Evaluation of the inhibitory effect of quercetin on the pharmacokinetics of tucatinib in rats by a novel UPLC–MS/MS assay

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ABSTRACT
Context: Tucatinib (CYP2C8 substrate) and quercetin (CYP2C8 inhibitor) are two common drugs for the treatment of cancer. However, the effect of quercetin on the metabolism of tucatinib remains unknown.

Objective: We validated a sensitive method to quantify tucatinib levels in rat plasma based on ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS), which was successfully employed to explore the effect of quercetin on tucatinib pharmacokinetics in rats.

Materials and methods: An Acquity UPLC BEH C18 column was applied to achieve the separation of tucatinib and internal standard (IS) talazoparib after protein precipitation with acetonitrile. Then, we used this assay to investigate the effect of different doses of quercetin (25, 50 and 100 mg/kg) on the exposure of orally administered tucatinib (30 mg/kg) in 24 Sprague-Dawley (SD) rats, which were randomly divided into three quercetin pre-treated groups and one control group (n = 6).

Results: Our developed assay was verified in all aspects of bioanalytical method validation, involving lower limit of quantification (LLOQ), selectivity, accuracy and precision, calibration curve, extraction recovery, matrix effect and stability. After pre-treatment with 100 mg/kg quercetin, AUC0–t, AUC0–∞ and Cmax of tucatinib were remarkably increased by 75.4%, 75.8% and 59.1% (p < 0.05), respectively, while CLz/F was decreased significantly by 47.3% (p < 0.05) when compared with oral administration of 30 mg/kg tucatinib alone. This change is dose-dependent.

Conclusions: This study will help better understand the pharmacokinetic properties of tucatinib with concurrent use with quercetin, and more clinical verifications were inspired to confirm whether this interaction has clinical significance in humans.

Introduction
Tucatinib (Figure 1(A)) is an oral tyrosine kinase inhibitor (TKI) highly selective for the kinase domain of human epidermal growth factor receptor 2 (HER2), with minimal inhibition of epidermal growth factor receptor (Moulder et al. 2017). It was developed for the treatment of HER2-positive solid tumours, including breast cancer (BC) and colorectal cancer (Cesca et al. 2020). On April 2020, a marketing authorization for tucatinib for the treatment of adult patients with HER2-positive locally advanced or metastatic BC was granted by US Food and Drug Administration (FDA) (Lee 2020; Shah et al. 2021).

Following oral administration after a high-fat meal, area under the plasma concentration–time curve from time zero extrapolated to infinite (AUC0–∞) of tucatinib increases 1.5-fold and the time to peak concentration (Tmax) is delayed from 1.5 h to 4 h (Kunte et al. 2020). Given that cancer patients often take multiple drugs, whether the combination of tucatinib and other drugs would cause drug–drug interactions (DDIs) must be explored. CYP2C8 was proved to be the major metabolic enzyme in vitro, in addition to CYP3A to a lesser extent (Lee 2020). It was reported that gemfibrozil (a strong CYP2C8 inhibitor) increased the exposure of tucatinib when they were given with concomitant administration (Lee 2020). Thus, it is essential to establish a quantitative method for tucatinib in biological fluids to investigate its pharmacokinetic characteristics and potential DDIs. To the best of our knowledge, there is no published analytical and bioanalytical method available for tucatinib determination in biological fluids.

Quercetin is one of the flavonoids which are natural polyphenolic compounds found in numerous components of the human daily diet, including red wine, onions, apples, tea and grapefruit juice (Formica and Regelson 1995). It exerts a broad range of fascinating clinical properties, such as anticancer (Yin et al. 2021), anti-inflammatory (Li et al. 2016), antimicrobial (Heinz et al. 2010) and antiallergic (Mlcek et al. 2016) activities. Quercetin has the potential to inhibit cytochrome P450 (CYP), especially CYP2C8, and subsequent studies have demonstrated that it significantly inhibits the metabolism of CYP2C8 substrates (Kajosaari et al. 2005; Kim et al. 2005; Gao et al. 2017). However, the effect of quercetin on the metabolism of tucatinib remains unknown.

