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Influence of limonene on the bioavailability of nicardipine hydrochloride from membrane-moderated transdermal therapeutic systems in human volunteers

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Abstract

The aim of the present study was to develop a membrane-moderated transdermal therapeutic system (TTS) of nicardipine hydrochloride using 2%w/w hydroxy propyl cellulose (HPC) gel as a reservoir system containing 4%w/w of limonene as a penetration enhancer. The permeability flux of nicardipine hydrochloride through ethylene vinyl acetate (EVA) copolymer membrane was found to increase with an increase in vinyl acetate (VA) 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 membrane 2825 (28% w/w VA) or membrane/skin composite was also studied. The results showed that nicardipine hydrochloride permeability through EVA 2825 membrane coated with TACKWHITE 4A MED/skin composite was higher than that coated with MA-31or MA-38. Thus a new TTS for nicardipine hydrochloride was formulated using EVA 2825 membrane coated with a pressure-sensitive adhesive TACKWHITE 4A MED and 2%w/w HPC gel as reservoir containing 4%w/w of limonene as a penetration enhancer. The bioavailability 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 20 h with improved bioavailability in comparison with the immediate release capsule dosage form.

Keywords: Nicardipine hydrochloride; Limonene; Transdermal permeability; Bioavailability studies

1. Introduction

Over the past few years, the field of transdermal drug delivery has experienced a rapid growth. One of the driving forces behind this growth is the increasing number of drugs that can be delivered to the systemic circulation, in clinically effective concentrations, via the skin portal. This is in spite of the inherent protective function of the stratum corneum, which is primarily one of the excluding foreign substances from entering the body. Success has been achieved in the administration of several drugs transdermally via transdermal therapeutic

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systems (TTS) with a view of maintaining a constant plasma concentration of the respective drug over a pre-determined time period (Toon et al., 1989; Jain et al., 1990; Hadgraft et al., 1993; Ghosh et al., 1995; Gabiga et al., 2000; Kim et al., 2001). Nicardipine hydrochloride, used in the treatment of angina pectoris and hypertension, meets many of the criteria required for transdermal delivery (Graham et al., 1985; Dow and Graham, 1986).

In a recent study, it was reported from our laboratory (Krishnaiah et al., 2002) that ethanol and 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 penetration enhancers. In the last two decades, several penetration enhancers have been recognized to induce some physiological problems in subcutaneous tissue (Aungst, 1991). Terpenes appear to be clinically acceptable penetration enhancers as indicated by high percutaneous enhancement ability, reversible effect on the lipids of stratum corneum and low cutaneous irritancy at lower concentrations (1–5%), and thus could be used as penetration enhancers for increasing the permeability of nicardipine hydrochloride (Pfister et al., 1990; Williams and Barry, 1991; Okabe et al., 1990; Zhao and Singh, 1999; Obata et al., 1991; Moghimi et al., 1996; El-Kattan et al., 2001; Krishnaiah et al., 2002a). In this context, Krishnaiah et al. (2002b) reported that 4%w/w of limonene in 2%w/w HPC gel provides the required permeability of nicardipine hydrochloride through the excised rat abdominal skin.

Besides the vehicle, gelling agent and penetration enhancer, 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 reservoir-type TTS 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 formulation parameters. Further, bioavailability study was conducted in human volunteers to find the ability of the 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 D-limonene 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. Hydroxy propyl cellulose (HPC) was a gift sample from M/s. Dow Chemical Company, USA. Release liner (3M™ Scotchpak™ 1022) and backing membrane (3M™ Scotchpak™ 9732) were the gift samples from 3M drug delivery systems, USA. Pressure-sensitive adhesives such as TACKWHITE A 4MED® and MA-31®/ MA-38® were gratis by M/s. Ichemco, Italy and M/s Adhesive Inc., UK respectively. Acetonitrile (HPLC grade) was obtained from M/s. Qualigens Fine Chemicals, Mumbai, India. Triple distilled water (TD) was used. 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 formation of gel. Nicardipine hydrochloride (1%w/w) and limonene (4%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 high performance

liquid chromatography (HPLC). A gradient HPLC (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps, variable wave length 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^{TM}, LC-18, 2 cm, Supelco, Inc., Bellefonte, PA.) and RP C-18 column (150 × 4.6 mm I.D., particle size 5 μm; Flexit Inc., Pune, India) was 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 in the ratio of 60:40 v/v. The mobile phase components 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. 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 formulation

