The effect of icotinib and apatinib on the pharmacokinetic profile of oxycodone and underlying mechanism

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This study aimed to investigate the interactions between icotinib/apatinib and oxycodone in rats, and to unveil the mechanism underlied. A UPLC-MS/MS method was developed and validated to determine oxycodone and its demethylated metabolite simultaneously. In vivo, SD male rats were administered oxycodone with or without icotinib and apatinib, respectively. The blood samples were collected, and subjected to UPLC-MS/MS analysis. Moreover, enzyme incubation assay was performed to investigate the mechanism of drug-drug interaction using both rat (RLM) and human liver microsomes (HLM). The results showed that icotinib decreased the $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of oxycodone. In addition, C_{max} of oxycodone increased obviously when coadministration of apatinib. In vitro, the K_m of oxycodone metabolism were 101.7±5.40 μM and 529.6±19.60 μM in rat and human liver microsomes (RLM and HLM), respectively. In RLM, icotinib and apatinib inhibited the disposition of oxycodone in a mixed mechanism with IC_{50} = 3.29 ± 0.090 μM and 0.95 ± 0.88 μM accordingly. While they have competitive and mixed mechanism in HLM with IC_{50} = 22.34 ± 0.81 μM and 0.48 ± 0.05 μM, respectively. In conclusion, icotinib and apatinib can inhibit the metabolism of oxycodone both in vitro and in vivo. Therefore, the dose of oxycodone should be reconsidered when co-administrated with icotinib and apatinib.

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Running Head: influence oxycodone metabolism

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Abstract

This study aimed to investigate the interactions between icotinib/apatinib and oxycodone in rats, and to unveil the mechanism underlied. A UPLC-MS/MS method was developed and validated to determine oxycodone and its demethylated metabolite simultaneously. In vivo, SD male rats were administered oxycodone with or without icotinib and apatinib, respectively. The blood samples were collected, and subjected to UPLC-MS/MS analysis. Moreover, enzyme incubation assay was performed to investigate the mechanism of drug-drug interaction using both rat (RLM) and human liver microsomes (HLM). The results showed that icotinib decreased the $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of oxycodone. In addition, C_{max} of oxycodone increased obviously when coadministration of apatinib. In vitro, the K_m of oxycodone metabolism were 101.7±5.40 μM and 529.6±19.60 μM in rat and human liver microsomes (RLM and HLM), respectively. In RLM, icotinib and apatinib inhibited the disposition of oxycodone in a mixed mechanism with IC_{50} = 3.29 ± 0.090 μM and 0.95 ± 0.88 μM accordingly. While they have competitive and mixed mechanism in HLM with IC_{50} = 22.34 ± 0.81 μM and 0.48 ± 0.05 μM, respectively. In conclusion, icotinib and apatinib can inhibit the metabolism of oxycodone both in vitro and in vivo. Therefore, the dose of oxycodone should be reconsidered when co-administrated with icotinib and apatinib.

Keywords: Icotinib; Apatinib; Oxycodone; UPLC-MS/MS; Interaction

Introduction

During the cancer therapy, comorbidities and multiple medicines are common. Therefore, the incidence of drug-drug interaction is high in clinic(Akbulut & Urun 2020; Millan et al. 2014; Yeung et al. 2018). However, medicine companied adverse reactions are often overlooked compared with the aim of prolonging life span for the late-stage patient. This situation undoubtedly reduces the quality life of patient(Fink & Gallagher 2019; Neufeld et al. 2017). To studying the drug interaction in the treatment of cancer is helpful for precise treatment.

Cancer pain is one of the most common comorbidities of cancer. The incident is 39.3% after curative treatment, 55% during anticancer treatment, and 66% in advanced or metastatic status(van den Beuken-van Everdingen et al. 2016). Oxycodone, a semisynthetic opioid, has been used as a potent analgesic more than a century(Kalso 2005; Kinnunen et al. 2019). CYP3A4 and CYP2D6 are the primary enzymes involved in the metabolism of oxycodone, producing main metabolite desmethyloxycodone(Cai et al. 2021; Söderberg Löfdal et al. 2013). Any drugs that can take effect on the activities of CYP3A4 and CYP2D6 would lead to stratification of oxycodone blood exposure.

Tyrosine kinase receptors have been widely used in clinical therapeutics(Ahn et al. 2017; Yoneda et al. 2019). Among them, icotinib and apatinib are two representative drugs(Du et al. 2021; Meng et al. 2020). Icotinib is the first oral inhibitor of epidermal growth factor receptor (EGFR) prescribing for EGFR positive non-small cell lung cancer(Guan et al. 2014). Common adverse reactions of icotinib include rash, diarrhea, pruritus, and so on(Shi et al. 2015). Apatinib is another representative drug that used in treatment of non-small cell lung cancer (NSCLC) and advanced gastric cancer(Xue et al. 2018). There had evident that icotinib takes a certain inhibitory effect on CYP3A in vitro(Sun et al. 2021; Xue et al. 2018). Furthermore, literatures showed that apatinib can inhibit the activity of many cytochrome P450 enzymes including CYP2D6(Bao et al. 2018b; Zhou et al. 2014). Therefore, the co-administration of icotinib/apatinib with oxycodone would result in fluctuation of drug response.

