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Research paper

Influence of menthol and pressure-sensitive adhesives on the in vivo performance of membrane-moderated transdermal therapeutic system of nicardipine hydrochloride in human volunteers

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Abstract

A membrane-moderated transdermal therapeutic system of nicardipine hydrochloride was developed using 2% w/w hydroxypropylcellulose (HPC) gel as a reservoir system containing 5% w/w of menthol as a penetration enhancer. The permeability flux of nicardipine hydrochloride through the ethylene vinyl acetate (EVA) copolymer membrane was found to increase with an increase in vinyl acetate content in the copolymer. The effect of various pressure-sensitive adhesives (MA-31®, MA-38® or TACKWHITE A 4MED®) on the permeability of nicardipine hydrochloride through EVA 2825 membrane (28% w/w vinyl acetate) or EVA 2825 membrane/skin composite was also studied. The results showed that nicardipine hydrochloride permeability through EVA 2825 membrane coated with TACKWHITE A 4MED®/skin composite was higher than that coated with MA-31® or MA-38®. Thus, a new transdermal therapeutic system for nicardipine hydrochloride was formulated using EVA 2825 membrane coated with a pressure-sensitive adhesive TACKWHITE A 4MED®, and 2% w/w HPC gel as reservoir containing 5% w/w of menthol as a penetration enhancer. In vivo studies in healthy human volunteers indicated that the TTS of nicardipine hydrochloride, designed in the present study, provided steady-state plasma concentration of the drug with minimal fluctuations for 26 h with improved bioavailability in comparison with the immediate release capsule dosage form.

Keywords: Nicardipine hydrochloride; Menthol; Pressure-sensitive adhesives; Transdermal therapeutic system; Ethylene vinyl acetate copolymer; In vivo performance

1. Introduction

Nicardipine hydrochloride, a dihydropyridine derivative, is widely used in the treatment of hypertension and angina pectoris [1]. The terminal half-life of nicardipine hydrochloride after single dose (30 mg) in human subjects is between 2 and 4 h. After oral administration, it undergoes extensive first-pass elimination, and exhibits inter- and intra-subject variation in plasma concentration. Because of the first-pass elimination, oral bioavailability of nicardipine hydrochloride in human subjects has been reported [2] to be low as 30–35%.

Several transdermal therapeutic systems (TTS) have been developed to achieve systemic medication for various drugs including nicardipine hydrochloride in the treatment of hypertension [3]. Avoidance of first-pass elimination, decrease in the side effects, and the relative ease of drug input termination in problematic cases, as well as maintaining suitable plasma concentration for longer duration through a non-invasive zero-order delivery are the well-documented advantages of this route of administration [4]. Nevertheless, transdermal drug delivery has always been challenged by the formidable barrier property of the intercellular lipid bilayer in the stratum corneum.

In a recent study, it was reported from our laboratory [5] that ethanol–water solvent system in the ratio of 70:30 v/v was a suitable vehicle for the transdermal delivery of nicardipine hydrochloride. However, it was necessary to improve the permeation rate of nicardipine hydrochloride by using suitable enhancers. Terpenes, the naturally occurring volatile oils, are considered as clinically acceptable penetration enhancers as indicated by high percutaneous enhancement ability [6–9], reversible effect on the

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lipids of stratum corneum and low cutaneous irritancy at lower concentrations (1–5%). Moreover, terpenes have been shown to increase the skin permeation of a number of drugs [10–16] and thus could be used as penetration enhancers for increasing the permeability of nicardipine hydrochloride. Krishnaiah et al. [17] reported that 5% w/w of menthol in 2% w/w hydroxypropylcellulose (HPC) gel provides the required permeability of nicardipine hydrochloride through the excised rat abdominal skin.

Besides the penetration enhancer, vehicle and gelling agent, the skin permeability of nicardipine hydrochloride could be affected by the rate-controlling membrane/or the pressure-sensitive adhesives. Thus, the present study was carried out to develop a membrane-moderated reservoir-type transdermal therapeutic system of nicardipine hydrochloride and to investigate the effect of rate controlling membrane and pressure-sensitive adhesives on the skin permeation rate of the drug so as to optimize the transdermal delivery of nicardipine hydrochloride. Further, bioavailability study in human volunteers was conducted to find the ability of the membrane-moderated reservoir-type TTS of nicardipine hydrochloride in providing a steady-state concentration of the drug.

2. Materials and methods

2.1. Materials

Nicardipine hydrochloride and (−)-menthol were obtained from M/s ICN Biomedicals, USA and M/s Merck-Schuchardt, Germany, respectively. Ethylene vinyl acetate (EVA) copolymer beads of various weight fractions (% w/w) of vinyl acetate (VA) were gift samples from M/s NOCIL India Ltd, India. Release liner (3M™ Scotchpak™ 1022) and backing membrane (3M™ Scotchpak™ 9732) were the gift samples from M/s 3M drug delivery systems, USA. The pressure-sensitive adhesive, TACKWHITE A 4MED®, is a water-based acrylic copolymer adhesive emulsion, and was a gratis from M/s Ichemco, Italy. The pressure-sensitive adhesive laminate, MA-31®, is an acrylic copolymer with moderate adhesion properties whereas MA-38® is also an acrylic copolymer with mild adhesion properties, and were gratis from M/s Adhesive Inc., UK. Acetonitrile and water used were of high performance liquid chromatography (HPLC) grade (M/s Qualigens Fine Chemicals, Mumbai, India). Other materials used in the study such as ethanol, propylene glycol and potassium dihydrogen phosphate were of analytical grade.

