# Concentration-dependent enantioselective transport of chiral timolol maleate across hairless mice skin upon using various concentrations of chiral terpene enhancer (D-Limonene).

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*Received:* October 2, 2013; *Accepted:* November 28, 2013

Original Article

## ABSTRACT

The objective of the present study was to examine the relationship between different concentrations of the chiral enhancer D-Limonene (D-LM) on the solubility of individual enantiomers and racemate Timolol maleate (TM). The preferential improvement of D-LM on S-TM, R-TM, and racemate transported across the hairless mice skin was also investigated. For solubility studies, excess of R-, S-, or racemate with different concentrations of D-LM were prepared. The samples were agitated, centrifuged, filtered and analyzed using HPLC with a chiral column at a wavelength of 294 nm. For skin transport studies, formulations containing 0.5% solutions of S-TM, R-TM, or racemate in a buffer solution with predetermined concentrations of D-LM were studied. Samples of 1 ml were withdrawn and quantitatively analyzed for their TM contents. Steady-state fluxes ($J_{ss}$), permeability coefficients and the enhancement factor were calculated. The studies showed that D-LM significantly increased the solubility of the enantiomeric, as well as, the racemate forms of TM in a concentration dependent manner. The permeation studies showed that the presence of D-LM significantly increased the flux values of both enantiomers and racemate. However, D-LM preferentially increased the permeability characteristics of the S-isomer compared to those of the R-isomer. Solubilities of the enantiomeric and the racemate forms of TM were found to be a single-valued function of D-LM concentration. Moreover, the addition of D-LM increased the transport of the S-TM, R-TM enantiomers and the racemate across the hairless mice skin. In conclusion, all the tested formulations, the overall permeability characteristics of the therapeutically active TM (i.e., S-TM) proved to be superior to those obtained with the R-TM either as enantiomer or racemate.

**KEY WORDS:** Timolol, terpene, chiral, enhancer, D-limonene, enantioselective

## INTRODUCTION

Timolol maleate (TM) exists as two optically active isomers, S-TM and R-TM. TM has a molecular weight of 432.50 Da. It is a white, odorless, crystalline powder which is soluble in water, methanol, and alcohol. TM is described chemically as (S)-1-[(1,1-dimethylethyl)amino]-3-[[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy]-2-propanol,( Z)-2-butenedioate. The empirical formula is C13H24N4O3S C4H4O4. TM is a potent non-selective β-adrenoreceptor blocking

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agent. It is marketed as the maleate salt of the levo (S-) isomer and is approved for the treatment of hypertension, myocardial infarction, angina, and glaucoma (1, 2). It has been reported that R-TM has only 3% of the potency of S-TM in blocking the isoproterenol-induced synthesis of adenosine 3,3,5-monophosphate. The R-enantiomer of TM is 49 times less potent than the S-enantiomer, with respect to $\beta_2$-adrenoceptor activity in animals, and 13 times less potent in constricting the airways of normal subjects. The R-isomer of TM has been found to be effective in lowering elevated intraocular pressure when applied topically to the eye. Yet it is only four times less potent in reducing the intraocular pressure in man (3). Comparison based on potency, the selectivity for $\beta_1$-versus $\beta_2$-receptor subtypes, partial agonist properties, and non-specific membrane-stabilizing effects shows that TM has a greater receptor binding affinity than the other available β-blockers including propranolol, metoprolol, nadalol, atenolol, and timolol (4).

It has been reported that TM is about 8 times more able than propranolol to block β-adrenoceptors (5). When administered orally, TM is well absorbed but it undergoes extensive first-pass metabolism (6). The half-life in plasma is about 4 hours. Plasma concentrations of TM may become sufficiently high to block pulmonary and cardiac β-adrenergic receptors leading to asthma and congestive heart failure. Hence, for long-term prophylactic use, the maintenance dose should be properly titrated to avoid risks associated with $\beta_2$-receptor blockade and recurrence of myocardial infarction. The ability of our biological system to discriminate between two enantiomers of a compound was recognized in 1971, when differences in organoleptic properties of the optical antipodes of structurally similar but stereochemically different organic compounds called R-carvone and S-carvone, were observed (7).