One gram of the drug reservoir (HPC gel formulation) was accurately weighed, placed in 100-ml volumetric flask containing 30 ml of mobile phase, stirred for 30 min and made upto 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 using 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, Kolkota, 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 (Zhao and Singh, 1999), 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 (Krishnaiah et al., 2002, 2002a,b,c) 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 was replaced with an equivalent amount of drug-free solvent (70% v/v ethanol) 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.

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 the place of skin sample.

The EVA 2825 membrane coated with a pressure sensitive adhesive such as TACKWHITE A 4MED® (water based pressure sensitive acrylic emulsion), MA-31® (moderate acrylic pressure sensitive adhesive) or MA-38® (mild acrylic pressure sensitive adhesive) 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 the place of EVA membrane.

2.7. Fabrication of experimental reservoir-type transdermal therapeutic system

An experimental reservoir-type TTS of nicardipine hydrochloride was fabricated by sandwiching the reservoir gel system between drug- impermeable backing laminate and a rate-controlling EVA membrane (25 cm^2) coated with pressure-sensitive adhesive. The reservoir system consisted of 1%w/w of nicardipine hydrochloride and a penetration enhancer (4%w/w of limonene) 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 acrylate adhesive emulsion (TACKWHITE A 4MED®), allowed to dry completely and a release liner (3M™ Scotchpak™ 1022, a polyester film coated with fluoropolymer) was pressed over the membrane. The HPC gel (3 g) containing the drug (1%w/w) and permeation enhancer (4%w/w of limonene) was placed over the EVA 2825 membrane/ adhesive composite placed on a slightly grooved surface, and then the backing laminate (3M™ Scotchpak™ 9732, a polyester film laminate with EVA heat-sealable layer) was placed on it. The composite was heat-sealed and cut to the appropriate sizes (25 cm^2). The TTS patch (with 4%w/w of limonene), thus prepared, was kept in a sealed aluminum pouch to minimize the loss of solvent (ethanol).

2.8. In vivo evaluation of the TTS patch (with 4%w/w of limonene) 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 nonsmokers and non-alcoholics. The biochemical examination of the volunteers revealed normal function of the kidney and liver. The nature and purpose of the study were fully explained to them. An informed written consent was obtained from every volunteer. None of the volunteers were on drug treatment 1 week prior to the participation of the study. The volunteers were divided into two groups (Group-I and -II), and a cross over 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 4%w/w of limonene) 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 4%w/w of limonene) 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.

2.9. HPLC analysis of nicardipine hydrochloride in human plasma

Standard graph was constructed by the addition of 0.1 ml of nifedipine (internal standard) spiking solution and an appropriate volume of nicardipine hydrochloride spiking solution to cover the concentration range from 10 to 150 ng/ml to 0.5 ml of human plasma. The mixture was vortexed for 15 s to ensure thorough mixing. After mixing, 5 ml of ethyl acetate was added, the resulting mixture was vortexed for 30 s, centrifuged at 3000 rpm for 10 min, the organic layer was separated out into a clean amber-colored vial and evaporated to dryness under vacuum. The residue was immediately reconstituted with 0.1 ml of mobile phase and filtered through 0.2-μm membrane filter. The filtrate (20 μl) was injected into the column (RP C-18, 250 X 4.6 mm I.D, particle size 5 μm; YMC Inc., USA) with 20 μl loop. The mobile phase (filtered through 0.2-μm PFTE membrane filter) comprised of 60:40 v/v of acetonitrile and 0.02M KH_2PO_4 and the flow rate of the mobile phase was maintained at 1 ml/min. which yielded a column back pressure of 74–75 kg/cm^2. The detection was by UV absorption at 239 nm. The range of the detector was set at 0.0001 AUFS. A good linear relationship ($r = 0.9987$) was observed between the concentration of nicardipine hydrochloride and area ratio of nicardipine hydrochloride to the internal standard. The required studies were carried out to find the inter- and intra-day variation, and accuracy. This method was found to be precise (CV less than 2%) and accurate (99.8–99.95% of recovery). The standard curve constructed, as described above, was used for estimating nicardipine hydrochloride in the samples of human plasma.

