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The impacts of CYP3A4 genetic polymorphism and drug interactions on the metabolism of lurasidone

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ABSTRACT

The aim of this study was to investigate the impacts of 24 variants of recombinant human CYP3A4 and drug interactions on the metabolism of lurasidone. *In vitro*, enzymatic reaction incubation system of CYP3A4 was established to determine the kinetic parameters of lurasidone catalyzed by 24 CYP3A4 variants. Then, we constructed rat liver microsomes (RLM) and human liver microsomes (HLM) incubation system to screen potential anti–tumor drugs that could interact with lurasidone and studied its inhibitory mechanism. *In vivo*, Sprague–Dawley (SD) rats were applied to study the interaction between lurasidone and olmutinib. The concentrations of the analytes were detected by ultra–performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). As the results, we found that compared with the wild-type CYP3A4, the relative intrinsic clearances vary from 355.77 % in CYP3A4.15 to 14.11 % in CYP3A4.12. A series of drugs were screened based on the incubation system, and compared to without olmutinib, the amount of ID–14283 (the metabolite of lurasidone) in RLM and HLM were reduced to 7.22 % and 7.59 %, and its IC_{50} were 18.83 ± 1.06 μM and 16.15 ± 0.81 μM, respectively. At the same time, it exerted inhibitory effects both through a mixed mechanism. When co-administration of lurasidone with olmutinib in rats, the $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of lurasidone were significantly increased by 73.52 % and 69.68 %, respectively, while $CL_{z/F}$ was observably decreased by 43.83 %. In conclusion, CYP3A4 genetic polymorphism and olmutinib can remarkably affect the metabolism of lurasidone.

1. Introduction

Lurasidone, an atypical antipsychotic, has been approved for the acute and maintenance treatment of schizophrenia and bipolar I depression in monotherapy [1] or in combination with lithium or valproate [2,3]. Lurasidone is unique in exhibiting high affinity for 5–HT_{2A}, dopamine D_2 receptors, and 5–HT_7 receptors (antagonists) and moderate affinity for 5–HT_{1A} receptors (partial agonists) [4], which may be associated with improved cognition and antidepressant effects [4]. However, it has been reported that high doses of lurasidone, whether used for acute treatment or long-term treatment, will greatly increase the probability of adverse reactions, such as nausea, drowsiness and

Abbreviations: AUC, area under the plasma concentration–time curve; CL_{int}, intrinsic clearance; $CL_{z/F}$;, plasma clearance; C_{max}, maximum plasma concentration; CO;, carbon monoxide; CYP3A4;, cytochrome P450 3A4; CYPOR;, cytochrome P450 reductase; DAS, Drug and statistics; EGFR TKI, epidermal growth factor receptor tyrosine kinase inhibitor; FDA, The United States Food and Drug Administration; HLM, human liver microsomes; IC_{50}, half-maximal inhibitory concentration; IS, internal standard; K_i, inhibition constant; K_m, Michaelis-Menten constant; MRM, multiple reaction monitoring; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NSCLC, non-small cell lung cancer; PBS, phosphate buffered saline; PCR, polymerase chain reaction; RLM, rat liver microsomes; S.D., standard deviation; SD, Sprague-Dawley; *Sf21*, Spodoptera frugiperda 21; $t_{1/2z}$, elimination half time; T_{max}, peak time; TQS, triple-quadruple tandem mass spectrometer; UPLC-MS/MS, ultra-performance liquid chromatography–tandem mass spectrometry; V_{max}, maximum velocity of the reaction; $V_{z/F}$, apparent volume of distribution; WHO, World Health Organization.

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extrapyramidal side effects [5]. And the individual differences in plasma drug concentrations may be mainly caused by genetic polymorphism of metabolic enzymes and drug interactions, which are the main reasons for efficacy stratification of clinical drugs.

Lurasidone is mainly metabolized into three active metabolites (ID–14283, ID–14326 and ID–14614) and two inactive metabolites (ID–20219 and ID–20220) by CYP3A4 enzyme in liver. In addition, the main metabolite (ID–14283) accounted for 26 % of the products of lurasidone [6]. However, the enzyme activity of CYP3A4 can vary up to 60-fold between individuals, leading to treatment failure and unpredictable toxic side effects [7]. Meanwhile, drug combination is common in clinical medication, and it is also one of the main reasons for the difference of drug plasma exposure.

According to Greenblatt and Harmatz's study, when healthy volunteers co-administration of lurasidone with posaconazole, a strong CYP3A inhibitor, the area under the plasma concentration curve (AUC) of lurasidone increased by 5.75 times and the maximum plasma concentration (C_{max}) increased by 4 times [8]. Many patients with serious diseases, such as cancer, leukemia, uremia and so on, usually have mental problems due to pathological, physiological or psychological factors. Therefore, in this study, we systematically screened a series of oncology drugs that might be used in combination with lurasidone to determine the effect of interaction on lurasidone metabolism. For instance, imatinib, research has shown that when patients take imatinib and simvastatin concomitantly, imatinib acts as a CYP3A4 inhibitor and increases the AUC of simvastatin by 3.5 times, thus greatly increasing the probability of adverse reactions to simvastatin [9]. In this study, we used rat liver microsomes (RLM), human liver microsomes (HLM) and Sprague–Dawley (SD) male rats to study the interaction between lurasidone and olmutinib. And we also evaluated the catalytic activity of CYP3A4 variants on the disposition of lurasidone. The experimental data possibly provided a range of reference values for therapeutic drug monitoring of lurasidone.

