European Journal of Pharmaceutics and Biopharmaceutics 72 (2009) 239–245

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Novel transdermal delivery of Timolol maleate using sugar esters: Preclinical and clinical studies

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ARTICLE INFO

Article history:
Received 20 August 2008
Accepted in revised form 5 December 2008
Available online 24 December 2008

Keywords:
Sugar esters
Transdermal delivery
Timolol maleate
Transepidermal water loss
Skin permeation

ABSTRACT

The feasibility of matrix controlled transdermal patch based on sugar fatty acid ester (SE) as penetration and absorption enhancer containing Timolol maleate (TM) was investigated. The influence of fatty acid type, chain length and hydrophile–lipophile balance (HLB) on the in vitro drug release as well as its permeation across hairless rat skin were studied and compared aiming to select a patch formula for clinical performance. Skin irritation induced by SE patch was evaluated by visual scoring, color reflectance measurements and non-invasive transepidermal water loss (TEWL) technique. The results indicated that among different SEs tried, laurate SE with shorter fatty acid chain length and higher HLB value significantly increased the amount of TM liberated from the patch (99 ± 2.1%) and its permeation across rat skin (86 ± 4.3%). The total drug permeation and flux values were approximately 5-fold greater compared to SE free patch. The extent of absorption of TM–SE patch expressed by AUC was 64% larger as compared to the oral solution with steady plasma concentration over 18 h and relative bioavailability (F_{rel}) of 163%. The developed patch was well tolerated by all the subjects with only moderate skin irritation, which was recovered in 24 h after patch removal. The results are very encouraging and offer an alternative approach to maintain higher, prolonged and controlled blood level profile of the drug over 18–24 h.

1. Introduction

Sugar esters (SEs) are non-ionic surfactants having a sugar substituent, sucrose, as the polar head group and fatty acids as non-polar groups. Properties of their hydrophilic and lipophilic balance (HLB) can be adjusted by varying fatty acid chain length (lauric, myristic, stearic, and oleic acid). They have HLB values from 1 to 16 depending on the type of fatty acid and the degree of esterification. Sucrose-based surfactants offer an attractive alternative to the generally more convenient ethylene oxide based non-ionic surfactant due to their low toxicity, biocompatibility, excellent biodegradability [1] and less dermatological damage [2]. The interest in using sugar ester in many areas is increased including pharmaceutical technology as emulsifiers, solubilizing agents, lubricants, penetrating enhancers and pore forming agents [1,3–8]. They can be applied in cosmetical applications [9] and as food additives as well [10].

Timolol maleate (TM) as a model drug (mol.wt. 332, log*p* 1.91, pK_a 9.2) is a non-selective beta-adrenergic blocking agent without membrane stabilizing or intrinsic sympathomimetic activities. TM is used in the management of hypertension, angina pectoris, myocardial infraction and glaucoma.

The main limitation of therapeutic effectiveness of TM is its short biological half-life, higher frequency of drug dosing, extensive first pass metabolism and poor bioavailability by oral route. It is rapidly absorbed from gastrointestinal tract with peak plasma concentration after 1–2 h., and metabolized up to 80% in liver with a half-life of 3–4 h. [11], thus necessitating frequent administration of larger doses (40–60 mg) daily to maintain therapeutic drug level. Therefore, the transdermal route is a better alternative to avoid hepatic first pass metabolism and to achieve constant plasma level over an extended period of time, which additionally warrants less frequent dose regime. Among different transdermal techniques investigated, iontophoretic delivery which is commonly used for the administration of ionic drugs through skin by the application of an electric current was extensively studied [12–16]. In the current work, a new transdermal matrix controlled strategy was adopted, in which TM and SEs as permeation and absorption enhancer were incorporated in a water insoluble but permeable polymer matrix without controlling the membrane. The TM liberation was controlled by diffusion through the matrix as the polymer cannot dissolve in the skin wrap. An example for an advanced transdermal system which is already on the market is Transtec® (35, 52.5 and 70 μg/h) transdermal patch containing 20, 30 and 40 mg buprenorphine incorporated into a polymer matrix which is able to control the drug delivery rate and produce stable plasma concentrations over a period of 96 h for the treatment of intermediate to severe pain [17].

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doi:10.1016/j.ejpb.2008.12.003

Thus, this study was designed to evaluate the suitability of different sugar fatty acid esters as permeation and absorption enhancer for transdermal matrix control delivery of TM. The influence of HLB of different SEs and their fatty acid type on the in vitro drug release and its permeation across hairless rat skin were also investigated. Skin irritation induced by SE patch was evaluated by visual scoring, color reflectance measurements and non-invasive transepidermal water loss (TEWL) technique. The second objective of this work was to select the best formulation for clinical study where in vivo TM plasma levels were measured after SE patch application.

