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Drug-Drug Interaction of Aldehyde Oxidase Inhibitor and Xanthine Oxidase Inhibitor with Favipiravir*

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ABSTRACT

Aim: Favipiravir is an effective antiviral used in the treatment of COVID-19. It is metabolized by aldehyde oxidase (AO) and xanthine oxidase (XO). This study investigated drug-drug interactions between favipiravir with both AO substrate and XO enzyme inhibitor, allopurinol, and an XO inhibitor, verapamil.

Material and Methods: 25 Sprague-Dawley female rats, 250-300 g, were divided into five equal groups. Blood samples were taken from the jugular vein at the end of 0, 15, 30, and 45 minutes, and at the end of the 1st, 2nd, 4th, 6th, and 8th hours after the drugs were administered. The drug-blood concentration was determined in the HPLC-UV device using plasma. The ELISA method measured AO and XO enzyme activities in rat liver tissue.

Results: Allopurinol prolonged the time taken for favipiravir to reach C_{max} (T_{max}), decreased maximum serum concentration (C_{max}), elimination half-life ($T_{1/2}$), area under the curve (AUC), and mean residence time (MRT). Allopurinol significantly reduced clearance per unit time (Cl/f) when co-administered with favipiravir. Verapamil accelerated the elimination of favipiravir, significantly reducing $T_{1/2}$, MRT, and AUC. On the other hand, Favipiravir decreased the absorption of verapamil and slowed its elimination. C_{max}, AUC, and Cl values of verapamil decreased. In addition, $T_{1/2}$, MRT, and volume of distribution (V_d) increased.

Conclusion: In conclusion, the concomitant use of favipiravir with other drugs that affect AO and/or XO enzyme activities may cause changes in the pharmacokinetic profiles of drugs and the levels of enzymes that metabolize drugs.

Keywords: Favipiravir; allopurinol; verapamil; pharmacokinetics; aldehyde oxidase

Aldehit Oksidaz İnhibitörü ve Ksantin Oksidaz İnhibitörünün Favipiravir ile İlaç-İlaç Etkileşimi

ÖZ

Amaç: Favipiravir, COVID-19 tedavisinde kullanılan etkili bir antiviraldir. Aldehit oksidaz (AO) ve ksantin oksidaz (KO) tarafından metabolize edilir. Bu çalışmada, favipiravir ile hem AO substratı hem de KO enzim inhibitörü allopurinol ve KO inhibitörü verapamil arasındaki ilaç-ilaç etkileşimleri araştırıldı.

Gereç ve Yöntemler: 250-300 g ağırlığındaki 25 adet Sprague-Dawley dişi sıçan beş eşit gruba ayrıldı. İlaç uygulandıktan sonra 0, 15, 30, 45 dakika sonunda ve 1., 2., 4., 6. ve 8. saatlerin sonunda jugular venden kan örnekleri alındı. İlaç-kan konsantrasyonu, plazma kullanılarak HPLC-UV cihazında belirlendi. Sıçan karaciğer dokusunda AO ve KO enzim aktiviteleri ELISA yöntemi ile belirlendi.

Bulgular: Allopurinol, favipiravirin C_{max}'a (T_{max}) ulaşması için geçen süreyi uzatmış, maksimum serum konsantrasyonunu (C_{max}), eliminasyon yarı ömrünü ($T_{1/2}$), eğrinin altındaki alanı (EAA) ve ortalama kalış süresini (MRT) azaltmıştır. Allopurinol, favipiravir ile birlikte uygulandığında birim zaman başına klirensi (Cl/f) önemli ölçüde azaltmıştır. Verapamil, favipiravirin eliminasyonunu hızlandırarak $T_{1/2}$, MRT ve EAA'yı önemli ölçüde azaltmıştır. Favipiravir ise verapamil emilimini azalttı ve eliminasyonunu yavaşlattı. Verapamilin C_{max}, EAA, Cl değerleri azaldı. Ayrıca $T_{1/2}$, MRT ve dağılım hacmi (V_d) arttı.

Sonuç: Sonuç olarak, favipiravirin AO ve/veya KO enzim aktivitelerini etkileyen diğer ilaçlarla birlikte kullanılması ilaçların farmakokinetik profillerinde ve ilaçları metabolize eden enzim düzeylerinde değişikliklere neden olabilir.

Anahtar Kelimeler: : Favipiravir; allopurinol; verapamil; farmakokinetik; aldehit oksidaz.

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INTRODUCTION

Favipiravir is a new anti-viral agent that selectively and potently inhibits the RNA-dependent RNA polymerase (RdRp) of RNA viruses. Favipiravir can inhibit the replication of flavi-, alpha-, phyllo-, bunya-, arena-, neuro-, and other RNA viruses (1). It is converted in cells into an active phosphorylated form and recognized as a substrate by viral RNA polymerase, hence inhibiting RNA polymerase action. Therefore, it was thought that favipiravir may have a potent antiviral effect on SARS-CoV2, which is an RNA virus (2). The oral bioavailability of favipiravir is more than 95%. Favipiravir is metabolized by aldehyde oxidase (AO) and xanthine oxidase (XO). However, as the favipiravir is both metabolized and inhibited by AO, an initial oral loading dose is required to achieve adequate blood levels. The plasma half-life is 4 hours in humans (3). Cytochrome P450 isoenzymes are not involved in the metabolism of favipiravir (4). Therefore, the use of favipiravir with other drugs that affect the XO and/or AO enzyme activities may cause the pharmacokinetic profiles of the drugs to change.

