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Original article

Effects of baicalein and fangchinoline on abemaciclib metabolism *in vivo* and *in vitro* and molecular docking analysis

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ABSTRACT

The primary objective of this study was to investigate the effects of baicalein and fangchinoline on the metabolism of abemaciclib. We hypothesized that these two natural compounds could significantly affect the metabolism of abemaciclib by inhibiting the activity of the CYP3A4 enzyme, thus potentially increasing its concentration in the body. *In vitro*, rat liver microsomes (RLM) and human liver microsomes (HLM) were employed to explore the inhibitory effects and mechanisms of baicalein and fangchinoline on abemaciclib. *In vivo*, twelve healthy male Sprague-Dawley (SD) rats were randomly assigned to three groups: Group A (control group), Group B (baicalein), and Group C (fangchinoline). The concentrations of abemaciclib and its metabolite N-desethylabemaciclib (M2) were evaluated using ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). Finally, molecular docking method was employed to understand the interaction between abemaciclib and baicalein. It was indicated by the *in vitro* findings that both baicalein and fangchinoline inhibited abemaciclib metabolism in RLM through a mixed mechanism of competitive and non-competitive inhibition pathway. In HLM, baicalein inhibited abemaciclib metabolism by employing a hybrid mechanism of uncompetitive and non-competitive inhibition, while fangchinoline exhibited its inhibition in a competitive manner. *In vivo*, pharmacokinetic experiments revealed significant increases for $AUC_{(0\text{-}t)}$ and $AUC_{(0\text{-}\infty)}$ of abemaciclib in Group B and Group C when compared to Group A, while the plasma clearance (CLz/F) of abemaciclib exhibited significant reductions. Moreover, molecular docking studies showed that both abemaciclib and baicalein docked to the active pocket of CYP3A4. This study demonstrated that the co-administration of baicalein or fangchinoline significantly affected the metabolism of abemaciclib, providing valuable insights for its clinical application.

1. Introduction

Globally, breast cancer is recognized as the predominant form of malignant neoplasm and constitutes the primary cancer-related mortality factor in females (Harbeck and Gnant, 2017; Kolak et al., 2017; Liang et al., 2020). Previous studies have shown metastatic heterogeneity in breast cancer, with bone, liver, lung and brain being the main organs targeted for breast cancer metastasis (Medeiros and Allan, 2019; Tahara et al., 2019; Liang et al., 2020). In metastatic breast cancer, endocrine therapy and combination therapy, as well as the development of targeted therapies for HER2, have made it increasingly possible to control the long-term disease of metastatic breast cancer (Cardoso et al., 2014; Lee et al., 2017; Swain et al., 2020; Hurvitz et al., 2023).

In recent years, significant advances have been made in the fields of drug properties, mechanisms of action, and drug interactions, particularly in cancer treatment and antimicrobial drug applications. (Ahmad et al., 2023; Farheen et al., 2023; Singh et al., 2023). Abemaciclib, an oral antineoplastic agent and a dual inhibitor of cyclin-dependent kinases 4 and 6 (CDK4/6), interrupts the growth and reproduction of malignant tumor cells by inhibiting the phosphorylation of retinoblastoma (RB) protein and the activation of the E2F transcription factor, and blocking the cycle transition from G1 phase to S phase (Goel et al., 2017; Praveenkumar et al., 2021; Huang et al., 2023). Abemaciclib was first approved by the US Food and Drug Administration (FDA) in 2017 combined with endocrine therapy (ET) for advanced or metastatic breast cancer patients with positive hormone receptors (HR) and negative human epidermal growth factor receptor 2 (HER2), and as a monotherapy for adult metastatic breast cancer patients with disease

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progression following endocrine therapy or chemotherapy (Kim, 2017; Royce et al., 2022). Abemaciclib has shown significant clinical efficacy and safety as a monotherapy or in conjunction with non-steroidal aromatase inhibitors (Dickler et al., 2017; Sledge et al., 2017; Johnston et al., 2020). Although the safety of abemaciclib has been confirmed, there are still many adverse reactions, such as diarrhea, fatigue, neutropenia, infection, thrombocytopenia, venous thromboembolism, etc. (Patnaik et al., 2016; Rugo et al., 2022).

Abemaciclib is widely metabolized by CYP3A4 in the human body, and forms three metabolites with similar potency and protein binding compared to abemaciclib, namely N-desethylabemaciclib (M2), hydroxyabemaciclib (M20) and hydroxy-N-desethylabemaciclib (M18) (Martínez-Chávez et al., 2021). Among these, M2 and M20 are the most abundant active metabolites, with their area under the curve (AUC) accounting for 39 % and 77 % of the parent drug, respectively. M18 is further metabolized from M2 and M20 through CYP3A4 (Martínez-Chávez et al., 2021).

In recent years, traditional Chinese medicine has aroused people's interests because of its curative effects on human diseases. For instance, flavonoids are being more and more frequently used as chemotherapeutic agents and dietary chemoprophylaxis agents (Ullah et al., 2020). Baicalein belongs to flavonoids and is one of the active components in the root of Scutellaria baicalensis Georgi (SBG). Moreover, an accumulating amount of *in vivo* and *in vitro* experiments have demonstrated that the anti-tumor effects of baicalein (Liu et al., 2016; Morshed et al., 2023). Furthermore, baicalein has a promising potential in inhibiting breast cancer metastasis, with its mechanism possibly associated with the downregulation of SATB1 and the Wnt/β-catenin pathway (Ma et al., 2016). Besides, fangchinoline is another promising drug for metastasis treatment of breast cancer. It is extracted from the roots of *S. tetrandra*, which is classified as a bisbenzylisoquinoline alkaloid (Wang et al., 2020). It exhibits inhibitory effects on the proliferation of breast cancer cells MDA-MB-231 and MCF-7 by inducing of apoptosis, arrest of G1 cycle and inhibition of migration (Xing et al., 2013). Radix Stephaniae Tetrandrine (RST), a compound characterized by a structural and pharmacological profile similar to that of fangchinolin, derived from the dried root of Stephania tetrandra S.Moore, has been successfully applied in clinical trials of traditional Chinese medicine and in preclinical trials of anti-breast cancer, to show that the active ingredient of RST has significant therapeutic potential against breast cancer (Guo et al., 2020).

