Studies on the influence of penetration enhancers on \textit{in vitro} permeation of carvedilol across rat abdominal skin

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Abstract

The aim of the investigation was to study the effect of penetration enhancers on the \textit{in vitro} permeation of carvedilol across excised rat abdominal skin and to select suitable penetration enhancer. Terpenes menthol, camphor, \textit{d}-limonene and carvone; surfactants, Transcutol and Labrasol at 5 % w/v, were used as penetration enhancers in the study. Skin permeation studies were conducted in Franz diffusion cells using excised rat abdominal skin. Solutions containing 5 % w/v camphor showed maximum permeation (451.20 µg?) in 24 hr with a flux of 5.23 µg/hr/cm² and was significantly different (p<0.05) to flux obtained with other permeation enhancers.

Control (phosphate buffer saline, pH 7.4 containing 40 % v/v polyethylene glycol) sample showed lowest permeation (59.18 µg?), with a flux of 0.67 µg/hr/cm². The flux of carvedilol obtained from the solutions containing camphor, Transcutol, \textit{d}-limonene, carvone, Labrasol and menthol (5 % w/v) were 7.81, 7.26, 6.52, 5.91, 4.21 and 2.28 times higher than that observed with control, respectively. The flux obtained with camphor was significantly higher (p<0.05) than the fluxes obtained with other penetration enhancers. The present study suggests that camphor, Transcutol and \textit{d}-limonene at 5 % w/v level may be used as penetration enhancers in the development of transdermal drug delivery systems.

Key words

Carvedilol, Terpenes, Transcutol, Labrasol, Rat skin.

Introduction

Carvedilol is a non-selective \textit{β}-adrenergic antagonist widely used in the treatment of mild to moderate essential hypertension and stable angina pectoris. It also possesses antioxidant and antiproliferative effects. That may enhance its ability to combat the deleterious effects of sympathetic nervous system activities in heart failure (1). It is well absorbed followed by oral administration. The systemic availability is approximately 25-35 % because of high first pass metabolism (2). To reduce its high first pass metabolism and enhance its bioavailability other routes of administration such as buccal (3), have been reported. The biological properties of carvedilol such as high first pass metabolism, low dose, need for long term treatment and repetitive dosing make this drug an interesting candidate for transdermal administration.

The transdermal route of administration has been recognized as one of the potential route for the local and systemic delivery of drugs. The advantages of transdermal delivery, include therapeutic benefits such as sustained delivery of drugs to provide a steady plasma profile,
particularly for drugs with short half lives and hence reduced systemic side effects; reducing the typical dosing schedule to once daily or even once weekly hence generating the potential for improved patient compliance and avoidance of the first pass metabolism effect for drugs with poor bioavailability (4). However, the highly organized structure of stratum corneum forms an effective barrier to the permeation of drugs, which must be modified if poorly penetrating drugs are to be administered. The use of chemical penetration enhancers would significantly increase the number of drug molecules suitable for transdermal delivery (5). Terpenes present in naturally occurring volatile oils appear to be clinically acceptable enhancers (6). Moreover, a wide variety of terpenes have been shown to increase the percutaneous absorption of number of drugs (7). In this investigation, the penetration enhancers camphor, carvone, menthol, d-limonene, Transcutol and Labrasol at 5 % w/v concentration were used. Excised rat abdominal skin was used for in vitro permeation studies. Previous studies, reported carvedilol transdermal therapeutic systems based on polymethacrylates (8) and membrane controlled matrix type patches using non ionic surfactants as penetration enhancers (9). The objective of present study was to investigate the effect of penetration enhancers on the permeation of carvedilol across rat abdominal skin and to select suitable penetration enhancer(s).

Materials and methods

Materials

Carvedilol was provided by Sun Pharmaceuticals, India. D-limonene, carvone menthol and poly ethylene glycol 400 (PEG 400) were purchased from Merck, India. Camphor was purchased from Sd fine chemicals India. Labrasol (PEG-8 caprylate/caprate), Transcutol (Diethylenglycol monoethyl ether) were gifts from Gattefosse (Cedex, France). All other chemicals and reagents used are of analytical grade.

