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*Original Article*

# Effects of quercetin on the pharmacokinetics of erlotinib in rats

Xiuwei Shen, Fan Chen, Fengwei Wang, Jipiao Liu, Peng Huang

*Ruian People's Hospital, The Third Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang Province, PR China*

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**Abstract:** The aim of this work was to research the effect of quercetin on the pharmacokinetics of erlotinib in rats. Twelve healthy male Sprague-Dawley rats were divided into two groups at random: A group (control group, received normal saline for 7 days), B group (tested group, 4 mg/kg quercetin for 7 days). All the rats were given a single dose of erlotinib with the concentration of 10 mg/kg after the last administration. The plasma concentration of erlotinib was detected using high performance liquid chromatography with diode array detector (HPLC-DAD) and different pharmacokinetic parameters were calculated by DAS 3.0 software. Compared to the control group, quercetin (B group) significantly increased the $C_{max}$ and AUC of erlotinib, but decreased $CL_z/F$ in rats. The results revealed that quercetin has a significant inhibition on the metabolism of erlotinib. Therefore, it is recommended that the concomitant use of erlotinib with quercetin should be avoided.

**Keywords:** Erlotinib, quercetin, HPLC, pharmacokinetics

## Introduction

Erlotinib is an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor; it has been approved for the first-line treatment of non-small cell lung cancer (NSCLC) with mutated EGFR, second-line treatment of NSCLC and first-line treatment of advanced pancreatic adenocarcinoma [1, 2]. The drug metabolism of Erlotinib may be altered by pharmacokinetic drug-drug interactions leading to high interpatient variability in plasma concentration [3]. It has been established that the magnitude of the pharmacological effect (tyrosine kinase inhibition) in vitro is concentration dependent. Moreover, in clinical researches though plasma concentrations of erlotinib seemed to correlate with treatment outcome [4, 5].

Quercetin is polyphenolic flavonoids easily found in vegetables, fruit, and various medical plants. Many studies have reported a broad range of pharmacological properties of quercetin, including benefits for neurodegeneration [6], hypertension [7], inflammation [8]. In addition to these beneficial effects, quercetin plays preventive and therapeutic roles in various types of cancer [9, 10].

Quercetin shows a significant inhibitory effect on CYP450 as dietary flavonoid. The potent inhibition of quercetin to CYP isoforms may influence metabolism process of many drugs. In the treatment of cancer, drug co-administration and individual differences in therapeutic effectiveness are commonplace. A vast variety of drugs that are metabolized by CYP450 should be used cautiously in clinical treatment. Quercetin has inhibitory effects on CYP3A2. CYP450 genes are highly promising for applications to enhance the sensitivity of tumor cells to cancer chemotherapeutic drugs [11].

For oral medications, drug metabolism can be influenced by drugs that inhibit the activity of hepatic enzyme. These drugs are often co-administered, there is an increasing issue of interaction between them. To date, it is still unclear whether quercetin lead to altering metabolism of erlotinib generally. Therefore, in the paper, a single rapid and sensitive high performance liquid chromatography (HPLC-VU)

method is described for the determination of erlotinib in rat plasma and to research the effect of quercetin on the pharmacokinetics of erlotinib in rats.

## Materials and methods

### *Chemicals materials*

Erlotinib (purity > 98%) and carbamazepine (purity > 98%, IS) were provided from Sigma (St. Louis, MO, USA). Quercetin (purity > 98%) were obtained from Xi An Kai Lai Biological Engineering Co., Ltd. (Xi An, China). Methanol and acetonitrile were HPLC grade and provided by Merck Company (Darmstadt, Germany). HPLC grade water was acquired using a Milli Q system (Millipore, Bedford, USA).

### *Instrumentation and conditions*

The analytes were performed using an Agilent 1100 liquid chromatographic system equipped with a G1379A vacuum degasser, a G1311A quaternary pump, a G1316A column oven, a G1313A autosampler and G1315B DAD detector.

