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ORIGINAL ARTICLE

Naringenin has an inhibitory effect on rivaroxaban in rats both in vitro and in vivo

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Abstract

Food–drug interactions are reported to have some impacts on the pharmacokinetics and pharmacodynamics of various oral drugs. To better understand the effects of naringenin, one natural product in many fruits, on the pharmacokinetics of rivaroxaban, drug–drug interactions (DDIs) between naringenin and rivaroxaban in vitro were investigated in Sprague–Dawley (SD) rat liver microsomes. For the DDIs in vivo, 12 male SD rats were randomly divided into the experimental group and the control group with six rats in each group. Rats in the experimental group were pre-treated with naringenin (10 mg/kg/day) for 2 weeks before the administration of rivaroxaban (10 mg/kg) by oral gavage, while the rats in the control group were given rivaroxaban (10 mg/kg) only once. The plasma concentration of rivaroxaban in rats was then measured by UPLC-MS/MS. In vitro data indicated that naringenin could decrease the metabolic clearance rate of rivaroxaban with the IC_{50} value of 38.89 μM, and exhibited a mixed inhibition to rivaroxaban (Ki =54.91 μM, aKi =73.33 μM, a = 0.74). In vivo data in rats revealed that as compared with that of the control group, the $AUC_{(0-t)}$ value of rats in the experimental group was increased from 2406.28 ± 519.69 μg/h/L to 4005.04 ± 1172.76 μg/h/L, the C_{max} value was increased from 310.23 ± 85.76 μg/L to 508.71 ± 152.48 μg/L, and the $V_{z/F}$ and $CL_{z/F}$ were decreased from 23.03 ± 4.81 L/kg to 16.2 ± 8.42 L/kg, 4.26 ± 0.91 L/h/kg to 2.57 ± 0.73 L/h/kg, respectively. These

Abbreviations: CYP, cytochrome P450; DOAC, direct oral anticoagulant; ESI, electrospray ionization; HDI, herb–drug interaction; MRM, multiple reaction monitoring; MRT, mean residence time; RLMs, rat liver microsomes; UPLC-MS/MS, ultra-performance liquid chromatography-tandem mass spectrometry; VKAs, Vitamin K antagonists; VTE, venous thromboembolism.

Hai-Feng Shi, Fang-Ling Zhao and Hao Chen are contributed equally to this work.

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data indicated that naringenin had an inhibitory effect on the pharmacokinetics of rivaroxaban in rats, suggesting that the DDIs between naringenin and rivaroxaban might occur when they were co-administered in the clinic.

KEYWORDS
drug–drug interactions, metabolism, naringenin, pharmacokinetic, rivaroxaban

1 | INTRODUCTION

Anticoagulants play an important role in many illnesses, and Vitamin K antagonists (VKAs), such as warfarin, have become the most frequently prescribed anticoagulant drugs for the prevention of thromboembolic events. However, VKAs still have many disadvantages which include multiple drug–drug interactions, high inter-individual variation, susceptibility to genetic polymorphism, narrow therapeutic window with unpredictable pharmacokinetics and pharmacodynamics.[1–4] Recently, direct oral anticoagulant (DOAC) has already overcome these limitations by targeting the single clotting factor (such as factor Xa or thrombin). Factor X is at the crossing point of the intrinsic and extrinsic coagulation pathway, which makes it plays a central role in the hemostasis and leads to the propagation and amplification of coagulation. At present, various anticoagulants have been extensively developed for the principle of acting on the free and clot bound factor X and thus made a success in anticoagulation.[5,6]

Rivaroxaban is an oral, reversible, and highly selective inhibitor for factor X. The structural formula of rivaroxaban is shown in Figure 1. It has been approved for the application in clinical settings in many countries for the prevention of venous thromboembolism (VTE) in adult patients after elective hip or knee replacement surgery, as well as for the treatment of deep vein thrombosis (DVT), pulmonary embolism (PE), and thromboembolic disorders.[7–10] Because of the predictable pharmacokinetics and pharmacodynamics characters, rivaroxaban has become more and more popular in clinics. It has been reported that rivaroxaban can be metabolized by two main routes: oxidative degradation of the morpholinone moiety (via CYP450-CYP3A4 and CYP2J2) and hydrolysis of the central amide bond and of the lactam amide bond in the morpholinone ring (CYP-independent).[11] Consequently, some inducers or inhibitors of CYP450 are reported to have the properties of affecting the pharmacokinetics of rivaroxaban.[12–14] The elimination of rivaroxaban is mainly through the kidney, and two proteins, the hepatobiliary routes-transport proteins P-glycoprotein (P-gp) and the breast cancer resistance protein (BCRP), are the main molecules responsible for the active renal secretion of rivaroxaban. Thus, inhibition of P-gp and BCRP can lead to the significantly decreased renal clearance rate and increased exposure of rivaroxaban.[10,15] Recent study has revealed that the combination of ciclosporin (a P-gp inhibitor) with fluconazole (a CYP3A4 inhibitor) could observably increase the AUC and C_{max} values of rivaroxaban through increasing the systemic exposure time and decrease in its renal clearance rate.[16]

Flavonoids are natural plant polyphenol compounds, which have many beneficial biochemical and pharmacological properties. Flavonoids can also be recognized as effective medicines for the prevention and treatment of many kinds of diseases. Naringenin, a representative of flavonoid, can be widely found in fruits, vegetables, and herbs. It possesses a great deal of biochemical and pharmacological activities such as antioxidant and anti-inflammatory, and it can also protect the liver,[17] induce cell apoptosis, and exhibit the anti-proliferation effect for cancer cells.[18] These characteristics have granted its broad application in the clinic.

