Pharmacokinetic Interaction of Favipiravir with Amlodipine in Local Iraqi Rabbits (*Oryctolagus cuniculus*)

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Abstract

Favipiravir is an effective anti-viral drug used to treat COVID-19, which is metabolized by aldehyde oxidase (AO) and xanthine oxidase (XO). This study investigated drug–drug interactions between Favipiravir and Amlodipine, an AO inhibitor, in healthy rabbits. A total of 20 local rabbits (Oryctolagus cuniculus), weighted between 2 and 2.5 kg, were divided into two equal groups: HFav. (dosed with distilled water for two weeks before administration of 40 mg/kg.BW of Favipiravir single oral dose) and HFav.+Am (dosed 0.5 mg/kg.BW of Amlodipine orally daily for two weeks before administration of 40 mg/kg.BW of Favipiravir single oral dose). Blood samples were taken from the marginal ear vein after 0.15, 0.30, 0.45, 1, 2, 4, 6, 8, 12, 24, 36, 48, and 72 hours. Thereafter, a high-performance liquid chromatograph (HPLC) technique assessed the drug plasma concentration. Amlodipine prolonged the time taken (Tmax) for Favipiravir to reach maximum concentration (Cmax) in the systemic circulation, decreased maximum serum concentration (Cmax), eliminated half-life, and increased the area under the curve (AUC). Furthermore, the results revealed that Amlodipine had reduced the clearance per unit time (Cl/f) when co-administered with Favipiravir. In conclusion, the concomitant administration of Amlodipine with Favipiravir caused substantial changes in the pharmacokinetic profile of Favipiravir. Therefore, the dosage rate adjustment of Favipiravir is recommended.

Keywords: *Favipiravir; Amlodipine; pharmacokinetics; aldehyde oxidase; rabbits.*

Introduction

Many patients on many medications have serious concerns about the potential for harmful drug-drug interactions (DDIs). The World Health Organization stresses that the severity and frequency of DDIs can be greatly reduced by catering to the needs of the people most likely to experience them1.Multiple illnesses, such as high blood pressure, arthritis, and diabetes, may necessitate the use of a combination of treatments2.Favipiravir is a novel antiviral drug that specifically and potently inhibits RNA viruses' RNA-dependent RNA polymerase (RdRp) 3.It is activated phosphorylated in cells and identified as a substrate by viral RNA polymerase, limiting RNA polymerase function. As a result, it is thought that Favipiravir may have a strong anti-viral impact on SARS-CoV2—an RNA virus 4.Favipiravir has an oral bioavailability of higher than 95%. It is mostly metabolized by aldehyde oxidase (AO). Human plasma half-life is four hours5. Furthermore, there is no involvement of Cytochrome P450 isoenzymes in the metabolism of Favipiravir 6.Therefore, the combination of Favipiravir with medications that modify AO enzyme activity may result in altered pharmacokinetic profiles.

Amlodipine is a peripheral artery vasodilator that operates directly on vascular smooth muscle, lowering blood pressure (BP) and decreasing peripheral vascular resistance7,8.Multiple medications are required to effectively treat COVID-19, particularly in people with underlying conditions (hypertension, diabetes, and cardiovascular diseases) 9,10. Drug-drug interaction is a topic that requires attention in clinical practice. The information about DDI concerning Favipiravir is scanty and/or not studied11.Therefore, in this study, drug interaction regarding pharmacokinetics was investigated between Favipiravir, which is used successfully to treat COVID-19, and Amlodipine in local Iraqi rabbits.

Materials and methods:

Animals: Twenty healthy adult male local rabbits (Oryctolagus cuniculus) were obtained from the local Iraqi farms in Baghdad. The ages of the rabbits ranged between 12 and 16 months, and their weight ranged from 2 to 2.5 kg. Before the initiation of the experiment, to guarantee the rabbits adapted without a hitch, they were housed in an area with a constant temperature of 20-25˚C for at least two weeks. Care was taken to avoid any unnecessary stress. The experiments of this study were conducted in the animal house of the College of Veterinary Medicine, University of Baghdad.

