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Design and *in vivo* evaluation of an indapamide transdermal patch☆

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ABSTRACT

The aim of the present study was to develop and evaluate a novel drug-in-adhesive transdermal patch system for indapamide. Initial *in vitro* experiments were conducted to optimize formulation parameters prior to transdermal delivery in rats. The effects of the type of adhesive and the content of permeation enhancers on indapamide transport across excised rat skin were evaluated. The results indicated that DURO-TAK® adhesive 87-2852 is a suitable and compatible polymer for the development of transdermal drug delivery systems for indapamide. The final formulation contained 4% N-dodecylazepan-2-one, 6% *l*-menthol and 3% isopropyl myristate. For *in vivo* studies patch systems were administered transdermally to rats while orally administered indapamide in suspension was used as a control. The PK parameters, such as the maximum blood concentration (C_{max}), time to reach the peak blood concentration (T_{max}), mean residence time (MRT), area under the curve (AUC_{0-t}) and terminal elimination half-life ($T_{1/2}$) were significantly ($p < 0.05$) different following transdermal administration compared with oral administration. In contrast to oral delivery, a sustained activity was observed over a period of 48 h after transdermal administration. This sustained activity was due to the controlled release of drug into the systemic circulation following transdermal administration.

1. Introduction

Mortality from heart diseases increases dramatically with age. Heart disease deaths that occur before the age of 65 are generally considered premature, preventable deaths and are, therefore, of particular public health significance. Hypertension is one of the main causes of heart disease and, in recent years, the age adjusted hypertension and hypertensive disease death rates have been increasing (Hoyert et al., 2005). Consequently, the prevention and treatment of hypertension is of major social significance (Kim and Kitakaze, 2004).

Transdermal patches are innovative drug delivery systems intended for skin application to achieve a systemic effect. Among the different types of systems, the drug-in-adhesive products, in which the drug is included in the adhesive layer contacting the skin, are very commonly used, being thin and comfortable to wear. More and more efficient systems have been introduced into the market, with the advantage of reducing the size of the patch to that of a postage stamp (DOT-Matrix®, Novogyne Pharmaceuticals).

A transdermal patch is a medicated adhesive patch that is placed on the skin to deliver a time-released dose of medication through the skin to treat systemic conditions. Since the early 1980s, this dosage form of transdermal therapeutic system (TTS) has been available commercially. Such a system offers a variety of significant clinical benefits over other systems, such as tablets and injections. For example, it provides controlled release of the drug, and produces a steady blood-level profile, leading to reduced systemic side effects and, sometimes, improved efficacy over other dosage forms (Ranade, 1991; Modamio et al., 2000; Ke et al., 2005). In addition, the transdermal patch dosage form is user-friendly, convenient, painless, and offers multi-day dosing, it generally leads to improved patient compliance (Audet et al., 2001). Consequently, the transdermal therapeutic system is of particular clinical significance for the prevention and long-term treatment of chronic diseases like hypertension.

Indapamide (Fig. 1) is a long-acting hypertensive with both diuretic and vasodilative actions and is defined by the 1999 WHO/ISH Hypertension Guidelines and JNC VII as a first-line drug for the treatment of hypertension (Jianliang et al., 2004). Indapamide (4-chloro-N-(2-methyl-1-indolinyl)-3-sulphamoyl benzamide) is a non-thiazide indole derivative of chlorosulphonamide, which has an anti-hypertensive action causing a drop in systolic, diastolic and mean blood pressure. This anti-hypertensive action is maximal at a dose of 2.5 mg/day and the diuretic effect is slight, usually without any clinical manifestation although at higher doses the diuretic effect becomes more prominent. The extra-renal antihypertensive action of 2.5 mg/day is demonstrated as a reduction in vascular hyperactivity and a reduction in total peripheral and arteriolar resistance. The extra-renal mechanism of action has also

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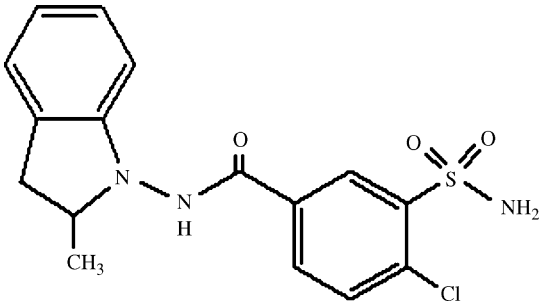

Fig. 1. The chemical structure of indapamide.

been demonstrated by the maintenance of the anti-hypertensive effect in functionally anephric patients. The extra-renal action is thought to be due to the inhibition of transmembrane ionic influx, essentially that of calcium, and the stimulation of the synthesis of the vasodilatory hypotensive prostaglandin PGE2 (Chaffman et al., 1984).

