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TOXICOKINETICS AND METABOLISM

Effects of drug-drug interactions and CYP3A4 variants on alectinib metabolism

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Abstract

In this study, the effects of 17 CYP3A4 variants and drug-drug interactions (DDI) with its mechanism on alectinib metabolism were investigated. In vitro incubation systems of rat liver microsomes (RLM), human liver microsomes (HLM) and recombinant human CYP3A4 variants were established. The formers were used to screen potential drugs that inhibited alectinib metabolism and study the underlying mechanism, and the latter was used to determine the dynamic characteristics of CYP3A4 variants. Alectinib and its main metabolite M4 were quantitatively determined by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). The results showed that compared with CYP3A4.1, only CYP3A4.29 showed higher catalytic activity, while the catalytic activity of CYP3A4.4, .7, .8, .12, .14, .16, .17, .18, .19, .20, .23, and .24 decreased significantly. Among them, the catalytic activity of CYP3A4.20 is the lowest, only 2.63% of that of CYP3A4.1. Based on the RLM incubation system in vitro, 81 drugs that may be combined with alectinib were screened, among which 18 drugs had an inhibition rate higher than 80%. In addition, nicardipine had an inhibition rate of 95.09% with a half-maximum inhibitory concentration (IC_{50}) value of 3.54 ± 0.96 μM in RLM and 1.52 ± 0.038 μM in HLM, respectively. There was a mixture of non-competitive and anti-competitive inhibition of alectinib metabolism in both RLM and HLM. In vivo experiments of Sprague–Dawley (SD) rats, compared with the control group (30 mg/kg alectinib alone), the $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, T_{max} and C_{max} of alectinib administered in combination with 6 mg/kg nicardipine were significantly increased in the experimental group. In conclusion, the metabolism of alectinib was affected by polymorphisms of the CYP3A4 gene and nicardipine. This study provides reference data for clinical individualized administration of alectinib in the future.

Keywords ALK-TKI · Alectinib · Nicardipine · CYP3A4 · Incubation system

Introduction

In the past few decades, lung cancer has been recognized as the most common malignant tumor in the world, and poses a great threat to human health and life (Sung et al. 2021). Among them, fusion positive non-small cell lung cancer (NSCLC) accounts for 85% of its incidence (Duma et al. 2019). Compared with other common tumor types, NSCLC is leading the way in terms of near-term improvements in life expectancy, with the use of targeted therapies for operable mutations resulting in improved overall survival (Howlader et al. 2020; Imyanitov et al. 2021). Anaplastic lymphoma kinase (ALK) gene fusion is an important therapeutic target in NSCLC, and its incidence is approximately 5% (Kwak et al. 2010). It is also called "diamond mutation" because of the high selectivity of targeted drugs against this target and the prolonged survival of patients (Duruisseaux et al. 2017; Kwak et al. 2010).

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Alectinib (Fig. 1A), a highly selective, second-generation ALK-tyrosine kinase inhibitor (ALK-TKI), has extremely high blood–brain barrier (BBB) permeability, and has demonstrated central nervous system efficacy and therapeutic safety in ALK fusion positive NSCLC patients (Kodama et al. 2014; Nishino et al. 2019; Sakamoto et al. 2011; Tomasini et al. 2019). Alectinib has been shown to be effective in patients with crizotinib (a first-generation ALK-TKI)-resistant NSCLC patients who are initially treated with crizotinib. It was also approved in the European Union as a first-line treatment for adult patients with advanced ALK-positive NSCLC and adults with advanced ALK-positive NSCLC who have previously received crizotinib therapy (Seto et al. 2013). Alectinib and its major pharmacologically active metabolite, M4 (Fig. 1B), have been studied both nonclinically and clinically, including drug-drug interactions (DDI) (Morcos et al. 2017a; Nakagawa et al. 2018; Sekiguchi et al. 2017). In vivo studies have shown that M4 is the main metabolite of oral alectinib in the human body, and alectinib is primarily metabolized by CYP3A4 and is also the substrate of CYP3A4 (Morcos et al. 2016). Similar to alectinib, M4 has anti-ALK activity in vitro and in vivo (Sato-Nakai et al. 2017). Previous studies have shown that alectinib and M4 only exhibit time-dependent inhibition of the CYP3A4 enzyme (Sekiguchi et al. 2017). This also suggests that alectinib and M4 have the potential to involve DDI through CYP3A4 in vitro (Zhou et al. 2004, 2005).

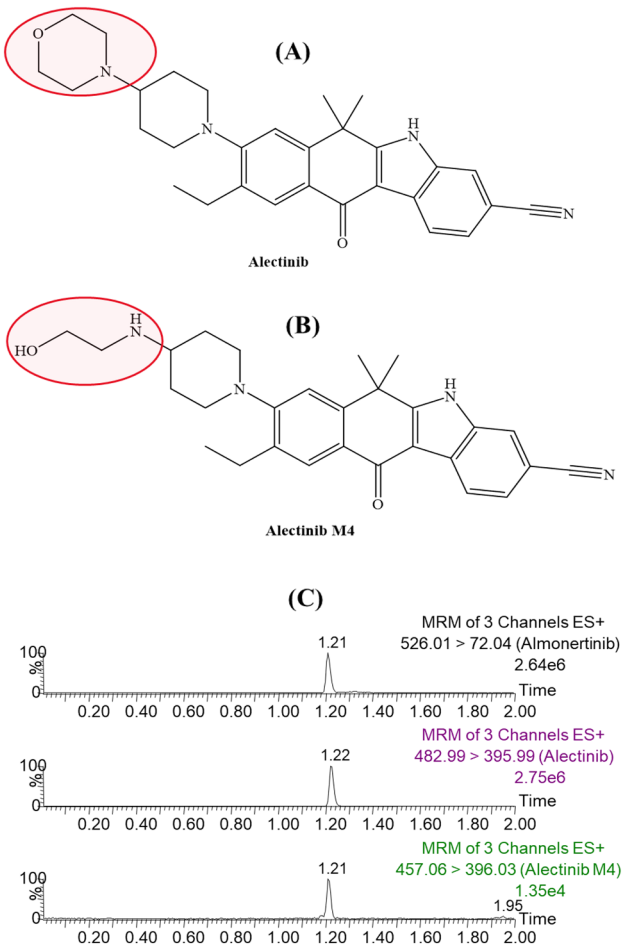

Fig. 1 Chemical structures of alectinib (**A**) and M4 (**B**), and the chromatographic information of alectinib, M4 and almonertinib (IS) (**C**)

