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ORIGINAL RESEARCH

Effects of Simvastatin on the Metabolism of Vonoprazan in Rats Both in vitro and in vivo

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Purpose: To study the potential drug–drug interactions between simvastatin and vonoprazan and to provide the scientific basis for rational use of them in clinical practice.

Methods: An incubation system was established with rat liver microsomes, and the main metabolite of vonoprazan M-I was detected by UPLC-MS/MS. The IC_{50} value of simvastatin was then calculated and its inhibitory mechanism against vonoprazan was also analyzed. Twelve SD rats were randomly divided into 2 groups, then they were given simvastatin or saline for 2 weeks continuously. On the day of the experiment, both groups were intragastrically administered with vonoprazan once, followed by the collection of blood at different time points. Then the plasma concentration of vonoprazan and M-I in rats were detected by UPLC-MS/MS.

Results: In vitro experiments revealed that simvastatin could inhibit the metabolism of vonoprazan, and its inhibition type belonged to the mixed non-competitive and competitive inhibition model. In vivo experiments in rats demonstrated that the area under concentration time curve (AUC) of vonoprazan was decreased but the clearance (CLz/F) of it was increased in the simvastatin administrated group, as compared to those of the control group. However, M-I in simvastatin treated group exhibited the higher AUC and lower CLz/F values compared to those in the control group. These data indicated that multiple doses of simvastatin administration could reduce the plasma concentration of vonoprazan and accelerate its metabolic rate in rats.

Conclusion: Simvastatin could inhibit the metabolism of vonoprazan in vitro but multiple doses of simvastatin exhibited the opposite effect In vivo. Altogether, our data indicated that an interaction existed between simvastatin and vonoprazan and additional cares might be taken when they were co-administrated in clinic.

Keywords: vonoprazan, simvastatin, drug–drug interactions, liquid chromatography-tandem mass spectrometry, rat liver microsomes

Introduction

As one of the drugs with wide clinical application, simvastatin is mainly used to treat dyslipidemia and it can reduce the concentrations of total cholesterol, triglyceride and low-density lipoprotein (LDL) in plasma, accomplished by increasing the concentration of high-density lipoprotein (HDL).[1] It has been reported that simvastatin is the substrates for cytochrome P450 (CYP) enzymes, P-glycoprotein (P-gp) and organic anion-transporting polypeptide transporter (OATP1B1), and it is mainly metabolized via CYP3A4/5, with CYP2C8/9/19 and CYP2D6 also involved in partial metabolism.[2] Recently, drug–drug interactions between simvastatin and other drugs were reported, such as simvastatin could inhibit the metabolism of verapamil and apatinib and increase their bioavailability by inhibiting CYP3A and P-glycoprotein (P-gp) efflux pumps.[3,4]

Vonoprazan, a potassium-competitive acid blocker (P-CAB), is a novel reversible proton pump inhibitor (PPI) that is used for the treatment or the prevention of gastric acid-related clinical conditions, such as erosive esophagitis, gastroesophageal reflux, gastroduodenal ulcer, aspirin or NSAID-induced peptic ulcer, and it can also be used for the eradication of Helicobacter

pylori.[5] It is reported that vonoprazan has a promising prospect for clinical application as it can be absorbed faster and has a longer half-life with stronger and longer-lasting antigastric acid secretion effect, compared to the traditional PPIs.[6]

Previous reports revealed that vonoprazan was mainly metabolized in two different pathways, the oxidization pathway by CYP enzyme isoforms (CYP3A4, CYP2B6, CYP2C19, CYP2D6) and the non-oxidization pathway by sulfotransferase (SULT2A1). With CYP enzyme, especially for CYP3A4, vonoprazan can be converted into 5-(2-fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrole-3-carboxylic acid (M-I) by the oxidative deamidation. We provided 3D models of chemical structure of vonoprazan and M-I for reference (Figure 1). As the main primary metabolite of vonoprazan, M-I can be further metabolized to the secondary metabolites, such as 5-(2-fluorophenyl)-1H-pyrrole-3-carboxylic acid (M-II), M-III and M-IV-Sul. However, none of these metabolites is pharmacologically active.[7–9]

Like other CYP3A4-mediated drugs, the metabolism of vonoprazan could be inhibited by some strong CYP3A4 inhibitor drugs. For example, voriconazole was reported to inhibit vonoprazan metabolism by inhibiting the enzymatic activity of cytochrome P450.[10] To date, no reports were found regarding the potential interactions between CYP3A4-mediated drugs simvastatin and vonoprazan. Considering for the wide usage of simvastatin and the possibility of co-administration of simvastatin and acid inhibitory drugs in clinic, we performed the drug-drug interaction study in rat model both in vitro and In vivo. Our data provide the first-hand evidence of the interactions between these two drugs and indicated that more cares might be taken when using them simultaneously in clinic.

Materials and Methods
Drugs and Reagents
Simvastatin and vonoprazan were purchased from Sunflower Technology Development Co. (Beijing, China). M-I was got from Wuxi Medical Technology Co. (Wuxi, China). Diazepam was purchased from Tianjin Golden York Pharmaceutical Co. (Tianjin China). Methanol and acetonitrile (chromatographic grade) were obtained from Merck GmbH (Darmstadt, Germany). Formic acid (chromatographic grade) was gotten from Sigma-Aldrich (St. Louis, Missouri, USA). Other chemical reagents were all analytically pure or guaranteed reagent.

