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Effect of Chiral Enhancers on the Permeability of Optically Active and Racemic Metoprolol across Hairless Mouse Skin

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ABSTRACT The stratum corneum, the rate-limiting barrier in transdermal drug delivery, is chiral in nature and enantiomers behave differently with respect to their transport across the skin, resulting in enantioselective permeation. The permeation characteristics of individual enantiomers of metoprolol free base (MB) were investigated using hairless mouse skin. The influence of chiral permeation enhancers, *l*-menthol and (±)-linalool, on the permeation of MB was also investigated. In the absence of enhancers, the permeation profiles of *R*- and *S*-MB from donor solutions containing either *RS*-MB or pure enantiomers are comparable ($p < 0.05$). In presence of enhancers, *l*-menthol and (±)-linalool, the flux values were increased 2.4- to 3.0-fold, respectively, and the permeation profiles of *R*- and *S*-MB from donor solutions containing *RS*-MB are comparable ($p < 0.05$). However, when donor vehicle contains pure enantiomers, the permeation enhancing effect of *l*-menthol on *S*-MB was significantly higher (by 25%) than on *R*-MB ($p < 0.05$). Further, in presence of *l*-menthol, the flux of *S*-MB from donor solution containing pure *S*-MB was 35% higher than the flux of *RS*-MB from racemate. No such effect was seen with (±)-linalool. In all the investigations, no enantiomeric inversion was observed during the permeation process. The lag times were shorter in the case of *l*-menthol compared with (±)-linalool. *Chirality 11:536–540, 1999.*

KEY WORDS: menthol; linalool; enantioselectivity; flux; lag time; transdermal delivery

INTRODUCTION

In recent years, transdermal delivery of drugs for systemic effect has gained considerable attention as they eliminate first pass effect, provide sustained plasma levels, and improve patient compliance. Stratum corneum, the rate-limiting barrier in transdermal permeation, is made up of keratin and ceramide which are chiral in nature.[1] Enantiomers may behave differently in chiral environments, exhibiting differences in pharmacokinetic and pharmacodynamic parameters.[2] However, little attention has been paid to the permeation characteristics of individual enantiomers of chiral compounds. Miyazaki et al. reported significant differences in the transport rates of the enantiomers of propranolol free base across rat skin.[3] Chiral permeation enhancers may cause enantioselective permeation. It was reported that the permeation enhancing effect of *l*-menthol on *S*-propranolol was about 2.5-fold higher compared to *RS*-propranolol across guinea pig skin.[4] Further, extrinsic factors such as differences in physicochemical properties between enantiomers and racemate were implicated in enantioselective permeation.[5] Recently, we have reported that the flux of *S*-ketoprofen was about 1.79-fold higher than that of *RS*-ketoprofen from their respective saturated donor solutions across hairless mouse skin.[6] In this case, the *S*-ketoprofen has lower melting point and higher solubility than that of *RS*-ketoprofen. In the present investigations, metoprolol, a β_1-selective adrenergic blocking agent, commercially available as racemic mixture was chosen as a model drug (Fig. 1). The biological activity of metoprolol resides entirely with the *S*-enantiomer while the *R*-enantiomer is therapeutically inactive.[7] The oral bioavailability of metoprolol was about 50% due to its extensive first-pass effect.[8] Transdermal delivery of metoprolol may offer a therapeutic advantage as well as patient compliance. Recently, a transdermal delivery system of metoprolol was reported to provide therapeutic levels for 24 hours in rats and also improved the absolute bioavailability.[9] However, no studies have been reported on the permeation characteristics of individual enantiomers of metoprolol. Recently, we have reported the potential use of terpenes as permeation enhancers in transdermal drug delivery systems.[10] The objective of the present study is to investigate the possible enantioselectivity in the permeation of metoprolol across hairless mouse skin in presence and absence of chiral enhancers such as *l*-menthol and (±)-linalool.

