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Transdermal absorption of celiprolol and bisoprolol in human skin in vitro[1]

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Abstract

Two β-blockers, celiprolol and bisoprolol, which have a priori interesting properties to be considered in the search of a possible candidate for a transdermal therapeutic system (TTS) were assayed. In vitro permeation studies were conducted at 32 ± 1°C across human abdominal skin. Franz glass diffusion cells were used in the static mode ($n = 7$). The amounts of drug permeated from receptor solution at predetermined times were analysed by reversed-phase HPLC with UV detection. From the penetration profiles obtained for each drug, the main permeation parameters, permeability coefficient (K_p), flow (J) and lag time (T_{lag}) were estimated as a measure of the intrinsic permeability across human skin. Mean K_p value was higher for celiprolol (0.59 ± 0.22 cm h^{-1}) than bisoprolol ($0.27 \pm 0.15 \times 10^{-3}$ cm h^{-1}), although both were very low. Mean J value was also higher for celiprolol (2.72 ± 0.92 μg h^{-1} cm^{-2}) than bisoprolol (1.19 ± 0.60 μg h^{-1} cm^{-2}). Mean T_{lag} value was 20.43 ± 8.43 h for celiprolol and 32.13 ± 39.34 h for bisoprolol. Both provide plasma concentrations at steady state that would be far from their therapeutic concentration. The results indicate the need for appropriate enhancers to improve their diffusion across human skin.

Keywords: Transdermal absorption; Celiprolol; Bisoprolol; Human skin; In vitro

1. Introduction

In recent years, topical application has been considered as a possible delivery route for drugs with systemic activity. Apart from advantages such as avoiding the hepatic first-pass effect or maintaining steady-state plasma levels to provide long-term therapy from a single dose (Barry,

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1983; Guy and Hadgraft, 1985; Asmussen, 1991; Ranade, 1991), the transdermal pathway is of potential interest in those patients who cannot take medicines by themselves, or when oral administration of drugs may be inadvisable such as in functional alterations of gastric motility or in nausea and vomiting.

Nevertheless, very few drugs are able, at least by passive diffusion alone, to penetrate the skin at a rate sufficient to be viable for drug delivery purposes. For this reason, it is desirable to evaluate the skin permeation characteristics of the drug in vitro before conducting studies in humans in vivo (Ritschel and Hussain, 1988; Chien et al., 1989; Wester and Maibach, 1992). Since a transdermal therapeutic system (TTS) containing cardiovascular agents is expected to have an important role in clinical practice, the present study focuses on the transdermal penetration of two β-blockers, celiprolol and bisoprolol. Although several β-blockers are under investigation for administration in TTS (Mey et al., 1989; Corbo et al., 1990; Ghosh et al., 1992; Melendres et al., 1993), there are no data available in the literature on percutaneous absorption in vitro of either of these two drugs. Both have properties that indicate that they may be suitable candidates for a TTS. Celiprolol has a short elimination half life (4–5 h) and bisoprolol is one of the most potent β-blockers whose therapeutic effects can be produced at low doses (5–20 mg daily) (Carretero-Cañigueral, 1991; Opie, 1991; Anonymus, 1995; Cardillo et al., 1992).

Here we study the permeability of celiprolol and bisoprolol across human skin in vitro. This will provide information of relevance to their possible development in TTS.

