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Effect of *Citrus suavissima* Hort. ex Tanaka on pharmacokinetics of erlotinib in rat plasma by UPLC-MS/MS

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ABSTRACT

Twelve Sprague-Dawley rats were randomly divided into two groups: *Citrus suavissima* Hort. ex Tanaka group and control group ($n = 6$). The rats in *Citrus suavissima* Hort. ex Tanaka group were given *Citrus suavissima* Hort. ex Tanaka juices (1 mL/100 g) by oral administration each day, continued for 14 days; the rats in control group were given Stroke-physiological saline solution (1 mL/100 g) by oral administration each day, continued for 14 days. The rats of these two groups were given a single oral administration of erlotinib (20 mg/kg) on the 15th day. After blood sampling at different time points and processing, the concentrations of erlotinib in rat plasma were determined by the established ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method. Chromatographic separation was achieved using a UPLC BEH C18 column (2.1 mm $\times$ 50 mm, 1.7 μ m) with erlotinib-d6 as an internal standard (IS). The initial mobile phase consisted of acetonitrile and water (containing 0.1% formic acid) with gradient elution. Multiple reaction monitoring (MRM) modes were utilized to conduct quantitative analysis. The sensitive, rapid and selective UPLC-MS/MS method was successfully applied to analyse the effect of *Citrus suavissima* Hort. ex Tanaka on pharmacokinetics of erlotinib in rat plasma. There were no significant differences in $AUC_{(0-t)}$, $t_{1/2}$, T_{max} , CL , C_{max} between the two groups ($P > 0.05$). While $MRT_{(0-t)}$ was decreased ($P < 0.05$) in *Citrus suavissima* Hort. ex Tanaka group, compared to the control group. It showed that *Citrus suavissima* Hort. ex Tanaka could not affect the metabolism of erlotinib.

KEYWORDS

erlotinib, pharmacokinetic, interaction, UPLC-MS/MS

INTRODUCTION

Citrus suavissima Hort. ex Tanaka is a cultivar of the genus Citrus in the genus Rutaceae, and is a special fruit of Wenzhou, Zhejiang [1, 2]. According to the historical records, Wenzhou citrus has been listed as a tribute since the Jin and Tang Dynasties [3, 4]. It has special medical effects such as phlegm-heating, phlegm and relieving cough, cooling and detoxification. It is known as the “Dragon Boat Festival in the Duanwu.” Through solvent extraction and column chromatography separation, limonin ($C_{26}H_{30}O_8$) and β -sitosterol ($C_{29}H_{50}O$) were extracted from the seeds and pulp of *Citrus suavissima* Hort. ex Tanaka, respectively [5]. Their structures were identified by spectral analysis (1H NMR, ^{13}C NMR, DEPT-NMR and 2D-NMR). The consumption of *Citrus suavissima* Hort. ex Tanaka in Zhejiang is very

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Fig. 1. Molecular structure of erlotinib

popular, and it contains a variety of biologically active substances [6–12], which may interact with many oral drugs, mainly to change the bioavailability of these drugs.

Erlotinib (Fig. 1) is a selective epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) for EGFR mutant non-small-cell lung cancer (NSCLC) patients having significant efficacy and is commonly used in the treatment of locally advanced or metastatic non-small cell lung cancer with two or more chemotherapy regimens failing [13–17]. Clinically, cancer patients are relatively scarce in nutrition. Patients often buy fruits for additional supplements. *Citrus suavisissima Hort. ex Tanaka* is rich in vitamins, has high nutritional value and good taste. It is one of the first choices for fruit supplementation. At present, there are few studies on the interaction of citrus fruits on clinical drugs. The effects of *Citrus suavisissima Hort. ex Tanaka* juice on the pharmacokinetics and adverse reactions of erlotinib are of great significance for rational and safe drug use.

Consequently, in the present study, we established an ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method for the quantification of erlotinib in rat plasma. Then the UPLC-MS/MS method was successfully applied to effect of *Citrus suavisissima Hort. ex Tanaka* on pharmacokinetics of erlotinib in rat plasma.

MATERIALS AND METHODS

Chemicals and reagents

Erlotinib (purity > 98%, Fig. 1), and erlotinib-d₆ (IS, purity > 98%) were purchased from Toronto Research Chemicals (North York, ON, Canada). HPLC-grade acetonitrile was purchased from Merck Company (Darmstadt, Germany). Ultra-pure water was prepared by Millipore Milli-Q water system (Bedford, MA, USA). Sprague Dawley (SD) rats (male, body weight 200–220 g) were obtained from Animal Experimental Center of Wenzhou Medical University.

Instrumentation and conditions

A UPLC-MS/MS system with ACQUITY H-Class UPLC and a XEVO TQS-micro triple quadrupole mass spectrometer (Waters Corp., Milford, MA, USA). Masslynx 4.1 software (Waters Corp.) was used for data acquisition and instrument control. Chromatographic separation was achieved using a UPLC BEH C18 column (2.1 mm × 50 mm,

1.7 μm) maintained at 40 °C. We followed the methods of Chen et al. [18].