2. Materials and methods

2.1. Materials

Timolol maleate was kindly provided by Epico Drug Company, Cairo, Egypt. Methacrylate ester copolymer (Eudragit® NE 30D) was purchased from Röhm Pharma GmbH, Germany. Different grades of sucrose esters; sucrose stearate S-1670 (HLB = 16), S-970 (HLB = 9), S-370 (HLB = 3), sucrose palmitate P-1670 (HLB = 15), sucrose myristate, M-1695 (HLB = 16), sucrose laurate L-1695 (HLB = 16) were purchased from Mitsubishi-Kagaku Foods Corporation, Japan. Disodium hydrogen phosphate and potassium dihydrogen phosphate were obtained from E. Merck, Darmstadt, Germany. All other reagents were of analytical reagent grade and were obtained from EL-Nasr Company, Cairo, Egypt.

2.2. Methods

2.2.1. Preparation of matrix controlled patches

TM matrix controlled patches were prepared using different SEs. Thirty micrograms of TM was dissolved in 5.0 ml distilled water. One hundred and fifty micrograms of different SEs was added and continuously mixed using magnetic stirrer (OFI Testing Equipment, Inc., model 152-45, Texas, USA) for 30 min at room temperature. Five percent of w/w aqueous polymeric dispersion (5.0 ml) diluted from Eudragit® NE 30D was added to the previous solution and the resulting dispersion was poured into a non-stick round container with a standard diameter of 55 mm to produce a thickness of about 0.15 ± 0.02 mm. Samples were dried at 25 °C, 50% RH for 48 h. Six patches, P1–P6, were prepared using L-1695, M-1695, P-1670, S-370, S-970 and S-1670, respectively. Drug-free patches (Pc_1) as well as SEs-free ones (Pc_2) were prepared as described above except that no drug or SEs were added, respectively, as control patches for comparative purposes in the release and skin permeation studies.

2.2.2. Dissolution studies

A USP dissolution tester (Hanson SR6, California, USA) was used to attain the dissolution profiles of TM-SEs different patches. The paddle over disk method was performed according to USP 29 apparatus 5. Five hundred milliliters of phosphate buffer (pH 5.5) was used as the dissolution medium. The release study was carried out at 32 ± 0.1 °C and the paddle rotation speed was adjusted to 50 rpm. Five milliliter samples were withdrawn periodically at predetermined time intervals of 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, and 5 h. Every withdrawal was followed by replacement with fresh medium to maintain a constant volume. The samples were analyzed spectrophotometrically (Shimadzu UV-1601 PC Double Beam, Kyoto, Japan) at 295 nm against the samples withdrawn at respective time interval from drug-free patches treated in a similar manner. The method was validated, the accuracy, repeatability (intra day and intermediate precision (inter day) and reliability were ensured. The recovery % was >98%. The dilution of the release medium due to replenishment following each aliquot withdrawal was taken into account in the calculation of the amount of TM released from the patch. The results were the mean values of three runs. The obtained release data were subjected to kinetic treatment according to zero, first and Higuchi diffusion models [18]. The correlation coefficient (r) and the order of release pattern were determined in each case.

2.2.3. In vitro skin permeation studies

Newly born Wistar albino rats (National Research Center, Dokki, Giza, Egypt) weighing between 80 ± 20 g were sacrificed and the full thickness skin, free of bites and scratches were excised. The study performed in this section was approved by the University Protection for Animal Care and Use Committee and the protocol was compliant with the "Principles of Laboratory Animal Care" [NIH Publication # 85-23, revised 1985]. The dermal surface was carefully cleaned to remove subcutaneous tissues without damaging the epidermal surface. When not used immediately, the skin was kept refrigerated (2–5 °C) and was used within 3 days. A Franz diffusion cell was first filled with 5 ml of phosphate buffer, pH 7.4, and the skin with a surface area of 3.14 cm^2 was placed across the ground glass joint with the stratum corneum facing the donor compartment. TM patch was mounted over the skin membrane and the Franz cell clamped together. The temperature of the receptor compartment was maintained at 37 ± 0.5 °C with an external constant temperature circulator water bath and the receiver medium was continuously stirred with a small magnetic bar in order to prevent any boundary layer effects. Control experiments (without patch) were carried out simultaneously to ensure the non-interference of skin leaching. At predetermined time intervals, samples (0.5 ml) were taken from the receptor compartment and the cell was refilled with an equivalent amount of fresh buffer solution. The samples were analyzed by HPLC method mentioned by Kubota et al. [19] and are described in Section 2.2.4. Each permeation experiment was replicated three times and from the concentration of TM in the receiving solution the amount permeated through the skin membrane was calculated. The cumulative amount of TM permeated into the receptor compartment was plotted against time to obtain a percentage permeation profile. The steady state flux, J_{ss} (μg/cm^2/h) was calculated from the linear portion of the plot of the cumulative amount permeated vs. time and expressed as

$$J_{ss} = Q/t = K_p C_{donor}$$

where Q is the amount of TM permeated through membrane in (μg/cm^2) in experimental time t in (h), C_{donor} is the concentration of TM in the donor chamber in (μg/cm^3) and K_p in (cm h^{-1}) is the permeability coefficient of TM through the membrane [20].