2. Materials and methods

2.1. Materials

Celiprolol chlorhydrate and bisoprolol fumarate were provided by Rhone-Poulenc Rorer (Madrid, Spain) and Merck (Barcelona, Spain), respectively. Acetonitrile and methanol, which were HPLC grade, were purchased from Scharlau (Germany). Triethylamine was purchased from Fluka (Alcobendas, Spain), phosphoric acid 85% from Probus (Barcelona, Spain), potassium phosphate mono-basic and all-sodium hydrogen phosphate anhydrous from Panreac Química (Montcada i Reixach, Spain), *n*-octanol from Farmaitalia Carlo Erba (Milan, Italy). All of them were of analytical grade and were 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 computerized integration system data output (model MT-450). Liquid chromatographic analyses were performed on a C18 Nucleosil column (5-μm particle diameter, 12.5 cm $\times$ 4 mm i.d.) (Teknokroma, Barcelona, Spain), operating at room temperature. The mobile phase was composed of acetonitrile/67 mM Sorensen's phosphate buffer (pH 5.0) (30/70, v/v) with 0.2% (w/v) of triethylamine and the pH is adjusted to 3 with ortophosphoric acid 85%. This was pumped at a flow rate of 0.8 ml min^{-1}. UV detection was performed at 225 and 232 nm for bisoprolol and celiprolol, respectively. Standard solutions of each drug 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). The concentration range of the calibration curves was 25–0.78 μg ml^{-1}. Intra- and interassay validation of the analytical methods indicated they were exact and precise. Accuracy, expressed as a percentage of the mean recovery, ranged from 100.32 to 102.94%. Precision, expressed as a relative standard deviation, ranged from 1.18 to 1.47%.

2.3. Stability determination

Accelerated studies of stability in solution were previously carried out with the drugs assayed (Modamio et al., 1994, 1996).

2.4. 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 high water solubility described in the literature (Mazzo et al., 1991; Anonymus 1995) and the limited quantity of drug available. 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 $3000 \times g$, 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.

2.5. 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 analyzed 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 ionizing at physiological pH, such as celiprolol and bisoprolol, the intrinsic partition coefficient (P') may be a more appropriate parameter to measure the partition coefficient of the non-ionized compound. This is in agreement with the pH-partition hypothesis, according to which the permeability of the non-ionized 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 pK values previously determined experimentally and considering a pH of 7.4 (Clarke, 1984; Betageri and Rogers, 1987):

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

2.6. pK determination

The pK 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). 5 ml of this was transferred to a battery of tubes (between 8 and 12) containing increasing volumes of 0.001 M NaOH standard solution. 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 (ΔpH/ΔV) versus the volume of NaOH initially added. The pK 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.

2.7. Permeation studies

The permeation in vitro was studied across abdominal human skin removed from two healthy women during plastic surgery. 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}. 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 parefilm 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, 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 analyzed by HPLC. Seven parallel experiments were conducted with each drug.

2.8. Data analysis

The penetration profiles were analyzed 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 following parametrized equation, which is derived according to Fick's second law of diffusion, was used:

$$Q_t = (\mathrm{AP}_2\ C_0) \times \left[P_1 t - \frac{1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \exp(-P_1\ n^2\ \pi^2\ t) \right] \quad (2)$$

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 $\mu g\ ml^{-1}$). 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:

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

$$P_2 = K \cdot L \quad (4)$$

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

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

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

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

The mean values of estimated permeation parameters between drugs assayed were compared by Mann–Whitney U-test (Williams et al., 1992).

On the basis of the parameters of permeation obtained, and from the values of 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:

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

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

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

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

3. Results and discussion

The stability of both drugs at 32°C, the temperature of this study, was previously established by extrapolation from Arrhenius equation (Modamio et al., 1994, 1996).

Table 1
Physicochemical parameters corresponding to bisoprolol and celiprolol (mean ± SD)

β-blocker	MW (g mol^{-1})	*mp* (°C)	S ($\times 10^4$) (μg ml^{-1})	$S_{Yalkowsky}$($\times 10^4$) (μg ml^{-1})	pK	P	P'
Bisoprolol	325.5	100	55.0 ± 11.0	41.1	8.03 ± 1.10	0.89 ± 0.08	4.69 ± 0.42
Celiprolol	379.5	200	22.8 ± 0.67	21.8	9.68 ± 0.03	0.20 ± 0.02	37.48 ± 4.32

MW, molecular weight; *mp*, melting point; S, solubility; $S_{Yalkowsky}$, obtained from the equation log S (M) = −0.01 (mp−25)− log P+0.8.
P and P', experimental and intrinsic values of the partition coefficient.

