Original Paper

Med Princ Pract 2008;17:37–42
DOI: 10.1159/000109588

Received: February 6, 2007
Revised: April 9, 2007

Transdermal Permeation of Trimetazidine from Nerodilol-Based HPMC Gel Drug Reservoir System across Rat Epidermis

Yellela S. Krishnaiah    Saleh M. Al-Saidan
Department of Pharmaceutics, Faculty of Pharmacy, Kuwait University, Kuwait

Introduction

Transdermal therapeutic systems (TTS) provide steady-state plasma concentration of the drug for prolonged periods when applied to the skin by avoiding first pass metabolism and thereby improve bioavailability of drugs. The most important advantage of TTS is that the unwanted effects, if any, could be terminated simply by removing them from the skin. Thus, TTS are attracting the attention of healthcare professionals as these transdermal formulations have high patient compliance [1]. However, the barrier function of the skin limits the diffusion of many drug molecules, and hence several active and passive techniques are being used to enhance the transdermal drug delivery [1–3]. Transdermal drug formulations using passive techniques appear to be promising in providing a constant drug delivery as reviewed by Williams and Barry [4]. One of the passive techniques involve the use of chemical penetration enhancers which include azone and its analogues, pyrrolidones, polyunsaturated fatty acids, alkanols, polymeric enhancers, nonionic surfactants, and terpenes [4]. The safety of chemical penetration enhancers is of primary consideration while selecting them for use in the development of TTS. In this context, terpenes such as nerodilol are generally regarded as safe (GRAS) with low cutaneous irritancy, providing excellent enhancement ability and, thus appear to be promising candidates for transdermal formulations [5].

Key Words
Trimetazidine · Transdermal · Nerodilol · In vitro permeation · HPMC gel

Abstract

Objective: To study the in vitro transdermal permeation of trimetazidine from hydroxypropylmethyl cellulose (HPMC) gel drug reservoir system using nerodilol as a penetration enhancer. Materials and Methods: An HPMC gel containing selected concentrations of nerodilol (0, 2, 4 or 5% w/v) and 2.5% w/v of trimetazidine was prepared, and subjected to in vitro permeation studies across rat epidermis. The amount of trimetazidine permeated at different time intervals (1, 2, 4, 8, 12, 18 and 24 h) was estimated, and the data were analyzed to calculate various permeation parameters. Results: There was an increase in the amount of trimetazidine (8,719.7 ± 153.3 μg/cm²) permeated across the rat epidermis up to 24 h (Q₄₋₆) with an increase in nerodilol concentration (5% w/v) in HPMC gel drug reservoir. However, no significant difference (p > 0.05) was observed in the amount of drug permeated (Q₄₋₆) with 5% w/v of nerodilol when compared to that obtained with 4% w/v of nerodilol (8,484.5 ± 165.8 μg/cm²). Nerodilol, at a concentration of 4% w/v enhanced the flux of trimetazidine across rat epidermis by about 1.96 times when compared to control. Conclusion: The HPMC gel drug reservoir containing 4% w/v of nerodilol showed optimal transdermal permeation of trimetazidine.
Trimetazidine, used in treatment of angina and hypertension, has a short half-life, and is administered 2–3 times a day with a dose ranging from 40 to 60 mg [6]. Chronic use of conventional oral controlled release dosage forms of trimetazidine is inconvenient and may result in unwanted side effects due to high fluctuation of drug concentration in blood [1]. A well-designed membrane-moderated TTS is an alternative pharmaceutical formulation that provides an effective and safe therapy with high patient compliance [7, 8]. A 2% w/v of HPMC gel containing 6% w/v limonene (a terpene penetration enhancer) has been shown to enhance in vitro transdermal permeation of trimetazidine [8]. In the present investigation, another terpene penetration enhancer (nerodilol) was substituted for limonene in formulating HPMC gel drug reservoir for in vitro study of transdermal permeation of trimetazidine.

