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Formulation and Biopharmaceutical Evaluation of a Transdermal Patch Containing Letrozole

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ABSTRACT: The purpose of this study was to formulate a drug-in-adhesive (DIA) transdermal patch containing letrozole, a third generation aromatase inhibitor for the treatment of breast cancer, using pressure-sensitive-adhesives (PSAs) and to evaluate the percutaneous penetration and pharmacokinetics of letrozole after transdermal administration, compared with that for the oral route. The formulation factors for such a patch, including the PSAs, enhancers and amount of drug loaded were investigated. Among the tested preparations, the formulation with DURO-TAK 87-4098, Azone and propylene glycol showed the highest letrozole permeation. The pharmacokinetic characteristics of an optimized DIA patch containing letrozole were determined using rats, while orally administered letrozole in solution was used as a control. The pharmacokinetic parameter, such as the mean residence time (MRT) was significantly ($p<0.05$) different following transdermal administration compared with oral administration. The *in vivo* results observed with the patches in rats were in good agreement with the plasma concentrations predicted from the *in vitro* penetration data. As a patient-friendly, convenient, high local drug concentration and sustained dosing therapeutic system, the transdermal patches incorporating letrozole provide a useful strategy for the prevention and treatment of breast cancer. Copyright © 2010 John Wiley & Sons, Ltd.

Key words: letrozole; transdermal patch; pharmacokinetic; *in vitro*; *in vivo*

Introduction

Transdermal application is an attractive method of drug administration, providing several various benefits: for example, avoidance of a potential hepatic first-pass effect, and the feasibility of constant drug delivery over a period up to one week. In addition, the dosage form of transdermal patches is user-friendly, convenient, painless, and offers multi-day dosing, it is generally accepted that it is associated with improved patient compliance [1–4]. Moreover, owing to high local tissue concentrations of drug in the application position, the transdermal therapeutic system is of particular clinical significance for the prevention and long-term treatment of many localized diseases.

Breast cancer is currently the second most lethal disease in women. In addition, the non-quantifiable effects of breast cancer in terms of quality of life and concerns about the potential for tumor recurrence and death should not be underestimated [5]. It is well known that estrogens are closely involved in the growth of human breast carcinoma, and that the great majority of breast carcinomas express estrogen receptor. Recent studies have demonstrated that estrogens are produced locally in breast carcinoma by several enzymes. Among these, aromatase is generally

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considered the most important enzyme, and aromatase inhibitors are currently used in the treatment of breast carcinoma in postmenopausal women as an estrogen deprivation therapy [6]. Letrozole is an FDA-approved third-generation aromatase inhibitor. On account of its being more potent, better tolerated and more selective among the inhibitors of aromatase, letrozole is now used widely as a first-line drug in the endocrine treatment of estrogen-dependent breast cancer in postmenopausal patients [7].

To date, a large proportion (approximately 75% before menopause and close to 100% after menopause) of the biologically active estrogen is considered to be produced locally in the breast carcinoma [8]. It is important to obtain a high concentration of the aromatase inhibitor in breast carcinoma in order to improve the clinical effects in breast carcinoma patients, because approximately 70% of breast carcinoma specimens show aromatase activities that are comparable to or greater than those found in other tissues [9].

In addition, at present the only commercial dosage form of letrozole is oral tablets, which must be administered once daily. Therefore, patient compliance is an issue and must be considered. For the above reasons, percutaneous administration of letrozole has been studied as a way to improve the problem. Fortunately, letrozole has a number of favorable physicochemical properties, such as a low molecular weight (MW 285.10), good lipophilicity (log $K_{o/w} = 1.73$, determined in a previous experiment) and only a small daily dose (2.5 mg/day) is required. Until now, there has been very little information about the transdermal delivery of letrozole.

In the present study, a single-layer drug-in-adhesive type of transdermal patch containing letrozole was developed, being the simplest of the various patches. Therefore, the selection of an appropriate pressure-sensitive-adhesive (PSA) is the most important factor in designing transdermal drug delivery systems (TDDS) [10]. Since the acrylic PSAs have been used widely in many transdermal formulations, several PSAs with different functional groups were chosen and their effects on the permeation of letrozole through the excised rat skin investigated. Permeation enhancers are commonly incorporated in TDDS in order to improve the penetration of the active ingredient. Some classic enhancers exert a potent enhancing effect. Moreover, in recent years researchers have also developed newer and relatively safer enhancers, exemplified by the series of O-acylmenthol derivatives that was synthesized in our laboratory using L-menthol and selected acids. Penetration experiments with five model drugs, 5-fluorouracil (5-FU), isosorbide dinitrate, lidocaine, ketoprofen and indomethacin, with log*P* values ranging from −0.95 to 3.8, were performed to investigate the promoting effect of these novel enhancers [11,12]. This study investigated the feasibility of its transdermal application with chemical enhancers, including three O-acylmenthol derivatives and a wide range of classic penetration enhancers. Furthermore, the pharmacokinetic characteristics of an optimized DIA patch containing letrozole were determined using rats, while orally administered letrozole in solution was used as a control. Moreover, the pharmacokinetic parameters for the intravenous administration of letrozole and permeability data from an *in vitro* study of rat skin were used to predict the steady-state plasma concentration. Following this, an *in vitro*/*in vivo* correlation was conducted.