In this study, considering that Sprague Dawley (SD) rats are common used in the experiment of pharmacokinetics and the homologous gene similarity with human, we evaluated the pharmacokinetics of oxycodone in SD rats. We selected a library of commonly used TKIs for screening, and based on the extent of inhibition, we identified icotinib and apatinib. Further drug-drug interaction experiments were conducted in vivo. We used RLM, HLM, and Sprague-Dawley (SD) rats to investigate the interaction between these two TKIs and oxycodone. The results would undoubtedly provide theoretical support for precise medicine, and can help to avoid adverse reaction or ineffective treatment.

Materials and methods

Chemicals and reagents

Noroxycodone and midazolam (internal standard, IS) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Icotinib was purchased from Inviwo Chemical Technology (Guangzhou, China). Apatinib was obtained from Beijing Sunflower Technology Development Co., Ltd. Liquid chromatography (LC) grade acetonitrile (ACN) were obtained from Merck (Darmstadt, Germany).

Ultrapure water was produced by a Milli-Q purification system. Human and rat liver microsomes were obtained from Corning Life Sciences Co., Ltd (Beijing, China).

UPLC-MS/MS conditions

A liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method was developed and used to determine oxycodone and noroxycodone as previous indicated by our group. A triple quadrupole mass spectrometer and electrospray ionization source (Waters XEVO TQD; Waters Corp., Millipore, Bedford, MA, USA) were equipped. The analytes were separated using a BEH C_{18} column (2.1 mm × 100 mm, 1.7 μm; Waters Corp.) which incubated at 40°C. The mobile phase was consisted of 0.1% formic acid (A) and ACN (B) with a gradient elution at 0.40 mL/min for 3.0 minutes. The gradient conditions were: 10–90 % B (0–1.0 minutes), 90–10 % B (1.0–2.0 min), and 10 % B (2.1–3.0 min). Multiple reaction monitoring in positive mode was used.

Microsome incubation assay

200 μL incubation system contains 1M PBS buffer, 0.2 mg/mL RLM or HLM, 1 mM NADPH, and 8–2000 μM oxycodone. Before the assay, the mixture without NADPH was pre-incubated at 37°C for 5 min. Then, 1 mM NADPH was added. After 30 minutes, the reaction is terminated by cooling to −80°C. Then, 400 μL acetonitrile and 20 μL midazolam (200 ng/mL, internal standard) were added to the mixture. Vortexing for 2 minutes and centrifuging at 13000 rpm for 10 minutes, the supernatant was obtained and subjected to UPLC-MS/MS analysis. Therefore, we obtain the K_m of oxycodone in RLM and HLM.

To evaluate the inhibitory effect of 24 TKIs on the metabolism of oxycodone, each drug at 100 μM was added into the incubation system as indicated above. Meanwhile, the concentration of oxycodone was set at 100 μM based on the K_m. Therefore, we obtained the inhibition rates of these 24 drugs on oxycodone metabolism and screened out the icotinib and apatinib with high inhibition rates.To determine the half maximal inhibitory concentration (IC_{50}), the concentration of icotinib and apatinib was set at 0.01, 0.1, 1, 10, 25, 50, and 100 μM, while the concentration of oxycodone was set at 100 μM in RLM and at 500 μM in HLM based on the corresponding K_m . To determine the inhibitory mode, the concentration of oxycodone was set at 25, 50, 100 and 200 μM in RLM, and 50, 250, 500 and 1000 μM in HLM. While the concentration of icotinib was set at 0, 0.75, 1.5 and 3 μM in RLM, and at 0, 5, 10 and 20 μM in HLM based on IC_{50} value. The concentration of apatinib was set at 0, 0.25, 0.5, 1 μM in RLM, and at 0, 0.125, 0.25, 0.5 μM in HLM. After incubation, the samples were prepared and determined by UPLC-MS/MS as mentioned above.

Animals and ethical statement

Sprague-Dawley male rats weighing 200 ± 20 g（6-7 week） were obtained from Viton Lever Laboratory Animal Center. License NO.:SCXK（zhejiang, China）2019-0001. Rats acclimated to the SPF animal room for one week. The room temperature was kept at 20-25℃ and the humidity was kept at 50%-65%. The change period of light and dark conditions was 12h, which simulate the change of day and night. During this period, the rats had ad libitum access to food and water. All SD rats were fed in the same environment. The study was conducted in accordance with the guidelines of the Ethics Committee of Wenzhou Medical University (wydw2020-0322). Prior to the experiment, food was withheld from the rats for 12 hours. Euthanasia was performed

by administering 2% and 5% isoflurane during and after the pharmacokinetic experiment, respectively.

