# Effect of moxifloxacin on pharmacokinetics of enalapril in rats

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**Abstract.** The purpose of this study was to investigate the effect of moxifloxacin on the pharmacokinetics of enalapril. Single administration experiment: 12 Wistar rats were randomly divided into two groups on average. Control group: enalapril (15mg/kg)+ normal saline combined group: enalapril (15mg/kg)+ moxifloxacin (80mg/kg), intragastric administration. Blood samples were measured by LC-MS/MS. Multiple administration experiment: 12 Wistar rats were randomly divided into two groups on average. The control group was given normal saline (day1-7)+ enalapril (day8,15mg/kg), and the combination group was given moxifloxacin (day1-7, 80mg/kg)+ enalapril (day8,15mg/kg) by intra-gastric administration. The treatments were the same as above. Pharmacokinetics parameters were calculated using Winnonlin 6.1 software and the two groups were compared using SPSS18.0 software. In single administration experiment compared with the control group, the $AUC_{(0-t)}$ and $C_{max}$ of enalapril were decreased by 31.99%（$P<0.05$）、42.57%（$P<0.05$）in the combined administration group. Quinolone antibiotics and ACEI drugs should not be taken at the same time during clinical treatment to avoid drug interactions that may occur when combined drugs are used.

## 1. Introduction

Angiotensin converting enzyme inhibitor (ACEI) is a class of antihypertensive drugs acting on renin-angiotensin-aldosterone system, which has a good positive effect on heart failure, angina pectoris, myocardial infarction at all levels, etc. ACEI drugs are generally administered by oral administration, and their absorption in the gastrointestinal tract is an important factor determining their bioavailability. Many studies have shown [1,2]that the absorption of drugs in the intestinal tract is not only through simple passive diffusion, but also may require the participation of uptake transporters. Among them, oligopeptide transporter 1(PEPT1) plays a very important role in the absorption of drugs due to the universality of its substrate. PEPT1 belongs to the proton-dependent oligopeptide transporters (POT) family, and is mainly expressed on the top side of the membranous base of the small intestinal epithelial cells. It can widely recognize molecules with oligopeptides and similar structures [3], such as ACEI drugs, peptid-like drugs, β-lactam antibiotics, immune modulators, etc., to promote the intestinal absorption of substrates [4]. Due to the high substrate tolerance and extensive substrate specificity of intestinal PEPT1, it becomes an important target molecule in drug delivery system. The prodrug is designed as a dipeptide and tripeptide analogs, and it is transported and absorbed through the PEPT1 carrier existing on the brush edge of intestinal tract, so as to improve the bioavailability of oral drugs. The antiviral drug valaciclovir is the most successful PEPT1-targeting prodrug currently on the market[5]. At present, studies on the uptake transporter PEPT1 mostly focus on the design and development of prodrugs, but less attention is paid to drug interactions. Hiroshiarakawa et al.[6]found that quinolones could inhibit the transport capacity of PEPT1 and significantly reduce the absorption of phenylalanine-(CN-S)-alanine (Phe-Ala), the classical substrate of PEPT1, in the intestine. In cell experiments, the absorption of Phe-Ala in lomefloxacin group and moxifloxacin group decreased by 72.4% and 63.2%, respectively. Compared with the control group (244±9 ng/ml), the peak plasma concentration ($C_{max}$) of Phe-Ala in moxifloxacin combination group was significantly decreased (171±1 ng/ml). This suggests that quinolones may affect the absorption of PEPT1 substrates in the intestinal tract, thereby increasing the potential risk of drug interactions.

During the treatment of cardiovascular diseases with ACEI drugs, the abnormal fluctuation of blood drug concentration caused by drug interaction will affect the safety and effectiveness of drug use in patients, especially for elderly patients taking drugs for a long time, which may induce adverse cardiovascular events and cause serious consequences. As a second-generation angiotensin converting enzyme-inhibitor, enalapril has been used early and widely in clinical practice. Enalapril is a precursor drug, and its active form enalapril has less than 10% absorption rate in the gastrointestinal tract. After enalapril is modified into monoethyl ester form, the gastrointestinal tract absorption rate increases to 60-70%[7]. After oral administration of enalapril, it is rapidly and completely hydrolyzed to enalaprilat in the

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liver, which can inhibit ACE, reduce angiotensin content and play a hypotensive role [8]. In this study, the effects of moxifloxacin, a quinolone, on the pharmacokinetics of enalapil, an ACEI drug, were investigated through animal experiments, and the mechanism of interaction between quinolones and ACEI drugs was discussed, so as to provide reference for clinical safe and rational drug use.