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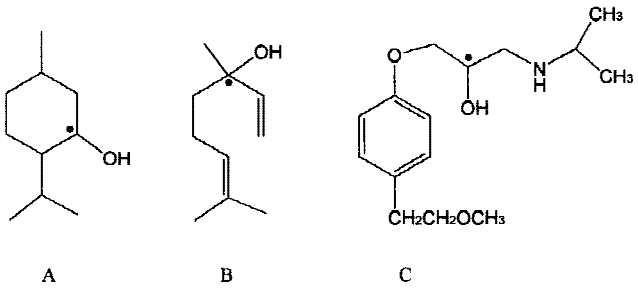

Fig. 1. Chemical structures of menthol (A), linalool (B), and metoprolol (C). The closed circles denote centers of chirality.

EXPERIMENTAL SECTION

Materials

S- and *R*-metoprolol hydrochloride were generously supplied by Astra Hassle AB, Sweden. The optical purity of these enantiomers as determined by stereoselective assay was greater than 99%. *RS*-metoprolol tartrate, *l*-menthol, and other chemicals were purchased from Spectrum (Gardena, CA); (±)-linalool was purchased from Sigma Chemical Co. (St. Louis, MO); male, 5–6 week old hairless mice (ncr-nufBR) were purchased from Taconic (German Town, NY).

Preparation of Metoprolol Free Base (MB)

Since free base form of metoprolol (MB) is expected to have higher flux values, the salts of *S*-, *R*-, and *RS*-metoprolol were converted to their free base form as follows. About 2.0 g of the salt was dissolved in 20.0 mL of water, and 10 N NaOH was added dropwise until no further precipitation occurred. The pH of the solution was adjusted to 10.0 and then extracted with ethyl acetate (25.0 mL × 3). Purity of the extract was checked by TLC to ensure no more salt form of metoprolol is present. The ethyl acetate extract was evaporated using rotary evaporator at 45°C and the resultant liquid was cooled to induce crystalization. No enantiomeric inversion was observed in this process when analysed by chiral HPLC.

Assay Method

Direct separation of metoprolol enantiomers by various chiral stationary phases was reported in the literature.[11,12] In the present studies, separation was achieved using Cellobiohydrolase (CBH) column (100 × 4.0 mm, Chrom Tech AB, Sweden) with a mobile phase of 10 mM phosphate buffer (pH 6.0), 50 µM disodium EDTA, and isopropyl alcohol (2.5%). The flow rate was 1.0 mL/min. The HPLC system consisted of ISCO-2350 pump and a ISCO-V^4 variable absorbance detector set at 225 nm. The retention times of *R*- and *S*-MB enantiomers were 7.0 and 16.5 min, respectively. Working equations were calculated by plotting the peak area as a function of concentration.

Permeation Studies

Skin permeation studies were carried out according to the procedures described in our previous report.[6] Hairless mice (5–7 weeks old) were sacrificed by cervical dislocation, and the full-thickness skin was carefully removed from the abdomen and dorsum. The excised skin was stored at −70°C until further studies. Prior to studies, the skin sample was hydrated in water at 37°C for 20 min and then mounted between two halves of the side-by-side diffusion cells. The stratum corneum was facing the donor solution and the available area for diffusion was 0.9 cm^2. Each diffusion cell had a volume of 3.5 mL and consisted of water jackets connected to a water bath circulator set at 37°C. The donor vehicle consisted of ethyl alcohol (95%)/water (1:1) mixture. Ethanol/water system was selected as donor vehicle for the following reasons. Ethanol was reported to enhance drug permeation rate with a linear relationship with that of ethanol up to 70% v/v of ethanol in ethanol/water system.[13] Further, ethanol helps in solubilization of MB in the donor vehicle. The donor vehicle contained either *R*-, *S*-, or *RS*-MB with or without *l*-menthol or (±)-linalool. The concentration of MB and the enhancers used was 0.1 M. The receiver compartment was filled with 5% propyleneglycol and both sides were stirred with magnetic stirrers. Propyleneglycol (5%) helps in maintaining the sink conditions in the receiver compartment. At predetermined time points (1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, and 12.0 h) a sample volume of 500 µL was taken from the receiver compartment and replaced with the same volume of fresh propyleneglycol (5%). The drug concentrations in the collected samples were determined by stereoselective HPLC. The cumulative amount of drug in the receiver compartment was corrected for the volume removed at various time points. The results were expressed as mean ± SD of three runs for each study.

