Enhancer aided in vitro permeation of atenolol and prazosin hydrochloride through mice skin

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Effect of penetration enhancers were studied on the permeation of antihypertensive drugs prazosin hydrochloride and atenolol through full thickness skin of Swiss albino mice. Atenolol was delivered to skin from saturated alcoholic solution containing 5% of 1-decanol and alcohol alone, while prazosin hydrochloride was saturated in dimethyl formamide (DMF; 5% w/v in water) and dimethyl sulfoxide (DMSO; 5% w/v in water). Atenolol permeation was augmented significantly in decanolic solution and also in pure alcohol. In case of prazosin hydrochloride, significant enhancement of permeation was shown by DMSO but not by DMF.

Materials and Methods

Prazosin hydrochloride (Sun Pharmaceutical Industries, Gujarat) and atenolol (Parke-Davies, Hyderabad) were received as gift samples. Chemicals used include ethanol (Qualigens, Mumbai), octanol (Rolex Laboratories, Mumbai), physiological saline (Core Healthcare, Gujarat), 1-decanol (Merck-Schuchardt, Hohenbrunn), dimethyl formamide (Central Drug House, Mumbai) and dimethyl sulfoxide (Central Drug House, Mumbai). Swiss albino mice (Mus musculus) were purchased from National Institute of Nutrition, Hyderabad.

Solubility measurements—Solubility determination was carried out by the method of Okumara et al.30. An excess amount of the drug was taken and dissolved in measured amount of distilled water in a glass vial to get a saturated solution. The system was kept at rest for 24 hr at 37°C to assist the attainment of equilibrium. The supernatant was filtered and assayed spectrophotometrically (Hitachi U-2000, Japan) at the suitable wavelength.

Partition coefficient determination—Octanol-water partition coefficient of the drugs was determined by the method described by Wells11. Octanol (10 mL) was added to equal volume of aqueous solution of the drug (known concentration) in a separating funnel. The system was kept for 24 hr at 37°C with intermittent shaking. Finally, the aqueous layer was separated, clarified by centrifugation at 100 g and assayed.

Skin permeation experiments—in vitro diffusion experiment of the drugs was carried out on excised abdominal mice skin. Removal of the surface hair was not attempted to avoid the accidental scratching of stratum corneum. Vertical type diffusion cell (Neutron Scientific, Calcutta) having a down stream volume of 20 mL was used. The excised skin was mounted on diffusion cell and receiver compartment was filled with 20 mL of normal saline containing 0.016% gentamicin sulphate to preserve the skin from deterioration. Aqueous drug suspension (3 mL) was placed in the donor compartment to maintain chemi-
cal potential. Temperature was maintained at 37°C. The sample solution was withdrawn at regular interval and assayed after suitable dilution using the standard curves. For prazosin hydrochloride standard curve was drawn at 246 nm (Sensitivity 0.1 meg) and atenolol was measured at 266 nm (Sensitivity 0.1 meg).

**Target flux calculation**—Target flux of the drugs was calculated using the available pharmacokinetic data\textsuperscript{12-15} utilizing the formula of Kim and Chien\textsuperscript{14} (\(F = \frac{C_{ss}}{C_{v}}\) / A; where A,F and BW are the surface area of transdermal delivery device, the desired steady-state permeation rate through skin and body weight of the subject respectively. C_{t} and C_{ss} are the total donor concentration and steady-state plasma concentration respectively]. Target permeation rate was calculated assuming the surface area of transdermal delivery device as 10 cm\(^2\) and body weight of the patient as 60 kg.

**Data analysis**—Cumulative amount of drugs permeated per unit skin surface area was plotted against time and the slope of the linear portion of the plot was estimated as the steady-state flux (J_{ss})\textsuperscript{15}. Permeability coefficient (Kp) was calculated [Kp = J_{ss} / C_{v}; where C_{v} is the total donor concentration of the solution].

Statistical analysis was performed by Student's t-test.

### Results and Discussion

Physicochemical parameters of the candidate drugs have been shown in Table 1. Overall solubility of the drug, prazosin hydrochloride, was also determined in DMF-water and DMSO-water system and the values were found to be 1.23 and 1.03 mg/mL respectively. Atenolol showed higher solubility both in pure alcohol and decanol-alcohol systems and the values were found to be 119.6 and 89.7 mg/mL respectively. Figure 1(A,B) depicts the rate of penetration of prazosin hydrochloride and atenolol with and without permeation enhancers through mice skin as a function of time. DMF (4-fold) and DMSO (5.1 fold) enhanced average flux compared to average flux of the drug from the saturated aqueous solution (Fig. 2A). The flux of atenolol from pure alcohol and decanol solution was significantly higher than that obtained from saturated aqueous solution (\(P = 0.02\) and \(P = 0.01\) respectively). Overall enhancement in permeation rate was 4 times in case of alcohol and 6.8 times with decanol (5\%, Fig. 2B). Desired steady-state flux (target flux) has been calculated utilizing the pharmacokinetic data and compared with the achieved steady-state flux obtained from the control experiments (Table 2).