Therefore, the present study establishes a stable, simple and hypersensitive ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) assay to detect plasma tucatinib
concentration in rats. Moreover, our newly developed method explores the impact of diverse doses of quercetin on tucatinib exposure and its pharmacokinetic alterations in the experiment of rats.

Materials and methods

Materials and chemicals

Tucatinib, quercetin (the purity of each compound >98%) and talazoparib [internal standard (IS), purity >99%, Figure 1(B)] purchased from Beijing Sunflower Technology Development Co., Ltd. (Beijing, China) were employed in this study. Both acetonitrile and methanol were LC grade, and were supplied from Merck Company (Darmstadt, Germany). A Water Purification System from Milli-Q (Millipore, Bedford, MA) was used to acquire ultrapure water.

Experimentation on animals

Sprague-Dawley (SD) male rats, weighing 190 ± 10 g, were purchased from Laboratory Animal Center, Wenzhou Medical University (Wenzhou, China). The rats were kept under appropriate environment, including proper humidity, temperature, light conditions, rodent diet and water. Before starting the experiment, all the rats were domesticated for 14 days under laboratory conditions to minimize their suffering. The animal studies were approved by the Animal Protection and Use Committee of Wenzhou Medical University (Wenzhou, China). The rats were kept under appropriate environment, including proper humidity, temperature, light conditions, rodent diet and water.

Protein precipitation was performed by adding 300 μL acetonitrile to 100 μL of plasma, followed by a 20 μL IS working solution. The mixture was then centrifuged for 10 min at 13,000 rpm at the temperature of 4 °C after mixing for 2.0 min. Finally, 100 μL supernatant of each sample was drawn into a sample vial, where only 2.0 μL of the sample was used to inject into the autosampler for analysis.

Sample treatment

Protein precipitation was performed by adding 300 μL acetonitrile to 100 μL of plasma, followed by a 20 μL IS working solution. The mixture was then centrifuged for 10 min at 13,000 rpm at the temperature of 4 °C after mixing for 2.0 min. Finally, 100 μL supernatant of each sample was drawn into a sample vial, where only 2.0 μL of the sample was used to inject into the autosampler for analysis.

Bioanalytical method validation

We conducted a series of confirmatory experiments following the principles of FDA based on the validation of bioanalytical...
assay (Wang et al. 2021; Zhou et al. 2021): the lower limit of quantification (LLOQ), selectivity, calibration curve, precision and accuracy, recovery rate, stability and matrix effect.

**Statistical analysis**

Origin 9.0 software (Originlab Company, Northampton, MA) in this study was applied to determine the average concentration versus time profile of tucatinib in plasma. The main pharmacokinetic parameters of tucatinib fitted with a non-compartmental model were calculated using DAS software (Drug and Statistics, Version 2.0, Shanghai University of Traditional Chinese Medicine, Shanghai, China). Pharmacokinetic parameters between groups were compared through one-way analysis of variance coupled with Dunnett’s test using Statistical Package for the Social Sciences (version 17.0; SPSS Inc., Chicago, IL). $p < 0.05$ is statistically significant.

**Results**

**Method validation**

**Selectivity and carry-over**

Figure 2 exhibits the representative MRM chromatogram peaks of six batches of blank rat plasma samples, the rat blank plasma sample pointed with the concentration of 0.5 ng/mL tucatinib at LLOQ and IS, along with the actual plasma sample after the oral administration of 30 mg/kg tucatinib in rats. Tucatinib and IS were identified at the retention periods of about 0.74 min and 0.73 min, respectively, without detectable endogenous interference. Consequently, the method has good selectivity and specificity to determine tucatinib in plasma. In addition, no carry-over was observed for either analyte or IS in rat plasma, since there was no interference peak detected following the injection of upper limit of quantification (ULOQ) samples.

**Standard curve and LLOQ**

The standard curve provided perfect linearity within the scope of 0.5–400 ng/mL for tucatinib in rat plasma. The typical linear regression formula of tucatinib was obtained as follows: $Y = 1.59031 \times X + 0.790294$ ($r^2 = 0.9976$). The LLOQ was 0.5 ng/mL, possessing enough precision and accuracy (Table 2).