**Materials and Methods**

**Materials**

Trimetazidine and nimodipine were gift samples from M/s Dai-Ichi Karkaria Limited, Hyderabad, India, and M/s Microlabs, Bangalore, India, respectively. Nerodilol was obtained from M/s Sigma-Aldrich Laborchemikalien GmbH, Steinheim, Germany. HPMC and ethanol were obtained from M/s Sigma-Aldrich Laborchemikalien GmbH, Seelze, Germany. Acetonitrile (HPLC grade) was obtained from M/s BDH Laboratory Supplies, Poole, England. Sodium dihydrogen phosphate and o-phosphoric acid were obtained from M/s Surechem Products Ltd., Suffolk, England. Water (HPLC grade) was produced directly from Millipore filter (Millipore, Molsheim, France).

**Preparation of Nerodilol-Based HPMC Gel Drug Reservoir System**

HPMC powder was added to 50% v/v ethanol-water while being stirred at 2,500 rpm (M/s Cole-Parmer Instrument Company, Ill., USA), and the resulting mixture was mixed continuously at 37°C until a gel was formed. Then, trimetazidine (2.5% w/v) followed by nerodilol (0, 2, 4 or 5% w/v) were added to the HPMC gel and mixed well for complete dispersion. The gel formulations were left overnight at room temperature (25–28°C).

**In vitro Permeation of Trimetazidine across Rat Epidermis**

Modified Keshary-Chien diffusion cells were used in the in vitro permeation studies [9]. The rat epidermis, prepared as reported earlier [10], was mounted between the two compartments of the diffusion cell with the stratum corneum facing the donor compartment. High vacuum silicone grease was applied onto the donor and receptor compartments and excessive epidermal membrane at the sides was trimmed off to minimize lateral diffusion. The effective diffusion area was 6.6 cm² and the volume of the receiver compartment was 35 ml. Two milliliters of 2% w/v HPMC gel drug reservoir containing the selected concentration of nerodilol (0, 2, 4 or 5% w/v) and 2.5% w/v of trimetazidine was placed in the donor cell and covered with parafilm and aluminum foil to minimize evaporation of the solution. Ethanol-water (50:50 v/v) solvent system was added to the receiver cell. The cells were maintained at 37 ± 0.5°C by placing them on a magnetic stirrer with heater. The permeate samples (0.5 ml) were withdrawn from the receiver compartments at predetermined time intervals (1, 2, 4, 8, 12, 18 and 24 h), and an equivalent volume of drug-free vehicle (50% v/v ethanol-water) was added to the receiver compartment to maintain a constant volume. The samples were assayed for trimetazidine by HPLC method. The amount of drug retained in the epidermis at the end of 24 h was also determined.

**Determination of Drug Retained in Rat Epidermis (DRE)**

At the end of the in vitro permeation study, the skin sample was removed from the cells and washed briefly in methanol (20 ml) for 15 s to remove the adhering HPMC gel drug reservoir. Following drying at room temperature for 10 min, the skin was cut into pieces and then homogenized in 4 ml of methanol for 10 min and sonicated for 30 min to leach out the drug. The samples were centrifuged, the supernatant liquid filtered through a 0.45-µm membrane filter, added with fixed quantity of internal standard (5 µg/ml of nimodipine) and analyzed for the drug content by HPLC method.

**HPLC Method for the Estimation of Trimetazidine**

The quantitative estimation of trimetazidine in skin permeates, drug reservoir formulation or skin samples was estimated by HPLC method. The chromatographic system consisted of Waters 2690 automatic sample injector with a loop of 250 µl and Waters 996 Photodiode Array Detector. A reversed-phase Waters Symmetry C₁₈ column (3.9 X 150 mm; 5 µm) and Waters Symmetry C₁₈ guard column (3.9 X 20 mm) were used. A standard curve was constructed for trimetazidine in the range of 0.2–20 µg/ml using nimodipine as an internal standard (5 µg/ml). The mobile phase was a mixture of acetonitrile, 0.005 M NaH₂PO₄ and 0.02 KH₂PO₄ in the ratio of 60:5:35 (pH adjusted to 4.0 with o-phosphoric acid). The filtered mobile phase components were pumped from the respective reservoirs at a flow rate of 0.9 ml/min. The column temperature was maintained at 40°C. The eluent was detected at 205 nm, and the data were acquired, stored and analyzed. Required studies were carried out to validate the HPLC method for its precision and accuracy in estimating trimetazidine in drug reservoir systems and samples of skin permeates and skin membranes. A good linear relationship was observed between the concentration of trimetazidine and the ratio of the peak area of drug to that of internal standard with a high correlation coefficient (r = 0.9999) in the range of 0.2–20 µg/ml. The method was found to be precise (as shown by less than 4% of intra- and inter-day variation) and accurate as shown by 98.8% of mean recovery.