Nowadays, the application of Nuclear Magnetic Resonance Spectroscopy (NMR) in metabolomics has made significant progress, particularly in metabolite identification and reaction mechanism studies. Improvements in sensitivity and resolution provide powerful support for in-depth analysis of drug metabolism and drug interactions (Emwas et al., 2019; Kijewska et al., 2021).

As we know, there have been no prior studies investigating the interaction between abemaciclib and baicalein or fangchinoline. In this study, we characterized the influences of baicalein and fangchinoline on the metabolism of abemaciclib in both rat liver microsomes (RLM) and human liver microsomes (HLM). Furthermore, we investigated the pharmacokinetic changes of abemaciclib in rats following the administration of abemaciclib combined with baicalein or fangchinoline by quantifying abemaciclib levels in rat plasma using ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). In addition, we performed molecular docking studies, which revealed the binding interactions between abemaciclib, baicalein, fangchinoline, and CYP3A4, providing insights into their inhibitory mechanisms. This study has significance in evaluating the clinical safety of abemaciclib.

2. Materials and methods

2.1. Chemical and reagents

Abemaciclib, its metabolite N-desethylabemaciclib (M2), alpelisib (internal standard, IS), baicalein and fangchinoline were supplied by Shanghai Canspec Scientific Instruments Co., Ltd. (Shanghai, China). The HLM was from iPhase Pharmaceutical Services Co., Ltd. (Jiangsu, China). Nicotinamide Adenine Dinucleotide Phosphate (NADPH) was offered by Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Additionally, all remaining chemical reagents and solvents employed in the experiment were of analytical grade. The chemical structures of abemaciclib, baicalein and fangchinoline are shown in Fig. 1.

2.2. Conditions of ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS)

UPLC-MS/MS was employed to measure the concentrations of abemaciclib and M2, which was equipped with a Waters Acquity UPLC BEH C18 column (2.1 mm × 50 mm, 1.7-μm particle size; Waters Corp., Millipore, Bedford, MA, USA). The autosampler rack and column temperatures were set at 10 °C and 40 °C, respectively. The mobile phase was comprised 0.1 % formic acid (solution A) and acetonitrile (solution B), employing a gradient elution with the rate of 0.40 mL/min. The procedure of gradient elution included the following stages: 0–0.5 min (10 % B), 0.5–1.0 min (10–90 % B), 1.0–1.4 min (90 % B), 1.4–1.5 min (90–10 % B), and 1.5–2.0 min (10 % B). Quantitation was carried out using the Waters XEVO TQS Triple Quadrupole Mass Spectrometer, equipped with an electrospray ionization source (ESI) which was in positive mode. The ion transitions used were m/z 507.09 → 393.04 for abemaciclib, m/z 479.05 → 393.00 for M2 and m/z 442.02 → 327.97 for alpelisib, respectively.

2.3. Method for obtaining RLM

Male SD rats were fasted overnight and then killed by cervical dislocation. Each liver was removed, washed with cold saline solution (0.9 % NaCl by weight/volume), weighed, and homogenized in an ice-cold 0.01 mM cold PBS buffer containing 0.25 mM sucrose solution. After homogenization, the mixture was centrifuged at 11,000 rpm at 4 °C for 15 min. After that, the precipitation was discarded, and the supernatant was transferred to a fresh tube and centrifuged once again

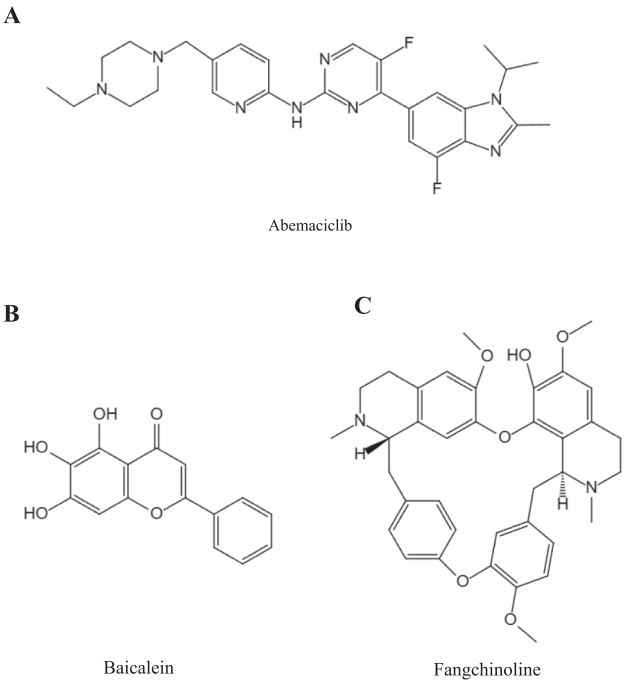

Fig. 1. Chemical structures of abemaciclib (A), baicalein (B) and fangchinoline (C).

at 11,000 rpm at 4 °C for 15 min. The clear liquid above was centrifuged again at 24,860 rpm at 10 °C for 120 min. The solid part that settled was mixed back into an ice-cold 0.01 mM cold PBS buffer containing 0.25 mM sucrose solution and kept frozen at −80 °C until it was needed. The protein content of RLM was measured using the Bradford Protein Assay Kit (Thermo Scientific, Waltham, MA, USA) (Kielkopf et al., 2020).