Animal experiments

AS the design method of related experiments reported previously. Eighteen SD rats were randomly divided into 3 groups (n=6): control group (group A), icotinib group (group B) and apatinib group (group C). Icotinib and apatinib were dissolved in 0.5% CMC-Na solution to make the final concentrations of 37.5 mg/mL and 40 mg/ml, respectively. Group B was given 37.5 mg/kg icotinib by gavage, and group C was given 40 mg/kg apatinib by gavage as well. 30 minutes later, three groups were subcutaneously injected with 3 mg/kg oxycodone. Oxycodone was diluted in physiological saline to the final concentration of 2 mg/mL prior to injection. At 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 10 hours post injection, blood samples were collected from the tail vein. Each sample was centrifuged at 13,000 rpm for 10 min. 100 μL of the supernatant was taken and mixed with 20 μL of 200 ng/mL midazolam and 200 μL acetonitrile. The sample was vortexed for 2 min, then centrifuged at 13,000 rpm for another 10 min. Finally, the supernatant was collected and subjected to UPLC-MS/MS determination.

Statistical Analysis

The kinetic curve was fitted using GraphPad Prism 5.0 software by log (inhibitor) vs. normalized response, Lineweaver-Burk double reciprocal plot method. The pharmacokinetic parameters were obtained using drug and statistics (DAS) software 3.0, fitting with non-compartmental model. The drug-time curves were drawn by Origin 8.0. All data are expressed as Mean ± SD. Statistical analysis was performed using independent samples t-test. $P < 0.05$ indicates a significant difference. The excessive deviation of data value was considered for exclusion.

Result

Development of UPLC-MS/MS assay to determine concentration of oxycodone and noroxycodone.

The monitoring transitions of oxycodone, noroxycodone and midazolam are m/z 316.2 → 241.1, m/z 302.2 → 187 and m/z 326.1 → 291.1, respectively. The chromatogram condition was optimized. As Figure 1 showed, there was no obvious endogenous interference. The retention time for oxycodone, noroxycodone, and IS were 1.06, 1.01, and 1.23 minutes, respectively.

Clarify the drug interaction profile of oxycodone

Next, we explored drugs that potentially interact with oxycodone. In the RLM incubation system, the K_m value of oxycodone was 101.7±5.40 μM, as shown in Figure 2A. In the HLM incubation system, the K_m value of oxycodone was 529.6±19.60 μM, as shown in Figure 2B. In order to determine the interaction between TKI and oxycodone, 24 TKIs were selected and incubated with oxycodone. As shown in Figure 2C, apatinib and icotinib were the most potent drugs in suppressing the oxycodone metabolizing with the inhibitory rate of 99.33% and 97.11%. Therefore, we selected these two as inhibitors to further study their inhibitory effect on oxycodone metabolism. In Table 1, 2 and Figure 3, We determined the IC_{50} of icotinib/apatinib in RLM and HLM. The results showed that oxycodone metabolizing was dose-dependently inhibited by apatinib in RLM with IC_{50} of 0.95±0.88 μM. Icotinib also inhibited the disposition of oxycodone

with IC_{50} = 3.29±0.09 μM. In HLM, the IC_{50} for apatinib and icotinib were 0.48 ± 0.05 μM and 22.34±0.81 μM, accordingly.

Effects of icotinib/apatinib on oxycodone metabolism in rats

To further evaluate the interaction between icotinib/apatinib and oxycodone in vivo, rats were given icotinib and apatinib with or without oxycodone, respectively. As shown in Figure 4 and Table 3, the $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of oxycodone were increased by 1.4- and 1.2-times in group B compared with the control group, respectively. In addition, the $CL_{z/F}$ was decreased by about 0.7 times. When coadministration of apatinib with oxycodone, the C_{max} of the oxycodone increased 1.5 times compared with the control group.

Icotinib/apatinib inhibit the metabolism of oxycodone with mixed mechanism

Furthermore, as shown in Figure 5, Table 1 and 2, both apatinib and icotinib inhibit oxycodone metabolism underlied mixed mechanism in the RLM. The K_i of apatinib is 0.65 μM, while it's 1.04 μM for icotinib. In HLM, the inhibition mode of apatinib is the same as that in RLM, with K_i = 1.04 μM. While icotinib competitively inhibits the metabolism of oxycodone with K_i = 9.42 μM.

Discussion

Cancer patients often take multiple drugs. The more drug takes, the more possibility drug interaction could happen(Kummer et al. 2011). Oxycodone is widely used in cancer patients, but its adverse effects are often overlooked comparing with prolongation of patients' life. It had been demonstrated that oxycodone is mainly metabolized by CYP3A4 and CYP2D6(Werk & Cascorbi 2014; Zhou 2008). Therefore, the plasma concentrations and tissue distribution of oxycodone may be affected by drug that could inhibit CYP3A4 and/or CYP2D6. Indeed, collective data had identified that many drugs can interact with oxycodone, for example voriconazole and diphenhydramine(Hagelberg et al. 2009; Sadiq et al. 2011).