Food–drug interactions have already become one of the most essential factors that have impacts on the pharmacokinetics and pharmacodynamics of various oral drugs.[19] It has been reported that naringenin can influence the pharmacokinetics and metabolism of other drugs when co-administrated with those drugs metabolized by CYP3A4 (such as tofacitinib, ibrutinib, and felodipine).[20–22] Considering that rivaroxaban can be influenced by

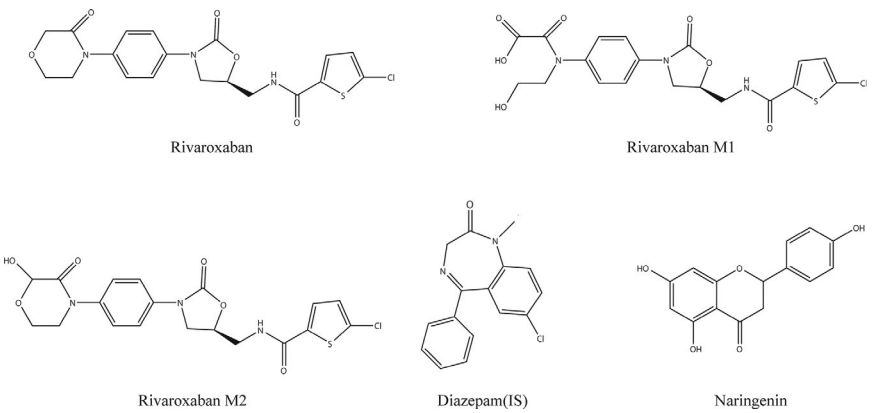

FIGURE 1 Chemical structures of rivaroxaban, rivaroxaban metabolite M2, M1, naringenin, and diazepam

inducers or inhibitors of CYP3A4,[12,23,24] the purpose of the current study is to evaluate whether some drug–drug interactions are existed between naringenin and rivaroxaban in rats both in vitro and in vivo.

2 | MATERIALS AND METHODS

The study was conducted in accordance with the BCPT policy for experimental and clinical studies.[25]

2.1 | Chemicals and reagents

Diazepam (purity >98%) and rivaroxaban (purity >99%) were obtained from J&K Scientific. Naringenin was purchased from the Beijing Sunflower. Reduced nicotinamide adenine dinucleotide phosphate (NADPH) was obtained from Roche Pharmaceuticals (Base). Chromatographic grade acetonitrile and methanol were purchased from Thermo Fisher Scientific. Purified water was collected through Milli-Q water purification system (Millipore). All other chemicals were of analytical grade or better.

2.2 | Animals and treatment

Twelve male Sprague–Dawley rats weighing 230–250 g were provided by the experimental animal center of Wenzhou Medical University. Rats were housed under normal conditions with 60% ± 5% humidity and a 12 h dark–light cycle at 25℃. Tap water and laboratory chow were provided ad libitum. Rats were handled daily for 1 week before initiating the experiment to minimize the nonspecific stress. All experimental produces were approved by the Animal Care and Use Committee of Wenzhou Medical University (approval No. wydw2019-650), and were in accordance with corresponding guidelines of committee.

2.3 | Instruments and operation conditions

UPLC-MS/MS system was used for the plasma sample separation and analysis. In detail, the chromatographic separation was performed with ACQUITY UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm) at 40℃. The mobile phase consisted of acetonitrile and water containing 0.1% formic acid with a flow rate of 0.40 ml/min. It applied a method of gradient elute, where the acetonitrile started with 30% (0–0.5 min), rapidly increased from 30% to 95% (0.5–1.0 min), maintained at 95% (1.0–2.0 min), and then decreased to 30% (2.0–2.6 min). The running time for one separation was 3 min.

The positive multiple reaction monitoring (MRM) mode was used for the mass scanning on XEVO TQD triple quadrupole mass spectrometer (Waters Corp.), which was equipped with electrospray ionization (ESI). Detailed scanning parameters can be found in our recently reported method.[26]

2.4 | In vitro pharmacokinetics

Incubation system was 190 μl and contained 100 mM of potassium phosphate buffer (pH 7.4), 1 mg/ml rat liver microsomes (RLMs, produced in the laboratory of Clinical Pharmacy of the Sixth Affiliated Hospital of Wenzhou Medical University[20,27–29]), and different concentrations of naringenin and rivaroxaban. The reaction was initiated after the 5 min pre-incubation at 37℃ in a shaking water bath and then launched by adding 10 μl NADPH with the final concentration of 1 mM. Thirty minutes later, the reaction was stopped by adding 200 μl acetonitrile and 20 μl diazepam (served as internal standard, IS). After the centrifugation at 16,060 *g* for 5 min, 150 μl of supernatant was taken out and put into the sample bottle. Then 2 μl of the sample was collected for the injection and detection by UPLC-MS/MS.

Detailed usages of naringenin and rivaroxaban were as following: nine concentrations of rivaroxaban (1, 2.5, 5, 10, 25, 50, 100, 200 μM) were used for the determination of its Km value; 10 μM of rivaroxaban and a series of different concentrations of naringenin (1, 2.5, 5, 10, 25, 50, 100 μM) were used for the calculation of its IC_{50} value; and the inhibition type was determined by Lineweaver-Burk Plot with a series concentrations of rivaroxaban (5, 10, 20, 40 μM) and a series concentrations of naringenin (0, 10, 20, 40, 80 μM).