Materials and Methods

Materials

The following reagents were used as purchased without further purification: letrozole (chemical purity: >99%) (Suzhou Everfortune IMP & EXP CO. Ltd, China), carbamazepine (chemical purity: >99%) (Zhejiang Jiuzhou Pharmaceutical Co. Ltd, China), DURO-TAK® adhesives 87-2852, 87-2677 and 87-4098 (National Starch and Chemical Company, USA), isopropyl myristate (IPM), *N*-methyl-2-pyrrolidone (NMP), Azone, propylene glycol (PG), Transcutol and Span 80 (China National Medicines Co. Ltd, China). In addition, 2-isopropyl-5-methylcyclohexyl 2-hydroxypanoate (M-LA), 2-isopropyl-5-methylcyclohexyl heptanoate (M-HEP) and 2-isopropyl-5-methylcyclohexyl tetradecanoate (M-TET) were synthesized in our laboratory [12].

A phosphate buffer solution at pH 7.4 was prepared according to the Chinese Pharmacopoeia

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(2005). The water used was deionized and distilled in an all-glass still, and is 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).

In vitro studies

Preparation of transdermal patches containing letrozole. The transdermal patches containing letrozole were prepared with various PSAs and enhancers. An appropriate amount of letrozole and enhancers was dissolved in a suitable amount of acetoacetate, which was later added to the PSA solution and mixed thoroughly with a mechanical stirrer. This solution was spread on a silicone-coated liner (Scotchpak^{TM}, 3M, St Paul, USA) with a wet film applicator (Sletrozole200, Shanghai Kaikai Co. Ltd, China) to give a film thickness of 100 μm, and kept at room temperature for 20 min and then at 60°C in an oven for 10 min (to remove any residual solvent). The patches were then covered with backing membrane (CoTran^{TM}, 3M, St Paul, USA), cut into appropriate sizes, packed in Al-foil and stored in a desiccator for further studies.

Preparation of rat skin. Male Wistar rats weighing 200 ± 20 g (6–8 weeks old) used in the *in vitro* penetration 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 using electric clippers. After the rats were killed by dislocating the spinal cord, the full thickness skin (i.e. epidermis with stratum corneum and dermis) was excised from the shaved abdominal site. Then fat and sub-dermal tissues were removed with surgical scissors. The integrity of the skin was confirmed under a microscope (Motic, American Megatrends. Inc) and any skin sample which was damaged was rejected. The skin was washed with normal saline and kept frozen at −20°C.

In vitro permeation of letrozole. Skin permeation studies were performed using a two-chamber side-by-side glass diffusion cell (effective diffusion 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 of pH 7.4 phosphate buffer solution and stirred continuously 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 bubble 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 letrozole passing across rat skin was calculated using the measured letrozole concentrations in the receiver solution.

In vivo studies

Preparation of intravenous and oral formulations. Letrozole (100 mg) was weighed into a sterile glass vial, followed by the addition of Tween 80 and ethanol 10 ml, respectively. The solution was then sonicated, diluted with saline solution (80 ml) to afford a 1.0 mg/ml solution and administered immediately.

In vivo study design. Female Wistar rats weighing 200 ± 40 g were used for these studies. Rats were anesthetized with urethane (1 g/kg, i.p.). For intravenous bolus experiments, a dose of 5 mg/kg (1.0 mg/ml) letrozole solution was infused into the tail vein over a period of 30 s. Blood samples of approximately 0.25 ml were withdrawn at 0.167, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96 and 120 h following the intravenous doses. For oral experiments, a dose of 5 mg/kg (1.0 mg/ml) letrozole solution was administered by gavage.

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Each rat received letrozole solution twice more at 24 h intervals immediately after blood retrieval. Blood samples of approximately 0.25 ml were withdrawn at 2, 4, 8, 12, 24, 26, 28, 32, 36, 48, 50, 52, 56, 60, 72, 96, 144 and 192 h following the intragastric doses. For transdermal delivery studies, patches (covering an area of 10 cm^2, containing 6 mg letrozole) were applied to the shaved abdomen of the rats. Blood samples were collected for 72 h while the patches remained on the animal, and until 192 h after the patches were removed. All animal studies were carried out in accordance with the guidelines for animal use published by the Life Science Research Center of Shenyang Pharmaceutical University. The blood samples were immediately centrifuged at 5000 rpm for 5 min, and the plasma was separated and stored at −20°C until required for HPLC analysis.

