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Chiral β-blockers for transdermal delivery

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Abstract

An investigation of the role of drug chirality on transport through the skin was conducted. It was shown that the ratio of enantiomer to racemate flux through the skin can be predicted from thermal analysis data. The melting temperature-membrane transport (MTMT) concept and an equation for calculating the enantiomer/racemic flux ratio through the skin, which uses the thermal characteristics of the compound, are presented. The concept predicts a significant difference in skin transport rates in those cases where there are large differences in melting temperatures between the pure enantiomers and racemate. Thermal analysis was carried out for three β-blocker chiral molecules: atenolol, alprenolol and propranolol. Propranolol free base showed a difference between racemate and enantiomer melting points of 21°C. By using the MTMT model, the predicted ratio of enantiomer/racemic fluxes through the skin was found to be 3.2. This predicted ratio was confirmed in experiments conducted on Testskin™ and human cadaver skin with solutions of propranolol base isomers and racemic compound in propylene glycol. The 3-fold greater skin permeation of the *S*-(−)-enantiomer vs the racemic compound, along with the 2 orders of magnitude greater pharmacological effect reported for this isomer, make this enantiomer the candidate of choice for transdermal delivery of propranolol.

Key words: Chiral isomer; β-Blocker; Propranolol; Alprenolol; Atenolol; Transdermal; Melting temperature-membrane transport; Skin

1. Introduction

For more than a decade a large number of publications have focused on the effect of stereochemistry on drug action, metabolism, disposition and bioequivalence after administration by the oral or parenteral route (Caldwell, 1992; Nerurkar et al., 1992; Wechter, 1992). A comprehensive review of chirality in drug development was recently published by Fassihi (1993).

The investigation of skin as a portal of entry for chiral molecules is an increasingly active and exciting field. Benezra and colleagues (1985) have shown enantiospecificity in allergic contact dermatitis. Amin et al. (1987) found stereoselective metabolic activation of carcinogenes in mouse skin. Loschmann et al. (1989) proposed transder-

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mal administration of the active enantiomer of a dopamine D-2 receptor agonist for prolonged reversal of parkinsonian motor deficits in man.

Lawter and Pawelchak (1989) showed that for nilvadipine, a calcium channel blocker, the flux through human cadaver skin of the pure enantiomer was about 7-fold higher than that of the racemate. The authors proposed that, for chiral substances such as nilvidapine which form racemic compounds and for which the melting point of the racemate is higher than that of the pure enantiomer, the enantiomer has a greater skin permeation. In this case, the melting point difference was 34°C,and the solubility of the enantiomers in several vehicles was about 10-fold greater than that of the racemate. Wearley et al. (1993) reported that the maximum flux through human cadaver skin of a new antifungal compound in saturated solution was one order of magnitude lower for the racemic compound than that for either of the enantiomers. The melting temperatures were 212 and 152°C for the racemic compound and enantiomers, respectively. However, a minimal difference between racemate and active enantiomer was observed in topical activity in a guinea pig dermatophyte model. This finding was explained by the authors to be due to the fact that the maximum effect of the drug occurred at a concentration lower than the saturation concentration of the racemic compound in the vehicle used. Propranolol is one of several β-adrenergic blockers which exhibit chirality. This nonselective β-blocker is a candidate for transdermal delivery, since it is subject to extensive first-pass hepatic metabolism (Corbo et al., 1990). The finding that enantiomers with a lower melting point than that of the racemate have higher skin permeation may be of special interest when one isomer is more potent than the other. Differences in potency of propranolol enantiomers have been reported. The (*S*)-(−)-enantiomer is 2 orders of magnitude more potent as a β-blocker than the (*R*)-(+)-isomer (Barett and Cullum, 1968; Takahashi et al., 1988). In the light of these properties, propranolol base (PL) was chosen for investigating the relationship between the melting characteristics, solubility and permeation fluxes of enantiomer and racemate.