We have recently investigated the feasibility of the transdermal use of indapamide, evaluating the transdermal delivery of indapamide *in vitro* and have successfully used a variety of permeation enhancers (Changshun et al., 2008). These experiments demonstrate that significant amounts of indapamide can be delivered across the skin from solution formulations. The aim of the present study was to investigate indapamide transport from a transdermal patch system and to determine whether therapeutically relevant delivery rates could be achieved under these conditions. After an initial investigation of formulation parameters their effect on indapamide transport across rat skin was also investigated *in vitro*. The effect of the type of adhesive and the content of permeation enhancers on indapamide transport across the skin was evaluated. For *in vivo* studies, patch systems were administered transdermally to rats while orally administered indapamide in suspension was used as a control. A sustained activity was observed over a period of 48 h after transdermal administration compared with oral administration. The sustained activity was due to the controlled release of drug into the systemic circulation following transdermal administration.

2. Materials and methods

2.1. Materials

Indapamide was a gift from the Kangya Pharmaceutical Co., Ltd. (Ningxia, China); acetaniline was purchased from the Beijing Xingjin Chemical Plant (Beijing, China); carbamazepine was obtained from Wanqing Pharmaceutical Co., Ltd. (Suzhou, China); isopropyl myristate (IPM), N-dodecylazepan-2-one, and *l*-menthol were supplied by China National Medicines Co., Ltd. (Shanghai, China); ethanol (EtOH), and polyethylene glycol 400 (PEG400) were obtained from the Bodi Drug Manufacturing Co., Ltd. (Tianjin, China). DURO-TAK® adhesives 87-2852, 87-2677 and 87-4098 were gifts from the National Starch and Chemical Company (Bridgewater, NJ, USA). Methanol was of HPLC grade and was obtained from the Yuwang Pharmaceutical Co., Ltd. (Shandong, China). All other chemicals were of the highest reagent grade available.

A phosphate buffer solution at pH 7.4 was prepared (1.36 g Na_2HPO_4 was added to 79 ml 0.1 mol/L NaOH, then add water to 200 ml). The water used was deionized and distilled in an all-glass still, and it is subsequently referred to as distilled water. A 0.1 mm cellulose nitrate 0.45-μm pore size membrane was obtained from FLM-Technology Development Co., Ltd. (Tianjin, China).

A Flying Pigeon centrifuge TGL-16G (Shanghai, China), SW-20C, water-bath, BRAUN InterFace 3731 shaver (Gillette Japan, Yokohama, Japan) and National ER804 electric clippers (Matsushita Electric Works, Ltd., Osaka, Japan) were used in the experiments.

2.2. Preparation of patches

Transdermal patches were prepared by the solvent evaporation technique. Adhesive patches containing indapamide (5% (w/w) based on polymer weight) were prepared using DURO-TAK® adhesives (National Starch and Chemical Company, USA). Appropriate amounts of pressure sensitive adhesive, permeation enhancers (viz. N-dodecylazepan-2-one, *l*-menthol, IPM) and drug were added to ethanol on a weight basis, agitated at room temperature for 24 h, spread on a silicone-coated liner (Scotchpak™, 3M, St. Paul, USA) with a wet film applicator (SLT200, Shanghai Kaikai Co., Ltd., China) to give a film thickness of 200 μm, and kept at room temperature for 1 h and then at 70 °C in an oven for 10 min (to remove any residual solvent). The patches were then covered with backing membrane (CoTran™, 3M, St. Paul, USA), cut into appropriate sizes, packed in Al-foil and stored in a desiccator for further studies.

2.3. Physicochemical evaluation of indapamide transdermal patch

2.3.1. The tack of the patches

Tack is the ability of a pressure-sensitive adhesive to bond under conditions of light contact pressure and a short contact time. The tack of the skin contact adhesive was measured by the rolling ball tack test using a CZY-G primary adhesive tester (Languang M&E Tech Development Center, Jinan, China). The patch with a width of 40 mm and a length of 50 mm was fixed on a plate. Different diameter steel balls were released from the top of the inclined plate (angle 45°). The number of the largest ball which did not roll down was reported as the tack value.

2.3.2. The shear adhesion of the patches

The shear adhesion test was carried out using a CZY-S lasting adhesive tester (Languang M&E Tech Development Center, Jinan, China). The patch sample was applied to a stainless-steel test panel that was mounted vertically, and subjected to a shearing force by a means of a given weight (1000 g) suspended from the patch. The time taken for the patch sample to detach from the test panel was recorded as the value of the shear strength.

2.3.3. The peel strength of the patches

Peel strength measures the force required to peel away a pressure-sensitive adhesive once it has been attached to a surface. The test was performed with a BLD-30S auto stripping tester (Languang M&E Tech Development Center, Jinan, China). Most currently used test methods for patch peel strength use a stainless-steel test panel as the substrate, a peel angle of 180°, with the sample cut into 3 cm widths and a test velocity of 300 mm/min.

