Evaluation of the Effects of Apatinib on the Pharmacokinetics of Tramadol and O-desmethyltramadol

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Abstract

Since the combination of anticancer drugs and opioids is very common, apatinib and tramadol are likely to be used in combination clinically. Based on this, it is particularly important to explore the effect of apatinib on the metabolism of tramadol. This study evaluated the effects of apatinib on the pharmacokinetics of tramadol and its main metabolite o-desmethyltramadol in SD rats and the inhibitory effects of apatinib on tramadol in rat liver microsomes (RLMs), human liver microsomes (HLMs) and recombinant human CYP2D6.1. The samples were determined by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). The in vivo results showed that compared with the control group, apatinib increased the AUC(0-t), AUC(0-[?]) and Cmax values of tramadol and O-desmethyltramadol, and decreased the values of VZ/F and CLz/F. In addition, the MRT(0-t), MRT(0-[?]) values of O-desmethyltramadol were increased. In vitro, apatinib inhibited the metabolism of tramadol by a mixed way with IC50 of 1.927μM in RLMs, 2.039μM in HLMs and 15.32μM in CYP2D6.1.

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT :

Clinically, it is very likely that apatinib and tramadol can be used in combination. In addition, a previous study found that apatinib can inhibit CYP2D6, CYP2C9, CYP3A4 and CYP2B6, tramadol is mainly metabolized by CYP2D6. This suggests that there may be an interaction between apatinib and tramadol.

WHAT THIS STUDY ADDS :

This study determined the effect of apatinib on the pharmacokinetics of tramadol and o-desmethyl tramadol in rats. The effects and mechanisms of apatinib on tramadol in human and rat liver microsomes (RLM and HLM) and recombinant human CYP2D6.1 were identified.

Abstract

Since the combination of anticancer drugs and opioids is very common, apatinib and tramadol are likely to be used in combination clinically. Based on this, it is particularly important to explore the effect of apatinib on the metabolism of tramadol. This study evaluated the effects of apatinib on the pharmacokinetics of tramadol and its main metabolite o-desmethyltramadol in SD rats and the inhibitory effects of apatinib on tramadol in rat liver microsomes (RLMs), human liver microsomes (HLMs) and recombinant human CYP2D6.1. The samples were determined by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). The in vivo results showed that compared with the control group, apatinib increased the $AUC_{(0\text{-}t)}$, $AUC_{(0\text{-}[?])}$ and C_{max} values of tramadol and O-desmethyltramadol, and decreased the values of V_Z/F and CLz/F. In addition, the $MRT_{(0\text{-}t)}$, $MRT_{(0\text{-}[?])}$ values of O-desmethyltramadol were increased. In vitro, apatinib inhibited the metabolism of tramadol by a mixed way with IC_{50} of 1.927μM in RLMs, 2.039μM in HLMs and 15.32μM in CYP2D6.1.

Key words: Apatinib; tramadol; O-desmethyltramadol; Drug Inhibition; Pharmacokinetics; Metabolism;

Introduction and Background

Tramadol is a centrally acting, fully synthetic opioid with an atypical mechanism of action, because it is not only a μ-opioid receptor agonist, but also a serotonin and norepinephrine reuptake inhibitor[1]. It was first synthesized in 1962 and it was made available to the foreign markets under the name Tramal for pain treatment in 1977[2]. Until 1995, it was approved by the US Food and Drug Administration for the treatment of moderate to moderately severe pain in adults[3]. In 2013, tramadol ranked second in total U.S. opioid market sales, accounting for 14.7%[4]. Typical side effects caused by opioids include gastrointestinal reactions (nausea, vomiting, constipation, etc.), itching, dizziness, hypogonadism, sleep disturbance, inattention, respiratory depression, etc. In addition to this, there are other effects related to tolerance, dependence, and addiction[5-8]. Tramadol is generally considered to be a "weak opioid" (its agonistic effect on μ-opioid receptors is only one-fourth to one-tenth of that of morphine), which makes it mistaken for better security[3]. However, due to the inhibition of serotonin and norepinephrine reuptake, tramadol has additional risks in addition to the side effects of opioids, including: epilepsy, tachycardia, serotonin syndrome, hypertension, and reports of mania[9, 10].