Enantiomers are identical in their physical and chemical properties but behave differently in a chiral environment, such as a biological system or in chiral medium. This feature of enantiomers is different from diastereomers and geometric isomers, which are chemically and physically distinct entities. Enantiomers usually differ in the nature and degree of their pharmacological and toxicological properties (8). A mixture of two enantiomers is called a racemic mixture or a racemate. Of drugs administered as racemates, the pharmacological activity often relies on a single enantiomer, while the other is either completely devoid of activity or is less active.

For a particular pharmacological action, the more active isomer is called the eutomer and the less active, the distomer. The eudismic ratio (the ratio of activity), is an indication of the degree of stereoselectivity. Chemically and biologically, enantiomers must be considered as different compounds often with greater pharmacological activity than homologous agents (9). The effect of chirality on the pharmacological behavior of a drug molecule is an interesting and active area in the field of drug design and drug delivery. The discovery that chiral drug molecules differ in their biological activities has been known for more than a 100 years. Since the initial observation of the existence of asparagine in two enantiomeric forms, numerous studies have been reported about the existence of stereoselectivity of enantiomeric drug substances (10). Molecular chirality originates because of the existence of configurational isomers, termed enantiomers (11). The interest in chirality is not only due to the advancement in medical sciences *per se*, but also due to the rapid progress in the different techniques that can be used to study individual enantiomers. It has become feasible to evaluate the biodistribution of enantiomeric drug molecules using chiral reagents, chiral stationary phases, chiral mobile phases, chiral catalysts, and chiral chromatographic separations (12).

For the last two decades, a number of studies have focused on the stereochemistry in drug action, metabolism, disposition, and bioequivalence (6, 13, 14). The issue of whether to use a racemate or a single enantiomer for therapy remains controversial. Manipulation of

the enantiomeric ratio or the use of only one enantiomer of a drug may minimize toxicity and maximize efficacy resulting in a significant increase in the therapeutic ratio and a more rational approach to therapeutics (15). The development of drugs with chiral centers presents specific challenges that must be addressed at various stages from discovery to clinical evaluation and finally when on the market (17).

Substances that promote drug diffusion through the skin by overcoming the resistance offered by the *stratum corneum* have been referred to as skin-penetration enhancers, permeation enhancers, accelerants, adjutants or sorption promoters (18-20). At present, there is a great deal of interest in the use of chiral terpenes as penetration enhancers. Terpenes, isolated from natural essential oils are used because they are safe, effective, and non-irritant skin penetration enhancers. The routes and mechanisms of enhancement effects of terpenes have been investigated using 5-Fluorouracil (5-FU) (21-23), zidovudine (24), ketoprofen (25), diclofenac sodium (26), and indomethacin (27, 28) as a model drugs.

Percutaneous penetration involves the dissolution of a drug in its vehicle, diffusion of the solubilized drug from the vehicle to the surface of the skin, and penetration of the drug through the *stratum corneum* (29-32). Previous studies have shown several mechanisms through which enhancers promote drug permeability including (1) solvent action to directly solubilize the skin-tissue components, (2) interaction with intercellular lipids to disrupt the structural integrity of stratum corneum thus increasing the diffusivity through the membrane, (3) interaction with intracellular protein to promote the permeation through the corneocyte layer, and (4) increase in the partitioning of a drug into the membrane.

Penetration enhancers exert their enhancing effect by one or more of the previous mechanisms. The terpene enhancers studied perviously include L-menthol, D-LM, carvacrol, menthone, carvone, and 1-8 cineole. It has been reported that, of the terpene enhancers studied, hydrocarbons (D-LM and alpha-pinene) are poor accelerants of alcohols, while ketones including menthone, carveol and carvone are more effective (23). The current study examined the solubility profile, enantioselectivity of TM transport individual isomers and racemate of TM across hairless mice skin, which has previously been shown to be a good model for human skin (15, 33, 34)

## MATERIALS AND METHODS

### Materials

Both S-TM and R-TM were kindly provided by, Merck Research Lab., Whitehouse, NJ (lot numbers not available). D-LM, monosodium phosphate, disodium phosphate, triethanolamine and propylene glycol, were purchased from Sigma Aldrich Chemical Co., St. Louis, MO. Sodium chloride, sodium hydroxide and phosphoric acid were obtained from Spectrum Chemical Co., Gardena, CA. Ethyl acetate, methanol, isopropyl alcohol, and ethyl alcohol were provided by Fisher Scientific Co., Fair Lawn, NJ. All chemicals were used as received and were of HPLC or reagent grade. Deionized distilled water was used throughout the study.