Experimental Animals
The experimental animals were healthy SPF-grade SD male rats with 250±20 g, provided by the Laboratory Animal Center of Wenzhou Medical University. Animals were fed rat chow once in the morning and once in the evening, and water was consumed freely at daily temperature of 20–25°C, 12 hours of light and relative humidity of 40–60% for 1
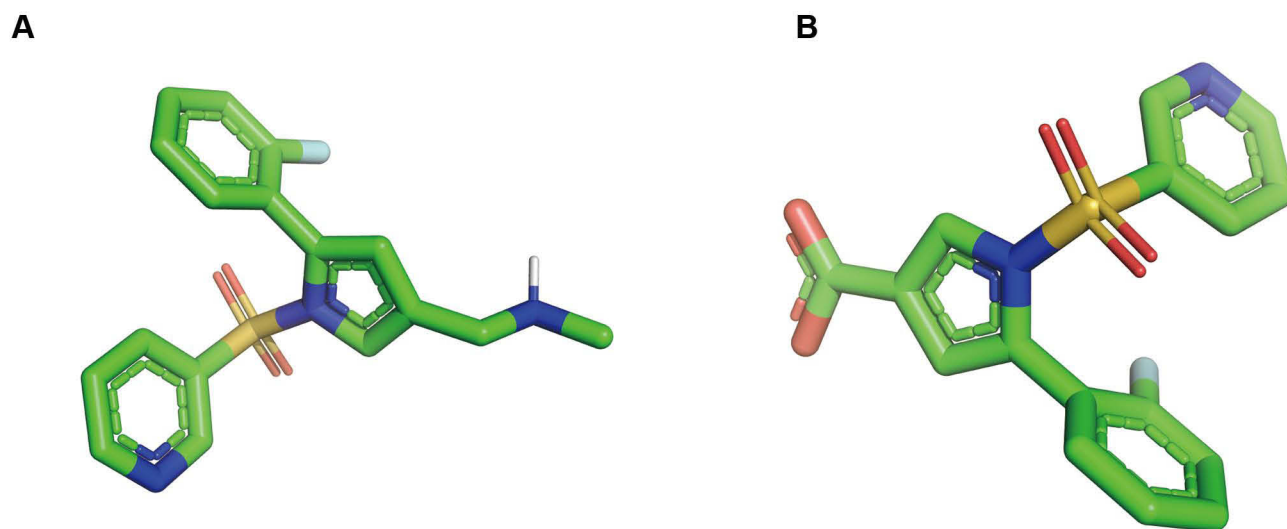
A

B

Figure 1 Structure displays of vonoprazan and M-I. (**A**) 3D model of chemical structure of vonoprazan; (**B**) 3D model of chemical structure of M-I.

week before the drug administrations. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals, the Animal Welfare Act, and the Office of Laboratory Animal Welfare.

All experimental procedures were approved by the Animal Experimental Ethics Inspection Department of the Laboratory Animal Center in Wenzhou Medical University (approval No. wydw2019-650).

Chromatography and Conditions

ACQUITY I-Class UPLC and Waters XEVO TQD MS (Milford, MA, USA) were used for the detection of vonoprazan and M-I with ACQUITY UPLC BEH C18 column (50 mm×2.1 mm, 1.7 μm) (Milford, MA, USA) at temperature of 40°C. The mobile phase consisted of acetonitrile and 0.1% formic acid in gradient proportion with running time of 3 minutes at flow rate of 0.4 mL/min. The injection volume of sample was 2 μL. The scanning method was multiple reaction monitoring (MRM) with detection in positive ion mode and an ESI+ ion source. Other mass spectrometry parameters were listed as following: capillary voltage 2.0 kV; ion source temperature 150 °C; argon flow rate: 0.15 mL/min. The other conditions of mass spectrometry were referred to Shen.[10]

In vitro Incubation Experiments

Rat liver microsomes (RLM) were prepared by differential centrifugation as described previously[9] and its protein concentration were determined as 28 mg/mL. The volume of incubation system was 190 μL, containing 10 μM vonoprazan (close to its Km, Figure 2), 2 μL RLM, 20 μL potassium phosphate buffer (1 M, pH = 7.4), 100, 50, 10, 5, 1, 0.1, 0.01, and 0 μM simvastatin. Three parallels were set up for each group and the above operations were performed on ice. Then the mixture was vortexed and pre-incubated in a water bath at 37°C for 5 min, and the reaction was initiated by adding 10 μL of NADPH (1 mM) and incubated at 37°C for 30 min with shaking in water bath. At the end, 200 μL acetonitrile and 20 μL internal standard diazepam (500 ng/mL) were added to terminate the reaction, following by vortex and centrifugation at 13,000 rpm for 5 minutes. Subsequently, 150 μL of supernatant was taken out and 2μL of sample was injected into LC-MS/MS machine for the detection and quantification of M1 which were used for calculation of IC_{50} value by GraphPad Prism 9.0.

