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International Journal of Pharmaceutics 350 (2008) 43–47
INTERNATIONAL JOURNAL OF PHARMACEUTICS
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Effect of permeation enhancers and organic acids on the skin permeation of indapamide

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Received 20 March 2007; received in revised form 29 June 2007; accepted 13 August 2007
Available online 22 August 2007

Abstract

The aim of present study was to investigate the transdermal properties of indapamide and to explore the efficacy of various permeation enhancers and organic acids with regard to the percutaneous absorption of indapamide. Permeation experiments were performed *in vitro*, using rat abdominal skin as a barrier. In the permeation studies, 2-chamber diffusion cells were used. The results obtained indicate that *N*-dodecylazepan-2-one, *N*-methyl-2-pyrrolidone, menthol and oleic acid had a strong enhancing effect on the permeation of indapamide and *N*-dodecylazepan-2-one exhibited the most potent enhancing effect. All eight of the organic acids chosen had a potent enhancing effect on the permeation of indapamide across rat abdominal skin. Among the organic acids examined, lactic acid had the greatest enhancing effect. The formation of an ion-pair between indapamide and organic acids may be responsible for the enhanced skin permeation of indapamide. Although the exact reason remains unknown, it is worth carrying out further investigations.

Keywords: Indapamide; Permeation enhancers; Organic acids; Percutaneous absorption; *In vitro*

1. Introduction

The skin has attracted much attention as an alternative route for administering systemically active drugs, but its potential use is often hindered by poor tissue permeability, predominantly attributed to the outermost layer of the skin, the stratum corneum (SC). This layer provides a protective barrier that prevents the loss of physiologically essential substances and limits the diffusion of potentially toxic chemicals from the external environment into the body. Different methods have been used to overcome the barrier property of the SC (Sloan et al., 1984; Mezel, 1985; Morimoto et al., 1992; Chang and Banga, 1998; Prausnitz et al., 2004; Hadgraft and Lane, 2006). One of the most widely used techniques involves chemical penetration enhancers (Smith and Maibach, 1995; Benson, 2005; Vavrova et al., 2005). Ideally, an enhancer should be chemically and pharmacologically inert, nontoxic, non-irritant and non-allergenic, have a rapid and reversible onset of action, be potent at low concentrations, compatible with the formulation ingredients, and cosmetically acceptable (Chattaraj and Walker, 1995).

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 clinical manifestation. At higher doses, the diuretic effect becomes more prominent. The extra-renal anti-hypertensive 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 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

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doi:10.1016/j.ijpharm.2007.08.020

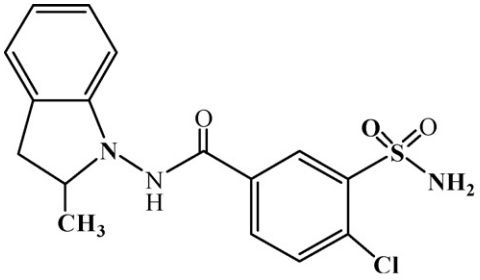

Fig. 1. The chemical structure of indapamide.

of calcium, and the stimulation of the synthesis of the vasodilatory hypotensive prostaglandin PGE2 (Chaffman et al., 1984).

Indapamide is marketed as immediate release pharmaceutical formulations containing 1.25 and 2.5 mg active substance per dose and as sustained-release coated tablets of 1.5 mg per dose. However, oral delivery of this drug has certain disadvantages, such as frequent administration and adverse drug reactions (allergic responses, sweats, sychnosphygmia and the induction of tremor cordis, hypopotassemia, increased blood sugar, extremitas inferior pain, sore neck) (Aiguo et al., 2003). Additionally, since indapamide is usually intended to be taken for a long period, patient compliance is also very important. Transdermal drug delivery offers many advantages, such as reduced side effects, less frequent administration to produce the desired constant plasma concentrations associated with improved patient compliance, elimination of the first-pass effect, sustained drug delivery and interruption of treatment when necessary. Since there are no published reports about the transdermal use of indapamide, we have investigated the feasibility of its transdermal application.