Preparation of Rat Abdominal Skin

Albino rats weighing 150-200 gm were selected for permeation studies and the study was conducted with the approval of institutional ethical committee. The animals were sacrificed using anesthetic ether, hair of test animals was carefully trimmed short (<2 mm) with a pair of scissors and the full thickness skin was removed from the abdominal region. The epidermis was prepared surgically by heat separation technique (10), which involved soaking the entire abdominal skin in water at 60° C for 45 sec, followed by careful removal of the epidermis. The epidermis was washed with water and used for ex vivo permeability studies. The thickness of the skin was measured with digital micrometer (Mitotoyo, Japan).

In vitro permeability studies

Franz diffusion cell with a surface area of 3.56 cm² was used for in vitro permeation studies. Rat abdominal skin with a thickness of about 1.0 mm was mounted between the compartments of the diffusion cell with stratum corneum facing the donor compartment. The receiver phase is 12 ml of phosphate buffer saline (PBS) pH 7.4 containing 40 % v/v of PEG 400, stirred at 500 rpm on a magnetic stirrer; the whole assembly was kept at 37 ± 0.5° C. PBS (pH 7.4), containing 40 % v/v PEG 400 and 3 mg of carvedilol (3 mL) was placed in the donor compartment. Carvedilol is practically insoluble in water hence a buffer PEG 400 system was used for solubilizing carvedilol. All individual solutions in donor compartment were prepared separately with and without (5% w/v) penetration enhancers. Menthol, camphor, Transcutol, Labrasol, d-limonene and carvone as penetration enhancers and PBS pH 7.4 containing 40 % v/v PEG 400
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as control were used in the study. The entire setup was placed over magnetic stirrer and temperature was maintained at about 37 ± 0.5°C by placing the diffusion cell in a water bath. The amount of drug permeated was determined by removing 1 mL of sample at appropriate time intervals up to 24 hr, the volume was replenished with an equal volume of PBS pH 7.4 containing 40 % v/v PEG 400. The drug content in the samples was determined by high performance liquid chromatography (HPLC) and the concentration was corrected for sampling effects according to the equation (11).

\[
C_n^1 = C_n (V_t/V_r - V_s) (C_{n-1}^1 / C_{n-1})
\]

Where \( C_n^1 \) is the corrected concentration of the \( n \)th sample, \( C_n \) is the measured concentration of carvedilol in the \( n \)th sample, \( C_{n-1}^1 \) is the measured concentration of the carvedilol in the \( (n - 1) \)th sample, \( V_t \) is the total volume of the receiver fluid and \( V_s \) is the volume of the sample drawn.

**Estimation of drug content in the sample by HPLC method**

The HPLC system (Shimadzu, Japan) consisted of a LC–10AT solvent module, and a model UV-Visible Spectrophotometric detector (SPD–10A) with LC 10 soft ware. Carvedilol was quantified according to a reported method (12). The column used was a Kromasil KR 100-5C8 (25 X 4.6 mm i.d, 5 µ). The mobile phase consisted of acetonitrile, 15 mM orthophosphoric acid (37:63), and 0.25 v/v % triethylamine mixture, and was adjusted to pH 2.5 with orthophosphoric acid. The elute was monitored at 238 nm with a flow rate of 1 mL/min. The sensitivity was set to 0.005 AUFS.

**Data analysis**

As described by Barry (13), the steady state flux (\( J_s \)), lag time (\( T_L \)), diffusion coefficient (\( D \)) and apparent permeation coefficient (\( P_{app} \)) are defined by

\[
J_s = \frac{(dQ/dt)_{ss}}{A} - (1)
\]

\[
D = \frac{h^2}{6T_L} - (2)
\]

\[
P = \frac{dQ}{Dt} \frac{1}{A} \frac{1}{C} - (3)
\]

Where, \( A \) is the effective diffusion area; \( h \), the thickness of skin; \( C_s \), the concentration in the saturated solution and \( (dQ/dt)_n \) is the steady state slope.