In order to investigate the inhibition mechanism of simvastatin on vonoprazan, these two drugs were incubated together at different concentration gradients, with 2.5, 5, 10 and 20 μM of vonoprazan and 0, 1.5, 3, 6 and 12 μM of simvastatin. The incubation procedure was the same as that mentioned above.
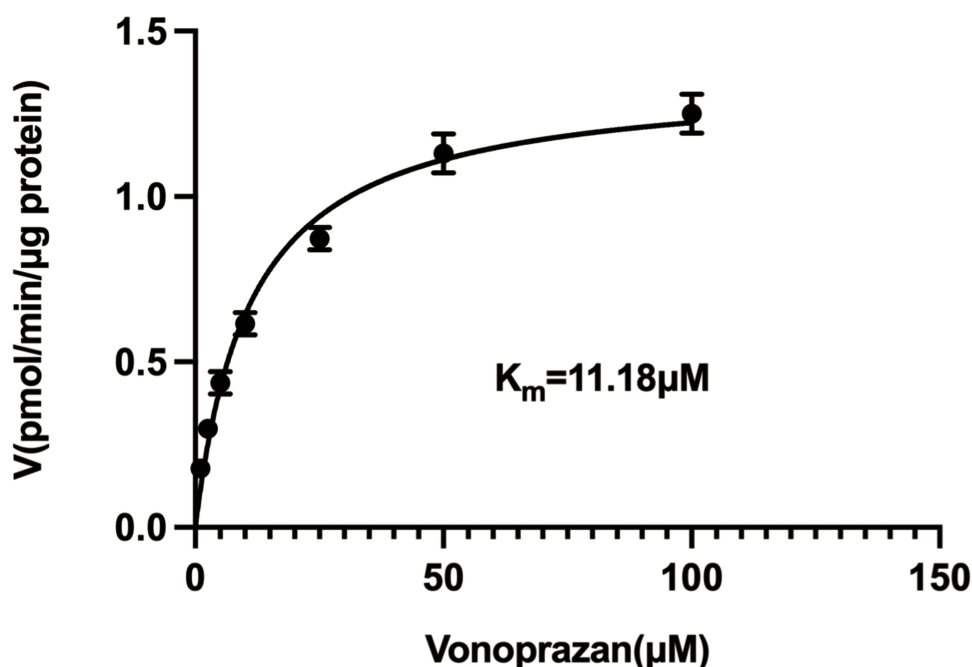

Figure 2 Michaelis-Menten kinetics of vonoprazan on cytochrome P450 enzymes in rat liver microsomes.

In vivo Pharmacokinetic Experiments

Twelve healthy SPF-grade SD male rats were randomly and equally divided into 2 groups: animals in the simvastatin group were injected intraperitoneally with 50 mg/kg of simvastatin solution once a day for 2 weeks, followed by 12 hours of fasting in advance then administrated by intragastrical gavage of vonoprazan (10 mg/kg); animals in the control group were injected with saline for 2 weeks, followed by gavage administration of the same dose of vonoprazan in the same period. Next, at 0.083h, 0.25h, 0.5h, 1h, 2h, 3h, 4h, 6h, 12h, and 24h after administration, 300 μL of blood was collected from the tail vein and placed into EP tubes containing 20 μL sodium heparin. After the centrifugation at 4°C, 4000 rpm for 10 minutes, 100 μL of plasma samples were taken out and mixed with 100 μL of acetonitrile and 20 μL of diazepam (500 ng/mL) to precipitate proteins. Then the mixtures were vortexed and centrifuged at 4°C, 4000 rpm for 5 minutes. 150 μL supernatant was transferred into the sample bottle and 2 μL of sample was analyzed by LC-MS/MS machine for metabolites quantification.

Molecular Docking Method

We retrieved the crystal structure of CYP3A4 from the RCSB PDB database (https://www.rcsb.org/) and the molecular structures of simvastatin and vornolazem from Pubchem (https://pubchem.ncbi.nlm.nih.gov/), and then we performed structure optimization using Pymol (Version 2.5. 2) and molecular docking by AutoDock Vina (Version 1.2.0), choosing the result with the highest affinity among the docked structures we simulated.[11,12] Finally, we visualized docking results on Pymol and found the sites where the drug interactions with CYP through hydrogen bonding.

Statistical Analysis

The IC_{50} value, Lineweaver-Burk plot and plasma concentration-time curves were obtained by GraphPad prism software (version 9.0, San Diego, CA, USA). DAS (Drug and Statistics) software (version 3.2.8; Lishui People's Hospital, Lishui, China) was used to plot the drug-time curves and to calculate the changes of vonoprazan and M-I in rat plasma over time with the non-atrial model of statistical moment parameters. Statistical analysis of the main pharmacokinetic parameters, such as area under the drug-time curve (AUC), clearance (CLz/F), and peak concentration (C_{max}), was performed using SPSS software (version 25.0; SPSS Inc., Chicago, IL, USA). All data were assessed distribution by using QQ plots. When comparing two independent samples, the Independent Samples *t*-test was used for normally distributed data (parametric test) and Mann–Whitney test was used for the non-normal distributed dataset (non-parametric test) and *$P<0.05$ indicates the statistical difference.