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); isopropyl myristate (IPM), *N*-dodecylazepan-2-one, *N*-methyl-2-pyrrolidone (NMP), menthol were supplied by China National Medicines Co. Ltd. (Shanghai, China); maleic acid, acetic acid, oxalic acid, adipic acid, succinic acid, were purchased from the Yuwang Pharmaceutical Co. Ltd. (Shandong, China); propylene glycol (PG), ethanol (EtOH), polyethylene glycol 400 (PEG400), oleic acid, lactic acid, citric acid, fumaric acid were obtained from the Bodi Drug Manufacturing Co. Ltd. (Tianjin, China). 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.

2.2. Drug analysis

The quantitative determination of indapamide was performed by HPLC with a methanol solution of acetanilide as internal standard and with reference to a calibration curve. A Hitachi instrument (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. 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 well defined separation between the drug and internal standard. There were no interferences from the endogenous components.

2.3. Determination of drug solubility

The saturation solubility of indapmide was determined in distilled water, pH 7.4 phosphate buffer solution containing 40% (v/v) PEG 400 (40% PEG400 PBS) and the individual donor vehicles. Analysis was carried out by placing an excess of the drug with 1 ml of the appropriate solvent in 1.5 ml sealed polypropylene micro-vials, shaking in a water bath at 32 °C for 48 h until equilibrium was achieved. The suspension was passed through a membrane filter (0.45 μm), and the concentration of indapamide in the filtrate was determined by HPLC with reference to a calibration curve after appropriate dilution with methanol when necessary. The experiments were performed in triplicate.

2.4. Skin sample preparation

Wistar male 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 (model 900, TGC, Japan). About 5 cm^2 (circle of 2.5 cm diameter) of skin on the left and right sides of the abdomen was excised and the skin membrane was checked to ensure that no obvious defects were present.

2.5. Permeation experiments

Permeation experiments were conducted at 32 °C in 2-chamber diffusion cells (with an effective diffusional area 0.95 cm^2 and receiver volume of 2.5 ml) (Fang et al., 2002). After removal of hair and subcutaneous fat, the rat abdominal skin membrane was mounted on a 2-chamber diffusion cell with the epidermal side facing the donor cell. The donor cell was filled with a 2.5 ml suspension of indapamide, about twice the solubility in each solvent system. The excess solid of indapamide in the suspension ensured drug saturation conditions in the donor phase throughout the experiment (clearly,

the drug solubility value will be different, depending on the vehicle composition), and the solubility of indapamide in donor phase was sufficient for the permeation. Since indapamide has a low solubility in water, the receptor cell was filled with 2.5 ml 40% PEG400 PBS to increase drug solubility and maintain sink conditions during the experiments. At predetermined time intervals, 2.0 ml of receptor solution was sampled 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.

2.6. Data analysis

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. The permeability coefficient P was calculated by dividing the flux by the solubility of indapamide in the individual donor vehicles. The enhancement ratio (ER) was calculated by dividing the flux of indapamide suspension (with enhancer) by that without enhancer. For comparison between two groups of data, significance was determined by t-test. Data were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Drug solubility

Indapamide, the structure of which appears in Fig. 1, has a molecular weight (MW) of 365.84. Its melting point is 160–162 °C and its solubility in water and 40% PEG400 PBS is 148.10 ± 11.10 μg/ml and 3.23 ± 0.12 mg/ml, respectively. Since indapamide has a low solubility in water, 40% PEG400 PBS was used to increase solubility and maintain sink condition during the experiments. The molecule of indapamide contains both a polar sulfamoyl chlorobenzamide moiety and a lipid- soluble methylindoline moiety which makes it a good candidate for transdermal delivery.