Advanced cancer patients often suffer from multiple complications, such as cardiovascular diseases, infections, and other diseases, which means that patients often need to be treated with multiple western medicines at the same time (Calvo et al. 2019). In addition, herbal products used by cancer patients have significantly risen in the last few decades, and have the potential for interactions with anticancer drugs (Yeung et al. 2018). The occurrence of DDI is often mediated by cytochrome P450 (CYP450). Therefore, when alectinib is combined with drugs and/or herbs that inhibit or induce alectinib-metabolizing enzymes, it is likely to cause DDI, thereby enhancing or attenuating the effect of the combined drugs. To investigate the DDI of alectinib, we screened a series of drugs on alectinib metabolism, including 36 monomers of traditional Chinese herbs, 28 cardiovascular drugs and so on. Then, we selected a cardiovascular drug (nicardipine) for animal experiments, and also explored the inhibitory mechanism of nicardipine on alectinib metabolism so as to provide data support for the clinical individualized medication of alectinib.

CYP3A4 is the most important drug metabolism enzyme in the CYP450 family, and plays an extremely meaningful role in the metabolism of exogenous and endogenous substances (Werk and Cascorbi 2014). To date, 47 variants have been identified in the Human CYP Allele Nomenclature Committee website (https://www.pharmvar.org/gene/CYP3A4) as released. Increasing evidence shows that CYP3A4 gene variation has a significant impact on the inter-individual variability of metabolic activity, which could be affected by genetic polymorphism, gender, environment, etc., in the catalytic process of drugs (Werk and Cascorbi 2014). However, the effects of multiple genetic variants of CYP3A4 on alectinib metabolism have not been studied, where genetic factors significantly influence the metabolism of alectinib and the toxicological outcomes that result from exposure (Tsatsakis 2021). In this study, we systematically evaluated the catalytic activities of wild-type CYP3A4.1 and 16 variants of CYP3A4, which is significant for establishing the association between genotype and metabolic phenotype of CYP3A4 for alectinib individualized precision medicine.

Through this study, we could better understand the gene polymorphism of CYP3A4 and DDI on alectinib metabolism, which provides data support for the precision administration of alectinib.

Materials and methods

Chemicals and reagents

Sources of investigational drugs: alectinib, M4, and almonertinib (internal standard, IS) were purchased from Shanghai Canspec Scientific Instruments Co., Ltd. (Shanghai, China). Nicardipine and 80 other drugs were also provided by Shanghai Canspec Scientific Instruments Co., Ltd. (Shanghai, China), and their information is presented in Supplementary Table S1. The rat liver microsome (RLM) and human liver microsome (HLM) used in the experiment were produced by Corning Life Sciences Co., Ltd. (Jiangsu, China), and recombinant human CYP3A4 and cytochrome b5 were prepared as described previously (Zhou et al. 2019). Reduced niacinamide adenine dinucleotide phosphate (NADPH) was purchased from Roche Pharmaceutical Ltd. (Basel, Switzerland). In addition, other chemical solvents used in the experiment were analytical grade.

Equipment and operating conditions

The concentrations of alectinib and M4 were determined using ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) technology. A Waters Acquity UPLC BEH C18 column (2.1 mm × 50 mm, particle size 1.7 μm) was used in the chromatographic system for separation, and the column temperature was set at 40 ℃. The mobile phase was consisted of 0.1% formic acid (A) and acetonitrile (B), and was eluted at a flow rate gradient of 0.4 mL/min for 3 min. Alectinib and M4 were quantified using a Waters Xevo TQS triple quadrupole mass spectrometer (Milford, MA, USA) with multiple reaction monitoring (MRM) selected in positive mode, with a monitoring transition of *m/z* 482.99 → 395.99 for alectinib, *m/z* 457.06 → 396.03 for M4, and *m/z* 526.01 → 72.04 for IS.

Enzyme reaction of alectinib using human recombinant CYP3A4

The incubation system for the enzyme reaction was validated and consisted of a 200 μL volume, including phosphate buffered saline (PBS, 1 M, pH 7.4), 0.5 pmol CYP3A4.1 or other CYP3A4 variants, 50 μg/mL cytochrome b5, 1 mM NADPH, and 0.05–5 μM alectinib. The above solutions, except for NADPH, were mixed and preincubated at 37 ℃ for 5 min before 1 mM NADPH was added to initiate the reaction, followed by incubation for 40 min and placed at − 80 ℃ to terminate the reaction. After the enzymatic reaction was completely terminated, 20 μL of 200 ng/mL IS working solution and 300 μL of acetonitrile (protein precipitant) were added to the mixture. Then, the mixture was vortexed for 2 min and centrifuged at 13,000 rpm for another 10 min, and 100 μL of the supernatant was placed into the injector for the quantification of alectinib metabolite M4 using UPLC-MS/MS.