2.4. Experimental procedure

2.4.1. In vitro

Male Wistar rats weighing 180–220 g (6–8 weeks old) used in all experiments were supplied by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). The experiments were performed in accordance with the guidelines for animal use published by the Life Science Research Center of Shenyang Pharmaceutical University. The rats were anesthetized with urethane (20% (w/w) i.p.) and the abdomen carefully shaved with a razor after removal of hair by electric clippers. After sacrificing the rats by dislocating the spinal cord, a full thickness skin (i.e. epidermis with stratum corneum and dermis) was excised from

the shaved abdominal site. The integrity of the skin was carefully ascertained by microscope observation, any skin which had low uniformity was rejected. After removing the fat and sub-dermal tissue, the skin was kept frozen at −20 °C and used within 1 week. Before starting the experiment it was allowed to reach room temperature for at least 10 h. Skin permeation studies were performed using a two-chamber side-by-side glass diffusion cell (effective diffusional area = 0.95 cm^2) equipped with star-head magnetic stirrers and a water-jacket. The excised abdominal skin was mounted between the cell halves so that the dermal side of the skin faced the receiver fluid. A circular transdermal patch was pressed on the skin with the adhesive side facing the stratum corneum. After securely clamping the cell assembly together, the receptor compartment was filled with 2.5 ml pH 7.4 phosphate buffer solution containing 40% (v/v) PEG400 (40% PEG400 PBS) to maintain sink conditions and continuously stirred at about 600 rpm. The temperature of the cell was maintained at 32 ± 1 °C using thermostatically controlled water which was circulated through a jacket surrounding the cell body throughout the experiments. Care was taken to ensure that no air bubbles remained in the water-jacket. At predetermined time intervals, 2.0 ml of receptor solution was taken for analysis and replaced with the same volume of fresh solution to maintain sink conditions. The drug concentration was determined by reversed phase HPLC with reference to a calibration curve. The cumulative amount of indapamide passing across rat skin was calculated using the measured indapamide concentrations in the receiver solutions.

2.4.2. In vivo

The study was conducted in accordance with the Ethical Guidelines for Investigations in Laboratory Animals and was approved by the Ethics Review Committee for Animal Experimentation of Shenyang Pharmaceutical University. Wistar rats were used after being allowed to acclimatize for 1 week. Before the day of administration, rats were fasted overnight but were allowed access to water ad libitum. Twelve 180–220 g (6–8 weeks old) rats were used in the study. Each rat received indapamide suspension twice (the second time was 24 h immediately after collection of the blood sample) or an indapamide transdermal patch in a randomized order, and the patch was removed after an interval of 48 h. The weight of the animals was measured and recorded before the start of each experiment. Rats were anesthetized with urethane (1 g/kg, i.p.) and, following anaesthesia, animals were secured in a supine position and placed on a surgical table. Thereafter, six rats were given intragastric doses of indapamide suspension (1.137 mg/mL in 0.5% (w/v) CMC-Na) 5.685 mg/kg. The hair of the other six rats at the abdominal site of the patch application was clipped before the experiment. Before applying the patches, the skin was gently wiped with warm water followed by an alcohol swab and patted dry. A single patch with an area of about 20 cm^2 and containing 10.0 mg indapamide was then applied to the shaved skin. Blood samples of approximately 0.15 ml were collected in dried heparinized tubes at 0, 0.08, 0.17, 0.5, 1, 2, 4, 8, 12, 24, 24.08, 24.17, 24.5,25, 26, 28, 32, 36, 48, 52, 56 and 60 h after oral administration and 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 28, 32, 36, 48, 52, 56 and 60 h after transdermal administration from the jugular vein and, as soon as possible, 0.1 ml of the whole blood was transferred accurately to another tube which was then frozen at −20 °C and stored until analysis.

2.5. Analytical methods

2.5.1. In vitro

The quantitative determination of indapamide was performed by HPLC with a methanol solution of acetaniline as internal standard and with reference to a calibration curve. A Hitachi chromatograph (pump L-2130, UV–vis detector L-2420, T2000L workstation) and a Hypersil ODS 5 μm 200 mm × 4.6 mm column (Dalian Elite Analytical Instruments Co., Ltd., DEAIC) were used. A mixture of methanol, distilled water and acetic acid (45:55:0.1, v/v/v) was used as a mobile phase, The column was maintained at 40 °C, and the flow rate was 1 ml/min while the UV detector was set at 240 nm. The retention times in this assay were found to be 3.87 and 6.07 min for internal standard and indapamide, respectively. The Hypersil ODS analytical column and the mobile phase used for the assay provided a clear separation between the drug and internal standard. There was no interference from any endogenous material.