The mobile phase consisted of acetonitrile and water (containing 0.1% formic acid) with gradient elution at a flow rate of 0.6 mL/min. The gradient elution was as follows: 0–0.2 min, acetonitrile 10%; 0.2–1.5 min, linear acetonitrile from 10% to 80%; 1.5–2.0 min, acetonitrile 80%; 2.0–2.1 min, linear acetonitrile from 80% to 10%; 2.1–3.0 min, acetonitrile 10%.

Nitrogen was used as the desolvation gas (900 L/h) and cone gas (50 L/h). Ion monitoring conditions were defined as capillary voltage of 1.5 kV, source temperature of 150 °C, and desolvation temperature of 450 °C [19]. Multiple reaction monitoring (MRM) modes of m/z 393.9 → 278.2 for erlotinib, and m/z 400.2 → 278.1 for erlotinib-d₆ (IS) were utilized to conduct quantitative analysis (Fig. 2).

Sample preparation

In a 1.5 mL centrifuge tube, 50 μL plasma, acetonitrile (200 μL, containing erlotinib-d₆ 50 ng/mL) was added into plasma, mixed by a vortexer for 1.0 min, and centrifuged at 13,000 rpm for 10 min at 4 °C. Two microliters of the supernatant was injected into the UPLC-MS/MS system for analysis.

Pharmacokinetic study

Twelve Sprague-Dawley rats (200–220 g) were randomly divided into two groups: *Citrus suavisissima Hort. ex Tanaka* group and control group ($n = 6$). First, the fresh *Citrus suavisissima Hort. ex Tanaka* were peeled off and the seeds were removed, and then a juicer was used to obtain the juice from pulp. The rats in *Citrus suavisissima Hort. ex Tanaka* group were given *Citrus suavisissima Hort. ex Tanaka* juice

Fig. 2. Mass spectrum of erlotinib and erlotinib-d₆

Fig. 3. Representative UPLC-MS/MS chromatograms of erlotinib and erlotinib-d6 in rat plasma

Table 1. Accuracy, precision, matrix effect and recovery of erlotinib in rat plasma

Concentration (ng/mL)	Precision (RSD%)		Accuracy (%)		Matrix effect (%)	Recovery (%)
	Intra-day	Inter-day	Intra-day	Inter-day		
1	11.2	12.8	105.9	106.5	105.5	93.2
3	7.3	5.6	99.4	99.0	100.4	94.6
400	3.6	3.2	103.4	105.5	105.6	95.4
1,800	6.5	4.7	102.1	97.5	107.0	94.5

(1 mL/100 g) by oral administration each day, continued for 14 days; the rats in control group were given Stroke-physiological saline solution (1 mL/100 g) by oral administration each day, continued for 14 days. The rats of two groups received a single oral administration of erlotinib (20 mg/kg) on the 15th day. Blood samples (0.3 mL) from the tail vein were collected into 1.5 mL heparinized polypropylene tubes at 0.085, 0.17, 0.33, 0.5, 1, 2, 3, 4, 6, 8, 12, 24 h, after oral administration. The samples were immediately centrifuged at 13,000 rpm for 10 min at 4 °C. Then the plasma was transferred to a new 1.5 mL tube and stored at –20 °C until analysis.

Plasma concentration versus time data for each rat was analyzed using DAS (Drug and Statistics) software (version 2.0, China Pharmaceutical University). Then, the main pharmacokinetic parameters such as area under the curve (AUC), half-life ($t_{1/2}$), peak time (T_{max}), clearance rate (CL), maximum plasma concentration (C_{max}), mean residence time (MRT) and apparent distribution volume (V) were obtained.

RESULTS AND DISCUSSION

Method development

The choice of positive and negative electrodes for electrospray ESI is often evaluated in methodology. Erlotinib is more suitable for ESI positive electrode detection [20, 21]. The ion fragments were further optimized, and the highest

peaks of fragment ion pairs were selected as quantitative ion pairs, m/z 393.9 $\rightarrow$ 278.2 for erlotinib, and m/z 400.2 $\rightarrow$ 278.1 for erlotinib-d₆ (IS) (Fig. 2). Methods selection of IS was also an important task in the process of establishment [22–25]. Finally, erlotinib-d₆ was selected as IS because erlotinib-d₆ and erlotinib have almost same structure and mass spectrometric ionization mode.