2. Materials and methods

2.1. Chemicals and reagents

Lurasidone (Fig. 1A), ID-14283 (Fig. 1A) and olmutinib were purchased from Shanghai Chuangsai Technology Co., Ltd. (Shanghai, China). A series of drugs (olmutinib, ponatinib, alectinib, tirabrutinib, lapatinib, abrocitinib, lenvatinib, ixazomib, givinostat, PF-07321332, oprozomib, voxtalisib, venetoclax, pilaralisib, napabucasin, parsaclisib, adagrasib, asciminib, abexinostat, sunitinib, imatinib, talazoparib, selinexor, apalutamide, fedratinib, duvelisib, fruquintinib, ivosidenib, larotrectinib, ACP196, pexidartinib, enasidenib, eliglustat, elagolix, gilteritinib, ripretinib, tazemetostat, adagrasib, avapritinib, trilostane, marimastat, tamoxifen, tofacitinib) were also purchased from Shanghai Chuangsai Technology Co., Ltd. (Shanghai, China). HLM was obtained from Corning Life Sciences Co., Ltd. (Jiangsu, China). Reduced nicotinamide adenine dinucleotide phosphate (NADPH) was got from Roche Pharmaceutical Ltd. (Basel, Switzerland). Recombinant human CYP3A4 and cytochrome b5 were prepared by Beijing Hospital (Beijing, China) [10].

2.2. Preparation of recombinant human CYP3A4 microsomes

The construction of the expression vectors, the expressions of CYP3A4 variant proteins and the determination of protein expression levels were carried out according to the previous procedures of our team [11]. Firstly, we subcloned CYP3A4 and CYPOR into pFastBac™ dual expression vector (Thermo Fisher, Waltham, MA, USA). Secondly, PCR was used to expand the vector of CYP3A4 variants. Thirdly, transfecting *Sf21* insect cells (Thermo Fisher, MA, USA) with the vectors to prepare baculoviruses. Fourthly, *Sf21* insect cells were transfected again to obtain recombinant CYP3A4 proteins. Finally, the total protein concentrations, protein expression levels, and the total amount of CYP of recombinant human CYP3A4 enzymes were determined by BCA kit (Thermo Fisher, MA, USA), immunoblotting assay, and carbonic monoxide (CO) differential spectrum assay after the cells were collected by ultra-centrifugation, respectively.

2.3. Preparation of rat liver microsomes [12]

Rat livers were weighed and homogenized with cold 0.01 mM phosphate buffered saline (PBS, pH 7.4) containing 0.25 mM sucrose. The homogenates were centrifuged at 11,000 rpm at 4 ℃ for 15 min, and the supernatants were centrifuged repeatedly. Then, the supernatants were moved to new tubes, centrifuged at 75,600 xg for 2 h, the supernatants were discarded, and 3 or 4 times the volume of cold 0.01 mM phosphate buffered saline was added to the precipitation for homogenization. Finally, the protein concentrations were determined by the Pierce™ BCA protein assay Kit (Thermo Scientific).

2.4. The analysis condition of UPLC-MS/MS

UPLC-MS/MS technology was applied to determine the concentrations of lurasidone, ID-14283 and midazolam (internal standard, IS). The liquid chromatographic separation was performed on a Waters Acquity UPLC BEH C18 column (2.1 mm × 50 mm, 1.7 μm; Milford, MA, USA) at 40 ℃. The mobile phase was consisted of 0.1 % formic acid (A) and acetonitrile (B). At the flow rate of 0.30 mL/min, the gradient elution process was 90 % A (0–0.5 min); 90–10 % A (0.5–1.0 min); 10 % A (1.0–1.4 min); 10–90 % A (1.4–1.5 min), and finally 90 % A (1.5–2.0 min). Mass quantification was performed using a Waters Xevo TQS triple-quadruple tandem mass spectrometer (Milford, MA, USA), and the analytes were detected by multiple reaction monitoring (MRM) in the positive mode. The ion transitions of lurasidone, ID-14283 and midazolam (IS) were *m/z* 493.05→165.99, *m/z* 509.03→182.00 and *m/z* 326.20→291.40, respectively.

2.5. Determination of enzymatic reactions

The enzymatic reaction of recombinant human CYP3A4 was performed in phosphate-buffered saline (1 M), which included CYP3A4.1 or other CYP3A4 variants (0.5 pmol), cytochrome b5 (50 μg/mL), lurasidone (0.5–50 μM), and NADPH (1 mM) in the final volume of 200 μL. After pre-incubation for 5 min at 37 °C, NADPH (1 mM) was added to initiate the reaction, followed by incubation for further 30 min and immediately terminated by freezing to − 80 °C. Next, 400 μL acetonitrile and 20 μL midazolam (IS, 200 ng/mL) were added to the mixture, fully vortexed for 2 min, centrifuged at 13,000 rpm for 10 min, and 100 μL supernatants were taken for UPLC-MS/MS determination.

To explore the potential drug interactions of lurasidone, 200 μL incubation system was consisted of PBS (1 M), RLM or HLM (0.3 mg/mL), NADPH (1 mM), lurasidone and other drugs. First of all, we measured the effect of other drugs on the metabolism of lurasidone by setting the concentration of other drugs at 100 μM and the concentration of lurasidone at 20 μM (the corresponding K_m value). Besides, the drugs with an inhibition ≥ 80 % were repeated in other independent experiment to verify the results. Then, we determined the half-maximum inhibitory concentration (IC_{50}) of olmutinib, and the concentration of olmutinib was set to 0, 0.01, 0.1, 1, 10, 25, 50 and 100 μM, while the concentration of lurasidone was set to 20 μM. Next, we intended to investigate the potential mechanism of the interaction between lurasidone and olmutinib. In the 200 μL incubation system, the concentration of lurasidone was 5, 10, 20, 40 μM (concentration range was determined according to the corresponding K_m value) and the concentration of olmutinib was 0, 10, 20, 40 μM in RLM and 0, 10, 30, 40 μM in HLM (concentration range was determined according to the corresponding IC_{50} value). Finally, the samples were prepared as described above.