**Statistical Analysis**

The \( Q_{24} \) (cumulative amount permeated in 24 hr) and flux values obtained from the various systems were tested for significant differences using a one-way analysis of variance (ANOVA) or unpaired t test. If the significant differences exist when ANOVA was used, the pair wise comparison of different systems was done to find out statistical significant difference in parameters using a Dunnet’s test. When the normality test failed, Kruskal-Wallis one-way ANOVA was used to find out if the significant differences exist between different systems. The statistical analysis was conducted using SigmaStat software version 1.0 (Jandel Corp., California).

**Results and Discussion**

**Effect of Penetration Enhancers**

The effect of enhancers on permeation of a drug usually depends upon physicochemical characteristics of both permeant as well as enhancer molecule. Among enhancers, various terpenes have been widely used for transdermal delivery of compounds (13). The effects of various penetration enhancers on the percutaneous penetration profile of carvedilol was shown in Table 1, Figs. 1 and 2. The thickness of isolated skin was found to be in between from 844 to 1234 microns.

Solutions containing 5 % w/v camphor showed maximum permeation of 451.20 µg in 24 hr with
a flux of 5.23 µg/hr/cm², was significantly higher (p<0.05) than the amount of carvedilol permeated by other permeation enhancers used in the study. Control sample showed lowest amount of permeation, 59.18 µg in 24 hr with a flux of 0.67 µg/hr/cm². Maximum flux (5.23 µg/hr/cm²) obtained with camphor at 5 % w/v, was significantly higher (p<0.05) than the flux obtained with other penetration enhancers, but was not significantly different (p<0.05) from the flux obtained with Transcutol. Transcutol (4.87 µg/hr/cm²) and d-limonene (4.37 µg/hr/cm²) showed similar flux values with lag time of 0.30 hr and 0.40 hr respectively.

The comparison of carvedilol flux obtained from drug solutions containing 5 % w/v penetration enhancer through excised rat skin was shown in Fig 3. The effect of the various enhancers on the flux of carvedilol followed the order: Camphor > Transcutol > d-limonene > Carvone > Labrasol > Menthol > Control. The flux of carvedilol obtained from the solutions containing camphor, Transcutol, d-limonene, carvone, Labrasol and menthol (5 % w/v) were 7.81, 7.26, 6.52, 5.91, 4.21 and 2.28 times higher than that observed with control, respectively. The main findings of the study are the accelerants enhance diffusion or partition and thus permeation of drugs. The lipid partitioning theory was proposed by Barry to describe the mechanism of action of permeation enhancers (14), whether by (i) disruption of the highly ordered structure of SC lipids, (ii) interactions with intracellular proteins or (iii) improvement in partitioning of the drug, co enhancers or co solvent in to the stratum corneum.

In this study, we found that diffusion of carvedilol through the skin was increased by terpenes. It is reported that terpenes enhance diffusion of drugs by extracting lipids from stratum corneum (15, 16), it results in reorganization of lipid domain and barrier disruption (17, 18). The mechanism of barrier disruption may be due to the competitive hydrogen bonding of oxygen-containing monoterpenes with ceramide head groups, thereby breaking the interlamellar hydrogen bonding network of lipid bilayer of stratum corneum and new polar pathways or channels are formed (19, 20). DSC proved the barrier disruptive action of terpenes, where there was a shift in endothermic transition temperature of stratum corneum lipids after treatment with terpenes (21). The other possible mechanism of action may be the lipid fluidizing activity of terpenes containing essential oils. Fourier transform infrared (FTIR) studies proved the lipid extractive action of terpenes from stratum corneum, where there was a decrease in heights and areas of both symmetric and asymmetric CH₂ stretching absorbance peaks of stratum corneum lipids (16, 22). Surfactants, Transcutol and Labrasol change lipid chain fluidity of the SC and improve drug partition (23).