2.5 | In vivo pharmacokinetics

Twelve SD rats were randomly divided into the experimental group (n = 6) and the control group (n = 6). Naringenin was dissolved in CMC-Na solution and used for the pretreatment of rats in the experimental group with 10 mg/kg/day by oral gavage for 2 weeks. For the control group, rats only received equal amounts of 0.5% CMC-Na solution in the pre-treating time. Dimethyl sulfoxide (DMSO) was used for the dissolution of rivaroxaban and polyethylene glycol 200 was used for the following dilution to get a final concentration of 5% DMSO within it. Rats in each group were administered at a dose of 10 mg/kg rivaroxaban with oral gavage, and 300 μl blood samples were then collected from tail veins at 0.083, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h. Blood samples were centrifuged at 1520 *g* for 10 min, and the supernatant was then collected and stored immediately at −80℃ before use.

2.6 | Plasma sample preparation

Collected rat plasma (100 μl) was mixed with 20 μl of 0.5 μg/ml IS and 200 μl acetonitrile, followed by vortexing for 30 s before the centrifugation at 16,060 *g* for 5 min. The supernatant was then used for sample injection and separation on XEVO TQD triple quadrupole mass spectrometer.

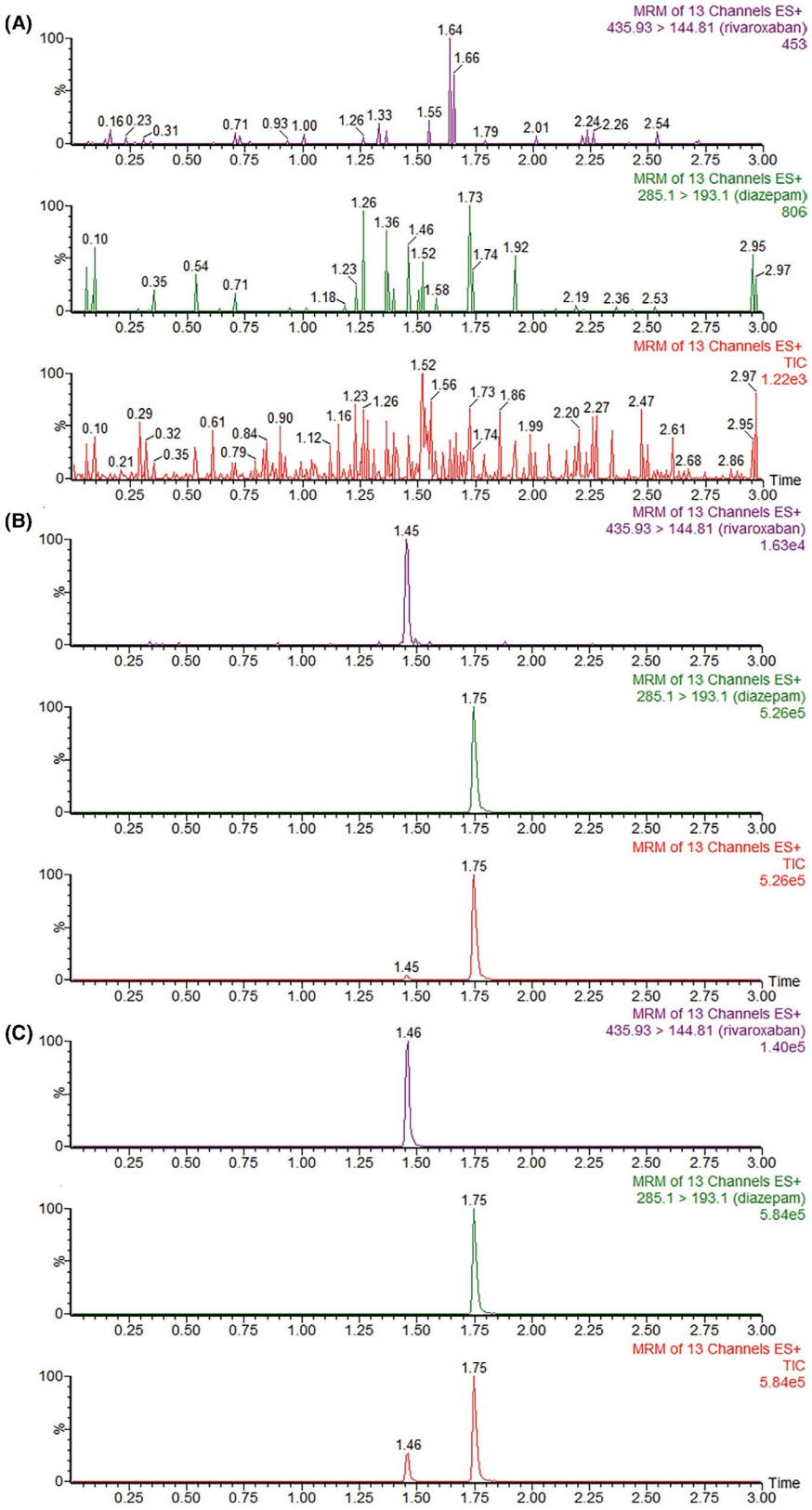

FIGURE 2 Typical MRM chromatograms of rivaroxaban and diazepam. Blank rat plasma (A), rat plasma spiked with Lower Limit of quantitation (LLOQ) rivaroxaban (B), rat plasma 0.5 h after the oral administration of rivaroxaban (C), IS, internal standard