Determination of DDI and inhibition mechanism of alectinib in vitro

The total volume of the 200 μL incubation system was consisted of PBS, 0.3 mg/mL RLM or HLM, 1 mM NADPH, and 0.01–10 μM alectinib. The K_m (Michaelis–Menten constant) value of alectinib in the RLM incubation system was 0.47 μM. To explore the potential DDI of alectinib, the 200 μL system was kept the same, and the K_m value was used as the concentration of alectinib in the RLM system to determine the inhibitory effect of 81 drugs (the concentration of each drug as the inhibitor was 100 μM) on alectinib metabolism. The subsequent reaction procedure was the same as that of the human recombinant CYP3A4 variants mentioned above. Drugs with inhibition rates ≥ 80% were needed to be repeated for verification.

The half maximal inhibitory concentration (IC_{50}) of nicardipine against alectinib was determined in RLM and HLM. The concentration gradient of nicardipine was 0, 0.01, 0.1, 1, 10, 25, 50 and 100 μM, and the concentration of alectinib was 0.47 μM in RLM and 5.9 μM in HLM, respectively. The subsequent processing steps were the same as above to obtain the IC_{50} values of nicardipine in RLM and HLM, respectively. To determine the underlying mechanism of the inhibitory effect of nicardipine on alectinib, according to the K_m and IC_{50} values, the concentrations of alectinib in the RLM were set at 0.12, 0.23, 0.47 and 0.94 μM, and the concentrations of nicardipine were set at 0, 0.88, 1.77, 3.54 and 7.08 μM. In the HLM system, the concentrations of alectinib were set at 0.58, 1.16, 2.90 and 5.80 μM, and the concentrations of nicardipine were set at 0, 0.15, 0.30, 0.76 and 1.52 μM. The subsequent processing steps were the same as above.

Effect of nicardipine on alectinib in SD rats in vivo

Eight Sprague–Dawley (SD) male rats (200 ± 10 g) were purchased from the Animal Experimental Center of the First Affiliated Hospital of Wenzhou Medical University and randomly divided into two groups (n = 4): group A (control group) and group B (experimental group). Animals were fasted for 12 h before experiments without restriction of water intake. Alectinib and nicardipine were prepared into suspension with 0.5% carboxymethyl cellulose sodium (CMC-Na) solution for oral administration. Group B was given nicardipine (6 mg/kg) by intragastric administration, and group A was given an equal volume of CMC-Na

solution. Thirty minutes later, both group A and B were given alectinib (30 mg/kg) by gavage. After administration, caudal vein blood was taken at 0.333, 0.667, 1, 1.5, 2, 4, 6, 8, 12, 24, 48, and 72 h. Blood samples (0.3 mL) were centrifuged at 8,000 rpm for 5 min, and 100 µL plasma was taken and frozen at − 80℃ for later treatment. Plasma samples were treated with protein precipitation method. Then, 300 µL acetonitrile and 20 µL almonertinib (IS working solution) were added to 100 µL plasma, thoroughly mixed and centrifuged at 13,000 rpm for 10 min. Finally, 100 µL supernatant was taken into the instrument to measure the concentrations of alectinib and M4.

Statistical analysis

The K_m, IC_{50}, Lineweaver–Burk plot, and mean plasma concentration–time curves were generated using GraphPad Prism 9.0 software. Drug and Statistics (DAS) software (version 3.0 software, Mathematical Pharmacology Professional Committee of China, Shanghai, China) was used to fit the pharmacokinetic curve to obtain the pharmacokinetic parameters of alectinib and M4 in rats. SPSS (version 24.0; SPSS Inc., Chicago, IL, USA) with one-way ANOVA Dunnett's test was used to statistically analyse whether there were significant differences between the parameters of wild type CYP3A4.1 and other variants, and a *t*-test was used to compare the pharmacokinetic parameters between two groups in rats. Generally, $p < 0.05$ was considered statistically significant.

Results

Determination of alectinib and M4 by UPLC-MS/MS

As shown in Fig. 1C, the retention times of alectinib, M4, and almonertinib (IS) were 1.22, 1.21, and 1.21 min, respectively. The analytes were well separated from each other, and no interfering peaks were observed affecting the analyte determination. The standard curve range of alectinib was 0.5–2000 ng/mL, and that of M4 was 0.5–20 ng/mL, and the lower limit of quantification of both was 0.5 ng/mL. For the validation of the bioanalytical method, quality control (QC) samples at low, medium, and high concentrations were prepared to assess the accuracy, precision, recovery, matrix effect and stability. The results are shown in Supplementary Tables S2–S4.

Effects of recombinant human CYP3A4 variants on the metabolism of alectinib

The Michaelis–Menten curves of alectinib catalyzed by CYP3A4.1 and other variants are shown in Fig. 2, and the

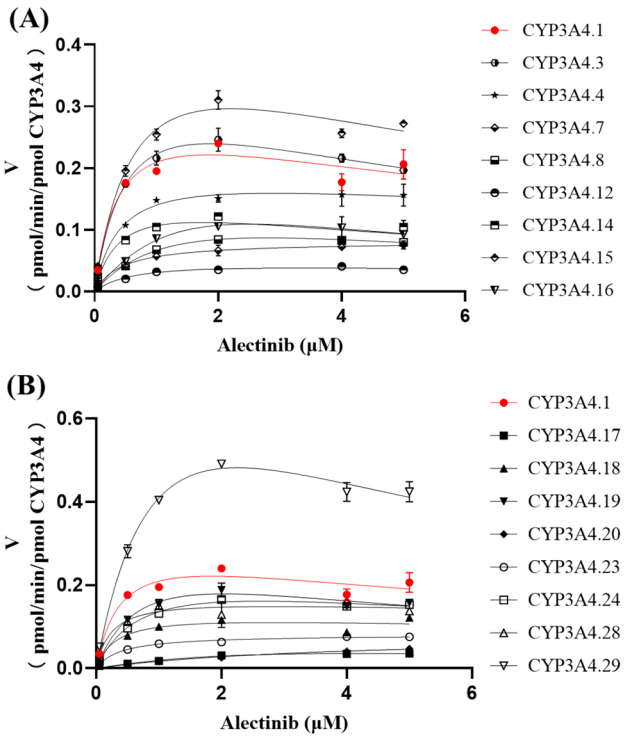

Fig. 2 Michaelis–Menten curves of the enzymatic activities of the wild-type CYP3A4 and other CYP3A4 variants on the metabolism of alectinib (**A**, **B**)