## 2. Materials and Methods

### 2.1 Material

#### *2.1.1 Chemicals Materials*

Enalapril maleate API (mass fraction 99%, Shijiazhuang Pharmaceutical Group), moxifixacin (mass fraction 99%, Chengdu Zhengkang Pharmaceutical Co. Ltd.), Enalapril Reference, Benalapril Reference (purity 99.2%, China Pharmaceutical and Biological Products Control Institute), Methanol (chromatographic pure, Dikma Company, USA).

#### *2.1.2 The instrument*

Agilent1260 high performance liquid chromatograph, Agilent6460 triple quadrupole mass spectrometer (equipped with electrospray ion source,Masshunter analysis operating software); Low temperature high speed centrifuge (USA Thermo Fay); XW-80A Whirlpool Mixer (Shanghai Medical University Instrument Factory); LT-224S Electronic Balance (Sedoris, Germany).

#### *2.1.3 Animals*

Twenty-four Wistar male rats, clean grade, body weight 200±20g, were obtained from the Experimental Animal Center of Lanzhou University, Certificate No.: SCXK (Gan 2013-04). The animals were housed at 25℃ and a relative humidity of 50±5%. A 12h light and 12h dark cycle was maintained with animals given free access to food and water.

### 2.2 Methods

#### *2.2.1 UPLC–MS/MS Conditions*

Liquid chromatography was performed on an UPLC unit(Waters Corp., Milford, MA, USA) with an Waters-$C_{18}$（100 mm×4.6 mm，2.7μm）. A gradient elution program was conducted for chromatographic separation with mobile phase A (25% methanol), and mobile phase B (75%water, 0.1% methanoic acid). The flow rate was 1.00 mL/min. Agilent6460 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source, was used for mass spectrometric detection. The mass spectrometer was operated in the positive-ion detection mode. The multiple reaction monitoring transitions were m/z 377.4→234.2 for enalapril, m/z 349.2→206.0 for enalaprilat，m/z 425.1→351.7 for benazepril.

#### *2.2.2 Preparation of reference solution*

160μg•$mL^{-1}$ benazepril internal standard reserve solution was prepared with methanol, and then diluted with methanol internal standard reserve solution to 16μg•$mL^{-1}$ internal standard working solution for use. 160μg•$mL^{-1}$ enalapril maleate reference working solution was prepared by dissolving in internal standard working solution and stored at 4℃ for reserve.

#### *2.2.3 Pharmacokinetic Study*

Single administration experiment in rats: 12 male Wistar rats were randomly divided into two groups, with 6 rats in each group. Control group: enalapril (15mg/kg)+ normal saline, combined group: enalapril (15mg/kg)+ moxifloxacin (80mg/kg), intragastric administration, the volume of administration in each group were equal. Blood samples were collected from the femoral artery 15, 30, 60, 90, 120, 180, 240, 360 and 600min after enalapril administration, about 0.2 mL were collected. At the same time, equal volume of normal saline was added. The blood samples were immediately transferred into a 1.5ml centrifuge tube coated with heparin and centrifuged for 10 000r/min, plasma 40μl was taken, internal standard solution 160μl was added, vortex oscillation 40s, centrifugation 10 000 r/min for 8 min to remove protein, and 10 μl was taken for sample analysis.

Multiple administration experiment in rats: 12 male Wistar rats were randomly divided into two groups, 6 rats in each group. The control group was given normal saline (day1-7d)+ enalapril (day8d,15mg/kg), and the combination group was given moxifloxacin (day1-7d, 80mg/kg)+ enalapril (day8d,15mg/kg) by intra-gastric administration, with the same dose volume in each group. Blood samples were collected from the femoral artery 15, 30, 60, 90, 120, 180, 240, 360 and 600min after enalapril administration on day 8, and the other treatments were the same as above.

#### *2.2.4 Data analysis*

Blood concentration data of enalapril and enalaprilat were processed by Winnonlin 6.1 statistical software, and non-atrioventricular model was used. SPSS13.0 software was used for statistical analysis of the data, which was expressed as $\bar{x}\pm s$. According to the analysis of variance, $P<0.05$ was considered statistically significant.

## 3. Results

### 3.1 Single administration experiment in rats

In the experimental group, the pharmacokinetic parameters of rats in the control group and the combined

administration group were statistically analyzed. Pharmacokinetic parameters of enalapril were shown in Table 1, and mean plasma concentration–time curve was shown in Figure 1. Compared with the control group, the $AUC_{(0-t)}$ and $C_{max}$ of enalapril were decreased by 31.99%（P＜0.05，42.57%（P＜0.05） in the combined administration group.