Tyrosine kinase inhibitors are the most commonly used tumor-targeted drugs nowadays. Herein, we found that icotinib and apatinib can significantly inhibit the metabolism of oxycodone. Previous studies have shown that icotinib has a certain inhibitory effect on CYP3A in vitro(Sun et al. 2021). Moreover, literatures reported that apatinib can inhibit the activities of cytochrome P450 enzymes, resulting in vary pharmacokinetics parameters(Bao et al. 2018a; Zhang et al. 2020). At the present study, we used bioequivalent dose of oxycodone, icotinib and apatinib based on clinical dosage. The results of present study demonstrated that icotinib increases the $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of oxycodone. Furthermore, the $CL_{z/F}$ is decreased by about 0.7 times. In addition, we found apatinib increase the C_{max} of the oxycodone. In vitro, both icotinib and apatinib take certain inhibitory effects on oxycodone metabolizing. In RLM, icotinib and apatinib inhibit the metabolism of oxycodone in a mixed mechanism. In HLM, icotinib is a competitive inhibitor of oxycodone, while apatinib is a mixed inhibitor of oxycodone. Interestingly, the inhibitory potency of them is inconsistence in in vivo and in vitro, especially apatinib. The pharmacokinetics parameters showed that the C_{max} of apatinib is 382.30 ± 46.70 ng/mL (0.96 ± 0.12 μM), which is close to IC_{50} and K_i obtained in RLM. Therefore, it's indicated that the blood exposure of apatinib insufficient to elicit the full DDI response. The doses we used at present study is bioequivalent to

that used in human. Therefore, we believed that the data can help guide the clinic practice. But the species difference between rats and humans may affect IC_{50} and inhibition type.

In summary, this study investigated the interaction between 2 TKIs and oxycodone. In vitro microsomal incubation experiments showed that icotinib and apatinib both have a metabolism inhibition rate of over 80% on oxycodone. Further in vivo experiments revealed that both icotinib and apatinib can significantly alter the pharmacokinetic parameters of oxycodone. Based on the combined in vivo and in vitro results, we have reason to believe that icotinib and apatinib may inhibit the metabolism of oxycodone. However, this study primarily focused on the impact of genetic polymorphisms and drug interactions on hydrocodone metabolism, and thus did not include gender as an additional variable. Therefore, the results of this experiment only apply to male individuals. Overall, our study can provide accurate application basis for the combination of oxycodone and tyrosine kinase inhibitors, such as analgesics.

Conflict of Interest

None.

Ethics statement

The animal study was reviewed and approved by Ethics Committee of Wenzhou Medical University (wydw2020-0322) .

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Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contribution

This work was carried out in collaboration with all authors. Bin Wu, Qi Zhou and Jianchang Qian contributed to the literature search and study design. Bin Wu, Qi Zhou, Jianchang Qian, and Feng Ye participated in drafting of the article. Zhize Ye, Nanyong Gao, Qi Zhou, Xiaoqin Hu, Qihui Kong carried out the experiments and analysis. Feng Ye and Jianchang Qian revised the manuscript. All authors contributed to data analysis, drafting, or revising the article; agreed on the journal to which the article will be submitted; and have agreed to be accountable for all aspects of the work.

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Figure 1

Representative chromatograms of oxycodone, noroxycodone and IS.

(A) The blank plasma sample; (B) The blank plasma sample spiked with oxycodone, noroxycodone and IS; (C) Rat plasma sample after administration.

Figure 1

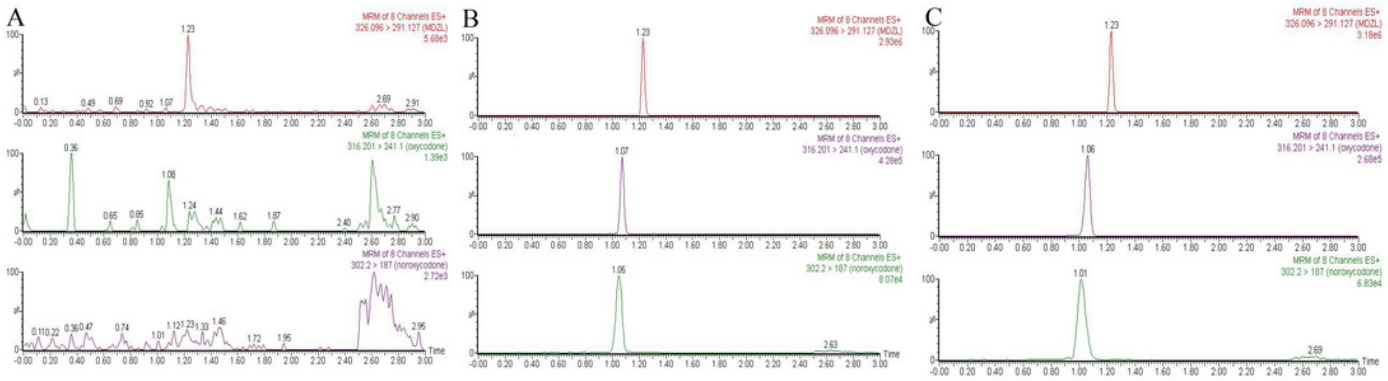

Figure 2

Michaelis constant (K_m) in RLM (rat liver microsomes) / HLM(human liver microsomes) and comparison of the inhibitory effects of drugs on the metabolism of oxycodone in RLM.

(A) K_m in RLM. (B) K_m in HLM. The incubition assay was performed as indicated as in the method. (C) Comparison of the inhibitory effects of drugs on the metabolism of oxycodone in RLM. Data are presented as the means ± SD, n = 3.