Analytical methods

Analytical methods for in vitro samples. The letrozole content of the samples was analysed by HPLC. The HPLC system involved an L-2420 variable-wavelength ultraviolet absorbance detector and an L-2130 pump (Hitachi High-Technologies Corporation, Tokyo, Japan). In addition, a Diamonsil® ODS 5 μm × 200 mm × 4.6 mm column (Dikma Technologies) was used with a mobile phase of methanol–water (60:40, v/v), delivered at a flow rate of 1.0 ml/min. The column was maintained at 40°C and the UV detector was set at 234 nm. Carbamazepine was used as the internal standard. The retention times in this assay were 4.57 and 7.15 min for letrozole and internal standard, respectively.

Analytical methods for in vivo samples. Plasma samples were first allowed to thaw at room temperature. Then 100 μl plasma and 10 μl carbamazepine solution (50 μg/ml) were pipetted into a 2 ml centrifuge tube and vortex-mixed for 30 s. Then, the mixture was extracted with 1 ml diethyl ether for 3 min using a vortex mixer. After centrifugation at 3000 rpm for 10 min, the supernatant was decanted into a clean test-tube and evaporated to dryness under nitrogen at 37°C. The residue was reconstituted with 100 μl mobile phase for *in vivo* analysis, vortexed and sonicated for 3 min. A sample of the clear solution (20 μl) was injected into the HPLC system. The extraction efficiency was 87.8 ± 2.29% for letrozole.

The HPLC analytical conditions were the same as those used in the *in vitro* studies except that the mobile phase was methanol–water (55:45, v/v).

Data treatment

In vitro data analysis. Each *in vitro* penetration experiment was repeated at least four times. All data were expressed as the mean value ± SE. Student's *t*-test was used to compare two group data with the level of significance set at $p < 0.05$.

In vivo pharmacokinetic analysis. The concentration–time data were subject to non-compartmental analysis using the statistical moment approach. The maximum measured plasma concentration (C_{max}) and the corresponding time (t_{max}) were taken directly from the raw data. The area under the curve ($AUC_{0-\infty}$) and the area under the first moment curve ($AUMC_{0-\infty}$) were calculated by the following equations:

$$AUC_{0-\infty} = AUC_{0-t} + \frac{C_n}{k} \quad (1)$$

$$AUMC_{0-\infty} = AUMC_{0-t} + \left(\frac{C_n}{k^2} + \frac{t_n C_n}{k}\right) \quad (2)$$

where C_n, t_n are the last measured plasma concentration and the corresponding time. AUC_{0-t} and $AUMC_{0-t}$ were calculated by the trapezoidal rule. Semilogarithmic plots of the plasma concentration vs time revealed a late linear slope for the curve and allowed estimation of the corresponding rate constant (k, which equals 2.303 × slope) [13,14].

The mean residence time (*MRT*) was calculated as $AUMC_{0-\infty}$ divided by the $AUC_{0-\infty}$. In addition, the mean absorption time (*MAT*) was obtained by the following equation:

$$MAT_{td} = MRT_{td} - MRT_{iv} \quad (3)$$

$$MAT_{po} = MRT_{po} - MRT_{iv} \quad (4)$$

where MRT_{td}, MRT_{po} and MRT_{iv} are the mean residence time after transdermal, oral and intravenous administration, respectively; MAT_{td}, MAT_{oral} and MAT_{iv} are the mean absorption time

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of transdermal, oral and intravenous administration, respectively.

The total body clearance (*CL*) determined from the intravenous pharmacokinetic study was calculated by the following equation:

$$CL = Dose_{\mathrm{iv}}/(AUC_{0-\infty})_{\mathrm{iv}} \quad (5)$$

The steady-state plasma concentration of letrozole after the application of patches containing letrozole was calculated using equation [15]:

$$C_{\mathrm{ss}} = AUC_{0-\mathrm{t}}/\mathrm{time} \quad (6)$$

where t or time is 72 h (the application of letrozole transdermal patches was for a 72 h period).

The absolute bioavailability of the letrozole patch, compared with that obtained following intravenous administration of letrozole, was calculated from the *AUC* values obtained.

$$F\% = \frac{AUC_{\mathrm{td}}/Dose_{td}}{AUC_{iv}/Dose_{iv}} \times 100\% \quad (7)$$

where AUC_{td} and AUC_{iv} are the area under the curve after transdermal and intravenous administration, respectively; $Dose_{\mathrm{td}}$ and $Dose_{\mathrm{iv}}$ are the dose of transdermal and intravenous administration, respectively.

Data for the concentration and pharmacokinetic parameters obtained from rats treated with oral solutions or transdermal patches containing letrozole were compared and statistical differences were analysed using the Student's *t*-test.