### Animals

Hairless mice, 6-7 weeks-old were provided by King Fahd Medical Research Center, Jeddah, Saudi Arabia. Animal use was approved by the Institutional Review Board for Animal Research/Studies who ensured that the care and use of animals conformed to the Declaration of Helsinki and the Guiding Principle in Care and Use of Animals (DHEW publication NIH 80-23).

### Equipment

A Hewlett Packard autosampler HPLC system with a chime station and variable wavelength UV detector was obtained from Ageilent Technology, St. Louis, MI. HPLC chiral column-AGP 100 x 4.0 mm, 5μm, was obtained from ChromTech, ChromTech International AB, Hägersten,

Sweden. The thermostatically controlled water bath, water bath shaker, sonicator, hot-plate/stirrer, Teflon magnetic bars (12.7x3.2 mm) and pH meter, were obtained from Fisher Scientific Co., Fair Lawn, NJ. Diffusion side-by-side cells (P1803) were purchased from Crown Glass Company Inc. Somerville, NJ. Millipore filter paper, (0.45mm, HA), was purchased from Millipore corporation, Bedford, MA.

## EXPERIMENTAL

### Preparation of racemic TM

A physical mixture of TM was prepared by mixing equimolar ratios of S-TM and R-TM. Equal amounts (0.43 gram) of S-TM and R-TM were weighed and placed into a mortar and were mixed well with a pestle. This physical mixture of racemate was used for the permeation studies.

### Solubility and *in vitro* permeation studies of timolol maleate

#### *Solubility of timolol maleate*

The solubilities of R-, S-, and racemate TM in phosphate buffer pH 7.4 and absolute ethyl alcohol (3:2) were determined using different concentrations of D-LM (0, 0.25, 0.5, 0.75, 1.0, and 1.5% v/v). Excess amounts of R-, S-, and racemic TM were added to the vials containing 1 ml of the vehicle. The vials were agitated for 24 hours in a water bath at 37°C. The resulting suspensions were centrifuged and the supernatant fluids filtered through a 0.45μm filter. The filtrates were diluted and analyzed using HPLC. The experiments were performed in triplicate.

### *In vitro* permeation studies of timolol maleate

0.01 M solutions of S-TM, R-TM, and racemate were placed into three suitable volumetric flasks containing a mixture of phosphate buffer at pH 7.4 and ethanol (3:2). The concentrations of the penetration enhancer D-LM were 0, 0.25, 0.5, 0.75, 1.0, and 1.5% v/v. The mice were euthanized by cervical dislocation and the abdominal skin was immediately carefully excised. The dermal side of the skin was cleaned of any adhering subcutaneous fat. The skin membranes were mounted on side-by-side diffusion cells with the *stratum corneum* facing the donor compartment. The cells were mounted in a thermostatically controlled diffusion system and maintained at 37 ± 0.5°C. The two chambers (3 ml/each chamber) of each cell set were clamped tightly. The donor compartments were filled with the donor phase of the test formulations and the vehicle was added to the receptor compartments using a micropipette. Teflon coated magnetic bars (12.7 x 3.2 mm) were placed inside both compartments and magnetically stirred at 600 RPM. The sampling ports of each cell were capped to prevent the evaporation of volatile vehicle and/or enhancers. The procedures were repeated in triplicate. Samples of 1 ml were withdrawn from the receptor compartments at predetermined time intervals. (0, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0 hours) using a micropipette. The withdrawn samples were replaced immediately with equal volumes of pre-heated vehicle in order to maintain sink conditions. The samples were collected from the donor compartments at the first and last time points and analyzed for thier drug contents to identify any chiral inversion during the permeation study. Additionally, to check the stability of the solution through the duration of the experiments, random samples from the test formulations were collected in light impermeable vials and were analyzed using HPLC for their TM content.