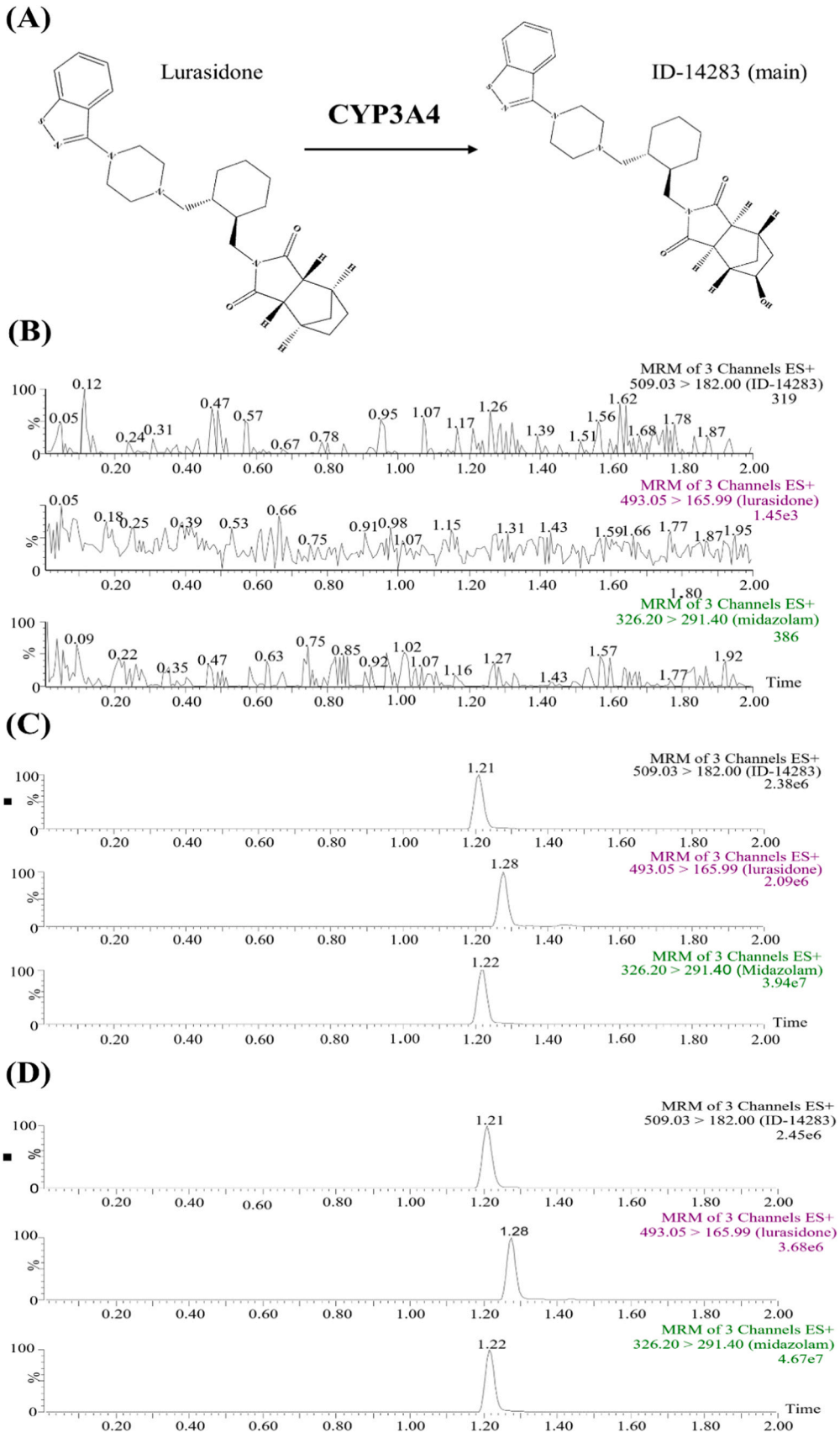

Fig. 1. The metabolic pathway of lurasidone (A). Chromatogram of a blank plasma sample (B). Chromatogram of a blank plasma sample supplemented with lurasidone, ID-14283 and midazolam (C). Chromatogram of a rat plasma sample at 1 h after the administration of lurasidone (D).

2.6. Animal experiments

Twelve SD male rats (200 ± 10 g) were purchased from Animal Experimental Center of The First Affiliated Hospital of Wenzhou Medical University (Zhejiang, China). SD rats were randomly divided into 2 groups (n = 6): intragastric administration of 4 mg/kg lurasidone (group A); intragastric administration of 4 mg/kg lurasidone and of 30 mg/kg olmutinib (group B). Before the formal experiment, the rats were fasted for 12 h with free access to drinking water, and at the beginning of the experiment, group B was given olmutinib. 30 min later, the two groups were both given lurasidone. Blood samples were collected from tail vein at 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 and 48 h. After mixing 100 μL of plasma with 300 μL of acetonitrile and 20 μL of midazolam (IS, 200 ng/mL), fully vortexing and centrifuging, the supernatants were obtained for UPLC–MS/MS analysis.

All experiments were approved by the Experimental Animal Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University, and performed in accordance with the Regulations on the Administration of Experimental Animals.

2.7. Statistical analysis

All data were presented as mean ± Standard Deviation (S.D.). We used GraphPad Prism 9.5 software to get the Michaelis-Menten constants, IC_{50} values, Lineweaver Burk plots, the secondary plots and the average plasma concentration-time curve. The major pharmacokinetic parameters in the non–compartmental model were obtained by Drug and Statistics (DAS) software ((Version 3.0, Bontz Inc., Beijing, China). One-way ANOVA Dunnett's test in GraphPad Prism 9.5 software was used to compare the kinetic parameters between the wild-type CYP3A4 and other variants, and *t*-test was used to compare the kinetic parameters between different groups. $P < 0.05$ was deemed to have statistically significant.