Allopurinol is widely used for the long-term treatment and prevention of chronic gout. It is in a class of medications called xanthine oxidase inhibitors. Allopurinol is metabolized to the corresponding xanthine analog, oxypurinol (alloxanthine), which also is an inhibitor of XO (5-7).

Verapamil is a phenylethylamine calcium channel blocker used for treating cardiovascular disease. It is a member of the non-dihydropyridine class of calcium channel blockers. Among this group of drugs, verapamil is the most effective inhibitor of AO (5).

Co-administration of favipiravir with a potent AO and/or XO inhibitor may reduce the antiviral efficacy. At the same time, drugs that are metabolized with the same enzyme can change each other's drug-blood concentrations. It is important to know both the antiviral effectiveness of favipiravir not decreasing and its interaction with frequently used drugs, to make drug-dose adjustments in terms of the effectiveness of treatments and prevention of toxicity. However, the interaction of favipiravir with an AO and/or XO inhibitor drug has not yet been investigated with pharmacokinetic parameters and enzyme activities.

Therefore, in this study, drug interaction at the pharmacokinetic and enzyme activity was investigated between favipiravir, which is used successfully in the treatment of COVID-19, with allopurinol and verapamil.

MATERIAL AND METHODS

Animals and treatment

All animals received humane care by the guidelines set by the Institutional Animal Use and Care Committee of Firat University and the protocol of the experiment was confirmed by the same committee (Ethical approval number; 2021/02). Female Sprague-Dawley rats (250-300 g) were obtained from the Laboratory of Experimental Animals of Firat University. The sample size was calculated using G Power software. Groups of animals were presented in Table 1. The animals were provided with a standard pellet diet and ad libitum water.

Table 1. Grouping and treatment schedule.

	Group (n=5)	Dose, route, and duration
1	Favipiravir	Favipiravir 0.5 % CMC, 50 mg/kg oral once on 1st. day, 30 mg/kg for 7 days.
2	Allopurinol	Allopurinol 0.5% CMC, 20 mg/kg oral for 7 days.
3	Allopurinol +Favipiravir	Allopurinol 0.5% CMC, 20 mg/kg oral for 7 days. Favipiravir 0.5% CMC, 50 mg/kg oral once on 6th day, 30 mg/kg on 7th day.
4	Verapamil	Verapamil 0.5% CMC, 10 mg/kg oral for 7 days.
5	Verapamil +Favipiravir	Verapamil 0.5% CMC, 10 mg/kg oral for 7 days. Favipiravir 0.5% CMC, 50 mg/kg oral once on 6th day, 30 mg/kg on 7th day.

CMC: Carboxymethyl cellulose

Blood sampling

Blood samples were collected at 0, 15, 30, and 45 min and 1, 2, 4, 6 and 8 h after ketamine (5 mg/kg)–xylazine (40 mg/kg) administration. Samples were collected into tubes containing EDTA from jugular veins and were separated to sera by centrifugation at 4 °C for 30 min at 3.500 rpm and the samples were stored at -80 °C until analysis. After the end of the experiment, rats were sacrificed according to animal use guidelines.

Preparation of stock solutions

Verapamil (100 µg/ml) and favipiravir (300 µg/mL) stock solutions were prepared in methanol. Allopurinol (200 µg/ml) stock solution was prepared in dichloromethane.

Determination of favipiravir level

Plasma was dissolved at +4 °C. 0.3 ml of methanol was added to 0.2 ml of plasma. The mixture was centrifuged at 4000 rpm for 5 minutes. A 20 µl of the supernatant was injected directly into the HPLC column. Chromatography conditions were adjusted according to the method suggested by Bulduk (8).

Determination of allopurinol level

Plasma was dissolved at +4 °C. 0.05 ml of 10% perchloric acid was added to 0.1 ml of plasma. After the mixture was kept in the refrigerator for 10 minutes, 0.2 ml of dichloromethane was added, vortexed, and centrifuged at 4000 rpm for 5 minutes. 60 µl of the supernatant was injected directly into the HPLC column (9).

Determination of verapamil level

Plasma was dissolved at +4 °C. 0.06 ml of 5 mol/l sodium hydroxide was added to 0.2 ml of plasma and the mixture was vortexed. 5 ml of n-hexane-ether (30:70, v/v) was added to the mixture and extracted for 10 minutes, and this process was repeated 3 times. After the obtained supernatant was dried under nitrogen gas, the residue was dissolved in 0.2 ml methanol. 160 µl of the solution was injected into the HPLC column (10).