2.5.2. In vivo

The whole blood samples were first allowed to thaw at room temperature. After vortexing, 100 μl of each whole blood sample was transferred to a 2 ml Eppendorf tubes and then 100 μl of an aqueous solution of carbamazepine (2.036 μg/ml) was added to the whole blood sample containing indapamide. The mixture was extracted with 0.6 ml diethyl ether by vortex for 1 min, and then centrifuged at 3000 rpm for 10 min in a microcentrifuge. The upper organic phase was transferred to another tube and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was then reconstituted in 100 μl mobile phase, and mixed by vortexing for 30 s, then a 20 μl aliquot of the solution was injected directly into the HPLC system for analysis. The analytical system and the mobile phase of the HPLC system were the same as that used in the *in vitro* studies. The column was also maintained at 40 °C, the flow rate was 1 ml/min while the UV detector was set at 240 nm. The retention times were found to be 6.07 and 9.63 min for indapamide and internal standard (carbamazepine), respectively. The Hypersil ODS analytical column and the mobile phase used for the assay provided a clear separation between the drug and internal standard. There was no interference from any endogenous material.

2.6. Statistical analysis

2.6.1. Data analysis in vitro

All experiments were replicated at least four times. The amount of each drug permeating through the skin during a sampling interval was calculated based on the measured receptor-phase concentration and volume. The cumulative amount of drug permeating per unit area *versus* time was plotted. All data were calculated and presented as mean ± S.E. The slope of the linear portion of the plot was calculated as the flux (μg/(cm^2 h)). The lag-time was determined by extrapolation of the linear portion of the cumulative amount of drug permeated *versus* time plot to the abscissa. For comparison between two groups of data, significance was determined by Student's *t*-test. Data were considered significant at $p < 0.05$.

2.6.2. Pharmacokinetic analysis

PK parameters such as the peak blood concentration (C_{max}) and time to reach this peak (T_{max}) were read directly from the individual blood concentration–time profiles. The AUC_{0-t} was calculated by the linear trapezoidal rule. The other PK parameters, e.g. biological half-life ($T_{1/2}$) and mean residence time were calculated using a computer programme 'DAS 2.0' which measures $T_{1/2}$ from the regression of the terminal phase of the concentration time plot. The MRT was calculated by dividing the $AUMC_{0-t}$ by AUC_{0-t}. The differences in various PK parameters were evaluated statistically by ANOVA.

3. Results

3.1. Effect of adhesives and permeation enhancers on skin permeation of indapamide in vitro

Choosing an ideal adhesive is important in designing a transdermal drug delivery system, since adhesion, chemical stability, and

Table 1
Chemistry and solvent composition of selected adhesives used in the study.

Adhesive	Chemical composition	Functional groups	Crosslinker	Solvent composition	Tack (Oz/in^2)
87-2852	Acrylate	–COOH	Present	Ethyl acetate (65%), isopropanol (19%), hexane (12%), toluene (2%), 2,4-pentanedione (<1%)	40
87-2677	Acrylate	–COOH	Present	Ethyl acetate 37%, isopropanol 37%, heptane 21%, toluene 5%, 2,4-pentanedione <1%	5
87-4098	Acrylate–vinylacetate	None	Not present	Ethyl acetate 100%	19

compatibility with other components of the system severely affect the transdermal properties of the drug candidate. *In vitro* skin permeation tests are very useful for selecting suitable adhesives for formulation of patches. In order to select a suitable adhesive for the indapamide patch, acrylic adhesives with or without carboxylate functional groups, and with or without crosslinking agents, were selected as listed in Table 1. These adhesives were selected based on their chemical composition, solvent compatibility, and tack properties, in order to design an ideal patch for indapamide. Initial studies were carried out by loading 5% (w/w) indapamide in different adhesives. The patches were smooth, uniform and flexible. The thickness of the patches was measured with a micrometer and was calculated by subtracting the combined thickness of the backing membrane and release liner from the thickness of the whole patch. The thickness of all the patches used was 100 ± 10 μm. Preliminary studies revealed that the drug was uniformly distributed throughout the film and that there were no interactions between the drug and carriers used, and the drug permeation properties were evaluated from these skin permeation studies. The cumulative permeation profiles of indapamide from the three different adhesives are shown in Fig. 2. A steady and continuous permeation of indapamide from all the patches was observed, indicating that indapamide was dissolved in all the adhesives with a uniform distribution. The cumulative amount of indapamide permeated from the 87-2852 adhesive was 1.55 and 1.54 times higher than the 87-2677, and 87-4098 adhesives, respectively. A *t*-test showed that the difference was statistically significant ($p < 0.05$). These results indicated that 87-2852 is a suitable and compatible polymer for the development of transdermal drug delivery systems for indapamide.