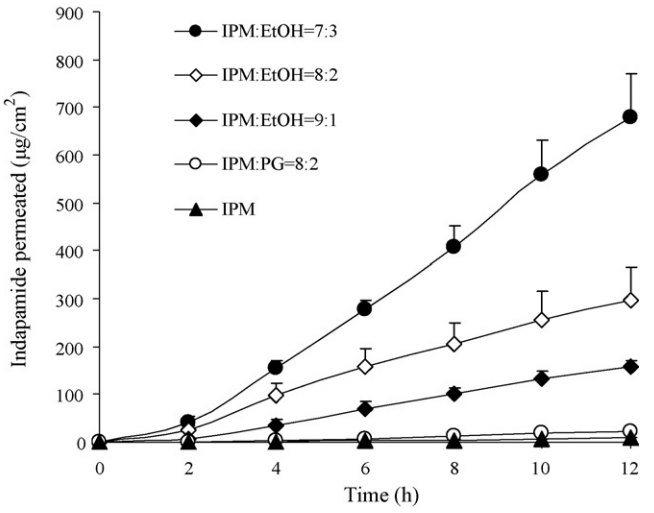

Fig. 2. Effect of PG and EtOH on the permeation of indapamide through rat abdominal skin (each point represents the mean ± S.E. of four experiments).

3.2. The effect of PG and EtOH on the permeation of indapamide

The effect of PG and EtOH on the permeation of indapamide through rat abdominal skin was examined. The permeation parameters are shown in Table 1 and the permeation profiles obtained are shown in Fig. 2.

As can be seen from Table 1, the solubility of indapamide, which was about 0.43 mg/ml in IPM, significantly increased after adding PG or EtOH. For example, indapamide solubility increased to 0.66 mg/ml in 20% (w/w) PG/IPM and 3.26, 8.23, 14.95 mg/ml in 10%, 20%, 30% (w/w) EtOH/IPM, respectively. Thus, EtOH is a more effective solvent than PG for improving indapamide solubility. The permeation of indapamide from IPM without PG or EtOH was extremely low, with a flux of about 0.87 μg/cm^2/h after a 12 h application. The fluxes of indapamide from 20% (w/w) PG/IPM and 10%, 20%, 30% (w/w) EtOH/IPM were 2.33, 15.59, 26.94 and 64.88 μg/cm^2/h, respectively, and the lag-time was shorter than that without PG or EtOH, which suggested some effect of PG and EtOH on lipid in the SC. As can be seen from Fig. 2, the permeation of indapamide increased with the increase of EtOH concentration. A t-test showed a significant difference between each other ($p < 0.05$). The resultant increase in the flux of indapamide can be attributed in part to the increased solubility of indapamide in the donor phase, in addition to the greater membrane fluidity and pore formation

Table 1
Permeation parameters of indapamide through rat abnominal skin using an identical receiver phase (40% PEG400 PBS) and donor phases consisting of IPM; IPM:PG (8:2) (w/w); IPM:EtOH (9:1); IPM:EtOH (8:2); IPM:EtOH (7:3) (w/w) ($n = 4$)

Vehicle	Solubility (μg/ml)	Flux (μg/cm^2/h)	T_{lag} (h)	$P \times 10^3$ (cm/h)	Enhancement ratio
IPM	427.37	0.87 ± 0.24	2.79	2.04 ± 0.56	1
IPM:PG = 8:2	657.21	2.33 ± 0.32	2.56	3.55 ± 0.49	2.68
IPM:EtOH = 9:1	3261.65	15.59 ± 0.99	1.65	4.78 ± 0.30	17.92
IPM:EtOH = 8:2	8226.55	26.94 ± 11.27	0.57	3.27 ± 1.37	30.97
IPM:EtOH = 7:3	14945.69	64.88 ± 19.84	1.55	4.34 ± 1.33	74.57

