AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Enantioselectivity Transport of Timolol Maleate Through Hairless Mice Skin is A Single-Valued Function of the Concentrations of Chiral Terpene Enhancer(D-Limonene)

Mohsen I. Afouna*

1.Department of Pharmaceutics, College of Pharmacy, Al-Azhar University, Nasr City, Cairo, Egypt

ABSTRACT

The purposes of the present study were: 1).To study the relationship between different concentrations of the chiral enhancer D-Limonene (D-LM) on the solubility of individual enantiomer and racemate Timolol maleate (TM). 2). To study the preferential enhancement of D-LM upon S-TM, R-TM, and racemate across the hairless mice skin. For solubility studies, excess of R-, S-, or racemate with wide-range of different concentrations of D-LM were prepared. Samples were agitated, centrifuged and filtered and analyzed by HPLC using chiral column at 294nm. For skin transport studies, formulations containing 0.5% solutions of S-TM, R-TM, or racemate in buffer solution with predetermined concentrations of D-LM were studied. Samples of 1-ml were withdrawn and quantitatively analyzed for their TM contents. The steady-state fluxes (Jss), permeability coefficients and the enhancement factor were calculated. In solubility studies, D-LM significantly enhances solubility of all forms of TM in a concentration dependent manner. In permeation studies, presence of D-LM significantly enhances the flux values of both enantiomers and racemate. However, D-LM immensely increased all permeability characteristics of the S-isomer compared to those of R-isomer. Solubilities of all forms of TM were found to be a single-valued function of D-LM concentration. Moreover, addition of D-LM has enhanced the transport of S-, R-TM enantiomers and that of racemate across hairless mice skin. For all tested formulations, the overall permeability characteristics of the therapeutically active TM (i.e., S-TM) were superior results obtained with the R-TM either as enantiomer or racemate.

Keyword: Timolol, Terpene, Chiral, Enhancer, D-Limonene, Enantioselective

*Corresponding Author Email: mafouna@paharma.asu.edu.eg/mafonah@kau.edu.sa
Received 02 October 2013, Accepted 06 November 2013

Please cite this article in press as: Afouna MI. Enantioselectivity Transport of Timolol Maleate Through Hairless Mice Skin is A Single-Valued Function of the Concentrations of Chiral Terpene Enhancer (D-Limonene). American Journal of PharmTech Research 2013.

INTRODUCTION

Timolol maleate (TM) exists as two optically active isomers, S-TM and R-TM. TM has a molecular weight of 432.50. 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 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 was 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 S-enantiomer, with respect to β_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 eye. Yet it is only four-times less potent in reducing intraocular pressure in man[3]. Comparison on the basis of potency, selectivity for β_1- versus β_2- receptor subtypes, partial agonist properties, and non-specific membrane-stabilizing effects shows that TM has the greatest 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 potent than propranolol with respect to β-adrenoceptor blockade[5]. When administered orally, TM is well absorbed but it subjects to 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 β_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, was observed[7].

Enantiomers are identical in their physical and chemical properties but behave differently in a chiral environment such as a biological system or a chiral medium. This feature of enantiomers is unlike 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]. An equimolar 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 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 is 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 over 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 with enantiomeric drug substances[10]. Molecular chirality originates because of the existence of configurational isomers, termed enantiomers[11]. The sudden surge in interest in chirality is not only due to advancement in medical sciences per se, but also due to rapid progress in the techniques employed to separate 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 up to two- decades, quite a number of publications have focused on stereochemistry in drug action, metabolism, disposition, and bioequivalence[6,13,14]. Controversies arose as to whether a racemate or a single enantiomer needs to be exploited for therapy. Manipulation of enantiomeric ratio or use of only one enantiomer of a drug may allow minimization of toxicity and efficacy and this may lead to a significant increase in 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 to the market[17].

Substances that promote drug diffusion through the skin by overcoming the resistance offered by 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 investigated as safe, effective, and non-irritant skin penetration enhancers. The routes and mechanisms of enhancement effects of terpenes have been hypothesized using 5-FU as a model drug[21-23]), zidovudine[24], ketoprofen[25], diclofenac sodium[26], and indomethacin[27,28].

