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Transdermal patches for site-specific delivery of anastrozole: *In vitro* and local tissue disposition evaluation

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ABSTRACT

Anastrozole is a potent aromatase inhibitor and there is a need for an alternative to the oral method of administration to target cancer tissues. The purpose of the current study was to prepare a drug-in-adhesive transdermal patch for anastrozole and evaluate this for the site-specific delivery of anastrozole. Different adhesive matrixes, permeation enhancers and amounts of anastrozole were investigated for promoting the passage of anastrozole through the skin of rats *in vitro*. The best *in vitro* skin permeation profile was obtained with the formulation containing DURO-TAK® 87-4098, IPM 8% and anastrozole 8%. For local tissue disposition studies, the anastrozole patch was applied to mouse abdominal skin, and blood, skin, and muscle samples were taken at different times after removing the residual adhesive from the skin. High accumulation of the drug in the skin and muscle tissue beneath the patch application site was observed in mice compared with that after oral administration. These findings show that anastrozole transdermal patches are an appropriate delivery system for application to the breast tumor region for site-specific drug delivery to obtain a high local drug concentration.

1. Introduction

Intratumoral production of estrogens is mainly responsible for the proliferation of breast cancer cells, particularly in post-menopausal women (Santner et al., 1984; Suzuki et al., 2003). Aromatase, which is mainly expressed in subcutaneous adipose, skin, and normal breast tissue as well as malignant tissue in post-menopausal woman, is the key enzyme estrogen biosynthesis of androgens to estrogens (Tilson-Mallett et al., 1983; Brodie et al., 1998; Bulun et al., 2007). Furthermore, intratumoral aromatase is the source of local estrogen production in breast cancer tissue (Brueggemeier et al., 2005). Therefore, intratumoral aromatase, which produces estrogens in situ, is a key target for successful inhibitor treatment (Tamulevicius and Streffer, 1995).

Anastrozole (Fig. 1), 2,2′-[5-(1H-1,2,4-triazol-l-ylmethyl)-1,3-phenylene] bis(2-methylpropiononitrile), is a potent, third-generation non-steroidal aromatase inhibitor for post-menopausal women in the treatment of breast cancer (Geisler et al., 1996, 2001; Goss and Wu, 2007). The problems associated with currently available oral therapies with aromatase inhibitors such as anastrozole are that they offer uncontrolled delivery and release, a lack of targeting and poor compliance as well as a number of common systemic side effects, e.g. hot flushes and bone damage and nausea (Wilkinson, 2004; Sarkar and Yang, 2008). It has been reported that hot flushes and bone damage are related to the circulating estrogen depletion caused by aromatase inhibitor treatment in post-menopausal breast cancer patients (Mom et al., 2006; Miki et al., 2007). In addition, the physicochemical characteristics (e.g. low molecular weight 293.4 g/mol, *n*-octanol/water partition coefficient 1.58, low melting point 83.3 °C) of anastrozole are similar to the ideal properties of a molecule able to effectively penetrate the stratum corneum (Barry, 2001; Zidan et al., 2006; AstraZeneca Canada Inc., 2008). The barrier function of the skin is well-known as well as where the breast is located. Moreover, the possibility to deliver a drug into the skin by patches is also well recognized; indeed in the main pharmacopoeias the monograph of medicated plaster, loco-regional patches, is reported over several years. It has been suggested that the breast can be used as an anchor for transdermal site-specific delivery of anastrozole to obtain high local drug concentration and reduce the risk of systemic side effects. However, to date, there have been no research papers on the delivery of anastrozole by the transdermal route in animals and patients.

The present research focused on formulating monolithic drug-in-adhesive patches containing anastrozole and the evaluation of the transdermal site-specific delivery of anastrozole from a trans-

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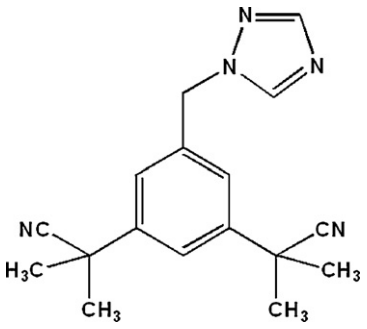

Fig. 1. The chemical structure of anastrozole.

dermal patch system *in vitro* as well as local tissue disposition studies. The effect of enhancers and the drug content on anastrozole transport across female rat skin was evaluated. This study investigated the concentration of anastrozole in the skin, muscle and plasma of female mice following application of transdermal patches in comparison with that following administration of the oral suspension.