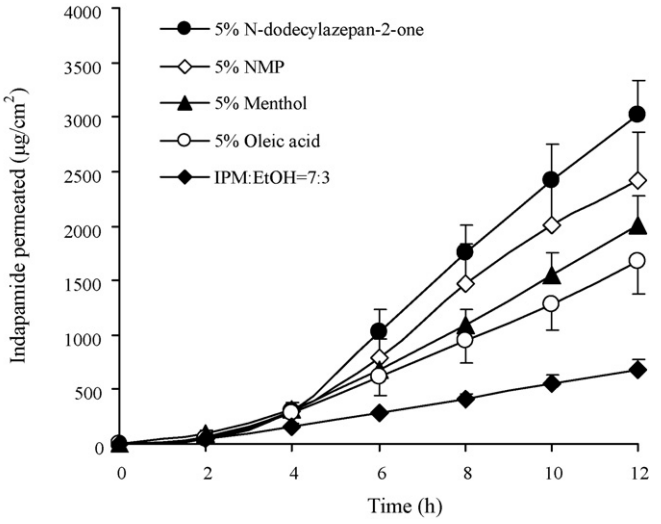

Fig. 3. Effect of different permeation enhancers on the permeation of indapamide through rat abdominal skin (each point represents the mean ± S.E. of four experiments).

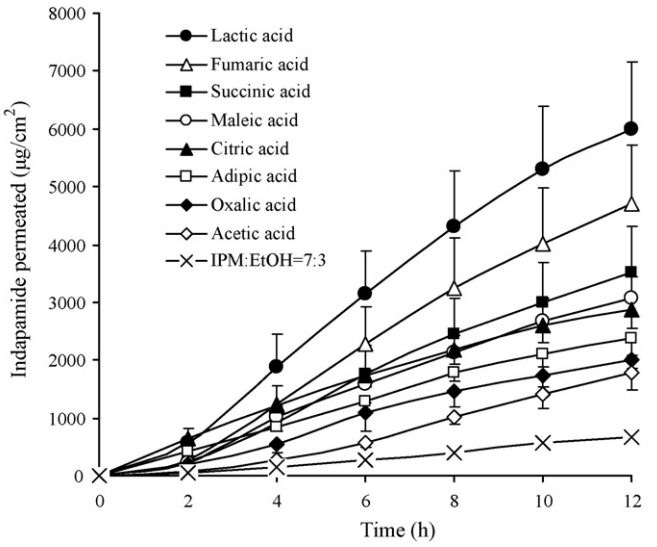

Fig. 4. Effect of various organic acids on the permeation of indapamide through rat abdominal skin (Each point represents the mean ± S.E. of four experiments).

associated directly with the increasing EtOH content (McDaid and Deasy, 1996).

3.3. The effect of different permeation enhancers on the permeation of indapamide

The effectiveness of various penetration enhancers on the transdermal transport of indapamide is shown in Fig. 3. The corresponding results, permeation parameters are given in Table 2.

As can be seen from Table 2, the solubility of indapamide was reduced slightly after adding most of the permeation enhancers except for 5% NMP, in which the solubility of indapamide increased from 14.95 mg/ml to 34.00 mg/ml. All the permeation enhancers studied significantly ($p < 0.05$) promoted the *in vitro* transport of indapamide across the stratum corneum which, in terms of flux, occurred in the order of 5% oleic acid <5% menthol <5% NMP <5% *N*-dodecylazepan-2-one. A *t*-test showed a significant difference between each other ($p < 0.05$). Five percent *N*-dodecylazepan-2-one increased the permeability coefficient by almost 6-fold, while 5% NMP, 5% menthol and 5% oleic acid increased it by a factor of approximately 2, 3 and 3, respectively. The permeability coefficient increased, consequently reducing resistance to the passage of indapamide through the skin.

3.4. The effect of various organic acids on the permeation of indapamide

Since a lipophilic multicomponent system consisting of L-lactic acid (1%), ethanol (10%) and isopropyl myristate system has been shown to selectively enhance the skin permeation of basic drugs (Nakamura et al., 1996, 1997; Kobayashi et al., 1997), we investigated the effects of eight different organic acids on the permeation of indapamide. The results obtained agree well with this research. The permeation parameters are shown in Table 2 and the permeation profiles obtained are shown in Fig. 4.