Results

Chromatograms of Vonoprazan Metabolite

Under conditions listed in the methods section, the peak emergence times of vonoprazan metabolite M-I and internal standard diazepam were 1.45 minutes and 1.70 minutes, respectively. As shown in Figure 3 that no interference could be detected among input drugs vonoprazan, M-I and diazepam, or in the blank rat liver microsomes.
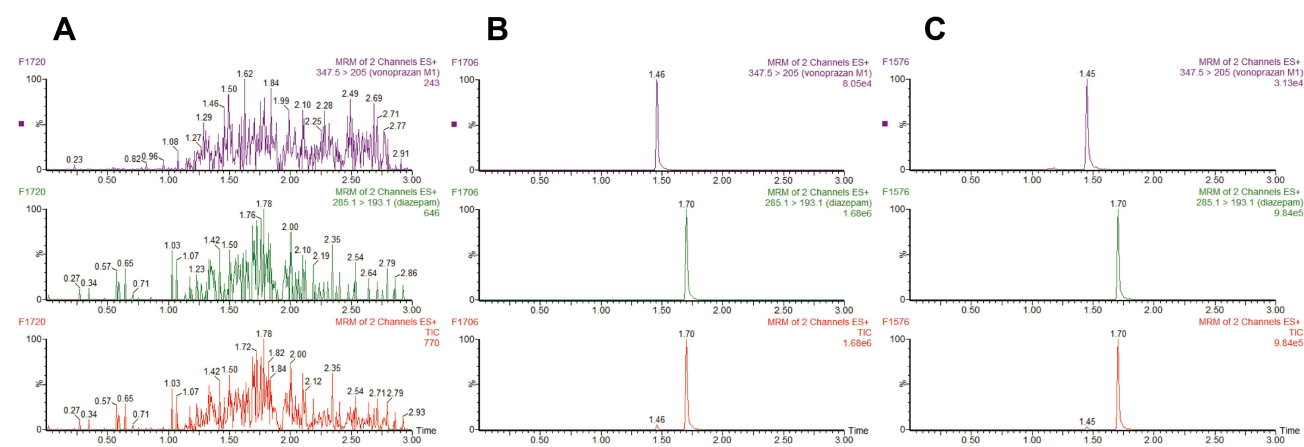

Figure 3 UPLC-MS/MS chromatograms of vonoprazan metabolite M-I and internal standard (IS) diazepam. (**A**) Blank rat microsomal system; (**B**) Standard M-I was mixed with denatured microsomes; (**C**) M-I and diazepam were detected after the incubation of rat liver microsomes with vonoprazan.

Inhibition of Vonoprazan by Simvastatin in vitro

In vitro pharmacokinetic data revealed that simvastatin could inhibit the metabolism of vonoprazan with IC_{50} value of 6.444 μM (Figure 4). Lineweaver-Burk plots for simvastatin inhibition of vonoprazan metabolism in rat liver microsomes indicated that the mechanism behind this inhibition was a mixture of non-competitive and competitive inhibition with $Ki=13.46$ μM and $\alpha Ki=5.54$ μM (Figure 5).

Effect of Multiple Doses of Simvastatin on Vonoprazan in vivo

The mean plasma concentration-versus-time curves of vonoprazan and its main metabolite M-1 in rat after administration by gavage of simvastatin are shown in Figure 6. Detailed pharmacokinetic parameters of drugs are illustrated in Table 1. As compared to those of control group, the $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of vonoprazan in the simvastatin pretreated group decreased to 33.4% and 34.5%, respectively; the peak concentration C_{max} of vonoprazan decreased to 25.4%, while the clearance CLz/F of vonoprazan increased by 1.65-fold. On the contrary, the $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of M-I in the simvastatin pretreated group were 1.28-fold and 1.35-fold higher than those in the control group, respectively, but the clearance CLz/F of M-I decreased to 77.9% of control group. These data indicated that multiple doses of simvastatin could significantly induce the metabolism of vonoprazan in rats by decreasing the plasma concentration of vonoprazan and increasing that of its metabolite.