Pharmacokinetic analysis

Chromatographic images obtained by injecting drugs into the high-performance liquid chromatography (HPLC-UV) (Shimadzu, Tokyo, Japan) device were calculated according to 6-point calibration curves prepared with different concentrations of standard solutions for each drug. The HPLC system consists of a pump (LC-20AT controlled by CBM-20A), an auto-sampler (SIL-20A), a degasser (DGU-20A), a column oven (CTO-20A), and a

UV-VIS (SPD- 20A) consisted of the detector. Pharmacokinetic parameters were calculated by using the PKSolver Non-Compartmental Analysis pharmacokinetics software program.

Determination of aldehyde oxidase and xanthine oxidase activity

Rats were sacrificed, and liver tissues were removed. To thoroughly remove excess blood, tissues were rinsed in ice-cold phosphate-buffered saline (PBS) (pH 7.4) and weighed before homogenization. Tissues were taken in the required quantity and homogenized in PBS (tissue weight (g): PBS (ml) volume=1:9) with a glass homogenizer on ice. The homogenates were then centrifuged at 5000g for 5 minutes to obtain the supernatant.

Liver tissue concentrations of AO and XO were determined by the ELISA technique using commercially available kits (ER0670, E1263Ra; Fine Test, Bioassay Technology Laboratory, BT Lab respectively) by the manufacturer's instructions. The results were evaluated in ng/ml for AO and XO.

Statistical Analysis

Descriptive data were analyzed using the IBM SPSS 22.0 package program (SPSS Inc., Chicago, IL, United States). Descriptive statistics of the data are presented as Mean±SD. Homogeneity of variance was analyzed using the Levene test and the assumption of normality was analyzed using the Shapiro-Wilk test. Mann-Whitney U test was used to compare pharmacokinetic parameters. One-way analysis of variance (ANOVA) and post hoc Tukey HSD test were used for statistical comparison of enzyme activities. $p<0.05$ was considered statistically significant.

The statistical power of the sample size was evaluated with an arbitrarily established effect size of 0.25(α=0.05). With the available sample size, the power was 0.998. Power calculations were made using the program G*Power 3.1.

RESULTS

Linearity and recovery

Calibration curves were created using HPLC-LC Solution 2013.

Calibration curve of favipiravir

Calibration curves were prepared with 6 different standard solutions of favipiravir in the range of 5-30 µg/ml. Each standard solution was injected three times into the HPLC device under appropriate chromatographic conditions. The linear equation of the generated calibration curve was as follows: y= 81995x + 448.3, R^2= 0.9999 [Y= peak area, X= concentration (µg/ml)]. As a result of repeated studies (n=5) at low, medium, and high concentrations within the calibration range, the recovery was more than 98%. Samples were studied for favipiravir (n=10), with a limit of detection (LOD) of 0.9 µg/ml and a limit of measurement (LOQ) of 2.7 µg/ml (Figure 1B, 2E, 2F).

Calibration curve of allopurinol

Calibration curves were prepared with 6 different standard solutions in the range of 20-120 µg/ml of allopurinol. Each standard solution was injected three times into the HPLC device under appropriate chromatographic conditions. The linear equation of the generated calibration curve was as follows: y= 939x-8304, R^2= 0.9999 [Y= peak area, X= concentration (µg/ml)]. As a result of repeated studies (n=5) at low, medium, and high concentrations within the calibration range, the recovery was more than 95%. Samples were studied (n=10), and the LOD was determined as 0.004 µg/ml and the LOQ as 0.016 µg/ml (Figure 1C, 2C, 2D).

Calibration curve of verapamil

Calibration curves were prepared with 6 different standard solutions of verapamil in the range of 1-40 µg/ml. Each standard solution was injected three times into the HPLC device under appropriate chromatographic conditions. The linear equation of the generated calibration curve was as follows: y= 4439x-4914.5, R^2= 0.9982 [Y= peak area, X= concentration (µg/ml)]. As a result of repeated studies (n=5) at low, medium, and high concentrations within the calibration range, the recovery was more than 90%. Samples were studied (n=10), with a LOD of 0.1 µg/ml and a LOQ of 0.3 µg/ml (Figure 1A, 2A, 2B).