2.2.4. HPLC analysis of TM

The concentrations of TM were determined by HPLC assay. The system consisted of a solvent delivery system comprised a pump 600 E multi, C-18 reverse-phase micro-particulate μBondapack column, particle size 10 μm, 25 cm × 4.6 mm (Waters Corp., Milford, MA, USA). The mobile phase was acetonitrile–water–triethylamine (18:81:1, v/v/v) adjusted to pH 3.0 with phosphoric acid. The flow rate was 2 ml/min. Effluents were monitored at 295 nm using UV detector (Waters Model 2487, Milford, MA, USA).

2.2.5. Measurement of transepidermal water loss (TEWL)

TEWL as non-invasive technique has been used in relation to the assessment of either the irritation [21–23] or the effect of penetration enhancers [24,25]. Four healthy volunteers without visible skin abnormalities participated (2 women aged 22, 25 years and 2 men aged 23–28 years). All gave an informed consent. They were not allowed to use soap, moisturizers or any other cosmetics and creams on the lower mid volar arms 48 h prior to and during the

course of the experiments. The experimental protocol was approved by the Ethical Committee of Cairo University protection of Human Subjects. In all the experiments only the median part of volar forearm was chosen for the sites of application and TEWL measurements. For each subject, three sites were marked, site 1 as a treatment site while site 2 as a control site where SE free patch (Pc_2) was applied in order to evaluate the patch occlusive effect. Site 3 was left open without any treatment (untreated area). Six hours after application, the patch was removed and TEWL was measured at 1, 2, 4, 6, 12, 24 and 48 h after patch removal using evaporimeter (VapoMeter SWL2g; Delfin Technologies Ltd., Kuopio, Finland). The VapoMeter uses a closed chamber system, i.e. it computes a TEWL value ($g\,m^{-2}\,h^{-1}$) from the progressive increase in relative humidity inside the chamber within 9 s. For at least 20 min before the measurements, the volunteers relaxed with their sleeves rolled up in an air conditioned measurement room at a temperature of 20–22 °C and a relative humidity of 50–60%. TEWL values of treated sites were directly compared to those obtained on the untreated area (application site 3), which was measured at the same time on the same subject to obtain the individual enhancement ratio (ER), which further used to calculate the overall average ER and the standard deviation.

2.2.6. Skin irritation

Directly after the removal of the patch and 2, 6, 12 and 24 h later, the application sites were evaluated for their irritation degree by visual scoring by the same investigator using a modified method of Draize et al. [26]. The erythema scores were given from 0 to 4 depending on the degree of erythema as follows: no erythema 0, slight erythema (barely perceptible light pink) 1, moderate erythema (dark pink) 2, moderate to severe erythema (light red) 3, severe erythema (extreme redness) 4. After visual evaluation of skin irritation, skin color reflectance was measured with Minolta colorimeter (Chromameter CR 200, Osaka, Japan) which recorded color reflectance in three dimensional space $L^*a^*b^*$ [27,28]. The L^* value (luminance) expresses the relative brightness of the color total black ($L^* = 0$) to pure white ($L^* = 100$). a^* is the red-green axis + 100 expressing full red and − 100 full green. b^* is the yellow-blue axis. Theoretically, skin irritation should decrease L^* value and increase a^* value. A base line color analysis was performed for each subject before the application of the patch. Skin irritation induced by SE free patch (Pc2) was also measured and compared with SE containing one (P1).