Experimental design: The College of Veterinary Medicine at the University of Baghdad gave their blessing to this study, which was conducted at their Department of Physiology, Biochemistry, and Pharmacology. All applicable regulations regarding the treatment of animals were considered.

The animals used in the experiments have been divided into two equal groups and assigned numbers as follows:

1-Group one (HFav.): Ten healthy rabbits received a 40 mg/kg dose.BW of Favipiravir (Awamedica® pharmaceutical company-Iraq) orally through gastric gavage needles.

2-Group two (HFav.+Am): Ten healthy rabbits received 5 mg/kg of Amlodipine orally for 14 consecutive days to inhibit AO production before Favipiravir administration. Subsequently, these rabbits received a single 40 mg/kg.bw/ dose of Favipiravir orally through gastric gavage needles.

Blood sampling and timing: Blood samples (1 ml) were obtained from the marginal ear vein using the cannulation technique (cannula gauge 27) from each animal using a plastic syringe of 3 ml. The sampling time periods were scheduled after 0.15, 0.30, 0.45, 1, 2, 4, 6, 8, 12, 24, 36, 48, and 72 hours after Favipiravir administration. The blood samples were collected with anticoagulant EDTA test tubes (Al-Hanoof® company-Jordan) and centrifuged for 10 minutes at 3000 rpm, and the plasma was collected in a 1 ml Eppendorf tube. The tubes were labeled with the time and date when blood had been drawn and stored in a freezer under -20ºC until the analysis could be performed 12. The plasma samples were diluted for Favipiravir concentrations and expressed as µg/ml for analysis with a high-performance liquid chromatography (HPLC) technique.

Preparation of stock solutions: The recommended human doses of Favipiravir (Awamedica® pharmaceutical company-Iraq) and Amlodipine (Actavis® UK) were 200 mg and 10mg in each tab respectively. According to 13 human dose was converted to animal dose by using the following equation

A-Amlodipine : Human effective dose of Amlodipine (10mg/person in day).Each tablet contains (10mg) was crushed well and

dissolved in 20ml of sterile distilled water to get a concentration of (0.5mg/ml). The oral dose for rabbit is (0.5 mg/kg BW) was prepared and administered orally by giving 1ml / Kg BW using a stomach tube.

B- Favipiravir :Each tablet of Favipiravir (contain 200 mg) was crushed and dissolved in 10 ml sterile distilled water. (The effective dose of Favipiravir 800mg/person in day), then converted to (40 mg/kg BW) for rabbit, was prepared and giving 2 ml to BW as a single dose PO via stomach tube.

Determination of plasma Favipiravir concentration: For five minutes, the mixture was centrifuged at 4000 rpm. We put 20 l of the supernatant into the HPLC column. Thereafter, we followed the procedure proposed by to optimize the chromatographic conditions 14.

Pharmacokinetic analysis: Drugs were injected into an HPLC (Shimadzu, Tokyo, Japan, serial No: L215056) and chromatographic curves were calculated using 6-point calibration curves generated with various concentrations of standard solutions. Pharmacokinetic parameters were computed using the PK-Solver, non-Compartmental Analysis pharmacokinetics software tool 15.

Calibration curve of Favipiravir: Six distinct Favipiravir standard solutions from 5-30 µg/ml were used to generate calibration curves. Three separate injections of each standard solution were made into the HPLC machine under optimal chromatographic conditions. Sample and standard injection volumes were 100 µl. Peak area was quantified at 360 nm. Y= peak area, X= concentration (µg/ml), R2= 0.9999. Repeated trials (n=5) at low, medium, and high concentrations within the calibration range yielded over 98% recovery. Favipiravir was tested in 10 samples at 0.9 and 2.7 µg/ml.