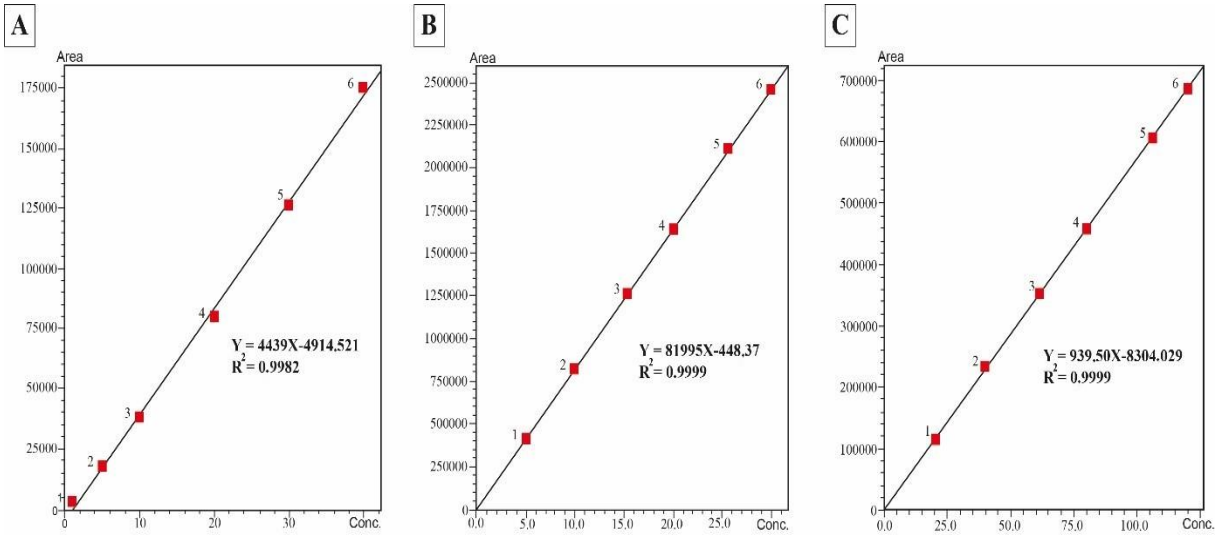

Figure 1. Six-point calibration curve and linear equation for verapamil, favipiravir, and allopurinol standards [A: Verapamil, B: Favipiravir, C: Allopurinol]

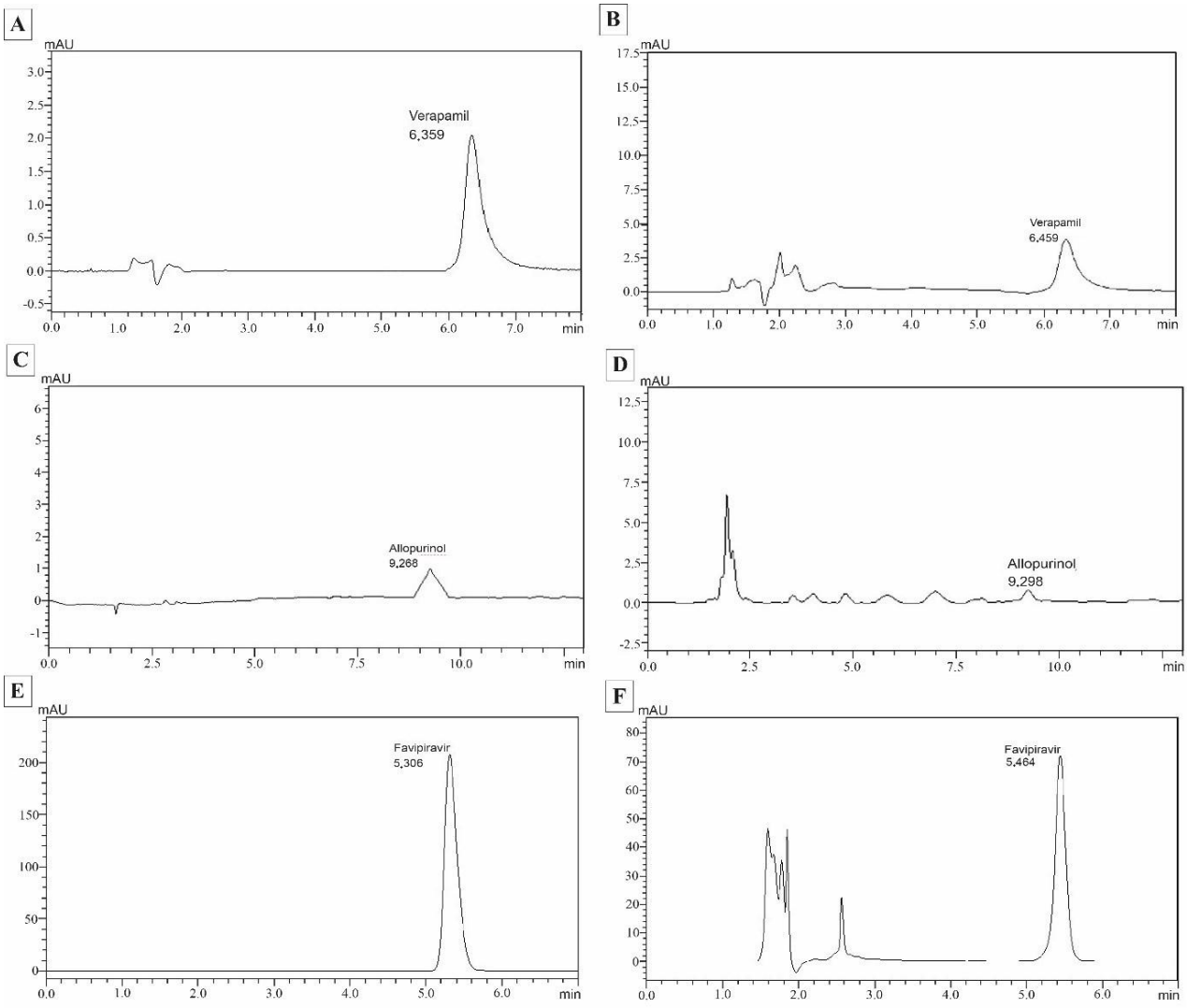

Figure 2. Chromatograms of verapamil, allopurinol, and favipiravir analysis in standard solution and plasma [A: Verapamil standard solution 10 µg/ml, B: Verapamil 4th hour plasma at; C: Allopurinol standard solution 20 µg/ml, D: Allopurinol 6th hour plasma; E: Favipiravir standard solution 20 µg/ml, F: Favipiravir 6th hour plasma]