Samples were separated on a ZORBAX Eclipse XDB-C18 (4.6 × 150 mm, 5 μm, Agilent, USA) and XDB-C18 protection column (4.6 × 12.5 mm, 5 μm, Agilent, USA) at 40°C. The mobile phrase consisted of acetonitrile-0.1% trifluoroacetic acid-water (33:20:47). All compounds were detected at an optimum wavelength of 346 nm, and the flow rate of mobile phrase was 1.0 mL/min.

### *Preparation of standard and quality control (QC) samples*

Individual stock solutions of Erlotinib (100.0 μg/mL) and carbamazepine (IS, 50.0 μg/mL) were dissolved in methanol. The working solutions for calibration and quality controls were made from the stock solution by diluting with methanol. All of the solutions were stored in a refrigerator at 4°C.

Calibration curve standards were prepared by spiking blank human plasma with appropriate amounts of the working solutions at final drug concentrations of 25, 50, 100, 250, 500, 1000, 2000 and 4000 ng/mL for Erlotinib. The preparation of QC samples was the same, with the three levels of plasma concentrations (50, 1000 and 3000 ng/mL).

### *Sample preparation*

Before analysis, the plasma samples were thawed to room temperature. In a 2 mL EP tube, an aliquot of 20 μL of the IS working solution (50 μg/mL) and 200 μL of NaOH (1 mol/L) were added to 200 μL of collected plasma sample followed by the addition of 1.0 mL ethyl acetate. The tubes were vortex mixed for 1.0 min. After standing for 30 min, the supernatant organic layer was transferred into a 1.5 mL EP tube and dried under nitrogen stream at 40°C. The dried residue was reconstituted in 50 μL of mobile phase and a 10 μL aliquot of this was injected into HPLC system for the analysis.

### *Method validation*

To evaluate the selectivity of the method, the blank human plasma and blank plasma spiked Erlotinib and IS were analyzed. Calibration curves were constructed and validated by analyzing spiked calibration samples on three days in a row. Peak area ratio of Erlotinib to IS was plotted against analyte concentrations, and standard curves were fitted by weighted ($1/\chi^2$) least squares linear regression in the concentration of 50-4000 ng/mL for Erlotinib. The lower limit of quantification (LLOQ) of Erlotinib in rat plasma was selected as the lowest concentration used in the calibration curve.

Accuracy and precision were assessed by the determination of QC samples at three concentration levels (50, 1000 and 3000 ng/mL) in six replicates. The precision was expressed by relative standard deviation (RSD) and the accuracy by the relative error (RE). In the same day, the Intra-precisions were calculated, and the Inter-precisions were calculated by continuous measurement within 3 days.

The extraction recoveries of Erlotinib at three QC levels (50, 1000 and 3000 ng/mL, n = 6) were determined by comparing peak area of the analytes in samples that were spiked with the analytes prior to extraction with those of samples to which the corresponding solution was added after extraction. The extraction recovery of the IS (50 μg/mL) was determined in a similar way as a reference.