### Calculation of permeability parameters

Factors influencing the penetration of a drug into the skin include the concentration of the dissolved drug ($C_s$) in the donor compartment, a partition coefficient ($K$) between the skin and the vehicle, and the diffusion coefficients of the vehicle and the skin ($P$). The flux ($J$), i.e., the amount ($M$), of a drug permeated through a membrane of unit cross-section ($S$) in unit time ($t$) was calculated in accordance to Fick's law (Equation 1):

$$J = dM/S \times dt \qquad \text{Eq. 1}$$

If a membrane of surface area ($S$) and thickness ($h$) separates donor and receptor compartments and if the concentrations in the donor and receptor compartments are ($C_1$) and ($C_2$), then Fick's law can be rewritten as shown in Equation 2:

$$J = dM/S \times dt = D(C_1-C_2)/h \quad \text{Eq. 2}$$

Under steady-state conditions ($C_1$) is much greater than ($C_2$) and thus,

$$J = DC_1/h$$

When the cumulative amount of the drug permeated per unit cross-sectional area is plotted with time, the slope of the linear portion of the graph is steady-state flux, from which a permeability coefficient can be calculated based on Fick's law as shown in Equation 3:

$$J = PSC_1 \quad \text{Eq. 3}$$

Where ($P$) is the permeability coefficient, and ($C_1$) is the donor cell concentration. The enhancement factors (EF) were calculated by dividing of the steady-state flux value of the test formulation by the corresponding value for the control. The statistical analyses were performed using a t-test.

### Assay technique

Enantioselective HPLC procedures were employed for the analysis of the samples using variable wave length UV at 294 nm wavelength. The column was maintained at 5°C using a built-in temperature control system. The mobile phase consisted of (ethyl acetate: methanol: isopropyl alcohol: 25% ammonia, in ratios of (80: 20: 2: 1, v/v/v/v). The mobile phase was filtered under vacuum using a 0.45 µm filter and degassed using a sonicator. The flow rate of the mobile phase was maintained at 1 ml/min throughout the analysis. Under these conditions, R-TM eluted between 6-8 minutes and S-TM, between 10 and 12 minutes.

## RESULTS AND DISCUSSION

### Solubility and *in vitro* permeation studies

Tables (1-3) show the solubility values of individual enantiomers and racemate of TM using various concentrations of D-LM. Addition of D-LM significantly increased the solubility of the individual R- and S-timolol maleate and in their racemic mixture as well. The solubility of the TM enantiomers significantly ($p<0.01$) and preferentially increased upon addition of various concentrations of D-L. At all concentrations of D-LM, especially at higher concentrations (i.e., 0.75, 1.0, 1.5%) the test formulations containing S-TM exhibited much greater solubility than the R-isomer regardless of the nature of the enantiomer. As can be seen in Tables (1-3) the solubility of TM in the test formulations were found to be a single-valued function of the enhancer concentration. The steady-state flux, the permeability coefficient and the enhancement factor (EF) were calculated for the individual enantiomers and racemate of the test formulations based on previously stated equations and calculations. The calculated average values of the studied permeation characteristics ± SD (n=3) are shown in Tables 1 to 3. Figures 1-4 show the cumulative amounts of drug penetrating a specific unit surface area of the hairless mice skin versus time (hr) using the test formulations. For each TM enantiomer, an analogous formulation containing the drug in the vehicle without enhancer (i.e., concentration of D-LM was 0%) served as controls.

For the formulations of the S-TM enantiomer, the differences in steady-state fluxes and permeability coefficient values were 2.7 and 2.5 fold, respectively more than those of the formulations of the R-TM form. Figure 5 shows the relationship between the D-LM and the EF values ($J_{ssTest}/J_{ssControl}$) for the test formulations.