2. Theoretical aspects

The flux of a compound across skin is related to its thermodynamic activity in a vehicle (Higuchi, 1960) by the relationship:

$$F = \mathrm{d}Q/\mathrm{d}t = \frac{a_v D}{\gamma_m h} = \frac{k_m}{h} CD \qquad (1)$$

where F is the flux of drug permeation through the membrane, $\mathrm{d}Q/\mathrm{d}t$ denotes the flux of drug permeation through the membrane, a_v represents the drug activity in the vehicle, D is the diffusion coefficient in the membrane, γ_m denotes the activity coefficient in the membrane, C is the effective membrane thickness, k_m represents the partition coefficient and C is the drug concentration in the vehicle.

The flux, F, is maximum, F_{max}, when the vehicle is saturated with drug, i.e., at a concentration of C_{max}. If it is assumed that D and k_m are the same for each enantiomer and for different mixtures of enantiomers, it can be shown from Eq. 1 that the ratio of maximum fluxes equals the ratio of the solubilities of the enantiomeric mixtures. These parameters are in fact identical only for achiral vehicles or membranes. The skin is, of course, comprised of chiral molecules. However, any difference in D and k_m would be expected to be small. The solubility, in terms of mole fraction solute, X, of a compound in a given solvent can be related to the melting temperature, T_t, and the enthalpy for fusion, ΔH, using the ideal solubility relation:

$$\ln X = -\frac{\Delta H}{R}\left(\frac{T_t - T}{T \cdot T_t}\right) \qquad (2)$$

where R is the gas constant and T denotes the temperature of solution. For the pure enantiomer, (*S*), and the racemic compound, (*RS*), Eq. 2 becomes Eq. 2a and 2b, respectively.

$$\ln X_S = -\frac{\Delta H_S}{R}\left(\frac{T_{tS} - T}{T \cdot T_{tS}}\right) \qquad (2a)$$

$$\ln X_{RS} = -\frac{\Delta H_{RS}}{R}\left(\frac{T_{tRS} - T}{T \cdot T_{tRS}}\right) \qquad (2b)$$

where X_S is the mole fraction of the (*S*) enantiomer in a saturated solution, ΔH_S represents the enthalpy of fusion of the (*S*)-enantiomer, T_{tS} is the melting temperature of the (*S*)-enantiomer, X_{RS} denotes the mole fraction of the racemate in a saturated solution, ΔH_{RS} is the enthalpy of fusion of the racemate, and T_{tRS} represents the melting temperature of the racemate.

The relationship between fluxes and solubilities for these two systems can be written

$$\ln\frac{F_{\max S}}{F_{\max RS}} = \ln\frac{X_{\max S}}{X_{\max RS}} = \frac{\Delta H_{RS}(T_{tRS}-T)}{R\cdot T_{tRS}\cdot T} - \frac{\Delta H_S(T_{tS}-T)}{R\cdot T_{tS}\cdot T} \quad (3)$$

Eq. 3 shows a simple dependence of the permeation flux ratio on melting temperatures and enthalpies of fusion. The dependence of the flux ratio on the melting behavior of chiral compound is the basis for the MTMT relationship or the 'melting temperature-membrane transport' concept.

3. Experimental

(*R*)-(+)-Atenolol and (*S*)-(−)-atenolol were purchased from Aldrich. Their chemical purities were 99% and $[\alpha]^{25}$ values were +16 and −16. (−)-Alprenolol d-tartrate salt hydrate and alprenolol hydrochloride, chemical purity 99%, were received from Sigma Chemical Co. The optically active free bases were prepared from the salts by precipitation from an aqueous solution by addition of 1 N NaOH. (*R*)-(+)-Propranolol HCl, (*S*)-(−)-propranolol HCl and DL-propranolol HCl were purchased from Sigma Chemical Co. The purity of each of these compounds was > 99% (tested by TLC). Their respective specific rotations, rotational angles, $[\alpha]^{20}$ measured in ethanol solutions, were + 29, −22 and 0°. The optically active and racemic propranolol bases (*R*)-(+)-propranolol, (*S*)-(−)-propranolol and DL-propranolol were prepared by precipitation of the base from aqueous solution with 1 N NaOH. The following designations will be used for propranolol bases (PL): RPL, SPL, and RSPL. Mineral oil (MO), propylene glycol (PG) and PEG 400 were NF grade. All other reagents were of analytical grade.