### Table 2. The precision and accuracy of tucatinib in rat plasma ($n = 6$).

| Analyte | Concentration (ng/mL) | Intra-day | Inter-day |
|---------|-----------------------|-----------|-----------|
|         | RSD% | RE% | RSD% | RE% |
| Tucatinib | 0.5  | 11.1 | 4.5 | 12.6 | 5.9 |
|          | 1    | 7.0 | 8.8 | 7.3 | 9.3 |
|          | 80   | 4.5 | 3.0 | 4.9 | 4.9 |
|          | 320  | 3.5 | -2.4 | 4.3 | -5.0 |

Figure 2. Representative chromatograms of tucatinib and IS in rat plasma: (A) blank plasma; (B) blank plasma spiked with standard solution at LLOQ (0.5 ng/mL) and IS; (C) sample obtained from a rat at 1.0 h after oral administration of 30 mg/kg tucatinib.
Therefore, this method is sensitive to determine the plasma concentration of tucatinib in rats.

**Accuracy and precision**
The accuracy and precision of tucatinib for inter-day and intra-day were quantified at LLOQ and three different QC concentrations on three separate days \((n = 6)\), and the summary are presented in Table 2. The RE range of the intra- and inter-assay accuracy was \(-5.0\%\) to \(9.3\%\), with the RSD of the precision \(<12.6\%\). These outcomes indicated that the well-established assay had good accuracy, precision and reproducibility and could be applied for the quantitative analysis of tucatinib in the plasma samples from rats.

**Matrix effect and extraction recovery**
Table 3 summarizes the extraction recovery and matrix effect of tucatinib. The recovery rate of tucatinib was from \(90.1\%\) to \(96.0\%\) at three QC concentrations in rat plasma, and the recovery of IS was \(93.3\%\), indicating few significant losses of both tucatinib and IS during the extraction process. Similarly, the matrix effects ranged from \(92.3\%\) to \(101.6\%\), and the matrix effect of IS was \(97.8\%\). The IS-normalized matrix factor of tucatinib had an RSD of \(6.0\%\) or less, which met the acceptance criteria (not more than \(15\%\)), manifesting that the matrix effect had no significant influence on rat plasma.

**Stability**
Stability experiments conducted in plasma samples at QC levels from Table 4 showed that tucatinib was durable and stable at ambient temperature for at least \(3\) h (short term stability), in the autosampler \((10\,^\circ\text{C})\) for \(6\) h thereafter extraction, three complete processes of freeze–thaw \((-80\,^\circ\text{C}\) to ambient temperature), and also at \(-80\,^\circ\text{C}\) within four weeks (long-term stability).

**Pharmacokinetics**
Using the novel developed bioanalytical assay based on UPLC–MS/MS technique, we detected the plasma concentrations of tucatinib in rats successfully, acquiring pharmacokinetics from different groups. Figure 3 exhibits the average concentration versus time curves of tucatinib in each rat group after taking \(30\,\text{mg/kg}\) tucatinib at a single oral dose. Table 5 sums up the essential pharmacokinetic parameters calculated under the mode of non-compartmental analysis.

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**Table 3. Recovery and matrix effect of tucatinib in rat plasma \((n = 6)\).**

| Analyte   | Concentration (ng/mL) | Recovery (%) | Matrix effect (%) |
|-----------|-----------------------|--------------|-------------------|
|           | Mean ± SD             | RSD (%)      | Mean ± SD         | RSD (%)       |
| Tucatinib | 1                     | 90.1 ± 3.6   | 4.0               | 101.6 ± 9.5   | 9.4           |
|           | 80                    | 93.7 ± 4.4   | 4.7               | 92.3 ± 3.9    | 4.2           |
|           | 320                   | 96.0 ± 5.3   | 5.5               | 99.9 ± 2.9    | 2.9           |