The EF values for the R-TM isomer ranged from 11 to 20 fold and for the S-TM isomer from 27 to 29 fold compared to the control (0% D-LM) depending upon the concentration of the D-LM

**Table 1** Effects of Different Concentrations of D-LM upon the Permeability Parameters and Solubilities of R-TM Formulations in Hairless Mice Skin.

| Formulation Code | D-LM % (v/v) | Mean Steady-State Flux ($J_{ss}$) (mg.cm$^{-2}$.sec$^{-1}$). | Permeability Coefficient (cm.s$^{-1}$) x 10$^{-6}$ | Solubility (mg/ml) | (EF) $J_{ss\text{-}Test}$ / $J_{ss\text{-}control}$ |
|---|---|---|---|---|---|
| R-TMControl | 0 | 3.91±1.37 | 2.6 | 15.8 0.4 | 1 |
| R-TM1 | 0.0025 | 4.41±4.74 | 4.3 | 59.3 ± 2.4 | 1.13 |
| R-TM2 | 0.005 | 23.24±4.11 | 2.9 | 72.6 ± 4.5 | 5.94 |
| R-TM3 | 0.0075 | 34.17±1.07 | 24.8 | 81.7 ± 5.9 | 8.74 |
| R-TM4 | 0.01 | 37.42±1.11 | 33.4 | 90.2 ± 6.1 | 9.57 |
| R-TM5 | 0.015 | 45.64±10.60 | 43.6 | 102.3 ± 9.3 | 11.16 |

**Table 2** Effects of Different Concentrations of D-LM upon the Permeability Parameters and Solubilities of S-TM Formulations in Hairless Mice Skin.

| Formulation Code | D-LM % (v/v) | Mean Steady-State Flux ($J_{ss}$) (mg.cm$^{-2}$.sec$^{-1}$) | Permeability Coefficient (cm.s$^{-1}$) x 10$^{-6}$ | Solubility (mg/ml) | (EF) $J_{ss\text{-}Test}$ / $J_{ss\text{-}control}$ |
|---|---|---|---|---|---|
| S-TMControl | 0.00% | 4.42±2.21 | 4.1 | 27.8 ± 0.6 | 1 |
| S-TM1 | 0.25% | 16.64±4.49 | 11.1 | 87.5 ± 2.6 | 3.76 |
| S-TM2 | 0.50% | 53.87±3.80 | 68.7 | 98.3 ± 3.1 | 12.19 |
| S-TM3 | 0.75% | 74.22±5.30 | 72.3 | 145.7 ± 5.7 | 16.79 |
| S-TM4 | 1.00% | 98.26±11.61 | 88.3 | 163.6 ± 6.3 | 23.82 |
| S-TM5 | 1.50% | 122.48±21.55 | 109.6 | 167.4 ± 9.4 | 27.71 |

**Table 3** Effects of Different Concentrations of D-LM upon the Permeability Parameters and Solubilities of Racemate TM Formulations in Hairless Mice Skin.

| Formulation Code | D-LM % (v/v) | Mean Steady-State Flux($J_{ss}$) mg.cm$^{-2}$.sec$^{-1}$ | | Permeability Coefficient (cm.s$^{-1}$) ´ 10$^{-6}$ | | Solubility(mg/ml) | | (EF)$J_{ssTest}$/$J_{sscontrol}$ | |
|---|---|---|---|---|---|---|---|---|---|
| | | R-TM | S-TM | R-TM | S-TM | R-TM | S-TM | R-TM | S-TM |
| RS-TMControl | 0.00% | 4.8±0.92 | 4.06±3.24 | 1.9 | 3.9 | 15.8 ± 1.1 | 13.20 ± 1.2 | 1 | 1 |
| RS-TM1 | 0.25% | 13.77±3.72 | 41.82±3.72 | 2.3 | 5.8 | 27.72±3.72 | 16.82±3.72 | 2.88 | 10.35 |
| RS-TM2 | 0.50% | 32.82±3.72 | 65.82±3.72 | 17.9 | 37.6 | 41.82±3.72 | 49.78±3.72 | 6.84 | 16.21 |
| RS-TM3 | 0.75% | 63.80±3.72 | 77.82±11.91 | 32.4 | 58.7 | 88.1 ± 7.3 | 89.50± 7.4 | 13.3 | 19.17 |
| RS-TM4 | 0.10% | 77.75±28.3 | 103.94±26.5 | 41.7 | 78.3 | 97.4 ± 9.5 | 122.6 ± 8.9 | 16.2 | 25.6 |
| RS-TM5 | 1.50% | 99.82±3.72 | 117.79±3.72 | 58.3 | 89.5 | 111.82±3.72 | 163.82±3.72 | 20.8 | 29.01 |

enhancer. For all the TM enantiomers, the EF values for the R-TM and the S-TM were dependent on the concentration of the chiral D-LM enhancer.