3.1. Skin membrane preparation

Testskin™ LSE (Organogenesis, MA, U.S.A.) was used as a model skin in the in vitro permeation studies. A number of experiments have been duplicated in human cadaver skin in order to compare the behavior of the two model skins.

Testskin™

Testskin™ LSE was recently introduced in in vitro permeation studies (Ernesti et al., 1992; Hager et al., 1992). It is an organotypic coculture of human dermal fibroblasts in a collagen-containing matrix and a stratified epidermis composed of human epidermal keratinocytes and a well differentiated stratum corneum. The thickness of this skin varies between 350 and 500 μm. Testskin™ was used on the day of receipt.

Human cadaver skin

Dermatomed (~ 300 μm) human cadaver thigh skin (male, age 37 years) pretreated with 50% w/w glycerol in water was kept frozen at −70°C until the day of the experiment. Before mounting into the diffusion cells the skin was defrosted at room temperature and rinsed with water.

3.2. Permeation experiments

The experiments were run at 37°C in Bronaugh flow-through diffusion cells with a diffusional area of 0.32 cm^2 at a flow rate of ~ 1.3 ml/h. The skin was placed in the diffusion cells at 37°C 30 min before the drug was applied to the skin. 100 μl of the test solution was applied to the skin in the donor compartment of the cell. The circulating receiver medium, maintained at 37°C, was composed of a 30% v/v mixture of PEG 400 in water. This mixture was selected in order to maintain pseudo-sink conditions. Samples were collected every 2 h. Drug concentration in the donor solutions and receiver samples was assayed by HPLC.

3.3. Skin permeation data analysis

A total of six replicates were run for each system. Analysis was performed on data obtained over a 12 h period. The skin permeation results were treated using the 'Transderm' software (Touitou and Wartenfeld, 1987). The statistical significance of the difference between systems was determined by a double-tailed Wilcoxon test using the 'Balance' (IBM) computer program.

3.4. Assay

PL concentrations were measured by injecting appropriate volumes of the samples in a Waters HPLC, equipped with a UV detector (detector, Waters 991PDA; pump, Waters 600E; injector, Waters 715 UltraWisp sample processor). The drug concentration was measured by a modified USP method (USP XXII, 1992).The assay was run at 290 nm on a C18 reverse-phase column (3×0.3 cm, Perkin Elmer Cartridge Pack), using external standard solutions of propranolol base. The flow rate of the mobile phase was 1 ml/min and its composition: methanol, 33% v/v; acetonitrile, 33% v/v; sodium lauryl sulfate, 0.2 w/v; and 0.15 M phosphoric acid aqueous solution, 33.8% v/v. Linear calibration curves over the concentration range of 118 ng/ml–47.2 μg/ml were generated. The standard deviation for replicate injections was not more than 2%.

3.5. Solubility measurements

Excess drug was suspended in the solvent of interest and shaken in a thermostated oven at 37°C for 24 h. The supernatant solution was filtered through a 0.45 μm Acrodisc (Gelman) filter preheated to 37°C. Samples were diluted and analyzed by HPLC.

3.6. Differential scanning calorimetry (DSC)

The melting temperatures of alprenolol and atenolol were measured in a Perkin Elmer DSC4 + TADS system. DSC measurements for propranolol were performed on a Seiko DSC 220 computerized system. DSC measurements were carried out in aluminium pans at a heating rate of 10°C/min under a nitrogen stream. The DSC curves were recorded and analyzed by the Seiko DSC or Perkin Elmer TADS software. The melting characteristics measured were the transition temperature, T_t, the peak temperature or the end of melting, T_m, and the enthalpy of fusion, ΔH, as shown in Fig. 1 for (*R*)-atenolol. The transition temperature, T_t, is the point at the intersection of the baseline and a line drawn tangentially to the ascending portion of the endotherm. Enthalpies of fusion, ΔH, were determined from the area of the DSC peak divided by the sample weight. Mixtures of SPL and RPL of different ratios containing mole fractions of SPL between 0.17 and 0.83 were prepared by disolving the enantiomers in methanol and evaporating the solvent under vacuum until a constant weight was attained. The mixture was then annealed at room temperature for at least 36 h. The total mass of each compound or mixture (2.2 ± 0.1 mg) was kept constant when T_m was measured in order to avoid mass-related artifacts.