Data Analysis

The steady-state skin flux was determined using Fick's law of diffusion:[14]

$$J_s = (1/A)\ (dM/dt) = P_e \Delta C,$$

where J_s is the steady state flux (µg cm^{-2} h^{-1}), dM/dt is the amount of drug permeated in unit time, A is the diffusion area (cm^2), P_e is the effective permeability coefficient (cm h^{-1}), and ΔC is the concentration gradient and can be assumed to be equal to donor cell concentration when the receiver cell concentration does not exceed 10% of the donor cell concentration. J_s was determined from the linear portion of the plot of cumulative amount permeated per unit area versus time. The lag time was determined by extrapolating the linear portion of the curve to the abscissa. Statistical differences were established by one way ANOVA and considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Control

The permeation profiles of the enantiomers of MB from donor solutions containing either pure enantiomers or racemate are shown in Fig. 2. The permeation parameters are summarised in Table 1. When donor solution contained *RS*-MB, the permeation profiles of *R*- and *S*-MB are found to be comparable with the flux values of 56.28 ± 14.8 and 55.82 ± 13.4 µg cm^{-2} h^{-1}, respectively ($p < 0.05$). When donor solution contains either pure *R*- or *S*-MB the perme-

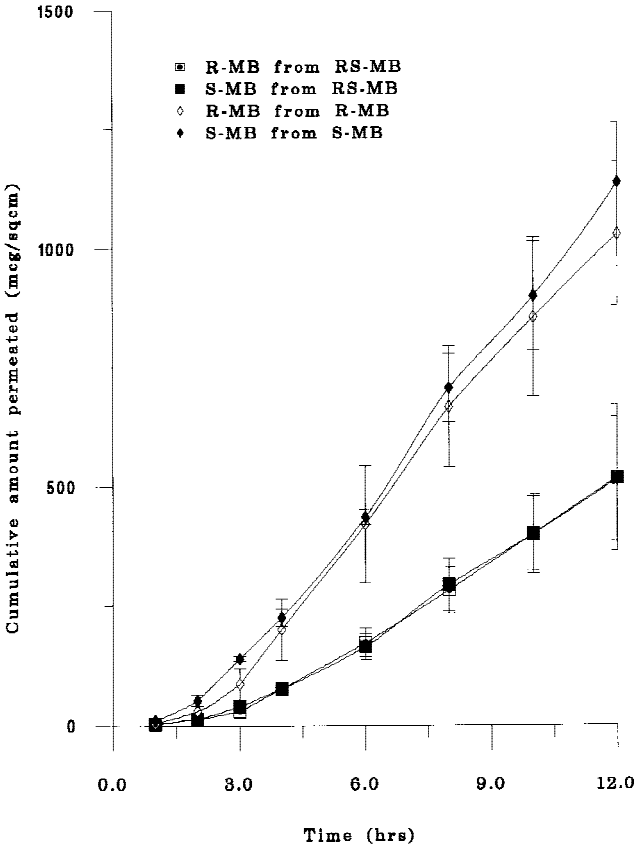

Fig. 2. Permeation profiles of the enantiomers of MB across excised mouse skin from donor solutions containing MB (0.1 M).