2. Methods

2.1. Materials

Anastrozole (the purity of anastrozole is up to 99%; ^{1}H NMR: 1.68 (12H, S, 4-CH_3), 5.48 (2H, S, –CH_2), 7.43 (2H, d, –Ar), 7.55 (1H, S, –Ar), 8.00 (1H, S, –triazole), 8.69 (1H, S, –triazole); MS: *m*/*z* 293) was synthesized in the laboratory of Prof. Cheng (Shenyang Pharmaceutical University, China); carbamazepine, Transcutol® P (TP), isopropyl myristate (IPM), oleic acid (OA) and *l*-menthol (MT) were supplied by China National Medicines Co. Ltd. (Shanghai, China); pressure sensitive adhesives (PSAs), DURO-TAK® 87-2852, 87-2677 and 87-4098, were kindly donated by the National Starch and Chemical Company (Bridgewater, NJ, USA). HPLC grade methanol was obtained from the Hanbon Science and Technology Co., Ltd. (Jiangsu, China). All other chemicals were of the highest reagent grade available.

2.2. Animals

Female Wistar rats weighing 180–220 g and female Kunming mice weighing 18–22 g were supplied by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). All experiments were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and also in accordance with the guidelines for animal use published by the Life Science Research Center of Shenyang Pharmaceutical University. All efforts were made to minimize animal suffering and to limit the number of animals used.

2.3. Preparation of patches

The drug-in-adhesive transdermal patches containing anastrozole were prepared by dissolving anastrozole, PSAs, and enhancers in ethyl acetate and mixed thoroughly with a mechanical stirrer to obtain a homogeneous coating formulation. A laboratory-coating unit (SLT200, Kaikai Co., Ltd., Shanghai, China) was used to prepare patches. The resulting formulation was coated onto a fluoropolymer-treated polyester release liner (ScotchPak® 1022, 3M, USA) at a thickness of 80 μm. The coated release liner was oven-dried at 60 °C for 10 min and after removal of the solvent, then it was laminated with a polyester backing film (ScotchPak® 9732, 3M, USA).

2.4. Drug content determination

The patch of 1 cm^2 ($n = 6$) was weighed accurately and dissolved in 100 mL methanol. Then the whole solution was sonicated for 60 min. After sonication and subsequent filtration, drug solution was appropriately diluted with *in vitro* mobile phrase and was determined by HPLC.

2.5. In vitro permeation experiments

Female rats were anesthetized with urethane (20%, w/v, i.p.). Fresh excised samples of full thickness skin (epidermis with SC and dermis) were obtained after hair on the abdominal area was removed by trimming with clippers (model 900, TGC, Japan) and the rats were shaved with an electrical shaver before being sacrificed by cervical dislocation. The integrity of the skin was carefully checked by microscopic observation, and any skin which was not uniform was rejected. The sub-dermal tissue was surgically removed and the dermal side was wiped with a cotton swab dipped in isopropyl alcohol for 1 min to remove adhering fat. The skin samples were stored at −20 °C until required (used within 1 week of preparation) (Zhao et al., 2009).

A two-chamber side-by-side glass diffusion cell (effective diffusion area = 0.95 cm^2) with a water jacket connected to a water bath at 32 °C was used for investigating the degree and rate of skin permeation of anastrozole from transdermal patches according to the method described in a previous study (Zhao et al., 2009). The excised rat abdominal skin was mounted between the cell halves so that the dermal side of the skin faced the receiver solution. 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 3 mL distilled water (the aqueous solubility of anastrozole 0.5 mg/mL at 25 °C) 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. Then, 2.0 mL of receptor medium was withdrawn at predetermined time intervals for analysis and replaced with the same volume of fresh receptor medium to maintain sink conditions. The drug concentration was determined by reversed phase HPLC with reference to a calibration curve. The cumulative amount of anastrozole passing across rat skin was calculated using the measured anastrozole concentrations in the receiver medium.

2.6. Local disposition study in mice

Before the day of administration, female mice were fasted overnight but were allowed access to water ad libitum. The hair on the abdomen of the mice was removed with electric hair clippers and a shaver before administration. The mice were divided in two groups, each of 20 mice and treated as follows:

Group I—Anastrozole suspension (1.5 mg/mL in 0.5%, w/v, CMC-Na, 15 mg/kg; p.o.);
Group II—Anastrozole patches (1 cm^2; 2 mg of drug).