2.7 | Data analysis

GraphPad Prism 7.0 software (GraphPad Software Inc.) was used to calculate the enzyme kinetic parameters of substrates, including IC_{50}, Ki, and αKi. Linear Lineweaver-Burk plots were used to obtain enzyme inhibition modes. DAS (Drug And Statistics) software (Version 3.2.8) was used for the calculation of pharmacokinetic characters which include the maximal plasma concentration (C_{max}), the time to peal plasma concentration (T_{max}), the apparent volume of distribution ($V_{z/F}$), the area under the plasma concentration-time

curve (AUC), the elimination half-life ($t_{1/2}$), the plasma clearance ($CL_{z/F}$), and the mean residence time (MRT). The independent sample Student's *t*-test was used for the statistical comparisons within groups with SPSS software (Version 25.0; SPSS Inc.), and $p < .05$ was regarded as statistically significant.

3 | RESULTS

3.1 | In vitro pharmacokinetics of naringenin and rivaroxaban

In this study, we successfully developed a measurement method for rivaroxaban with UPLC-MS/MS technique. Figure 2 illustrates the typical UPLC-MS/MS chromatogram of blank plasma spiked with rivaroxaban and IS (diazepam). RLMs, endogenous substances, or metabolites in plasma exhibited no interference with the determination, indicating that the assay showed good specificity and well anti-interference performance. As shown in Figure 3, the V_{max} and K_m values of rivaroxaban were 4.35 pmol/min/mg and 18.40 μM, respectively. After adding various concentrations of naringenin, the metabolism of rivaroxaban was inhibited to some extent and in a dose-dependent manner with the IC_{50} value of 38.89 μM.

3.2 | Inhibitory kinetics of naringenin on the rivaroxaban in RLMs

After the incubation of RLMs with different concentrations of rivaroxaban and naringenin, we characterized the main inhibition effects of naringenin on rivaroxaban. As shown in Figure 4, all the inhibition effects of naringenin were in dose-dependent manners in all concentration gradients. The Lineweaver-Burk Plot result indicated that naringenin exhibited a mixed inhibition effect on rivaroxaban with Ki and αKi (α = 0.74) values were 54.91 μM and 73.77 μM, respectively.

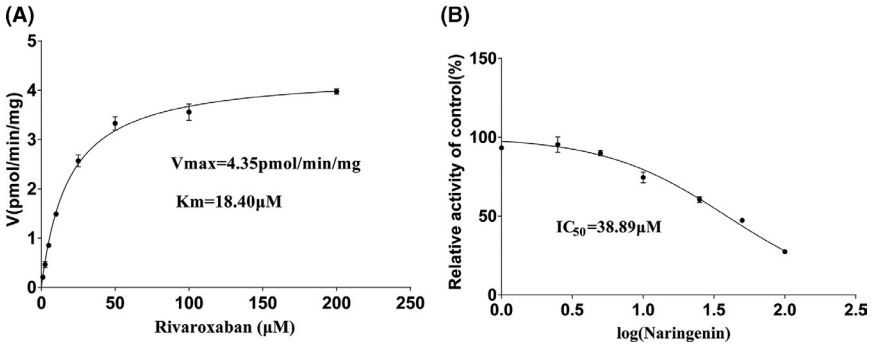

FIGURE 3 Michaelis–Menten curve of rivaroxaban metabolism in RLMs (A) and IC50 curve of naringenin inhibition on the rivaroxaban metabolism (B). (values are means ± standard deviations, *n* = 3)

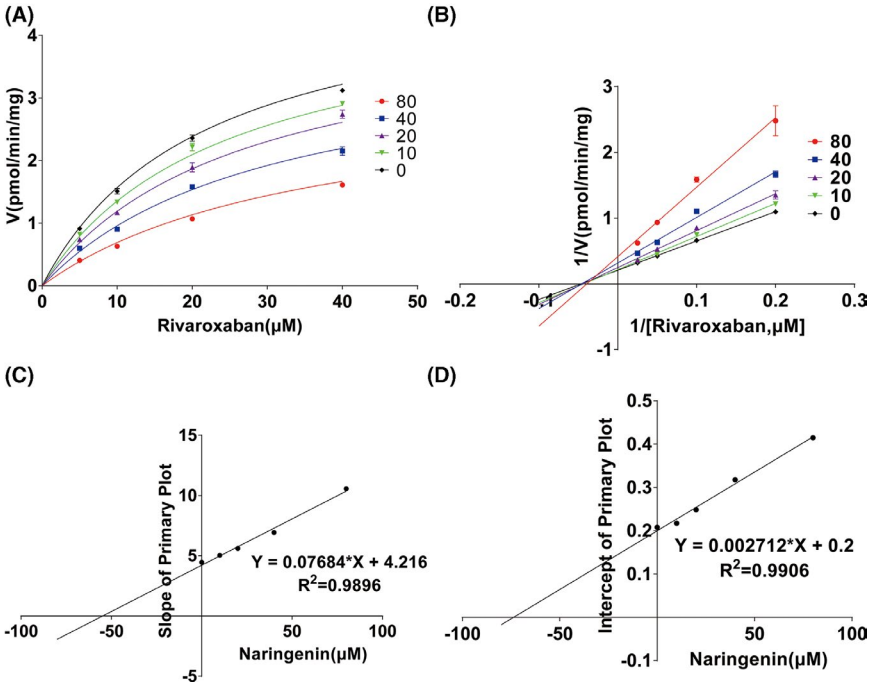

FIGURE 4 Inhibitory kinetics of naringenin on the rivaroxaban in RLMs. Michaelis–Menten curve of naringenin inhibition on rivaroxaban metabolism in the rat (A). Lineweaver-Burk plots of naringenin inhibition on rivaroxaban in RLMs (B). Slope of Primary Plot (C). Intercept of Primary Plot (D). Data are shown as the mean ± standard deviation of triplicate experiment. (values are means ± standard deviations, *n* = 3)