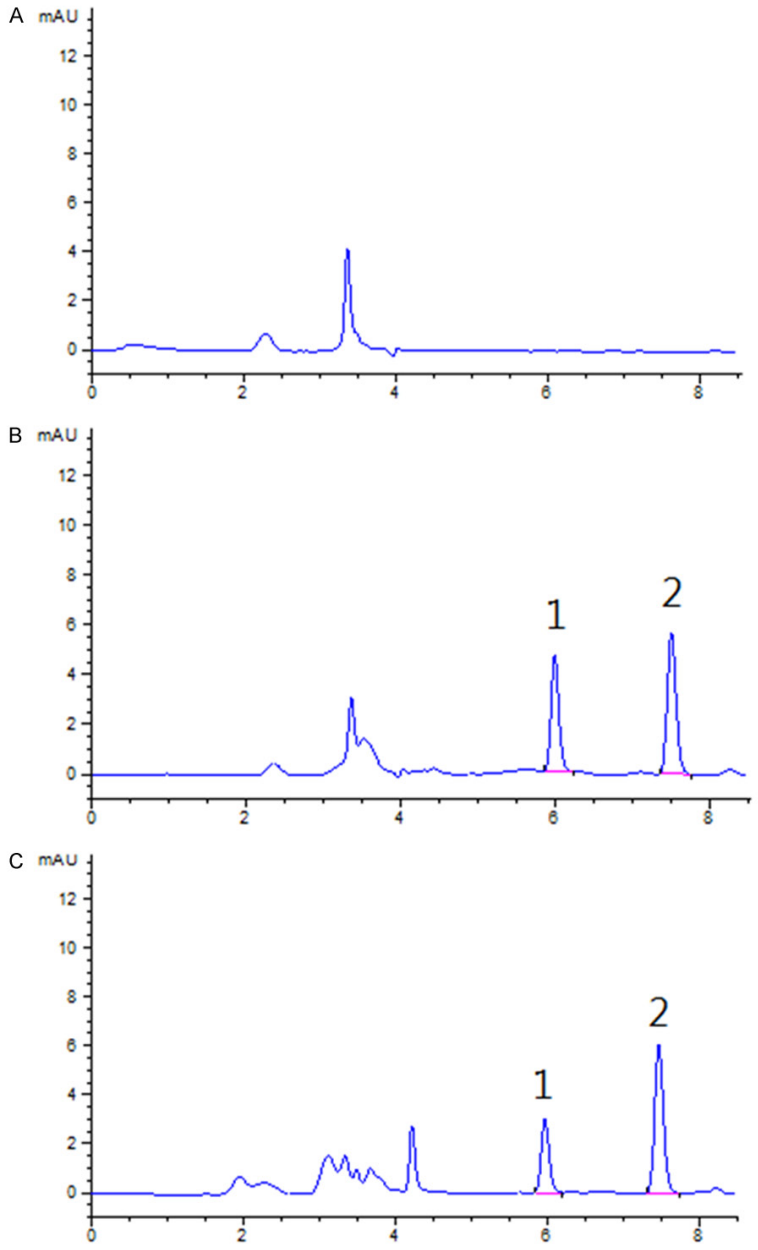


**Figure 1.** Representative HPLC for erlotinib and carbamazepine (IS) in rat plasma. A. Blank plasma sample; B. Blank plasma sample spiked with erlotinib2 (1000 ng/mL) and IS; C. Rat plasma sample. 1-Erlotinib; 2-carbamazepine (IS).

The stability of Erlotinib in rat plasma was tested by analyzing five replicates of plasma samples at three concentration levels (50, 1000 and 3000 ng/mL) in different conditions. The short-term stability was determined after the exposure of the spiked samples at room temperature for 12 h. The freeze-thaw stability was evaluated after three complete freeze-thaw cycles (-20°C) on consecutive days. The long-term stability was assessed after storage of the standard spiked plasma samples at -20°C for 21 days.

*Animals*

Male Sprague-Dawley rats with body weight of 220 ± 20 g were obtained from Henan University of Science and Technology. The rats were adapted to the new environment for 7 days in laboratory conditions in order to minimize the influence of any animal suffering before the experiment started. Necessary approval from the Institutional Animal Ethics Committee was obtained to carry out the experiments.

*Study design*

Twelve Sprague-Dawley male rats were randomly divided into 2 groups: A group (the control group received normal saline for 7 days), B group (long-term administered with 4 mg/kg quercetin for 7 days). All the rats were given a single dose of erlotinib with the concentration of 10 mg/kg. All the blood samples (0.3 mL) were collected from the tail-vein into heparinized 1.5 mL polythene tubes at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h after oral administration. The samples were immediately centrifuged at 4000 × g for 8 min. The plasma obtained (100 μL) was stored at -20°C until analysis.

*Statistical analysis*

The mean and standard deviation (SD) was used for the results. The noncompartmental analysis was used to calculate the pharmacokinetic parameters by DAS (Drug and statistics) software (Version 3.0, Shanghai University of Traditional Chinese Medicine, China). The statistical analyses were evaluated by unpaired

**Table 1.** Precision and accuracy for erlotinib of QC samples in rat plasma (n = 6)

| Compound | Added (ng/mL) | Intra-day | | | Inter-day | | |
|---|---|---|---|---|---|---|---|
| | | Found (ng/mL) | RSD% | RE% | Found (ng/mL) | RSD% | RE% |
| Erlotinib | 50 | 49.55 ± 2.39 | 4.83 | -0.90 | 49.53 ± 2.88 | 5.81 | -0.94 |
| | 1000 | 1010.83 ± 50.11 | 4.96 | 1.08 | 1016.80 ± 51.57 | 5.07 | 1.68 |
| | 3000 | 3012.55 ± 96.25 | 3.19 | 0.42 | 2990.27 ± 87.08 | 2.91 | -0.32 |