3. Results

3.1. The chromatographic and mass spectrometric characteristics of lurasidone and ID–14283 were determined by UPLC–MS/MS

As the results, the retention times of lurasidone, ID–14283 and midazolam (IS) were 1.28, 1.20 and 1.22 min, respectively (Fig. 1). All analytes were well separated from each other without any interference. The ranges of calibration standard curves of lurasidone and ID–14283 were both 0.1–500 ng/mL, and the same of the LLOQ were 0.1 ng/mL. The analytes precision and accuracy values were less than 15 %, and the stability, extraction recovery and matrix effect were met the requirements.

3.2. Effect of recombinant human CYP3A4 on metabolism of lurasidone in vitro

Fig. 2 and Table 1 show the Michaelis–Menten curves and kinetic parameters of lurasidone metabolized by 24 variants of recombinant human CYP3A4 *in vitro*. According to the change of V_{max}, we could mainly get two cases: compared with the wild type CYP3A4.1, most of variants were obviously decreased, the lowest to 4.61 %, while CYP3A4.15 and CYP3A4.29 were increased to 130.41 % and 150.23 %, respectively. According to the change of K_m, we found that compared with CYP3A4.1, CYP3A4.12 had no obvious difference, while the other variants showed significantly reduced K_m values, with a decline range from 14.64 % to 54.87 %. Intrinsic clarity (V_{max}/K_m) is usually used as a standard to evaluate CYP3A4 enzyme activity. According to Table 1, compared with CYP3A4.1, 8 variants (CYP3A4.15,.19,.23,.24,.28,.29,.32,.33) were significantly increased (180.95–347.62 %); CYP3A4.12 was significantly decreased to 14.29 %, and the other variants had no significant difference. In addition, the concentration of ID–14283 could not be detected in CYP3A4.16,.17 and.18 variants.

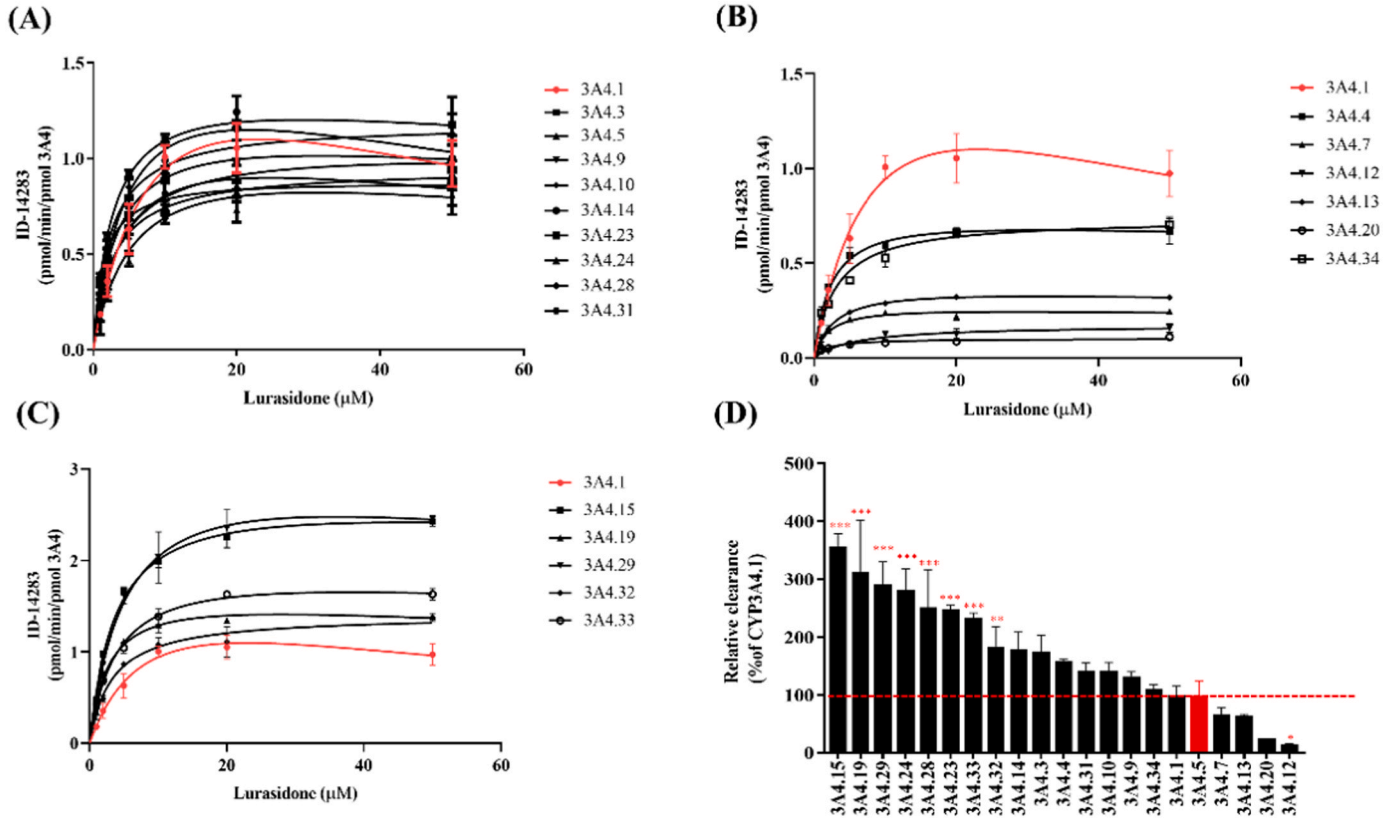

Fig. 2. Michaelis-Menten curves of lurasidone metabolism by the wild-type CYP3A4 and other variants (A-C). The relative clearance of CYP3A4 variants toward the metabolism of lurasidone compared with the wild-type CYP3A4 (D). Data are presented as mean ± S.D., n = 3. *P < 0.05, * *P < 0.01, * **P < 0.001.

Table 1
Kinetic parameters of lurasidone metabolism through 24 CYP3A4 variants.