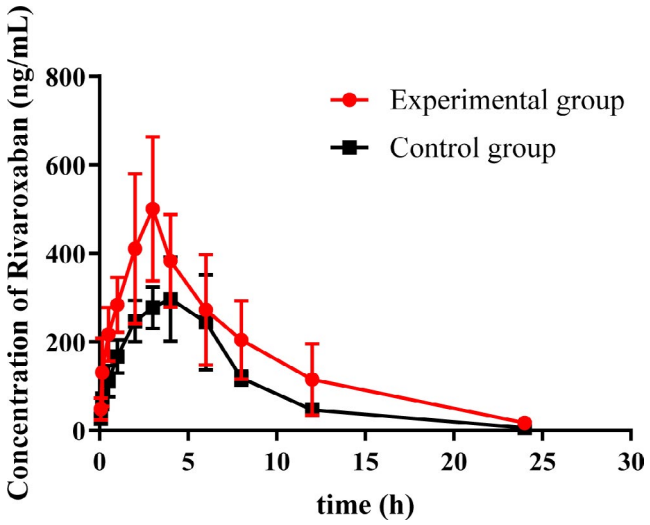

FIGURE 5 Mean plasma concentration–time curves of rivaroxaban in rats in Control group and Experimental group (values are means ± standard deviations, $n = 6$)

TABLE 1 The main pharmacokinetic parameters of rivaroxaban in Control group and Experimental group ($n = 6$, mean ± SD)

Parameters	Control group	Experimental group
$AUC_{(0-t)}$ ($ng \cdot ml^{-1} \cdot h^{-1}$)	2406.28 ± 519.69	4005.04 ± 1172.76*
$AUC_{(0-\infty)}$ ($ng \cdot ml^{-1} \cdot h^{-1}$)	2438.52 ± 529.29	4142.16 ± 1150.79*
$MRT_{(0-t)}$ (h)	5.97 ± 0.34	6.53 ± 1.41
$MRT_{(0-\infty)}$ (h)	5.28 ± 0.44	7.43 ± 2.32
$t_{1/2z}$ (h)	3.77 ± 0.36	4.44 ± 1.95
T_{max} (h)	3.4 ± 0.548	2.8 ± 0.45
$V_{z/F}$ ($L \cdot kg^{-1}$)	23.03 ± 4.81	16.2 ± 8.42*
$CL_{z/F}$ ($L \cdot h^{-1} \cdot kg^{-1}$)	4.26 ± 0.91	2.57 ± 0.73*
C_{max} ($ng \cdot ml^{-1}$)	310.23 ± 85.76	508.71 ± 152.48*

Data are expressed as mean ± SD.

*Significantly different from control, $p < .05$.

3.3 | Effect of naringenin on the in vivo pharmacokinetics of rivaroxaban

To better understand the in vivo inhibition effect of naringenin on rivaroxaban, six SD rats were pre-treated with continuous administration of naringenin in 10 mg/kg/day for 2 weeks, and then gained 10 mg/kg rivaroxaban by oral gavage. Compared with the control rats, experimental rats exhibited elevated plasma concentration-time curves and significantly decreased $V_{z/F}$ and $CL_{z/F}$ values (Figure 5 and Table 1). The values of $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, and C_{max} of rivaroxaban in the experimental group decreased significantly by about 0.6-fold. On the contrary, the $V_{z/F}$ and $CL_{z/F}$ values decreased significantly and there was no obvious change on the other parameters. These data inferred that naringenin could inhibit the metabolism of rivaroxaban in rat in vivo.

4 | DISCUSSION

Rivaroxaban has been widely used in clinical practice recently, and co-administration of rivaroxaban with other drugs has become more and more popular than ever. To better evaluate its pharmacokinetic characters, several LC-MS/MS methods have been developed for the determination of rivaroxaban in human blood samples.[30–32] Recently, one liquid chromatography-tandem mass spectrometry method for the simultaneous detection of 3 oral anticoagulants, including rivaroxaban, has been reported with the run-time of 5 min and LOD of 0.025 ng/ml.[33] As compared, the running time for one separation in this study was validated with the retention time of rivaroxaban and diazepam (IS) was only 1.45 and 1.75 min, respectively, which is more time-saving than any previously proposed methods.