**Table 4. Stability results of tucatinib in plasma under different conditions \((n = 5)\).**

| Analyte   | Added (ng/mL) | Room temperature, 3 h | Autosampler 10 °C, 6 h | Three freeze–thaw | 80 °C, 4 weeks |
|-----------|---------------|-----------------------|------------------------|-------------------|---------------|
|           |               | RSD (%) | RE (%) | RSD (%) | RE (%) | RSD (%) | RE (%) | RSD (%) | RE (%) |
| Tucatinib | 1             | 6.5     | 13.2    | 11.1    | 9.1     | 9.1     | 13.9    | 12.6    | 7.5     |
|           | 80            | 4.8     | 4.3     | 4.8     | 4.9     | 7.2     | 5.0     | 4.9     | 5.3     |
|           | 320           | 3.9     | -8.4    | 2.8     | -6.6    | 4.1     | -6.8    | 4.0     | -5.0    |

**Figure 3.** Mean plasma concentration–time curves of tucatinib in different treatment groups of rats. Group A: the control group (0.5% CMC-Na); group B: 25 mg/kg quercetin; group C: 50 mg/kg quercetin; group D: 100 mg/kg quercetin \((n = 6, \text{mean ± SD})\).
Discussions

The settings for UPLC–MS/MS were optimized for tucatinib and IS as to obtain maximal sensitivity, the product spectrums of tucatinib and IS are presented in Figure 1. In the method development, acetonitrile and water were first selected as the mobile phase. However, the supernatant of precipitated samples produced asymmetric peaks with poor sensitivity, which did not satisfy the method requirement. Therefore, 0.1% formic acid in water was adopted as mobile phase B. The optimized condition improved the ionization of the analyte and produced higher sensitivity and symmetric peaks.

A quick and straightforward extraction method is also essential due to the large number of plasma samples involved in pharmacokinetics or DDI-associated experiments (Wang et al. 2021; Zhou et al. 2021). In the present study, on the basis of the advantages of simplicity and rapidity, protein precipitation with acetonitrile was first assessed for sample preparation. The preliminary experiments indicated that acetonitrile method to precipitate proteins resulted in a higher recovery rate than other methods. However, acetonitrile was first assessed for sample preparation. The preliminary experiments indicated that acetonitrile method to precipitate proteins resulted in a higher recovery rate than other methods. Therefore, 0.1% formic acid in water was adopted as mobile phase B. The optimized condition improved the ionization of the analyte and produced higher sensitivity and symmetric peaks.

Quercetin is a naturally occurring flavonoid and is mainly present as a glycoside in several components of the daily diet. It has been proved that quercetin is a potent CYP2C8 inhibitor and inhibits CYP2C8-catalysed metabolism in vivo (Kim et al. 2005). From the results of our study, when tucatinib was co-administered with 25 mg/kg quercetin in group B, the main pharmacokinetic parameters (AUC0→t, AUC0→∞, t1/2, Tmax, CLz/F, and Cmax) of tucatinib had no significant differences compared with the control group A. However, compared with group A, groups C and D raised the AUC0→t, AUC0→∞ and Cmax of tucatinib (p < 0.05), while decreased CLz/F (p < 0.05), indicating that the total tucatinib systemic exposure increased. Also, 100 mg/kg quercetin exhibited a more substantial inhibition on tucatinib metabolism than 50 mg/kg quercetin. Therefore, the concurrent use of tucatinib with high dose of quercetin should be treated with extreme caution. If their combined use is unavoidable, our data suggested dose reduction or interruption of tucatinib should be taken. Otherwise, the patient might suffer from some severe side effects (such as diarrhea, palmar-plantar erythrodysesthesia syndrome and nausea) caused by increased tucatinib plasma levels (Murthy et al. 2020). The limitation of our research lies in the small number of rats used in the experiment.

Conclusions

In the present experiment, we established a sensitive and accurate bioanalytical assay based on UPLC–MS/MS to determine tucatinib concentrations in plasma samples from rats. The optimized method had been carefully verified under FDA guidelines. In addition, we found that a high dose of quercetin exhibited inhibitory effect on the metabolism of tucatinib in rats. While considering the complex and varied clinical factors of cancer patients, further human clinical trials on tucatinib metabolism should be investigated to confirm the accuracy of the interaction and the significance.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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