Each mouse received an anastrozole patch or suspension once. All patch formulations were applied to abdominal area after removal of abdominal hair and then removed immediately before sampling (for mice sacrificed at 12 h, patches were removed at 8 h). After removal of the patches, the residual adhesive on the skin surface was carefully wiped off using cotton soaked in ethyl acetate, then skin was processed in accordance with rat skin treatment. Samples were collected at 0.17, 1, 4, 8 and 12 h after adminis-

tration. Four mice at different time intervals in every group were anesthetized. Blood samples were collected in dried heparinized tubes by cardiac puncture. Plasma was separated by centrifugation at 600 × *g* for 10 min, using a XiangYi H2050R instrument (XiangYi Centrifuge Instrument Co. Ltd., Changsha, China). The abdominal skin and muscle beneath the drug application site were collected from mice after sacrifice. Plasma, skin and muscle samples were stored at −70 °C until further analysis.

2.7. Samples extraction procedure

For plasma samples, 100 μL plasma and 10 μL internal standard solution (equal to 0.2 μg of carbamazepine) were pipetted into a 1.5 mL centrifuge tube and vortex-mixed for 30 s. The mixture was then extracted with 1 mL diethyl ether for 3 min using a vortex mixer. After centrifugation at 7000 × *g* for 5 min, the diethyl ether layer was decanted into a clean test-tube and evaporated under nitrogen at 37 °C. The residue was reconstituted in 100 μL mobile phase, vortexed, and centrifuged at 7000 × *g* for 5 min. A sample of the clear solution was injected into the HPLC system (Mendes et al., 2007).

For skin and muscle samples, the collected abdominal skin and muscle samples were weighed exactly and added to 5 μL internal standard solution (equal to 2 μg and 0.1 μg of carbamazepine, respectively) and then extracted with 1 mL diethyl ether for 10 min with ultrasonication. The mixture was centrifuged at 7000 × *g* for 5 min and the diethyl ether layer was decanted into a clean test-tube and evaporated under nitrogen at 37 °C. The residue was reconstituted in 1 mL and 100 μL mobile phase, respectively, vortexed, and centrifuged at 7000 × *g* for 5 min. A sample of the clear solution was injected into the HPLC system.

2.8. Quantitative analysis

The concentrations of anastrozole in the receptor medium, plasma, skin or muscle were determined using a HPLC method. The HPLC system was equipped with an L-2420 variable-wavelength ultraviolet absorbance detector and an L-2130 pump (Hitachi High-Technologies Corporation, Tokyo, Japan). The reversed phase stainless-steel column (200 mm × 4.6 mm) was packed with Diamonsil C-18 (5 μm particle size; Dikma Technologies, Beijing, China). The mobile phase was a mixture of methanol and water (57:43) for *in vitro* and (50:50) containing 0.1% acetic acid and triethylamine for plasma and tissue samples at a flow-rate of 1 mL/min. The wavelength was set at 220 nm for all studies. The external standard method was used for the permeation studies and the injection volume was 60 μL. For local tissue disposition studies, the internal standard was carbamazepine and the injection volume was 20 μL.

2.9. Data treatment

All *in vitro* experiments were carried out in triplicate. The amount of drug permeated through the skin during a sampling interval was calculated based on the measured receptor-phase concentration and volume. The cumulative amount of drug permeated per unit area (*Q*) versus time was plotted. The slope of the linear portion of the plot was calculated as the steady-state flux (J_{ss}, 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.

All data were calculated and presented as mean ± standard deviation (S.D.). Statistical analyses of the data were performed using ANOVA and the Student's *t*-test. The level of significance was taken as $P \leq 0.05$.

3. Results and discussion

3.1. Physical appearance of the anastrozole patch system

The adhesive layer was observed visually for completeness and surface texture. 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 80 ± 10 μm. No anastrozole crystals were found in any formulation by microscopic observation.

3.2. In vitro permeation study

The *in vitro* permeation experiments were tested for the optimization of formulation potency. The optimization process was based on determining a steady-state flux and Q_{24} for different formulations.