2.2. Preparation of HPC gel

To prepare 2% w/w HPC gel, HPC powder was added to 70% v/v ethanol while being stirred by means of a stirrer (M/s Remi Motors, India) at 2500 rpm, and the resulting mixture was mixed continuously at 37°C for about 1 h until the gel formation. Nicardipine hydrochloride (1% w/w) and menthol (5% w/w) were added to HPC gel and mixed well for complete dissolution. The gel formulations were left overnight at ambient temperature.

2.3. HPLC analysis of nicardipine hydrochloride

The quantitative determination of nicardipine hydrochloride was performed by HPLC. A gradient HPLC (Shimadzu HPLC Class-VP series) with two LC-10AT VP pumps, variable wavelength programmable UV/VIS Detector SPD-10A VP, CTO-10AS VP Column oven (Shimadzu), SCL-10A VP system controller (Shimadzu), a disposable guard column LC-18 (Pelliguard™, LC-18, 2 cm, Supelco, Inc., Bellefonte, PA.) and RP C-18 column (250 mm × 4.6 mm ID, particle size 5 μm; YMC, Inc., Wilmington, NC, USA) were used. The HPLC system was equipped with the software "Class-VP series version 5.03 (Shimadzu)".

The mobile phase used was a mixture of acetonitrile and 0.02 M KH_2PO_4. The components of the mobile phase were filtered and pumped in the ratio of 60:40 v/v at a flow rate of 1 ml/min. The column temperature was maintained at 40°C. The eluent was detected by UV detector at 239 nm, and the data were acquired, stored and analyzed with the software Class-VP series version 5.03 (Shimadzu). A standard curve was constructed for nicardipine hydrochloride in the range of 0.01–2 μg/ml. A good linear relationship was observed between the concentration of nicardipine hydrochloride and area of nicardipine hydrochloride with a high correlation coefficient ($r = 0.9999$). The required studies were carried out to estimate the precision and accuracy of this HPLC method of analysis of nicardipine hydrochloride. This HPLC method was found to be precise and accurate as indicated by less than 2% CV (inter- and intra-day variation) and high recovery (99.98%). The standard curve constructed, as described above, was used for estimating nicardipine hydrochloride either in the skin permeates or in HPC gel formulations.

2.4. Quantitative determination of nicardipine hydrochloride in HPC gel formulations

One gram of the HPC gel formulation was accurately weighed, placed in 100-ml volumetric flask containing 30 ml of mobile phase, stirred for 30 min and made up to volume. The resultant mixture was filtered through 0.45-μm membrane filter and injected into the HPLC system. The amount of nicardipine hydrochloride was estimated from the standard curve as described above.

2.5. Preparation of rat abdominal skin

The animals used for the preparation of skin were male albino rats (150–200 g) obtained from M/s Ghosh Enterprises, Kolkata, India. They could have a free access to food and water until used for the study. The care of the rats was in

accordance with the institutional guidelines. The rats were euthanized using carbon dioxide asphyxiation before the experiments. The dorsal hair was removed with a clipper and full thickness skin was surgically removed from each rat. The epidermis was prepared by a heat separation technique [11], which involved soaking of the entire abdominal skin in water at 60°C for 45 s, followed by careful removal of the epidermis. The epidermis was washed with water and used for the in vitro permeability studies.

2.6. In vitro skin permeability studies

Modified Keshary–Chien diffusion cells [5,17] were used in the in vitro permeation studies. The epidermis prepared, as above, was mounted between the two compartments of the diffusion cells with stratum corneum facing the donor compartment. The effective diffusional area was 3.5 cm^2. The volume of receiver compartment was 24 ml. The HPC gel (2 g) containing 20 mg of nicardipine hydrochloride was added to the donor cell. Ethanol and water in the ratio of 70:30 v/v was added to the receiver cell in order to maintain the sink conditions. The cells were placed on a magnetic stirrer with heater (Remi Equipments, Mumbai, India) and temperature maintained at 37 ± 0.5°C. The contents in the receiver compartment were stirred with the help of a magnetic bar at 500 rpm. At predetermined times (1, 2, 4, 6, 12 and 24 h) 0.5 ml permeate samples were withdrawn from the receiver compartment and an equivalent amount of drug-free solvent (70% v/v ethanol) was replaced to maintain a constant volume. The samples of the skin permeates were assayed for nicardipine hydrochloride by HPLC method as per the conditions described above.

2.7. Preparation of EVA membranes

EVA membranes with VA (vinyl acetate) content ranging from 9 to 28% were prepared to evaluate suitable membrane for the development of membrane-moderated TTS. The membranes were prepared by solvent extrusion using glass–substrate technique. 'Membrane-casting apparatus' fabricated in our laboratory was utilized for the preparation of the membranes. Briefly, the procedure involves pouring of 5% w/v EVA polymer solution on to a glass frame (100 cm^2) and allowing the solvent to evaporate slowly. After complete evaporation of the solvent, the membrane was removed carefully.