Variants	V_{max} (pmol/min/pmol P450)	K_m (μM)	V_{max}/K_m (μL/min/pmol P450)
CYP3A4.1	2.17 ± 0.23	10.79 ± 2.81	0.21 ± 0.03
CYP3A4.3	1.25 ± 0.09 * **	3.51 ± 0.67 * **	0.36 ± 0.06
CYP3A4.4	0.77 ± 0.02 * **	2.37 ± 0.07 * **	0.33 ± 0.01
CYP3A4.5	1.10 ± 0.35 * **	5.92 ± 3.35 * **	0.20 ± 0.05
CYP3A4.7	0.28 ± 0.03 * **	2.11 ± 0.62 * **	0.14 ± 0.02
CYP3A4.9	1.23 ± 0.02 * **	4.56 ± 0.30 * **	0.27 ± 0.02
CYP3A4.10	1.13 ± 0.06 * **	3.93 ± 0.31 * **	0.29 ± 0.03
CYP3A4.12	0.17 ± 0.02 * **	6.01 ± 1.06	0.03 ± 0.00 *
CYP3A4.13	0.38 ± 0.02 * **	2.92 ± 0.15 * **	0.13 ± 0.01
CYP3A4.14	1.58 ± 0.27 * **	4.44 ± 1.46 * **	0.37 ± 0.06
CYP3A4.15	2.83 ± 0.24 * **	3.88 ± 0.58 * **	0.73 ± 0.05 * **
CYP3A4.16	ND	ND	ND
CYP3A4.17	ND	ND	ND
CYP3A4.18	ND	ND	ND
CYP3A4.19	1.72 ± 0.27 * *	2.89 ± 1.20 * **	0.65 ± 0.18 * **
CYP3A4.20	0.10 ± 0.00 * **	2.14 ± 0.12 * **	0.05 ± 0.00
CYP3A4.23	1.44 ± 0.09 * **	2.82 ± 0.24 * **	0.51 ± 0.01 * **
CYP3A4.24	0.90 ± 0.02 * **	1.58 ± 0.26 * **	0.58 ± 0.08 * **
CYP3A4.28	1.19 ± 0.16 * **	2.45 ± 0.83 * **	0.52 ± 0.14 * **
CYP3A4.29	3.26 ± 0.15 * **	5.52 ± 0.87 * *	0.60 ± 0.08 * **
CYP3A4.31	0.95 ± 2.13 * **	3.31 ± 0.43 * **	0.29 ± 0.03
CYP3A4.32	1.41 ± 2.85 * **	3.79 ± 0.56 * **	0.38 ± 0.07 * *
CYP3A4.33	2.00 ± 3.83	4.19 ± 0.09 * **	0.48 ± 0.02 * **
CYP3A4.34	0.73 ± 2.38 * **	3.22 ± 0.02 * **	0.23 ± 0.01

Notes: Compared with CYP3A4.1, *P < 0.05, * *P < 0.01, * **P < 0.001. ND, not determine.

3.3. Olmutinib inhibits the metabolism of lurasidone in RLM/HLM by a mixed mechanism

The Michaelis-Menten curve of lurasidone in RLM/HLM were conducted. The V_{max} and K_m values in RLM were 0.23 ± 0.01 pmol/min/μg protein and 19.74 ± 0.55 μM, and 0.21 ± 0.01 pmol/min/μg protein and 22.21 ± 1.24 μM in HLM, respectively. Fig. 3 shows the effects of various anti–tumor drugs on the metabolism of lurasidone, and we found that five anti-tumor drugs with an inhibition ≥ 80 %, among which olmutinib inhibited the metabolism of lurasidone by 92.78 %. According to Fig. 4, the IC_{50} values of olmutinib in inhibiting lurasidone metabolism in RLM and HLM were 18.83 ± 1.06 μM and 16.15 ± 0.81 μM, respectively. And the IC_{50} values of tamoxifen, adagrasib, asciminib and pilaralisib in RLM were 21.56 ± 0.25 μM, 25.73 ± 0.49 μM, 35.38 ± 0.22 μM and 43.02 ± 0.64 μM, respectively (Supplementary Fig. S1). Fig. 5 shows the Lineweaver-Burk plot of the impact of olmutinib on lurasidone metabolism. The results showed that olmutinib inhibited the metabolism of lurasidone in RLM and HLM both by a mixed mechanism, with a K_i = 21.08 μM and αK_i = 9.01 μM in RLM and with a K_i = 32.60 μM and αK_i = 11.04 μM in HLM, respectively.

3.4. Olmutinib increased the drug exposure of lurasidone in SD rats

The mean concentration-time curves of lurasidone and ID-14283 are shown in Fig. 6, and the corresponding pharmacokinetic parameters are shown in Table 2 and Table 3. Compared with group A, the $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of lurasidone in group B increased to 173.52 % and 169.68 %, respectively, while the $CL_{z/F}$ decreased to 56.17 %. The C_{max} of ID-14283 was down to 69.97 %. The results show that olmutinib increased the plasma exposure of lurasidone in SD rats.

4. Discussion

Bipolar disorder is a chronic psychiatric disorder with a calculated global lifetime prevalence of 0.2–1.0 % [13,14], which is not only a seriously disabling disease for patients, but also a challenge for clinical doctors [15]. The World Health Organization (WHO) has identified bipolar disorder as one of the top 20 causes of disability worldwide [16]. The United States Food and Drug Administration (FDA) has approved lurasidone for the treatment of bipolar I depression as a monotherapy in children and adolescents (ages 10–17) and in combination with lithium or valproate to treat adults in 2013 [2,3].