2.10. In vitro and in vivo data analysis

The nicardipine hydrochloride concentration in the skin permeate samples was corrected for sampling effects according to the equation described by Hayton and Chen (1982):

$$C_n^1 = C_n\left(\frac{V_T - V_S}{V_T}\right)\left(\frac{C_{n-1}^1}{C_{n-1}}\right)$$

where C_n^1 is the corrected concentration of the nth sample, C_n is the measured concentration of nicardipine hydrochloride in the nth sample, C_{n-1} is the measured concentration of the nicardipine hydrochloride 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.

The flux (μg/cm^2 per 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 (Julraht et al., 1995; Ho et al., 1998). The steady state permeability coefficient (k_p) of the drug through rat epidermis was calculated by using the following equation (Yamune et al., 1995):

$$k_p = \frac{J}{C}$$

where J is the flux and C is the initial concentration of nicardipine hydrochloride in the donor compartment.

The plasma concentration of nicardipine hydrochloride at different time intervals was subjected to pharmacokinetic analysis to calculate various parameters such as maximum plasma concentration (Cmax), time to reach maximum concentration (Tmax) and area under the curve ($AUC_{0-\infty}$). The values of Cmax and Tmax 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 4%w/w of limonene) when compared with reference formulation (immediate release dosage form) was calculated by dividing its $AUC_{0-\infty}$ with that of immediate release capsule dosage form (reference formulation).

2.11. 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 the 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 using analysis of variance (ANOVA) and Duncan's multiple range test with the help of STATISTICA™ computer program (Release 4.5, StatSoft Inc., 1993). The observed difference in mean pharmacokinetic parameters of nicardipine hydrochloride after application of TTS patch (with 4%w/w of limonene) 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

Nicardipine hydrochloride, a calcium antagonist, is used in the treatment of angina pectoris and hypertension (Graham et al., 1985; Dow and Graham, 1986). It is subjected to an extensive hepatic first-pass metabolism following oral administration with systemic bioavailability ranging from 20 to 33% (Graham et al., 1985). Since its short biological half-life (2–4 h), the drug has to be given frequently (30 mg three times daily). Thus, the conventional therapy may result in higher fluctuation in plasma concentration of the drug resulting in unwanted side effects. Hence, the development of a TTS for nicardipine hydrochloride that could provide a predetermined constant drug delivery is beneficial for an effective and safe therapy of hypertension.

In the earlier report (Krishnaiah et al., 2002b) form our laboratory, HPC gel formulations containing nicardipine hydrochloride and selected concentrations of limonene (1–12%w/w) were prepared, and evaluated for in vitro permeation of nicardipine hydrochloride through excised rat epidermis. As limonene concentration increased from 0 w/w to 4%w/w, the permeability of nicardipine hydrochloride was found increased. On increasing the limonene concentration further from 4 to 12%w/w, the increase in the permeability was insignificant ($P > 0.05$). The flux of nicardipine hydrochloride was found to be 240.31 ± 1.57 μg/cm^2 per h with an enhancement ratio of about 7.52 when limonene was incorporated at a concentration of 4%w/w in HPC gels in comparison with the control (31.95 ± 2.74 μg/cm^2 per h). The DSC and FT-IR data indicated that limonene increased the drug permeability through the rat skin by disrupting the highly-ordered intercellular lipid structure of the stratum corneum. Based on these studies, HPC gel (2%w/w) containing 4%w/w of limonene, as a penetration enhancer, was chosen for further studies to design the TTS of nicardipine hydrochloride.

The HPLC method used in quantitative determination of nicardipine hydrochloride either in the permeate samples or HPC gel samples was found to be precise and accurate as indicated by less than 2% of CV (inter- and intra-day variation) and high recovery (99.98%). The HPC gel formulations were found to contain 99.8–100.1% of nicardipine hydrochloride showing the uniformity of drug content in the gel formulation.