4. Results and discussion

4.1. Melting characteristics of some β-blockers

The first stage in this investigation was the determination of pure enantiomer and racemate

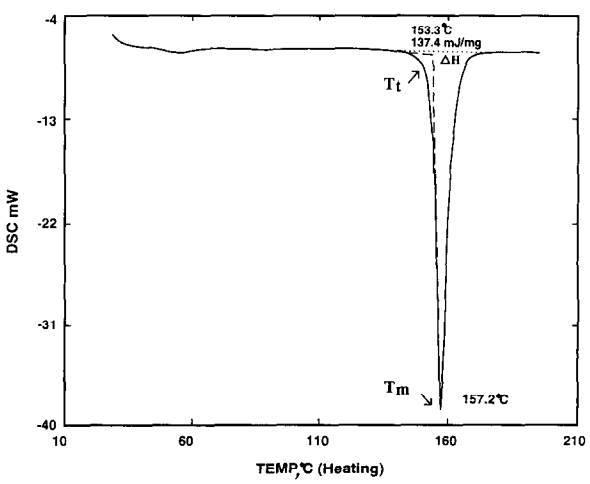

Fig. 1. Thermal characteristics measured on DSC curves: T_t, T_m and ΔH shown on the DSC curve for (*R*)-atenolol.

Table 1
Melting temperatures (T_t) for some chiral β-blocker drugs

Compound	T_t (°C) ($\pm \sigma_{n-1}$)	ΔH (cal/mol)
Atenolol		
(*S*)	150.2±1.9	8789
(*R*)	151.1±2.5	8747
(*RS*)	147.2±1.6	8523
Propranolol base		
(*S*)	71.5±0.5	8665
(*R*)	71.5±0.3	8573
(*RS*)	92.3±0.3	10385
Alprenolol base		
(*S*)	25.3±2.6	5684
(*RS*)	58.5±0.9	8512

melting temperatures for alprenolol, atenolol and propranolol. Their melting transition temperatures are presented in Table 1. As can be seen from these data, the difference in melting points between the atenolol enantiomers and racemate was very small. Larger differences were found for propranolol and alprenolol, for which the enantiomers had lower melting points. The thermal data for alprenolol and propranolol base (*S*)-enantiomers and racemates were substituted in Eq. 3 to calculate the ratio of skin permeation fluxes. Values of 3.2 and 3.5 were found for propranolol and alprenolol, respectively. Propranolol was chosen for further studies. The rationale for this choice was the known difference in pharmacological effect of the enantiomers.

4.2. Melting behavior and phase diagram for PL enantiomers and racemate

The melting behavior of SPL, RSPL and mixture M1 containing SPL : RPL (0.17 : 0.83) can be seen from the DSC data presented in Fig. 2 and Table 1. The pure enantiomers and the racemic compound exhibited single endotherms which are due to melting. The DSC data in Table 1 show melting transition temperatures (T_t) values of 71.5 ± 0.3, 71.5 ± 0.5 and 92.3 ± 0.3°C for RPL, SPL and RSPL, respectively. The difference between the melting points of the enantiomers and the racemate is 21°C. The fact that the melting point of RSPL is greater than that of RPL or SPL indicates that propranolol free base enantiomer mixture may form either a racemic compound or a solid solution in the solid state. Confirmation of the former case was obtained from the DSC curves of additional disproportionate mixtures of the enantiomers. These mixtures exhibited two