Pharmacokinetic parameters

Allopurinol, favipiravir prolonged time taken to reach C_{max} (T_{max}), decreased the maximum serum concentration (C_{max}), elimination half-life ($T_{1/2}$), area under the curve (AUC), and mean residence time (MRT). When allopurinol was used in combination with favipiravir, its clearance per unit time (Cl/f) was significantly reduced (p=0.008) (Table 2). Verapamil speeds ups the clearance of favipiravir and significantly reduces the $T_{1/2}$, MRT, and AUC values (p=0.008) (Table 2). However, favipiravir reduced the absorption of verapamil and slowed its elimination. Verapamil C_{max}, AUC, and Cl values decreased. $T_{1/2}$, MRT, and volume of distribution (V_d) increased (Table 2, Figure 3).

Aldehyde oxidase and xanthine oxidase activities

Favipiravir, an XO substrate, decreased XO activity more than inhibition of allopurinol, and this activity was not changed in the combined group (p<0.0001) (Figure 4A). Administration of favipiravir and allopurinol separately and together did not significantly affect AO activity (p<0.0001) (Figure 4B). Favipiravir, which binds to XO as a substrate, significantly reduced enzyme activity compared to verapamil. In the group administered verapamil and favipiravir together, the XO activity approached the group in which only favipiravir was administered (p<0.0001) (Figure 4C). Verapamil administration did not cause a significant change in XO activity. AO activity was significantly decreased in the favipiravir and combined group compared to the verapamil group (p<0.0001) (Figure 4D).

Table 2. Pharmacokinetic parameters of favipiravir, allopurinol, and verapamil (Mean±SD) (n=5)

Concentration (µg/ml)	*Favipiravir*			*Allopurinol*		*Verapamil*	
Groups	*Fav*	*Fav+Allo*	*Fav+Ver*	*Allo*	*Fav+Allo*	*Ver*	*Fav+Ver*
$t_{1/2}$ *(h)*	16.17±0.41	13.96±0.34[a]	13.52±0.54[a]	4.78±0.26	5.02±0.21	8.82±0.25	46.51±2.94[c]
T_{max} *(h)*	0.50±0	1.00±0[a]	0.50±0	6.00±0.01	4.00±0.01[b]	1.00±0	1.00±0
C_{max} *(µg/ml)*	921.75±0.95	794.30±0.90[a]	946.23±20.03	2413.52±7.02	2102.70± 9.02[b]	33.47±1.00	15.40±4.02[c]
*AUC 0-t (µg/ml*h)*	5146.31±4.12	5390.99±230.53	4855.47±36.74[a]	16428.86±306.02	16886.95± 227.49	160.71± 2.29	74.00±6.82[c]
*AUC 0-∞ (µg/ml*h)*	18042.86±12.75	16796.15±10.87[a]	14722.94±42.56[a]	22573.71±12.47	23045.14± 17.81[b]	353.69± 7.68	620.19±29.25[c]
AUC 0-t/0-∞	0.29±0.02	0.32±0.06	0.33±0.12	0.73±0.01	0.73±0.01	0.45±0.04	0.12±0.03[c]
*AUMC 0-∞ (µg/ml*h^2)*	423483.5±154.15	341733.9±146.16[a]	289354.8±384.58[a]	219311.50±9.52	217459.90± 8.14[b]	4597.11± 6.86	41299.06± 17.37[c]
MRT 0-∞ (h)	23.47±0.57	20.35±0.18[a]	19.65±0.65[a]	9.7±0.01	9.44±0.01[b]	13.00±0.72	66.59±1.02[c]
V_d *(mg)/(µg/ml)*	0.039±0.0021	0.030±0.01[a]	0.040±0.01	0.0061±0.0001	0.0063± 0.0001	0.36±0.02	1.08±0.09[c]
Cl (mg)/(µg/ml)/h	0.0017±0.0002	0.0015±0.0011	0.0020±0.0003[a]	0.0089±0	0.0087±0[b]	0.028±0.002	0.016±0.002[c]

[a]: Significant compared to Favipiravir group, [b]: Significant compared to Allopurinol group, [c]: Significant compared to Verapamil group (p=0.008) (Mann Whitney U test was used.) (Fav: Favipiravir, Allo: Allopurinol, Ver: Verapamil, $T_{1/2}$: Elimination half-life, T_{max}: Time taken to reach C_{max}, C_{max}: Maximum serum concentration, AUC: Area under the curve, MRT: Mean residence time, V_d: Volume of distribution, Cl: Clearance)