The metabolism of tramadol is very complex. At present, 23 different metabolites (active and inactive) of tramadol have been identified in humans[2, 11]. Among them, O-desmethyltramadol is the main active metabolite, and tramadol selectively activates μ opioid receptors through it. The affinity of O-desmethyltramadol is 700 times higher than its parent compound tramadol and 5.5 times lower than morphine[12]. Tramadol is metabolized to O-desmethyltramadol by CYP2D6, and to N-desmethyltramadol by CYP 3A4 and 2B6. N-desmethyltramadol is pharmacologically inactive[2, 13]. Therefore, changes in CYP450 will affect the metabolism of tramadol and the accumulation of metabolites.

Apatinib (Aitan, brand name in China) is a small molecule tyrosine kinase inhibitor, independently developed by China and approved for the subsequent-line treatment of advanced gastric or gastroesophageal junction adenocarcinoma[14]. As one of the latest oral anti-angiogenic drugs, apatinib has been applied to various types of malignant tumors, such as breast cancer, non-small cell carcinoma and epithelial ovarian cancer etc., and has obvious survival benefits and tolerable toxicity[14-19]. Cancer and pain are inseparable. Moderate to severe pain is common in cancer patients, affecting 70-80% of patients with advanced disease[20]. Opioids are still the basic means to control cancer-related pain and are promoted in international guidelines[21]. It

seems that the combination of anticancer drugs and opioids is very common. Therefore, it is very likely that apatinib and tramadol can be combined clinically. In addition, a previous study found that apatinib can inhibit CYP2D6, CYP2C9, CYP3A4 and CYP2B6[22], and tramadol is mainly metabolized by CYP2D6. This suggests there may be an interaction between apatinib and tramadol.

In this study, in vivo, we determined the effect of apatinib on the pharmacokinetics of tramadol and o-desmethyltramadol in rats. In vitro, we identified the effect and mechanism of apatinib on tramadol in human and rat liver microsomes (RLM and HLM) as well as recombinant human CYP2D6.1.

2.Materials and Methods

2.1. Chemical and reagents

Tramadol, O-desmethyltramadol, apatinib and midazolam (used as internal standard, IS) were bought from Shanghai Canspec Scientific & Technology Co., Ltd. LC–MS grade acetonitrile (ACN) and methanol were purchased from Merck (Darmstadt, Germany). Formic acid of HPLC grade (FA, purity 99.9%) was obtained from J&K scientific Ltd. (Shanghai, China). The reduced nicotinamide adenine dinucleotide phosphate (NADPH) was purchased from Roche Pharmaceutical Ltd. (Basel, Switzerland). Carboxymethylcellulose sodium salt (CMC-Na) was from Sigma-Aldrich Company (Shanghai, China). Pooled RLM and HLM were bought from Corning Life Sciences Co., Ltd. Recombinant human CYP2D6.1 and cytochrome b5 were kind gifts from Beijing Hospital (Beijing, China).

2.2. Equipment and operation conditions

The concentration of tramadol and O-desmethyltramadol was determined by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Mass spectrometer contained an Acquity UPLC XEVO TQD triple quadrupole (Waters Corp., Milford, MA) and an electrospray ionization source. The chromatographic separation was performed on a Waters ACQUITY UPLC BEH C18 column (2.1×50 mm, 1.7μm, Waters Corp.) at 40°C. The transitions were m/z 264.2-58.0 for tramadol, m/z 250.2-58.2 for O-desmethyltramadol, m/z 326.1-291.1 for IS. The mobile phase consisted of solvent A (0.1% formic acid) and solvent B (ACN) with gradient elution at a flow rate of 0.4 mL/min, total run time was 2 min. A gradient elution program was as follows: 0–1.4 min, 60%–10% A, 1.4–2.6 min, 10%–60% A.