ation profiles of the enantiomers are comparable with the flux values of 119.99 ± 20.6, and 129.71 ± 18.1 μg cm^{-2} h^{-1}, respectively ($p < 0.05$). Further, the lag times of *R*- and *S*-MB are also comparable with the values of 2.69 ± 0.6 and 2.74 ± 0.5 h, respectively ($p < 0.05$). No racemization was observed during the permeation process. These results suggest that the chiral nature of the stratum corneum and epidermis did not give rise to enantioselective permeation. These findings are in agreement with the reported studies on propranolol,[15] ephedrine,[1] and ketoprofen[6] where no enantioselective permeation across the skin was observed. The lack of enantioselectivity may be attributed to various factors such as (a) high drug concentration, (b) presence of organic solvent in the donor solution which causes conformational changes in keratin/lipid molecules, and (c) a different pH of the donor solution from that of the skin.[1,15] In fully hydrated skin, the intercellular region becomes more fluid and ethanol up to 60% in the donor vehicle increases the fluidity of the lipid domain and also forms new pores in the skin.[13] Under these conditions, therefore, enantiomers may overcome the enantioselectivity by diffusing through lipid and protein domains aided by the solvent flow. It was reported in the transdermal permeation of several ester prodrugs of propranolol that the enantioselective permeation was not due to the chiral nature of the skin, but due to the differences in the rates of hydrolysis between the enantiomers.[16] Further, the lipophilicity of prodrug is significantly higher than that of propranolol.[16] Extrinsic factors such as differences in physicochemical properties of enantiomers and racemate[5,6] and presence of chiral excipients[17] were reported to cause enantioselective permeation. In the present studies, the melting point of the pure enantiomers of MB (43.9°C) was slightly lower than *RS*-MB (49.9°C). However, these differences in physicochemical properties did not give rise to any significant difference in the flux values of pure enantiomers and the (*R*+*S*) racemate. It is expected that such differences may be seen only when donor solutions are saturated with the compound since the flux values depend on the thermodynamic activity of the solute in the donor vehicle.[5,6]

Effect of Permeation Enhancers

Figure 3 shows the permeation profiles of the enantiomers of MB from donor solutions containing either pure enantiomers or racemate and in presence of *l*-menthol (0.1 M). The permeation parameters are listed in Table 1. As in the case of control experiments, where donor solution contained RS-MB, the permeation profiles of the enantiomers are comparable ($p < 0.05$). However, when donor solution contained pure enantiomers, the permeation enhancing effect of *l*-menthol on *S*-MB was significantly higher (by 25%) than on *R*-MB. Further, the flux of *S*-MB from donor solution containing pure *S*-MB was 35% higher than the flux of *RS*-MB from racemate. Such effect was not observed with (±)-linalool (Fig. 4). These observations are in agreement with our earlier report where the permeation enhancing effect of *l*-menthol on *S*-propranolol was about 2.5-fold higher compared to *RS*-propranolol across guinea pig skin.[4] These observations may indicate the existence of stereospecific interactions between ceramides in stratum corneum and *l*-menthol/MB leading to enantioselective permeation. The ceramides contain multiple hydroxyl functional groups with well defined stereochemistry.[1] Further, stereospecific interactions were reported between ceramide IV and the diastereomers of ephedrine and were rationalised interms of differential hydrogen bonding.[1] The permeation enhancing effect of *l*-menthol on *RS*-MB, in comparison with control, was about 2.5-fold. The mechanism of permeation enhancing effect of *l*-menthol/alcohol system is as follows. Alcohol present in the donor solution, promotes the penetration of *l*-menthol into the stratum corneum where it disrupts the highly ordered structure of intercellular lipids that are made up of ceramides.[18,19] In presence of *l*-menthol, the lag times were reduced significantly.