2.8. Permeability studies across EVA membranes

The experimental conditions to study the permeation of nicardipine hydrochloride across the EVA copolymer membranes were the same as those outlined above for the skin permeation studies, except that the EVA copolymer membrane with various weight factions of VA was used in place of the skin sample.

The EVA 2825 membrane, coated with a pressure-sensitive adhesive such as TACKWHITE A 4MED®, MA-31® or MA-38®, was mounted on the skin and the permeation of nicardipine hydrochloride across the membrane/skin composite was also determined. The experimental conditions were the same as those outlined above, except that the membrane/skin composite was used in place of the EVA 2825 membrane.

2.9. Fabrication of experimental membrane-moderated reservoir-type transdermal therapeutic system

An experimental membrane-moderated reservoir-type transdermal therapeutic system of nicardipine hydrochloride was fabricated by sandwiching HPC gel drug reservoir system between drug-impermeable backing laminate and a rate-controlling EVA 2825 membrane (25 cm^2) coated with pressure-sensitive adhesive. The reservoir system consisted of 1% w/w of nicardipine hydrochloride and a penetration enhancer (5% w/w of menthol) in 2% w/w of HPC gel prepared with ethanol–water (70:30 v/v) solvent system. The rate-controlling membrane was EVA copolymer containing 28% w/w VA (EVA 2825). To ensure intimate contact of the transdermal patch to the skin, a pressure-sensitive adhesive polymer was coated on to the EVA 2825 membrane.

The EVA 2825 membrane was coated with a water-based acrylic adhesive emulsion (TACKWHITE A 4MED®), allowed to dry completely and a release liner (3M™ Scotchpak™ 1022) was pressed over EVA 2825 membrane. The HPC gel (3 g) containing the drug (1% w/w) and permeation enhancer (5% w/w of menthol) was placed on the reverse side of the EVA 2825 membrane/adhesive/release liner composite placed on a slightly grooved surface, and then the backing laminate (3M™ Scotchpak™ 9732) was placed on it. The composite was heat-sealed and cut to the appropriate sizes (25 cm^2). The TTS patch (with 5% w/w of menthol), thus prepared, was kept in a sealed aluminum pouch to minimize the loss of solvent (ethanol).

2.10. In vivo evaluation of the TTS patch (with 5% w/w of menthol) of nicardipine hydrochloride in healthy human volunteers

After approval of the ethics committee, the study was conducted at M/s Sipra Labs Pvt. Ltd, Hyderabad, India. Six healthy male volunteers (60–70 kg, age between 25 and 30 years) participated in the study and all were non-smokers and non-alcoholics. The nature and purpose of the study were fully explained to them. An informed written consent was obtained from every volunteer. They were withheld from any drugs for 1 week prior to the participation of the study. The volunteers were divided into two groups (Group I and Group II) and a crossover study was carried out. An immediate release capsule dosage form containing 30 mg of nicardipine hydrochloride was chosen as a reference

formulation, and was administered to three volunteers (Group I). Group II ($n = 3$) volunteers applied TTS patch (with 5% w/w of menthol) of 25 cm^2 to the anterior surface of the forearm near the elbow. After a washout period of 10 days, Group I volunteers applied TTS patch (with 5% w/w of menthol) and Group II received the reference formulation (immediate release capsule dosage form). The volunteers were allowed to remove the patch, in case of any sign of irritation at the application site. Blood samples were collected from the volunteer's cubical vein of the forearm via a hypodermic syringe (rinsed with dilute heparin solution) over a period of 48 h (0, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 24, 36 and 48 h). The blood samples were immediately centrifuged at 5000 rpm, plasma was separated and stored at −20°C until analysis by HPLC.

2.11. HPLC analysis of nicardipine hydrochloride in human plasma

The quantitative determination of nicardipine hydrochloride in human plasma was performed by HPLC using the equipment described above. An aliquot (0.5 ml) of plasma sample was measured into a glass tube with a teflon-lined cap, followed by the addition of 100 ng of nifedipine (internal standard). To the above plasma samples, 5 ml of ethyl acetate was added, vortexed for 5 min and centrifuged at 3000 rpm for 10 min. The organic layer was removed, evaporated to dryness under vacuum, reconstituted with 100 μl of acetonitrile and 20 μl injected into the HPLC column (RP C-18, 150 × 4.6 mm ID, particle size 5 μm; Flexit Inc., India) through which the mobile phase components (acetonitrile and 0.02 M KH_2PO_4) were pumped from the respective solvent reservoirs in the ratio of 70:30 v/v at a flow rate of 1 ml/min. The column back pressure was 74–75 kg/cm^2 and the eluents were detected by UV detector at 239 nm. The sensitivity of the detector was set at 0.0001 AUFS. The data were acquired, stored and analyzed with the software "Class-VP series version 5.03 (Shimadzu)". The peak area ratio of nicardipine hydrochloride to that of internal standard was determined, and this was used to find the plasma concentration of nicardipine hydrochloride from the regression equation obtained after constructing the calibration curve. The calibration curve was obtained by spiking drug-free plasma with varying amount of nicardipine hydrochloride (10–150 ng/0.5 ml) and fixed quantity of internal standard (100 ng of nifedipine) and treating the plasma samples as described above. A good linear relationship was observed between the plasma concentration of nicardipine hydrochloride and the peak area ratio of nicardipine hydrochloride to that of internal standard with a high correlation coefficient ($r = 0.9987$) in the range of 10–150 ng/0.5 ml. However, the minimum detection limit was found to be 10 ng/0.5 ml. The method was found to be precise (intra- and inter-day variation was found to be less than 2%) and accurate (recovery ranged from 99.8 to 99.95%). Whenever a lower amount of drug less than the minimum detection limit was observed, the HPLC analysis was repeated with larger quantity of plasma sample.