2.2.7. In vivo absorption study

2.2.7.1. Study design. Based on the in vitro performance, one patch formulation (F1) was selected to be tested in vivo. The in vivo study was carried out to compare the pharmacokinetics of TM from F1 patch containing 30 mg TM (Treatment A) to an oral aqueous solution containing the same dose of TM (Treatment B) using a non-blind, two-treatment, two-period, randomized, crossover design. Twelve healthy non-smoking male volunteers (25–35 years, 64–75 kg, 165–182 cm in height) participated in the study and were randomly assigned to one of the two treatment groups of equal size. Each subject read, understood and signed an informed written consent and was well informed about the risks and objectives of the study. All the subjects showed no clinically relevant positive findings from standard physical examination at entry; normal clinical and laboratory tests within 24 h prior to the start of the study. No history of cardiovascular, renal or hepatic disease and no history of hypersensitivity, asthma, urticaria or other allergic symptoms. The study protocol was approved by Cairo University protection of Human Subjects Committee and the protocol complies with the declarations of Helsinki and Tokyo for humans. The study was performed on two phases. Phase I, half the number of volunteers received treatment A and the remainder received treatment B which is considered as a standard. A washout period of one week separated the phases. On the second phase, the reverse of randomization took place. In the transdermal absorption study, one TM patch was applied to the arm of each subject using a non-irritating adhesive tape. While for oral administration, aqueous solution of TM (30 mg in 5 ml water) was administered to subjects of group 2 fasted for at least 10 h before the study day [29]. Each group was supervised by a physician who was also responsible for their safety and collection of samples during the trial. At 8:00 a.m. the assigned treatment was given and no food was allowed for 4 h after dosing. Venous blood samples (5 ml) were withdrawn and were collected into heparinized tubes at the following time points: 0 (predose), 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 18, 24, 36, 48 and 72 h after the administration of a treatment. Plasma was obtained by centrifugation at 6000 rpm for 10 min (Centurion Scientific Ltd., West Sussex, UK). The plasma was pipetted into glass tubes and then frozen at 22 °C until ready to be analyzed using HPLC conditions described previously.

2.2.7.2. Standard solutions. Blank plasma samples (1 ml) were spiked with TM aqueous stock solution (10 μg/ml) to contain 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 μg/ml. Plasma samples were then mixed with 100 μl of 4 M sodium hydroxide and 5 ml of dichloromethane in a 10-ml glass centrifuge tube. The tubes were capped and shaken by hand for 10 s, followed by vortex-mixing for 30 s. After centrifugation for 5 min at 5000 rpm, the upper aqueous layer was discarded, and the organic layer was transferred to a small glass tube and evaporated to dryness with a gentle air stream generated from a pump at room temperature. The residue was reconstituted with 100 μl of mobile phase, and 50 μl of the resulting solution was injected onto the HPLC column [19]. A plasma sample, without the addition of TM, was also treated in the same way. Retention time of TM was about 3 min. A standard curve was constructed by plotting the peak area of TM against TM concentrations in plasma. All the assays were performed in triplicate and the percent drug recovery after plasma treatment was 94.27 ± 2.46. The lower limit of quantification was 0.02 μg/ml and a linear response across the full range of concentrations from 0.05 to 1.2 μg/ml ($r^2 = 0.992$) was obtained. The analysis of quality control samples showed a precision below 10% relative standard variation and accuracy below ±5% for intra-batch analysis.

2.2.7.3. Plasma analysis. The plasma obtained after receiving treatment A and treatment B was assayed as described above without the addition of TM. For low concentration plasma samples, the samples were spiked with the known concentrations of TM before analysis.

2.2.7.4. Pharmacokinetic analysis. Pharmacokinetic characteristics from plasma data following the two treatments were estimated for each subject by using a computer program, WinNonlin® (version 1.5, Scientific consulting, Inc., Cary, NC, USA). Non-compartmental analysis was used. C_{max} (ng/ml) and t_{max} (h) were the observed maximal drug concentration and its time, respectively. The area under the curve, $AUC_{(0-t)}$ (ng h/ml), was calculated using the trapezoidal rule from zero time to the last time of blood sample. The area under the curve from zero to infinity, $AUC_{(0-\infty)}$ (ng h/ml), was calculated as $AUC_{(0-\infty)} = AUC_{(0-t)} + C_t/k$, where C_t is the last measured concentration at the time t, and k is the terminal elimination rate constant estimated by log-linear regression analysis on data visually assessed to be a terminal log-linear phase. Apparent terminal elimination half-life ($t_{1/2}$) was calculated as $t_{1/2} = 0.693/k$. The relative bioavailability F for transdermal drug delivery was calculated by comparing the transdermal and oral AUCs.

2.2.8. Statistical analysis

The data obtained from different formulations were analyzed for statistical significance by one-way analysis of variance (ANOVA) followed by post hoc multiple comparisons using the least square difference (LSD). The analysis was performed on untransformed and log-transformed data for the pharmacokinetic parameters, C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ and untransformed data for $t_{1/2}$. Differences between series were considered to be significant at $p \leqslant 0.05$. For the pharmacokinetic parameters C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ and $t_{1/2}$, statistical analyses were performed using a two-sample t test assuming unequal variances wherein p values <0.05 were considered statistically significant. Statistical inferences were based on untransformed and log transformed values for the C_{max} and AUC parameters and observed values for $t_{1/2}$. All the statistical tests were performed using the software SPSS statistics program (version 14, SPSS Inc., Chicago, USA).