Figure 2

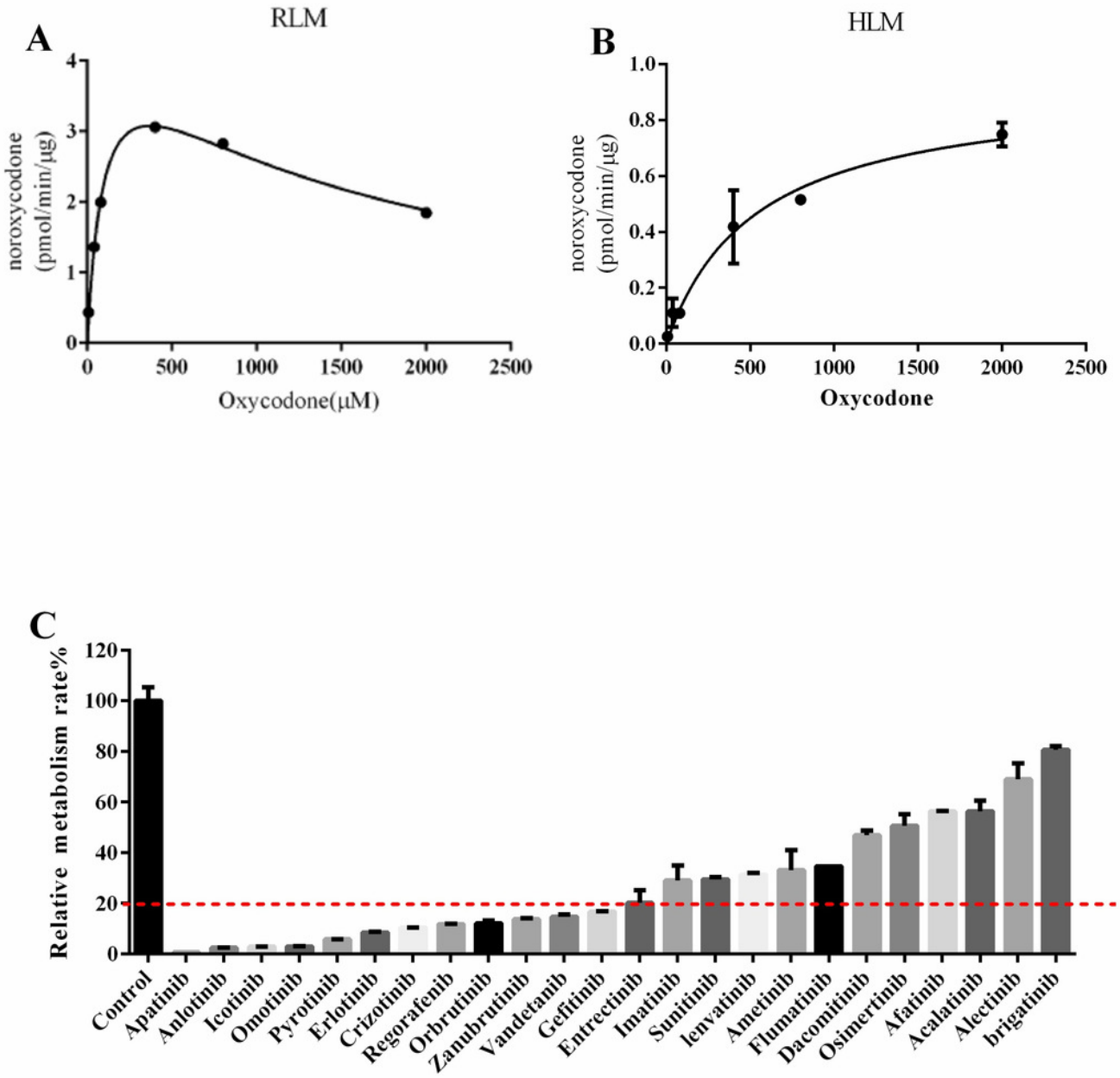
A
RLM
noroxycodone (pmol/min/μg)
Oxycodone(μM)
B
HLM
noroxycodone (pmol/min/μg)
Oxycodone
C
Relative metabolism rate%
Control
Apatinib
Anlotinib
Icotinib
Omotinib
Pyrotinib
Erlotinib
Crizotinib
Regorafenib
Orbrutinib
Zanubrutinib
Vandetanib
Gefitinib
Entrectinib
Imatinib
Sunitinib
lenvatinib
Ametinib
Flumatinib
Dacomitinib
Osimertinib
Afatinib
Acalatinib
Alectinib
brigatinib

Figure 3

Half-maximal inhibitory concentration (IC_{50}) value in RLM / HLM.

(A) Apatinib with various concentrations for IC_{50} in the activity of RLM. (B) Apatinib with various concentrations for IC_{50} in the activity of HLM. (C) Icotinib with various concentrations for IC_{50} in the activity of RLM. (D) Icotinib with various concentrations for IC_{50} in the activity of HLM. Data are presented as the means ± SD, n = 3.