Percutaneous penetration involves dissolution of a drug in its vehicle, diffusion of solubilized drug from the vehicle to the surface of the skin, and penetration of the drug through the stratum corneum[29-32]. Several authors have proposed several mechanisms by which enhancers promote drug permeability including: ***(a)*** solvent action to directly solubilize the skin-tissue components, ***(b)*** interaction with intercellular lipids to disrupt the structural integrity of stratum corneum thus increasing the diffusivity through the membrane, ***(c)*** interaction with intracellular protein to promote permeation through corneocyte layer, and ***(d)*** increase in the partitioning of a drug into the membrane. Penetration enhancers exert their enhancing effect through one or more of the above mechanisms. The terpene enhancers studied earlier 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 a-pinene) were found to be poor accelerants with alcohols while ketones including menthone, carveol and carvone were found to be more effective[23]. In the current study, the solubility profile, enantioselectivity of TM transport individual isomers and racemate of TM were evaluated across hairless mice skin, which was shown to be a good model for human skin[15,33,34]

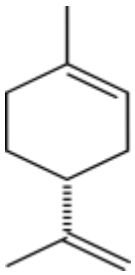

Figure 1: Chemical Structure of D-LM

MATERIALS AND METHODS

Drug and Chemicals

Both S-TM and R-TM were kindly provided by, Merck Research Lab., Whitehouse, NJ. D-LM, monosodium phosphate, disodium phosphate, triethanolamine and propylene glycol, were purchased from Sigma Aldrich Chemical Co., St. Louis. 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.

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 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

Hewlett Packard autosampler HPLC system with chime station, variable wave length UV detector was obtained from Ageilent Technology, SL, MI. HPLC chiral column-AGP 100 x 4.0 mm, 5µm, was obtained from ChromTech, ChromTech International AB, Hägersten, Sweden. Thermostatically controlled water bath, water bath shaker, sonicator, hot-plate/stirrer, pH meter, were obtained from Fisher Scientific Co., Fair Lawn, NJ. P1803. Diffusion cells are purchased from Crown Glass Company Inc. Somerville, NJ. Millipore filter paper, (0.45mm, HA), were purchased from Millipore corporation, Bedford, MA.

Preparation of Racemic TM

A physical mixture of TM was prepared by mixing equimolar ratios of S-TM and R-TM. Equal amounts (250-mg) of S-TM and R-TM were weighed and taken into a mortar. The two components were mixed well with a pestle. This physical mixture of racemate was used for permeation studies.

Solubility and *In Vitro* Permeation Studies

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 vials containing 1ml of the vehicle. The vials were agitated at 37 °C for 24 hours in a shaking water bath. The resulting suspensions were centrifuged and the supernatant fluids filtered through a 0.45mm filter. The filtrates were diluted and analyzed by HPLC. The experiment was performed in triplicate.

5% solutions of S-TM, R-TM, and racemate in a mixture of phosphate buffer pH 7.4 and ethanol (3:2) were prepared. 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 sacrificed by cervical dislocation and the abdominal skin was carefully excised immediately after sacrifice. The dermal side of the skin was cleaned of any adhering subcutaneous fat. The skin membranes were mounted on side-by-side diffusion cells in a manner where the stratum corneum facing the donor compartment. The cells were mounted in thermostatically controlled diffusion system and maintained at 37 ± 0.5°C. The two half-of each cell set were clamped tightly. The donor compartments were filled out with the donor phase of the test formulations and the plane vehicle was added into the receptor compartments using micropipette. A suitable Teflon coated magnetic bars were placed inside

both compartments. The sampling ports of each cell were capped to avoid evaporation of volatile vehicle and/or enhancers. The procedures were repeated in triplicate. Samples of 1ml were withdrawn from the receptor compartments at a 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 condition. 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 (if exists). Also, to check the solution stability during the time of experiments, random samples from the test formulations were collected in dark vials (to avoid exposure to light) and were analyzed by HPLC for their TM contents.