Results

Effect of PSAs on skin penetration of letrozole in vitro

The effect of various PSAs on rat skin permeation of letrozole was investigated. The letrozole concentration in PSA was fixed at 1.5% (w/w) and each patch was prepared with a thickness of 100 ± 10 μm. The patches were smooth, uniform and flexible. As illustrated in Figure 1, among the PSAs examined, DURO-TAK 87-4098 exhibited the highest permeation rate of letrozole through rat skin, followed by 87-2852 and 87-2677. Application of the Student's *t*-test showed that the difference was statistically significant ($p < 0.05$). Since the highest permeation rate was achieved with DURO-TAK 87-4098, it was chosen for further studies to develop a transdermal delivery system for letrozole.

Effect of penetration enhancers on skin penetration of letrozole in vitro

Penetration effect of a single enhancer. The skin permeation profiles of letrozole patches with various enhancers are illustrated in Figure 1. All the enhancers used in the experiments increased the penetration of letrozole through the rat skin. The cumulative amount after a 24 h application of letrozole with enhancers was found to be significantly higher than that without penetration enhancers ($p < 0.05$).

Generally, Azone, Span 80 and M-LA exhibited significant enhancing effects on letrozole percutaneous absorption, with the cumulative permeated amount being 88.7 ± 7.68, 84.4 ± 8.22 and $80.2 \pm 3.55\ \mu g/cm^2$ after 24 h, respectively. Although not as good as Azone, M-TET, IPM and M-HEP also exhibited potent enhancing effects, with the cumulative amounts being 55.4 ± 8.54, 42.2 ± 4.63 and $34.3 \pm 0.97\ \mu g/cm^2$ after 24 h, respectively. NMP and Transcutol

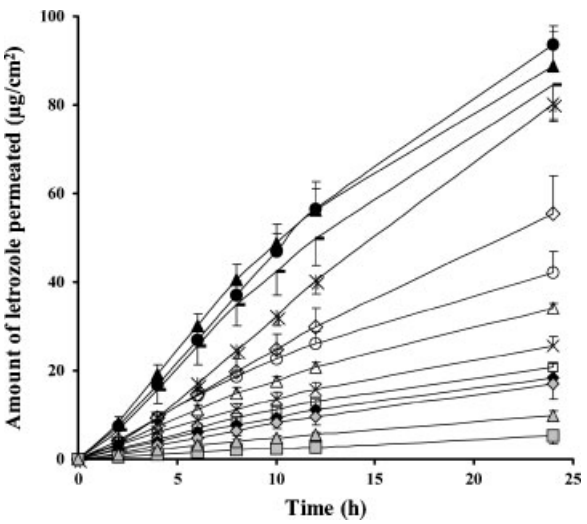

Figure 1. The penetration profiles of letrozole patches in the presence of different PSAs, different enhancers and different percentages of Azone. 15% Azone (●); 10% Azone (▲); Span 80 (▬); M-LA (✳); M-TET (◇); IPM (○); M-HEP (△); NMP (×); Transcutol (□); 5% Azone (◆); 87-4098 (◈); 87-2677 (△); 87-2852 (▣). Each point represents the mean ± SE, $n = 3$–4

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exhibited only a minor promoting effect. Overall, different chemical enhancers exhibited significantly different promoting activity on letrozole transport across rat skin.

In vitro penetration experiments indicated that Azone was the most potent penetration enhancer. Further investigation of the effect of the concentration of Azone on the penetration letrozole patches was carried out (Figure 1). The cumulative amounts of letrozole from the patches with 5%, 10% and 15% (w/w) Azone were 17.2 ± 3.54, 88.7 ± 7.68 and $89.2 \pm 4.34\,\mu g/cm^2$ respectively, after 24 h. Increasing the Azone concentration (5%–15%) resulted in an increase in the cumulative amount of letrozole. However, there was no significant difference ($p > 0.05$) between 10% and 15% Azone. The apparent 'optimal' Azone concentration was 10% (w/w). Thus, the addition of 10% Azone seems to be optimum for increasing the skin permeation rate.

Synergistic effect of enhancers. Single chemicals, however, offer limited enhancement of skin permeability. Mixtures of chemicals can overcome this limitation owing to their synergistic interactions. As seen above Azone exhibited a maximum enhancement of letrozole transdermal permeation. Whether there was a synergistic effect between Azone and other enhancers, the two most effective enhancers from the *in vitro* experiments, Azone and Span 80, was investigated. Surprisingly, Azone and different percentages of Span 80, led to a reduction in the cumulative amounts when used simultaneously in patches which was even lower than that when either enhancer was used alone.