The use of chemical penetration enhancers seems to be an effective way to reduce the barrier properties of the stratum corneum. Current research focuses particularly on non-irritant and non-toxic enhancers (Akimoto and Nagase, 2003). The incorporation of permeation enhancers into transdermal drug delivery systems is essential to increase the permeation of indapamide from the patches. In our study, various permeation enhancers known to be potent and safe were selected, and their effects on the permeation of indapamide through excised rat skin were investigated.

Further studies were carried out by preparing 87-2852 adhesive patches with different loadings of permeation enhancers. Fig. 3 shows the permeation profiles of indapamide from transdermal patches containing 2%, 4% and 6% N-dodecylazepan-2-one alone. At 4% concentration, the cumulative amount of indapamide permeated was the highest followed by concentrations of 6%, 0% and 2%. Correspondingly, the fluxes were 0.30, 0.15, 0.42 and 0.36 μg/(cm^2 h) for 0%, 2%, 4% and 6%, respectively. There was a significant difference ($p < 0.05$) between each of these except for between 0% and 6% ($p > 0.05$). Since 2% N-dodecylazepan-2-one can significantly inhibit the permeation of indapamide, while there was no significant difference at a concentration of 6% ($p > 0.05$) compared with the 0% concentration, 4% N-dodecylazepan-2-one was chosen.

N-dodecylazepan-2-one is known to be safe and has been used to increase the skin permeation of a large number of drugs. N-dodecylazepan-2-one is non-irritant to human skin, even in undiluted form (Stoughton, 1982), reversible in its action (Adachi et al., 1988) and very poorly absorbed through human skin (Wiechers et al., 1988). N-dodecylazepan-2-one is also included in the Chinese Pharmacopoeia (2005) and is an ideal permeation enhancer for clinical use. This enhancing activity of N-dodecylazepan-2-one is due to the direct effects on the barrier properties of the skin. It has been reported that N-dodecylazepan-2-one affects the lipid

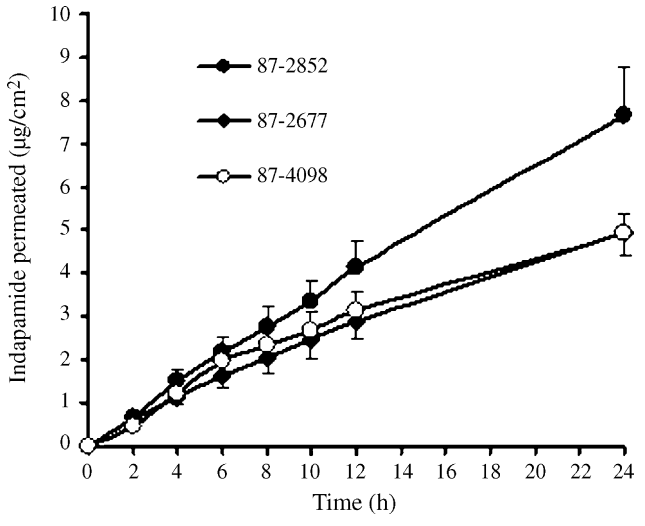

Fig. 2. The penetration profiles of indapamide patches in the presence of different adhesives (each point represents the mean ± S.E. of four experiments).

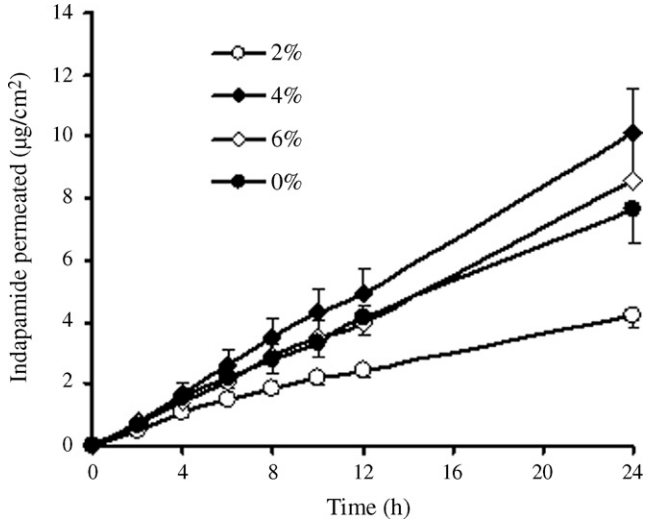

Fig. 3. The penetration profiles of indapamide patches in the presence of different percentages of N-dodecylazepan-2-one (each point represents the mean ± S.E. of four experiments).