Calculation of the Permeability Parameters

The factors influencing the penetration of a drug into the skin include: concentration of the dissolved drug, (C_s) in the donor compartment, 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:

$$J = dM/S \times dt$$

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:

$$J = dM/S \times dt = D(C_1-C_2)/h$$

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 permeability coefficient can be determined employing the suitable mathematical treatment and Fick's law:

$$J = PSC_1$$

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 over the corresponding value for the control. The statistical analyses were done by t-test.

Assay Technique

Enantioselective HPLC procedures were employed for 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 0.45µm filter and degassed using a sonicator. The flow rate of the mobile phase was maintained at 1ml/min throughout the analysis procedures.

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 increases the solubilities of the individual R- and S-timolol maleate and in their racemic mixture as well. The solubility of TM enantiomers significantly ($p<0.01$) and preferentially increased upon addition of various concentrations of D-L. At all concentrations of D-LM, especially with higher concentrations (i.e., 0.75, 1.0, 1.5%) the test formulations containing S-TM exhibited much greater solubility parameters than that of the R-isomer irrespective 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 in accordance to the previously explained mathematical treatments and equations. The calculated average values of the studied permeation characteristics ± SD (n=3) are represented in Tables (1-3). Figures (2-6) show the cumulative amounts of drug penetrate 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 used vehicle without enhancer (i.e., concentration of D-LM was 0%) were served as controls.

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}) µg.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-TM$_{Control}$	0%	3.91±1.37	2.6	15.8 ± 0.4	1
R-TM1	0.25%	4.41±4.74	4.3	59.3 ± 2.4	1.13
R-TM2	0.5%	23.24±4.11	12.9	72.6 ± 4.5	5.94
R-TM3	0.75%	34.17±1.07	24.8	81.7 ± 5.9	8.74
R-TM4	1.0%	37.42±1.11	33.4	90.2 ± 6.1	9.57
R-TM5	1.5%	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}) µg.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-TM$_{Control}$	0%	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	0.5%	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	1.0%	98.26±11.61	88.3	163.6 ± 6.3	23.82
S-TM5	1.5%	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}) µg.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-TM$_{Control}$	0%	4.8±0.92	4.06±3.24	1.9	3.90	15.8 ± 1.1	13.20 ± 1.2	1	1
RS-TM1	0.25%	13.77±3.72	41.82±3.72	2.3	5.80	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	13.30	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	16.20	25.60
RS-TM5	1.5%	99.82±3.72	117.79±3.72	58.3	89.5	111.82±3.72	163.82±3.72	20.80	29.01