The correlation coefficient between the EF and the D-LM concentration of 0.25, 0.5,.0.75,1.0, 1.5 % were found to be >0.88, >0.86, >0.92, >0.95 respectively. This linearity indicated that the EF ratio is a single-valued function of the D-LM under experimental conditions. Statistically, the permeability parameters of individual enantiomers of the TM demonstrated significant difference ($p<0.01$) in the steady-state flux values. Nonetheless, all test formulations for the S-isomer demonstrated greater permeability than

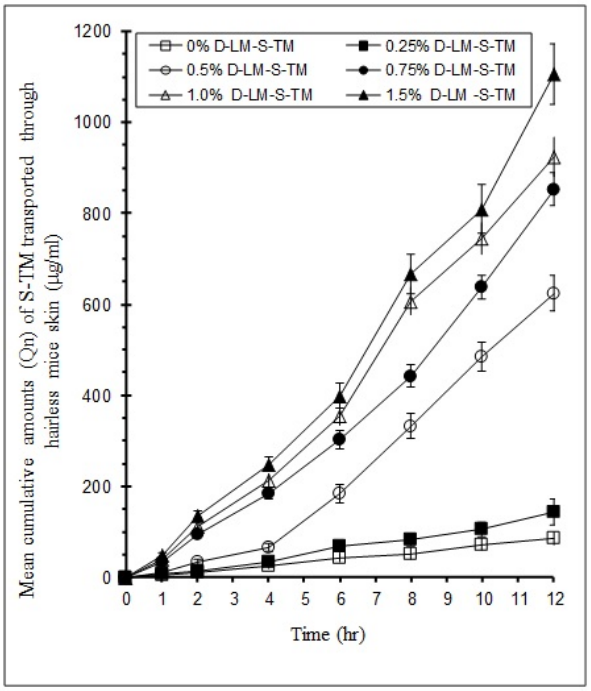


**Figure 1** The mean cumulative amounts (±SD) of pure S-TM delivered from formulations containing different concentrations of D-LM through hairless mice skin into the receiver chamber of Franz diffusion cell as a function of time, (n=3).

the R-isomer at all data points.

Transport studies of the TM enantiomers and the racemate across hairless mouse skin showed enantioselective permeation of the R- and S-isomers, with S-isomer demonstrating greater

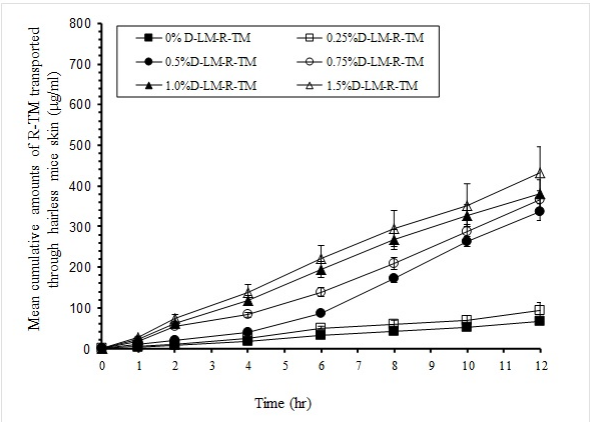


**Figure 2** The mean cumulative amounts (±SD) of pure R-TM delivered from formulations containing different concentrations of D-LM through hairless mice skin into the receiver chamber of Franz diffusion cell as a function of time, (n=3).

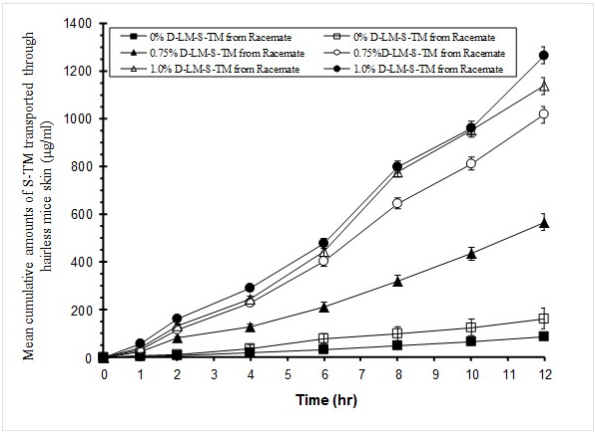


**Figure 3** The mean cumulative amounts (±SD) of S-TM in racemate delivered from formulations containing different concentrations of D-LM through hairless mice skin into the receiver chamber of Franz diffusion cell as a function of time, (n=3, see text for details).