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

To control the release of 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%w/w to 28w/w (EVA 2825) were prepared by glass substrate technique and the average thickness of the membrane was found to be 25.8 ± 1.95 μm. The permeation profiles of nicardipine hydrochloride from the HPC gel (3 g) containing 1%w/w of drug and 4%w/w of limonene across the various EVA membranes are shown in Fig. 1. The membrane permeation rate of nicardipine hydrochloride increased with an increase in the VA content of EVA copolymer membrane. This might be due to the increased water vapor transmission EVA copolymer with an increase in VA content of the EVA polymers (9–28%w/w). Thus, EVA2825 copolymer with 28% VA content has more water vapor

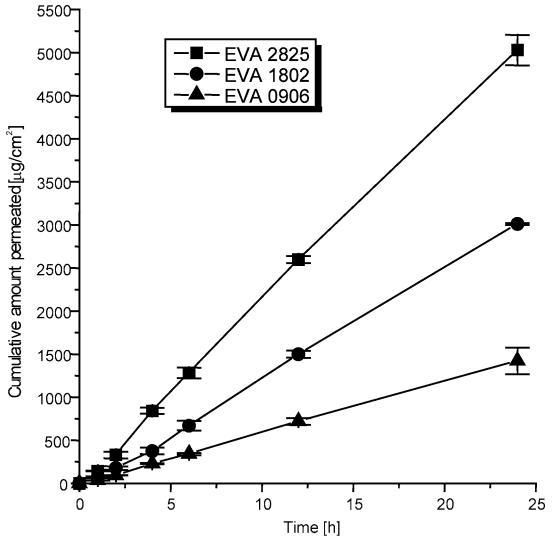

Fig. 1. Mean (±S.D.) cumulative amount of nicardipine hydrochloride permeated from 2% HPC gel containing 4% w/w of limonene across the EVA membranes with 28%w/w (EVA 2825), 18%w/w (EVA 1802) and 9%w/w (EVA 0906) of VA.

transmission than EVA copolymer with 9% (EVA 0906) or 18% (EVA1802) of VA content. Also increase in VA content (9–28%w/w) in the EVA membranes increases the polarity of the EVA copolymers. Thus EVA2825 containing 28% of VA has low lipophilicity and provided high permeability of nicardipine hydrochloride in the present study.

The maximum steady-state permeation rate of nicardipine hydrochloride was observed (212.34 ± 6.98 µg/cm^2 per h) through the EVA membrane containing 28% VA (EVA 2825), and found to be significant ($P < 0.001$) when compared with 18%w/w VA (EVA 1802) or 9%w/w VA (EVA 0906). The maximum permeability flux obtained with 4%w/w limonene across the rat abdominal skin was 240.31 ± 1.57 µg/cm^2 per h wherein EVA2825 provided only 212.34 ± 6.98 µg/cm^2 per h indicating that the EVA2825 membrane controlled the release of nicardipine hydrochloride. The other membrane EVA1802 provided a permeability flux of 133.8 ± 0.7 µg/cm^2 per h, which was nearer to the required permeability flux (116 µg/cm^2 per h). Subsequently the rate controlling membrane needs a coat of pressure-sensitive adhesive layer, which offers its own resistance to the permeability of the drug. In such a case, EVA1802 membrane with an adhesive coat may not be able to provide the required permeability. 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 was 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 2825 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. The cumulative amount of nicardipine hydrochloride permeated through EVA membrane coated with various adhesives (TACKWHITE A 4MED®, MA-31® or MA-38®) is shown in Figs. 2 and 3 shows the cumulative amount of drug permeated through membrane/ skin composite. When the EVA membrane was coated with MA-31, the permeation rate (Table 1) of nicardipine hydrochloride was slightly a lower (167.08 ± 3.80 µg/cm^2 per h) than that with TACKWHITE A 4MED (170.96 ± 1.84 µg/cm^2 per h), or MA-38® (192.99 ± 2.99 µg/cm^2 per h). But there was no significant difference ($P > 0.05$) in the permeation rate of nicardipine hydrochloride between MA-31 and TACKWHITE A 4MED. However, the permeation rate of nicardipine hydrochloride across the membrane/ skin composite (Table 2) was significantly ($P < 0.01$) higher when the EVA membrane was coated with TACKWHITE A 4MED (136.29 ± 2.72 µg/cm^2 per h) than that