2.12. In vitro and in vivo data analysis

The nicardipine hydrochloride concentration in the permeates was corrected for sampling effects according to the equation described by Hayton and Chen [18]:

$$C_n^1 = C_n[V_T/(V_T - V_S)](C_{n-1}^1/C_{n-1})$$

where C_n^1 is the corrected concentration of the nth sample, C_n the measured concentration of nicardipine hydrochloride in the nth sample, C_{n-1} the measured concentration of the nicardipine hydrochloride in the $(n-1)$th sample, V_T the total volume of the receiver fluid and V_S the volume of the sample drawn.

The flux (μg/cm^2/h) of nicardipine hydrochloride (J) was calculated from the slope of the plot of the cumulative amount of nicardipine hydrochloride permeated per cm^2 of skin at steady state against the time using linear regression analysis [19,20]. The steady-state permeability coefficient (K_p) of the drug through rat epidermis was calculated by using the following equation [21]:

$$K_p = J/C$$

where J is the flux and C the concentration of nicardipine hydrochloride in the gel.

The plasma concentration of nicardipine hydrochloride at different time intervals was subjected to pharmacokinetic analysis to calculate various parameters including maximum plasma concentration (C_{max}), time to reach maximum concentration (T_{max}) and area under the curve ($AUC_{0-\infty}$). The values of C_{max} and T_{max} were directly read from the arithmetic plot of time versus plasma concentration of nicardipine hydrochloride. The area under the curve of time versus plasma concentration of nicardipine hydrochloride ($AUC_{0-\infty}$) was calculated by using trapezoidal rule. The relative bioavailability of nicardipine hydrochloride from TTS patch (with 5% of menthol) was calculated by dividing its $AUC_{0-\infty}$ with that of immediate release capsule dosage form (reference formulation).

2.13. Statistical analysis

The in vitro permeation data involving the effect of VA content on the permeation of the drug through EVA copolymer membranes were subjected to Student's t-test to find the statistical significance of observed differences. The statistical significance of the observed difference in the permeability of the drug through EVA 2825 membrane coated with various types of pressure-sensitive adhesives and membrane/skin composite was tested by analysis of variance (ANOVA) with post hoc Benedicts t-test. The observed difference in mean pharmacokinetic parameters of

nicardipine hydrochloride after application of TTS patch (with 5% of menthol) and immediate release capsule dosage form was subjected to paired *t*-test to find the statistical significance. In all the cases, a value of $P < 0.05$ was considered statistically significant.

3. Results and discussion

In the earlier report [17] from our laboratory, HPC gel formulations containing nicardipine hydrochloride and selected concentrations of menthol (1–12% w/w) were prepared, and evaluated for in vitro permeation of nicardipine hydrochloride through excised rat epidermis. As menthol concentration increased from 0 w/w to 8% w/w, the permeability of nicardipine hydrochloride was found increased. On increasing the menthol concentration further from 8 to 12% w/w, the increase in the permeability was insignificant ($P > 0.05$). The flux of nicardipine hydrochloride was found to be 204.06 ± 2.31 μg/cm^2/h with an enhancement ratio of 6.38 when menthol was incorporated at a concentration of 5% w/w in HPC gels in comparison with the control (Table 1). The differential scanning calorimetry (DSC) and Fourier transform infrared (FT-IR) data revealed that the increased transdermal permeability of nicardipine hydrochloride might due to the disruption of highly ordered lipid bilayers of stratum corneum [17]. Based on these studies, HPC gel (2% w/w) containing 5% w/w of menthol as a penetration enhancer was chosen for further studies to design membrane-moderated TTS of nicardipine hydrochloride. The HPC gel formulations were found to contain 99.6–100.2% of nicardipine hydrochloride showing the uniformity of drug content in the gel formulation.