It has been reported that plenty of drugs, especially metabolized by CYP3A4 or P-gp, have impacts on the pharmacokinetics of rivaroxaban because rivaroxaban is also the substrate of CYP3A4 or P-gp. For instance, when 10 mg of rivaroxaban was administered in combination with 200 mg ketoconazole once daily, the AUC value and C_{max} value of rivaroxaban increased by 82% and 53%, respectively. Whereas the body clearance rate was significantly reduced by 45%.[34] Another example is that the systemic exposure of single-dose rivaroxaban increased significantly with AUC and C_{max} values increased by 150% and 60%, respectively, when co-administrated rivaroxaban with ritonavir. On the other hand, some inducers of CYP3A4 and P-gp could decrease the systemic exposure of rivaroxaban and reduce its anticoagulant effect. Co-administration of rivaroxaban with rifampicin, one combined strong inducer for both CYP3A4 and P-gp, could decrease the value of AUC and C_{max} of rivaroxaban by 50% and 22%, respectively.[35]

As a combined inhibitor for CYP3A4 and P-gp, naringenin exhibits some interactions with many drugs when co-administered. It was reported that naringenin could inhibit the metabolism of tofacitinib by suppressing the CYP3A4 activity. Compared with the control group, pre-treatment of rats with naringenin for 2 weeks could increase the AUC, MRT, and T_{max} values of tofacitinib by 1.76-fold, 1.60-fold, and 4.00-fold, respectively, but decreased its $CL_{z/F}$ by 1.69-fold.[20] Similarly, metabolisms of both ibrutinib and felodipine were inhibited, and their systemic exposures were also increased when co-administrated them with naringenin. It was supposed that naringenin might affect these two drugs by inhibiting the CYP3A4-mediated metabolism.[21,22] On the other hand, naringenin owns many biochemical benefits for human health. It shows strong antioxidation effects by enhancing the antioxidant defenses and scavenging the reactive oxygen species. It also has a great ability to modulate signaling pathways related to fatty acids metabolism, thus favors the oxidation of the fatty acids, and exhibits effects on anti-inflammatory, anticancer, and anti-proliferation.[36] In addition, naringin has great potential for the treatment of cardiovascular disease because many in vitro studies and in vivo animal models have demonstrated that naringenin played an active role in the inhibition of atherosclerotic.[37–40] Moreover, naringenin could protect the recovery of ischemia/reperfusion (I/R) injury by inhibiting atherosclerotic and could

improve the cardiac function and structure in the diabetic mice model.[41] Based on above biochemical and pharmacological characteristics of naringenin, it is possible to have a co-administration of naringenin with rivaroxaban in clinic, especially for the treatment of cardiovascular disease. Therefore, investigating the DDIs between naringenin and rivaroxaban might have significant theoretical and clinical application value.

In this study, we investigated the DDIs between naringenin and rivaroxaban both in vitro and in vivo. In vitro pharmacokinetics data illustrated that the inhibition of naringenin on rivaroxaban was identified as a mixed inhibition with an IC_{50} value of 38.87 μM. After the pretreatment of rat with naringenin for 14 days, the experimental group showed that the AUC value and C_{max} value of rivaroxaban increased by 1.7 times and 1.6 times, but the values of both $V_{z/F}$ and $CL_{z/F}$ were significantly decreased, as compared with those of the control group (Table 1). The in vitro and in vivo data were consistent and indicated that naringenin has a relatively strong inhibition effect on the metabolism of rivaroxaban. Rivaroxaban has been reported to be a combined substrate for both CYP3A4 and P-gp, and naringenin is known as an inhibitor of CYP3A4 and P-gp. We speculated that naringenin might affect the metabolism of rivaroxaban by inhibiting the function of CYP3A4 or P-gp, which is similar to the activities of tofacitinib, ibrutinib, and felodipine. Recently, we have reported the interactions between rivaroxaban and ticagrelor, which are another kind of widely used drugs metabolized by CYP3A4 and as well as a substrate and inhibitor of CYP3A4 and P-gp.[26] Similar to naringenin, ticagrelor could increase the AUC and C_{max} values of rivaroxaban by more than twice and decrease its metabolic clearance rate in rats. Taken together, we speculated that DDIs might occur with increased incidence rate of adverse reactions when rivaroxaban is co-administered with drugs metabolized by CYP3A4 in the clinic.

Our experiment also has a few limitations. As this study was based on animals, it cannot mimic the true state of the DDIs in the human body completely. More work needs to be carried out in the future to investigate the putative interactions between naringenin and rivaroxaban in the human body.

In conclusion, with UPLC-MS/MS detection method for rivaroxaban in RLMs and blood plasma, we determined the impact of naringenin on the pharmacokinetic and pharmacodynamic parameters of rivaroxaban both in vitro and in vivo. Our data indicated that naringenin has a suppressive impact on the rivaroxaban metabolism by increasing the systemic exposure time of rivaroxaban and reducing its body clearance rate in rats. These data suggest that the full consideration of drug–drug interactions might be taken when prescribing the co-administration of naringenin and rivaroxaban in the clinic.