permeation than R-isomer. Incorporating the chiral terpene enhancer D-LM resulted in a significant increase in the permeation of the individual enantiomers. This could be of clinical importance since it has been reported that the R-enantiomer of TM is less potent than the S-enantiomer with respect to the $\beta_2$-adrenoceptor activity, and much less potent in constricting the airways of normal subjects (3). The influence and significance of stereochemical aspects in percutaneous absorption of chiral drugs, such as TM, is based on the understanding of the intrinsic ability of the components of *stratum*

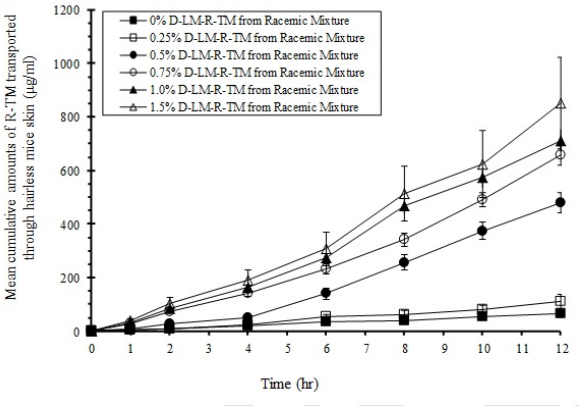


**Figure 4** The mean cumulative amounts (±SD) of R-TM in racemate delivered from formulations containing different concentrations of D-LM through hairless mice skin into the receiver chamber of Franz diffusion cell as a function of time, (n=3, see text for details).

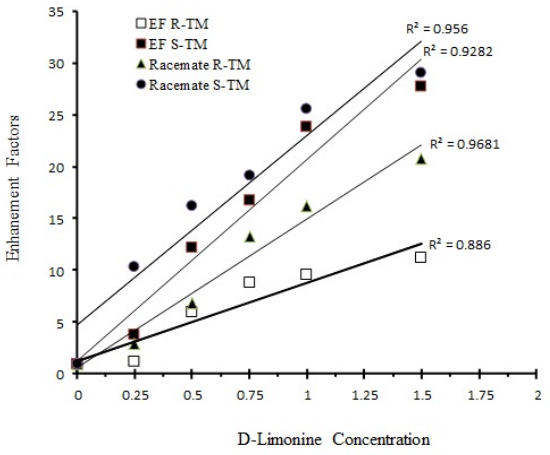


**Figure 5** Correlation between the concentration of D-LM as a chiral enhancer and the enhancement factor using various formulations of all TM Enantiomers.

*corneum* to discriminate between two optical isomers (34). This is attributed to the presence of ceramides in the skin, which have hydroxyl functional groups that are stereochemical in nature. It was hypothesized that stereospecific interaction of the components of skin with the isomers might result in preferential permeation of one isomer over the other. Extrinsic factors such as differences in physico-chemical properties of the enantiomers and the racemate are also known to cause enantioselective permeation (20, 25, 30).

The present study showed that increasing concentrations of D-LM resulted in increasing solubilities of the individual isomers and the racemate of TM, which in turn increased the latters' steady-state flux values. D-LM showed at 0.75% v/v a difference in the solubilities of the two isomers, the S-isomer being more soluble than the R-isomer. This is indicative of a preferential solubilizing ability of the D-LM on the two isomers. At this stage, as reported previously, the enantioselective permeation depends on the thermodynamic activity, as well as, the solubility of the solutes in the donor vehicle (20, 30, 31). Therefore, the influence of the extrinsic factors cannot be predicted and the thermodynamic properties of the enantiomers and the racemate must be well understood to explain the dependence of the physico-chemical parameters on the membrane transport. Of the proposed mechanisms through which the chiral terpene enhancer D-LM may facilitate the enantioselective permeation of a chiral drug, such as TM, is the interaction with the drug and the chiral recognition sites of the membrane resulting in the alteration or disruption of the lipo-protein structure of the *stratum corneum*. Lipid-protein partitioning was assumed to be the mechanism which explains the action of the chiral terpenes (23). The current study showed that, as the enhancer concentration was increased from 0.25 to 1.5% v/v, the enantioselectivity in the TM transport increased significantly. However, this effect was relatively lower for higher concentrations (i.e., 1.0 and 1.5%) of D-LM. It is been suggested that with time and high concentrations of the enhancer in the donor vehicle, conformational changes might occur in the *stratum corneum* making the skin fully hydrated. This could lead to an increase in the fluidity of the lipid domain and the formation of new pores in the membrane leading to a decrease in the stereoselective nature of the ceramide moieties of the *stratum corneum*. This might result in the loss of the stereoselective interaction of enantiomers with highly fluidized ceramide moieties. Enantiomers would then simply be driven by the solvent flow.