3.1. Effect of VA content of EVA copolymer membranes on the permeability of nicardipine hydrochloride

To control the release nicardipine hydrochloride from the reservoir system, EVA copolymer membrane was selected as a rate-controlling membrane. The EVA copolymer membranes with VA contents ranging from 9% to 28 w/w were prepared by solvent extrusion using glass–substrate technique. The mean thickness of the EVA membranes was found to be 25.8 ± 1.95 μm. The permeation profiles of nicardipine hydrochloride from the HPC gel (2 g) containing 1% w/w of drug and 5% w/w of menthol across the various EVA membranes were shown in Fig. 1. The membrane permeation rate of nicardipine hydrochloride increased with an increase in the VA content of EVA copolymer membrane. The maximum steady-state permeation rate ($P < 0.001$) of nicardipine hydrochloride was observed (211.53 ± 6.41 μg/cm^2/h) through the EVA membrane containing 28% VA (EVA 2825). Hence, it was planned to carry out further studies using EVA 2825 copolymer membrane as a rate-controlling layer in the design of TTS for nicardipine hydrochloride.

3.2. Effect of pressure-sensitive adhesives on the permeation of nicardipine hydrochloride through membrane/skin composite

A suitable pressure-sensitive adhesive is necessary in the design of TTS to ensure intimate contact of the transdermal patch to the skin. However, these polymeric adhesives exhibit their own influence on the permeability of the drug through both rate-controlling EVA membrane and skin. Hence, it is planned to study the influence of various pressure-sensitive adhesives on the permeability of nicardipine hydrochloride through EVA 2825 membranes coated with adhesives and adhesive-coated EVA membrane/skin composite. Such a study is necessary to choose a suitable adhesive polymer for optimal transdermal delivery of the drug through the proposed TTS. In the present study, three types of pressure-sensitive adhesives were chosen. Two of them were adhesive laminates (MA-31® and MA-38®) wherein the adhesive layer from the laminates was transferred onto the rate-controlling EVA2825 membrane. The mean thickness of the adhesive coat of laminates was 37.5 μm. The third adhesive polymer was a water-based acrylic adhesive emulsion (TACHWHITE A 4MED®) wherein this was coated uniformly over the EVA 2825 membrane. The mean thickness of the adhesive coat was 21.6 μm. This was evident from subtracting the mean

Table 1
Effect of menthol on the percutaneous parameters of nicardipine hydrochloride in HPC gels

Concentration of menthol (% w/w)	% drug permeated	J (μg/cm^2/h)[a]	K_p (cm/h × 10^3)[a]	ER[a]	DCS (μg/g)[a]
0 (control)	12.77 ± 0.12	31.95 ± 2.74	3.20 ± 0.27	1	1200.10 ± 201.56
1	30.26 ± 0.59	73.49 ± 5.04	7.35 ± 0.50	2.30 ± 0.15*	1469.25 ± 115.36
2	46.75 ± 0.25	117.90 ± 1.48	11.79 ± 0.14	3.68 ± 0.05*	1869.63 ± 156.01
5	79.27 ± 0.30	204.06 ± 2.31	20.41 ± 0.23	6.38 ± 0.07*	2069.98 ± 100.9
8	86.47 ± 0.13	227.70 ± 1.30	22.77 ± 0.13	7.12 ± 0.04*	2290.56 ± 169.67
10	87.32 ± 0.04	223.78 ± 3.57	22.38 ± 0.36	6.99 ± 0.11**	2285.00 ± 165.99
12	87.00 ± 0.35	224.48 ± 19.63	22.45 ± 0.10	7.01 ± 0.03**	2298.56 ± 98.58

ER, enhancement ratio of nicardipine hydrochloride; DCS, drug content in skin after 24 h; *significant at $P < 0.001$ when compared to control; **insignificant at $P > 0.05$ when compared 8% w/w menthol.

[a] Mean ± s.d., $n = 3$.

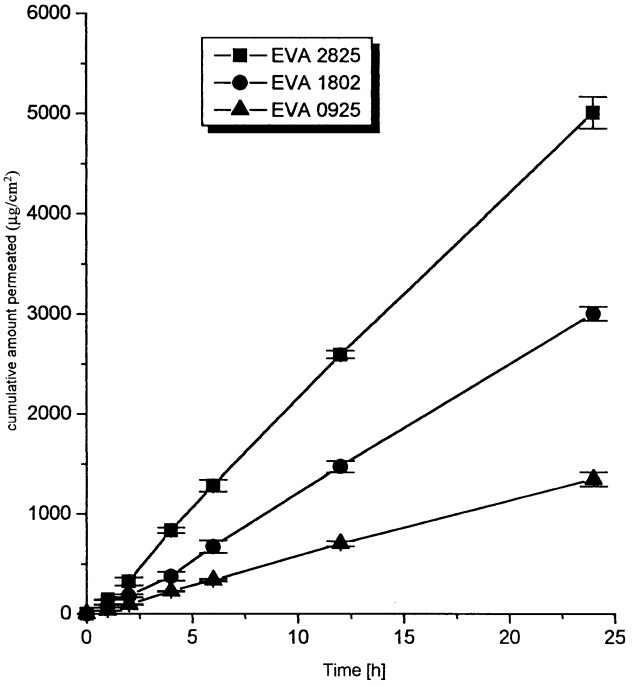

Fig. 1. Mean (±s.d.) cumulative amount of nicardipine hydrochloride permeated from 2% HPC gel containing 5% w/w of menthol across the EVA membranes with 28% w/w (EVA 2825), 18% w/w (EVA 1802) and 9% w/w (EVA 0925) of VA.