2.3. In vitro experiments

The 200μL incubation system contained 1.6μL apatinib, 3.0μL tramadol, 5μL Rat liver microsomes (RLM) or Human liver microsomes (HLM) or 5μL CYPD6.1 (along with 3.7μL b5), and10μL NADPH (1mM), and180.4μL 1M potassium phosphate buffer (PBS)for HLM and RLM, 176.7μL PBS for CYP2D6.1. In the experiment of IC50 determination, the concentration of apatinib was designed at 0.01, 0.1, 1, 10, 50 and 100μM, while that of tramadol was 80μM for RLM, 50 μM for HLM, 40 μM for CYP2D6.1, which were close to their Km value correspondingly. In the experiment of the inhibitory effect of apatinib on tramadol, the concentration gradient of apatinib (0-32μM) and tramadol (10-160μM) was set according to the IC50 value and the Km value. The incubation system was carried out at 37°C, and after incubating for 30 minutes, it was cooled to -80°C. Then 400 μL ACN and 20 μL IS (20ng mL^{-1}) were added to the mixture. After vortex mixing for 2 minutes and high-speed centrifugation at 13000 rpm for 10 minutes, the supernatant was taken for testing.

2.3. In Vivo Experiments

12 male Sprague–Dawley (SD) rats purchased from the Shanghai Animal Experimental Center were randomly divided into 2 groups (n=6): group A was apatinib group (taking apatinib with tramadol), group B was the control group (taking tramadol alone). Before the formal experiment, the 12 rats were fasted for 12 hours. At the beginning of the experiment, the group A was given 40mg kg^{-1} apatinib orally, and the group B was given the same volume of 0.5% CMC-Na. After 30 minutes, both groups were given 20 mg kg^{-1} tramadol.

The time points of blood collection from the tail vein of rats were 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hours after administration of tramadol.

The above blood was centrifuged at 13,000 rpm for 10 minutes to obtain the required plasma. 100μL of the collected plasma was taken and mixed with 20μL IS (300ng mL^{-1}) and 200μL ACN in a 1.5mL centrifuge tube. After being vortexed for 2 minutes and centrifuged for 10 minutes, the supernatant was diluted with pure water (1:1) for UPLC-MS/MS analysis.

2.4. Statistical Analysis

IC_{50} and Lineweaver-Burk Plot are calculated by GraphPad Prism 5.0. The average concentration-time curve is drawn by Origin 8.0. The pharmacokinetic parameters were evaluated by DAS software (version 3.0), using non-compartmental analysis. The statistical analysis of all data is expressed as mean ± standard deviation and analyzed by SPSS 19.0. $P<0.05$ represents statistical significance.

3.Results

3.1. UPLC-MS/MS

The correlation coefficients of the calibration curves of tramadol and o-desmethyltramadol were both greater than 0.99. Tramadol is used in a concentration range of 0.25 to 500 ng mL^{-1}, and o-desmethyltramadol is used in a concentration range of 1 to 1000 ng mL^{-1}. The chromatograms of tramadol, O-desmethyltramadol and IS under different conditions are shown in Figure 1.

3.2. Effects of apatinib on the metabolism of tramadol in vitro

The IC_{50} curve and Lineweaver-Burk Plot of apatinib on tramadol in HLM, RLM and CYP2D6.1 are shown in Figure 2 and Figure 3, respectively, and the corresponding values are shown in Table 1. According to Table 1, The IC_{50} values of RLM and HLM are very close (Table 1). The inhibitory strength of apatinib on tramadol is greater in RLM and HLM than in CYP2D6.1. The results indicate that apatinib inhibits tramadol in a mixed way.