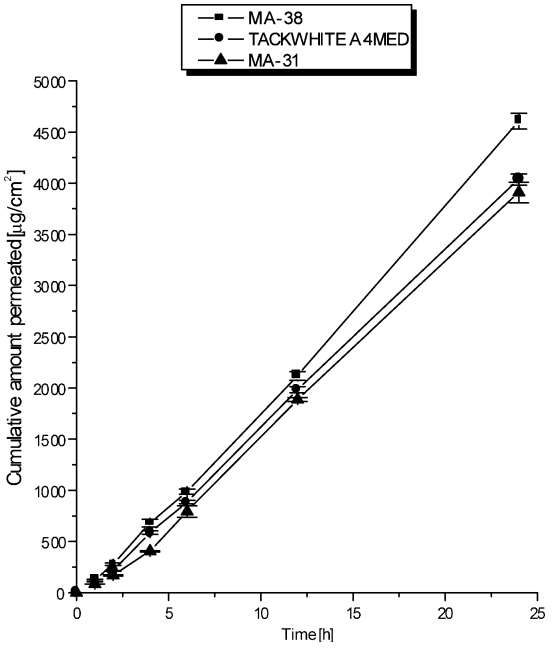

Fig. 2. Mean (±S.D.) cumulative amount of nicardipine hydrochloride permeated from 2% w/w HPC gel containing 4% w/w of limonene across the EVA membranes (28% w/w of VA) coated with various adhesives.

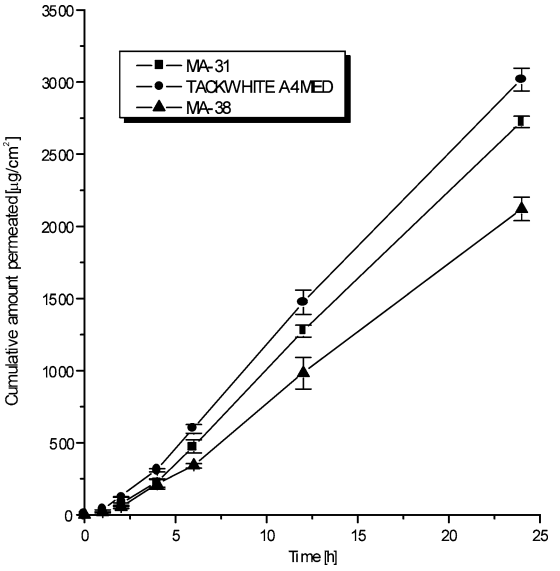

Fig. 3. Mean (±S.D.) cumulative amount of nicardipine hydrochloride permeated from 2% HPC gel containing 4% w/w of limonene across the EVA 2825 membrane/skin composite (membrane coated with MA-31, TACKWHITE A 4MED or MA-38).

with MA-31 (123.11±0.85 μg/cm^2 per h) or MA-38 (95.78±4.31 μg/cm^2 per h). This may be due to the stronger adhesion of TACKWHITE A 4MED to the skin than the other adhesives as reported by Kim et al. (2001). The greatest permeability showed by TACKWHITE A 4MED might be also due to lower thickness (21.6 μm) of adhesive layer TACKWHITE A 4MED on EVA 2825 membrane than that with other adhesives (37.5 μm).

The lower permeability of nicardipine hydrochloride through EVA/ skin than that through EVA membrane coated with an adhesive layer may be due to the incomplete adhesion of EVA membrane to the skin resulting in some air pockets that act as barriers between the membrane and the skin (Kim et al., 2001). 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* bioavailability studies of TTS patch of nicardipine hydrochloride