A similar finding was also observed for the stereoselective interaction of propranolol enantiomers with the lipid membranes. The interaction was subtle and was seen only at low solute concentrations (32). One of the possibilities suggested for the non-stereoselective penetration of propranolol enantiomers at high solute concentration was the limited number of stereoselective sites available in the lipid membrane of the skin, so that enantioselective permeation may be possible only at low concentrations of the enhancer. Consistent with this earlier finding, there are two possible scenarios that could explain the effects of the D-LM on the TM transport shown in this study. First, increasing the concentrations of D-LM might result in either a change in the conformation of the ceramide moieties resulting in a decrease in the stereoselective interaction with the chiral enhancer. Second, the D-LM

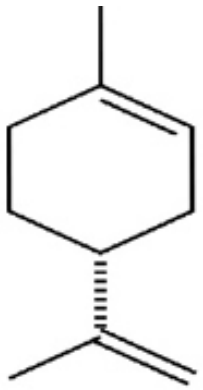

**Figure 6** Chemical Structure of D-LM

might induce a state of saturation of the available chiral recognition sites on the lipo-protein membrane (33). One or both of these scenarios could answer the question of why higher concentrations of D-LM show a decrease in the enantioselective permeation of TM, particularly in its racemate form. Theoretically, as seen in Figure (6), the presence of the double-bond in this cyclic terpene D-LM makes it more hydrophobic (log P 4.58 +/- 0.23) (34). Such moderate positive value of log P indicates that D-LM processes hydrophilic/lipophilic characteristics that are balanced with regard to its solubilizing capacity for hydrophilic molecules and its penetration effectiveness in the skin. This might explain the overall significant effect of the D-LM as a permeation enhancer. It was also observed that the action of the terpenes was transient and reversible. From these studies it could be inferred that if a hydrophilic drug was selected as the permeant, then extrinsic factors like physico-chemical properties of the chiral enhancer studied might play a role in promoting the transport of a hydrophilic chiral drug across a biomembrane.

## CONCLUSION

The influence and significance of stereochemistry aspects of a chiral drug such as TM is based on the understanding of the inherent ability of the *stratum corneum* components to discern between two different optical isomers intended for percutaneous absorption, resulting in differential diffusion rates (9, 35, 36). Hence it is important to find out whether these factors are extrinsic (i.e., related to the chirality of the drug molecule, enhancer and/or other excipients) or intrinsic (i.e., due to the chiral environment of the skin) or if both types of effects are involved in the enantioselective interaction of the components of the skin with the R- and/or S-isomers of TM. The net result would be a preferential permeation of one isomer. The rates of permeation of individual enantiomers and isomers of racemate were influenced by the presence of a chiral terpene enhancer D-LM. Finally, in pharmaceutical drug delivery, the relevant expansion of the specification of a chiral drug substance is its stereochemistry in terms of permeation across biological membranes, pharmacokinetic disposition, and enantiomeric stability, all of which need to be considered both *in vitro* and *in vivo*. The decision to develop a single enantiomer should be made only if it provides a genuine therapeutic benefit and is economically feasible. In the future, it is expected that drug stereoisomerism is likely to gain greater attention in clinical practice, research, and drug regulation.

## ACKNOWLEDGEMENTS

The author wishes to thank Merck Research Lab., Whitehouse, NJ for providing samples of both S-TM and R-TM for the use in non-clinical studies.

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