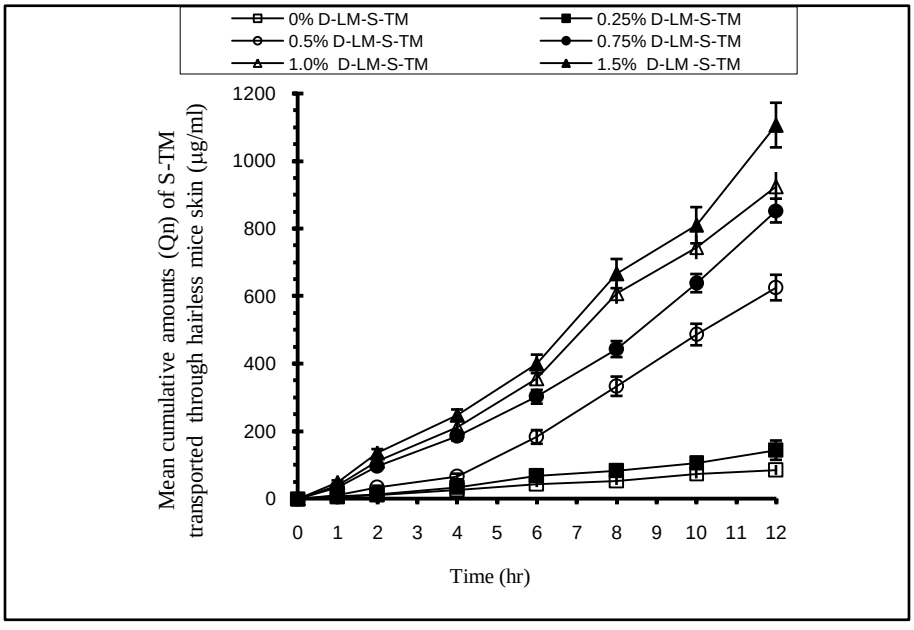

Figure 2: The mean cumulative amounts (±SD) of pure S-TM delivered from formulations containing different concentrations of D-LM through hairless mice skin into the receiver chamber of Franz diffusion cell as a function of time. (n=3).

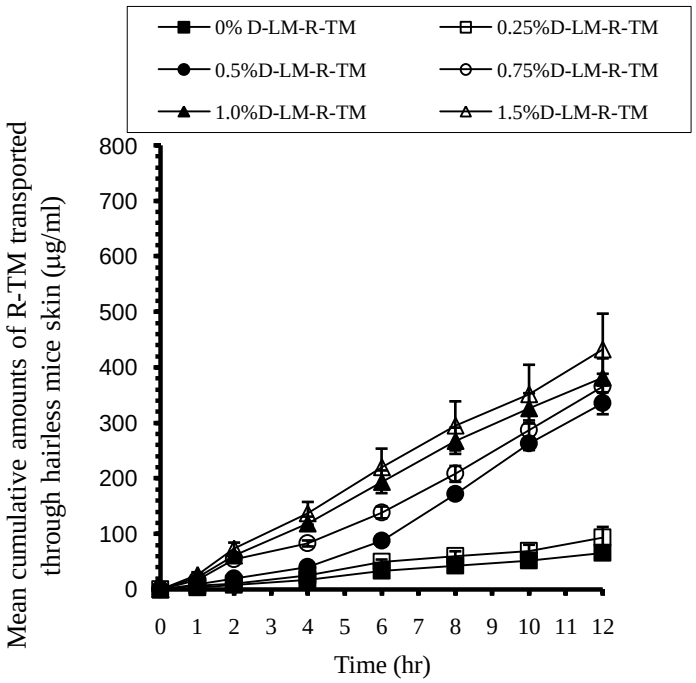

Figure 3: The mean cumulative amounts (±SD) of pure R-TM delivered from formulations containing different concentrations of D-LM through hairless mice skin into the receiver chamber of Franz diffusion cell as a function of time. (n=3).

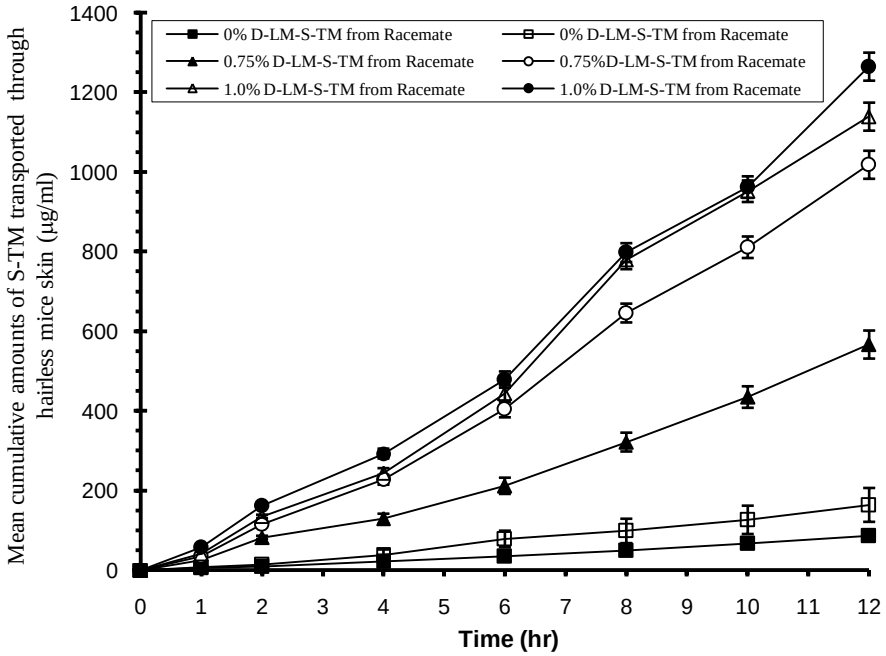

Figure 4: The mean cumulative amounts (±SD) of S-TM in racemate delivered from formulations containing different concentrations of D-LM through hairless mice skin into the receiver chamber of Franz diffusion cell as a function of time. (n=3, see text for details).