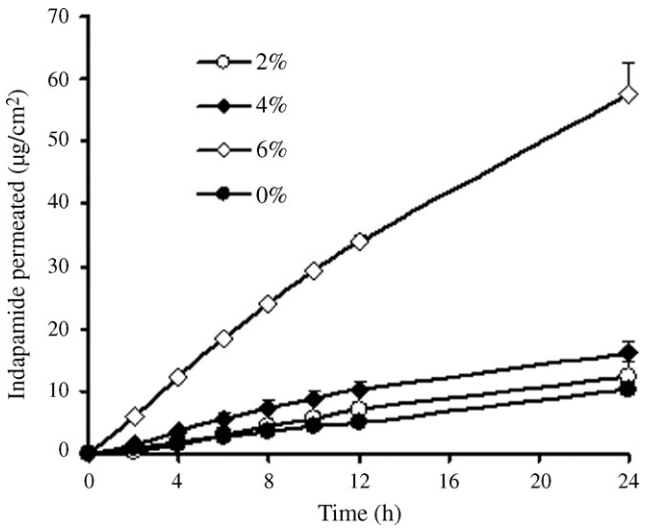

Fig. 4. The penetration profiles of indapamide patches in the presence of 4% N-dodecylazepan-2-one and different percentages of *l*-menthol (each point represents the mean ± S.E. of four experiments).

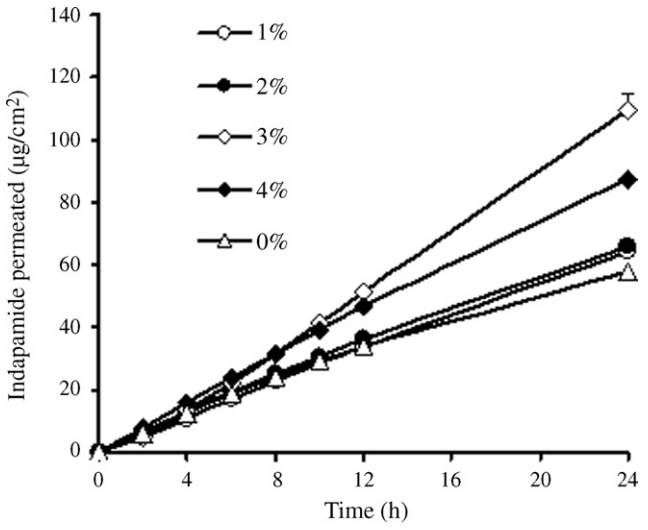

Fig. 5. The penetration profiles of indapamide patches in the presence of 4% N-dodecylazepan-2-one, 6% *l*-menthol and different percentages of IPM (each point represents the mean ± S.E. of four experiments).

structure of the stratum corneum (Barry, 1987), and reduces transition temperatures within lipid bilayers to induce formation of a liquid phase with a resultant increase in lipid fluidity (Beastall et al., 1988).

Fig. 4. presents the penetration profiles of indapamide from transdermal patches containing 2%, 4% and 6% *l*-menthol in addition to 4% N-dodecylazepan-2-one. Although a moderate increase in permeation of the drug was achieved by incorporating N-dodecylazepan-2-one in the patch, a clear increase in the permeation of the drug was found following the co-application of *l*-menthol and N-dodecylazepan-2-one. One possible hypothesis to explain such an increase in the cumulative amount and flux is that *l*-menthol could work synergistically with N-dodecylazepan-2-one (Williams and Barry, 2004), while *l*-menthol acts by disrupting the lipid structure of the stratum corneum, thereby increasing the diffusion coefficient of the drug in the membrane (Williams and Barry, 1991; Kunta et al., 1997). The maximum accumulated permeation was observed at a 6% *l*-menthol concentration, and was approximately 5.67-fold that obtained with 4% N-dodecylazepan-2-one alone, while the corresponding figures were 1.22- and 1.60-fold for 2% and 4% *l*-menthol concentrations compared with 4% N-dodecylazepan-2-one alone. A *t*-test showed that these differences were statistically significant ($p < 0.05$). The fluxes were 0.49, 0.54 and 2.07 μg/(cm^2 h) for 2%, 4% and 6% *l*-menthol in addition to 4% N-dodecylazepan-2-one, respectively. On account of the flavour of *l*-menthol, the 6% concentration was selected for further research.

Fig. 5. illustrates the penetration profiles of indapamide from transdermal patches containing 1%, 2%, 3% and 4% IPM on the basis of 4% N-dodecylazepan-2-one and 6% *l*-menthol. The fluxes of indapamide from patches were 2.56, 2.53, 4.89 and 3.49 μg/(cm^2 h) following the addition of 1%, 2%, 3% and 4% IPM, respectively. 3% IPM had the greatest enhancing effect on the permeation of indapamide, in terms of the accumulated amount permeated, followed by 4%, 2% and 1% IPM in that order. Addition of 1% and 2% IPM appeared to increase the transport of indapamide, but this increase was not statistically significant ($p > 0.05$). IPM enhances the skin permeation rate of progesterone, estradiol, indomethacin and methyl nicotinate (Leopold and Maibach, 1996), tacrine (Kim et al., 1992) and benztropine (Gorukanti et al., 1999). IPM is known to act as a fluidizer of intercellular lipids, and affects the lipid-rich phase in the stratum corneum, thereby reducing its barrier function. The final formulation contained 4% N-dodecylazepan-2-one, 6% *l*-menthol and 3% IPM.