Molecular Docking Prediction of Simvastatin and Vonoprozan

To better understand the mechanism behind the interaction of simvastatin and vonoprazan, molecular docking analysis were also conducted in this study according to the methods previously reported.[13–15] Our simulation results on Pymol showed that simvastatin interacted with arginine (ARG) at position 106 of CYP3A4 via hydrogen bonding, while vonoprazan interacted with heme (HEM) at position 601 of CYP3A4, with the sites of action 9.2 Å apart (Figure 7). Probably, this spatial positional proximity may be one of the reasons for the ease of interaction between the two drugs.
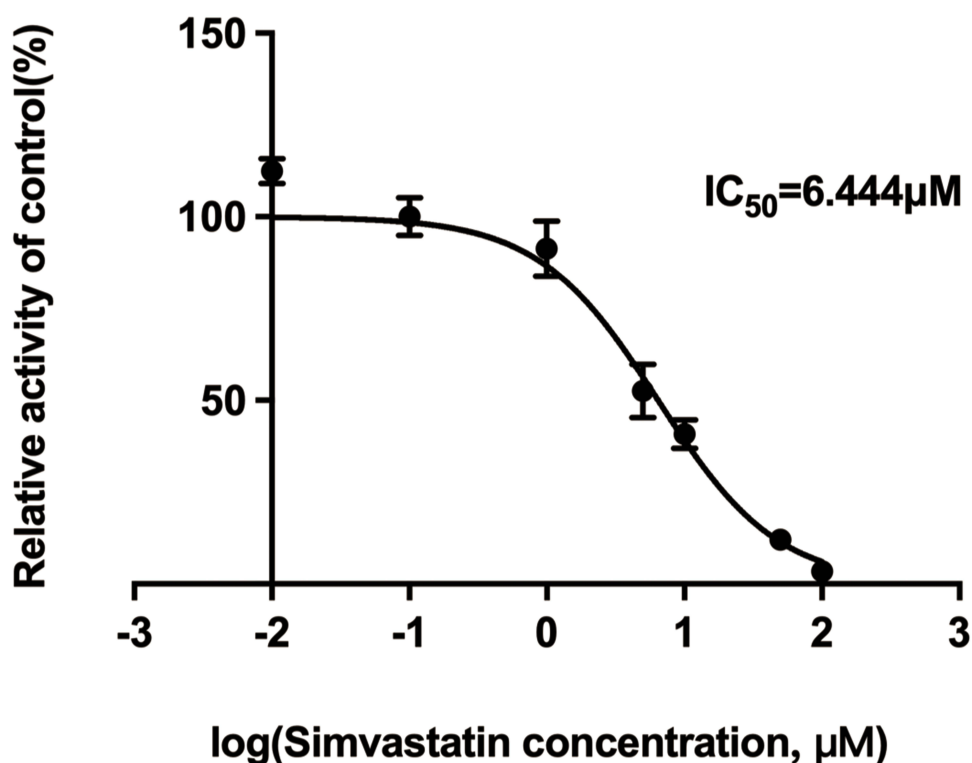

Figure 4 IC_{50} profile of simvastatin on cytochrome P450 enzymes in rat liver microsomes.
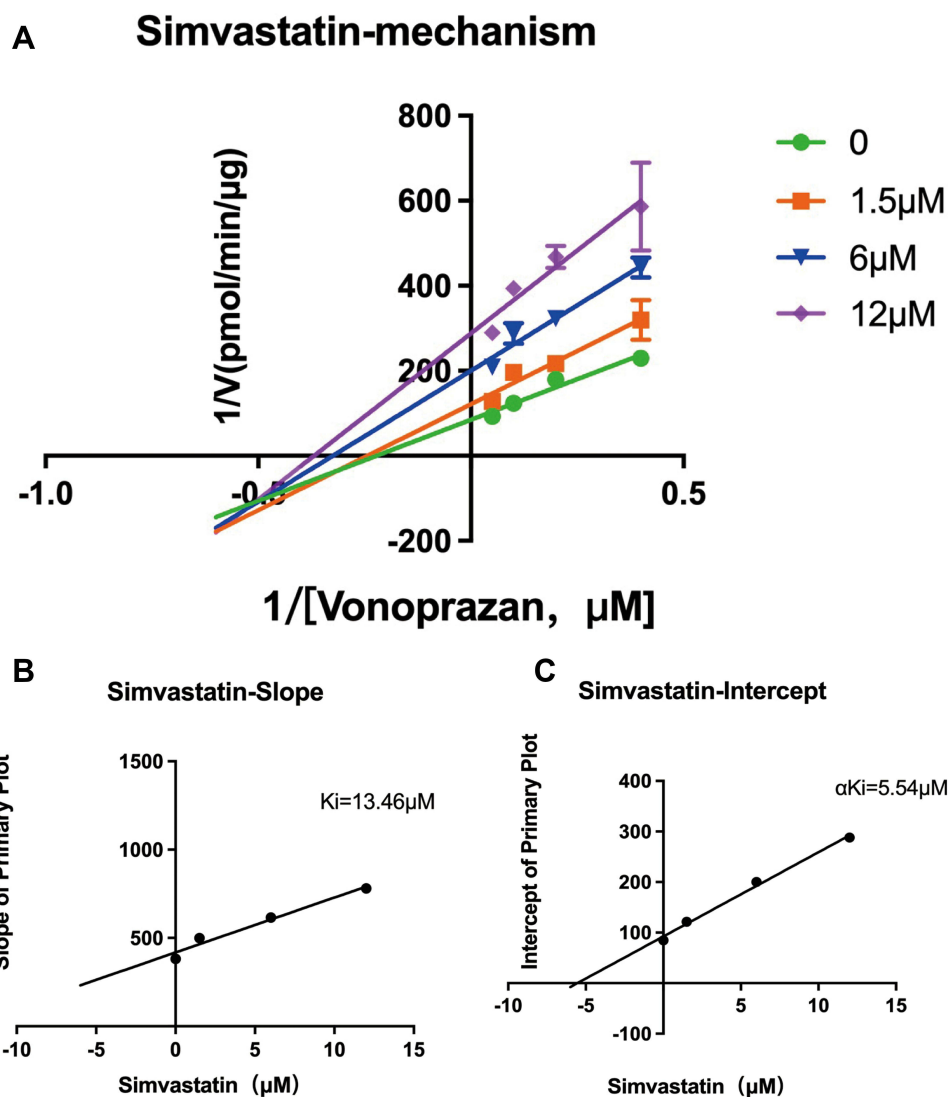
A