In the present study prazosin hydrochloride was chosen as a model for the poorly water soluble drug and atenolol as a moderately water soluble drug. Since the intrinsic penetration rate of prazosin hydrochloride from saturated aqueous solution is not adequate to meet the demands of maintenance dose (Table 2), DMF and DMSO were used as enhancers which increased the drug permeation significantly (Fig. 1A). As the saturation solubility remains essentially same in pure water, DMF-water and DMSO-water system, the permeation enhancing effect can only be attributed to the increase of drugs diffusivity due to alteration of skin properties. Vollmer et al.\textsuperscript{15} have also found 30 fold increase in the permeability coefficient of prazosin using ethanolic solution of DMSO as permeation enhancer. Solubility parameters of DMF and DMSO have been reported to be 12.1 and 13 respectively\textsuperscript{19}, whereas, solubility parameters of the skin of most rodent species have been assigned a value of 10 (Ref. 17). This matching of solubility parameters may have influenced the permeation process.

In case of atenolol, we have found significantly enhanced permeation of ethanolic solution in which it has much higher solubility than in water (Fig. 1B). Ethanol, with its solubility parameter 12.1 has been

<table>
<thead>
<tr>
<th>Drug</th>
<th>Solubility mg/mL(^{-1})</th>
<th>Octanol/water partition coefficient</th>
<th>Melting point, °C</th>
<th>Steady-state flux (J_{ss}) mg cm(^{-2}) hr(^{-1})</th>
<th>Permeability coefficient (Kp) cm hr(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prazosin hydrochloride</td>
<td>1.00</td>
<td>0.173</td>
<td>279</td>
<td>2.169(\pm)0.46</td>
<td>0.0021(\pm)0.00045</td>
</tr>
<tr>
<td>Atenolol</td>
<td>31.2</td>
<td>0.374</td>
<td>159</td>
<td>321.44(\pm)48.88</td>
<td>0.0087(\pm)0.00050</td>
</tr>
</tbody>
</table>
shown to increase the permeation rate of nitroglycerin, propranolol hydrochloride, diazepam etc.\textsuperscript{2,18.} The experimentally determined partition coefficient of atenolol (log$P$ = -0.45) is close to that of ethanol, reported by Hori et al.\textsuperscript{18} (log$P$ = -0.31). Hence, the possibility of vehicle mediated enhancement of drug permeation can not be ruled out. However, experiments performed without enhancers in donor compartment reveal that the actions of enhancers were taking place in the skin and enhanced permeation was not solely due to alteration of solute activity in the vehicle. Hori et al.\textsuperscript{18} have shown that decanol though permeates through the skin minimally, could enhance

![Graph](image-url)

**Fig. 1(A)**—Comparison of permeation profiles of prazosin hydrochloride from saturated aqueous solution DMF (5\% v/v in water) and DMSO (5\% v/v in water). (Each data point represents the mean ± SE of three experiments. * indicates significance at 5\% level).

**Fig. 1(B)**—Comparison of permeation profiles of atenolol from saturated aqueous solution with saturated alcoholic solution and decanol (5\% v/v in alcohol). (Each data point represents the mean ± SE of three experiments. * * indicates the significance at 1\% and 5\% levels respectively).

**Fig. 2**—First hour flux and average flux of (A) prazosin hydrochloride; and (B) atenolol obtained from different systems. First hour flux (■) average flux (●). (Each data point represents the average of three experiments).
the permeation rate of propranolol hydrochloride from a similar vehicle. Solubility parameter of 1-decanol is found to be 9.4 as calculated from Fedor’s group contribution value. It is possible that, due to matching of solubility parameters decanol got partitioned into skin at much higher extent and caused perturbation of membrane properties which are responsible for augmented diffusion of the drug.

Permeation kinetics of hydrophilic drug substances usually display a low initial permeation rate and an enhanced steady-state flux. However, in our study, we have noticed the opposite trend (Fig. 2A & B). Though, the solute activity in the donor compartment was kept constant by supplying drugs in the form of suspension, the permeation pattern in all the cases displays higher initial flux values followed by stabilization to comparatively lesser steady-state values. Two reasons have been suggested for the phenomenon that these moderately soluble drugs after forming a depot in the tissue, retards the downward movement of unbound drug molecules, resulting in lesser permeation rate in the later hours. The other reason may be the higher follicular density of haired skin in contrast to hairless skin, usually used for these experiments.

This study suggests that ethanol, a protic solvent, enhances the drug permeation by increasing solute activity, and the putative permeation enhancers 1-decanol, DMSO and DMF can influence the skin permeability rate of atenolol and prazosin respectively.

Acknowledgement

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References


Table 2—Comparison of desired skin permeation rate (target flux) calculated from pharmacokinetic data and achieved steady-state flux obtained from the enhancer unaided permeation study

<table>
<thead>
<tr>
<th>Drug</th>
<th>Desired plasma concentration (C)</th>
<th>Clearance (clt) mL min⁻¹ kg⁻¹</th>
<th>Target flux mg cm⁻² hr⁻¹</th>
<th>Steady-state flux (Jss) mg cm⁻² hr⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prazosin hydrochloride</td>
<td><strong>6.7±2.9 ng/mL</strong></td>
<td>3.0±0.3</td>
<td>7.2</td>
<td>2.169±0.46</td>
</tr>
<tr>
<td>Atenolol</td>
<td>0.1-1.0 mg/mL</td>
<td>2.0±0.2</td>
<td>360.0</td>
<td>0.0087±0.0005</td>
</tr>
</tbody>
</table>

**The values have been taken from the reference of Grahame et al.**

All other data appear in Reg. [12]