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Rapid Communication

The Transdermal Patches for Site-Specific Delivery of Letrozole: A New Option for Breast Cancer Therapy

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Abstract. The aim of this work was to evaluate capability of site-specific delivery of a transdermal patch through determination of letrozole in local tissues disposition in female mice. After transdermal administration, the letrozole levels in skin, muscle, and plasma were 10.4–49.3 μg/g, 1.64–6.89 μg/g, and 0.35–1.64 μg/mL, respectively. However, after the mice received letrozole suspension, the drug concentration of plasma and muscle were 0.20–4.80 μg/mL and 0.15–2.38 μg/g. There was even no drug determined in skin through all experiments. Compared with oral administration, the transdermal patch for site-specific delivery of letrozole could produce high drug concentrations in skin and muscle and meanwhile obtain low drug level in plasma. These findings show that letrozole transdermal patch is an appropriate delivery system for application to the breast tumor region for site-specific drug delivery to obtain a high local drug concentration and low circulating drug concentrations avoiding the risk of systemic side effects.

KEY WORDS: letrozole; local tissue disposition; site-specific drug delivery; transdermal patch.

INTRODUCTION

Estrogens play important roles in the development of hormone-dependent breast carcinomas. While ovarian estrogen synthesis ceases at menopause, peripheral and local tissue's aromatization of androgens to estrogens continues and becomes a main source of estradiol. The development of aromatase inhibitors represents a new strategy in the treatment of breast cancer. As the third-generation aromatase inhibitors (AIs), letrozole has been approved by FDA to be widely used as a first-line drug in the endocrine treatment of estrogen-dependent breast cancer in postmenopausal patients [1].

However, it has been reported that plasma estrogen levels do not necessarily reflect tissue estrogen concentrations [2]. Several studies have found that tissue estrogen levels may be ten- to 20-fold higher compared to plasma levels in postmenopausal women [3–5]. Furthermore, recent studies [6, 7] have demonstrated that a large proportion (close to 100%) of the biologically active estrogen is considered to be produced locally in the breast carcinoma after menopause. Therefore, it is a more effective method to inhibit estrogen of breast tissue than that of systemic circulation.

In addition, AIs have some adverse events in common that are caused by the circulating estrogen depletion, for example, hot flushes and bone damage [8–10]. Otherwise, at present, the only commercial dosage form of letrozole is oral tablets, which must be administered once daily. Accordingly, developing an alternative dosage form that increases local tissue concentrations in the application position and decreases drug level in plasma, is easy to administer, and is easy to comply is worthwhile. The transdermal route encompasses all the above advantages. To date, no research has been done which focused on the transdermal preparation of letrozole in particular site-specific delivery.

The purpose of this study is to evaluate the capability of site-specific delivery in local tissue for the transdermal patch of letrozole.

MATERIALS AND METHODS

Materials

Letrozole (Suzhou Everfortune IMP & EXP CO. Ltd., China), carbamazepine (Zhejiang Jiuzhou Pharmaceutical Co. Ltd., China), diethyl ether, and methanol (China National Medicines Co. Ltd., China) were used in this study.

In Vivo Local Tissue Disposition Studies

Female mice weighing 20±2 g were used for these studies. 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 hair on the abdomen of mice was removed with electric hair clippers and a shaver before administration. The

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Table I. Mean Plasma, Muscle, and Skin Concentrations (Micrograms Per Milliliter or Micrograms Per Gram) in Mice Compared Oral Administration of Letrozole Suspension (5.0 mg/mL, 50 mg/kg) with Letrozole Transdermal Patch (3 mg/5 cm^2, the Patch was Removed after an Interval of 24 h; $n=4$)