The anastrozole concentration in the PSAs was fixed at 2% (w/w, based on adhesive weight). Table 1 contains a summary of the permeation data for adhesive screening. In the drug-in-adhesive design, the simplest form for a transdermal patch system, as the drug and any excipients are directly loaded or dispersed into the PSA polymer, the PSA used has a unique influence on this very simple design. Acrylic PSAs have been known for a long time because of their favorable properties (Tan and Pfister, 1999). Therefore, DURO-TAK® acryl adhesives were selected and evaluated for the *in vitro* studies. To investigate the effect of different acrylic PSAs on the permeation of anastrozole across rat skin, DURO-TAK® 87-2852 and 87-2677 with carboxylic acid groups, and DURO-TAK® 87-4098 without functional groups, were tested. The cumulative amount of anastrozole permeated from the 87-4098 adhesive was 2.26 and 3.84 times higher than the 87-2677 and 87-2852 adhesives,

Table 1
Summary of data for the permeation of anastrozole from the patches with different formulations through excised rat skin.

Formulation			J_{ss} (μg/cm^2/h)	Q_{24} (μg/cm^2)
PSAs	Enhancers (w/w)	Anastrozole content (w/w)		
87-2852	–	2%	0.17 ± 0.09	7.19 ± 3.61
87-2677	–	2%	0.52 ± 0.22	12.19 ± 5.06
87-4098	–	2%	1.35 ± 0.51	27.61 ± 2.67
87-4098	5% MT	2%	3.78 ± 0.87	58.38 ± 18.58
87-4098	5% TP	2%	2.83 ± 0.35	72.28 ± 10.10
87-4098	5% OA	2%	4.48 ± 0.35	102.31 ± 9.54
87-4098	5% IPM	2%	7.13 ± 2.76	168.64 ± 36.78
87-4098	2% IPM	2%	4.10 ± 0.62	86.45 ± 12.69
87-4098	8%IPM	2%	11.34 ± 2.67	262.45 ± 61.18
87-4098	8% IPM	4%	17.35 ± 5.45	389.49 ± 71.94
87-4098	8% IPM	8%	26.13 ± 6.75	599.36 ± 85.72

Data are given as mean ± S.D. (n = 3).

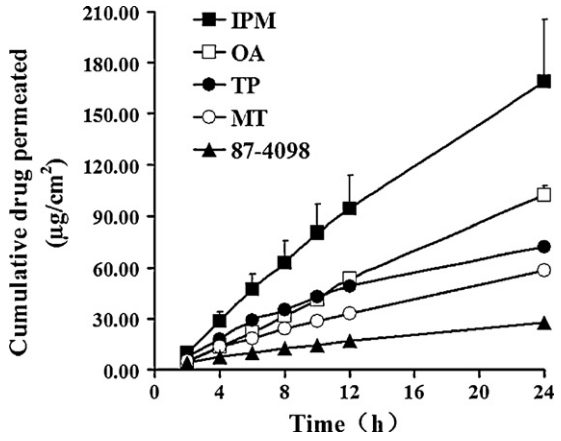

Fig. 2. The penetration profiles of anastrozole through excised rat skin from the patches containing 2% (w/w) anastrozole and 5% (w/w) of different enhancers in PSA 87-4098. Each point and bar shows the mean and S.D. ($n = 3$).

respectively. A *t*-test showed that the difference was statistically significant ($P < 0.05$). The data indicated that the patch using 87-4098 without any functional groups produced the highest flux of anastrozole and the patches using 87-2677 and 87-2852 produced very low flux of anastrozole. This result also showed that different functional groups in acrylic PSAs could have different effect on drug release, since the diffusivity of a drug molecule is restricted by the variation of the three-dimensional network of the polymer chains (Ouano et al., 1977). In addition, the five H-bonding acceptors in the chemical structure of anastrozole (obtained from Science Finder database) interact with the carboxylic acid group of the PSAs and produce a clear reduction in penetration. Therefore, 87-4098 was selected as the PSA of choice for the patch containing anastrozole.

The concentration of anastrozole and enhancers in the adhesive 87-4098 was fixed at 2% (w/w) and 5% (w/w), respectively. The use of chemical penetration enhancers seems to be an effective way to reduce the barrier properties of the stratum corneum. Therefore, it is important to select a suitable enhancer for drug permeation. IPM, OA and MT are considered by the FDA to be GRAS enhancers. TP has been shown to have an enhancing effect in previous studies (Harrison et al., 1996; Mura et al., 2000). The log *P* of IPM, OA, MT and TP are 7.43, 7.70, 3.20 and −0.62, respectively (obtained from Science Finder Database). The cumulative permeation profiles and data of all the formulations are shown in Table 1. This allowed a linear relationship to be obtained for each formulation ($r \geq 0.96$), and showed that there was a steady-state flux of anastrozole across the excised rat abdominal skin over the entire period of the experiment. Moreover, the drug permeation profiles (Fig. 2) followed zero-order kinetics suggesting that these formulations could provide a sustained and controlled permeation of the drug.