**Conclusion**

The present study suggests that camphor, Transcutol and d-limonene at 5 % w/v concentration may be used as penetration enhancers, for the transdermal delivery of carvedilol. The data obtained in the present study using rat abdominal skin cannot be translated to *in vivo* delivery in humans as other factors such as cutaneous microvasculature, which prevents the accumulation of the drug in the skin and the cutaneous metabolism of the drug which significantly alter the permeation profile of the drug. The results obtained from this study will be helpful in the development of transdermal drug delivery systems. Further work is recommended to evaluate the optimum concentration of camphor, Transcutol and d-limonene to meet the target flux.

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References
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Table 1: Permeation parameters of Carvedilol through excised rat abdominal skin from PBS pH 7.4 containing 40 % v/v of PEG 400 and 5 % w/v of Penetration enhancer

<table>
<thead>
<tr>
<th>Penetration enhancer</th>
<th>$Q_{24}^*$ (µg/cm²/hr)</th>
<th>$J_s^b$ (µg/cm²/hr)</th>
<th>$P_{app}^c$ (cm/hr)</th>
<th>$T_L^d$ (hrs)</th>
<th>$D^e$ (cm²/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>59.18 ± 13.30</td>
<td>0.67 ± 0.095</td>
<td>0.22 ± 0.013</td>
<td>0.50 ± 0.042</td>
<td>0.13 ± 0.045</td>
</tr>
<tr>
<td>Menthol</td>
<td>135.06 ± 35.84</td>
<td>1.53 ± 0.125</td>
<td>0.51 ± 0.029</td>
<td>0.31 ± 0.029</td>
<td>0.08 ± 0.009</td>
</tr>
<tr>
<td>Camphor</td>
<td>451.20 ± 70.57</td>
<td>5.23 ± 0.331</td>
<td>1.87 ± 0.011</td>
<td>0.41 ± 0.034</td>
<td>0.11 ± 0.031</td>
</tr>
<tr>
<td>D-limonene</td>
<td>353.49 ± 12.52</td>
<td>4.37 ± 0.403</td>
<td>1.45 ± 0.014</td>
<td>0.30 ± 0.014</td>
<td>0.08 ± 0.002</td>
</tr>
<tr>
<td>Carvone</td>
<td>324.13 ± 27.32</td>
<td>3.96 ± 0.328</td>
<td>1.32 ± 0.021</td>
<td>0.35 ± 0.023</td>
<td>0.09 ± 0.006</td>
</tr>
<tr>
<td>Transcutol</td>
<td>384.18 ± 45.97</td>
<td>4.87 ± 0.571</td>
<td>1.62 ± 0.019</td>
<td>0.40 ± 0.032</td>
<td>0.10 ± 0.010</td>
</tr>
<tr>
<td>Labrasol</td>
<td>222.60 ± 45.21</td>
<td>2.82 ± 0.177</td>
<td>0.93 ± 0.059</td>
<td>0.34 ± 0.016</td>
<td>0.09 ± 0.003</td>
</tr>
</tbody>
</table>

$^a$ Cumulative amount (µg) of drug permeated per cm², results are mean ± SD (n=3)

$^b$ $J_s$ Transdermal flux, values represent mean ± SD (n=3).

$^c$ $P_{app}$ Permeability Coefficient, values represent mean ± SD (n=3).

$^d$ $T_L$ Lag Time, values represent mean ± SD (n=3).

$^e$ $D$ Diffusion Coefficient, values represent mean ± SD (n=3).
Fig 1: Effect of terpenes as penetration enhancers on in vitro permeation of carvedilol through rat abdominal skin, values represent mean ± S.D (n=3)

Fig 2: Effect of surfactants as penetration enhancers on in vitro permeation of carvedilol through rat abdominal skin, values represent mean ± S.D (n=3)
Fig 3: Comparison of carvedilol flux obtained from drug solutions containing 5% w/v penetration enhancer through excised rat skin, values represent mean ± S.D (n=3)