3.2. Evaluation of the adhesive properties of the patches

Generally, patches must be permanently tacky at room temperature; they must adhere spontaneously to the surfaces they contact following only light finger pressure; and they must possess sufficient cohesive strength that, upon removal from a surface, they leave no adhesive residue. Three important performance tests of patches are tack, shear strength and peel strength.

Tack is a composite response of the material surface (energy and roughness) and bulk (viscoelastic and thickness) properties. The effects of skin permeation enhancers on the tack of the patches were investigated. It was observed that the tack value of the samples containing permeation enhancers was that of ball number 29. The tack test results confirmed the good adhesive properties of the patches on the skin.

Shear strength or creep resistance is regarded as a measure of cohesive strength. If a patch has adequate cohesive strength, it will not slip after application and will leave no residue upon removal. The longer the time it takes to pull the patch off the panel, the greater the shear strength. It was observed that the shear strength values of the patches containing permeation enhancers were between 20 and 30 min.

Peel strength is the force required to remove the patch from a test substrate. It is important in transdermal drug delivery system applications because the bond must provide adequate adhesion to the skin, yet allow non-traumatic removal. Peel strength is the force per unit width required to break the bond between the patch and the stainless-steel. In other words, peel values are reported as force per unit width (e.g. kN/m), with higher values indicating greater bond strength. The value for the peel strength is independent of the length but is dependent on the width of the sample. The force data were gathered as a function of time, and the average value was used as a measure of the peel strength, from 0.4 to 0.6 kN/m. If the pulled patch did not leave any residue on the panel, this indicated "adhesive failure", which is desirable for a transdermal drug delivery system. During the test, the patches were stripped cleanly from the plate and left no visually noticeable residue. This proved that the anchorage of the matrix to the backing membrane and the cohesion of the matrix were good.

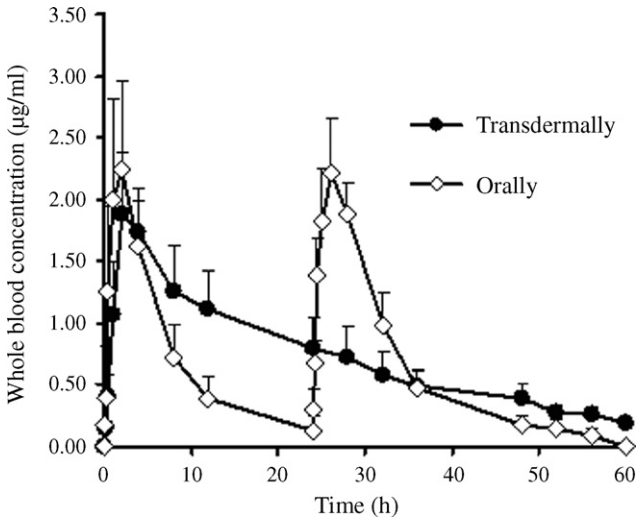

Fig. 6. Whole blood concentration–time profiles of indapamide after two (the second time is at 24 h immediately after the blood sample was collected) oral administrations of indapamide suspension (1.137 mg/mL in 0.5% (w/v) CMC-Na) 5.685 mg/kg and transdermal administration as a single patch (containing indapamide 10.0 mg, and the patch was removed from the rat after an interval of 48 h) (each point represents the mean ± S.D.; $n = 6$).

3.3. Effect on pharmacokinetics

3.3.1. Concentration–time profiles

The mean whole blood concentration–time profiles of indapamide after oral and transdermal administration are shown in Fig. 6. The results for the oral administration of indapamide indicated that it was rapidly absorbed from the rat gastrointestinal tract with a C_{max} of 2.25 ± 0.73 μg/ml at a T_{max} of 2.17 ± 0.98 h after the first administration, and with a C_{max} of 2.22 ± 0.44 μg/ml at a T_{max} of 2.33 ± 0.82 h after the second administration. Transdermal administration of indapamide achieved a C_{max} of 1.97 ± 0.43 μg/ml at a T_{max} of 2.67 ± 1.03 h, and the whole blood concentration of indapamide declined more slowly than that following oral administration. Furthermore, it was observed that there were no significant changes to the skin surface after removal of the indapamide transdermal patch. Upon removal of the transdermal patch, a mild reservoir effect was observed for about 12 h followed by normal elimination similar to that after oral administration.