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DISCLOSURE

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

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

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REFERENCES

1. Toorop MMA, Rein N, Nierman MC, et al. Self-reported therapy adherence and predictors for nonadherence in patients who switched from vitamin K antagonists to direct oral anticoagulants. *Res Pract Thromb Haemost*. 2020;4(4):586-593. https://doi.org/10.1002/rth2.12316
2. Elsebaie MAT, van Es N, Langston A, Buller HR, Gaddh M. Direct oral anticoagulants in patients with venous thromboembolism and thrombophilia: a systematic review and meta-analysis. *J Thromb Haemost*. 2019;17(4):645-656. https://doi.org/10.1111/jth.14398
3. Huisman MV, Ferreira M, Feuring M, Fraessdorf M, Klok FA. Less abnormal uterine bleeding with dabigatran than warfarin in women treated for acute venous thromboembolism. *J Thromb Haemost*. 2018;16(9):1775-1778. https://doi.org/10.1111/jth.14226
4. Zirlik A, Bode C. Vitamin K antagonists: relative strengths and weaknesses vs. direct oral anticoagulants for stroke prevention in patients with atrial fibrillation. *J Thromb Thrombolysis*. 2017;43(3):365-379. https://doi.org/10.1007/s11239-016-1446-0
5. Turpie AG. New oral anticoagulants in atrial fibrillation. *Eur Heart J*. 2008;29(2):155-165. https://doi.org/10.1093/eurheartj/ehm575
6. Denas G, Pengo V. Investigational anticoagulants for hematological conditions: a new generation of therapies. *Expert Opin Investig Drugs*. 2013;22(10):1281-1294. https://doi.org/10.1517/13543784.2013.821463
7. Burghaus R, Coboeken K, Gaub T, et al. Evaluation of the efficacy and safety of rivaroxaban using a computer model for blood coagulation. *PLoS One*. 2011;6(4):e17626. https://doi.org/10.1371/journal.pone.0017626
8. Turpie AG. Advances in oral anticoagulation treatment: the safety and efficacy of rivaroxaban in the prevention and treatment of thromboembolism. *Ther Adv Hematol*. 2012;3(5):309-323. https://doi.org/10.1177/2040620712453067
9. Misselwitz F, Berkowitz SD, Perzborn E. The discovery and development of rivaroxaban. *Ann N Y Acad Sci*. 2011;1222:64-75. https://doi.org/10.1111/j.1749-6632.2011.05971.x
10. Bratsos S. Pharmacokinetic properties of rivaroxaban in healthy human subjects. *Cureus*. 2019;11(8):e5484. https://doi.org/10.7759/cureus.5484
11. Antoniou S. Rivaroxaban for the treatment and prevention of thromboembolic disease. *J Pharm Pharmacol*. 2015;67(8):1119-1132. https://doi.org/10.1111/jphp.12387
12. Mueck W, Stampfuss J, Kubitza D, Becka M. Clinical pharmacokinetic and pharmacodynamic profile of rivaroxaban. *Clin Pharmacokinet*. 2014;53(1):1-16. https://doi.org/10.1007/s40262-013-0100-7
13. Kreutz R. Pharmacodynamic and pharmacokinetic basics of rivaroxaban. *Fundam Clin Pharmacol*. 2012;26(1):27-32. https://doi.org/10.1111/j.1472-8206.2011.00981.x

