

Favipiravir inhibits acetaminophen sulfate formation but minimally affects systemic pharmacokinetics of acetaminophen

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Favipiravir has potent antiviral activities against influenza virus and RNA viruses.
- Acetaminophen (paracetamol) plays an important role in treatment of seasonal influenza virus infection. As such, co-administration of favipiravir and acetaminophen is likely to happen in clinical practice.

WHAT THIS STUDY ADDS

- Favipiravir slightly but significantly inhibits acetaminophen sulfate formation both *in vitro* and *in vivo*, but does not impact acetaminophen glucuronide formation.
- The effect on systemic pharmacokinetics of acetaminophen itself is statistically significant, but small in magnitude.
- The acetaminophen–favipiravir interaction is unlikely to be clinically important. However, a conservative clinical recommendation would be to limit maximum daily acetaminophen dosage to 3 g in patients taking favipiravir.

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AIMS

The antiviral agent favipiravir is likely to be co-prescribed with acetaminophen (paracetamol). The present study evaluated the possibility of a pharmacokinetic interaction between favipiravir and acetaminophen, *in vitro* and *in vivo*.

METHODS

The effect of favipiravir on the transformation of acetaminophen to its glucuronide and sulfate metabolites was studied using a pooled human hepatic S9 fraction *in vitro*. The effect of acute and extended administration of favipiravir on the pharmacokinetics of acetaminophen and metabolites was evaluated in human volunteers.

RESULTS

Favipiravir inhibited the *in vitro* formation of acetaminophen sulfate, but not acetaminophen glucuronide. In human volunteers, both acute (1 day) and extended (6 days) administration of favipiravir slightly but significantly increased (by about 20 %) systemic exposure to acetaminophen (total AUC), whereas C_{max} was not significantly changed. AUC for acetaminophen glucuronide was increased by 23 to 35 % above control by favipiravir, while AUC for acetaminophen sulfate was reduced by about 20 % compared to control. Urinary excretion of acetaminophen sulfate was likewise reduced to 44 to 65 % of control values during favipiravir co-administration, while excretion of acetaminophen glucuronide increased to 17 to 32 % above control.

CONCLUSION

Favipiravir inhibits acetaminophen sulfate formation *in vitro* and *in vivo*. However the increase in systemic exposure to acetaminophen due to favipiravir co-administration, though statistically significant, is small in magnitude and unlikely to be of clinical importance.

Introduction

Outbreaks of seasonal epidemics caused by influenza viruses have become a public health concern in the United States. For the specific treatment of influenza infection, two classes of drugs are available: the ion channel blockers and the neuraminidase inhibitors. The ion channel blockers amantadine and rimantadine are of limited value due to lack of activity against influenza B virus, adverse side effects, and the rapid emergence of resistant virus strains [1]. Neuraminidase inhibitors are effective against both influenza A and B viruses, and their clinical value has been reported [2, 3]. However viral resistance still is a problem, and new treatments are needed.

Favipiravir (T-705) is an RNA-dependent RNA polymerase inhibitor acting on viral genetic copying to prevent viral replication [4–7]. The active form of favipiravir, T-705RTP (T705-4-ribofuranosyl-5'-triphosphate), is produced by intracellular enzymes (human hypoxanthine guanine phosphoribosyltransferase). T-705RTP selectively inhibits the virus RNA polymerase, but does not influence cellular RNA or DNA synthesis, thereby reducing the potential for toxicity. Favipiravir has potent antiviral activity against not only influenza virus (A, B and C families) but also RNA viruses such as flaviviruses, bunyaviruses, arenaviruses, and noroviruses. Favipiravir is also a candidate for drug combinations with the neuraminidase inhibitors, oseltamivir and zanamivir, in the therapy and management of human influenza virus infections [4–7].

Favipiravir (Avigan®) is approved in Japan for treatment of novel or re-emerging influenza virus infections, against which neuraminidase inhibitors or other anti-influenza drugs might be ineffective or not sufficiently effective. Favipiravir has entered Phase III clinical trials in the United States for the treatment of influenza virus infections.

Many individuals with seasonal influenza virus infection take acetaminophen (paracetamol) to treat fever, myalgia, and other symptoms. As such, concurrent administration of favipiravir and acetaminophen is likely to happen in clinical practice. This raises the possibility of pharmacokinetic drug interactions, which could be of concern if acetaminophen exposure was increased to potentially unsafe levels.