2.4. The inhibitory effects of baicalein and fangchinoline on abemaciclib in RLM and HLM

RLM and HLM were used to examine the inhibitory effects of baicalein and fangchinoline on abemaciclib *in vitro*. The formation of M2 was served as an indicative marker of the inhibitory effects. The 200 μL incubation system was comprised of potassium phosphate buffer (PBS, 100 mM, pH = 7.4), NADPH (1 mM), 0.3 mg/mL of RLM or HLM, abemaciclib, and inhibitor (baicalein or fangchinoline). At first, various concentrations of abemaciclib (0.1, 1, 2, 5, 10, 20, and 50 μM for RLM; 0.1, 1, 2, 5, 10, 15, and 20 μM for HLM) were added into the reaction buffer to establish the Michaelis constant (K_m) values. Subsequently, different concentrations of baicalein or fangchinoline, ranging from 0, 0.01, 0.1, 1, 10, 25, 50 to 100 μM, were employed to determine the corresponding half-maximal inhibitory concentration (IC_{50}) values. At last, to uncover the inhibitory mechanisms of baicalein and fangchinoline on abemaciclib, a variety concentration of baicalein were generated, guided by the IC_{50} values (RLM: 0, 3.99, 7.98 and 15.95 μM; HLM: 0, 4.60, 18.38, and 36.76 μM). The concentrations of fangchinoline were also prepared based on IC_{50} values (RLM: 0, 0.50, 1.01, and 2.02 μM; HLM: 0, 3.06, 6.12, and 12.24 μM). Meanwhile, a set of abemaciclib concentrations were established based on the K_m values (RLM: 0.32, 0.64, 1.28, and 2.56 μM; HLM: 0.90, 1.80, 3.60, and 7.20 μM). The reaction was initiated by adding 10 μL of NADPH after pre-incubation at 37 °C for 5 min. After incubating for 30 min at 37 °C and terminating at −80 °C, 20 μL of IS working solution (500 ng/mL) was added, as well as 400 μL of acetonitrile. The samples were spun at 13,000 rpm for 10 min to obtain 100 μL of the supernatant for UPLC-MS/MS analysis.

2.5. Pharmacokinetic interactions of baicalein and fangchinoline with abemaciclib in rats

Sprague–Dawley (SD) male rats (200 ± 20 g) were sourced from the Animal Experimental Center of the First Affiliated Hospital of Wenzhou Medical University (Zhejiang, China). The day before the experiment, the SD rats underwent a 12 h fasting period, during which water was unrestricted but food was restricted. After the fasting period, 12 SD rats were randomly distributed into three groups (n = 4), group A (control group) and group B, C (experimental group). Groups B and C were received an oral administration of baicalein (50 mg/kg), and fangchinoline (50 mg/kg), respectively (Lai et al., 2003; Shi et al., 2018; Wu et al., 2019), and Group A was given an equal volume of solvent. After 30 min, all rats were received an oral administration of 30 mg/kg of abemaciclib. Blood samples (300 μL each) were collected from the tail vein at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, and 48 h after administration of abemaciclib, and were promptly centrifugated at 8,000 rpm for 8 min. For sample preparation, 10 μL of the IS working solution (500 ng/mL) was added to 100 μL of collected plasma. After that, 300 μL of acetonitrile was added, and the mixture was thoroughly vortexed before centrifugation at 13,000 rpm for 10 min. Then, we collected 100 μL of the supernatant to quantify the concentration of abemaciclib by UPLC-MS/MS.

2.6. Molecular docking simulations

Molecular docking simulations were conducted with resources from the Protein Data Bank (PDB, https://www.rcsb.org/pdb). X-ray crystal structures of human CYP3A4 (PDB ID: 5ET8) were accessed, and 3D sdf format files of baicalein and abemaciclib were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Initially, the 3D protein conformation was optimized using PyMOL, which involved the removal of the protein crystal water and the original ligand, and hydrogen atoms were added using Auto Dock Tools 1.5.6. A grid box measuring 40 Å × 40 Å × 40 Å with a grid spacing of 0.375 Å was set. The molecular docking process was conducted using the AutoDock 4.2.6 program, employing the Lamarckian Genetic Algorithm (LGA) for ligand tethering of targeted protein, producing a maximum output of 50 gestures (Shivanika et al., 2022). Subsequently, further visualization of the best scoring conformation was performed using PyMOL.

2.7. Statistical analysis

The kinetic parameters of abemaciclib in both RLM and HLM, including K_m and IC_{50}, were analyzed. Lineweaver-Burk graphs and mean plasma concentration–time curves were produced by GraphPad Prism 9.0. Pharmacokinetic parameters, including $AUC_{(0\text{-}t)}$, $AUC_{(0\text{-}\infty)}$, time to peak plasma concentration (T_{max}), maximal plasma concentration (C_{max}), plasma clearance (CLz/F), and elimination half-life ($t_{1/2}$) were calculated with Drug and Statistics (DAS) 3.0 software. Statistical analysis was performed using SPSS 23.0 software, employing a one-way analysis of variance (ANOVA) with Dunnett's test to assess differences in dynamic parameters among the three rat groups. All parameters from experiments were presented as mean ± standard deviation (SD). $P < 0.05$ was considered to have statistical significance.