3. Results and discussion

3.1. Drug release from different SE patches

The general features of the release profile of TM from different SE patches showed an initial fast release phase followed by a slower release one (Fig. 1). The kinetic analysis of the release data followed diffusion controlled mechanism. The initial fast release phase was due to a release of the drug incorporated at or near the surface of the patch while the drug release in the second phase was regulated by diffusion through the swollen patch matrix. This profile was advantageous if we took into account the possibility of epidermis saturation with initial fast drug released in the first 10–15 min. As depicted, the incorporation of SEs resulted in remarkable increase in TM release from permeable patches compared to SE free one Pc_2. It was clear that not only the HLB value but also the type of fatty acid used as well as its chain C number greatly influenced the release pattern. In general, stearate-based SEs (P4, P5 with HLB of 3, 9, respectively) were less efficient than laurate (P1), Myristate (P2) and palmitate (P3) with HLB of 16, 16 and 15, respectively. Regardless of HLB value, significant increase ($p < 0.05$) in the percentage of drug liberation was observed with SEs of shorter fatty acid chains length having the same HLB value. This was evidenced by comparing percentages of drug released from stearate-based matrix P6, prepared with 18 c number fatty acid chain (70.7 ± 3.5%) with P1 and P2 prepared with 12 and 14 c number lauric and palmatic acids (99.7 ± 2.3 and 90 ± 1.9%), respectively. A possible explanation of these findings was related to the ability of shorter fatty acid chain to be built more completely into the polymeric chain, thus increasing the free volume holes in the polymer which in turn increased polymer permeability and consequently increase the drug release [30,4]. The improvement due to SEs incorporation was in the respective order L-1695 (P1) > M-1695 (P2) > P 1570 (P3) > S-1670 (P6) > S-970 (P5) > S-370 (P4). Therefore, based on drug release behavior TM patches P1, P2 and P3 were chosen to progress to skin permeability study.

3.2. In vitro skin permeation studies

Timolol is a weak base (pK_a 9.2) having intermediate lipophilicity ($\log p_{oct/buffer\ pH\ 7.4} = 1.9$) [31]. At pH range of 5.5–8.0, Timolol was predominantly in the ionized form and due to the polarity of these species most of the drug did not partition into the skin [32]. Though newly born rat skin was not a precise model for human skin for percutaneous absorption studies, it could be used to gain insight into the general pattern and mechanisms. The absence of hair on the skin would avoid introduction of artifacts due to the methods used for hair removal in regular rats. As depicted in Fig. 2, although skin had some control on transdermal TM delivery and despite the small percentage of TM in the non-ionized form (1.5% at pH 7.4), yet the extent of drug permeation through the skin from SE patches was still quite high. The skin slowed down TM release by approximately 13–18% only than its direct release to buffer solution in sink condition with a similar trend as in drug release study (shorter fatty acid SE enhances permeation better than longer fatty acid SE having a similar HLB). The calculated flux values of TM across rat skin were presented in Fig. 3 with laurate SE being the maximum (143.43 μg/cm^2/h) which was approximately 5-fold greater compared to SE free patch (Pc_2). In this sense, the author postulated that the overall flux was due to the permeation of both the ionized and unionized species. The permeability coefficient of the two species was estimated from the flux data using the approach of Hayashi et al. [33], where the effect of pH on the permeability of TM through the rat skin was measured, and the contribution of each species to the steady state skin permeation rate was estimated by determining the permeability coefficient of each species. The permeability coefficient of the ionized form resulted in 1.8×10^{-5} cm/s while the value for non-ionized was 6.4×10^{-9} cm/s. The latter is in good agreement with the permeability coefficient value (0.5×10^{-8} cm/s) predicted by Sutinen et al. [32]. This result indicates that the permeability of the two forms is in agreement with the pH partition theory where

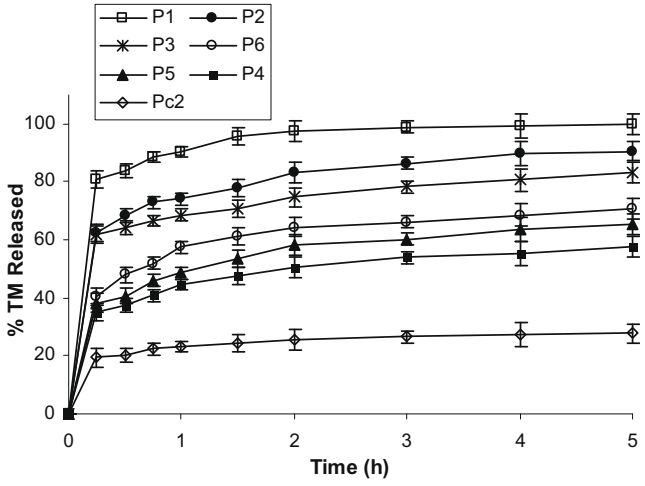

Fig. 1. In vitro drug release of Timolol from different SE patches in phosphate buffer (pH 5.5) at 32 ± 0.1 °C (mean + SD, $n = 3$).