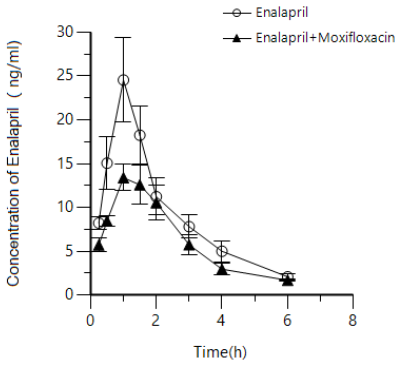


**Fig.1.**Mean plasma concentration–time curve of enalapril in rats after oral a single administration of 15.0 mg/kg enalapril alone or in combination with 80.0 mg/kg moxifloxacin （n=6）

**Table 1.** Pharmacokinetic parameters of enalapril in rats after a single dose（n=6）

| Pharmacokinetic parameter | Enalapril | Enalapril+Moxifloxacin |
|---|---|---|
| $AUC_{(0-t)}$( ng.mL.h$^{-1}$) | 54.93±7.67 | 37.36±3.52* |
| $C_{max}$（ng/ml） | 24.97±3.97 | 14.34±0.57* |
| $t_{max}$（h） | 0.88±0.25 | 1.25±0.29* |
| $t_{1/2}$（h） | 1.51±0.17 | 1.698±0.31 |

*Indicates a significant difference from the control ($p < 0.05$) by Student's t-test.

Pharmacokinetic parameters of enalaprilat were shown in Table 2, and mean plasma concentration-time curve was shown in Figure 2. Compared with the control group, the $AUC_{(0-t)}$ and $C_{max}$ of enalapril were decreased by 12.82%（P＜0.05）, 44.68%（P＜0.05）in the combined administration group.

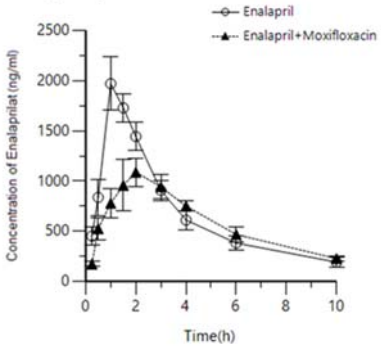


**Fig.2.** Mean plasma concentration-time curve of enalaprilat in rats after oral administration of 15.0 mg/kg enalapril alone or in combination with multiple doses of 80.0 mg/kg moxifloxacin （n=6）

**Table 2.** Pharmacokinetic parameters of enalaprilat in rats after a single dose（n=6）

| Pharmacokinetic parameter | Enalapril | Enalapril +Moxifloxacin |
|---|---|---|
| $AUC_{(0-t)}$ ( ng.mL.h$^{-1}$) | 6723.40±784.61 | 5861.56±770.44* |
| $C_{max}$（ng/ml） | 2011.25±204.63 | 1112.57±168.975* |
| $t_{max}$（h） | 1.13±0.25 | 1.88±0.25* |
| $t_{1/2}$（h） | 3.59±0.36 | 3.46±0.03 |

*Indicates a significant difference from the control ($p < 0.05$) by Student's t-test.

### 3.2 Multiple administration experiment in rats

After 7 days of moxifloxacin administration, the pharmacokinetic parameters of rats in the control group and the combined administration group were statistically analyzed. Pharmacokinetic parameters of enalapril were shown in Table 3, and mean plasma concentration–time curve was shown in Figure 3. Compared with the control group, the $AUC_{(0-t)}$ and $C_{max}$ of enalapril were decreased by 3.01%（P＞0.05）, 4.63%（P＞0.05）in the combined administration group.

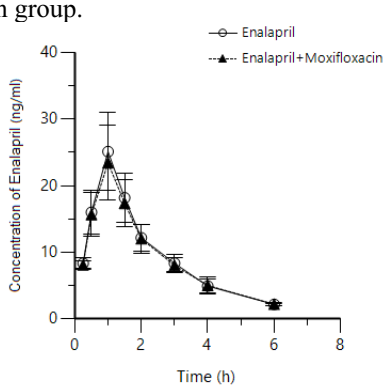


**Fig.3**. Seven days after moxifloxacin（80.0 mg/kg）was given, Mean plasma concentration-time curve of enalapril in rats after oral administration of 15.0 mg/kg enalapril alone or in combination with moxifloxacin （n=6）