3. Results

3.1. Evaluation of the influences of baicalein and fangchinoline on abemaciclib metabolism in vitro

The Michaelis-Menten curves and K_m values of abemaciclib in RLM and HLM are depicted in Fig. 2 and Table 1. Ultimately, the K_m values of abemaciclib were determined to be 1.28 μM for RLM and 3.60 μM for HLM, respectively. Fig. 3 and Table 1 illustrate that baicalein had inhibitory effect on abemaciclib metabolism, exhibiting IC_{50} values of 15.95 μM in RLM and 18.38 μM in HLM, respectively. As shown in Table 2, fangchinoline also displayed inhibition on abemaciclib metabolism, showing IC_{50} values of 2.02 μM in RLM and 12.24 μM in HLM, respectively. These results suggested that baicalein exhibited a weak inhibition on abemaciclib metabolism in both RLM and HLM. Besides, fangchinoline demonstrated a weak inhibition of abemaciclib in HLM, and a moderate inhibition in RLM, respectively. For a more comprehensive understanding of how baicalein and fangchinoline inhibited abemaciclib, Figs. 4 and 5 present the Lineweaver-Burk plots. In RLM, both baicalein and fangchinoline inhibited abemaciclib metabolism through a mixed mechanism of competitive and non-competitive inhibition, exhibiting K_i values of 10.22 and 1.84, and α values of 2.73 and 3.90, respectively. In addition, in HLM, baicalein inhibited the metabolism of abemaciclib through a mixed way of uncompetitive and non-competitive mechanism with K_i and α values of 27.24 and 0.18, while fangchinoline exhibited its inhibition in a competitive way with K_i value of 6.52.

3.2. Evaluation of the influences of baicalein and fangchinoline on abemaciclib pharmacokinetics in vivo

The pharmacokinetic parameters of abemaciclib were determined in Group A (30 mg/kg abemaciclib alone), Group B (50 mg/kg baicalein + 30 mg/kg abemaciclib), and Group C (50 mg/kg fangchinoline + 30 mg/kg abemaciclib). The results of the statistical tests are displayed in Table 3. Fig. 6 illustrates the mean plasma concentration–time curves of abemaciclib in different groups. Following pretreatment with a single dose of baicalein, the $AUC_{(0\text{-}\infty)}$ and $AUC_{(0\text{-}t)}$ of abemaciclib were 1.99-fold and 1.84-fold of the control group accompanied by a 48.90 % reduction in CLz/F. Moreover, fangchinoline significantly elevated the

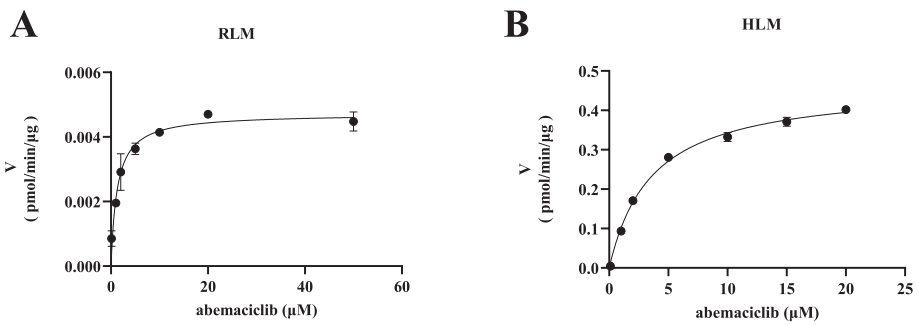

Fig. 2. Michaelis-Menten plots of abemaciclib in RLM (A) and in HLM (B). Data are expressed as mean ± SD, n = 3.

Table 1
The IC_{50} values and inhibitory effect of baicalein on abemaciclib metabolism in RLM and HLM.

	IC_{50} values (μM)	K_m (μM)	Inhibition type	K_i (μM)	$αK_i$ (μM)	α
RLM	15.95	1.28	Mixed inhibition	10.22	27.88	2.73
HLM	18.38	3.60	Mixed inhibition	27.24	4.79	0.18

$AUC_{(0-\infty)}$ of abemaciclib to 1.54-fold, with the decreasing CLz/F of 37.63 %. However, the parameters of T_{max}, C_{max} and $t_{1/2}$ in both Groups B and C did not show significant differences. These findings suggested that both baicalein and fangchinoline could inhibit the metabolism of abemaciclib in rats.

3.3. Molecular docking studies of abemaciclib and baicalein on CYP3A4

To further understand the interactions between abemaciclib and baicalein, both compounds were co-docked into the active catalytic cavity of the target enzyme CYP3A4. As illustrated in Fig. 7, it was observed that abemaciclib established a hydrogen bond interaction with HEM-601 (3.2 Å) of CYP3A4, presenting a binding energy of −9.34 kcal/mol. Additionally, with the binding energy of −5.8 kcal/mol, baicalein interacted with the active amino acid residues ALA-305 and SER-119 of enzyme through conventional hydrogen bonds at distances of approximately 2.2Å and 2.7 Å, respectively. Importantly, baicalein can also undergo hydrogen bonds of 2.5 Å and 3.5 Å linking with HEM-601. These results indicated a significant overlap in the binding sites of abemaciclib and baicalein with CYP3A4, with HEM-601 being a shared binding site. Therefore, when abemaciclib and baicalein are administered together, they may compete for the identical binding site and spatial position. However, the binding sites are not exactly coincident, which is essentially consistent with the mixed inhibition pattern of baicalein against abemaciclib.