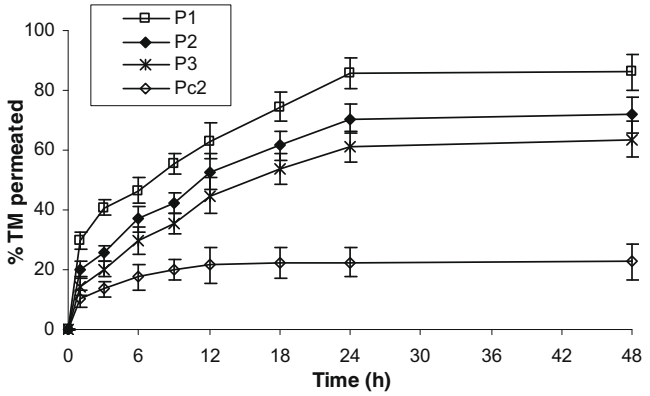

Fig. 2. Effect of skin on the in vitro release of Timolol from SE patches in phosphate buffer (pH 7.4) at 37 ± 0.5 °C (mean ± SD, $n = 3$).

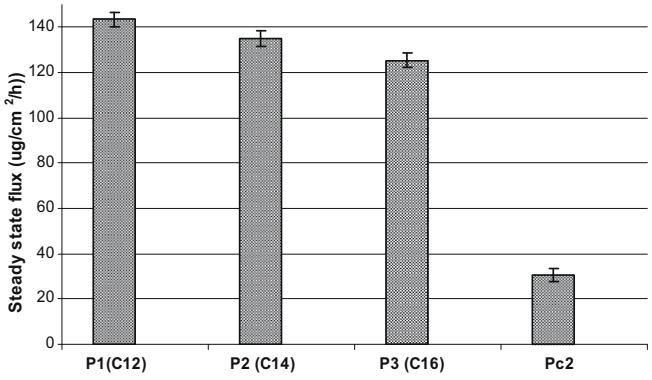

Fig. 3. Effect of SE carbon chain length on the steady state flux of Timolol in vitro release in phosphate buffer (pH 7.4) at 37 ± 0.5 °C across hairless rat skin (mean ± SD, $n = 3$).

at the pH value of the skin permeation experiment (7.4), the contribution of the ionized form prevailed over that of the unionized form owing to the pK_a value of TM (9.2).

The mechanism by which SEs increase skin permeability may involve disruption of the densely packed lipids that fill the extra cellular spaces of the stratum corneum. This was substantiated by the evidence that lauryl SE of 12 carbon chain lengths was the most effective one which corresponds to the chain length of the steroid nucleus of cholesterol, suggesting that these may act by disrupting ceramide–cholesterol or cholesterol–cholesterol interaction [34]. As the carbon chain length of the fatty acids increased, their molecular weights increased and their mobility within the skin layers was also expected to decrease and this may explain their lower permeation enhancement effect. Similar relationship between carbon length of fatty alcohols and permeation enhancement was also observed for melatonin [2] and theophylline [35]. Of the tested patches, laurate SE patch (P1) that achieved maximum permeation and flux enhancement was chosen for further studies.

3.3. TEWL and skin irritation

It is known that drug penetration across the skin increases with decreased barrier function, mostly due to the damage of stratum corneum. A high TEWL indicates defects in the barrier function of the stratum corneum. As the skin barrier function was believed to be primarily located in the intercellular domains [36], the lipid phase acts as a barrier against water loss. It was logical to correlate the increase in TEWL with the perturbation of the intercellular lipids of the skin by sugar esters as penetration enhancer. Fig. 4 shows the result of TEWL measurements on human skin after the application of SE patch represented as TEWL ERs. Control SE free patch (Pc_2) increased TEWL slightly while application of laurate SE patch (P1) led to steady significant increase in TEWL compared with the control up to 12 h with a considerable interindividual variation and then started decreasing. A clear relationship was found between TEWL and in vivo amount of drug penetrated through the skin.

The erythema scores upon exposure to SE patch (P1) have been presented in Fig. 5. No cutaneous reactions were evidenced by visual observation and SE patch was well tolerated by all the subjects. As the time progressed, the erythema level increased and the maximal irritation was graded as moderate (2) at 6 h. Within 24 h after the patch removal erythema decreased and the skin recovery took place. The erythema visual scoring level was supported by reflectance measurements where increased skin redness (high a^* value which has been demonstrated to be the most useful parameter in describing irritant dermatitis) and decreased skin brightness (low L^* value) were recorded up to 6 h after patch removal then the a^* value returned to the base line by 24 h [37,27,28]. It is not surprising that, SE free patch was as irritating as SE containing one (slight to moderate erythema with a^* value of 0.54 ± 0.21 at 6 h) if one consider the alkaline nature of the drug [38] and occlusion effect that has been shown to cause skin irritation [39]. In the previous studies, transdermal administration of Timolol was reported to cause minimal skin irritation in human [28,38,40] and in animals [41]. Based on our results, a clear correlation was found between TEWL measurements, skin irritation and amount of drug penetrated through the skin [2,25,42].