14. Kanuri SH, Kreutz RP. Pharmacogenomics of novel direct oral anticoagulants: newly identified genes and genetic variants. *J Pers Med*. 2019;9(1):7. https://doi.org/10.3390/jpm9010007
15. Mueck W, Kubitza D, Becka M. Co-administration of rivaroxaban with drugs that share its elimination pathways: pharmacokinetic effects in healthy subjects. *Br J Clin Pharmacol*. 2013;76(3):455-466. https://doi.org/10.1111/bcp.12075
16. Brings A, Lehmann M-L, Foerster KI, et al. Perpetrator effects of ciclosporin (P-glycoprotein inhibitor) and its combination with fluconazole (CYP3A inhibitor) on the pharmacokinetics of rivaroxaban in healthy volunteers. *Br J Clin Pharmacol*. 2019;85(7):1528-1537. https://doi.org/10.1111/bcp.13934
17. Rashmi R, Bojan Magesh S, Mohanram Ramkumar K, Suryanarayanan S, Venkata SM. Antioxidant potential of naringenin helps to protect liver tissue from streptozotocin-induced damage. *Rep Biochem Mol Biol*. 2018;7(1):76-84.
18. Lu WL, Yu CR, Lien HM, Sheu GT, Cherng SH. Cytotoxicity of naringenin induces Bax-mediated mitochondrial apoptosis in human lung adenocarcinoma A549 cells. *Environ Toxicol*. 2020;35(35):1386-1394. https://doi.org/10.1002/tox.23003
19. Zha W. Transporter-mediated natural product-drug interactions for the treatment of cardiovascular diseases. *J Food Drug Anal*. 2018;26(2S):S32-S44. https://doi.org/10.1016/j.jfda.2017.11.008
20. Wang B, Shen J, Zhou Q, et al. Effects of naringenin on the pharmacokinetics of tofacitinib in rats. *Pharm Biol*. 2020;58(1):225-230. https://doi.org/10.1080/13880209.2020.1738504
21. Liu J, Liu H, Zeng Q. The effect of naringenin on the pharmacokinetics of ibrutinib in rat: a drug-drug interaction study. *Biomed Chromatogr*. 2019;33(5):e4507. https://doi.org/10.1002/bmc.4507
22. Surya Sandeep M, Sridhar V, Puneeth Y, Ravindra Babu P, Naveen BK. Enhanced oral bioavailability of felodipine by naringenin in Wistar rats and inhibition of P-glycoprotein in everted rat gut sacs in vitro. *Drug Dev Ind Pharm*. 2014;40(10):1371-1377. https://doi.org/10.3109/03639045.2013.819885
23. Gopalakrishnan L, Kumar V, Kohli P, Singh P, Rastogi U, Gibson CM. Pharmacokinetic evaluation of rivaroxaban for the treatment of acute coronary syndromes. *Expert Opin Drug Metab Toxicol*. 2012;8(7):889-900. https://doi.org/10.1517/17425255.2012.688026
24. Walenga JM, Adiguzel C. Drug and dietary interactions of the new and emerging oral anticoagulants. *Int J Clin Pract*. 2010;64(7):956-967. https://doi.org/10.1111/j.1742-1241.2009.02286.x
25. Tveden-Nyborg P, Bergmann TK, Lykkesfeldt J. Basic & clinical pharmacology & toxicology policy for experimental and clinical studies. *Basic Clin Pharmacol Toxicol*. 2018;123(3):233-235. https://doi.org/10.1111/bcpt.13059
26. Chong J, Chen H, Dai D, et al. Effects of ticagrelor on the pharmacokinetics of rivaroxaban in rats. *Pharm Biol*. 2020;58(1):630-635. https://doi.org/10.1080/13880209.2020.1785510
27. Wang S, Lin Z, Su KE, et al. Effect of curcumin and pirfenidone on toxicokinetics of paraquat in rat by UPLC-MS/MS. *Acta Chromatographica*. 2018;30(1):26-30. https://doi.org/10.1556/1326.2017.00175
28. Wang Y, Wang C, Wang S, et al. Cytochrome P450-based drug-drug interactions of vonoprazan in vitro and in vivo. *Front Pharmacol*. 2020;11:53. https://doi.org/10.3389/fphar.2020.00053
29. Zhou Y, Meng D, Chen F, et al. Inhibitory effect of imperatorin on the pharmacokinetics of diazepam in vitro and in vivo. *Front Pharmacol*. 2020;11:01079. https://doi.org/10.3389/fphar.2020.01079
30. Kuhn J, Gripp T, Flieder T, et al. Measurement of apixaban, dabigatran, edoxaban and rivaroxaban in human plasma using automated online solid-phase extraction combined with ultra-performance liquid chromatography-tandem mass spectrometry and its comparison with coagulation assays. *Clin Chim Acta*. 2018;486:347-356. https://doi.org/10.1016/j.cca.2018.08.017
31. Schellings M, Boonen K, Schmitz E, et al. Determination of dabigatran and rivaroxaban by ultra-performance liquid chromatography-tandem mass spectrometry and coagulation assays after major orthopaedic surgery. *Thromb Res*. 2016;139:128-134. https://doi.org/10.1016/j.thromres.2016.01.012
32. Foerster KI, Huppertz A, Meid AD, et al. Dried-blood-spot technique to monitor direct oral anticoagulants: clinical validation of a UPLC–MS/MS-based assay. *Anal Chem*. 2018;90(15):9395-9402. https://doi.org/10.1021/acs.analchem.8b02046
33. Schmitz EMH, Boonen K, van den Heuvel DJA, et al. Determination of dabigatran, rivaroxaban and apixaban by ultra-performance liquid chromatography - tandem mass spectrometry (UPLC-MS/MS) and coagulation assays for therapy monitoring of novel direct oral anticoagulants. *J Thromb Haemost*. 2014;12(10):1636-1646. https://doi.org/10.1111/jth.12702
34. Gelosa P, Castiglioni L, Tenconi M, et al. Pharmacokinetic drug interactions of the non-vitamin K antagonist oral anticoagulants (NOACs). *Pharmacol Res*. 2018;135:60-79. https://doi.org/10.1016/j.phrs.2018.07.016
35. Hellwig T, Gulseth M. Pharmacokinetic and pharmacodynamic drug interactions with new oral anticoagulants: what do they mean for patients with atrial fibrillation? *Ann Pharmacother*. 2013;47(11):1478-1487. https://doi.org/10.1177/1060028013504741
36. Salehi B, Fokou P, Sharifi-Rad M, et al. The therapeutic potential of naringenin: a review of clinical trials. *Pharmaceuticals (Basel)*. 2019;12(1):11. https://doi.org/10.3390/ph12010011
37. Burke AC, Sutherland BG, Telford DE, et al. Naringenin enhances the regression of atherosclerosis induced by a chow diet in Ldlr(-/-) mice. *Atherosclerosis*. 2019;286:60-70. https://doi.org/10.1016/j.atherosclerosis.2019.05.009
38. Assini JM, Mulvihill EE, Sutherland BG, et al. Naringenin prevents cholesterol-induced systemic inflammation, metabolic dysregulation, and atherosclerosis in Ldlr(-)/(-) mice. *J Lipid Res*. 2013;54(3):711-724. https://doi.org/10.1194/jlr.M032631
39. Mulvihill EE, Assini JM, Sutherland BG, et al. Naringenin decreases progression of atherosclerosis by improving dyslipidemia in high-fat-fed low-density lipoprotein receptor-null mice. *Arterioscler Thromb Vasc Biol*. 2010;30(4):742-748. https://doi.org/10.1161/atvbaha.109.201095
40. Zhao Q, Yang H, Liu F, et al. Naringenin exerts cardiovascular protective effect in a palmitate-induced human umbilical vein endothelial cell injury model via autophagy flux improvement. *Mol Nutr Food Res*. 2019;63(24):e1900601. https://doi.org/10.1002/mnfr.201900601
41. Heidary Moghaddam R, Samimi Z, Moradi SZ, Little PJ, Xu S, Farzaei MH. Naringenin and naringin in cardiovascular disease prevention: a preclinical review. *Eur J Pharmacol*. 2020;887:173535. https://doi.org/10.1016/j.ejphar.2020.173535

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