Propylene glycol (PG) has been used widely as an enhancer in transdermal delivery systems either alone or in combination with other agents. Various formulations of the transdermal patches were prepared for screening a suitable amount of PG at a fixed Azone concentration (10%). Figure 2 shows that Azone and different percentages of PG had an apparent synergistic effect on the letrozole transdermal penetration. The maximum accumulated permeation was observed at a 5% PG concentration, and 10% PG markedly increased letrozole penetration while 15% PG had only a slight enhancing activity. No significant skin permeation enhancing effect on letrozole was observed with 15% PG compared with 10%

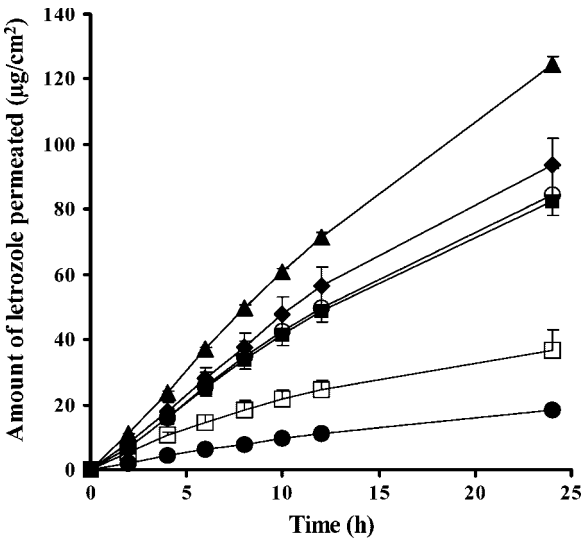

Figure 2. The penetration profiles of letrozole patches in the presence of 10% Azone and different percentages of PG. Azone (10%) + PG (5%) (▲); Azone (10%) + PG (10%) (♦); Azone (10%) + PG (15%) (■); Azone (10%) (○); PG (10%) (□); control (●): without any enhancer. Each point represents the mean ± SE, $n = 3$–4

Azone alone ($p > 0.05$). Therefore, the 5% concentration was selected for combination with Azone.

Effect of loading amount on skin penetration of letrozole in vitro

In order to evaluate the effect of the loading amount of the drug in the patch on the skin permeation of letrozole, patches containing different amounts of letrozole were prepared. From Figure 3, it can be seen that the permeation rate of letrozole increased sharply on increasing the amount of letrozole in the patch from 0.75% to 1.5%. Nevertheless, when the loading amount of letrozole patch was 3%, there was a rapid decrease in the penetration rate. Thus, 1.5% letrozole was selected for the optimum patches.

Figure 3 shows that with 10% Azone and 5% PG in each formulation as enhancer, the cumulative amount of letrozole penetrating from the patches increased from 24.1 ± 2.06 to $119 \pm 10.3\,\mu g/cm^2$ after 24 h following an increase in the loading dose from 0.75% to 1.5% ($p < 0.05$). However, no further increase, and even a decrease of the cumulative amount, was observed on increasing the letrozole load above 1.5%. Figure 4 shows patches with letrozole loads

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of 0.75%, 1.5% and 3% captured by optical microscopy (Motic, American Megatrends. Inc). Crystallization was not observed in the patch containing 0.75% letrozole nor in that containing 1.5% before penetration of patches *in vitro* was examined (after storage for 24 h), whereas the patch with 3% letrozole showed crystal formation in the matrix, even though no crystals were immediately observed in any of the formulations after their preparation. The result suggests that letrozole may be supersaturated in the patch when the loading is 3%.

Pharmacokinetic study of the letrozole patch and the oral formulation

Concentration–time profiles. The mean plasma concentration–time profiles of letrozole after oral and transdermal administration are shown in Figure 5. Following multiple oral administrations, there were three absorption peaks in the concentration–time profile of letrozole, i.e. $1.73 \pm 0.16\,\mu g/ml$ at 8 h, $2.71 \pm 0.25\,\mu g/ml$ at 36 h and $3.63 \pm 0.35\,\mu g/ml$ at 52 h. However, a single peak ($4.18 \pm 0.27\,\mu g/ml$ at 62 h) was obtained after transdermal application. The plasma concentration of letrozole after transdermal administration declined more slowly than that following oral administration. Furthermore, it was observed by eye that there were no significant changes to the skin surface after removal of the letrozole transdermal patch.

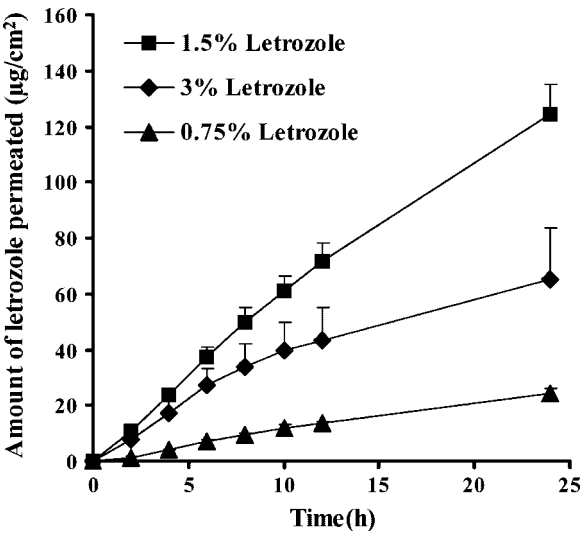

Figure 3. The penetration profiles of letrozole patches with the different loading percentages of letrozole. Each point represents the mean ± SE, $n = 3$–4