3.4. In vivo studies

The mean plasma concentration–time curves following the administration of TM transdermal patch and oral solution are shown in Fig. 6. Remarkable differences in the shape of the concentration–time courses between the two treatments were found, expressed by rapid sharp peak of TM absorption from the oral solution at 1 h followed by a fast decline in plasma drug levels. The value was in good agreement with those obtained by Sutinen et al. [28] and Ishizaki et al. [43] of 0.9 ± 0.3 and 1.5 ± 0.3 h, respectively. In the case of TM SE transdermal patch, the absorption was much slower and extended over a longer period of time. The patch delivered large fraction of its drug content during the first 12 h of the application period and produced relatively steady plasma concentration over 18 h. After 24 h, TM absorption slowed down and

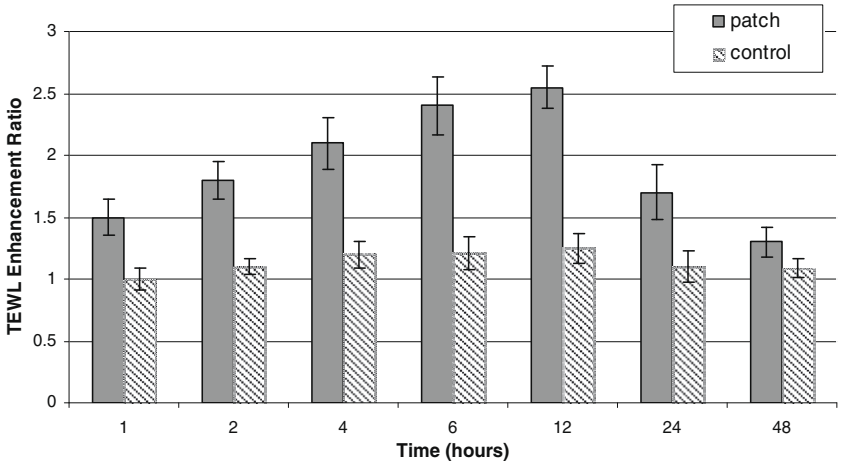

Fig. 4. Enhancement ratios of TEWL measurements after the application of P1 patch (mean ± SD, $n = 4$).

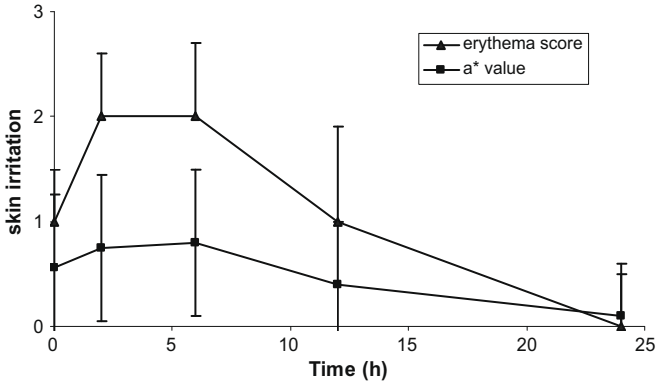

Fig. 5. Skin irritation induced by P1 SE patch (mean ± SD, $n = 4$).

its plasma concentration decreased. Moreover, the patch exhibited higher drug levels in the plasma from 8 to 24 h compared to the drug solution. The average C_{max} was significantly lower (20.2 ± 4.24 ng/ml) for the patch compared with the oral solution (125 ± 23.16 ng/ml) while the t_{max} was significantly higher ($p = 0.01$) in the case of the transdermal patch (12 h) compared with the oral solution (1 h). The extent of absorption of TM from the patch expressed by AUC_{0-t} was determined to be about 64% larger and statistically significantly different as compared to the oral solution.

The relative bioavailability (F_{rel}) of TM SE transdermal patch to the oral solution was estimated to be on average 163%. The elimination half-lives of TM after oral and transdermal administration were 1.5 ± 0.3 h and 16.3 ± 2.7 h, respectively, and were statistically significant ($p = 0.001$). Since these results were inconsistent with the pharmacokinetic theory in which absorption should not alter elimination, flip flop kinetics which referred to the situation in which the absorption half-life was much longer than the elimination half-life should be considered. The half-life of the decline of drug in the body from the patch now corresponds to the absorption half-life. Because the absorption was so slow such that much of the drug remained to be absorbed well beyond the peak time, the decay phase represented the drug being eliminated as fast as it was absorbed such that absorption is now the rate limiting step. Thus, the decay phase in this situation did not represent the elimination half-life of the drug but the rates absorption and elimination. Therefore, the elimination half-life for an absorption rate limiting situation cannot be estimated from this situation. The estimates of the mean untransformed pharmacokinetic parameters obtained by non-compartmental fitting of the concentration-time data from the biostudy of average parameters are given in table 1. The statistical analysis comparing the pharmacokinetic parameters between the two treatments is also summarized in Table 1 with p values.