enzyme kinetic parameters are shown in Table 1. Statistical analysis of the parameters with CYP3A4.1 as the reference revealed the following changes. For V_{max}, we observed significant differences between CYP3A4.1 and CYP3A4.7, 0.12, 0.20, 0.23, and 0.29. Among them, CYP3A4.7, 0.12, 0.20, and 0.23 showed a downward trend, with a decrease of 65.63%—84.38%, and CYP3A4.29 greatly increased, with an increase of 221.88% ($p < 0.001$). There was no noticeable difference between other variants. In K_m, we found that the K_m values of CYP3A3.16, 0.17, and 0.20 were largely increased compared with CYP3A4.1, with an increase of 672.50%–1317.50%. In addition, we observed the autometabolic inhibition of M4 in 13 variants of CYP3A4.1, 0.3, 0.8, 0.12, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.24, 0.28, 0.29, and the Michaelis–Menten curve showed a significant downward trend after the plateau phase. We calculated the K_i value and made statistics as shown in Table 1. Intrinsic clearance ($CL_{int} = V_{max}/K_m$) was considered as the evaluation criterion of CYP3A4 activity. The CL_{int} of CYP3A4.4, 0.7, 0.8, 0.12, 0.14, 0.16, 0.17, 0.18, 0.19, 0.20, 0.23, and 0.24 was particularly dissimilar to CYP3A4.1, and all showed a significant downward trend, with a decrease of 38.75%—97.50%. However, CYP3A4.3, 0.15, 0.28, 0.29 were not meaningfully different from CYP3A4.1. The statistical results of relative clearance were the same as those of CL_{int} (Fig. 3).

Table 1 Kinetic parameters for M4 activity of CYP3A4.1 and other CYP3A4 variants on alectinib metabolism

Variants	V_{max} (pmol/min/pmol CYP)	K_m (μM)	Ki (μM)	CL (μL/min/pmol CYP)	Relative clearance (% of wild type)
3A4.1	0.32 ± 0.03	0.40 ± 0.09	9.42 ± 4.32	0.80 ± 0.10	100.00 ± 12.96
3A4.3	0.39 ± 0.03	0.59 ± 0.11	5.97 ± 0.49	0.68 ± 0.09	84.08 ± 10.69
3A4.4	0.21 ± 0.03	0.44 ± 0.11	ND	0.49 ± 0.05**	61.00 ± 5.85**
3A4.7	0.11 ± 0.04*	0.78 ± 0.58	ND	0.16 ± 0.05***	19.65 ± 5.77***
3A4.8	0.20 ± 0.05	1.76 ± 0.67	4.98 ± 2.03	0.12 ± 0.01***	14.43 ± 1.49***
3A4.12	0.05 ± 0.00**	0.66 ± 0.09	18.84 ± 4.84	0.08 ± 0.01***	9.95 ± 0.71***
3A4.14	0.18 ± 0.07	0.53 ± 0.39	11.08 ± 11.12	0.43 ± 0.18***	52.92 ± 21.97***
3A4.15	0.50 ± 0.09	0.74 ± 0.22	7.30 ± 3.00	0.70 ± 0.10	86.49 ± 12.13
3A4.16	0.38 ± 0.09	3.09 ± 1.10*	2.15 ± 0.40	0.13 ± 0.02***	15.65 ± 1.87***
3A4.17	0.14 ± 0.08	5.67 ± 3.69***	4.43 ± 2.66	0.02 ± 0.00***	3.06 ± 0.23***
3A4.18	0.14 ± 0.02	0.34 ± 0.08	80.24 ± 107.92	0.41 ± 0.05***	51.25 ± 6.01***
3A4.19	0.35 ± 0.11	0.95 ± 0.52	5.30 ± 2.37	0.40 ± 0.08***	49.20 ± 9.42***
3A4.20	0.08 ± 0.01**	3.91 ± 0.63**	ND	0.02 ± 0.00***	2.63 ± 0.18***
3A4.23	0.08 ± 0.00**	0.40 ± 0.05	ND	0.20 ± 0.01***	25.43 ± 1.79***
3A4.24	0.26 ± 0.03	0.81 ± 0.17	9.59 ± 4.73	0.33 ± 0.03***	40.97 ± 4.08***
3A4.28	0.19 ± 0.06	0.33 ± 0.23	85.48 ± 108.07	0.71 ± 0.27	88.33 ± 34.14
3A4.29	1.03 ± 0.23***	1.27 ± 0.45	4.49 ± 1.88	0.84 ± 0.10	104.21 ± 12.26