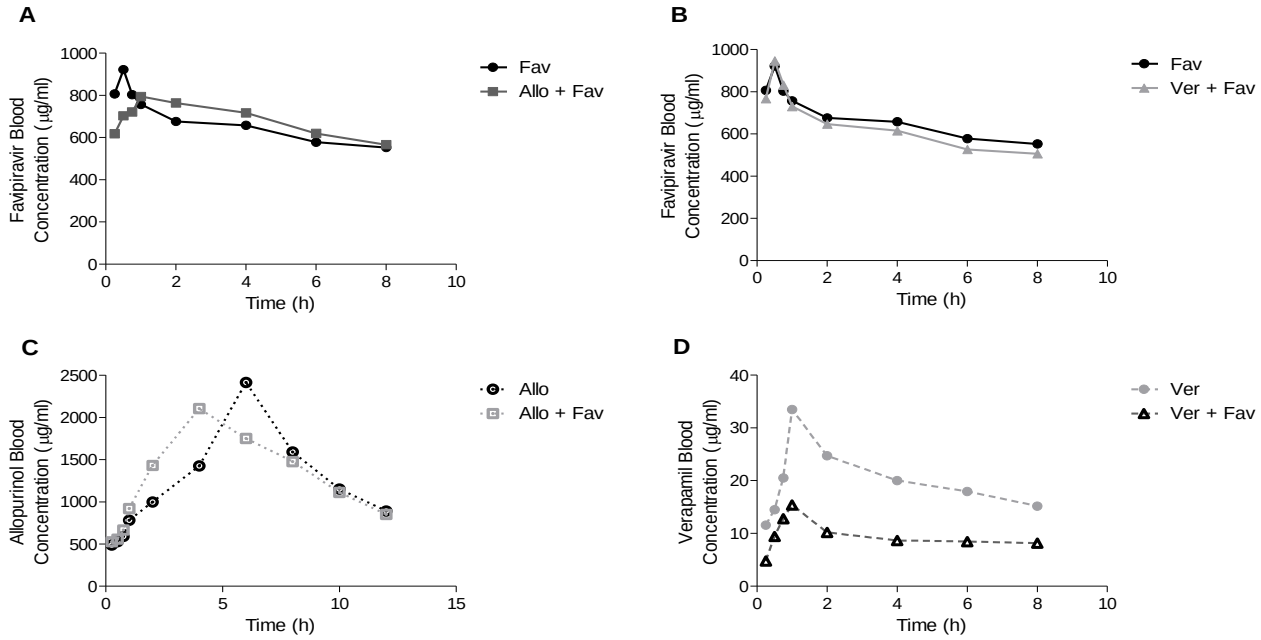

Figure 3. Time-dependent blood concentrations of favipiravir, allopurinol, and verapamil

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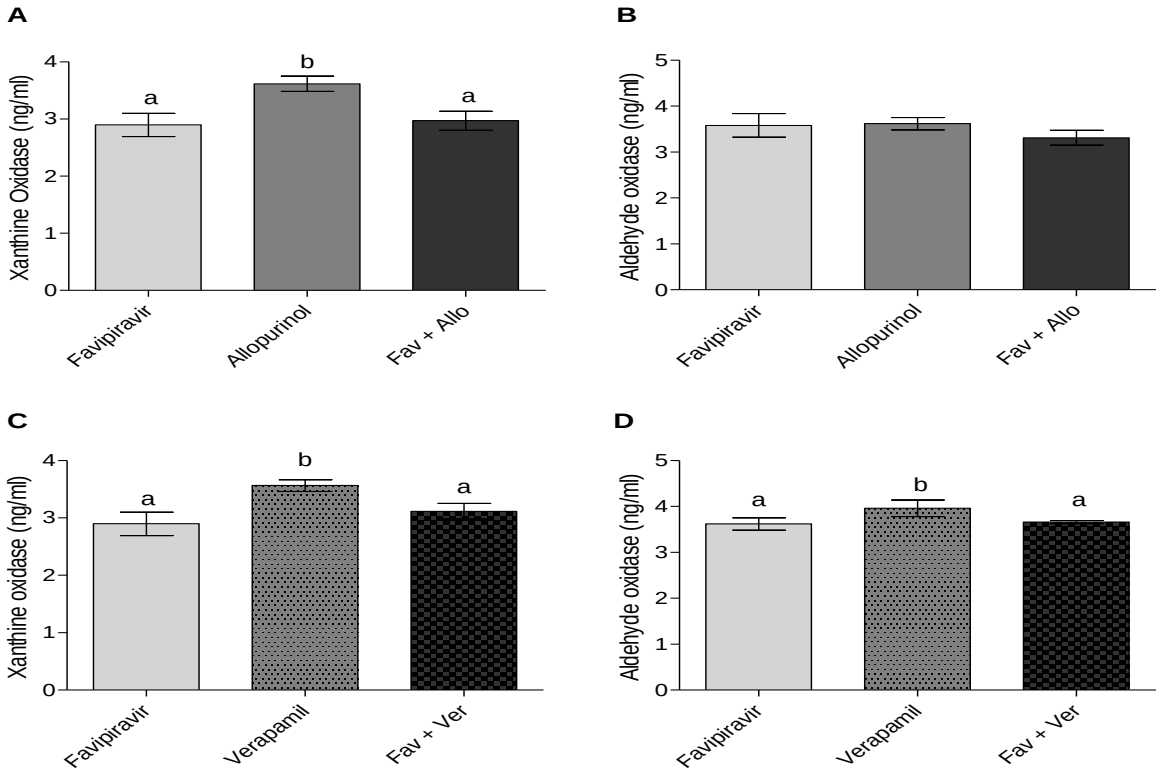