Figure 3

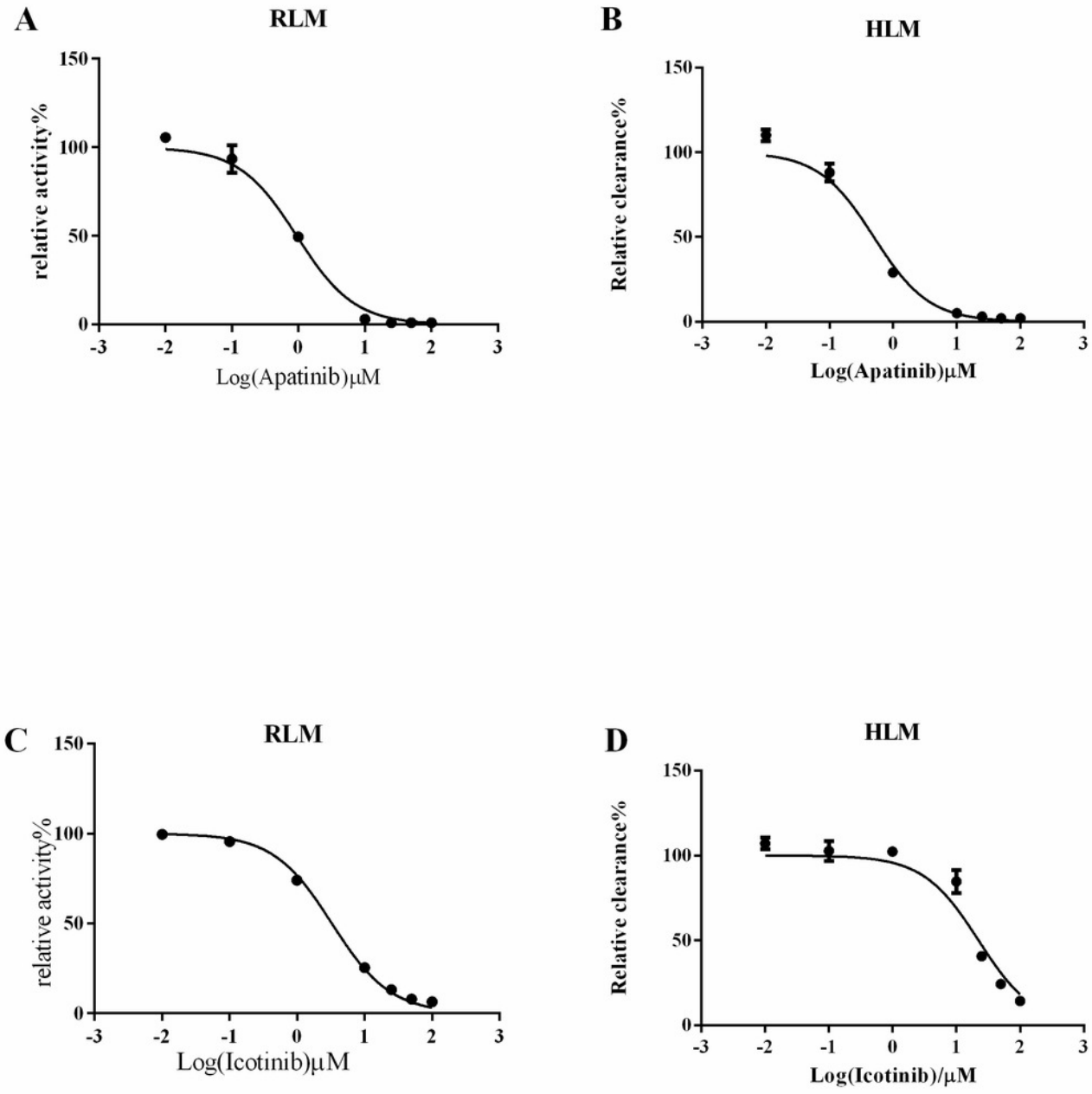
A
RLM
relative activity%
Log(Apatinib)μM
B
HLM
Relative clearance%
Log(Apatinib)μM
C
RLM
relative activity%
Log(Icotinib)μM
D
HLM
Relative clearance%
Log(Icotinib)/μM

Figure 4

The concentration-time curve of oxycodone in the experimental and control groups.

(A) Icotinib and (B) apatinib. Data are presented as the means ± SD, n = 6.