Lurasidone is principally metabolized into three active metabolites (ID-14283, ID-14326, and ID-14614) through oxidative N-dealkylation,

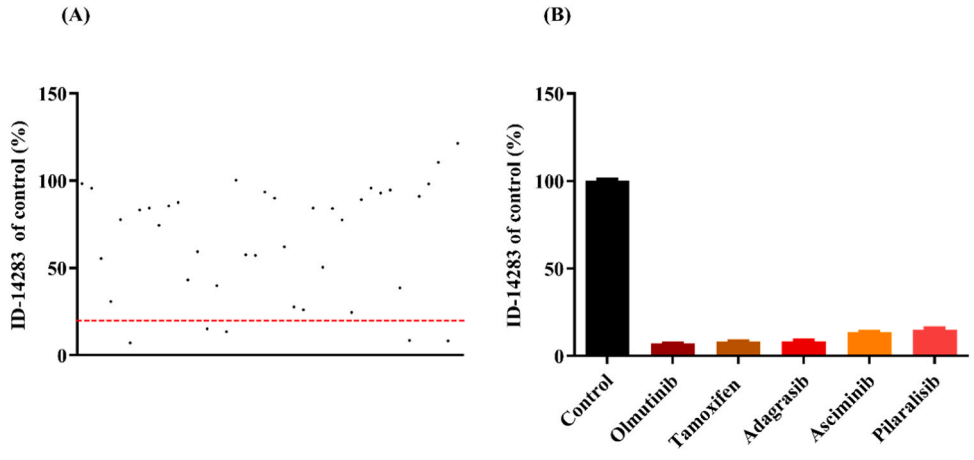

Fig. 3. Comparison of inhibitory effects of anti-tumor drugs in RLM on metabolic formation of the lurasidone metabolite ID-14283 (A). The drugs with an inhibition ≥ 80 % in RLM (B). Data are presented as mean ± S.D.

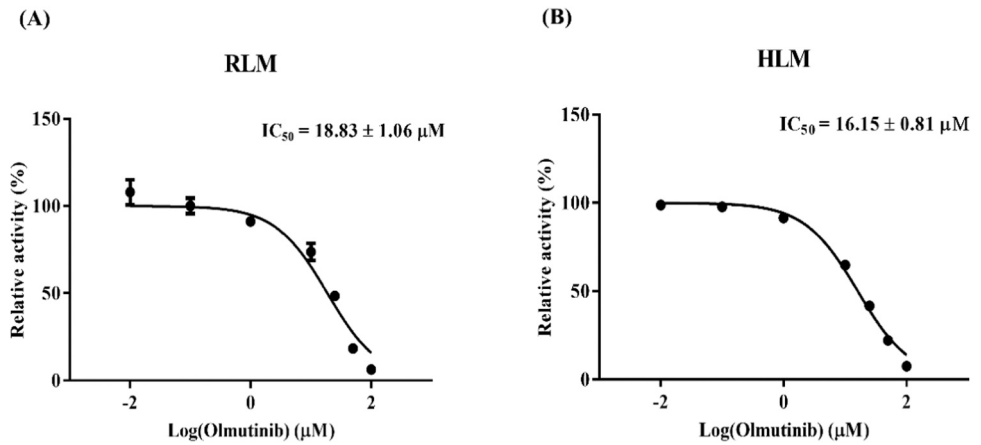

Fig. 4. Concentrations (0, 0.01, 0.1, 1, 10, 25, 50, 100 μM) of olmutinib to determine the half-maximal inhibitory concentration (IC_{50}) for the metabolism of lurasidone by the RLM (A) and HLM (B), respectively. Data are presented as mean ± S.D., n = 3.