**Data Analysis**

The flux (µg/cm²-h) of trimetazidine was calculated from the slope of the plot of the cumulative amount of trimetazidine permeated per cm² of skin at steady state against the time using linear regression analysis [11, 12]. The steady state permeability coefficient (kₑ) of the drug was calculated by using the following equation [13]; kₑ = J/C, where ‘J’ is the flux and ‘C’ is the concentration of trimetazidine in the gel. The penetration enhancing effect of nerodilol was calculated in terms of enhancement ratio (ER), and

---

Med Princ Pract 2008;17:37–42

Krishnaiah/Al-Saidan
was calculated by using the following equation [14]: $ER = \frac{\text{flux with penetration enhancer}}{\text{flux without penetration enhancer}}$ as previously described [14]. The difference in the permeation of trimetazidine with selected concentrations of nerodilol was tested for statistical significance by analysis of variance (ANOVA) with a post hoc test such as Bonferroni test for multiple comparison using SPSS® computer program (PC Version 14.0, SPSS Inc., 1989–2005).

Results

The cumulative amount of trimetazidine permeated from HPMC gel drug reservoir containing selected concentrations (0, 2, 4 and 5% w/v) of nerodilol is shown in figure 1. There was a steady-state permeation of the drug with a mean lag period ranging from 2.1 to 3.0 h. The amount of trimetazidine permeated ($Q_{24}$) increased with increased concentration of nerodilol in HPMC gel drug reservoir. The value of $Q_{24}$ without nerodilol (control) was 4,247.2 ± 82.5 μg/cm$^2$, and on adding 4% w/v of nerodilol it increased to 8,484.5 ± 165.8 μg/cm$^2$. However, there was no significant increase in the value of $Q_{24}$ (8,719.7 ± 153.3 μg/cm$^2$) with 5% w/v of nerodilol when compared to that obtained with 4% w/v of nerodilol in the drug reservoir. The flux of trimetazidine was enhanced to 380.5 ± 7.9 μg/cm$^2$/h with 4% w/v of nerodilol, which is about 1.96 times compared to control (194.1 ± 7.4 μg/cm$^2$/h). There was no significant increase ($p > 0.05$) in the flux of the drug with 5% w/v nerodilol (395.4 ± 7.6 μg/cm$^2$/h) compared to that obtained with 4% w/v of nerodilol (380.5 ± 7.9 μg/cm$^2$/h). The permeability of the drug without nerodilol was 7.77 ± 0.30 cm/h × 10$^{-3}$. On adding 4% w/v of nerodilol, there was a significant ($p < 0.001$) increase in the permeability coefficient of the drug compared to control (table 1). However, there was no significant difference ($p > 0.05$) in the permeability coefficient of the drug with 5% w/v of nerodilol compared to that obtained with 4% w/v of nerodilol.

Without nerodilol in HPMC gel drug reservoir, the amount of drug retained in the epidermis was 1,220.1 ± 46.9 μg/g. The DRE increased with an increase in the concentration of nerodilol in the drug reservoir up to 2,324.3 ± 368.5 μg/g with 5% w/v. However, there was