Compared to wild type, *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

ND not determined

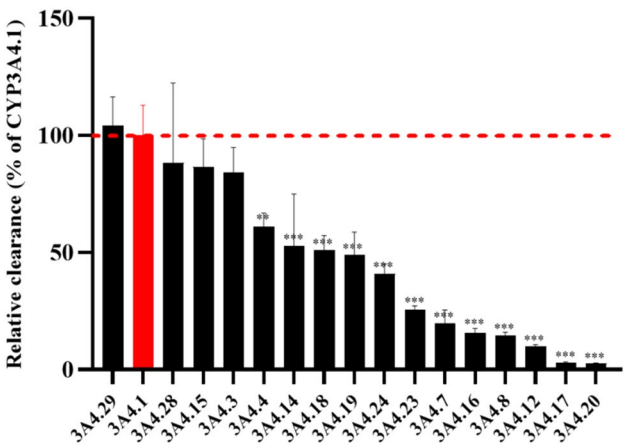

Fig. 3 Relative clearance of CYP3A4 variants toward the metabolism of alectinib compared with the wild type

Screening drugs with the possibility of DDI when combined with alectinib

The V_{max} and K_m values of alectinib were 0.0016 ± 0.00 pmol/min/μg protein and 0.47 μM in RLM, respectively, and 0.0184 ± 0.00 pmol/min/μg protein and 5.86 μM in HLM, respectively. A total of 81 drugs with potential combination with alectinib were screened in this experiment, including cardiovascular drugs, traditional Chinese medicine, psychotropic drugs and so on. The information on 81 drugs is presented in Supplementary Table S1. The inhibition results of these drugs on alectinib are shown in Fig. 4A. In total, 18 drugs with inhibition rates greater than 80% were found, and the inhibition profiles of these drugs are supplemented in Fig. 4B. Among cardiovascular drugs in Fig. 4C, the inhibition rate of nicardipine was 95.09%, indicating that the drug combined with alectinib had a high possibility of DDI.

Nicardipine inhibited alectinib metabolism through a mixed mechanism of non-competitive and anti-competitive inhibition in RLM/HLM

The Michaelis–Menten curves of alectinib catalyzed by RLM and HLM are displayed in Fig. 5A and B. In addition, the IC_{50} curves of nicardipine on alectinib metabolism in RLM and HLM are shown in Fig. 5B and 6B, with values of 3.54 ± 0.96 μM and 1.52 ± 0.038 μM, respectively (Tables 2). The IC_{50} values were all less than 10, indicating that nicardipine had a moderate inhibitory effect on alectinib metabolism in vitro. According to Lineweaver–Burk plots, nicardipine inhibited alectinib metabolism through a mixed type of non-competitive and anti-competitive inhibition mechanism in RLM (Fig. 5C) and HLM (Fig. 6C), with K_i values of 10.300 and 2.668, and α values of 0.549 and 0.302, respectively (Table 2).

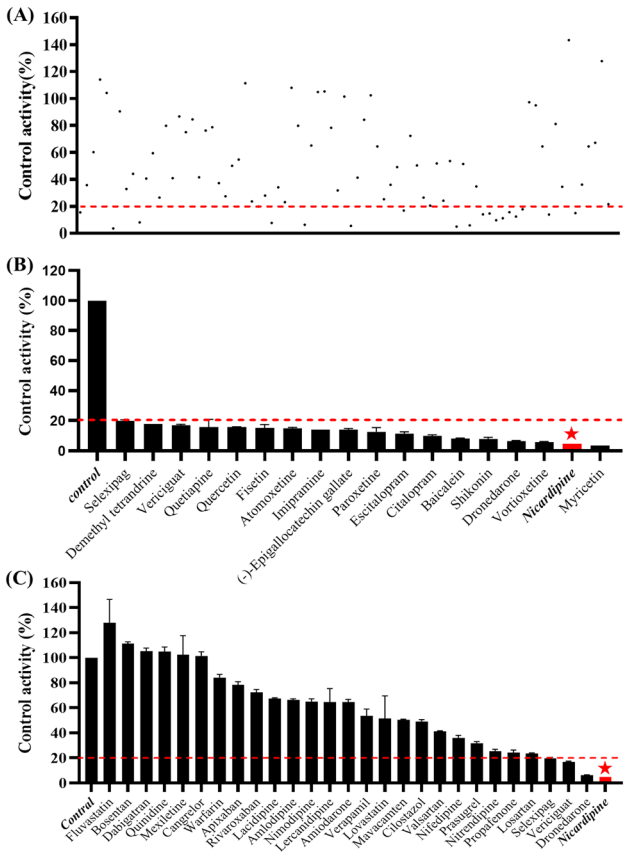

Fig. 4 Comparison of the inhibitory effects of different drugs (100 μM) on the metabolism of alectinib in RLM. For all screened drugs (**A**, the red line represents 20%), 18 drugs with metabolic rates less than 20% of the control (**B**) and cardiovascular drugs (**C**), the data are expressed as mean ± SD

Nicardipine inhibited alectinib metabolism in SD rats

The average concentration–time curves of alectinib and M4 in rats are shown in Fig. 7, and the corresponding pharmacokinetic parameters are shown in Table 3 and 4. When nicardipine was combined with alectinib, the $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, T_{max} and C_{max} of alectinib increased significantly, while $t_{1/2}$ and $C_{Lz/F}$ had no significant changes. T_{max} of M4 was significantly increased, while other pharmacokinetic parameters were not significantly different. These results showed that nicardipine increased the plasma exposure of alectinib in SD rats, indicating that nicardipine had a potential DDI with alectinib.

Discussion

Oral alectinib is mainly metabolized by CYP3A4 and converted to M4 in the liver; meanwhile, both alectinib and M4 are substrates of CYP3A4 (Morcos et al. 2016). The significant interindividual differences in CYP3A4 activity are mainly due to genetic polymorphisms, which also suggests that CYP3A4 genetic polymorphisms have an important effect on alectinib metabolism (Zhou et al. 2004, 2005). Alectinib has a highly positive curative effect in the clinical treatment of ALK-positive advanced NSCLC patients and is widely used as a first-line treatment in patients with crizotinib resistance (Kodama et al. 2014; Sakamoto et al. 2011). However, there is a lack of reports on the effects of CYP3A4 variants on alectinib metabolism. Against this background, we evaluated the effects of 17 CYP3A4 variants on alectinib metabolism in vitro using human recombinant microsomes, where wild-type CYP3A4.1 was used as the control.