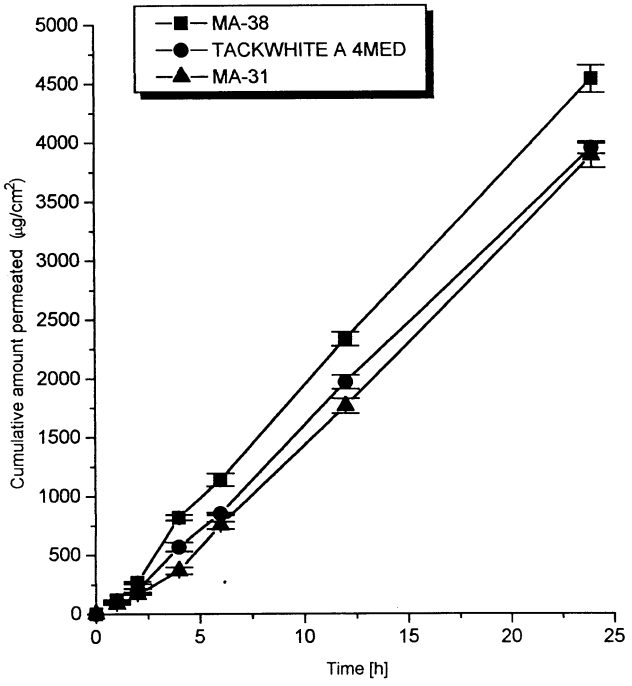

Fig. 2. Mean (±s.d.) cumulative amount of nicardipine hydrochloride permeated from 2% HPC gel containing 5% w/w of menthol across the EVA membranes (28% w/w of VA) coated with an adhesive.

thickness of EVA 2825 membrane from the mean thickness of EVA 2825 membrane coated with pressure-sensitive adhesives. The cumulative amount of nicardipine hydrochloride permeated through EVA 2825 membrane coated with various adhesives (TACKWHITE A 4MED®, MA-31® or MA-38®) was shown in Fig. 2, and Fig. 3 shows the cumulative amount of drug permeated through adhesive-coated EVA 2825 membrane/skin composite. When EVA 2825 membrane was coated with MA-38®, the permeation rate (Table 2) of nicardipine hydrochloride was slightly higher (183.53 ± 2.83 μg/cm^2/h) than that obtained with TACKWHITE A 4MED® (165.17 ± 1.42 μg/cm^2/h), or MA-31® (162.07 ± 6.41 μg/cm^2/h). But there was no significant difference ($P > 0.05$) in the permeation rate of nicardipine hydrochloride between MA-31® and TACKWHITE A 4MED®.

The thickness of the adhesive coat on EVA 2825 with MA-31® or MA-38® remained the same (37.5 μm), yet resulted in significant difference in the permeability of nicardipine hydrochloride. Since there was a difference in the adhesion properties of MA-31® and MA-38®, it is possible that the difference in chemical/physical nature of these adhesive coats with different partition coefficients and solubility properties of the drug might have provided a different permeability. The thickness of the adhesive coat with TACKWHITE A 4MED® was less when compared to that coated with MA-31® or MA-38®, yet showed less permeability than that obtained with MA-38® (mean thickness 21.6 μm). Such a difference might be due to difference in chemical composition of MA-38® and TACKWHITE A 4MED®. However, the permeation rate (Table 3) of nicardipine hydrochloride across the EVA 2825 membrane/skin composite was significantly ($P < 0.001$)

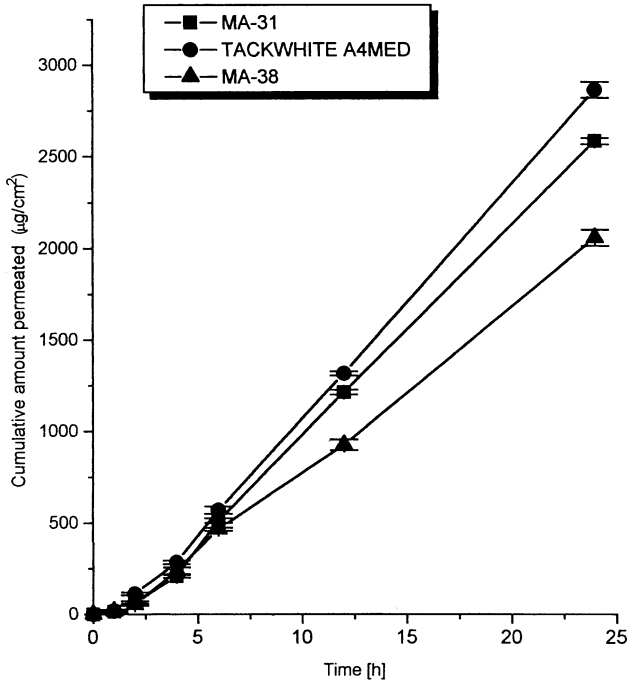

Fig. 3. Mean (±s.d.) cumulative amount of nicardipine hydrochloride permeated from 2% HPC gel containing 5% w/w of menthol across the EVA 2825 membrane/skin composite (membrane coated with MA-31®, TACKWHITE A 4MED® or MA-38®).