As far as possible, the internal interfering substances are separated from the retention time by HPLC, mobile phase and chromatographic column determine the chromatographic behavior [26–28]. We tried acetonitrile-0.1% formic

Fig. 4. Mean plasma concentration–time curves of erlotinib in rat plasma

Table 2. Primary pharmacokinetic parameters of erlotinib in rats ($n = 6$)

Parameters	Unit	<i>Citrus suavisissima Hort. ex Tanaka</i>	Control
$AUC_{(0-t)}$	ng/mL*h	90,132.5 $\pm$ 19,817.7	115,228.4 $\pm$ 25,477.9
$AUC_{(0-\infty)}$	ng/mL*h	186,824.9 $\pm$ 140,455.2	298,811.2 $\pm$ 170,006.6
$MRT_{(0-t)}$	h	7.0 $\pm$ 2.7*	10.1 $\pm$ 1.0
$MRT_{(0-\infty)}$	h	26.9 $\pm$ 32.4	45.2 $\pm$ 23.4
$t_{1/2z}$	h	18.7 $\pm$ 22.5	31.6 $\pm$ 16.1
T_{max}	h	2.2 $\pm$ 0.4	2.6 $\pm$ 0.5
$CL_{z/F}$	L/h/kg	2.4 $\pm$ 0.9	3.1 $\pm$ 0.8
$V_{z/F}$	L/kg	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1
C_{max}	ng/mL	10,612.6 $\pm$ 5,421.3	8,934.9 $\pm$ 1,795.2

Compared to control group, * $P < 0.05$.

acid, acetonitrile-10 mmol/L ammonium acetate solution (containing 0.1% formic acid), methanol-0.1% formic acid, methanol-10 mmol/L ammonium acetate solution (containing 0.1% formic acid) and gradient elution. The results showed that acetonitrile-0.1% formic acid obtained the most satisfactory chromatographic peak shape and retention time (t_R was 1.29 min for erlotinib and IS) (Fig. 3).

UPLC-MS/MS method verification

Typical UPLC-MS/MS chromatograms of blank plasma spiked with erlotinib and IS were shown in Fig. 3, with reasonable peak time of 1.29 min and sharp peak shape. No interference of visible impurity and endogenous substances was observed, indicating that the analyte of interest was efficiently separated by the optimized gradient elution procedure.

The concentration of erlotinib standard curve in rat plasma was within the range of 1–2,000 ng/mL. The equation of standard curve is $y = 0.0018x - 0.0007$, $r = 0.9983$, where y is the peak area ratio of erlotinib to internal standard and x represents erlotinib concentrations in plasma. The LLOQ of erlotinib in rat plasma was 1 ng/mL.

As shown in Table 1, intra-day and inter-day precision RSD of erlotinib in rat plasma was lower than 13%. Accuracy range was between 97.5 and 106.5%, and matrix effect was between 100.4 and 107.0%.

Pharmacokinetic study

The mean plasma concentration–time curve of erlotinib was shown in Fig. 4; compared with the control group, the AUC and C_{max} of *Citrus suavisissima Hort. ex Tanaka* group are larger, but there is no statistical difference, and there may be larger individual differences in rats, which needs further research to confirm. The primary pharmacokinetic parameters based on non-compartmental model analysis are summarized in Table 2. There were no significant differences in the $AUC_{(0-t)}$, $t_{1/2}$, T_{max} , CL , C_{max} between the two groups ($P > 0.05$). While $MRT_{(0-t)}$ was decreased ($P < 0.05$) in *Citrus suavisissima Hort. ex Tanaka* group compared to the control group.

Another new additive effect of drugs and foods in the body, that is, changes in pharmacokinetic characteristics or pharmacodynamic properties or effects on the body's

nutrition, is called food–drug interaction (FDI) [29–32]. FDI may cause changes in the effects of drugs, increasing or decreasing the occurrence of adverse drug reactions. For example, grapefruit juice can significantly inhibit the activity of CYP450 3A4 in the liver and intestinal wall, thereby increasing the exposure of drugs metabolized by 3A4 *in vivo*, which may increase the probability of adverse reactions caused by drugs. Lin et al. gave rats a single dose and multiple doses of *Citrus suavisissima Hort. ex Tanaka* juice [33] and found that a single dose of *Citrus suavisissima Hort. ex Tanaka* juice had no effect on the activity of CYP450 in rats, while multiple doses of *Citrus suavisissima Hort. ex Tanaka* juice could inhibit CYP1A2 and CYP2C19, and induce the activity of CYP2C9; however, the effect on CYP3A was not investigated in their study. Erlotinib is mainly metabolized in the body by the drug metabolism enzyme CYP3A in the liver, and a small amount is metabolized by the CYP1A2, 2C8 and 2D6 pathways [34–36]. However, the $AUC_{(0-t)}$, $t_{1/2}$, T_{max} , CL , C_{max} were not significantly changed, after *Citrus suavisissima Hort. ex Tanaka* could not affect the metabolism of erlotinib.

CONCLUSION

In the present study, we investigated the effect of *Citrus suavisissima Hort. ex Tanaka* on pharmacokinetics of erlotinib in rats by UPLC-MS/MS method, which showed that *Citrus suavisissima Hort. ex Tanaka* could not affect the metabolism of erlotinib.

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