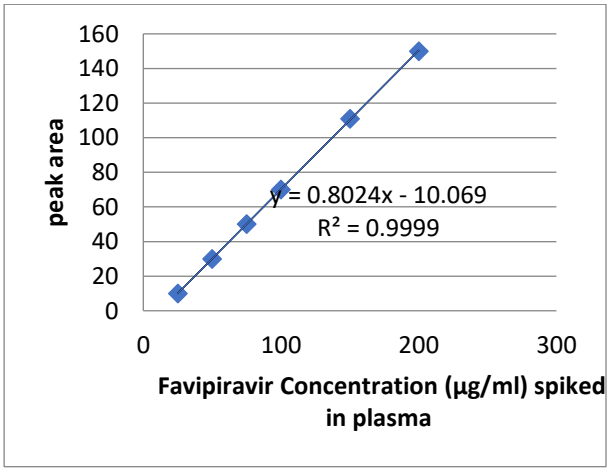
Statistical analysis: To determine the influence of numerous factors on research parameters, the Statistical Analysis System- SAS (2012) program was employed. The T-test was performed to compare means. The Chi-square test was performed to compare percentages (0.05 probability). Correlation coefficient estimation for the variables in this research has been done 16.

Results and Discussion:

Chromatographic analysis: The sample exhibited identifiable, well-resolved peaks of Favipiravir under the chromatographic conditions. The retention time of Favipiravir was found to be 4.87 ± 0.18 minutes, whereas the retention time of the internal standard was 5.00 ± 0.07 minutes. The method developed was validated for Linearity, LOD, LOQ, specificity, dilution of integrity, and recovery.

Calibration curve linearity: The calibration function (peak area ratio versus concentration) was linear over a 25–200µg/ml working range, with six points of calibration used for quantification by linear regression. The regression equation for the analysis was Y = 0.8024x–10.069 with the coefficient of correction (r2) = 0.9999 (Figure 1).

Figure 1. Calibration curve of Favipiravir in plasma as standard.



Pharmacokinetics of Favipiravir:

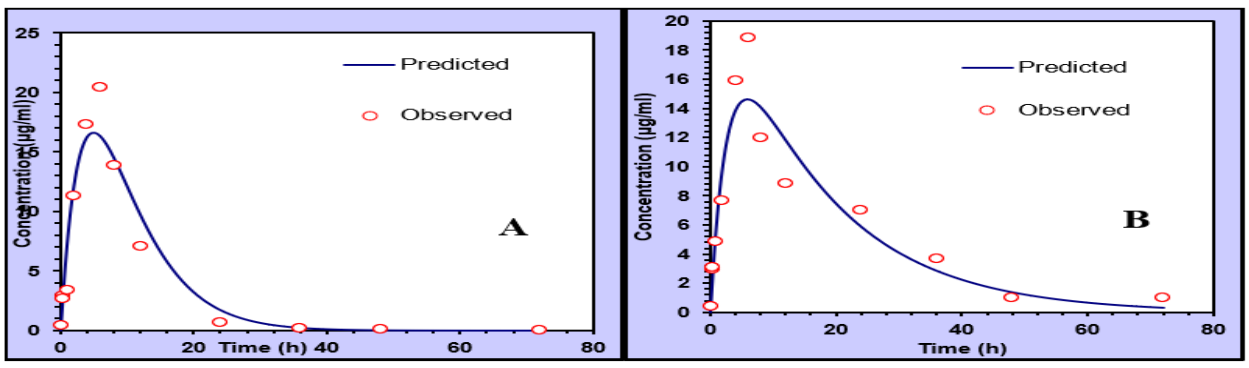
Taking into consideration a single oral 40 mg/kg B.W. administration of the Favipiravir to the rabbits, the plasma concentration of Favipiravir versus the time curve is shown in Table 2 and Figure 2. Favipiravir appeared to be highly absorbed, leading to maximal peak concentration Cmax (16.66µg/ml) in the HFav. group, which was higher than those observed in the HFav.+Am group. The absorption of the drug was evidenced by the observed absorption rate constant and absorption half-life. The HFav.+Am group showed a longer half-life and absorption rate constant (5.84 ± 0.018 and 5.41 ± 0.021 hr) than the HFav. group. Furthermore, the apparent volume of distribution in HFav.+Am (1.34 ± 0.092L) is higher than Vd in the HFav. group. In addition, the elimination half-life of Favipiravir in rabbits was found to be highly affected with Amlodipine in the HFav.+Am group compared with the HFav. group. The higher AUC appeared in the HFav.+Am group (655.08 ± 2.75). Finally, the inhibition of the AO enzyme by Amlodipine strongly affected Favipiravir clearance CL, so the results of the pharmacokinetic profile demonstrated that the healthy rabbits in HFav. group significantly have P < 0.05 higher clearance (0.179 ± 0.790) than the HFav.+Am group.