Table 2
Effect of pressure-sensitive adhesives on the permeability of nicardipine hydrochloride through 2% w/w HPC gel containing 5% w/w of menthol across EVA 2825 membrane

Adhesive	Permeability parameters		
	J (μg/cm^2/h)[a]	K_p (cm/h × 10^3)[a]	Q_{24} (μg/cm^2)[a]
Without adhesive (control)	211.53 ± 6.41	21.15 ± 0.64	5006.52 ± 158.26
MA-38®	183.53 ± 2.81*	18.35 ± 0.28*	4380.97 ± 74.67*
MA-31®	162.07 ± 2.93*	16.21 ± 0.30*	3813.39 ± 76.80*
TACKWHITE A 4MED®	165.17 ± 1.42*	16.52 ± 0.15*	3898.38 ± 27.69*

Q_{24}, cumulative amount of nicardipine hydrochloride after 24 h; *significant at $P < 0.001$ when compared to without adhesive (control).
[a] Mean ± s.d., $n = 3$.

higher from EVA 2825 membrane was coated with TACKWHITE A 4MED® (122.25 ± 1.87 μg/cm^2/h) when compared to that obtained with MA-31® (111.65 ± 0.59 μg/cm^2/h) or MA-38® (87.71 ± 1.38 μg/cm^2/h). This may be due to the stronger adhesion of TACKWHITE A 4MED® than the other adhesives as reported by Kim et al. [22]. The permeability flux of nicardipine hydrochloride through rat abdominal skin was 204.06 μg/cm^2/h whereas the permeability flux across adhesive-coated EVA 2825 was found to be 165.17 ± 1.42 μg/cm^2/h indicating that the patch is controlling the permeation of the drug. But the permeability flux further decreased to 122.25 ± 1.87 μg/cm^2/h when the patch was applied to rat abdominal skin indicating that the skin is also controlling the permeation. Thus, it is likely that both the patch and the skin are controlling the transdermal permeation of nicardipine hydrochloride. Based on these results, TACKWHITE A 4MED® was selected as an adhesive for further study, and was used for coating the EVA 2825 membranes.

3.3. In vivo evaluation of membrane-moderated TTS patch of nicardipine hydrochloride

The mean plasma concentration of nicardipine hydrochloride at different time intervals following the application of TTS patch (with 5% w/w of menthol) or oral administration of immediate release capsule dosage form was shown in Fig. 4. The plasma concentration of nicardipine hydrochloride gradually increased and attained average steady-state level of 21.39 ± 1.15 ng/ml at about 3.9 h (lag period). However, the steady-state concentration of the drug declined gradually after 28 h. Thus, the steady-state concentration of nicardipine hydrochloride (21.39 ± 1.15 ng/ml) was maintained for 26 h. The pharmacokinetic parameters such as C_{max}, T_{max}, $AUC_{0-\infty}$ and relative bioavailability were given in Table 4. The pharmacokinetic parameters of nicardipine hydrochloride after the application of TTS patch (containing 5% w/w of menthol) were significantly different from that obtained after oral administration of immediate release capsule. Unlike oral dosing, the plasma concentration of the drug after the application of TTS (with 5% w/w of menthol) was constant over 26 h. It took about 8.12 h (T_{max}) to reach maximum concentration of 26.65 ± 0.41 ng/ml (C_{max}). However, on oral administration as an immediate release capsule, the C_{max} (90.09 ± 5.758 ng/ml) of nicardipine hydrochloride reached within 0.83 h and declined rapidly after 3.5 h.

The inter-subject variation in plasma nicardipine hydrochloride level observed on oral administration of immediate release capsule was significant ($P < 0.05$). The low variation (less than 2.5% coefficient variation) in peak plasma levels following transdermal application of nicardipine hydrochloride could be accounted for uniformity in the skin permeation characteristics of the drug, which are possibly the same for all subjects. The inter-subject variation in plasma levels observed in the volunteers

Table 3
Effect of pressure-sensitive adhesives on the permeability of nicardipine hydrochloride through 2% w/w HPC gel containing 5% w/w of menthol across EVA membrane/skin composite

Adhesive	Permeability parameters		
	J (μg/cm^2/h)[a]	K_p (cm/h × 10^3)[a]	Q_{24} (μg/cm^2)[a]
MA-38®	87.71 ± 1.38	8.77 ± 1.38	2056.96 ± 43.92
MA-31®	111.65 ± 0.59*	11.16 ± 0.06*	2583.99 ± 16.42*
TACKWHITE A 4MED®	122.53 ± 1.87*	12.25 ± 0.18*	2864.59 ± 44.35*

Q_{24} cumulative amount of nicardipine hydrochloride after 24 h; *significant at $P < 0.001$ when compared to MA-38.
[a] Mean ± s.d., $n = 3$.