The values of physicochemical parameters corresponding to celiprolol and bisoprolol, such as molecular weight of the free base (MW) and melting point (mp) taken from the literature (The Merck Index, 1989; Mazzo et al., 1991; Opie, 1991; Anonymus, 1995), the experimental solubility in Sorensen's phosphate buffer (pH 7.4) (S), pK and partition coefficient (P) and the intrinsic partition coefficient (P'), are listed in Table 1. An idea of the reliability of our estimation of S data was obtained from the theoretical predictions of the Yalkowsky equation (Yalkowsky and Valvani, 1980; Yalkowsky and Barnerjee, 1992), shown at the foot of Table 1. This equation enables the estimation of aqueous solubility of compounds, expressed in moles per liter, from the partition coefficient and the melting point data. As it can be seen, the magnitude of theoretical and experimental solubility data of drugs assayed keeps the same order, above all in the case of celiprolol. The facts that first, phosphate buffer (pH 7.4) as aqueous phase was used instead of water in the estimation of both experimental solubility and partition coefficient and, second, the temperature of the study was 32°C, could explain some of the differences found. Nevertheless, this prediction confirm the experimental solubility data obtained.

The permeation profiles for bisoprolol and celiprolol, that is the values of accumulated amounts of drug permeated versus time are shown in Fig. 1. Experiments were finished after 2 days (72 h). After the estimation of P_1 and P_2, the K_p, J, and T_{lag} parameters for each drug were calculated by the method described above. Table 2 summarizes mean and standard deviation values of these permeation parameters. Significant differences ($P < 0.05$) between drugs for K_p and J values, but not for T_{lag} values ($P > 0.05$) were found.

Mean K_p and J values were very low for both drugs but greater for celiprolol. The results obtained for K_p values suggested that this parameter by itself represented an obstacle for transdermal absorption in comparison with those of other drugs (Calpena et al., 1994).

Drugs that have the potential to be formulated in TTS should reach a suitable J, which is directly related to the solubility of the drug in the vehicle. Despite their appropriate solubility, J value was very low in both drugs.

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 was also a constraining factor in the formulation of a TTS for both drugs.

In a further attempt to explain the mechanisms of permeation through human skin, the molecular weight and the influence of lipophilicity on the permeability coefficient were taken into account (Roy and Flynn, 1988; Green et al., 1989). MW directly affects the diffusion across simple or complex membranes, mainly in an inverse relationship, and should be considered when the passage of drugs across skin is studied. The differences in permeability of the two drugs may not be attributable to the differences in their molecular weight (Table 1). Lipophilicity is also a determinant factor in permeation studies and this was measured as the intrinsic partition coefficient *n*-octanol/Sorensen's phosphate buffer (pH 7.4). The greater value found for celiprolol seems to be consistent with its influence on the penetration across skin.

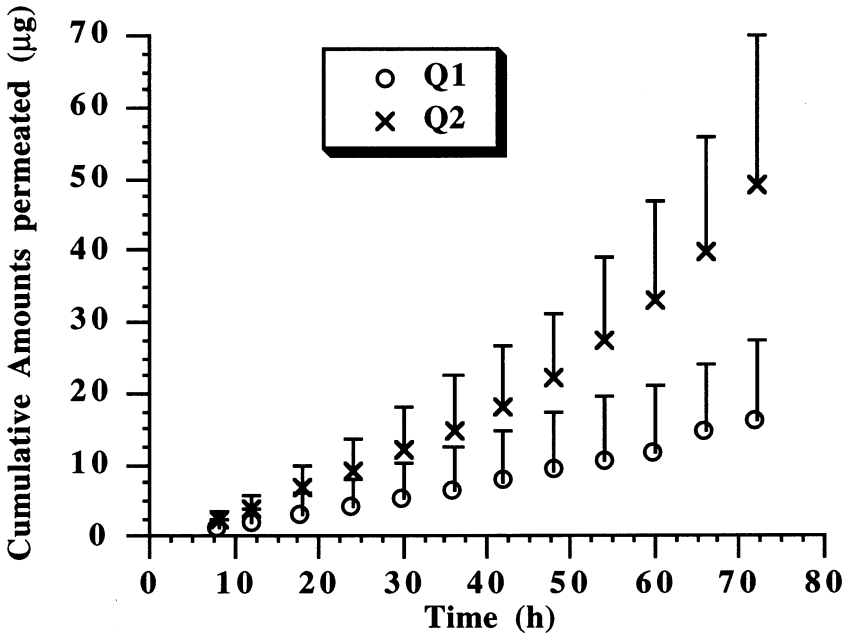

Fig. 1. Time course of cumulative amount permeated of bisoprolol (Q_1) and celiprolol (Q_2) ($n = 7$)