<table>
<thead>
<tr>
<th>Concentration of nerodilol</th>
<th>Flux, $J$, μg/cm$^2$/h</th>
<th>Mean, ER</th>
<th>$Q_{24}$, μg/cm$^2$</th>
<th>Lag time, h</th>
<th>$k_p$, cm/h × 10$^{-3}$</th>
<th>DRE, μg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 % w/v (control)</td>
<td>194.1 ± 7.4</td>
<td>–</td>
<td>4,247.2 ± 82.5</td>
<td>3.0 ± 0.3</td>
<td>7.77 ± 0.30</td>
<td>1,220.1 ± 46.9</td>
</tr>
<tr>
<td>2 % w/v</td>
<td>328.5 ± 2.4*</td>
<td>1.69</td>
<td>7,377.5 ± 123.8*</td>
<td>2.6 ± 0.2</td>
<td>13.14 ± 0.10</td>
<td>1,612.2 ± 194.6</td>
</tr>
<tr>
<td>4 % w/v</td>
<td>380.5 ± 7.9*</td>
<td>1.96</td>
<td>8,484.5 ± 165.8*</td>
<td>2.4 ± 0.2</td>
<td>15.22 ± 0.32</td>
<td>2,256.1 ± 482.2**</td>
</tr>
<tr>
<td>5 % w/v</td>
<td>395.4 ± 7.6*</td>
<td>2.04</td>
<td>8,719.7 ± 153.3*</td>
<td>2.1 ± 0.2**</td>
<td>15.82 ± 0.31*</td>
<td>2,324.3 ± 368.5**</td>
</tr>
</tbody>
</table>

* $p < 0.001$ when compared to control; * $p > 0.05$ when compared to 4% w/v nerodilol; ** $p < 0.05$ when compared to control.
no significant difference \( (p > 0.05) \) in the value of DRE obtained with 4\% w/v of nerodilol compared to that obtained with 5\% w/v of nerodilol.

**Discussion**

Based on our earlier reports \([7, 8]\), 50\% v/v ethanol-water was chosen as a vehicle for formulating a terpene-based HPMC gel drug reservoir system. This is because of the ability of ethanol-water solvent system to act as penetration enhancer on its own as well as its ability to dissolve the chosen terpene enhancer (nerodilol). Ethanol permeates rapidly through human skin \([15]\), increases the solubility of poorly soluble drugs in the donor phase \([16]\), alters the solubility properties of skin tissue \([17]\), changes the lipoidal pathway of stratum corneum \([18]\) and thereby enhances transdermal drug permeation of several drugs such as estrone, β-estradiol, and hydrocortisone.

The flux of trimetazidine with 50\% v/v of ethanol-water was 194.1 ± 7.4 \(\mu g/cm^2\cdot h\) whereas that reported with water in our earlier study was 52.0 ± 0.6 \(\mu g/cm^2\cdot h\) \([7]\). Thus, there was about 3.7 times increase in the flux of trimetazidine with the ethanol-water vehicle. This means that 50\% v/v ethanol is acting as a penetration enhancer on its own. With 4\% w/v of nerodilol, the flux of the drug increased to 380.5 ± 7.9 \(\mu g/cm^2\cdot h\), which is about 2 times of that obtained with the control (2\% w/v HPMC gel prepared with 50\% v/v ethanol-water).

Although there was an apparent increase in the flux of trimetazidine with an increase in nerodilol concentration, such an increase in the flux was insignificant \( (p > 0.05) \) with 5\% w/v nerodilol when compared to that with 4\% w/v nerodilol. Based on this result, 4\% w/v of nerodilol was considered as optimal concentration for providing an optimal permeation of trimetazidine. The optimal flux (380.5 \(\mu g/cm^2\cdot h\)), obtained with 4\% w/v nerodilol was about 4 times the required flux (94.7 \(\mu g/cm^2\cdot h\)) of trimetazidine. The required flux \( (J) \) was calculated as per the method described earlier \([7, 8]\). Hence, permeation study was not continued with a higher concentration \((>5\% w/v)\) of nerodilol. The higher flux obtained with 4\% w/v of nerodilol was necessary to account for the difference in the permeability of the rat epidermal membrane used in the present study and human skin and that of the resistance offered by the components of the transdermal patch \([1]\).