In the incubation system, only CYP3A4.29 showed higher catalytic activity than CYP3A4.1. As shown in Fig. 2B, we clearly observed that the V_{max} of CYP3A4.29 was largely higher than that of CYP3A4.1, but there was no statistically significant difference in the clearance rate between them, which may be caused by the large K_m of CYP3A4.29. The catalytic activities of the 12 variants in CYP3A4.4, 0.7, 0.8, 0.12, 0.14, 0.16, 0.17, 0.18, 0.19, 0.20, 0.23, and 0.24 were typically lower than that of CYP3A4.1. Patients with these variants were poor metabolizers. Compared with other variant carriers, we should pay more attention to the occurrence of DDI when using alectinib. Among them, the catalytic activity of CYP3A4.20 was the lowest, only 2.63% of that of CYP3A4.1, which may be caused by carrying a premature stop codon and producing a truncated protein (Zhou et al. 2011). According to Fig. 2, it could be observed that alectinib had self-inhibition in most CYP3A4 variants, which may be related to the fact that alectinib and M4 are both substrates of CYP3A4. Alectinib is metabolized to produce M4 under the catalytic action of enzymes. It has been reported that M4 accounts for approximately 40% of the parent drug AUC (Morcos et al. 2017b), and a large number of M4 may compete with alectinib for binding enzyme sites. This may lead to the situation that the Michaelis–Menten curve reaches a plateau and then declines. Therefore, our data indicated that CYP3A4 gene polymorphism had different degrees of influence on alectinib metabolism. Although this experiment was only verified in vitro, it could still provide a reference for clinical trials.

Before conducting alectinib in vivo, nicardipine was selected after 81 potential drugs were screened. Cancer patients often have complications or underlying diseases that are likely to be used in combination with cardiovascular drugs. Nicardipine, a calcium channel blocker, is widely used in the treatment of cardiovascular disease, and is clinically approved for the treatment of hypertension. It was reported that nicardipine was a relatively potent inhibitor of CYP3A4 in vitro, suggesting that DDI between nicardipine and other drugs metabolized mainly by CYP3A4 appears to occur in vivo (Nakamura et al. 2005; Wallin 1990). To

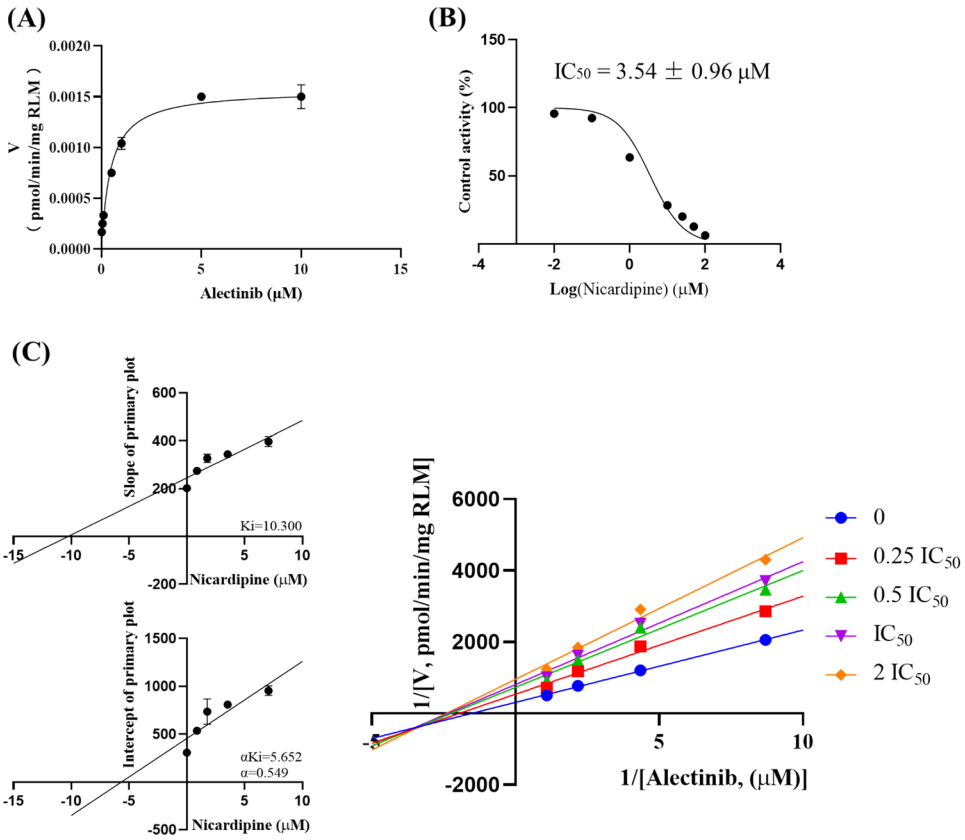

Fig. 5 In RLM, Michaelis–Menten plot (**A**), IC_{50} of Nicardipine (**B**), linewever-burk plot, secondary diagram of Ki and secondary diagram of αKi inhibiting alectinib metabolism at different concentrations of nicardipine (**C**). Data are expressed as mean ± SD, n = 3