The mean plasma concentration of nicardipine hydrochloride at different time intervals following the application of TTS (with 4%w/w of limonene) or oral administration of immediate release capsule dosage form (30 mg) is shown in Fig. 4. The plasma concentration of nicardipine hydrochloride gradually increased and attained average steady state level of 32.11±0.76 ng/ml at about 5.67 h (lag period). However, the steady state concentration of the drug declined gradually after 26 h. Thus, the steady state concentration of nicardipine hydrochloride (29.34±1.21 ng/ml) was maintained for 20 h. The pharmacokinetic parameters of nicardipine hydrochloride such as *C*max, *T*max, $AUC_{0-\alpha}$ and relative bioavailability following oral administration of immediate release capsule dosage form and application of TTS patch are given in Table 3. The pharmacokinetic parameters of nicardipine hydrochloride (*C*max, *T*max, $AUC_{0-\alpha}$ and relative bioavailability) from TTS patch (containing 4% w/w of limonene) were significantly different ($P < 0.001$) than those

Table 1
Effect of pressure-sensitive adhesives on the permeability of nicardipine hydrochloride through 2%w/w HPC gel containing 4%w/w of limonene across EVA 2825 membrane

Adhesive	Permeability parameters		
	J (μg/cm^2 per h)[a]	k_p (cm/h X 10^3)[a]	Q_{24} (μg/cm^2)[a]
Without adhesive (control)	212.34 ± 6.98	22.24 ± 1.85	5028.67 ± 176.92
MA-38	192.99 ± 2.99*	19.30 ± 0.30*	4607.74 ± 76.84*
MA-31	170.96 ± 1.84*,#	17.10 ± 0.18*,#	3908.26 ± 101.87*,#
TACKWHITE A 4MED	167.08 ± 3.80*,#	16.71 ± 0.38*,#	4037.71 ± 54.59*,#

Q_{24} cumulative amount of nicardipine hydrochloride after 24 h. *, Significant at $P < 0.001$ when compared without adhesive (control). #, Significant at $P < 0.001$ when compared with MA-38.

[a] Mean ± S.D., $n = 3$.

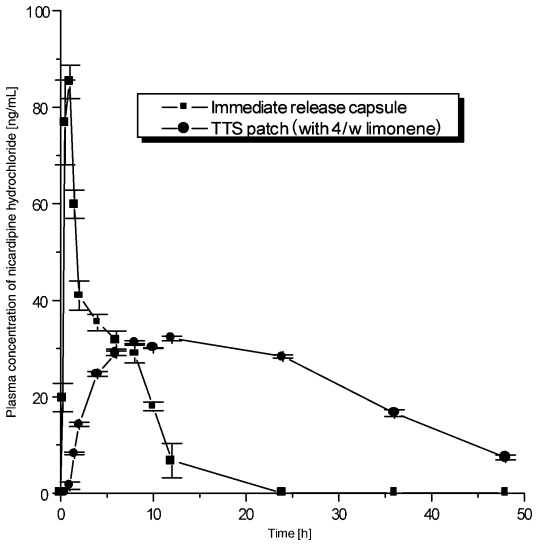

Fig. 4. Mean (±S.D.) plasma concentration of nicardipine hydrochloride following the oral administration of immediate release capsule dosage form and application of TTS patch (with 4% w/w limonene) in human volunteers.

from immediate release capsule dosage form. Unlike oral dosing, the drug levels after the application of TTS (with 4%w/w of limonene) were constant for about 20 h. It took about 11 h (Tmax) to reach maximum concentration of 32.11 ± 0.76 ng/ml (Cmax). However, on oral administration as an immediate release capsule, the Cmax (90.09 ± 5.758 ng/ml) of nicardipine hydrochloride reached within 0.83 h and declined rapidly after 3.5 h.

The intersubject variation in plasma nicardipine hydrochloride level observed on oral administration of immediate release capsule was significant ($P < 0.05$). The low variation (less than 3% of CV) 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 intersubject variation in plasma levels observed in the volunteers receiving immediate release capsule could be due to the high

Table 2
Effect of pressure-sensitive adhesives on the permeability of nicardipine hydrochloride through 2% w/w HPC gel containing 4% w/w of limonene across EVA membrane/skin composite

Adhesive	Permeability parameters		
	J (μg/cm^2 per h)[a]	k_p (cm/h X 10^3)[a]	Q_{24} (μg/cm^2)[a]
MA-38	95.78 ± 4.31	9.58 ± 0.43	2236.64 ± 107.67
MA-31	123.11 ± 0.85*	12.30 ± 0.08*	2871.21 ± 41.85*
TACKWHITE A 4MED	136.29 ± 2.72*,#	13.63 ± 0.27*,#	3167.07 ± 57.88*,#

Q_{24} cumulative amount of nicardipine hydrochloride after 24 h. *, Significant at $P < 0.001$ when compared with MA-38. #, Significant at $P < 0.01$ when compared with MA-31.