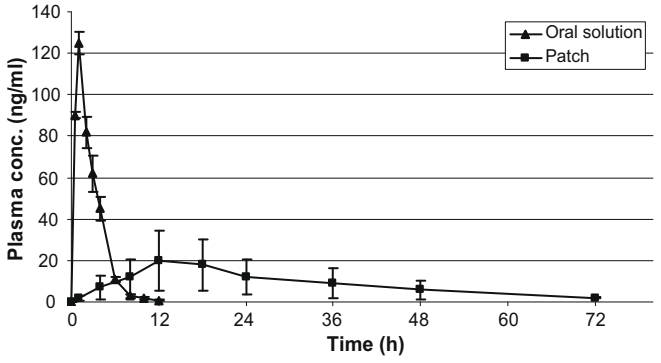

Fig. 6. Plasma concentration–time profile following administration of 30 mg Timolol from SE transdermal patch or oral solutions (mean ± SD, $n = 12$).

Table 1
Mean pharmacokinetic parameters of TM after the administration of 30 mg oral solution or transdermal patch to 12 volunteers.

Parameter	Oral	Patch	Statistical tests
C_{max} (ng/ml)	125.1 ± 23.16	20.22 ± 4.24	$p = 0.0001$
AUC_{0-t} (ng h/ml)	387.05 ± 62.3	632.5 ± 62.6	$p = 0.002$
$AUC_{0-\infty}$ (ng h/ml)	388.79 ± 51.29	679.7 ± 71.9	$p = 0.001$
t_{max}* (h)	1.0	12.0	$p = 0.0002$
$t_{1/2}$ (h)	1.5 ± 0.3	16.3 ± 2.7	$p = 0.0001$
MRT (h)	2.57 ± 0.7	26.9 ± 2.5	$p = 0.0001$
	% Bioavailability = 163%		

Data are mean values ($n = 12$) ± SD.
* Medians.

Table 2
Geometric mean and 95% confidence intervals (in parentheses) for log-transformed pharmacokinetic parameters of TM after administration of 30 mg oral solution or transdermal patch to 12 volunteers.

Parameter	Oral	Patch	Statistical tests
C_{max} (ng/ml)	4.78 ± 0.12 (4.61–4.96)	2.95 ± 0.13 (2.77–3.12)	$p = 0.0001$
AUC_{0-t} (ng h/ml)	5.63 ± 0.26 (5.52–6.13)	6.14 ± 0.15 (6.14–6.75)	$p = 0.014$
	% Bioavailability = 166%		

With regard to the extent parameters for TM from the two treatments, the ANOVA model for log-transformed data indicated significant treatment effects for C_{max} and $AUC_{(0-t)}$ with $p = 0.0001$ and $p = 0.014$, respectively. The ANOVA model also indicated no significant sequence effect. The estimates of the geometric mean of C_{max} and $AUC_{(0-t)}$ parameters along with the 95% CI are presented in Table 2. The bioavailability of TM from transdermal patch determined from log-transformed data was about 166% relative to the oral solution. Due to the small variability encountered in the study both untransformed and log-transformed analyses for C_{max} and $AUC_{(0-t)}$ gave similar results and conclusions.

Considering that the bioavailability of TM from the transdermal patch was increased with minimal cutaneous reaction, a lower dose of TM might be used in TM therapy which could reduce the various side effects associated with the usual higher clinical dose of the drug. Further, delayed t_{max} and maintenance of higher TM plasma levels (compared to effective topical ocular dose for adequate reduction of IOP which is one drop of 0.5% TM solution twice daily to obtain plasma concentration of 0.35 ng/ml) over 12–24 h as well as less blood levels fluctuation (i.e., the absence of sharp peak and sharp fall in blood levels) from the transdermal patch compared to drug solution, clearly indicated that the developed transdermal dosage form not only improved the bioavailability of TM, but also gave prolonged and controlled blood level profile of the drug.

In conclusion, the data reported here opened the door for future use of sugar esters as promising absorption and penetration enhancer in transdermal drug delivery. On the basis of in vitro, skin permeation and clinical results, TM-laurate SE patch containing 1.5% SE and 5% Eudragit NE 30D was successfully used as controlled transdermal TM delivery, where TM steady state plasma level was achieved with improved bioavailability and negligible skin irritation.

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