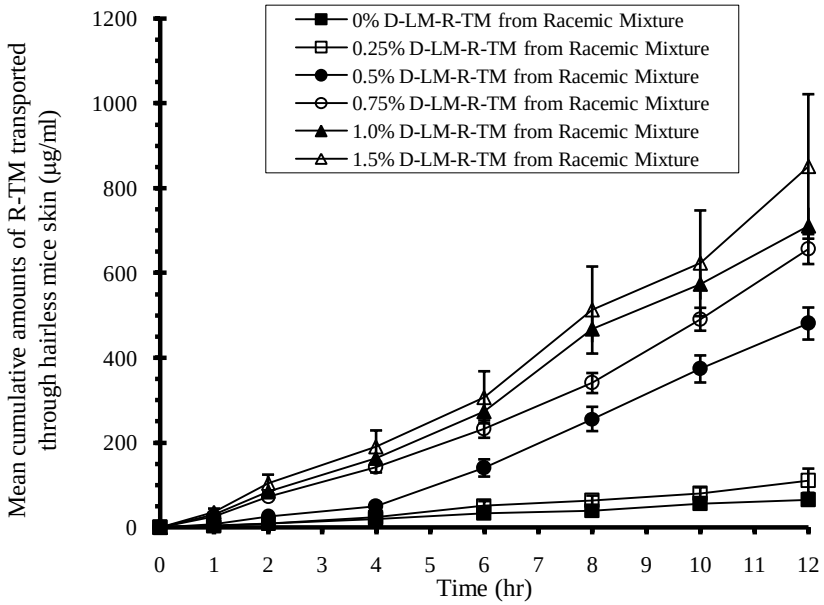

Figure 5: The mean cumulative amounts (±SD) of R-TM in racemate delivered from formulations containing different concentrations of D-LM through hairless mice skin into the receiver chamber of Franz diffusion cell as a function of time. (n=3, see text for details).

For the formulations of the S-TM enantiomer, the differences in steady-state fluxes and permeability coefficient values were 2.7- and 2.5-folds, respectively more than those of the formulations of the R-TM form. Figure 5 shows the relation between the D-LM and the EF values $(J_{ssTest}/J_{ssControl})$ for the test formulations. The EF values for R-TM isomer were ranged between 11- to-20-fold, and for S-TM isomer between 27- to-29-fold when compared to that of the control (0% D-LM) depending upon the concentration of the concentration of D-LM enhancer. For all TM enantiomers, the EF values for R-TM and S-TM were dependent upon 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 (0.8860, 0.8681, 0.9282, 0.9560) respectively. This good correlation clearly proves that the EF ratio is a single-valued function of the D-LM at the experimental conditions. Statistically, the permeability parameters of individual enantiomers of TM have shown significant difference ($p<0.01$) in the steady-state flux values. Nonetheless, all test formulations for the S-isomer has shown greater permeabilities than those of the R-isomer at all data-time points.