Optimizing the design of the transdermal patches is made more complex when an enhancer is incorporated into the device because of many potential interactions among drug, enhancer, system components and skin (Pfister and Hsieh, 1990). According to the data in Table 1, the skin permeation studies showed that the patches containing an enhancer could markedly enhance the skin penetration of anastrozole ($P < 0.05$) with respect to the patches containing no enhancer. In particular, the patch containing IPM provided the highest flux and enhancement. This result showed that the cumulative amount of drug permeated from the formulations, in general, was related to the structures and high lipophilicities of enhancers. Because of the chemical structure of the enhancers possessing a large polar head group and lipid alkyl chain, this may cause a reduction in cholesterol–cholesterol interference and cholesterol–ceramide interactions and increase the fluidity of the stratum corneum lipid bilayers, facilitating permeation of the hydrophilic permeants through the membrane and forming a reservoir in the skin (Williams and Barry, 2004; Trommer and Neubert, 2006). In addition, the semipolar microenvironment of the enhancer site of action is closely mimicked by liquid *n*-octanol (He et al., 2004; Ibrahim and Li, 2009). It is reckoned that the potency of enhancers is attributed to their partitioning between the aqueous phase and the intercellular lipid domain. Therefore, it has been suggested that the enhancers with a high *n*-octanol/water partition coefficient (log *P*) distribute well into the modified stratum corneum intercellular lipid domain.

To investigate the enhancement effect of the loading of IPM, further studies were carried out by preparing patches with different loadings of IPM alone. The flux of the formulation with 8% IPM was greater than those with 2%, 5% IPM. A *t*-test showed that these differences were statistically significant ($P < 0.05$). The fluxes of the formulations containing IPM showed a linear increase as the loading of IPM changed from 2 to 8% ($r = 0.9956$). Therefore, an IPM loading of 8% was used for the subsequent studies.

The penetration data from transdermal patches containing 2, 4 and 8% (w/w) anastrozole on the basis of 8% IPM are shown in Table 1. Addition of 4 and 8% anastrozole appeared to increase significantly the transport of drug compared with addition of 2% ($P > 0.05$). When anastrozole was loaded up to a level of 4 and 8%, the Q_{24} was 1.48 and 2.28 times higher, respectively, than with an anastrozole loading of 2%. In other words, since the loading of anastrozole increased, keeping rest of the formulation the same, it showed clearly a concentration-dependent effect in promoting the transdermal permeation of anastrozole. Therefore, the formulation containing 8% anastrozole and 8% IPM was the optimum for further investigations.

3.3. Disposition of anastrozole in mice

Since the principal source of estrogen in post-menopausal women is synthesized from adrenally generated androgen by aromatase in breast tumor and peripheral tissues, it is expected that a high drug concentration would be provided by site-specific delivery of transdermal patches to local tissues but not to the systemic circulation. To examine this, the drug concentrations in skin, muscle and plasma of mice were investigated after transdermal and oral administration. According to preliminary experiment, the same sampling points were designed for a point-to-point comparison of drug concentration in local tissue after oral and transdermal administration. The aim to the removal of the patches at 8 h was to investigate anastrozole reservoir deposition in local tissue.

Fig. 3 shows the concentrations of anastrozole in skin, muscle and plasma following application of transdermal patches and an oral suspension. Fig. 3A shows that anastrozole concentrations in skin, muscle and plasma increased following the application of the patches, then at 8 h reached average maximum concentrations. The profile indicates that the majority of the penetrated drug was retained in the skin following use of the transdermal patches. Following the removal of the patches at 8 h, the drug concentration in skin, muscle and plasma at 12 h was observed to exhibit a reduction of 28.4, 29.1 and 42.6%, respectively. A significant concentration gradient of anastrozole was observed between skin, muscle and plasma after transdermal administration at various intervals, compared with that after oral administration. Furthermore, the phenomenon that the anastrozole concentrations in skin, muscle and blood produced a relatively constant concentration between 0.17 and 12 h was due to rapid permeation of drug and the slow depletion of the drug accumulated in skin and muscle. It suggested an accumulation of anastrozole in the skin and muscle layers and its depot-forming ability after transdermal administration (Rao et al., 2003). This phenomenon demonstrated that permeated anastrozole would reach the site of action due to transport from the