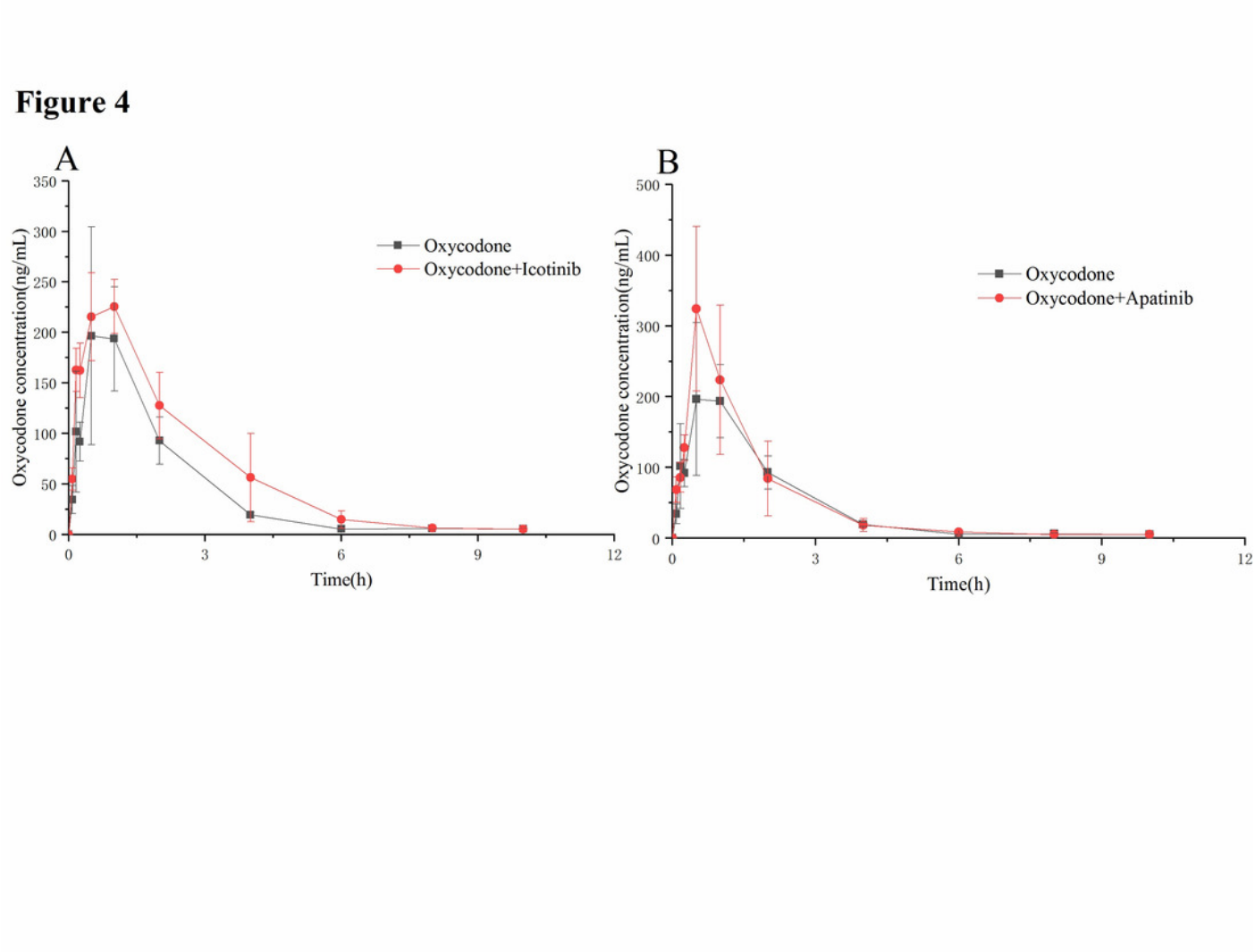

Figure 5

Lineweaver-Burk plot and the secondary plot for K_i in RLM/HLM. Lineweaver-Burk plot and the secondary plot for K_i in the inhibition of oxycodone.

(A) icotinib and (B)apatinib with various concentrations in RLMs. Lineweaver-Burk plot and the secondary plot for K_i in the inhibition of oxycodone metabolism by (C)icotinib and (D)apatinib with various concentrations in HLMs. Data are presented as the means ± SD, n = 3.