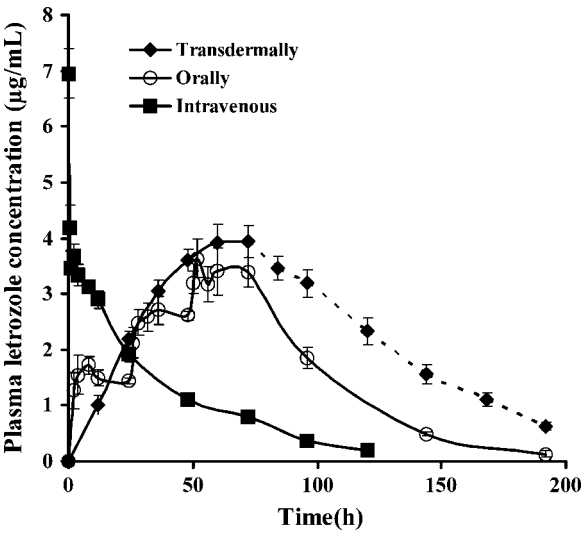

Figure 5. Mean plasma concentration-time profiles of letrozole after the different administration routes into rats. The dotted line (···) indicates the plasma concentration after the removal of patches. Each point represents the mean ± SE, $n = 6$

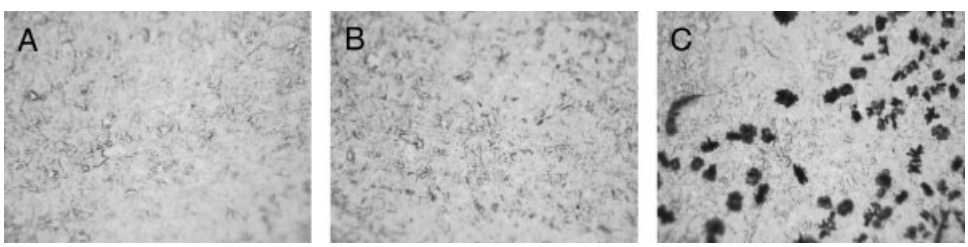

Figure 4. Photographs of the letrozole patches containing different amounts of the drug after storage for 24 h. (A) 0.75%; (B) 1.5%; and (C) 3%. Magnification × 40

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Pharmacokinetic parameters. The main pharmacokinetic parameters were also calculated by the statistical moment method. The results from the pharmacokinetic analysis are summarized in Table 1. Compared with oral application, the MRT values were clearly extended (97.5 ± 2.49 h for transdermal versus 68.6 ± 1.27 h for oral application). Essentially, the MAT values were markedly increased and the MAT obtained from the transdermal patches was 2-fold higher compared with oral administration. In addition, the calculated absolute bioavailability of letrozole after transdermal application of the patch compared with intravenous administration was approximately 53.5%.

Intravenous administration of letrozole

Pharmacokinetic parameters after intravenous administration of letrozole to rats were required in order to predict the *in vivo* plasma levels of letrozole in the rats from the *in vitro* skin permeability data. Hence, pharmacokinetic parameters were calculated after intravenous administration of letrozole to rats (Table 1). After intravenous administration, an $AUC_{0-\infty}$ of 148 ± 6.43 (μg h)/ml, an MRT of 39.8 ± 1.93 h and a *CL* value of 40.9 ± 1.80 ml/(h kg) were obtained.

Discussion

In the present study, a single-layer drug-in-adhesive type of transdermal patch was developed, in which the adhesive layer not only serves as an adherent layer to the skin but is also responsible for the release of drug. Many formulation factors, for example PSAs, enhancers and the amount loaded, were investigated.

Effect of adhesive on the skin penetration of letrozole in vitro

The selection of a suitable PSA is crucial in developing a transdermal delivery system since its adhesion, stability characteristics and compatibility with other patch components have a great influence on the delivery efficacy of the drug [16]. In this study, the effect of three PSAs with different functional groups on the permeation of letrozole through excised rat skin was evaluated. The DURO-TAK® adhesive 87-4098 without carboxyl group had the higher letrozole

Table 1. Pharmacokinetic parameters of letrozole after oral administration of letrozole solution (5 mg/kg) thrice at 24 h intervals, transdermal administration as a single patch (6.0 mg, removed after 72 h) and intravenous injection of letrozole (5 mg/kg) via the tail vein ($n = 6$)