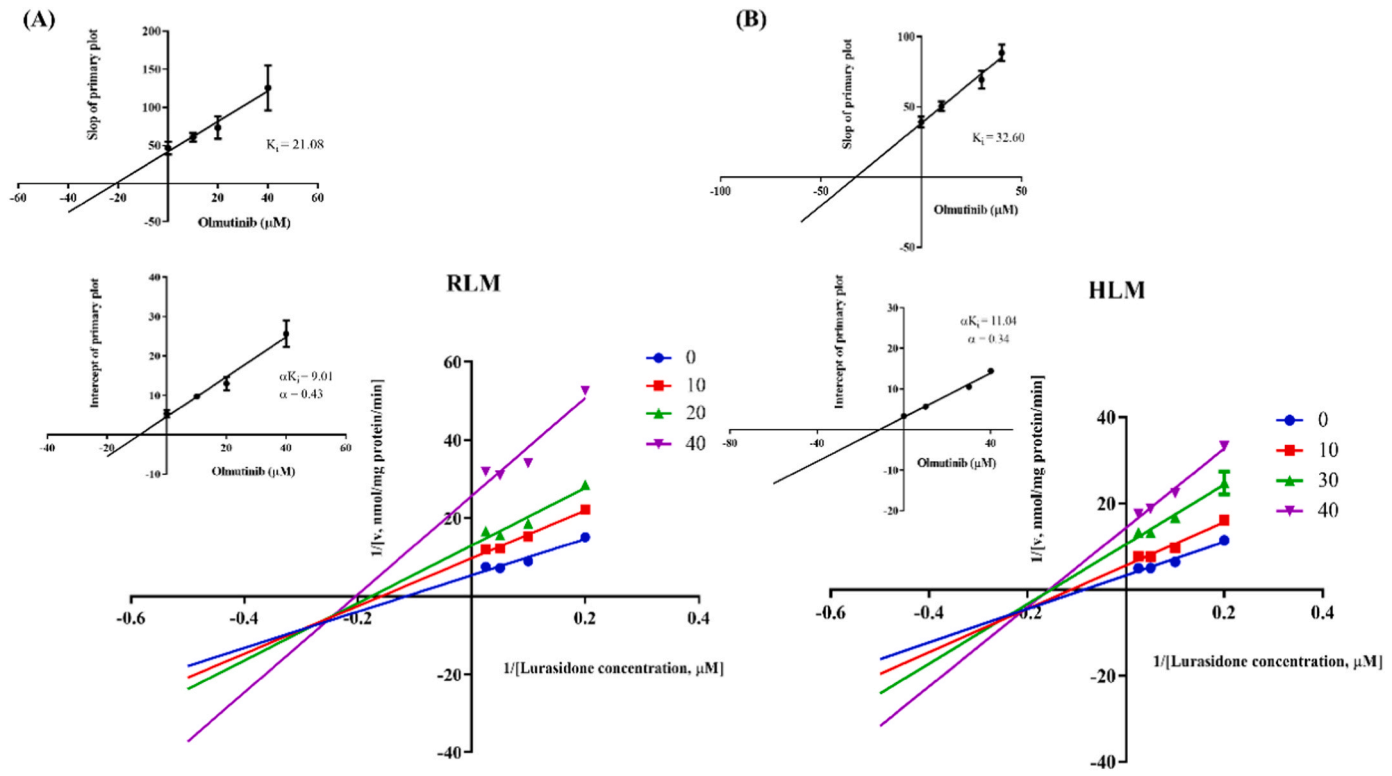

Fig. 5. Lineweaver-Burk plot, the secondary plot for K_i and the secondary plot for αK_i in the inhibition of lurasidone metabolism by olmutinib with different concentrations in RLM (A) and HLM (B), respectively. Data are presented as mean ± S.D., n = 3.

hydroxylation of norbornane ring, and S-oxidation by the CYP3A4 enzyme in the liver. The ID-14283 is the main metabolite, accounting for 26 % of the breakdown products of lurasidone, with a shorter half-life (7.48–10 h) than lurasidone. By contrast, the detectable amount of the other two active metabolites were very low, accounting for a combined 4 % [5].

To date, our team has found that the pharmacological effect of many drugs can be affected by genetic polymorphism of their metabolic enzymes, such as methadone [17], osimertinib [18], risperidone [19]. Besides, genetic polymorphism accounted for 90 % of the variation of CYP3A4 activity among individuals [20], indicating that genetic polymorphism of CYP3A4 may have an impact on the metabolism of lurasidone. Even so, there is no human data available to indicate that the pharmacokinetics of lurasidone, or the clinical response to lurasidone treatment, is in any way influenced by variants in CYP3A genotype and the present work is solely a study of *in vitro* metabolism. In this study, the wild-type CYP3A4.1 was used as the control group to evaluate the effects of 23 other variants on lurasidone metabolism *in vitro*. According to the results, the catalytic activity of 8 variants (CYP3A4.15,.19,.23,.24,.28, .29,.32,.33) was significantly increased compared with CYP3A4.1, and their K_m values were significantly lower than the wild type, which may be the main reason for their increased catalytic activity. The activity of CYP3A4.12 was significantly lower than CYP3A4.1, which may be caused by its V_{max} values being significantly lower than the wild-type. The remaining CYP3A4 variants (CYP3A4.3,.4,.5,.7,.9,.10,.13,.14,.20, .31,.34) showed similar catalytic activity to the wild-type. In addition, CYP3A4.16,.17, and.18 had no catalytic activity to lurasidone, and the concentration of ID–14283 could not be detected. Based on the study of

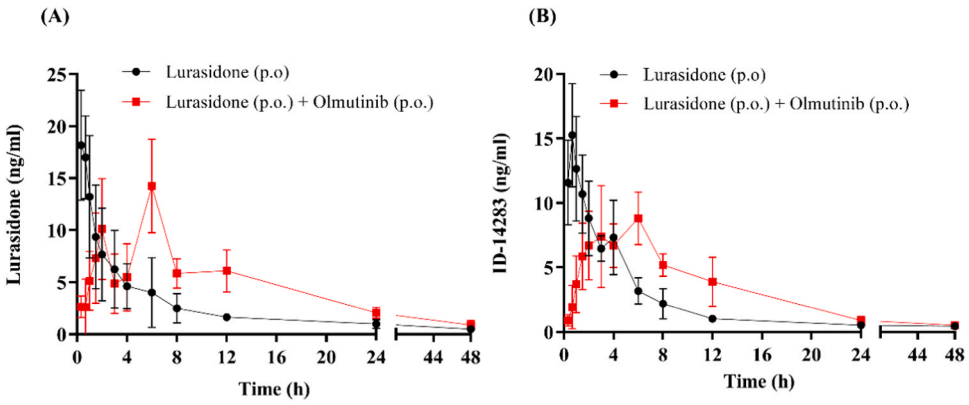

Fig. 6. Effect of olmutinib on the pharmacokinetics of lurasidone in SD rats. Mean concentration-time curve of lurasidone (A) and ID-14283 (B) in two groups: single group (lurasidone), combined group (lurasidone with olmutinib). Data are presented as mean ± S.D., n = 6.

Table 2
The main pharmacokinetic parameters of lurasidone in two groups of rats (N = 6).

Parameters	Lurosidone (p.o.)	Lurosidone (p.o.) + Olmutinib (p.o.)
$AUC_{(0-t)}$ (μg/L*h)	94.15 ± 32.98	163.37 ± 19.52 * *
$AUC_{(0-\infty)}$ (μg/L*h)	107.12 ± 33.67	181.76 ± 34.20 * *
$t_{1/2z}$ (h)	19.26 ± 2.77	13.15 ± 4.97 *
T_{max} (h)	0.5 ± 0.18	5.58 ± 3.77 * *
$V_{z/F}$ (L/kg)	1144.13 ± 420.49	413.67 ± 100.86 * *
$CL_{z/F}$ (L/h/kg)	40.22 ± 11.17	22.59 ± 3.80 * *
C_{max} (μg/L)	19.95 ± 3.94	13.96 ± 3.84 *

Notes: AUC, area under the plasma concentration–time curve; $t_{1/2z}$, elimination half time; T_{max}, peak time; $V_{z/F}$, apparent volume of distribution; $CL_{z/F}$, plasma clearance; C_{max}, maximum plasma concentration. *P < 0.05, * *P < 0.01, * **P < 0.001, to compare with the control group.

Table 3
The main pharmacokinetic parameters of ID-14283 in two groups of rats (N = 6).