Table 2
The IC_{50} values and inhibitory effect of fangchinoline on abemaciclib metabolism in RLM and HLM.

	IC_{50} values (μM)	K_m (μM)	Inhibition type	K_i (μM)	$αK_i$ (μM)	α
RLM	2.02	1.28	Mixed inhibition	1.84	7.17	3.90
HLM	12.24	3.60	Competitive inhibition	6.52	/	/

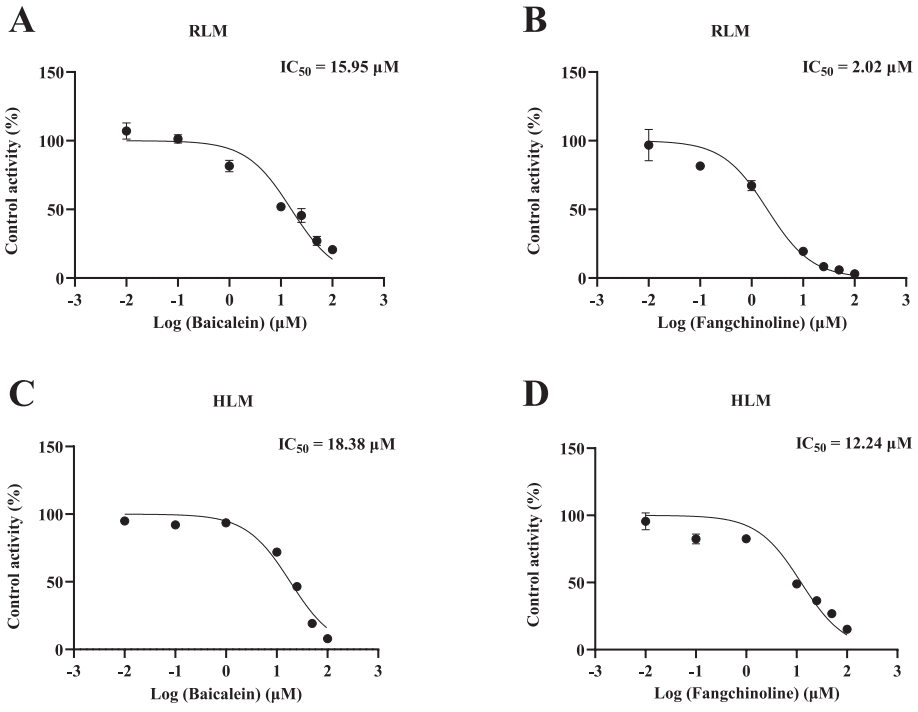

Fig. 3. IC_{50} plots of the effects of baicalein and fangchinoline on abemaciclib in RLM (A, B) and HLM (C, D). Data are expressed as mean ± SD, n = 3.