In this sense, Potts and Guy (1992) proposed a simple theoretical model to predict skin permeability on the basis of physicochemical properties. This model requires the multiple regression of log K_p as a function of logarithm of intrinsic octanol-water partition coefficient (log P') and MW, and appears to apply equally well to human and mouse skin permeability data of compounds spanning a broad range of physicochemical properties and structures. Thus, for compounds ranging in molecular weight from 18 to >750 and in log P' from -3 to $+6$, such as celiprolol and bisoprolol, the permeability through human skin can be predicted by the following equation:

$$\log K_p(\text{cm s}^{-1}) = -6.3 + 0.71 \log P' - 0.0061\ \text{MW} \quad (11)$$

Table 2
Main permeation parameters for bisoprolol and celiprolol, shown as mean and standard deviation ($n = 7$)

β-blocker	K_p ($\times 10^{-3}$) (cm h^{-1})	J (μg h^{-1} cm^{-2})	T_{lag} (h)
Bisoprolol	0.27 ± 0.15	1.19 ± 0.60	32.13 ± 39.34
Celiprolol	0.59 ± 0.22	2.72 ± 0.92	20.43 ± 8.43

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

The log K_p (cm s^{-1}) values obtained experimentally and after the application of Eq. (11) were -5.0 and -7.5 for celiprolol and -5.4 and -7.8 for bisoprolol, respectively. These experimental and predictive parameter data are similar to both drugs. Nevertheless, considering this equation, the relevance of lipophilicity and molecular size as predictors of β-blockers permeability across human skin is restricted in this work, due to the limited number of β-blockers studied. Probably a greater number of β-blockers could have confirmed its usefulness as it has happened with other series of drugs.

The permeation parameters obtained were used for predictive purposes. Table 3 shows the oral daily doses (D_0) for the treatment of essential hypertension, the theoretical daily transdermal doses (D_t), predicted range of amounts permeated daily in steady-state conditions (D_{ss}) and predicted range of 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

Table 3
Relevant parameters for bisoprolol and celiprolol used to formulate a therapeutic transdermal system

β-blocker	D_0 (mg)	D_t (mg)	D_{ss} (mg/day)	C_{ss} ($\times 10^{-3}$) (μg.ml^{-1})	C_t (μg ml^{-1})	F (%)	Cl_p (ml min^{-1})
Bisoprolol	5–20	4.5–18	0.46	1.27	0.01–0.1	90	250
Celiprolol	600	330	1.05	4.03–5.58	0.05–0.4	55	130–180

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

pharmacokinetic parameters, such as the bioavailability (F) and plasmatic clearance (Cl_p) taking from the literature (Opie, 1991; Anonymus, 1995) are also shown.

The predicted D_{ss} values were lower than those corresponding to the theoretical transdermal dose (D_t) and C_{ss} values were lower than those corresponding to their therapeutic index in both drugs (Table 3). These findings show foreseeable difficulties in their possible development in TTS to be used in clinical practice.

Since the tactics to improve the diffusion are either reducing the barrier properties of the skin or increasing the diffusion properties of the drugs, the use of appropriate penetration enhancers seemed to be necessary to increase their permeation capacity across human skin (Walters, 1989).

Finally, there was considerable variability to transdermal drug permeation found in the parameters obtained from permeation profiles across human skin, which was obtained in spite of using skin from the same anatomical side (abdominal) and from the same person. This fact reveals the need to increase the number of replicates and to assay drugs with a reasonably wide therapeutic index. Interindividual variability in skin absorption, which is assumed to be greater, would not then constitute an obstacle for refining dosage adjustment.

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