Figure 4 shows the permeation profiles of the enantiomers of MB in presence of (±)-linalool (0.1 M) and the permeation parameters are listed in Table 1. As in case of control experiments, the permeation profiles of the enantiomers of MB are comparable ($p < 0.05$) when donor solution contains either pure enantiomers or racemate. These findings are in agreement with the reported study on ketoprofen where (±)-linalool did not give rise to any enantioselective permeation.[6] Further, the presence of (±)-linalool did not affect the lag time of metoprolol permeation across the skin. Similar results were reported with other lipophilic permeation enhancers such as long chain fatty

TABLE 1. Permeation parameters of the enantiomers of MB across excised hairless mouse skin

Donor composition[a]	Permeant	Flux (μg cm^{-2} h^{-1})	Lag time (h)	Permeability coefficient (cm h^{-1}) × 10^{-4}
		Control		
(0.1 M) *RS*-MB	*R*-MB	56.28 ± 14.8	2.69 ± 0.6	42.09 ± 11.1
	S-MB	55.82 ± 13.4	2.74 ± 0.5	41.74 ± 10.0
(0.1 M) *R*-MB	*R*-MB	111.99 ± 20.6	2.62 ± 0.6	41.88 ± 7.7
(0.1 M) *S*-MB	*S*-MB	129.71 ± 18.1	2.28 ± 0.6	48.50 ± 6.7
		l-Menthol (0.1 M)		
(0.1 M) *RS*-MB	*R*-MB	134.54 ± 20.2	0.68 ± 0.6	100.62 ± 7.6
	S-MB	149.42 ± 38.9	0.99 ± 0.6	111.74 ± 14.5
(0.1 M) *R*-MB	*R*-MB	317.81 ± 41.0	0.93 ± 0.4	118.84 ± 15.3
(0.1 M) *S*-MB	*S*-MB	386.96 ± 16.9	1.24 ± 0.8	143.21 ± 6.3
		(±)-Linalool (0.1 M)		
(0.1 M) *RS*-MB	*R*-MB	132.4 ± 25.3	2.37 ± 1.0	99.02 ± 18.9
	S-MB	134.03 ± 25.6	2.40 ± 0.8	100.24 ± 19.2
(0.1 M) *R*-MB	*R*-MB	265.48 ± 19.3	2.93 ± 0.4	99.27 ± 7.2
(0.1 M) *S*-MB	*S*-MB	292.73 ± 36.2	2.40 ± 0.2	109.46 ± 13.5

[a]The donor phase consisted of 0.1 M MB in water/alcohol (1:1) mixture.

acids and sesquiterpene compounds where gradual increase in the membrane permeability was observed due primarily by slow redistribution of the enhancers with in the stratum corneum.[20] In comparison with control experiments, (±)-linalool increased the flux values of both *R*- and *S*-MB by 2.4 and 2.3-fold, respectively. The permeation enhancing effects of *l*-menthol and (±)-linalool on *RS*-MB and *R*-MB are comparable ($p < 0.05$). However, the permeation enhancing effect of *l*-menthol on *S*-MB is significantly higher than that of (±)-linalool ($p < 0.05$). However, some studies have reported that acyclic terpenes had higher permeation enhancing effect than cyclic terpenes.[6,21] The acyclic terpenes have suitable structure of linear hydrocarbon tails with double bonds and polarhead groups. They ex-