Simvastatin-mechanism

B

Simvastatin-Slope

Ki=13.46µM

C

Simvastatin-Intercept

αKi=5.54µM

Figure 5 Lineweaver-Burk plots for simvastatin inhibition of vonoprazan metabolism in rat liver microsomes. (**A**) Lineweaver–Burk plots for simvastatin (0, 1.5, 6, 12 µM) inhibition of vonoprazan (2.5, 5, 10, 20 µM) in rat liver microsomes. Data are shown with the mean standard deviation of three parallel experiments; (**B**) Slope of primary plot; (**C**) Intercept of primary plot.
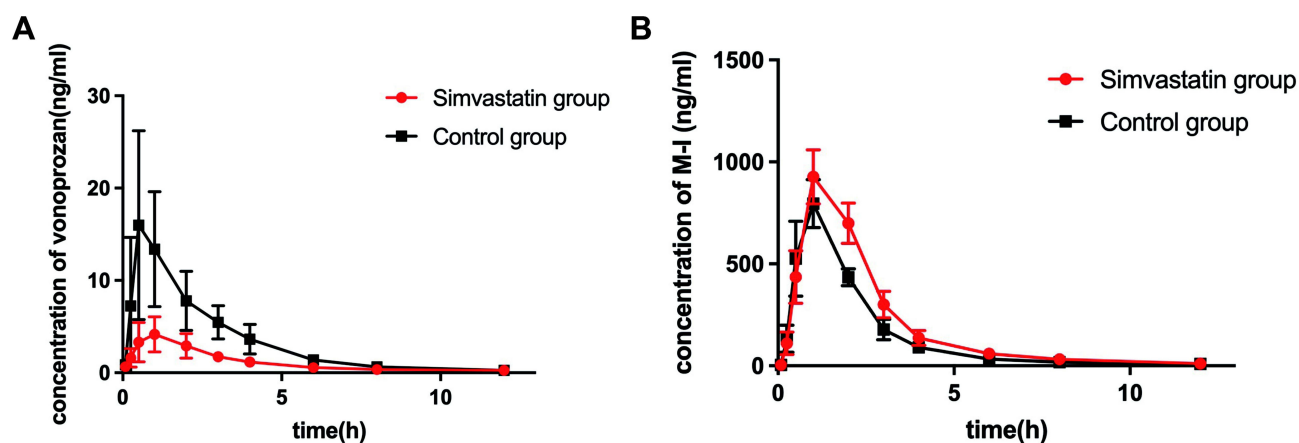
A

B

Figure 6 The concentration-time curves of vonoprazan (**A**) and M-I (**B**) in rat (n = 6). The simvastatin group was injected with simvastatin intraperitoneally for 2 weeks and then administered with vonoprazan intragastrically; The control group was injected with saline only for 2 weeks and followed by administrated with same dose of vonoprazan to that of simvastatin pretreated group.

Table 1 Main Pharmacokinetic Parameters of Vonoprazan and M-I in vivo

Pharmacokinetic Parameters	Vonoprazan		M-I	
	Simvastatin Group[a]	Control Group	Simvastatin Group[a]	Control Group
$AUC_{(0-t)}$ (μg/L*h)	13.87±4.96*	41.544±18.51	2186.23(2027.74–3109.85) *	1711.08±109.64
$AUC_{(0-∞)}$ (μg/L*h)	14.46±4.80*	41.93±18.59	2351.96(2052.43–3170.09) *	1737.64±115.58
$MRT_{(0-t)}$ (h)	3.21±0.39*	2.62±0.27	2.40±0.16*	2.17±0.17
$MRT_{(0-∞)}$ (h)	3.47(2.70–6.43) *	2.74±0.34	2.54±0.23	2.40±0.27
$t_{1/2}$ (h)	1.97(1.79–5.41)	1.79±0.41	2.02±0.33	2.58±1.21
T_{max} (h)	1(1–1) *	0.67±0.26	1±0	1±0
Vz/F (L/kg)	569.6±330.37*	149.13±83.89	2.51±0.48	4.27±1.86
CLz/F (L/h/kg)	151.05±48.77*	56.98±27.31	0.9040(0.63–0.97) *	1.16±0.08
C_{max} (μg/L)	4.17±1.91	16.45±9.66	926.29±133.45	796.11±118.16

Notes: n=6 per group; data are expressed mean ± SD; *P < 0.05 indicates statistical difference between simvastatin group and control group. [a]All data were assessed distribution by using QQ plots. Skewed data were presented as median (min - max) while normal distribution data were presented as mean ± SD.
Abbreviations: AUC, the area under concentration time curve; MRT, the mean residence time; $t_{1/2}$, half-life period; T_{max}, maximum plasma time; Vz/F, apparent volume of distribution; CLz/F, clearance; C_{max}, peak plasma concentration.