3.3. Effects of apatinib on the metabolism of tramadol in vivo

The average plasma concentration-time curves of tramadol and O-desmethyltramadol and their corresponding pharmacokinetic parameters are shown in Figure 4 and Table 2 and 3. Compared with group B (control group), the $AUC_{(0\text{-}t)}$, $AUC_{(0\text{-}[?])}$ and C_{max} values of tramadol and O-desmethyltramadol increased, while V_Z/F and CLz/F decreased. In addition, the $MRT_{(0\text{-}t)}$, $MRT_{(0\text{-}[?])}$ values of O-desmethyltramadol increased. Other parameters have no significant difference.

4. Discussion

Tramadol is mainly metabolized by CYP2D6, CYP3A4 and CYP2B6. Since the phase I metabolic reaction mediated by CYP450 is slower than the phase II binding reaction, they become the rate-limiter for the overall metabolic disposal of CYP substrate drugs[4]. CYP enzymes are easily induced and inhibited by other substrates, which means that the plasma concentration and tissue distribution of tramadol and its main metabolite o-desmethyltramadol may be affected by drugs that affect the activities of CYP2D6, CYP3A4 and CYP2B6. Since apatinib is metabolized by CYP3A4/5, CYP2D6, CYP2C9 and CYP2E1[23], sharing two CYP metabolic pathways with tramadol, and the fact that tramadol and apatinib are likely to be combined clinically, whether apatinib affects the metabolism of tramadol is worth studying.

The results of in vivo experiments show that apatinib does influence the metabolism of tramadol. Compared with the control group, the $AUC_{(0\text{-}t)}$, $AUC_{(0\text{-}[?])}$ and C_{max} of tramadol increased, Vz/F and CLz/F decreased, which proves that apatinib inhibits the metabolism of tramadol in rats, increasing the side effects of tramadol. The inhibitory effect of tramadol on the neuronal reuptake of norepinephrine and 5-HT increases its side effect compared with other opioids[24]. This in vivo result corresponds to the subsequent in vitro result.

Apatinib strongly inhibited the metabolism of tramadol by a mixed way with IC_{50} <5 μM in RLMs. The results in HLMs are close to those in RLMs, indicating that the effects of apatinib on the metabolism of tramadol in rat can be analogized to humans to a certain extent. Studies have pointed out that tramadol is metabolized much faster in animals than in humans: 1% and 25-30% of the oral dose are excreted in urine as prototypes respectively[2, 25]. This also implies that when apatinib and tramadol are used in combination in humans, the accumulation of tramadol is more serious than in rats.

Although CYP2D6 only accounts for 2-4% of all liver CYP enzymes, it metabolizes about 25% of clinically used drugs, and about 80% of tramadol is metabolized by CYP2D6[4, 26-28]. Based on this, we studied the potential inhibitory effects of apatinib on tramadol in CYP2D6.1. Apatinib inhibits tramadol by a mixed way in CYP2D6.1, suggesting the complexity of the inhibition way. The inhibitory intensity of apatinib in 2D6.1 is much smaller than that of RLM and HLM, the reason may be that the inhibition of tramadol by apatinib in vitro is not only through CYP2D6. Studies have shown that apatinib can strongly inhibit CYP3A4 and CYP2B6[22] while the N-demethylation process of tramadol is through these two enzymes[29]. Therefore, apatinib may also inhibit the metabolism of tramadol through CYP3A4 and CYP2B6.

In addition, compared with the control group, the exposure of O-desmethyltramadol to $AUC_{(0\text{-}t)}$, $AUC_{(0\text{-}[?])}$, $MRT_{(0\text{-}t)}$, $MRT_{(0\text{-}[?])}$ and C_{max} was increased significantly, This may be because apatinib significantly reduced tramadol's first pass elimination,which greatly increased the amount of absorption. Besides, O-desmethyltramadol will subsequently be metabolized into N,O-didesmethyl-tramadol through CYP3A4 and CYP2B6[30], and this process may be hindered by apatinib[22]. Since O-desmethyltramadol is the main active metabolite, and its affinity is 700 times higher than that of its parent compound, exaggerated effects or even opioid intoxication may occur.