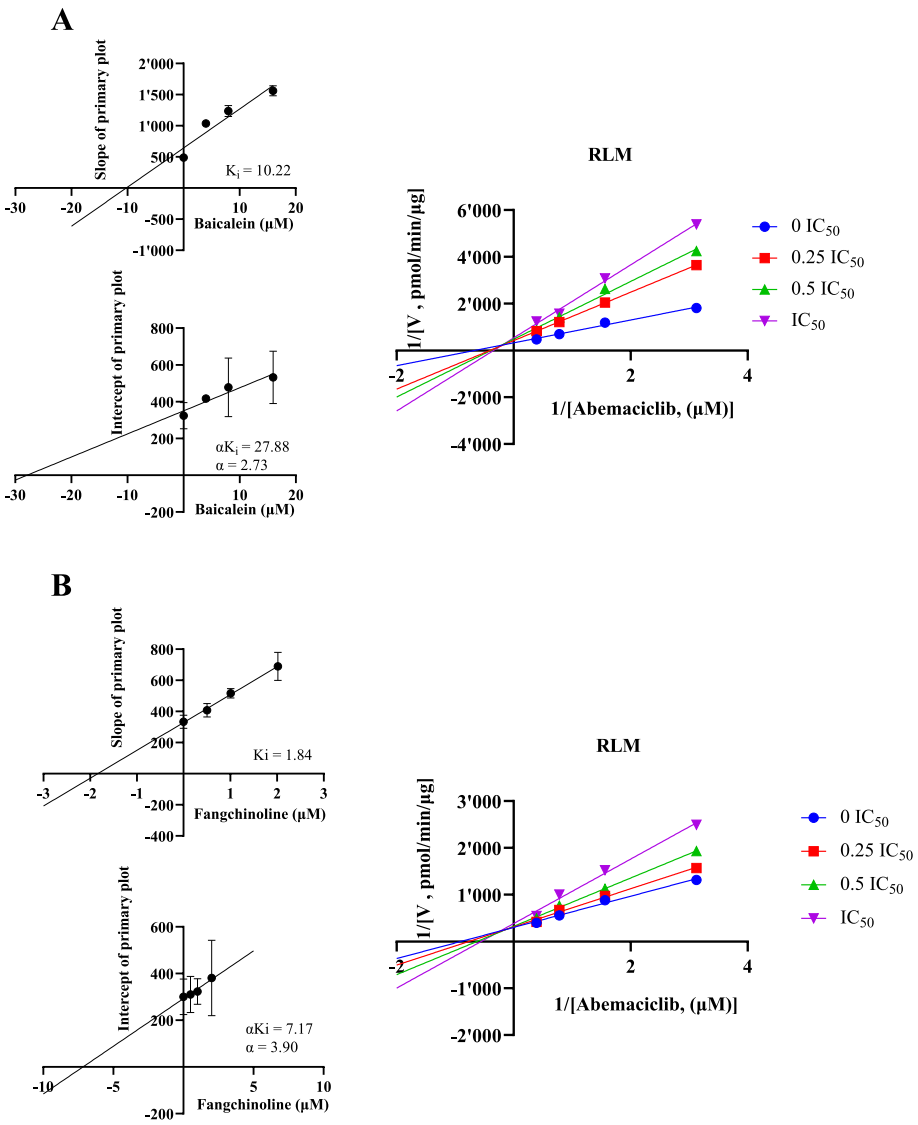

Fig. 4. In RLM, Lineweaver-Burk plots, secondary diagrams of K_i and secondary diagrams of αK_i inhibiting abemaciclib metabolism at different concentrations of baicalein (A) and fangchinoline (B). Data are expressed as mean ± SD, n = 3.

4. Discussion

The global impact of breast cancer is rapidly increasing, with notable differences among countries. Specifically, China has witnessed a sharp rise in both the incidence and mortality rates of breast cancer (Lei et al., 2021; Xia et al., 2022).

In 2017, the FDA granted its first approval for abemaciclib, to be employed alongside ET for patients diagnosed with metastatic or advanced breast cancer who are HR+ and HER2− (Kim 2017). Additionally, it was approved as a monotherapy treatment in adult patients with metastatic breast cancer who had experienced disease progression after endocrine therapy or chemotherapy (Royce et al., 2022). Abemaciclib undergoes extensive metabolism in the human body primarily by CYP3A4 (Martínez-Chávez et al., 2021).

In recent years, traditional Chinese medicine has been a hot spot due to its efficacy in treating various human diseases. Baicalein is classified as a flavonoid. It is possibly applied for treatment of metastatic breast cancer because of the downregulation of SATB1 and the Wnt/β-catenin pathway (Ma et al., 2016). Fangchinoline belongs to bisbenzylisoquinoline alkaloids (Wang et al., 2020). It demonstrates an inhibitory effect on the proliferation of breast cancer cells MDA-MB-231 and MCF-7 by inducing apoptosis, arresting the G1 cell cycle, and inhibiting migration (Xing et al., 2013).

The research findings indicated a growing trend in the utilization of herbal remedies by patients undergoing cancer treatment. It aims to reduce the side effects of chemotherapy and improve their overall health (Yin et al., 2017). Therefore, it is crucial to evaluate potential herb-drug interactions.

To explore the impacts of baicalein and fangchinoline on the *in vitro* metabolism of abemaciclib, enzyme kinetic studies were performed using both RLM and HLM. Our findings indicated that baicalein demonstrated a weak inhibition of abemaciclib in both RLM and HLM. Fangchinoline functioned as a weak inhibitor in HLM, and it moderately inhibited the metabolism of abemaciclib in RLM. Fangchinoline appeared to have a stronger inhibitory effect in RLM than in HLM. Significant biological and physiological differences exist between rats and humans, including variations in the expression and activity levels of liver metabolic enzymes. These differences may contribute to the different responses in drug metabolism between the two species. Further investigation confirmed the inhibitory type of baicalein and fangchinoline on abemaciclib. The Lineweaver-Burk plots indicated that the set of straight lines met in the second quadrant, suggesting that both baicalein and fangchinoline inhibited abemaciclib metabolism through a mixed way of competitive and non-competitive inhibition in RLM.