[a] Mean ± S.D., $n = 3$.

Table 3
Pharmacokinetic parameters of nicardipine hydrochloride following oral administration of immediate release capsule dosage form (30 mg) and application of TTS patch (4%w/w of limonene) in human volunteers ($n = 6$)

Formulation	C_{max} (ng/ml)	T_{max} (h)	$AUC_{0-\alpha}$ (ng/h per ml)	Relative bioavailability (%)	Lag period (h)
Immediate release capsule dosage form	90.09 ± 5.58	0.83 ± 0.17	456.17 ± 12.01	–	0.21 ± 0.02
TTS patch (with 4% w/w of limonene)	32.11 ± 0.76*	11.33 ± 0.56*	1196.31 ± 16.46*	262.43 ± 10.31*	5.67 ± 0.57*

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

variation in gastric emptying and GI absorption etiology of individual subjects (Hunt, 1951; Hunt and Spurgel, 1951; Jain et al., 1990).

The relative bioavailability of nicardipine hydrochloride with the TTS patch was found to be highly significant ($P < 0.001$) indicating the improved bioavailability on transdermal delivery of the drug (Table 3). The reported (Dow and Graham, 1986) low bioavailability of nicardipine hydrochloride (32%) might be due to the extensive first pass metabolism. However, TTS patch (with 4%w/w of limonene), designed in the present study, was found to enhance the bioavailability of nicardipine hydrochloride by 2.62 times (mean relative bioavailability 262.43 ± 10.31) with reference to an immediate release capsule dosage form. Considering the reported (Dow and Graham, 1986) mean oral bioavailability as 32%, the TTS patch, used in the present study, provided 83.84% 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 4%w/w of limonene), designed in the present study, was found to provide prolonged steady state concentration of nicardipine hydrochloride with minimal fluctuations and improved bioavailability. The TTS application sites, in volunteers, were examined visually for signs of local irritation after wearing the device for 2 days. No local irritation was observed at application site indicating that the device was well tolerated on dermal application after 2 days of patch application.

Krishnaiah et al. (2002c) studied the effect of menthol on the in vivo performance of the TTS of nicardipine hydrochloride in human volunteers. The permeation rate of nicardipine hydrochloride across the rat abdominal epidermis was found to increase from 31.95 ± 2.74 to 204.06 ± 2.31 μg/cm^2 per h with the incorporation of 5% w/w of menthol to 2% w/w HPC gel, where as in the present study the in vitro skin permeability was increased to 240.31 ± 1.57 μg/cm^2 per h with the addition of low concentration of limonene (4%w/w). The in vitro permeability of nicardipine hydrochloride through EVA membrane (coated with TACKWHITE A 4MED)/ skin composite was 122.53 ± 1.87 μg/cm^2 per h, but in the present study (4%w/w of limonene), it was 136.29 ± 2.72 μg/cm^2 per h. The observed increase in the steady state plasma concentration of the drug from 21 (with 5%w/w menthol) to 29 ng/ml (with 4%w/w limonene) might be because of this increased permeability with limonene. But the steady state plasma concentration of the drug (29 ng/ml), in the present study, was maintained for about 20 h when limonene was incorporated as penetration enhancer. Thus the study shows that 4%w/w of limonene appears to be a better penetration enhancer than 5%w/w of menthol in providing higher plasma concentration of nicardipine hydrochloride. This may be due to the difference in their physico–chemical nature of the penetration enhancers (El-Kattan et al., 2000).

The successful outcome of the present study warrants for further studies in patient volunteers to assess the ability of the above TTS formulations of nicardipine hydrochloride in providing an effective and safe therapy of hypertension, and such studies are in progress.

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