Discussion

Traditional PPIs are widely used for the treatment of gastric acid-related diseases, however, a series of deficiencies have been reported recently, such as instability under acidic conditions, relatively slow onset of action, and unstable and short duration of acid-suppressive effects. Specially, traditional PPIs are mainly metabolized by the hepatic CYP2C19, and about 63.75% of the Chinese population are poor metabolizers who carry the typical defective alleles *CYP2C19*2* or *CYP2C19*3*. These two variants can significantly reduce the drug metabolic activity of CYP2C19, and that is the main reason for the individual differences in pharmacokinetics of PPIs in the oriental Asian population.[16,17] In contrast, vonoprazan is mainly metabolized by CYP3A4 and is more stable than traditional PPIs that exhibits a promising prospect in clinic, especially for Asian populations.[18]

The main advantages of vonoprazan over conventional PPIs include: (1) Vonoprazan has stronger and more lasting anti-gastric acid secretion effect than common PPIs. As a lipophilic weak base with high pKa value (9.3), vonoprazan can immediately be protonated when exposing it to the acidic environment. Due to the highly selective and slow dissociation rate for binding to H^+-K^+-ATPase in the range of weak acid to neutral pH, vonoprazan exhibited 358–2357 times greater H^+-K^+-ATPase activity than that of lansoprazole. It is not only less affected by pH value, but also can better control of nocturnal acid secretion; (2) Vonoprazan absorbs faster than traditional PPIs, and it can reach maximum plasma concentration within 1.5–2.0 hours of oral administration; (3) Vonoprazan has shorter onset time than conventional PPIs, with acid-suppressive effects occurring within 24 hours of administration of 20 mg; (4) Vonoprazan exhibits longer half-life of approximately 7.7 hours, compared with that of 1–1.5 hours for conventional PPIs; (5) Vonoprazan has high stability, whereas the activation of conventional PPIs is acid-dependent; (6) Vonoprazan has higher population tolerance.[19–22]

In spite of the advantages listed above, vonoprazan still has some potential to interact with other drugs, especially for CYP3A4 mediated drugs. As the most highly expressed metabolic enzyme in the liver, CYP3A4 is involved in the in vivo metabolism of most of the clinically used drugs. It has been reported that many drugs can also inhibit or induce changes in CYP3A4 activity or expression, thus cause drug toxicity reactions or make the treatment ineffective.[23,24] However, little is known about the drug-drug interactions between vonoprazan and other drugs metabolized by CYP3A4.

In this study, the influence of simvastatin on the metabolism of vonoprazan was investigated for the first time. Our data indicated that simvastatin could inhibit the metabolism of vonoprazan in a mixed model of non-competitive and competitive inhibition in vitro (Figure 5). However, in vivo experiments showed that plasma concentration of vonoprazan was decreased and its clearance was increased significantly in the simvastatin pretreated group. These data inferred that simvastatin could significantly induce the metabolism of vonoprazan in rats. Wang et al also reported similar results when they investigated the interactions between simvastatin and sinomenine. They found that simvastatin could inhibit the
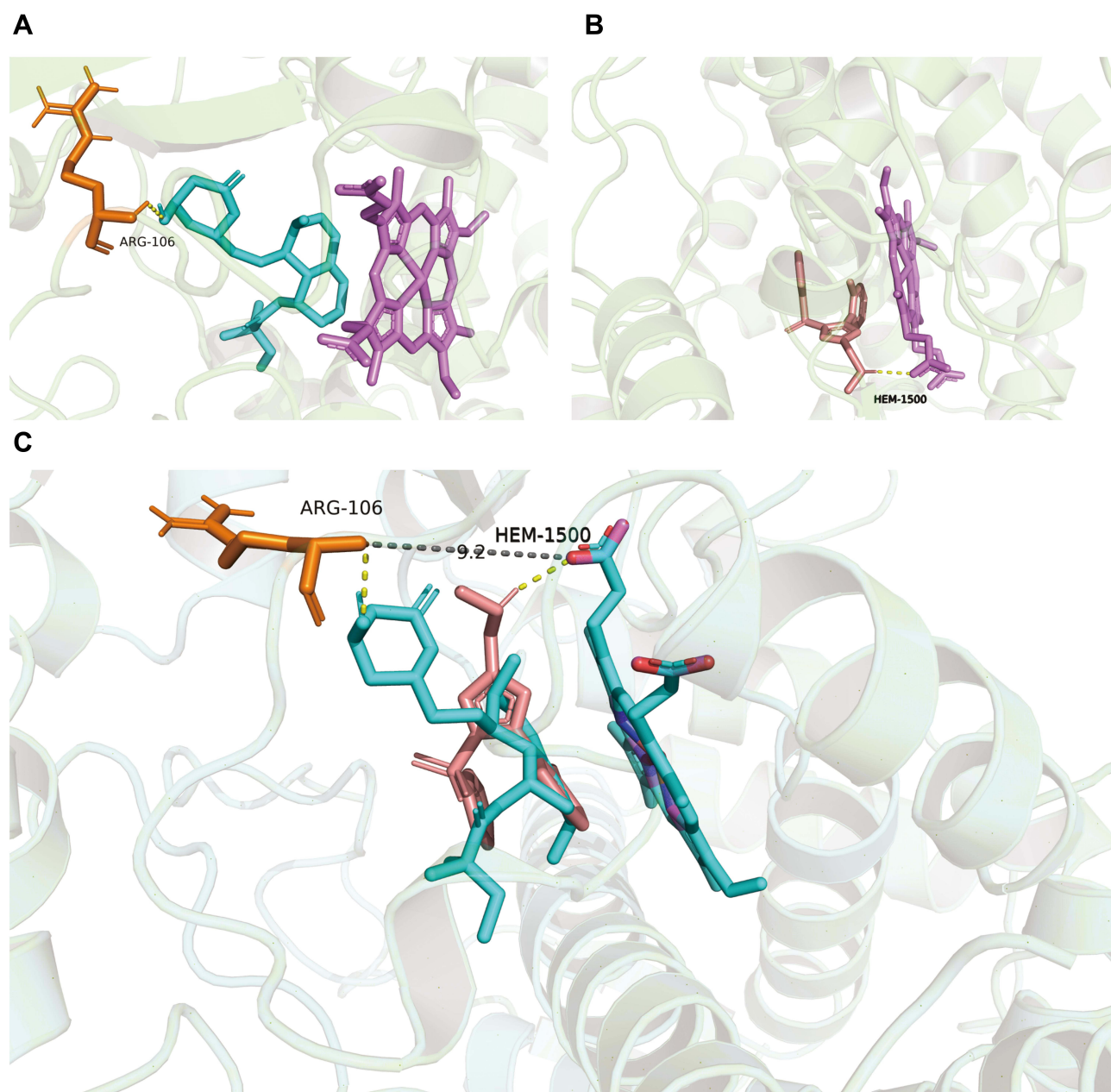
Figure 7 Molecular docking scheme of simvastatin and vonoprazan. (**A**) Action site between simvastatin and CYP3A4 via hydrogen bonding; (**B**) Action site between vonoprazan and CYP3A4 via hydrogen bonding; (**C**) Aligned docking results of the two groups.