	Transdermal			Oral		
Time (h)	Plasma	Muscle	Skin	Plasma	Muscle	Skin
4	0.35±0.05[a]	1.64±0.22[a]	32.9±3.15[a]	4.80±0.67	2.38±0.21	ND
12	1.64±0.12[a]	6.89±0.36[a]	49.3±8.55[a]	3.22±0.37	1.40±0.11	ND
24	1.29±0.12	4.19±0.51[a]	27.5±1.63[a]	1.88±0.28	0.24±0.03	ND
36	0.90±0.09	2.53±0.10[a]	22.5±1.85[a]	0.81±0.15	0.15±0.01	ND
48	0.64±0.03[a]	1.79±0.17[a]	10.4±1.44[a]	0.20±0.01	ND	ND

Each value represents average of four measurements, mean value±SE
ND no detection
[a] Value is significantly different from oral administration group at same time point, $P<0.05$

mice were divided in two groups, each of 20 mice and treated as follows:

Group I: Letrozole suspension (5 mg/mL in 0.5% *w/v* CMC-Na, 50 mg/kg; p.o.);
Group II: Letrozole patches (5 cm^2, 3 mg of drug).

All patch formulations were applied to the abdominal area (surrounding breast tissue) after removal of abdominal hair. At different time intervals, four mice in every group were anesthetized (diethyl ether). Blood samples were collected in dried heparinized tubes by cardiac puncture. After the mice were sacrificed by dislocating the spinal cord, the abdominal skin and muscle beneath the drug application site were collected. It is noted that the residual adhesive on the skin surface was carefully wiped off using cotton soaked in ethyl acetate after removal of the patches. In addition, the skin and muscle of the surrounding breast tissue in the oral group were also taken as control. The samples were stored at −20°C until analysis.

Treated Method for Samples

Plasma Samples

Firstly, 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 3,000 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 analysis.

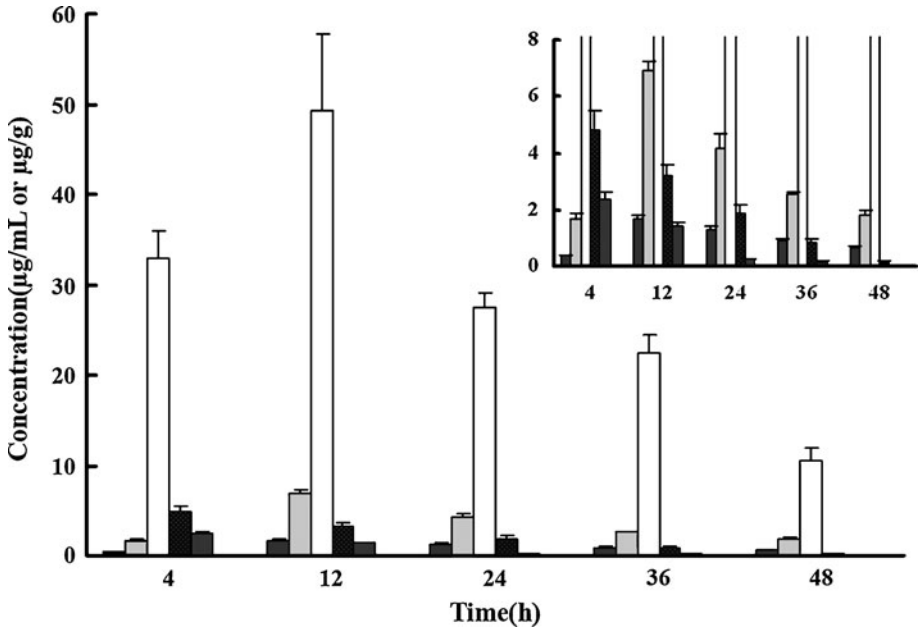

Fig. 1. Drug plasma, muscle, and skin samples concentrations (micrograms per milliliter or micrograms per gram) in female mice given letrozole transdermal patch (3 mg/5 cm^2, removed at 24 h) and oral administration of letrozole suspension (50 mg/kg). Transdermal administration: plasma (■), muscle (□), skin (□); oral administration: plasma (▩), muscle (▩), skin (▨). The *small graph* located in the *upper right* is a magnified version of the main graph in order to clearly see the relative concentrations of letrozole in muscle, skin, and plasma following both routes of administration. Each column represents the mean±SE of four mice

Muscle Samples

One milliliter of diethyl ether and 10 μL carbamazepine solution (50 μg/mL) were added to the comminuted muscle (about 0.04 g) following sonicating for 10 min. The sample was vortexed and centrifuged for 5 min at 10,000 rpm. The diethyl ether fraction was removed and dried, and the residue was reconstituted in 100 μL mobile phase for analysis.