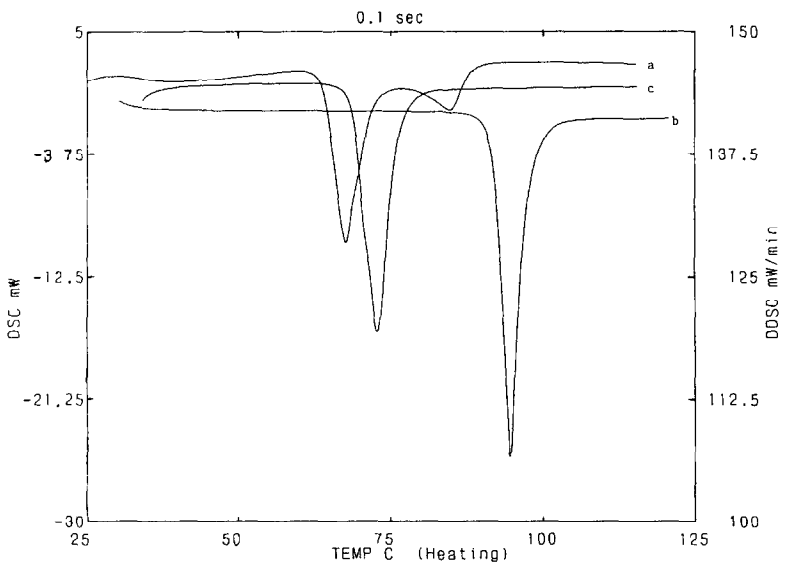

Fig. 2. Representative DSC curves for: (a) SPL : RPL (0.172 : 0.828); (b) RSPL and (c) SPL.

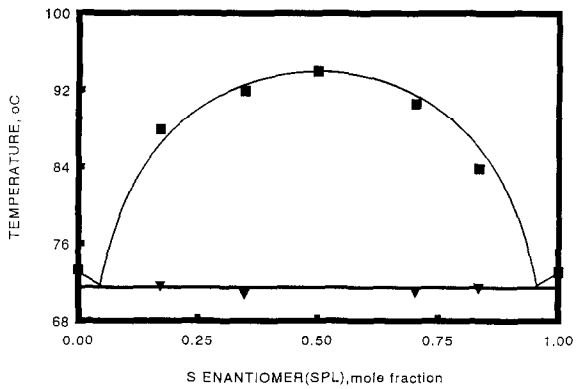

Fig. 3. Binary phase diagram for propranolol base enantiomers and mixtures.

endotherms as is shown in Fig. 2 for mixture M1. The form of the melting point diagram corresponds to that of a racemic compound (Jacques et al., 1991). A phase diagram was constructed for propranolol by plotting the peak temperatures (T_m) of pure enantiomers, their racemate and mixtures of various compositions (Fig. 3). The liquidus curve (upper solid curve) in Fig. 3 was computed from the Schroeder-Van Laar and Prigogine-Defay equations (Eq. 4 and 5, respectively) (Jacques et al., 1991).

$$\ln x = \frac{\Delta H_S}{R}\left(\frac{1}{T_{mS}} - \frac{1}{T_m}\right) \quad (4)$$

$$\ln 4x(1-x) = \frac{2\Delta H_{RS}}{R}\left(\frac{1}{T_{mRS}} - \frac{1}{T_m}\right) \quad (5)$$

For the Schroeder-Van Laar segments of the curve (0–0.054 and 0.946–1.0 mole fraction of the (*S*)-enantiomer), the enthalpy of fusion, ΔH_S (Table 1), and peak temperature, T_{mS}, were taken from the DSC curve of the (*S*)-enantiomer. For the Prigogine-Defay segments (0.054–0.946 mole fraction), the enthalpy of fusion, ΔH_{RS} (Table 1), and peak temperature, T_{mRS}, were taken from the DSC curve of the racemate. The solidus line was drawn through the eutectic points which were taken as the points of intersection of the Prigogine-Defay and Schroeder-Van Laar curves.