metabolism of sinomenine in vitro, while multiple doses of simvastatin could induce the metabolism of sinomenine In vivo.[25] They proved that CYP3A1/2 was responsible for the metabolism of sinomenine in rats and the mRNA and protein expression levels of CYP3A1/2 in rat livers were significantly increased in the simvastatin pretreated group.[25] Because CYP3A1/2 in rats is highly homologous to CYP3A4 in humans, we believe that the induction effect of simvastatin to vonoprazan metabolism in rats might also be due to the higher expression level of CYP3A1/2 in multidose simvastatin pretreated animals.

In addition to simvastatin, some other statins like atorvastatin and lovastatin were also reported to increase the enzymatic activity of CYPs to different degrees, in which the capacity of this induction is CYP2C8>CYP3A4>CYP2C9>CYP2C19~CYP2D6.[26] Schuetz and Kocarek also reported the induction of CYP3A4 by simvastatin. They found that the expression levels of CYP3A4 mRNA and protein were increased around 24–36 times and 6–20 times,

respectively, after taking some statins like simvastatin 24 hours later.[27,28] The explanation for the induction effect of simvastatin is that it can affect lipids metabolism and may trigger the activation of pregnane X receptor PXR, that is the main nuclear receptor mediating the effects of exogenous drugs on CYP3A4 expression.[29,30]

In addition to the hypolipidemic effect, simvastatin also exhibits certain anti-inflammatory effects by reducing the mRNA expression level and decreasing the production of the famous pro-inflammatory marker interleukin-6 (IL-6).[31] Machavaram et al reported that upregulated IL-6 levels could decrease the metabolic activity of CYP3A4, whereas blocking the IL-6 signaling pathway could restore the enzyme activity.[32] Accordingly, we believed that simvastatin may also induce the enhancement of CYP3A4 activity in this study, by inhibiting the production of IL-6 in simvastatin group.

It was reported that statins have different affinities for the membrane transport proteins that associated with intestinal absorption, hepatic metabolism, biliary excretion, and renal clearance. Whether this association can impact the extra-hepatic metabolism of vonoprazan also needs further investigation, because vonoparazan can also be metabolized by some extrahepatic pathway, such as renal clearance.[33–35]

What is noteworthy is that, in clinic, both simvastatin and vonoprazan are well generally tolerated and most of the adverse reactions are mild and transient if take them solely. However, data in this study indicated that simvastatin exhibited strong inhibitory effect on the metabolism of vonoprazan, and some of the side effects of vonoprazan in digestive system might be stronger when co-administrated these two drugs together in clinic, such as diarrhea, constipation, nausea and abdominal distension. In addition, diseases of other systems such as headache, rash, edema, eosinophilia, and muscle injury may also be present rarely.

Conclusion

In vitro incubation experiments indicated that simvastatin had a metabolic inhibitory effect on vonoprazan. However, In vivo pharmacokinetic experiments in rats revealed that multiple doses of simvastatin could accelerate the metabolism of vonoprazan in rats. Considering for the wide usage of simvastatin and bright prospects of vonoprazan, our data suggest that the interactions between vonoprazan and simvastatin may need to be considered when they are combined clinically, so as to avoid or reduce the occurrence of some adverse reactions.

Acknowledgments

Funding

Disclosure

The authors report no conflicts of interest in this work.

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