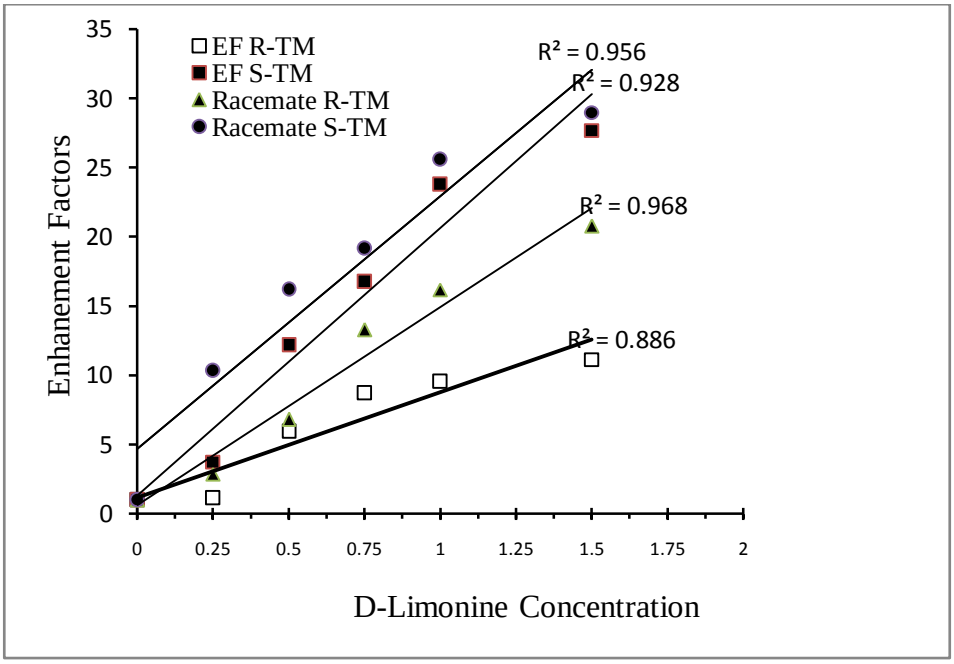

Figure 6: Correlation between the concentration of D-LM as a Chiral Enhancer and the Enhancement Factor Using Various Formulations of all TM Enantiomers

Transport studies of TM enantiomers and racemate across hairless mouse skin showed enantioselective permeation of R- and S-isomers, with S-isomer having greater permeation than R-isomer. Incorporation of chiral terpene enhancer D-LM resulted in significant increase in permeation of individual enantiomers. This might be of great clinical significance since it has been reported that R-enantiomer of TM is less potent than S-enantiomer with respect to β_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 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 assumed that stereospecific interaction of the components of skin with the isomers might result in preferential permeation of one isomer over the other. Extrinsic factors like differences in physico-chemical properties of enantiomers and racemate are also known to cause enantioselective permeation[20,25,30].

In the present investigation, increasing concentrations of D-LM led to increase in solubilities of individual isomers and racemate, which in turn increased the steady-state flux values. At 0.75% v/v D-LM, there was a difference in solubilities between the two isomers. The S-isomer has showed greater solubility than R-isomer that indicates a preferential solubilizing ability of the D-

LM on the two isomers. At this stage, as reported in earlier studies, the enantioselective permeation would depend on the thermodynamic activity as well as the solubility of the solutes in the donor vehicle[20,30,31]. Therefore, the influence of extrinsic factors thus cannot be predicted and thermodynamic properties of enantiomers and racemate need to be well understood in order to explain the dependence of membrane transport on physico-chemical parameters. Of the proposed mechanisms by which chiral terpene enhancer D-LM facilitated the enantioselective permeation of a chiral drug such as TM is by interacting with the drug as well as with the chiral recognition sites of the membrane resulting in alteration or disruption of the lipo-protein structure of stratum corneum. This was suggested in the lipid-protein partitioning theory set forth to establish the mechanism of action of chiral terpenes[23]. In the current study, as the enhancer concentration was increased from 0.25 to 1.5%v/v the enantioselectivity in the TM transport is significantly increased. However, this was relatively lower in case of higher concentrations (i.e., 1.0 and 1.5%) of D-LM. It is been proposed that with time and high concentrations of enhancer in the donor vehicle, conformational changes occur in the stratum corneum that make the skin fully hydrated. This would lead to an increase in fluidity of the lipid domain and 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 loss of stereoselective interaction of enantiomers with highly fluidized ceramide moieties. Enantiomers would then be simply driven by solvent flow.