Skin Samples

One milliliter of methanol and 10 μL carbamazepine solution (5 μg/mL) were added to 0.05 g of comminuted skin sample in a 2-mL centrifuge tube and vortex-mixed for 30 s. Letrozole was extracted with methanol by sonicating for 10 min. Then, the mixture was vortexed for 30 s and centrifuged for 5 min at 10,000 rpm. The clear solution was placed into a clean vial for analysis.

Analytical Method

The letrozole content of the samples was analyzed by high-performance liquid chromatography (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 (55:45, *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.

To validate the current analytical method, extraction efficiency, intra-day precision, and inter-day precision for letrozole in samples were determined. The extraction efficiency for letrozole in plasma, muscle, and skin was 84.5±2.64%, 91.4±2.48%, and 87.8±2.79%, respectively. The intra-day precision for letrozole, expressed as relative standard deviation (RSD%), was 2.07%, 2.51%, and 1.47% for plasma, muscle, and skin samples, respectively. The inter-day precision for letrozole was 2.81%, 2.48%, and 1.99% for plasma, muscle, and skin samples, respectively.

Statistical Analysis

All data were expressed as the mean value±SE. Student's *t* test was used to compare two group data (transdermally and oral administration) with the level of significance set at $P<0.05$.

RESULT AND DISCUSSION

Aiming to increase the drug concentration in breast tissue, avoid the system side effect, and improve multiple daily dose regimens associated with the oral administration, we designed a letrozole transdermal patch for site-specific delivery. In order to examine the role of localization, it is critical to study drug local tissue disposition (*e.g.*, plasma, muscle, and skin concentrations) after transdermal and oral administration. Table I and Fig. 1 show the concentrations of letrozole in skin, muscle, and plasma following application of transdermal patches and oral suspension.

After oral administration, most of letrozole concentrate in plasma, and less or none exist in muscle and skin. In the case of letrozole concentration, the level in muscle was only 0.1–0.5-fold higher than that in plasma. There was even no drug determined in muscle at 48 h. Neither are all samples of skin. However, an opposite result was found in the transdermal patches. Letrozole levels in skin and muscle were 16–95-fold and two- to fivefold greater than levels detected in plasma, respectively. It indicated that transport of letrozole from the patch to the action site had the potential to maintain higher letrozole levels in local tissues. These results also proved that it was feasible to produce site-specific delivery of letrozole without producing high plasma concentrations as required in breast cancer by applying transdermal patches.

When the patches were applied to the site of action, the letrozole is released from the patches to the skin, and majority are retained in the stratum corneum, then some of it may enter from skin to muscle, and last, only few can enter the blood. Moreover, aromatase, which catalyzes estrogen biosynthesis of androgens to estrogens, is mainly expressed in subcutaneous adipose, skin, and normal breast tissue as well as malignant tissue in a postmenopausal woman [11–13]. Based on the above findings, if the transdermal patch of letrozole is adhered on the breast, a high local drug concentration in skin and muscle can inhibit aromatase efficiently and accordingly decrease the level of estrogens in postmenopausal patients. Otherwise, low drug concentrations in plasma can avoid the risk of systemic side effects caused by the circulating estrogen depletion.

CONCLUSION

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 and reduce systemic side effects. Therefore, it is a new option for breast cancer therapy to inhibit aromatase activity via the transdermal patches for site-specific delivery of letrozole.

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