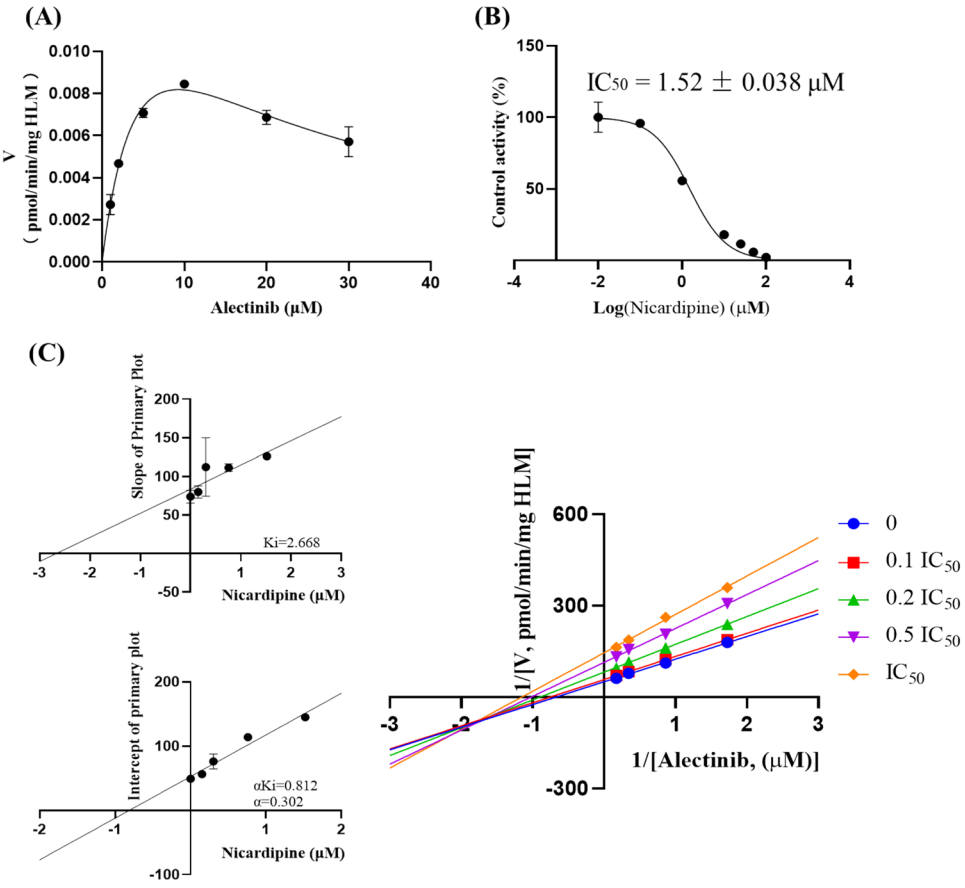

Fig. 6 In HLM, Michaelis–Menten plot (**A**), IC_{50} of Nicardipine (**B**), linewever-burk plot, secondary diagram of Ki and secondary diagram of αKi inhibiting alectinib metabolism at different concentrations of nicardipine (**C**). Data are expressed as mean ± SD, n = 3

Table 2 The IC_{50} values and inhibitory effects of nicardipine on alectinib metabolism in RLM and HLM

	IC_{50} values (μM)	Inhibition type	Ki (μM)	αKi (μM)	α
RLM	3.54 ± 0.96	Non-competitive and anti-competitive inhibition	10.300	5.652	0.549
HLM	1.52 ± 0.038	Non-competitive and anti-competitive inhibition	2.668	0.812	0.302

Table 3 The main pharmacokinetic parameters of alectinib in two groups of SD rats ($n = 4$)

Parameters	Alectinib	Alectinib + Nicardipine
$AUC_{(0\to t)}$ (μg/L*h)	13,174.44 ± 3167.43	26,174.84 ± 1760.45**
$AUC_{(0\to\infty)}$ (μg/L*h)	14,616.25 ± 3394.47	27,445.11 ± 1719.15**
$t_{1/2}$ (h)	21.58 ± 6.82	14.56 ± 3.89
T_{max} (h)	4.50 ± 1.92	8.00 ± 0*
$CL_{z/F}$ (L/h/kg)	2.14 ± 0.49	1.10 ± 0.07

AUC area under the plasma concentration–time curve, $t_{1/2}$ elimination half time, T_{max} peak time, $CL_{z/F}$ plasma clearance, C_{max} maximum blood concentration

*$P < 0.05$, **$P < 0.01$, compared with the group A

Table 4 The main pharmacokinetic parameters of M4 in two groups of SD rats (n = 4)

Parameters	Alectinib	Alectinib + Nicardipine
$AUC_{(0\to t)}$ (μg/L*h)	296.77 ± 91.25	209.23 ± 120.17
$AUC_{(0\to\infty)}$ (μg/L*h)	409.38 ± 178.58	457.92 ± 133.25
$t_{1/2}$ (h)	35.08 ± 11.35	58.83 ± 31.85
T_{max} (h)	11.00 ± 2.00	38.00 ± 20.00**
$CL_{z/F}$ (L/h/kg)	83.06 ± 30.70	69.77 ± 20.05
C_{max} (μg/L)	8.05 ± 1.69	3.89 ± 1.82

AUC area under the plasma concentration–time curve, $t_{1/2}$ elimination half time, T_{max} peak time, $CL_{z/F}$ plasma clearance, C_{max} maximum blood concentration

*$P < 0.05$, **$P < 0.01$, compared with the group A

the best of our knowledge, there has been no study on the combination of nicardipine and alectinib, and relevant data are insufficient. Previous studies of alectinib metabolism by CYP450 enzyme and prediction using PBPK model have found that alectinib has weak CYP3A4 induction potential, and there are multiple scavenging enzymes and mechanisms besides CYP3A4 involved in total metabolism, which is considered as a weak interactor, so the possibility of DDI is low (Cleary et al. 2018; Nakagawa et al. 2018; Sekiguchi et al. 2017). However, our pharmacokinetic results in SD rats showed the possibility of DDI when alectinib and nicardipine were combined. Nicardipine is primarily metabolized by CYP2C8, 2D6 and 3A4, and is a relatively potent inhibitor of these metabolic enzymes (Nakamura et al. 2005). These three CYP450 isoforms are also the metabolic enzymes of alectinib, which means that nicardipine may inhibit the metabolism of alectinib from a variety of metabolic pathways in vivo.