Pharmacokinetic parameter	Administration route		
	Transdermal	Oral	Intravenous
Dose (mg/kg)	30	15[a]	5
k[b] (10^2) h^{-1}	1.78 ± 0.11	3.34 ± 0.57	2.36 ± 0.09
AUC_{0-t} (μg · h/ml)	439 ± 29.2	309 ± 16.9	114 ± 6.43
C_n/k (μg · h/ml)	36.6 ± 5.40	8.12 ± 5.69	7.95 ± 1.52
$AUC_{0-\infty}$ (μg · h/ml)	475 ± 32.8	317 ± 18.1	122 ± 7.23
$AUC_{0-\infty}$[c] (μg · h/ml) per mg/kg	15.8 ± 1.09	21.1 ± 1.21	24.4 ± 1.29
$AUMC_{0-t}$ (μg · h^2/ml)	37 143 ± 2838	19 548 ± 1599	3631 ± 374
C_n/k^2 (μg · h^2/ml)	2148 ± 402	639 ± 546	269 ± 85.1
t_nC_n/k (μg · h^2/ml)	7020 ± 1037	1559 ± 1092	954 ± 182
$AUMC_{0-\infty}$ (μg · h^2/ml)	46 312 ± 3955	21 746 ± 1265	4855 ± 558
$AUMC_{0-\infty}$[d] (μg · h^2/ml) per mg/kg	1541 ± 132	1454 ± 84.4	971 ± 112
MRT (h)	97.5 ± 2.49*	68.6 ± 1.27	39.8 ± 1.93*
MAT[e] (h)	57.7*	28.8	N.A.
F (%)	53.5*	71.4	100*

[a]Total dose of oral administration.
[b]Late linear slope for the semilogarithmic plots of the plasma concentration vs time revealed a curve, allowing estimation of the corresponding rate constant (k, or 2.303 × slope).
[c]*AUC* is normalized to a 1.0 mg/kg dose.
[d]*AUMC* is normalized to a 1.0 mg/kg dose.
[e]*MAT* is mean absorption time. *MAT*trans = *MRT*trans−*MRT*iv; *MAT*po = *MRT*po−*MRT*iv.
*$p < 0.05$, significantly different from oral group.

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penetration rate than 87-2677 and 87-2852 with a carboxyl group. The poor skin permeation from the patches made from the PSA containing a carboxylic acid might be due to an interaction between the triazole moiety of letrozole and the carboxylic acid of the PSA. Other studies have produced similar results [17,18]. The results obtained indicate that DURO-TAK® adhesive 87-4098 is a suitable and compatible adhesive for the development of transdermal drug delivery systems for letrozole.

Effect of enhancers on the penetration of letrozole patches in vitro

Effect of single penetration enhancers. The stratum corneum is regarded as the main barrier in the permeation process which limits the transdermal penetration of a wide range of substances. The use of chemical penetration enhancers seems to be an effective way to reduce the barrier properties of the stratum corneum. The incorporation of permeation enhancers into transdermal drug delivery systems is essential to increase the permeation of letrozole from the patches. In the present study, several conventional chemical enhancers (Azone, Span 80, IPM, NMP and Transcutol) were selected and three O-acylmenthol derivatives were synthesized in our laboratory (M-LA, M-HEP and M-TET) as penetration enhancers of letrozole from patches. As shown in the results section, all the selected enhancers increased the penetration of letrozole. In particular, Azone and Span 80 exhibited the most potent enhancing effect on letrozole. Azone is known to be safe and has been used to increase the skin permeation of a large number of drugs. Azone is non-irritant to human skin, even in undiluted form [19], reversible in its action [20] and very poorly absorbed through human skin [21]. Azone is also included in the Chinese Pharmacopoeia (2005) and is an ideal permeation enhancer for clinical use. The enhancing effect of Azone on skin permeability has been attributed to a disruption of the organized lipid structure in the intercellular region of the stratum corneum, resulting in increased lipid fluidity and enhanced drug diffusivity [22,23]. In our experiments, there was concentration dependence in the permeation-enhancing effect of Azone, showing that above a 10% concentration of Azone, the cumulative amount of letrozole did not increase markedly ($p > 0.05$ between 10% and 15% Azone). This may be due to the fact that at low concentrations, the main effect of Azone is to increase the permeability by disordering or 'fluidizing' the lipid structure of the stratum corneum, while a high concentration of Azone exerts an effect on the hydration of the stratum corneum. As the concentration of Azone increases, the water content also increases. This condition makes it difficult for lipophilic compounds to partition into the 'hydrated' stratum corneum and, consequently, the penetration is reduced.

According to a previous report [12], M-LA, M-TET and M-HEP were synthesized in our laboratory to investigate the promoting effect on five model drugs with a wide range of log *P*. Their activities depended on the local disorder induced by their insertion within the stratum corneum intercellular lipid lamella [24–26]. The synthetic O-acylmenthol derivatives have exhibited both a high enhancing potency and a low toxicity [27–29]. However, the three derivatives had an enhancing effect on letrozole that was inferior to Azone and Span 80 in our experiment. Therefore, no further investigation of these synthetic O-acylmenthol derivatives was carried out in our experiments.