Table 1. Pharmacokinetics parameters of single oral administration of 40 mg/kg/BW Favipiravir in local rabbits.

Parameters / **Groups**	**HFav.** **Mean** ± S.E	**HFav.+Am** **Mean** ± S.E	**p-value** **t-test**
$t_{1/2}$ a (hr)	3.58 ± 0.037A	4.81 ± 0.012A	0.046
$t_{1/2}$ e (hr)	3.89 ± 0.006B	22.34 ± 0.012A	0.036*
$t_{1/2}$ka (hr)	3.26 ± 0.007A	4.36 ± 0.010A	0.041*
CL/F (L/kg/hr)	0.170 ± 0.007A	0.073 ± 0.001B	0.022*
T_{max} (hr)	4.92 ± 0.082B	8.99 ± 0.071A	0.908
C_{max} (µg/ml)	16.66 ± 0.016A	14.09 ± 0.032B	0.152
AUC 0-inf (µg/ml*hr)	223.16 ± 0.191B	525.33 ± 1.41A	0.045*
Vd (L)	0.88 ± 0.054B	1.34 ± 0.092A	0.048*
F (%)	0.95 ± 0.081A	0.96 ± 0.023A	0.019*
CL (L/hr)	0.179±0.790A	0.076±0.006B	0.067*

*(P<0.05).N=10

Figure2. Concentration of Favipiravir versus time, A: HFav. (without amlodipine).B: HFav.+Am (with amlodipine).



Multiple medicines are often used to treat COVID-19 in individuals suffering from chronic conditions such as hypertension, diabetes, and cardiovascular disease, as well as consequences such as acute respiratory distress syndrome, shock, arrhythmia, and acute renal damage 9,17. Favipiravir-related DDI data are few, unfortunately. In the present investigation, the pharmacokinetics of Favipiravir were investigated in rabbits with and without the AO inhibitor amlodipine. In rabbits, this is the first report of an Amlodipine pharmacokinetic interaction with Favipiravir. Favipiravir is extensively metabolized in the liver by AO and to a lesser level by XO to the inactive metabolite favipiravir-M1 (F-M1) and eliminated via the renal pathway 6,18. There is no involvement of cytochrome P450 isoenzymes in the metabolism of Favipiravir 6,19. Therefore, only drug with effects on AO Favipiravir were studied for their interactions. The clearance of Favipiravir is predicted to be reduced when co-administered with an AO inhibitor like Amlodipine, leading to elevated Favipiravir plasma concentrations and lower M1 concentrations. However, no studies in the literature investigate the impact of the concomitant use of an AO inhibitor on the plasma concentration of Favipiravir 20. Clinical practice should not ignore potential drug interactions resulting from AO 21. This study investigated the DDI when Favipiravir is co-administered with Amlodipine in local Iraqi rabbits. A study by PMDA (20) revealed the relationship between AO activities and Favipiravir metabolites (F-M1) in the liver cytosol of 16 people, 8 men and 8 women. There was a link between how much F-M1 formed and how much AO activity there was. In an in vitro study using human hepatic cytosol, it was found that menadione, isovanillin, and allopurinol all stopped F-M1 from forming by 73.6%, 52.6%, and 27.3%, respectively 19. These findings indicate that Favipiravir is less impacted by XO inhibition and is metabolized by AO, which is consistent with the findings of our investigation. In addition, this is the first research to offer pharmacokinetic and enzyme data about the co-administration of Favipiravir with an AO inhibitor in rabbits. According to the present study, when Favipiravir and an AO inhibitor were used together, the metabolic clearance of Favipiravir was reduced, leading to elevated plasma concentrations of the drug. When Favipiravir plasma concentration was raised, irreversible inhibition of AO occurred at a higher concentration. The plasma concentration of Favipiravir increased more quickly as a result of this inhibition compared to that seen in the absence of Amlodipine 5. From these data, we infer that the AO inhibitor has minimal effect on the plasma levels of Favipiravir in individuals with modest AO activity. So, it's reasonable to assume that when blood concentrations of AO-inhibiting medicines drop, so does their inhibitory action 19. Demir et al. 18 AO inhibition suggests that Favipiravir may limit methotrexate elimination. Contrary to predictions, co-administration of Favipiravir with Amlodipine lowered Cl, t1/2, and Cmax while increasing AUC. The reduced clearance, Cmax, and t1/2 values of Favipiravir may be due to the fact that Amlodipine dramatically lowers enzyme activity. According to a research of animals, the AO activity of Favipiravir was lower in female mice than in male mice 19. There may be changes in Favipiravir metabolism and enzyme activity based on species, race, and gender 22. In addition, the outcomes of this study may have been influenced by the provided dosage, the variation across species, and the substantial impact on pharmacokinetic parameters. The current study's findings are congruent with those of Obach et al. 23, who discovered that