3.3.2. Pharmacokinetic parameters

The pharmacokinetic parameters of indapamide are presented in Table 2. Compared with two oral administrations without dose normalization, the AUC_{0-t} values were significantly increased: 44.16 ± 10.82 μg/(ml h) for indapamide transdermally *versus* 35.43 ± 6.37 μg/(ml h) for indapamide orally, and the MRT values were prolonged: 20.77 ± 1.03 h for indapamide transdermally *versus* 18.01 ± 2.35 h for indapamide orally. Similarly, the apparent $T_{1/2}$ values were also markedly increased: 18.17 ± 2.45 h for indapamide transdermally *versus* 7.19 ± 2.37 h for indapamide orally.

Table 2
Pharmacokinetic parameters of indapamide after two (the second time is at 24 h immediately after the blood sample was collected) oral administrations of indapamide suspension (1.137 mg/mL in 0.5% (w/v) CMC-Na) 5.685 mg/kg and transdermal administration as a single patch (containing indapamide 10.0 mg, and the patch was removed from the rat after an interval of 48 h) ($n = 6$).

Parameters	Transdermal	Oral
C_{max1} (μg/ml)	1.97 ± 0.43	2.25 ± 0.73
T_{max1} (h)	2.67 ± 1.03	2.17 ± 0.98
C_{max2} (μg/ml)		2.22 ± 0.44
T_{max2} (h)		2.33 ± 0.82
$AUC_{0-t\ h}$ (μg/(ml h))	44.16 ± 10.82	35.43 ± 6.37
MRT (h)	20.77 ± 1.03	18.01 ± 2.35
$T_{1/2}$ (h)	18.17 ± 2.45	7.19 ± 2.37

4. Discussion

Transdermal delivery offers several advantages over oral routes for controlled drug delivery (Berner and John, 1994) viz., avoidance of hepatic first-pass metabolism, the ability to control drug delivery for a longer time than the gastrointestinal transit of oral dosage forms, the ability to avoid a changing physiological environment and chemical or metabolic degradation, the ability to discontinue administration by removal of the system. Dermally applied materials, absorbed in quantities large enough to elicit a pharmacological effect, have been used for years (Hadgraft, 1996).

In the present study, we developed a single-layer drug-in-adhesive type of trandermal patch, in which the adhesive layer not only serves as an adherent layer to the skin but is also responsible for the release of drug. On both sides of the adhesive layer, there is a temporary liner-layer and a permanent backing.

Drug-in-adhesive type transdermal patches of indapamide were prepared and the effects of adhesives and permeation enhancers on the permeation of indapamide were studied. The results indicated that DURO-TAK® adhesive 87-2852 is a suitable and compatible polymer for the development of transdermal drug delivery systems for indapamide. The use of chemical penetration enhancers seems to be an effective way to reduce the barrier properties of the stratum corneum. It was proved that N-dodecylazepan-2-one has the best permeation enhancing effect only at the appropriate concentration. Some people considered that the best concentration of N-dodecylazepan-2-one is between 2% and 6%, but it differs with the drug. There is a concentration dependent on the permeation enhancing effect of N-dodecylazepan-2-one. The results of our research proved that at 4% concentration N-dodecylazepan-2 one alone showed higher permeation enhancing effect compared to that of 6% N-dodecylazepan-2 one. This is because when at low concentration, the main effect of N-dodecylazepan-2-one maybe is to increase permeability by disordering or 'fluidising' the lipid structure of the stratum corneum, while at high concentration, N-dodecylazepan-2-one will hamper the permeation of drug because of its high hydrophobicity. The incorporation of permeation enhancers into transdermal drug delivery systems is essential to increase the permeation of indapamide from the patches. In our study, various permeation enhancers known to be potent and safe were selected, and their effects on the permeation of indapamide through excised rat skin were investigated. The final formulation contained 4% N-dodecylazepan-2-one, 6% *l*-menthol and 3% isopropyl myristate. The prepared patches showed good uniformity with regard to drug content.

The low T_{max} and high C_{max} values following oral administration are due to rapid absorption from the gastrointestinal tract. In contrast, the low C_{max} and prolonged T_{max} after transdermal administration of the transdermal patch are due to the barrier properties of the skin which lead to an early accumulation of drug in the skin followed by its sustained release into the systemic circulation. The reservoir effect after removal of the transdermal patches is due to the slow depletion of the drug accumulated in skin tissues and the long elimination half-life of indapamide. The higher MRT values following transdermal delivery compared with the oral route may be due to the continuous replenishment of drug in the systemic circulation by constant and controlled delivery of drug from the transdermal patch.

The low activity after oral administration is in good agreement with PK data, which indicates that the whole blood concentration of indapamide declined rapidly and, hence, produced a shorter

therapeutic action. The sustained response following transdermal administration was due to controlled and continuous release of drug into the systemic circulation over an extended period.

In conclusion, the present data confirm the feasibility of developing indapamide transdermal patches. Further studies, now in progress, will deal with the application of the presently reported findings to human skin permeation, involving *in vivo* tests.

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