Similar finding was also observed that stereoselective interaction of propranolol enantiomers with the lipid membranes was subtle and was seen only at low solute concentrations[32]. One of the possibilities put forth 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 faced only at low concentrations of the enhancer. Therefore, consistent with this earlier finding, there are two possible scenarios that explain such effect of D-LM. First, increasing the concentrations of D-LM might have led to either change in conformation of ceramide moieties leading to decrease in stereoselective interaction with the chiral enhancer. The second scenario is, 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 racemate. Theoretically, as can be seen in Figure, the presence of the double-bond in this cyclic terpene D-LM has increased the *log P* value. Such moderate positive value of *log P* indicates that D-LM

processes very reasonable hydrophilic/lipophilic characters. This might explain the overall substantial effect of D-LM as permeation enhancer. It was also observed that the action of 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 like TM is based on the previous understanding of the inherent ability of stratum corneum components to discern between any two optical isomers intended for percutaneous absorption, leading to differential diffusion rates[35]. 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 both types 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 over the other. The rates of permeation of individual enantiomers and isomers of racemate were influenced in the presence of 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 is made only if it gives genuine therapeutic benefit and becomes economically feasible. In the future, it appears that drug stereoisomerism is likely to be the focus of greater attention in clinical practice, research, and drug regulation.

ACKNOWLEDGEMENT

The author is very grateful to Merck Research Lab., Whitehouse, NJ for providing samples for non-clinical studies from both S-TM and R-TM.

REFERENCES

1. Vlasses PH, Ribeiro LG, Rotmensch HH, Bondi JV, Loper AE, Hichens M et al. Initial evaluation of transdermal timolol: Serum concentrations and β-blockade. J Cardiovas. Pharmacol. 1985;7(2):245-50.
2. Vliestra ER, McGoon MD. Beta-adrenergic blockers: choosing among them. Postgraduate Medicine. 1984;76(3):71-80.

3. Richards R, Tatterfield AE. Comparison of the airway response to eye drops of timolol and its isomer L-714,465 in asthmatic subjects. J Clin Pharmacol. 1987;24:485-91.
4. Frishman WH. Atenolol and timolol, two new systemic p-adrenoreceptor antagonists. The New Eng J Med. 1982;306(24):1456-61.
5. Singh BN, Williams FM, Whitlock RM, Collett J, Chew C. Plasma timolol levels and systolic time intervals. Clin Pharmacol Therap. 1980;28(2):159-66.
6. Nerurkar SG, Dighe SV, Williams RL. Bioequivalence of racemic drugs. Clin Pharmacol. 1992;32:935-43.
7. Russell GF, Hills JI. Odor differences between enantiomeric isomers. Science. 1971;172: 1043-44.
8. Drayer DE.. Pharmacodynamic and pharmacokinetic differences between drug enantiomers in humans: An overview. Clin Pharmacol Therap. 1986;40(2):125-33.
9. Ariens E J. Chirality in bioactive agents and its pitfalls. Trends in Pharmacol Sci. 1986;200-5.
10. Millership JS, Fitzpatrick A. Commonly used chiral drugs: A Survey. Chirality. 1993;5:573-6.
11. Eliel EL. Stereochemistry of Organic Compounds. New York: Wiley & Son, p. 1994;140-66.
12. Durrheim H, Flynn GL, Higuchi WI, Behl CR. Permeation of hairless mouse skin I: Experimental methods and comparison with human epidermal permeation by alkanols. J Pharm Sci. 1980;69(7):781-9.
13. Caldwell J. The importance of stereochemistry in drug action and disposition. Clin Pharmacol. 1992;32:5-929.
14. Wechter WJ. From controversy to resolution: Bioequivalency of racemic drugs-A symposium on the dynamics, kinetics, bioequivalency, and analytical aspects of stereochemistry. Clin Pharmacol. 1992;32:915-6.
15. Williams K, Lee E. Importance of drug enantiomers in clinical pharmacology. Drugs. 1985;30:333-54.
16. Afouna MI, Fincher TK, Khan M, A. Reddy IK. "Percutaneous permeation of optically active and racemic mixtures of chiral drugs and prediction of their flux ratios using thermal data: A pharmaceutical perspective", Chirality. 2003a;22(1,2):101-14.
17. Cayen MN. Racemic mixtures and single stereoisomers: Industrial concerns and issues in drug development. Chirality. 1993;3:94-8.