It has been reported that terpenes increase the transdermal drug permeation mainly by disrupting the intercellular packing of the stratum corneum lipids \([19–21]\). It is then presumed that nerodilol might have disrupted the intercellular packing of the stratum corneum and thereby enhanced the permeation of trimetazidine in the present study. The findings of Fourier transform infrared studies reported earlier with nerodilol on rat stratum corneum support this hypothesis \([10]\). Terpenes exert their penetration enhancing activity due to their ability to permeate across the skin \([21]\) and thereby modify the solvent nature of the stratum corneum with a resultant increase in the drug partitioning into the tissue \([4]\). Thus, it appears that both the 50\% v/v ethanol-water vehicle and nerodilol (terpene) are enhancing the transdermal permeation of trimetazidine in a similar way, which resulted in an additional penetration enhancing effect. This in turn produced a higher mean enhancement ratio of about 7.3 with an ethanolic solution of 4\% w/v nerodilol compared to that obtained with water.

The increase in the value of DRE with an increase in the concentration of nerodilol in HPMC gel drug reservoir shows that the drug retained in the skin diffused steadily. The solubility of trimetazidine has been reported to increase with an increase in the concentration of ethanol in water while optimizing the ethanol-water vehicle for designing HPMC gel drug reservoir system \([7]\). The enhanced flux of trimetazidine has been suggested to be due to the increased solubility of the drug in the ethanol-water vehicle \([15–17]\). Similarly, it is possible that nerodilol, on permeating across the skin, increased the DRE most probably by increasing the solubility of trimetazidine in the skin layers with an increase in the concentration of nerodilol in the 50\% ethanol-water vehicle from 457.5 ± 2.7 to 876.9 ± 11.9 \(mg/ml\) in 0 and 5\% w/v nerodilol, respectively. This indicates that nerodilol, after permeating through the skin, enhanced the DRE levels in the skin and thereby increased the transdermal flux of trimetazidine \([4, 21]\). It is possible that trimetazidine diffused at a steady state due to the increased quantity of drug retained in the skin such that the skin was controlling the release of trimetazidine. However, in an ideal TTS, the device is expected to control the drug release across the skin. In the present study it appeared that the skin was controlling drug release from the nerodilol-based HPMC gel drug reservoir system instead of the transdermal drug delivery system.

The lag period for producing steady state permeation of the drug across the rat epidermis decreased with an increase in nerodilol (table 1). However, the decrease in the lag period was significant \( (p < 0.05) \) only with 5\%
w/v of nerodilol in the drug reservoir. This indicates that nerodilol is interacting with the skin more or less at the same rate as an increase in the nerodilol concentration. However, there was a significant increase in the extent of nerodilol-skin interaction as evidenced by the increase in the flux of trimetazidine. Terpenes act as penetration enhancers due to their ability to modify the solvent nature of the stratum corneum and thereby improve drug partitioning into the tissue [4]. Mostly for this reason, the quantity of drug retained in the skin membrane (DRE) at the end of in vitro permeation study increased, with an increase in the concentration of nerodilol in the HPMC drug reservoir (table 1). Many terpenes permeate human skin well [21], which in turn increase the thermodynamic activity of the drug in the formulation. Small angle X-ray diffraction studies indicated that nerodilol reinforces the bilayers, possibly by orientating alongside the stratum corneum lipids [22]. Nerodilol is a saturated secondary alcohol (lipophilicity as denoted by log P is 5.36) and, the highly lipophilic nerodilol might have produced a high penetration enhancing activity of weakly lipophilic trimetazidine (log P = 0.60) across the skin membrane [23].

Our earlier reports [10, 24] showed that nerodilol at a concentration of 10% w/w enhanced the transdermal permeation of nicorandil in human volunteers with no signs of damage or irritation when nerodilol-based patch was applied to the skin. This indicates that the optimal concentration of nerodilol (4% w/v) is unlikely to cause any damage to the stratum corneum on application of the transdermal patch to humans. Though nerodilol showed enhanced permeation, the transdermal components of the proposed membrane-moderated TTS, such as rate-controlling membrane (e.g. EVA2825) and adhesive coat, may exhibit their own resistance to the permeation of trimetazidine across them. This has to be taken into account before fabricating the proposed membrane-moderated TTS of trimetazidine. Such studies were carried out and reported separately.

**Conclusion**

The results of the in vitro permeation studies across the rat epidermis showed that HPMC gel drug containing 4% w/v of nerodilol provided optimal transdermal permeation of trimetazidine.

**Acknowledgements**

This research work was supported by Kuwait University Research Grant No. PP01/04.

---

**References**