In conclusion, based on our research results, apatinib can enhance the analgesic effect of o-desmethyltramadol, but also greatly increase the toxic and side effects caused by the accumulation of tramadol and o-desmethyltramadol. In order to avoid the risk of greatly increased adverse reactions, we do not recommend simultaneous administration of apatinib and tramadol clinically. Due to the high probability of combined application of apatinib and tramadol in cancer patients, our study provides a contribution to the rational use of drugs in this regard.

5.Acknowledgments

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6. Conflict of interest statement

The authors report no conflicts of interest in this work.

7. Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Table.1. The IC50 values and inhibitory effects of apatinib on tramadol in HLMs, RLMs and CYP2D6.1

	IC50 values (μM)	Inhibition type	Ki(μM)	αKi(μM)	α
RLMs	1.927	mixed	0.801	3.952	4.934
HLMs	2.039	mixed	2.672	13.45	5.034
CYP2D6.1	15.32	mixed	28.38	64.25	2.264

Table.2. The main pharmacokinetic parameters of tramadol in 2 groups (n =6)

Parameters	Group A	Group B
AUC(0-t) (ug/L*h)	7273.187±2680.883**	710.148±127.326
AUC(0-[?]) (ug/L*h)	7278.701±2683.291**	724.84±121.467
MRT(0-t) (h)	3.6±0.377	3.389±0.853
MRT(0-[?]) (h)	3.617±0.367	4.293±1.619
t1/2z (h)	2.32±1.072	7.554±7.124
Tmax (h)	1.5±0.548	1.792±1.346
Vz/F (L/kg)	9.824±4.61*	324.58±313.568

Parameters	Group A	Group B
CLz/F (L/h/kg)	3.076±1.151**	28.327±5.318
Cmax (ug/L)	1555.169±450.828**	162.063±50.829

*p < 0.05, **P<0.005 in comparison with group B.

Table.3. The main pharmacokinetic parameters of O-desmethyltramadol in 2 groups (n =6)

Parameters	Group A	Group B
AUC(0-t) (ug/L*h)	755.152±208.119**	246.079±56.316
AUC(0-[?]) (ug/L*h)	790.146±187.052**	281.735±72.841
MRT(0-t) (h)	7.172±0.693**	5.289±0.461
MRT(0-[?]) (h)	7.776±0.409*	6.603±0.896
t1/2z (h)	3.159±0.768	2.925±0.593
Tmax (h)	6.333±0.816	4.125±2.906
Vz/F (L/kg)	117.125±23.395**	309.179±58.566
CLz/F (L/h/kg)	26.373±5.473**	77.049±28.893
Cmax (ug/L)	97.216±23.528**	34.456±8.107

*p < 0.05, **P<0.005 in comparison with group B.

Fig.1 UPLC-MS/MS chromatographs of tramadol, O-desmethyltramadol and IS (midazolam). (A) Blank plasma sample. (B) Blank plasma spiked with 100 ng mL^{-1}tramadol, 25 ng mL^{-1} O-desmethyltramadol and 300 ng mL^{-1} IS. (C) Apatinib-treated rat plasma sample at 4h after tramadol.

Abbreviations: TIC, total ionic chromatography.

Fig.2. Apatinib with various concentrations for half-maximal inhibitory concentration (IC_{50}) in the activity of (A) RLM, (B) HLM and (C) CYP2D6.1. Values are the mean ± SD, N = 3.

Fig.3. Primary Lineweaver–Burk plot, the secondary plot for Ki and the secondary plot for αKi in the inhibition of the metabolism of tramadol by various concentrations of apatinib in (A) RLM, (B) HLM and (C) CYP2D6.1. Values are mean ± SD, N = 3.

Fig.4. Mean concentration-time curve of tramadol and O-desmethyltramadol in the control group (tramadol alone) and the apatinib group (tramadol with apatinib) (N = 6).