Figure 5

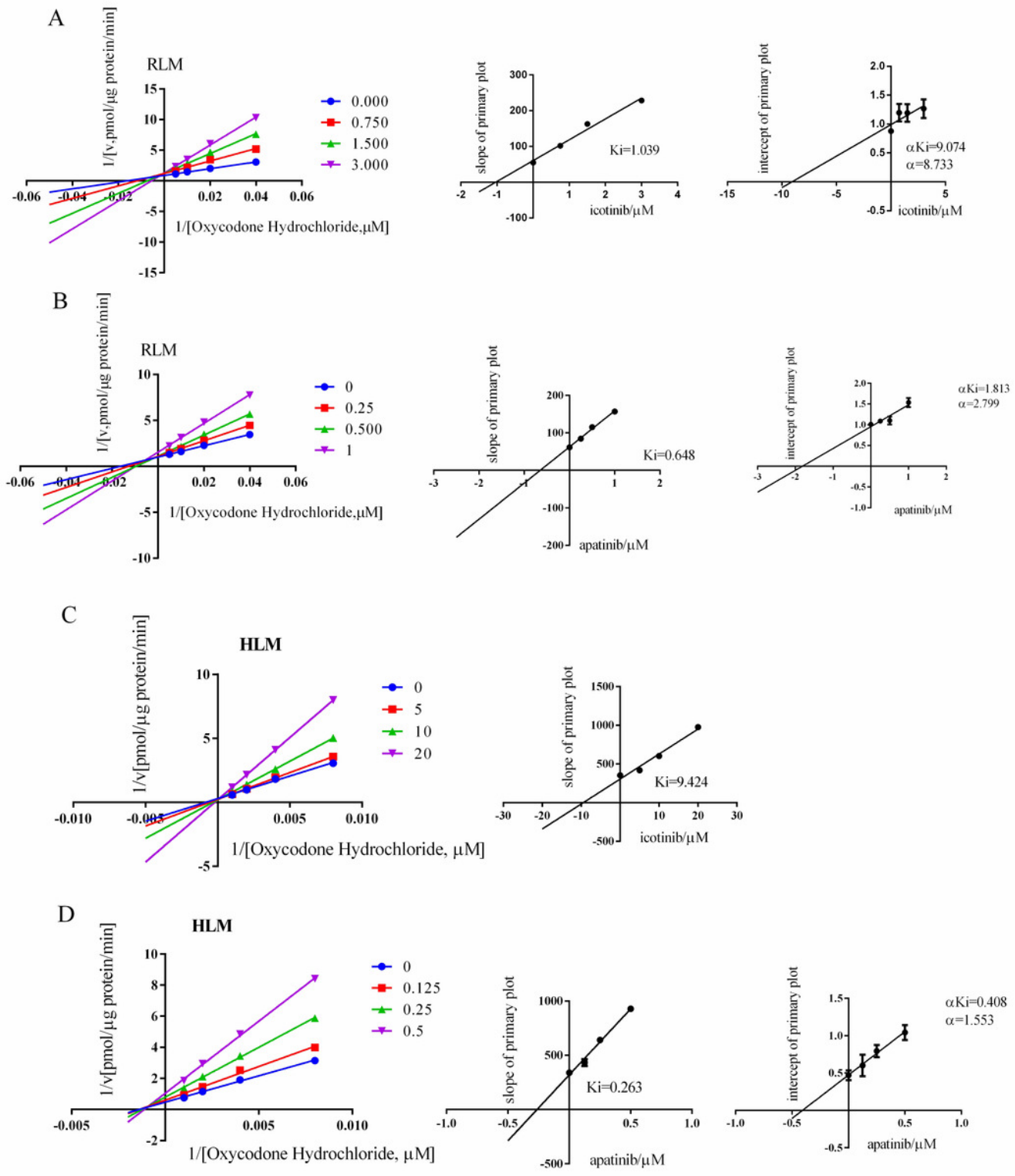
A
RLM
1/[v,pmol/μg protein/min]
1/[Oxycodone Hydrochloride,μM]
0.000
0.750
1.500
3.000
slope of primary plot
Ki=1.039
icotinib/μM
intercept of primary plot
αKi=9.074
α=8.733
icotinib/μM
B
RLM
1/[v,pmol/μg protein/min]
1/[Oxycodone Hydrochloride,μM]
0
0.25
0.500
1
slope of primary plot
Ki=0.648
apatinib/μM
intercept of primary plot
αKi=1.813
α=2.799
apatinib/μM
C
HLM
1/v[pmol/μg protein/min]
1/[Oxycodone Hydrochloride, μM]
0
5
10
20
slope of primary plot
Ki=9.424
icotinib/μM
D
HLM
1/v[pmol/μg protein/min]
1/[Oxycodone Hydrochloride, μM]
0
0.125
0.25
0.5
slope of primary plot
Ki=0.263
apatinib/μM
intercept of primary plot
αKi=0.408
α=1.553
apatinib/μM

Table 1(on next page)

The IC_{50} values and inhibitory effects of apatinib and icotinib on oxycodone in RLMs.

Table 1. The IC_{50} values and inhibitory effects of apatinib and icotinib on oxycodone in RLMs.

RLM	IC_{50} (μM)	Inhibition type	K_i(μM)	αK_i(μM)	α
Apatinib	0.95±0.88	Mixed inhibition	0.65	1.81	2.71
Icotinib	3.29±0.09	Mixed inhibition	1.04	9.07	8.73

Table 2(on next page)

The IC_{50} values and inhibitory effects of apatinib and icotinib on oxycodone metabolism in HLMs.

Table 2. The IC_{50} values and inhibitory effects of apatinib and icotinib on oxycodone metabolism in HLMs.

HLM	IC_{50} values(μM)	Inhibition type	K_i(μM)	$αK_i$(μM)	α
Apatinib	0.48±0.05	Mixed inhibition	1.04	0.41	0.26
Icotinib	22.34±0.81	Competitive inhibition	9.42		

Table 3(on next page)

Pharmacokinetic parameters of oxycodone in three groups.

Table 3. Pharmacokinetic parameters of oxycodone in three groups.

		control	oxycodone+apatinib	oxycodone+icotinib
$AUC_{(0-t)}$	ug/L*h	452.10±88.11	521.20±114.10	647.00±167.50*
$AUC_{(0-\infty)}$	ug/L*h	467.30±83.00	543.40±110.30	658.60±156.10*
$MRT_{(0-t)}$	h	1.87±0.19	1.69±0.23	2.08±0.33
$MRT_{(0-\infty)}$	h	2.31±0.46	2.29±0.43	2.38±0.39
$t_{1/2z}$	h	2.66±0.55	3.32±1.29	2.04±1.38
T_{max}	h	0.67±0.26	0.58±0.20	0.83±0.26
$V_{z/F}$	L/kg	25.91±9.22	27.80±12.93	15.79±14.25
$CL_{z/F}$	L/h/kg	6.60±1.22	5.69±1.04	4.77±1.12*
C_{max}	ug/L	231.80±56.22	350.20±86.25*	239.00±33.98

Note: Compared to control group, *$P < 0.05$.