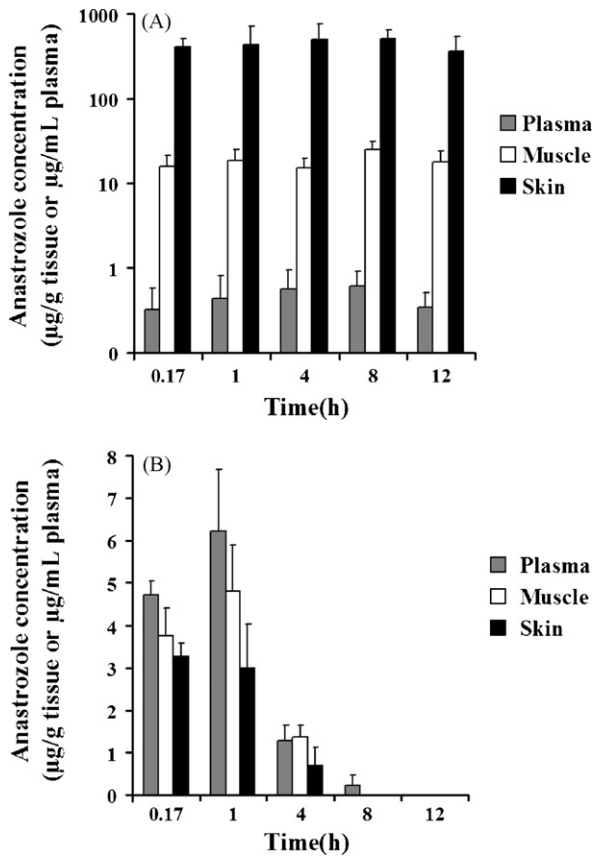

Fig. 3. (A) Mean plasma, skin and muscle concentration (μg/mL or μg/g) in mice after anastrozole transdermal patch (2 mg/1 cm^2, the patch was removed at 8 h); (B) mean plasma, skin and muscle concentration (μg/mL or μg/g) in mice after oral administration of anastrozole suspension (1.5 mg/mL in 0.5%, w/v, CMC-Na, 15 mg/kg) ($n = 4$).

PSA and retention in the stratum corneum, i.e. permeation through the stratum corneum being rate-limiting and/or reservoir deposition in the stratum corneum. In addition to the high and sustained drug concentrations in the layers of skin and muscle, the absence of significant systemic absorption of the drug is also desirable for the treatment of breast cancer without significant systemic side effects.

The maximum concentration in skin, muscle and plasma appeared at approximately 1 h following oral administration (Fig. 3B). The drug concentration in skin, muscle and plasma showed obvious fluctuations, falling sharply after 1 h following oral treatment. This was possibly due to the rapid and well absorption of anastrozole (AstraZeneca Canada Inc., 2008). The muscle–plasma concentration ratio (transdermal: 49.06, 43.02, 26.91, 41.48, 51.29; oral: 0.79, 0.77, 1.09) at these sampling times following transdermal administration were obviously higher than those following oral administration. This result indicated that sustained, controlled transport of anastrozole from the patch to the action site had the potential to maintain higher levels in local tissues and lower levels in the systemic circulation after application of patches compared with oral treatment, and the reduced fluctuations between peaks and troughs that might be associated with side effects (Ye et al., 2008). These results also proved that it was feasible to produce site-specific delivery of anastrozole without producing high serum concentrations as required in breast cancer by applying transdermal patches.

4. Conclusion

The present work was carried out to optimize the formulation to maximize the skin permeability of anastrozole using transdermal patches. The optimum formulation for *in vitro* skin permeation contained DURO-TAK® 87-4098, IPM 8% and anastrozole 8%. As expected, the local tissue disposition studies revealed that, compared with oral administration, transdermal administration could produce high local drug concentrations and low circulating drug concentrations increasing efficacy and reducing of systemic side effects. The results presented in this study suggest that applying transdermal patches containing anastrozole can achieve targeted anastrozole by sustained, controlled site-specific delivery to local tissues without producing high serum concentrations. These findings show that anastrozole transdermal patches will has a good prospect for treating breast cancer.

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