4.3. Solubility data for propranolol in various vehicles

The experimental solubility values for PL enantiomers and racemate in various vehicles are

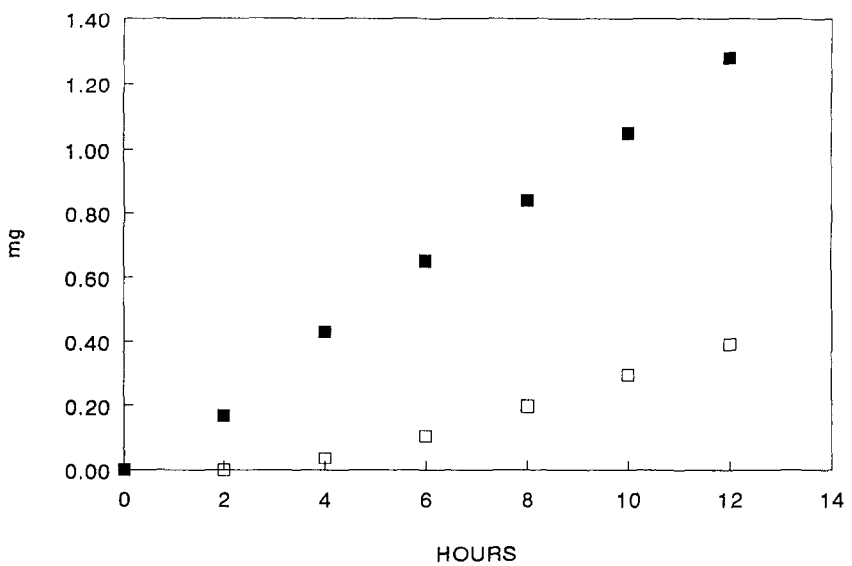

Fig. 4. Permeation profiles of SPL from saturated solution in PG through Testskin™ and human cadaver skin.

Table 2
Solubility of propranolol base enantiomers and racemate in various vehicles at 37°C

	C_s (mg/ml)		
	SPL	RPL	RSPL
Vehicle			
Saline	0.55	0.44	0.22
Buffer, pH 7.4	0.70	0.73	0.47
Mineral oil	6.22	5.93	2.19
30% v/v PEG 400 in H_2O	5.99	4.36	2.35
Propylene glycol	367.6	371.8	91.2

given in Table 2. These data clearly indicate that the enantiomers are more soluble than the racemate in all the vehicles used in this study. The (*S*)-enantiomer/racemate solubility ratios in the various vehicles were: 2.5, 1.5, 2.8, 2.6 and 4.0, for saline, buffer pH 7.4, MO, 30% PEG aqueous solution and PG, respectively. The difference in solubility in aqueous solvents was expected, taking into consideration the pK_a (9.2) of this molecule. In the buffer solution the protonated form of propranolol is predominant while in PG the drug exists in its free base form. This behavior may change on contact with the skin according to the composition of the liquid strata present on the surface of the membrane.

4.4. Permeation fluxes of S propranolol vs RS propranolol from saturated solutions in propylene glycol through Testskin™ and human cadaver skin

Permeation experiments were first run with saturated propylene glycol solutions of SPL and RSPL. The permeation profiles of SPL from saturated solutions in PG through Testskin™ and cadaver human skin are given in Fig. 4. Although it appears that a steady-state regime was reached in the two membranes, the lag time values are different, namely, zero for Testskin™ and 2.75 h for human skin. The permeation fluxes of SPL vs RSPL from saturated solutions of PG were measured in paired experiments on Testskin™ and human cadaver skin. The diagrams in Fig. 5 show that SPL flux through Testskin is significantly higher ($0.01 < P < 0.05$) than RSPL flux. The enantiomer flux (335 $\mu g/cm^2$ per h) is 3.1-times greater than that of the racemate (108 $\mu g/cm^2$ per h). The human cadaver skin results clearly indicate that SPL flux is significantly higher ($P \leq 0.001$) than the RSPL flux, reaching values of 149 and 45 $\mu g/cm^2$ per h, respectively. Although, in comparison with Testskin, the absolute values of the fluxes in human skin are lower, the ratio of fluxes is 3.3. This value is very close to the flux ratio measured in Testskin™.

