationship between drug lipophilicity and permeability coefficient across pig buccal mucous membrane of four β-blockers (bupranolol, propranolol, oxprenolol and acebutolol), but not an optimum or plateau value of intrinsic partition coefficient. After this, Wang et al. (1991) compared the influence of lipophilicity on isolated pigmented rabbit conjunctiva and cornea penetration of eleven β-blockers also grouped into three categories. A sigmoidal relationship, rather than the parabolic one, described best the penetration of the two membranes. Ghosh et al. (1993) investigated the permeation of nine β-blockers across hairless mouse skin and found linear correlation. Although the intrinsic partition coefficient varied over a 4-fold log range, data did not show a plateau region for the very lipophilic compounds. Following this line, Sasaki et al. (1994) also found linear correlations across intestinal and cornea membranes of albino rabbits for the six β-blockers tested.

In this study, the greatest value found for propranolol seems to be consistent with its influence on penetration magnitude across skin, followed by lipophilic oxprenolol, metoprolol and lastly hydrophilic atenolol. If data of two lipophilic drugs (bisoprolol and celiprolol) previously obtained in the same experimental conditions (Modamio et al., 1998) are considered, their permeation range maintains direct correlation with their intrinsic partition coefficients.

Once seen that correlation coefficients increased when MW were excluded from the multiple regression analysis, $\log K_p$ through abdominal human skin membrane and $\log P'$ were regressed in order to establish the function that would relate both variables. The best fit, judged by the highest correlation coefficient (r), is represented by the following equation (Fig. 2):

$$\log K_p \text{ (cm s}^{-1}) = -4.16 + 0.91 \log P' - 0.14 (\log P')^2 \quad (11)$$

$$r = 0.9892 \quad P = 0.003 \quad n = 6$$

Fig. 2 shows that permeability coefficients increase with the corresponding increase in $\log P'$, reaching a plateau at a $\log P'$ of 2.7 (case of propranolol). This was consistent with the cornea permeation study of Schoenwald and Huang (1983), although in our study *n*-octanol–water partitioning varied only over a threefold logarithmic range. Nevertheless, the plateau was reached for propranolol, the unique very lipophilic β-blocker from the group of six assayed. However, inclusion of a linear relationship reduced the correlation coefficient to 0.9256. As a result, more very lipophilic β-blockers are required to be able to verify that an optimum value of $\log P'$ for in vitro percutaneous permeation across human skin has been found.

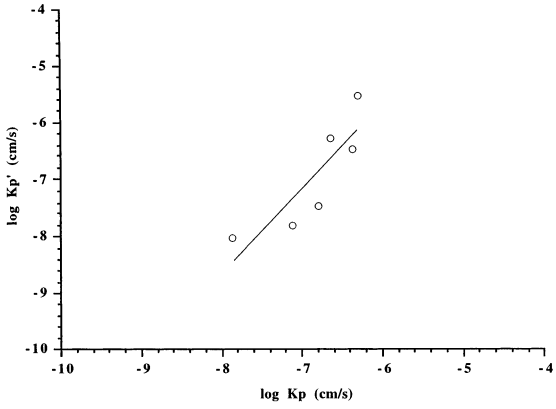

Fig. 3. Linear relationship between experimental (log K_p) and theoretical* (log K'_p) logarithmic permeability coefficients for all six β-blockers. * Obtained from the equation log K_p (cm s^{-1}) = $-6.3 + 0.71 \log P' - 0.0061$ MW (Potts and Guy, 1992).

In a previous article (Modamio et al., 1998), the theoretical model proposed by Potts and Guy (1992) to predict skin permeability on the basis of intrinsic octanol–water partition coefficient and MW was applied to bisoprolol and celiprolol, since these drugs were included within the wide range of physicochemical properties and structures considered. It was said that a greater number of β-blockers could be used to predict percutaneous absorption on the basis of their physicochemical characteristics, as with other series of drugs. So, this theoretical model is applied now to all six β-blockers assayed. Fig. 3 shows a linear relationship between experimental (log K_p) and theoretical (log K'_p) logarithmic permeability coefficients. The equation obtained by linear regression, considering log K_p as independent variable and log K'_p as dependent one, is: log K'_p (cm s^{-1}) = 2.88 + 1.44 log K_p (cm s^{-1}) with a correlation coefficient of 0.8515. This result seems to confirm that the Potts and Guy theoretical model can predict drug percutaneous absorption capacity, which implies that lipophilic stratum corneum alone can fully characterise the barrier properties of mammalian skin.

The permeation parameters obtained are used to predict the potential systemic capacity of the individual drug studied after transdermal administration. Table 4 shows oral daily doses (D_0) for the treatment of essential hypertension, theoretical daily transdermal doses (D_t), predicted amount permeated daily in steady-state conditions (D_{ss}) and predicted plasma level at steady state (C_{ss}), assuming that the area of the patch (TTS_{area}) was 16 cm^2. The therapeutic index (C_t) and some pharmacokinetic parameters, such as bioavailability (F) and plasmatic clearance (Cl_p) taken from the literature (Riddell et al., 1987), are also shown.

Predicted D_{ss} and C_{ss} values are lower than values for the theoretical transdermal dose (D_t) and their therapeutic index, respectively in all drugs (Table 4). These findings show foreseeable difficulties in their possible development in the transdermal therapeutic system to be used in clinical practice, since these drugs would not permeate enough to reach therapeutic plasma levels.

Although the stratum corneum layer is known to be a major barrier to drug permeation, other factors besides lipophilicity, such as skin protein binding or first-pass metabolism into the skin (Ademola et al., 1993), may affect drug permeability behaviour and may be important during the development of potential transdermal drugs.

Table 4
Relevant parameters for β-blockers assayed used to formulate a transdermal therapeutic system[a]

β-blocker	D_0 (mg)	D_t (mg)	D_{ss} (mg day^{-1})	C_{ss} ($\times 10^{-3}$) (μg ml^{-1})	C_t (μg ml^{-1})	F (%)	Cl_p (ml min^{-1})
Propranolol	160–320	56–112	3.42	2.37	0.05–0.1	35	1000
Oxprenolol	160–480	80–240	2.61	9.06	0.04–0.1	50	200
Metoprolol	100–400	50–200	1.43	0.99	0.05–0.1	50	1000
Atenolol	50–100	25–50	0.09	0.35–0.63	0.2–0.6	50	100–180

[a] D_0, oral daily dose; D_t, theoretical daily transdermal dose; D_{ss}, predicted amount permeated daily in steady-state conditions; C_{ss}, predicted plasma level at steady state; C_t, therapeutic index; F, bioavailability; Cl_p, plasmatic clearance.

Nevertheless, the transdermal route of drug administration continues to be attractive. As a consequence, there have been an increasing number of attempts to reduce the resistance of stratum corneum to β-blocker permeation capacity, and the long lag time. While there is a growing interest in physical approaches such as iontophoresis, sonophoresis or electroporation (Vanbever et al., 1994), most attention has focused on the use of chemical penetration enhancers or vehicles that can improve effectively the permeability of drugs with different lipophilicity and different drug formulations for transdermal delivery (Hori et al., 1991; Kemken et al., 1991; Ghosh et al., 1992a,b; Melendres et al., 1993; Krishna and Pandit, 1996; Kobayashi et al., 1997; Kunta et al., 1997; Ktistis and Niopas, 1998), although combined approaches have also been studied (Hirvonen et al., 1993). In addition, in vitro skin permeation of β-blocker enantiomers and evaluation of stereoselective transdermal transport (Heard et al., 1993; Touitou et al., 1994; Ahmed et al., 1997) have also been studied.

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