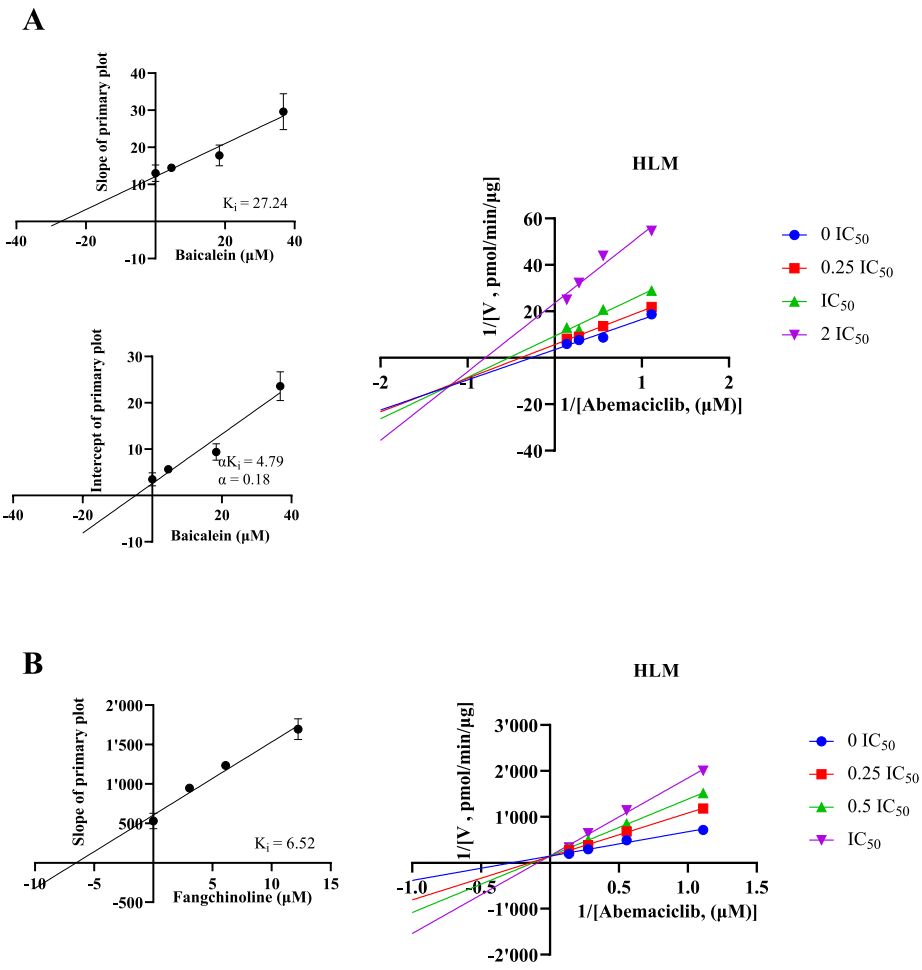

Fig. 5. In HLM, Lineweaver-Burk plots, secondary diagrams of K_i and secondary diagram of αK_i inhibiting abemaciclib metabolism at different concentrations of baicalein (A) and fangchinoline (B). Data are expressed as mean ± SD, n = 3.

Table 3
The main pharmacokinetic parameters of abemaciclib taken alone and with baicalein or fangchinoline in SD rats (n = 4, Mean ± SD).

Parameters	Group A	Group B	Group C
$AUC_{(0-t)}$ (ng/mL*h)	3366.66 ± 659.02	6192.14 ± 927.26*	5186.52 ± 1220.69*
$AUC_{(0-\infty)}$ (ng/mL*h)	3529.86 ± 606.72	7030.12 ± 1635.16*	6154.86 ± 2428.65
$t_{1/2z}$ (h)	9.81 ± 6.45	15.33 ± 7.10	19.57 ± 15.59
T_{max} (h)	10.50 ± 9.00	21.00 ± 17.32	24.00 ± 0.00
CLz/F (L/h/kg)	8.69 ± 1.49	4.44 ± 1.00*	5.42 ± 1.88*
C_{max} (ng/mL)	164.88 ± 40.33	201.11 ± 14.26	224.48 ± 82.59

Compared with Group A, *P < 0.05.

Additionally, in HLM, baicalein and fangchinoline inhibited abemaciclib metabolism through mixed inhibition and competitive inhibition, respectively. These results implied that baicalein and fangchinoline may exhibit mixed inhibition through multiple pathways.

Abemaciclib exhibited significant metabolic differences across species, particularly in the generation of its metabolites. For example, the CYP enzymes in humans and rats demonstrated different metabolic pathways and the formation of isomers when producing abemaciclib metabolites (Thakkar and Kate, 2020; Martínez-Chávez et al., 2021). Consequently, baicalein and fangchinoline showed varying inhibition types in RLM and HLM, most likely due to structural differences in the CYP enzymes across species. These differences led to distinct binding modes of the inhibitors at the active sites and thus affected their inhibition mechanisms in RLM and HLM.

To comprehend the potential influences of baicalein and fangchinoline on the *in vivo* pharmacokinetics of abemaciclib, SD rats were divided into control and co-treatment groups. In the presence of baicalein, there were significant changes in the $AUC_{(0-\infty)}$, $AUC_{(0-t)}$ and CLz/F of abemaciclib compared to those of the control group. After pretreatment with fangchinoline, significant changes were observed in $AUC_{(0-t)}$ and CLz/F of abemaciclib compared to the control group. However, there were no significant differences in the T_{max}, C_{max} and $t_{1/2}$ in both Groups B and C. The *in vivo* findings were consistent with the *in vitro* study, suggesting that both baicalein and fangchinoline had inhibitory effects on abemaciclib metabolism.

Based on *in vivo* results, baicalein had relatively a stronger inhibitory effect than fangchinoline. Moreover, a molecular docking analysis was performed to model the binding and possible docking poses of abemaciclib and baicalein to the active cavity of the human CYP3A4 enzyme. The molecular docking results revealed the binding mechanism of baicalein and abemaciclib with CYP3A4. The rigid benzene ring and hydroxyl groups of baicalein are key structural features that enhance its binding stability. These hydroxyl groups (5-, 6-, and 7-hydroxyl groups) form stable hydrogen bonds with key residues SER-119 and ALA-305 in the CYP3A4 active cavity at 2.2 Å and 2.7 Å, respectively, as well as two hydrogen bonds at the HEM-601 site at 2.5 Å and 3.5 Å. This hydroxyl group distribution and its precise docking within the active cavity significantly strengthen the enzyme-ligand interaction while competitively preventing the binding of other substrate molecules. In comparison, abemaciclib primarily binds to CYP3A4 through a hydrogen bond at

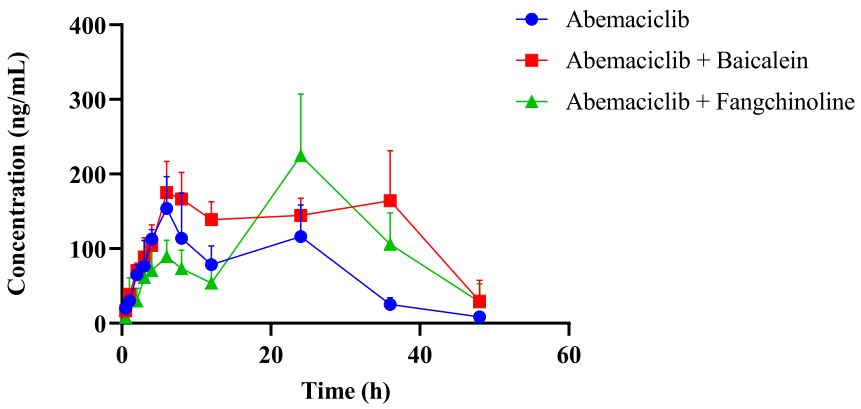

Fig. 6. The average plasma concentration–time curves of abemaciclib in the single group (abemaciclib alone) and the combined groups (abemaciclib with baicalein or fangchinoline). Data are expressed as mean ± SD, n = 4.