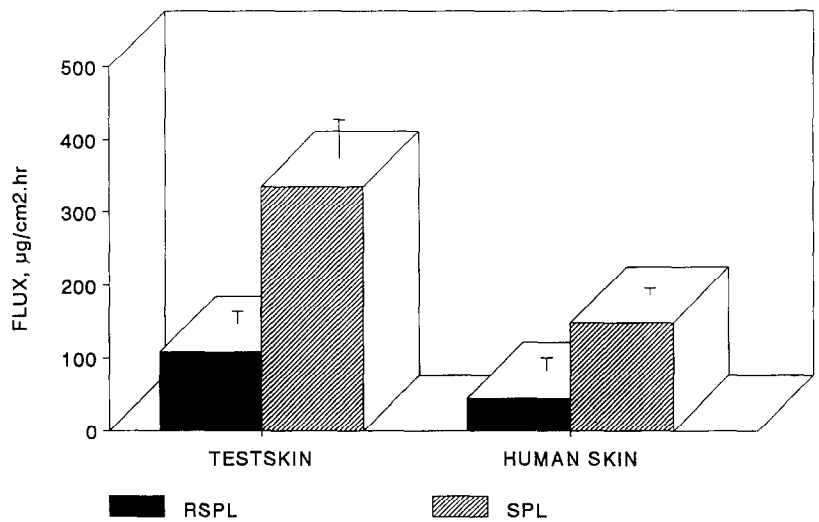

Fig. 5. RSPL vs SPL fluxes from saturated solution in propylene glycol through Testskin™ and human cadaver skin. $0.01 < P < 0.05$ Testskin™; $P < 0.001$ human skin.

Table 3
Melting temperatures, solubility ratio and flux ratio for S and RS propranolol base

	SPL	RSPL
Mol. Wt	259.3	259.3
pK_a	9.2	9.2
T_t (°C) [a]	71.5	92.3
Ratios	Theoretical [b]	Experimental [c]
$C_{max\ SPL} / C_{max\ RSPL}$	3.2	4.0
$F_{max\ SPL} / F_{max\ RSPL}$	3.2	3.3 [d] and 3.1 [e]

[a] Data from DSC measurements.
[b] Calculated by means of Eq. 3.
[c] Propylene glycol systems.
[d] Human cadaver skin.
[e] Testskin™.

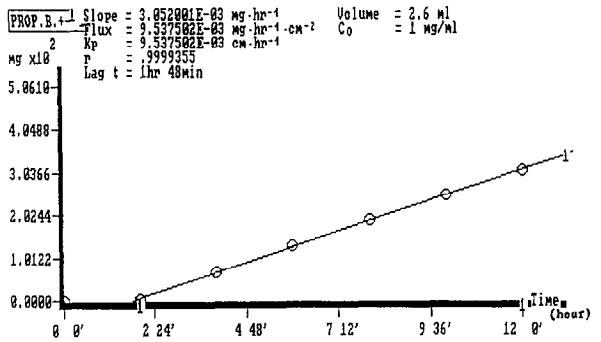

Fig. 6. Skin permeation profile of RSPL through Testskin™ from 1 mg/ml solution in mineral oil.

The experimental data were further compared with the theoretical values obtained by introducing into Eq. 3 the experimental values of melting temperatures and enthalpies of fusion measured in this study: $T = 310$ K, $T_{tRS} = 365$ K, $T_{tS} = 344$ K; $\Delta H_{RS} = 10385.4$ cal/mol and $\Delta H_S = 8665.3$ cal/mol. For SPL and RSPL, Eq. 3 predicts a ratio of 3.2 for enantiomer/racemate solubility and skin permeation fluxes. This value is close to the experimental value for the solubility ratio in PG, 4.0 and is similar to the ratio of the experimental fluxes of SPL and RSPL from saturated solutions (Table 3).