**Table 3**. Pharmacokinetic parameters of enalapril 7 days after moxifloxacin administration

| Pharmacokinetic parameter | Enalapril | Enalapril +Moxifloxacin |
|---|---|---|
| $AUC_{(0-t)}$( ng.mL.h$^{-1}$) | 56.78±7.92 | 55.07±8.06 |
| $C_{max}$（ng/ml） | 25.92±4.4 | 24.72±3.2 |
| $t_{max}$（h） | 0.88±0.25 | 0.88±0.25 |
| $t_{1/2}$（h） | 1.58±0.135 | 1.60±0.21 |

Pharmacokinetic parameters of enalaprilat were shown in Table 4, and mean plasma concentration–time curve was shown in Figure4. Compared with the control group, the Cmax of enalapril were decreased by 7.13%（P＞0.05）in the combined administration group.

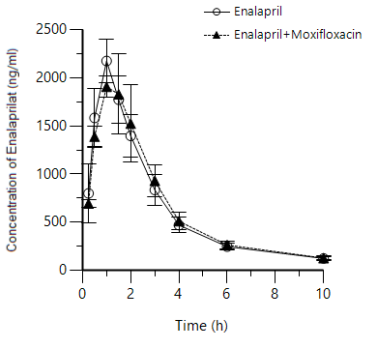


**Fig.4.** Seven days after moxifloxacin（80.0 mg/kg）was given, Mean plasma concentration-time curve of enalaprilat in rats after oral administration of 15.0 mg/kg enalapril alone or in combination with moxifloxacin （n=6）

**Table 4.** Pharmacokinetic parameters of enalaprilat 7 days after moxifloxacin administration

| Pharmacokinetic parameter | Enalapril | Enalapril +Moxifloxacin |
|---|---|---|
| $AUC_{(0-t)}$ ( $ng.mL.h^{-1}$) | 6347.53±799.38 | 6453.95±977.90 |
| $C_{max}$（ng/ml） | 2173.31±227.04 | 2018.72±191.85 |
| $t_{max}$（h） | 1.00±0.00 | 1.25±0.289 |
| $t_{1/2}$（h） | 3.26±0.15 | 3.02±0.785 |

## 4. Discussion

ACEI drugs are the substrates of PEPT1, which, as a transporter of intestinal intake, has a wide range of substrates[9]. If PEPT1 substrates or inhibitors are taken at the same time, theoretically, this may increase the risk of drug interactions.

It has been reported that quinolones are inhibitors of PEPT1[6]. In mice, when moxifloxacin is combined with PEPT1 substrate phenylalanine-Ψ alanine (Phe-Na-Ala), the AUC is reduced by nearly 60 ng/ (ml·h) compared with Phe-Ψ-Ala alone. At the same time, moxifloxacin decreased the uptake of cefalexin and valaciclovir in HeLa/ PEPT1 cells. Therefore, attention should be paid to clinical drug interactions when quinolone antimicrobials are combined with PEPT1 substrates.

In this study, enalapril, a widely used ACEI second-generation drug, was selected, and its metabolite was only enalaprilat[10]. The effects of moxifloxacin on the absorption of enalapril were investigated in rats. The main pharmacokinetic parameters obtained in the experiment were basically consistent with those reported in the literature [11,12]. In the single administration experiment, compared with the control group, the $AUC_{(0-t)}$ and $C_{max}$ of enalapril in the combined administration group were significantly decreased by 12.82%($P<0.05$), 44.68% ($P<0.05$). $T_{max}$ was increased from (0.875±0.25) h to (1.25±0.29) ht. This suggests that moxifloxacin may reduce the absorption of enalapril and delay the absorption rate of enalapril by inhibiting PEPT1. At the same time, moxifloxacin and enalapril may be used as substrates to compete for PEPT1, thus affecting the absorption of enalapril.

In multiple dosing studies, if moxifloxacin is only a PEPT1 inhibitor and not a substrate, parameters such as AUC or $C_{max}$ should decrease in the combination group compared to the control group. However, there was no significant difference in the major pharmacokinetic data between the two groups, suggesting that the effect of moxifloxacin on enalapril absorption in rats was more likely due to competition with PEPT1.

## 5. Conclusions

This study is the first to report the inhibition effect of moxifloxacin on the absorption of enalapril, which may be due to the competitive inhibition caused by both of them being PEPT1 substrates. Therefore, it is suggested that quinolone antibiotics and ACEI drugs should not be taken at the same time during clinical treatment to avoid drug interactions that may occur when combined drugs are used.

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