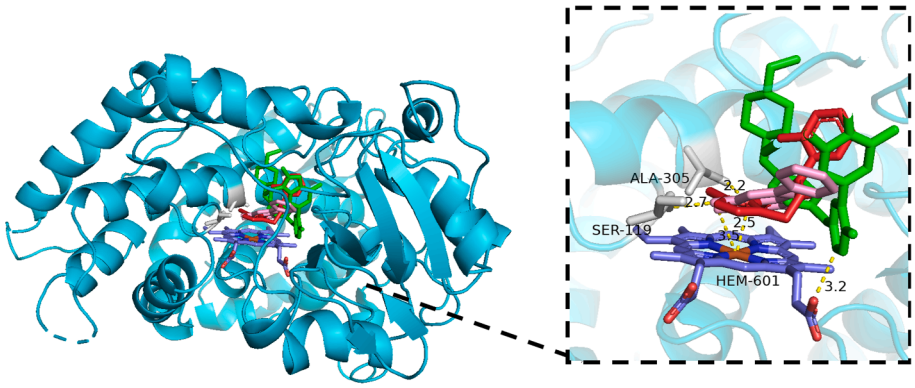

Fig. 7. Stereo views of molecular docking results of CYP3A4 with abemaciclib and baicalein. The structures of abemaciclib, baicalein and the reference ligand are shown in green, pink, and red respectively, and the amino acid residues interacting with them are shown in gray. Hydrogen bonds are indicated by yellow dash lines.

the HEM-601 site with a bond length of 3.2 Å. Since both baicalein and abemaciclib bind to the HEM-601 site, a competitive mechanism may exist between the two, which leads to baicalein inhibiting the catalytic activity of CYP3A4 on abemaciclib. Thus, it was concluded that baicalein may increase the plasma exposure of abemaciclib by inhibiting the enzymatic activity of CYP3A4.

In a study involving the screening of 50 individual herbal preparations using HLM to assess CYP3A4 activity for *in vitro* probe reactions, Huang Qin (Scutellaria baicalensis Georgi) had been demonstrated to exhibit significant inhibitory effects on the metabolism of CYP3A4 (Pao et al., 2012). Additionally, it had been demonstrated that baicalein significantly increased the bioavailability of nimodipine in rats, possibly due to its inhibition of CYP3A4 (Cho et al., 2011). Moreover, baicalein increased the oral bioavailability of tamoxifen, mainly due to its inhibitory effect on CYP3A4-mediated metabolism of tamoxifen, occurring either in the small intestine or in the liver (Li et al., 2011). Consequently, the suppressive impact of baicalein on the metabolism of abemaciclib could be ascribed to its inhibition of CYP3A4. However, there are no prior reports of fangchinoline interactions with other herbs or drugs.

Abemaciclib inhibited tumor cell proliferation by targeting CDK4/6, but over time, tumor cells may develop resistance. Common resistance mechanisms included alterations in the RB1 gene, overexpression of Cyclin E, and activation of the PI3K/AKT/mTOR pathway (Pandey et al., 2019; Huang et al., 2022; Zhu and Zhu, 2023). Co-administration of baicalein and fangchinoline could inhibit CYP3A4 enzyme activity, then slowing the metabolism of abemaciclib, and helping to decrease the risk of resistance associated with prolonged high-dose exposure. This could delay resistance mechanisms such as RB1 gene loss or Cyclin E overexpression (Pandey et al., 2019; Huang et al., 2022; Zhu and Zhu, 2023). Additionally, abemaciclib therapy often caused untoward effects such as diarrhea, fatigue, and hematological toxicity (Rugo et al., 2022; Tong et al., 2024). By reducing the necessary dose through metabolic inhibition, baicalein and fangchinoline may help to mitigate these adverse effects and thereby improve patient tolerance.

So far, no previous studies have investigated the interaction between abemaciclib and baicalein or fangchinoline. Our results suggested that if baicalein or fangchinoline is taken together with abemaciclib, it might increase abemaciclib levels in the body, and adjusting abemaciclib dosage may be necessary.

5. Conclusion

Our findings in this study indicated that both baicalein and fangchinoline not only significantly inhibited abemaciclib metabolism *in vitro*, but also demonstrated inhibitory effects in rats. Besides, molecular docking studies simulated the interaction of two small molecules with CYP3A4. Future research may involve investigating potential interactions between other flavonoids or alkaloid compounds from traditional Chinese medicine and abemaciclib. Considering abemaciclib is processed differently in various species, further studies in cell-based models or disease-specific rat models will be essential to deepen our understanding of how baicalein and fangchinoline impact on abemaciclib metabolism. These findings could then inform subsequent human studies, providing a foundation for medical applications.

CRediT authorship contribution statement

Xiaohai Chen: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Fengsheng Hong:** Writing – original draft, Visualization, Methodology, Investigation. **Hualu Wu:** Writing – review & editing, Conceptualization. **Yuxin Shen:** Writing – review & editing. **Hailun Xia:** Methodology, Investigation, Formal analysis. **Ren-ai Xu:** Writing – review & editing, Validation, Supervision, Software, Resources, Project administration, Conceptualization. **Lu Shi:** Writing – review & editing, Validation, Supervision, Software, Resources, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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