Table 2
Permeation parameters of indapamide through rat abnominal skin using an identical receiver phase (40% PEG400 PBS) and 30% (w/w) EtOH/IPM added 5% (w/w) *N*-dodecylazepan-2-one, 5% (w/w) NMP, 5% (w/w) menthol, 5% (w/w) oleic acid or 30% (w/w) EtOH/IPM added organic acids which molar quantities were equal to indapamide dissolved in 30% (w/w) EtOH/IPM were used as vehicles ($n = 4$)

Vehicle	Solubility (μg/ml)	Flux (μg/cm²/h)	T_{lag} (h)	$P \times 10^3$ (cm/h)	Enhancement ratio
IPM:EtOH = 7:3	14945.69	64.88 ± 19.84	1.55	4.34 ± 1.33	1
5% *N*-dodecylazepan-2-one	12722.58	313.48 ± 65.92	2.45	24.64 ± 5.18	4.83
5% Menthol	13985.93	195.31 ± 40.22	2.10	13.96 ± 2.88	3.01
5% NMP	33997.77	251.68 ± 95.27	2.39	7.40 ± 2.80	3.88
5% Oleic acid	12102.28	163.39 ± 47.14	2.05	13.50 ± 3.90	2.52
Acetic acid	11945.49	177.89 ± 61.31	2.20	14.89 ± 5.13	2.74
Maleic acid	12725.57	281.47 ± 66.29	0.66	22.12 ± 5.21	4.34
Oxalic acid	12791.00	184.62 ± 23.83	0.64	14.43 ± 1.86	2.85
Adipic acid	11890.04	201.39 ± 74.14	0.00	16.94 ± 6.24	3.10
Lactic acid	13854.31	549.14 ± 159.21	0.56	39.64 ± 11.49	8.46
Citric acid	12767.33	224.83 ± 55.96	0.00	17.61 ± 4.38	3.47
Succinic acid	15172.11	333.13 ± 127.56	1.04	21.96 ± 8.41	5.13
Fumaric acid	19401.41	449.50 ± 170.74	1.16	23.17 ± 8.80	6.93

The results obtained indicate that all 8 organic acids significantly ($p < 0.05$) promoted the permeation of indapamide across rat abdominal skin, and the permeability coefficient increased from more than 3-fold to 9-fold. Lactic acid had the greatest enhancing effect on the permeation of indapamide, in terms of flux, followed by fumaric acid, succinic acid, maleic acid, citric acid, adipic acid, oxalic acid and acetic acid in that order. There are significant differences ($p < 0.05$) between the effects of acids except for between maleic acid and citric acid ($p = 0.18$), between citric acid and succinic acid ($p = 0.25$). The formation of an ion-pair between indapamide and an organic acid may be responsible for the enhanced skin permeation of indapamide.

3.5. General discussion

It appears from the work presented here that the formulation of an effective transdermal drug delivery system (TDDS) for the delivery of indapamide may be possible. Further work is needed in order to achieve clinical levels in humans. Among the various vehicles and permeation enhancers used in this study, a combination of organic acids could be used for the design of a suitable indapamide TDDS. Certainly, there are a number of options available such as the inclusion of penetration enhancers or an increase in the percentage of EtOH in the donor system.

As yet, the only treatment with indapamide involves oral drug delivery systems that require frequent administration and the development of successful TDDS for the delivery would have many advantages over traditional therapy. This research has attempted to show that the formulation of an effective TDDS for the delivery of indapamide may be possible. This avenue of drug delivery should be further investigated so that the successful treatment of hypertension and other clinical conditions for which indapamide is indicated may become possible, resulting in increased benefit to patients. The development of an effective TDDS for the delivery of indapamide is now in progress.

Acknowledgements

The author wishes to thank Professor Yasunori Morimoto, Faculty of Pharmaceutical Sciences, Josai University, Japan, for providing the 2-chamber diffusion cell. The author is grateful to Kangya Pharmaceutical Co. Ltd. (Ningxia, China), for providing the sample of indapamide to carry out this research.

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