**Table 2.** Recovery of erlotinib from rat plasma after extraction (n = 6)

| Compound | Concentration (ng/mL) | Recovery (%) | RSD (%) |
|---|---|---|---|
| Erlotinib | 50 | 78.16 ± 3.14 | 4.02 |
| | 1000 | 81.38 ± 1.93 | 2.37 |
| | 3000 | 80.56 ± 1.68 | 2.08 |

*t*-test (SPSS 19.0, Chicago, IL). A value of $P < 0.05$ was considered to be statistically significant.

## Results

*Sensitivity*

Under the experimental conditions described above, Erlotinib and IS were well separated from endogenous materials. Representative chromatograms of a blank plasma sample, a plasma sample spiked with Erlotinib and IS, and a rat sample are shown in **Figure 1**.

*Linearity of calibration curve*

The linear regressions of the peak area ratios versus concentrations were fitted over the concentration range of 25-4000 ng/mL for erlotinib in rat plasma. The typical equations of the calibration curve was as follow: $y = 0.3174x - 0.0084$, $r = 0.9995$, where y represents the ratio of peak area of erlotinib to that of IS, and x represents the plasma concentration. The LLOQ for the determination of erlotinib in rat plasma was 25 ng/mL.

*Precision and accuracy*

The precision of the method was evaluated by calculating RSD for QCs at three concentration levels (50, 1000 and 3000 ng/mL) over three validation days. The intra-day RSDs were 4.83%, 4.96%, 3.19% and inter-day RSDs were 5.81%, 5.07%, 2.91% respectively at three concentrations.

The accuracy of the method ranged from -0.94% to 1.68% for erlotinib at three QC levels. Assay performance data is presented in **Table 1**. The results demonstrated that the values were within the acceptable range and the method was accurate and precise.

*Recovery*

Mean extraction recoveries of erlotinib were (78.16 ± 3.14)%, (81.38 ± 1.93)% and (80.56 ± 1.68)% (n = 6) at the concentrations of 50, 1000 and 3000 ng/mL, respectively (**Table 2**).

*Stability*

The RSDs of three quality control plasma samples (50, 1000 and 3000 ng/mL) spiked erlotinib were less than 10%, and erlotinib have shown good stability in plasma for 12 h at room temperature, during three freeze-thaw cycles, and for 21 days at -20°C.

*Effect of quercetin on the pharmacokinetic study of erlotinib*

**Figure 2** reveals the mean plasma concentration-time profiles of erlotinib after oral administration of erlotinib (15 mg/kg) in different treatment group, including the control, quercetin group. The corresponding pharmacokinetic parameters are revealed in **Table 3**. As shown in **Table 3** and **Figure 2**, quercetin significantly altered the pharmacokinetic parameters of erlotinib. Compared with group A, the $C_{max}$ of erlotinib was significantly increased by 57.4% by quercetin. Moreover, the $AUC_{(0-\infty)}$ of erlotinib was increased by 64.3%. In addition, the $CL_z/F$ of erlotinib with quercetin was reduced by 54.3%. According to the data, it showed that quercetin have significant influence on the absorption of erlotinib in rats.

## Discussion

Quercetin is a bioflavonoid with antiproliferative and proapoptotic activity in various cancer