4.5. *Permeation fluxes through Testskin™* of (S)-, (R)-, and (RS)-propranolol from solutions containing 1 mg/ml drug in mineral oil

Two contradictory reports were recently published on the enantioselective rat skin permeation of propranolol (*S*)- and (*R*)-isomers. Heard et al. (1993) were unable to detect enantioselective transport of propranolol from PG solutions containing 100 μg/ml drug as was formerly reported

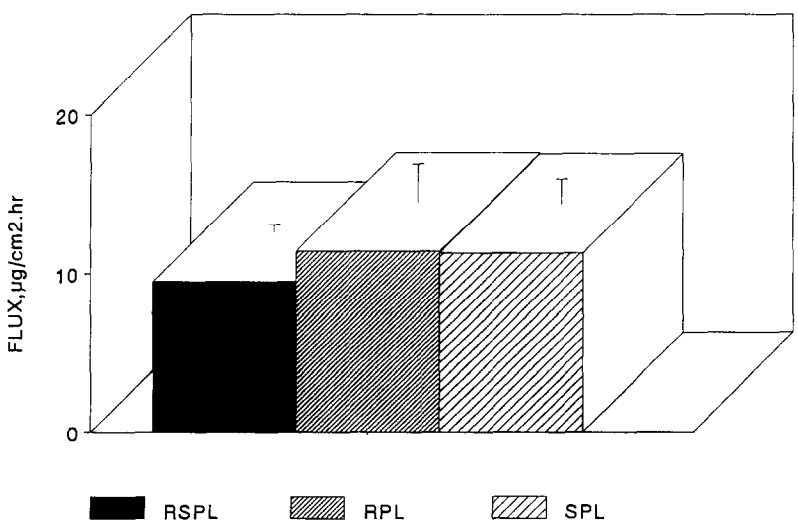

Fig. 7. Propranolol base enantiomers and racemic: fluxes through Testskin™ from 1 mg/ml solution in mineral oil (not significantly different (*R*) vs (*S*) and (*R*) or (*S*) vs (*RS*) at the level of 5%).

(Miyazaki et al., 1992). Heard et al. (1993) showed that the rate of transport across human skin of propranolol from aqueous solutions was the same for both isomers. In the present study we tested the enantioselectivity of propranolol permeation through Testskin™ by using solutions of an equal concentration of (*S*), (*R*) or (*RS*) compound in mineral oil. The plot of the cummulative amount of drug permeating the skin as a function of time and the related kinetic parameters were obtained with the Transderm program. A typical skin permeation profile can be seen (Fig. 6) for these systems: a lag time followed by a steady-state regime. The fluxes given in Fig. 7 indicate that the three compounds permeated the skin at the same rate. The flux values were statistically analyzed for (*S*) vs (*R*) and (*S*) or (*R*) vs (*RS*) differences and none were found at a level of 5% significance. The fact that the fluxes for SPL, RPL and RSPL are equal also supports our previous assumption that k_m and D are the same for the two enantiomers and the racemates.

5. Conclusions

The melting temperature-membrane transport (MTMT) concept and an equation for calculating the enantiomer/racemic flux ratio through the skin which uses thermal analysis data have been presented. This concept predicts significant differences in skin transport rates for enantiomers and racemate having large differences in melting temperatures. Thermal analysis was carried out for three β-blocker chiral compounds: atenolol, alprenolol and propranolol. Propranolol base shows a racemate and enantiomer melting point difference of 21°C. By using the MTMT model, the predicted ratio for enantiomer/racemic fluxes through the skin is 3.2. This behavior was confirmed in experiments on Testskin™ and human cadaver skin with solutions of propranolol base isomers and racemic compound in propylene glycol. The 3-fold greater skin permeation of the (*S*)-(−)-enantiomer vs the racemic compound, along with the 2 orders of magnitude larger pharmacological effect reported for this isomer, make this enantiomer the candidate of choice for transdermal delivery. The MTMT relationship can be used as a valuable tool in screening chiral compounds as candidates for transdermal drug delivery.

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