Parameters	Lurosidone (p.o.)	Lurosidone (p.o.) + Olmutinib (p.o.)
$AUC_{(0-t)}$ (μg/L*h)	88.91 ± 26.18	112.94 ± 14.51
$AUC_{(0-\infty)}$ (μg/L*h)	92.91 ± 25.89	119.42 ± 16.34
$t_{1/2z}$ (h)	13.23 ± 9.82	10.40 ± 2.96
T_{max} (h)	0.72 ± 0.14	5.67 ± 3.83 *
$V_{z/F}$ (L/kg)	878.33 ± 589.52	501.67 ± 139.42
$CL_{z/F}$ (L/h/kg)	46.20 ± 13.81	34.04 ± 4.81
C_{max} (μg/L)	16.25 ± 3.62	9.19 ± 2.93 * **

Notes: AUC, area under the plasma concentration–time curve; $t_{1/2z}$, elimination half time; T_{max}, peak time; $V_{z/F}$, apparent volume of distribution; $CL_{z/F}$, plasma clearance; C_{max}, maximum plasma concentration. *P < 0.05, * *P < 0.01, * **P < 0.001, to compare with the control group.

Kumondai et al., we found that the activity of these three isoforms to midazolam (a classical CYP3A4 substrate) was not high, and CYP3A4.17 had no catalytic activity either [21]. For CYP3A4.17, it was due to the exchange of an amino acid substitution: F189S in exon 7, which leads to the reduction of its catalytic activity [22,23], while the reasons for the low enzyme activities of CYP3A4.16 and CYP3A4.18 needs to be further studied.

Drug combinations are widely used in clinical practice, and are also one of the main reasons for the fluctuation of plasma drug concentration. We took into account that many patients with serious diseases, such as cancer, leukemia and uremia and so on, usually have mental problems due to pathological, physiological or psychological factors. Thus, we systematically screened a series of oncology drugs that might be used in combination with lurasidone to determine the effect of interaction on lurasidone metabolism. As a result, 5 drugs (olmutinib, tamoxifen, adagrasib, asciminib and pilaralisib) with inhibition rates greater than 80 % were found, and among them, the inhibition rate of olmutinib was 92.78 %, indicating that the drug combined with lurasidone had a high possibility of drug interactions. In addition, we found that the IC_{50} values of olmutinib, tamoxifen, adagrasib, asciminib and pilaralisib in RLM were 18.83 ± 1.06 μM, 21.56 ± 0.25 μM, 25.73 ± 0.49 μM, 35.38 ± 0.22 μM and 43.02 ± 0.64 μM, respectively, which used in combination with lurasidone, may have an impact on exposure to its plasma levels. Among them, olmutinib had the strongest inhibitory effect on the metabolism of lurasidone. Nevertheless, the IC_{50} values of olmutinib was far exceeding C_{max} of humans with olmutinib (1327.2 ± 380.3 ng/mL) [24], such that the *in vitro* IC_{50} should have no clinical significance. More in-depth research is required for the treatment of humans with olmutinib concomitantly with lurasidone. *In vivo* in rats, compared with the group A, the $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of lurasidone, when co-administered with olmutinib, were significantly increased to 173.52 % and 169.68 %, respectively, and the $CL_{z/F}$ was reduced to 56.17 %. However, $t_{1/2}$ and C_{max} of lurasidone were decreased by 31.72 % and 30.03 %, respectively. Thus, olmutinib may also affect absorption while slowing the metabolism of lurasidone. In addition, the pharmacokinetics of ID-14283 (Table 3 and Fig. 6) indicated that olmutinib also increased the $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of ID-14283 although not significant. In general, the AUC should decrease as the metabolism was inhibited by olmutinib. However, it is unknow if the clearance of ID-14283 may also have been impacted by olmutinib.

Approximately 1.6 million people die of cancer each year, of which lung cancer is the leading cause of cancer death around the world [25]. Moreover, around 85 % of patients with lung cancer are non-small cell lung cancer (NSCLC) [26]. Olmutinib is an oral third-generation representative epidermal growth factor receptor tyrosine kinase inhibitor (EGFR TKI) for the treatment of non-small cell lung cancer (NSCLC) [27]. However, olmutinib has been removed from clinical development due to the occurrence of toxic epidermal necrolysis in human patients, and our research only studied the effect of olmutinib on the metabolism of lurasidone *in vitro* and in rats. Furthermore, olmutinib is actually a weak or negligible CYP3A inhibitor, based on the anticipated C_{max} values in humans in relation to the IC_{50} or K_i. However, the impact of olmutinib on intestinal metabolism may be more pronounced due to the higher olmutinib concentration in the gut lumen. Hence, our study was not applicable to the interaction of lurasidone and olmutinib in humans, while the data may provide some reference for the factors affecting lurasidone metabolism.

5. Conclusion

Different variants of CYP3A4 have different effects on the metabolism of lurasidone due to their different enzymatic activities. *In vitro*, olmutinib inhibited the metabolism of lurasidone by a mixture

mechanism. All in all, this study can provide some basis data for further pharmacokinetic research of lurasidone.

CRediT authorship contribution statement

Qingqing Li: Investigation, Writing – original draft, Conceptualization, Data curation, Formal analysis. **Jing Wang:** Investigation, Methodology, Visualization, Writing – original draft. **Zheng-lu Wang:** Writing – review & editing. **Yuxin Shen**: Writing – review & editing. **Qi Zhou:** Investigation, Methodology, Data curation, Formal analysis. **Yanan Liu:** Investigation, Methodology. **Guo-xin Hu:** Supervision, Writing – original draft, Writing – review & editing. **Jian-ping Cai:** Project administration, Resources, Software, Supervision, Writing – review & editing, Validation. **Ren-ai Xu:** Project administration, Resources, Software, Supervision, Writing – review & editing, Validation.

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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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2023.115833.

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