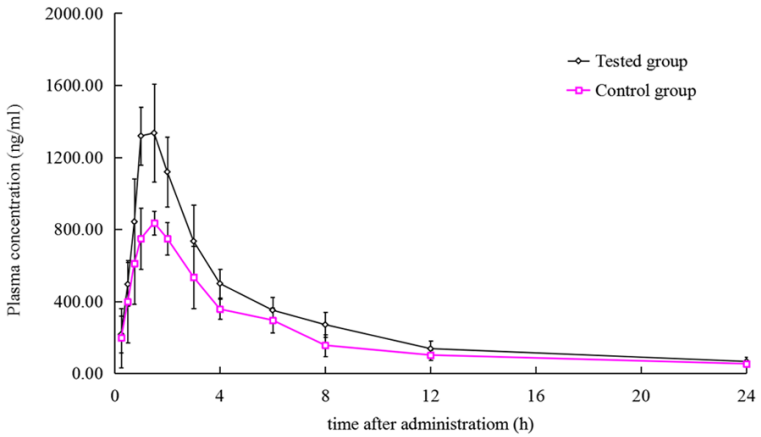


**Figure 2.** Mean plasma concentration time profiles of erlotinib in 2 groups after oral administration of 10 mg/kg erlotinib.

**Table 3.** The main pharmacokinetic parameters of erlotinib in 2 groups (n = 12)

| Parameters | B (Tested group, Erlotinib + quercetin) | A (Control group, Erlotinib) |
|---|---|---|
| $t_{1/2}$ (h) | 10.83 ± 4.72 | 8.37 ± 3.17 |
| $T_{max}$ (h) | 1.25 ± 0.27 | 1.29 ± 0.46 |
| $C_{max}$ (ng/mL) | 1421.04 ± 188.28 | 902.70 ± 91.18 |
| $AUC_{(0-t)}$ (ng·h/mL) | 7401.83 ± 925.74 | 4504.45 ± 727.31 |
| $AUC_{(0-\infty)}$ (ng·h/mL) | 8441.22 ± 1485.25 | 5242.82 ± 744.81 |
| $CL_z/F$ (L/h/kg) | 21.18 ± 6.91 | 46.35 ± 24.75 |
| $Vd_z/F$ (L/kg) | 1.82 ± 0.32 | 2.91 ± 0.40 |

cells. Based on previous analysis, it can be estimated that a similar proportion of participants with advanced NSCLC take quercetin. This possibly inhibit the activity of CYP450 and have the potential to increase the concentration of the medication. There was previous research has demonstrated that quercetin could inhibited the activity of CYP1A2 in healthy volunteers [12]. Quercetin is a globally recognized and relatively safe drug as a complementary or alternative medicine. The potential herb-drug interaction (HDI) is the major problem in the co-administration of quercetin and other medicines [13-16]. This co-administration poses a common clinical concern given by the inhibited of quercetin that may require therapy by decreasing the dosage of medication.

In our study, we evaluated the potential impact of concurrent administration of quercetin on erlotinib pharmacokinetics in rats. It was not a surprise that coadministration of the quercetin resulted in a substantial increase in erlotinib plasma concentration. The absence of any impact on erlotinib $t_{1/2}$ or $T_{max}$ supported that this observed increase in plasma concentration was due to inhibited reduced metabolism and elimination and not a change in absorption.

There are studies have been revealed the effect of antipeptic ulcer medicine on the erlotinib. One study demonstrated that sucralfate had no significant effect on the absorption of erlotinib or little increased the quantity of metabolite OSI-420 [17]. And another revealed that esomeprazole and omeprazole could reduce the absorption of erlotinib causing a reduction in exposure of drug in rats. So, it is necessary to avoid concomitant administration of PPIs with erlotinib [18].

In summary, quercetin increased exposure to erlotinib in rats by inhibiting the metabolism of erlotinib. The impact of quercetin on erlotinib's pharmacokinetic parameters is higher and is expected to be of similar significance on metabolite. In light of the current limited evidence, it is prudent and reasonable to avoid concomitant administration of erlotinib with quercetin if it is possible. Any clinical decision should be made by weighing the uncertain benefit of changing against the potential negative impact that such actions may have on the patient.

## Conclusion

Clinicians should be conscious that administration of quercetin can decrease the metabolism, thereby resulting in a significant increase in the plasma concentration of erlotinib. The combination of erlotinib and quercetin requires careful therapeutic drug monitoring of the erlotinib plasma concentration to ensure effective patient exposure to the drug.

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## Disclosure of conflict of interest

None.

**Address correspondence to:** Peng Huang, Ruian People's Hospital, The Third Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang Province, PR China. E-mail: hp2014032@126.com

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