amlodipine had a strong inhibitory effect on AO in human cytosol. Additionally, Barr et al 24. dietary elements such as green tea revealed weak to moderate suppression of AO 25. Finally, Asken et al. 5 concluded that when using allopurinol as an AO inhibitor, simultaneous use of Favipiravir with other medications that impact AO and/or XO enzyme activity may produce changes in the pharmacokinetic profiles of pharmaceuticals and the levels of enzymes that metabolize drugs.

Conclusion:

It could be concluded that the simultaneous administration of Amlodipine with Favipiravir caused significant changes in the pharmacokinetics of Favipiravir; therefore, dosage rate adjustment is strongly recommended.

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Conflict of Interest: The authors declare no conflict of interest.

Reference

Subramanian, A., Adhimoolam, M., & Kannan, S. (2018). Study of drug–Drug interactions among the hypertensive patients in a tertiary care teaching hospital. Perspectives in clinical research, 9(1), 9.

AL-Rekabi, F. M. K., Asker, S. J., & Shwaish, M. M. (2018). Acute and subchronic interaction between metformin and meloxicam in mice. Research Journal of Pharmacy and Technology, 11(6), 2336-2344.

Baranovich T, Wong SS, Armstrong J, Marjuki H, Webby RJ, Webster RG, Govorkova EA. T-705 (favipiravir) induces lethal mutagenesis in influenza A H1N1 viruses in vitro. Journal of virology. 2013 Apr 1;87(7):3741-51.

Kiso M, Takahashi K, Sakai-Tagawa Y, Shinya K, Sakabe S, Le QM, Ozawa M, Furuta Y, Kawaoka Y. T-705 (favipiravir) activity against lethal H5N1 influenza A viruses. Proceedings of the National Academy of Sciences. 2010 Jan 12;107(2):882-7.

Özek da, keskin z, Hande yü, Türkmen nb, Aslan S, Unüvar S. Drug-Drug Interaction of Aldehyde Oxidase Inhibitor and Xanthine Oxidase Inhibitor with Favipiravir. Sağlık Bilimlerinde Değer. 2022,12(3):520-8.

Madhloom BN, Diajil AR. Oxidative stress status in hypertensive patients on amlodipine treatment. Journal of Baghdad College of Dentistry. 2020 Mar 15;32(1):1-8.

Mishima E, Anzai N, Miyazaki M, Abe T. Uric acid elevation by favipiravir, an antiviral drug. The Tohoku journal of experimental medicine. 2020;251(2):87-90.

Alhamdani FY. Possible beneficial effects of amlodipine, lisinopril, and their combination on lipid profile in hypertensive patients. The Iraqi Journal of Veterinary Medicine. 2009 Dec 31;33(2):126-37.

Shirley M, McCormack PL. Perindopril/amlodipine (Prestalia®): a review in hypertension. American Journal Of Cardiovascular Drugs. 2015 Oct;15:363-70.

Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y. Clinical characteristics of 138 hospitalized patients with 2019 novel

coronavirus–infected pneumonia in Wuhan, China. jama. 2020 Mar 17;323(11):1061-9.