Available data indicate that cancer patients use herbal products (along with standard treatments) more often than the general population. The reasons cited for such use include improving health, reducing the risk of recurrence, and reducing the side effects of cancer treatments. Herbs, however, contain biologically active compounds and can potentially interact with prescription medications, including chemotherapy drugs (Yeung et al. 2018). Notably flavones such as apigenin, luteolin, diosmetin and tangeretin, flavonols such as myricetin, quercetin and kaempferol and

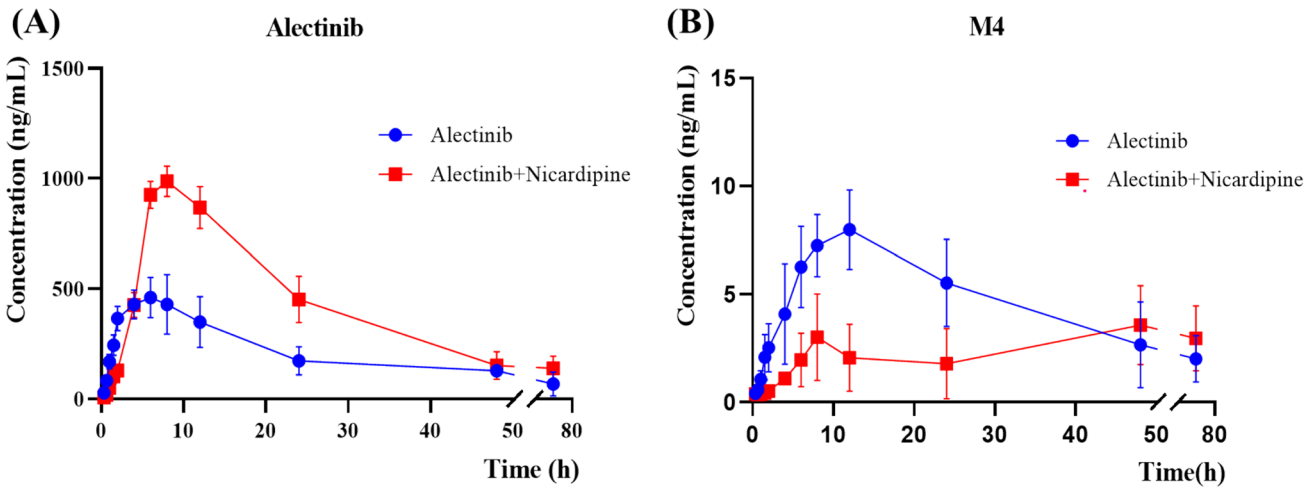

Fig. 7 Mean concentration–time curve of alectinib (**A**) and M4 (**B**) in rats. Data are presented as the means ± SD, n = 4

isoflavones such as genistein and daidzein, are compounds abundant in dietary nutrients that are consumed daily worldwide. Thus, these compounds have been predominantly investigated, in terms of their abilities to interact with the cytochrome P450 CYP1 family of enzymes for cancer prevention (Androutsopoulos et al. 2010). In addition, it was reported that CYP1 family enzymes enhanced the antiproliferative activity of dietary flavonoids in breast cancer cells, through bioconversion to more active products (Androutsopoulos et al. 2009a). Moreover, it turned out that the contribution of CYP1A1 to cancer progression or prevention may depend on the balance of procarcinogen activation/detoxication and dietary natural product extrahepatic metabolism (Androutsopoulos et al. 2009b).

To date, several ALK-TKIs have been developed and are widely available in clinical practice, some of which have received approval by the US Food and Drug Administration (FDA), such as alectinib. Coadministration of esomeprazole (Morcos et al. 2017c), and posaconazole (a strong CYP3A inhibitor) (Morcos et al. 2017a) had no clinically relevant effect on the exposure of alectinib and M4 in humans. However, CYP inhibitors and inducers can significantly alter the exposure of ALK-TKIs and can lead to clinically relevant DDI (Zhao et al. 2020). Interestingly, TKI treatments also increase the risk of DDI, which may result in changes in pharmacokinetics or pharmacodynamics of TKIs or their concomitant treatments, with subsequent risks of increasing their toxicity and/or reducing their effectiveness (Libiad et al. 2022). The results in our study showed that nicardipine inhibited alectinib metabolism in vitro and in vivo, indicating the potential DDI is a real possibility. Thus, nicardipine may lead to high interpatient variability of alectinib and subsequent risks for increased toxicity and serious adverse events, indicating that clinical intervention is necessary.

Conclusion

In conclusion, the present study evaluated the effects of CYP3A4 variants and drug interactions on alectinib metabolism. According to our results, CYP3A4 gene polymorphisms exerted differential effects on alectinib metabolism, which could provide some reference for the subsequent establishment of genotype–phenotype relationships in clinical settings. In addition, we found that the inhibitory effect of nicardipine on alectinib metabolism was consistent in vitro and in vivo. Since alectinib and nicardipine are widely used in clinical practice, our study could provide a basis for the appropriate and reasonable combination of alectinib and nicardipine in clinical practice, and provide support for clinical individualized precision medicine.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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