18. Pfister RW, Hsieh DST. Permeation enhancers compatible with transdermal drug delivery systems. Pharma Techn. 1990;132-40.
19. Touitou E, Chow DD, Lawter JR. Chiral β-blockers for transdermal delivery. Int. J. Pharm. 1994;104:19-28.
20. Afouna MI, Fincher TK, Zaghloul, AA. Reddy IK. Effect of azone upon the in vivo antiviral efficacy of cidofovir or acyclovir topical formulations against cutaneous hsv-1 infections and its correlation with skin target site free drug concentration using hairless mice. Int J Pharm, 2003b;253(1-2):159–68.
21. Cornwell AP, Barry BW. Sesquiterpene components of volatile oils as skin penetration enhancers for the hydrophilic permeant 5-fluorouracil. J Pharm Pharmacol. 1994;46(4):261-9.
22. Ghosh KP, Banga KA. Hydrogel-based iontotherapeutic delivery devices for transdermal delivery of peptide/protein drugs. Pharm Res. 1993;10(5):697-702.
23. Williams, C. A., and B. W. Barry. Terpenes and the lipid-protein partitioning theory of skin penetration enhancement. Pharm Res. 1991;8(1):17-24.
24. Kararli TT, Kirschhoff FC, Penzotti, Jr. SC. Enhancement of transdermal transport of azidothymiine (AZT) with novel terpene and terpene-like enhancers: in vivo-in vitro correlations. J Cont Rel. 1995;34:43-51.
25. Kommuru TR, Khan AM, Reddy IK. Racemate and enantiomers of ketoprofen: Phase diagram, thermodynamic studies, skin permeability, and use of chiral permeation enhancers. J Pharm Sci. 1998;87(7):833-40.
26. Arellano, A. Enhancing effect of terpenes on the in vitro percutaneous absorption of diclofenac sodium. Int J Pharm. 1996;130:141-5.
27. Takayama K, Nagai T. D-LM and related compounds as potential skin penetration promoters. Drug Devel Indus Pharm. 1994;20(4):677-84.
28. Martin A. Physical Pharmacy. 4ed. Williams and Wilkins, Waverly Co.; 1993, p.324-29.
29. Touitou E, Biana G, Kommuru TR, Afouna MI, Reddy IK., Skin Transport of Optically Active Stereoisomers, In: Chirality in Drug Design and Development, Reddy I. K. (Editor), Marcel Dekker, Inc., New York; (2004).
30. Afouna MI, Mehta SC, Ghanem A-H, Higuchi WI, Kern ER, DeClercq E et al. Influence of the treatment protocol upon the in-vivo efficacy of cidofovir (HPMPC) and acyclovir

(ACV) formulations in topical treatment of cutaneous HSV-1 infections in hairless mice, J Pharm Sci. 1999;88(5):530-34.

31. Barry BW. Mode of action of penetration enhancers in human skin. J Cont Rel. 1987;6:85-97.
32. Suedee R, Brain KR, Heard CM. Differential permeation of propranolol enantiomers across human skin in vitro from formulations containing an enantioselective excipient. Chirality. 1999;11(9):680-3.
33. Testa B. Mechanisms of chiral recognition in pharmacology. Acta Pharm. Nord. 1990;2(3):137-44.
34. Afouna MI, Mehta SC, Ghanem A-H, Higuchi WI, Kern ER, DeClercq E et al. Assessment of correlation between skin target site free drug concentration and the in-vivo topical efficacy in hairless mice for (E)-5-(2-Bromovinyl)-2-Deoxyuridine and acyclovir formulations. J Pharm Sci, 1998;87(8):917-21.
35. Heard CM, Brain KR. Does solute stereochemistry influence percutaneous penetration? Chirality. 1995;1:305-09.