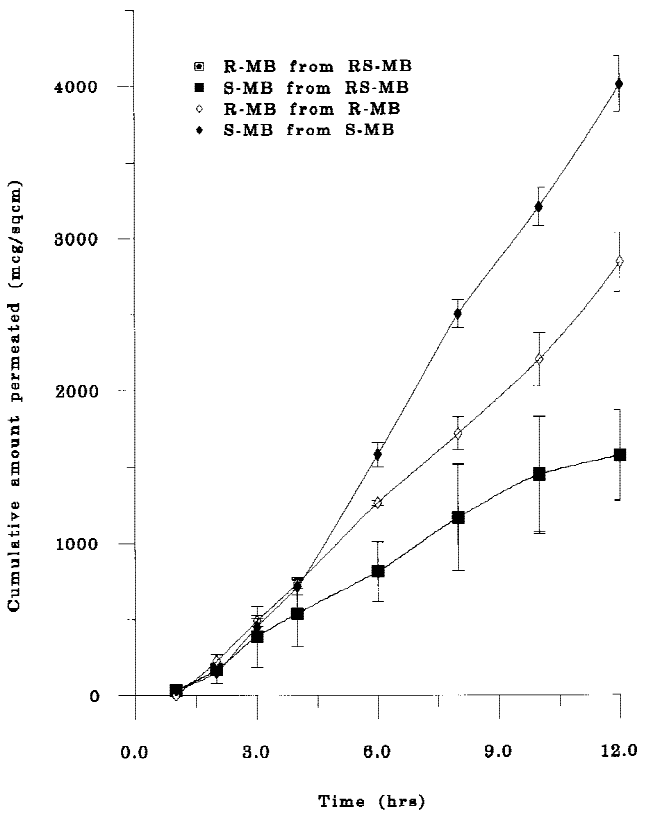

Fig. 3. Permeation profiles of the enantiomers of MB across excised mouse skin from donor solutions containing MB (0.1 M) with *l*-menthol (0.1 M).

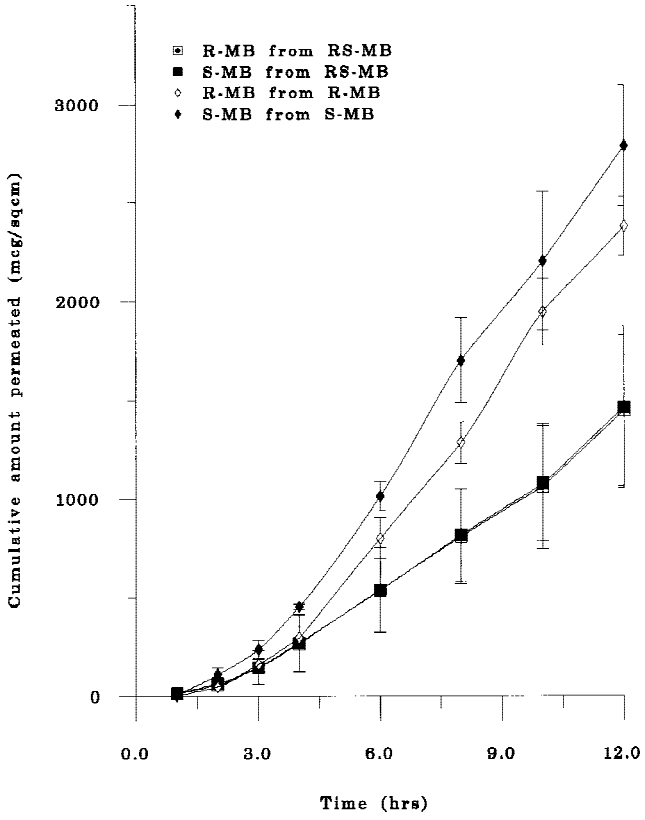

Fig. 4. Permeation profiles of the enantiomers of MB across excised mouse skin from donor solutions containing MB (0.1 M) with (±)-linalool (0.1 M).

pected to accomodate well in the lipid bilayers and disrupting the lipid packing and there by increasing the fluidity.[6]

CONCLUSIONS

Differences in the pharmacokinetic and pharmacodynamic properties of enantiomers is well documented and justify the development of transdermal delivery systems for certain chiral compounds. Use of stereoselective methods is essential in developing transdermal delivery systems of chiral drugs particularly when differences exists between pharmacodynamics of the enantiomers. In the present studies, no intrinsic enantioselectivity in the transdermal permeation of the enantiomers of metoprolol across hair less mice skin was observed. The presence of chiral enhancers, *l*-menthol and (±)-linalool, increased the transdermal flux of metoprolol. No enantioselectivity was seen when donor solution contained *RS*-MB. However, *l*-menthol had higher permeation enhancing effect on *S*-MB when solution contained pure *S*-MB than when donor solution contained either *R*-MB or *RS*-MB. Such effects were not observed with (±)-linalool. The present investigations suggest the use of chiral enhancers in the transdermal delivery of potential *S*-metoprolol which is a pharmacologically active enantiomer.

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