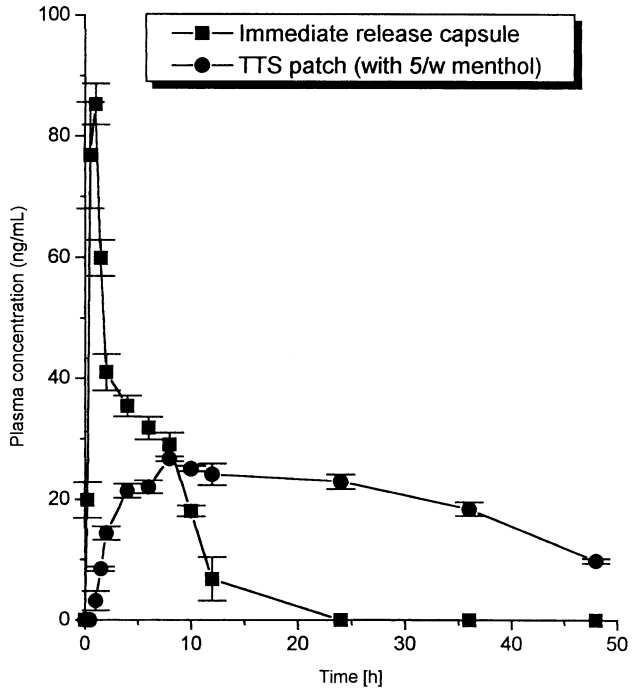

Fig. 4. Mean (±s.d.) plasma concentration of nicardipine hydrochloride following the oral administration of immediate release capsule dosage form and application of TTS patch (with 5% w/w of menthol) in human volunteers.

receiving immediate release capsule could be due to the high variation in gastric emptying and gastrointestinal (GI) absorption etiology of individual subjects [23–25].

The area under the curve ($AUC_{0-\infty}$) of nicardipine hydrochloride with the TTS patch was found to be significantly ($P < 0.001$) higher indicating the improved bioavailability on transdermal delivery of the drug (Table 4). The reported [2] low bioavailability of nicardipine hydrochloride (32%) is due to the extensive first-pass metabolism. However, TTS patch with 5% w/w of menthol, designed in the present study, was found to enhance the bioavailability of nicardipine hydrochloride by 2.82 times (mean relative bioavailability 282%) with reference to an immediate release capsule dosage form. Assuming the reported [2] mean oral bioavailability as 32%, the TTS patch used in the present study provided 90.24% of bioavailability. This increased bioavailability may be due to the elimination of hepatic first-pass metabolism on transdermal delivery of the drug. Thus, the TTS patch (with 5% of menthol), designed in the present study, was found to provide prolonged steady-state concentration of nicardipine hydrochloride with minimal fluctuations and improved bioavailability. The TTS patch application sites, in human volunteers, were examined visually for signs of local irritation after wearing the patch for 2 days. It was observed that there were no signs of skin irritation or sensitization after 48 h of application at the site. Also none of the human volunteers reported any signs of skin irritation or sensitization at the end of 48 h of study indicating that the patch was well tolerated on dermal application. The successful outcome of the present study warrants for further studies in patient volunteers to assess their ability in providing an effective and safe therapy of hypertension, and such studies are in progress.

4. Conclusions

The present study was carried out to develop a membrane-moderated transdermal therapeutic system of nicardipine hydrochloride using 5% w/w of menthol in HPC gel as a reservoir system. The permeability flux of nicardipine through the EVA membrane was found to increase (56.99 ± 2.98–211.526 ± 6.41 μg/cm^2/h) as the VA content in the copolymer increased (9%–28% w/w). The permeability of drug through EVA 2825 membrane (coated with TACK WHITE 4A MED®)/skin composite was significantly ($P < 0.001$) higher (122.53 ± 1.87 μg/cm^2/h) than that coated with MA-31® (111.65 ± 0.59 μg/cm^2/h) or MA-38® (87.71 ± 1.38 μg/cm^2/h). In vivo performance of the TTS patch (containing 5% w/w of menthol) of nicardipine hydrochloride was studied in healthy human volunteers against an immediate release oral capsule dosage form. The menthol-based membrane-moderated TTS of nicardipine hydrochloride provided a steady-state concentration of the drug for 26 h in comparison with the immediate release capsule dosage form, and the relative bioavailability from TTS was found to be 282.01 ± 24.67%. The TTS patch (containing 5% w/w of menthol) may be useful for long-term constant drug delivery with minimum fluctuations. The expected in vivo performance of the menthol-based membrane-moderated TTS of nicardipine hydrochloride designed in the present study, prompted for further studies in patient volunteers.

Table 4
Pharmacokinetic parameters of nicardipine hydrochloride following oral administration of immediate release capsule dosage form (30 mg) or application of TTS patch (5% w/w of menthol) in human volunteers ($n = 6$)

Formulation	C_{max} (ng/ml)	T_{max} (h)	$AUC_{0-\infty}$ (ng/h/ml)	Relative bioavailability (%)	Lag period (h)
Immediate release oral capsule dosage form	90.09 ± 5.58	0.83 ± 0.17	456.17 ± 12.01	–	0.21 ± 0.02
TTS patch (with 5% w/w of menthol)	26.65 ± 0.41*	8.12 ± 0.05*	1284.51 ± 81.97*	282.01 ± 24.67	3.72 ± 0.53

*Significant at $P < 0.001$ when compared to immediate release capsule dosage form.

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