Figure 4. AO and XO enzyme activities in liver tissue (p=0.008) All values were stated as mean ± SD (n = 5). The probability value is p<0.0001, and values with different superscripts in the same column are statistically significantly different, using one-way ANOVA, followed by Tukey's test as a post-hoc analysis. (Fav: Favipiravir, Allo: Allopurinol, Ver: Verapamil)

DISCUSSION

Favipiravir is extensively metabolized to an inactive metabolite favipiravir-M1 (F-M1) by AO and lesser extent by XO in the liver and excreted by the renal route (11-13). Cytochrome P450 isoenzymes do not contribute to the metabolism of favipiravir (4, 11). For this reason, only the interaction of drugs that have effects on AO and XO with favipiravir was investigated in this study. When favipiravir is co-administered with an AO inhibitor such as verapamil or a XO inhibitor such as allopurinol, it is expected that the clearance of favipiravir will decrease, resulting in increased favipiravir plasma concentration and decreased M1 concentration. However, there are no studies in the literature investigating the effect of concomitant use of an AO inhibitor or a XO inhibitor on the plasma concentration of favipiravir (12). Potential drug interactions resulting from AO and XO inhibition should not be ignored in clinical practice (14). In this study, the drug−drug interaction when co-administered favipiravir with allopurinol, a XO inhibitor, and an AO substrate, was investigated for both drugs.

As a result of XO inhibition by allopurinol, the plasma concentration of favipiravir is expected to increase. However, according to our findings, although there was no significant change in favipiravir clearance, $T_{1/2}$, and MRT values were decreased. When this situation is evaluated together with enzyme activity, we show that favipiravir is a substrate for XO and that allopurinol inhibition does not significantly affect the substrate drug-enzyme relationship. When favipiravir, also an AO substrate, was used with allopurinol, the amount of AO did not change. In this result, it can suggest that favipiravir competitively binds to AO. $T_{1/2}$, MRT, and AUC values of favipiravir metabolized by AO decreased in the combined group. For favipiravir, prolongation of T_{max} and decreased C_{max} and V_d may also indicate interaction at the level of absorption and distribution. However, more research is needed to reach this conclusion.

In a study, the correlation between AO and XO activities and F-M1 was investigated in human liver cytosol samples from 8 men and 8 women. There was a relationship between F-M1 formation level and AO activity, but no relationship was found between F-M1 formation and XO activity. In an *in vitro* study using human hepatic cytosol, it was reported that menadione and isovanillin (AO inhibitors), and allopurinol (XO inhibitor) inhibited F-M1 formation by 73.6%, 52.6%, and 27.3%, respectively (4). The less inhibition by allopurinol may be the result of favipiravir being metabolized mainly by AO and not by XO. These results show that favipiravir is less affected by XO inhibition and is metabolized by AO, consistent with our study. In addition, our study is the first to present pharmacokinetic and enzyme data on the co-administration of favipiravir with an AO substrate and XO inhibitor.

The decreased clearance of allopurinol in the combined group may be explained by the competitive binding of favipiravir to AO. The decrease in T_{max}, $C_{max,}$ and AUC for allopurinol suggests that it may also interact with favipiravir during the absorption and distribution phases.

Concomitant use of favipiravir with an AO inhibitor decreased the metabolic clearance of favipiravir, resulting in increased plasma concentrations of favipiravir. Increasing plasma concentration of favipiravir increased irreversible inhibition to AO. This inhibition resulted in a more rapid increase in plasma favipiravir concentration than (without a concomitant AO inhibitor) and XO is metabolized differently by non-AO enzymes such as aldehyde dehydrogenase. Based on these results, we can suggest that in subjects with low AO activity, plasma favipiravir concentration is not much affected by the AO inhibitor. Therefore, it can be thought that the inhibitory effect against AO decreases depending on the decrease in blood concentrations of drugs that inhibit AO (4). Demir et al. suggested that favipiravir may inhibit methotrexate elimination by inhibiting aldehyde oxidase (13). Contrary to expectations, we found that when favipiravir was co-administered with verapamil, an AO inhibitor, Cl increased and $t_{1/2}$, MRT, $C_{max,}$ and AUC values decreased. However, we found that verapamil Cl decreased and $t_{1/2}$, MRT values increased.

In addition, the metabolism of favipiravir was not affected, since verapamil did not cause a significant change in XO activity. The increased clearance of favipiravir and the decreased $t_{1/2}$, MRT, $C_{max,}$ and AUC values may be explained by the fact that the enzyme activities are not significantly reduced by verapamil. The decrease in C_{max} and AUC of both drugs was the result of interaction at the level of absorption. It has been observed that favipiravir irreversibly inhibits the enzyme AO, which is mainly metabolized, depending on concentration and time (3).