Naser NH, Alibeg AA. Exacerbation of COVID 19 in Hypertensive Patients (A review). Iraqi Journal of Pharmaceutical Sciences. 2021 Dec 9;30(2):23-30.

Negru PA, Radu AF, Vesa CM, Behl T, Abdel-Daim MM, Nechifor AC, Endres L, Stoicescu M, Pasca B, Tit DM, Bungau SG. Therapeutic dilemmas in addressing SARS-CoV-2 infection: Favipiravir versus Remdesivir. Biomedicine & Pharmacotherapy. 2022 Mar 1;147:155.

Harahap Y, Diptasaadya R, Purwanto DJ. Volumetric absorptive microsampling as a sampling alternative in clinical trials and therapeutic drug monitoring during the COVID-19 pandemic: a review. Drug Design, Development and Therapy. 2020 Dec 31:5757-71.

Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. Journal of basic and clinical pharmacy. 2016 Mar;7(2):27-31.

Bulduk İ. HPLC-UV method for quantification of favipiravir in pharmaceutical formulations. Acta Chromatographica. 2021 Apr 28;33(3):209-15.

Al-Rikabi, F. M. K. Evaluation of selected parameters of rat liver injury following repeated administration of oseltamivir for different periods. The Iraqi Journal of Veterinary Medicine, 2012.36(1), 145–156.

Zhang Y, Huo M, Zhou J, Xie S. PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. Computer methods and programs in biomedicine. 2010 Sep 1;99(3):306-14.

SAS Institute. SAS user's guide: statistics. Sas Institute; 2012.

Yue H, Bai X, Wang J, Yu Q, Liu W, Pu J, Wang X, Hu J, Xu D, Li X, Kang N. Clinical characteristics of coronavirus disease 2019 in Gansu province, China. Ann Palliat Med. 2020:1404-12.

Demir E, Sütcüoğlu O, Demir B, Ünsal O, Yazıcı O. A possible interaction between favipiravir and methotrexate: Drug-induced hepatotoxicity in a patient with osteosarcoma. Journal of Oncology Pharmacy Practice. 2022 Mar;28(2):445-8.

Pharmaceuticals and Medical Devices Agency (PMDA). Report on the deliberation results: avigan. Japan; Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau. 2014.

Ahmad RM, Ibrahim OM. Effect of erythromycin on pharmacokinetics of Amlodipine in hypertensive rabbits. Online Journal of Veterinary Research. 2019;23(6):555-67.

Madelain V, Nguyen TH, Olivo A, De Lamballerie X, Guedj J, Taburet AM, Mentré F. Ebola virus infection: review of the pharmacokinetic and pharmacodynamic properties of drugs considered for testing in human efficacy trials. Clinical pharmacokinetics. 2016 Aug;55(8):907-23.

Du YX, Chen XP. Favipiravir: pharmacokinetics and concerns about clinical trials for 2019‐nCoV infection. Clinical Pharmacology & Therapeutics. 2020 Aug;108(2):242-7.

Hanioka N, Saito K, Isobe T, Ohkawara S, Jinno H, Tanaka‐Kagawa T. Favipiravir biotransformation in liver cytosol: Species and sex differences in humans, monkeys, rats, and mice. Biopharmaceutics & Drug Disposition. 2021 May;42(5):218-25.

Obach RS, Huynh P, Allen MC, Beedham C. Human liver aldehyde oxidase: inhibition by 239 drugs. The Journal of Clinical Pharmacology. 2004 Jan;44(1):7-19.

Barr JT, Jones JP, Oberlies NH, Paine MF. Inhibition of human aldehyde oxidase activity by diet-derived constituents: structural influence, enzyme-ligand interactions, and clinical relevance. Drug Metabolism and Disposition. 2015 Jan 1;43(1):34-41.

Mahmood MK, Anwer ZM. The Prevalence of Potentially Inappropriate Prescribing in Geriatric Patients with Psychiatric Disorders in Iraq. Iraqi Journal of Pharmaceutical Sciences. 2020 Jun 25;29(1):166-73.