Synergistic effect of enhancers. In designing a transdermal system, more than one enhancer can be incorporated. These enhancers could exhibit different enhancing mechanisms and release kinetics, and a synergistic effect in enhancing the permeation of a drug could be obtained. The ability of PG to increase transdermal permeability has been attributed to several effects. For example, PG has been shown to reduce drug/tissue binding by occupying hydrogen bonding sites and solvating alpha-keratin within corneocytes, and may also marginally increase lipid fluidity, leading to increased drug permeability [30,31]. However, the main effect of PG appears to involve a solvent-drag effect where, by partitioning into the stratum corneum itself, PG improves the solubility of drugs within the stratum corneum, resulting in increased drug partitioning into the skin [23].

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A synergistic action between 10% Azone and 5% PG was observed when they were applied together in the present study. A synergistic effect on the skin penetration rate with PG in combination with Azone has been reported by other researchers [22]. Azone enhances intercellular drug diffusion and intracellular proteins, offering considerable resistance to drug permeation, are not affected by it. PG does not completely penetrate the cells (corneocytes), but remains in the intercellular spaces dehydrating intracellular protein structures due to its hygroscopicity and competes with water for hydrogen bonding sites. In combination with Azone, PG gains access to the intercellular lipids and may occupy their inner hydrophilic regions. Conversely, Azone being solubilized in PG, partitions more readily into the intercellular domain. This mechanism can explain the significant enhancement of letrozole penetration through rat skin from a formulation containing Azone in PG.

Effect of the loading amount on skin penetration of letrozole in vitro

Roy *et al.* [32] showed a linear increase in the steady-state flux of fentanyl from polyisobutylene matrices through human cadaver skin for drug loads up to saturation concentrations in the formulation. They suggested that drug crystals in highly loaded matrices lead to a reduction in drug thermodynamic activity, and this may reduce the drug flux through the stratum corneum as a result. Therefore, our present result is consistent with previous findings, and it is concluded that 3% letrozole is supersaturated in the adhesive matrix. This indicates that the thermodynamic activity, the driving force for the permeation process, reached its maximum at a drug load of approximately 1.5%, which is also the optimal loading for the letrozole patch.

Biopharmaceutical evaluation

As shown by the experimental results, upon removal of the transdermal patch, a mild reservoir effect was observed for about 120 h (from 72 h to 192 h) followed by normal elimination similar to that after oral administration. After transdermal administration, the mean absorption time (MAT) of letrozole was prolonged compared with that after oral administration. This may be due to the longer duration of absorption provided by the transdermal patch. Also, prolonged duration of action is possible with a single application of a transdermal patch. Moreover, the absolute bioavailability of the dermal patch is 53.5%, which is obviously low. The main reason for this is that the drugs in patches can not be absorbed fully into the systemic circulation and the part of the drug is left in the patch. These results suggest that the transdermal patch of letrozole can prolong the duration of activity and offer a lower dosing frequency and consequently improve patient compliance.

In vitro/in vivo correlation. To predict the steady-state plasma concentrations of letrozole after the application of transdermal patches from the *in vitro* permeation data, the following equation was used [15]:

$$C_{SS}^{P} = \frac{J_{SS}A}{CL} \tag{8}$$

where C_{SS}^{P} is the predicted steady-state plasma concentration, J_{ss} is the steady-state flux across rat skin, A is area of the applied patch (10 cm^2) and CL is the total body clearance (40.9 ± 1.8 ml/(h kg)) determined from the intravenous pharmacokinetic study. The mean steady-state flux of letrozole following the application of patches was estimated to be 5.24 $\mu g/cm^2/h$. The observed steady-state plasma concentration of letrozole calculated by Equation (6) (6.09 ± 0.40 $\mu g/ml$) after the application of patches was comparable to the predicted steady-state plasma concentrations (6.41 ± 0.52 $\mu g/ml$) obtained from the *in vitro* permeation data. This excellent *in vitro/in vivo* correlation indicates that further formulation studies for the transdermal patch of letrozole can be developed with the help of *in vitro* experiments.

Conclusion

A novel patch formulation with DURO-TAK 87-4098, 10% Azone and 5% PG resulted in the highest permeation of letrozole in our experiments. In the *in vivo* experiments in rats, the

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pharmacokinetic parameters after transdermal administration changed compared with those after oral administration, suggesting that the dosing frequency may be reduced and the time of administration may be extended. The observed steady-state plasma levels were in good agreement with the predicted steady-state plasma levels from the *in vitro* data and a good *in vitro*/*in vivo* correlation was established. These results confirm the feasibility of developing a patient-friendly, convenient, high local concentration and multi-day dosing therapeutic system using these transdermal patches incorporating letrozole.

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Conflict of Interest

All authors have no financial/commercial conflicts of interest. They have read and approved this version of the article, and due care has been taken to ensure the integrity of the work. No part of this paper has published or submitted elsewhere.

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