In a study conducted on experimental animals, it was reported that the AO activity of favipiravir was lower in female mice than in males (4). There may be species, race, and sex differences in favipiravir metabolism and enzyme activities (15). The results of this study may have been affected by the dose administered, the difference between species, and the fact that the study was conducted under anesthesia.

In the treatment of COVID-19, the use of multiple drugs is common in patients with chronic diseases such as hypertension, diabetes, and cardiovascular diseases and complications such as acute respiratory distress syndrome, shock, arrhythmia, and acute kidney injury (16, 17). Unfortunately, information on drug-drug interactions caused by favipiravir is limited. Favipiravir is metabolized in the liver by AO in the cytosol, but not by enzymes in microsomes. Published data on whether favipiravir and its active metabolite T-705-RTP affect the activities of hepatic drug-metabolizing enzymes are not yet available. A study in healthy volunteers found that co-administration of favipiravir with acetaminophen increased the AUC of acetaminophen and acetaminophen glucuronide by 20% and 23-34%, respectively, but decreased the AUC of acetaminophen sulfate by 29-35%. In addition, acetaminophen and acetaminophen glucuronide excretion increased in urine as a result of the combined use of these two drugs. Co-incubation of favipiravir with human liver cell S9 inhibits the formation of acetaminophen sulfate with an IC_{50} of 24 μg/ml. It was thought that this inhibition resulted from the inhibition of sulfate transferase (18).

In vitro studies have shown that selective estrogen receptor modulators such as raloxifene, tamoxifen, and estradiol, H2 receptor antagonists such as cimetidine, calcium
channel blockers such as felodipine, amlodipine and verapamil, anti-arrhythmic drugs such as propafenone, and tricyclic antidepressants such as amitriptyline are potent AO inhibitors (19). Potential drug-drug interactions between these drugs and favipiravir should be carefully monitored. Various drugs such as citalopram, zaleplon, famciclovir, and sulindac are also metabolized by AO (20). In vitro studies have shown that favipiravir is a concentration and dose-dependent inhibitor of AO at concentrations between 3.14 and 942 μg/mL (21). Therefore, potential drug-drug interactions between favipiravir and these second drugs should be carefully monitored at both pharmacokinetic and pharmacodynamic levels (22).

In preclinical studies, the efficacy, toxicity, and pharmacokinetics of favipiravir were evaluated using animal models such as mice, rats, dogs, and monkeys (23). Common side effects when favipiravir was used in the treatment of influenza in clinical studies included mild to moderate diarrhea, increases in blood uric acid and transaminases, and decreases in neutrophil and leukocyte counts (24). Although the cause and mechanism of these adverse events have not yet been elucidated, the incidence of adverse events is thought to be related to AO, which catalyzes the inactive metabolism of favipiravir. In addition, properties such as enzymatic activity and substrate specificity vary greatly between humans and preclinical species (25).

A recent study was conducted investigating the pharmacokinetics of favipiravir in 21 patients with COVID-19 (26). Based on the study results, a higher serum concentration of effective favipiravir is expected for the treatment of COVID-19. Although favipiravir has proven to be effective in the treatment of SARS-CoV2 in humans and its effective doses have been determined, it has been suggested that higher values than these values are needed (27). Pharmacokinetic data from previous studies have shown that favipiravir is 50% bound to plasma proteins (28). Based on this assumption, the total plasma concentration of the drug must be >20 μg/ml to reach the EC_{50} value (9.4 μg/ml) for the free drug. Based on clinical trial results, it was observed that after 3 days, 52% of patients did not receive a sufficient dose of medication to produce a serum concentration above 20μg/mL at any time point (29, 30).

Studies on drug-drug interactions are very important in terms of determining blood concentrations of drugs, evaluating the effectiveness of the drug, and preventing toxicity. Favipiravir is frequently used in the treatment of COVID-19. Especially people with chronic diseases often use different drugs together with favipiravir. Therefore, it is very important to determine the drug interaction and safety of favipiravir. This study, in which the safety of the use of favipiravir with AO and XO inhibitors with the highest risk of drug interaction, was determined by measuring both the change in drug-blood concentration and the change in enzyme activities in experimental animals, will contribute to the prediction of possible other drug interactions for future studies. This is the first study

to show the effect of co-administration of favipiravir with drugs that are AO and XO inhibitors on pharmacokinetics and enzyme activities.

CONCLUSION

In favipiravir treatment, it is important to make appropriate dose adjustments and to know the interaction with the drugs prescribed together with favipiravir to increase the treatment efficacy and reduce drug toxicity. Concomitant use of favipiravir with other drugs that affect AO and/or XO activities causes changes in the pharmacokinetic and pharmacodynamic profiles of both favipiravir and the drug with which it is used. In this study, we presented preliminary data on possible drug-drug interactions due to pharmacokinetic changes as a result of concomitant use of favipiravir with XO and/or AO inhibitors. The pharmacokinetic complexities and conflicting data regarding the efficacy of favipiravir require further studies to understand the pharmacokinetic variables that influence clinical outcomes. Therefore, this prospective study aimed to understand the pharmacokinetics of favipiravir and the drug interactions between allopurinol and verapamil.

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