Acetaminophen is mainly metabolized to glucuronide and sulfate conjugates by human liver [8]. In human hepatic cytosol, favipiravir is metabolized to an oxidized metabolite T-705M1 (not the same as T-705RTP) by aldehyde oxidase. The present study evaluated the extent to which favipiravir inhibits acetaminophen biotransformation to its glucuronide and sulfate metabolites *in vitro*. A clinical study was also performed to assess the pharmacokinetic interaction of favipiravir and acetaminophen in human healthy volunteers.

Methods

In vitro study

The effect of favipiravir and its principal metabolite (T-705M1) on the transformation of acetaminophen to acetaminophen glucuronide and acetaminophen sulfate was evaluated *in vitro*. The phosphorylated metabolite (T-705RTP) was not available for study.

Incubation mixtures contained pooled human hepatic S9 (1 mg protein ml⁻¹) from 15 donors, (eight male and seven female), obtained from KAC Co., Kyoto, Japan and manufactured by Tissue Transformation Technologies™ Inc., NJ, USA. Acetaminophen (2 mM), alamethicin (25 μg ml⁻¹), MgCl₂ (5 mM) and a Tris-HCl buffer (100 mM) with or without favipiravir (0 and 30–3000 μM) or T-705M1 (0 and 3–300 μM). The mixtures were pre-incubated for 5 min at 37°C. The reaction was initiated by adding 50 μl of a mixture of uridine 5'-diphosphoglucuronic acid trisodium salt (40 mmol l⁻¹) and adenosine 3'-phosphate 5'-phosphosulfate lithium salt hydrate (400 μmol l⁻¹) in a total volume of 200 μl. After incubation for 15 min at 37°C, the reaction was terminated by adding 200 μl of internal standard solution (50 μg ml⁻¹ ketoprofen in methanol) and centrifuged at 12 000 rev min⁻¹ (approximately 11 000 g) for 10 min at 4°C (Kubota 1710). The supernatant was evaporated to dryness under reduced pressure, and the residue was reconstituted with 200 μl of mobile phase (A:B = 97:3, v/v). The solution was transferred to a protein precipitation plate (Sirocco™, Waters) and filtered by a liquid handling-pipetting system under reduced pressure.

Acetaminophen glucuronide and sulfate were analyzed by a high performance liquid chromatography tandem mass spectrometer (Waters® 2795 separation module and Thermo Fisher Scientific TSQ mass spectrometer) in the negative electron spray ionization (ESI) mode. The separation was achieved by a Waters Atlantis® T3 (2.1 × 150 mm, 3 μm) column at 30°C, and eluted with a 26 min gradient mobile phase at a flow rate of 0.3 ml min⁻¹. The mobile phase was composed of A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) with a linear gradient 0–5 min 3–13% B, 5–10 min 13–65% B, 10–15 min 65% B, 15–16 min 65–3% B, and 16–26 min 3% B. The MRM mass transitions m/z 326 →150 and 230 →150 were used for acetaminophen glucuronide and acetaminophen sulfate, respectively.

The calibration curve ranges were 0.075–3 μM of acetaminophen glucuronide and acetaminophen sulfate, with correlation coefficients consistently exceeding 0.969. The precision of quality control samples at concentrations of 0.15, 0.75 and 1.5 μM were within ±20.0% of their respective nominal values.

The 50% inhibitory concentration (IC₅₀) values for favipiravir and T-705M1 inhibition of acetaminophen glucuronide and acetaminophen sulfate formation were analyzed by nonlinear regression using SAS PROC NLIN [9].

Clinical study design

The study protocol and consent form were reviewed and approved by the Institutional Review Board serving Seaview Research, Miami, FL, USA. All subjects provided written informed consent prior to participation.

The study was a single-centre, open-label, pharmacokinetic interaction study of favipiravir and acetaminophen in healthy adult volunteers. The volunteers (22 male and six female) were between 23 and 51 years of age. The ethnic ratio was White:Asian at 23:5. The body mass weight index range was from 20 to 29 kg m⁻². All subjects were healthy, active, non-smoking adults with no history of significant medical disease and taking no prescription medicines.

Subjects received a single 650 mg oral dose of acetaminophen in the fasting state on three occasions. For the first trial (day 1), acetaminophen was administered alone. This served as the baseline control trial. For the next two trials, the same dose of acetaminophen was given with acute co-administration of favipiravir (day 2), and again during extended co-administration of favipiravir (day 6).

The specific doses and dosage times for the study medications were as follows:

Day 1: 650 mg acetaminophen once (2 × 325 mg tablets in the morning)

Day 2: 1200 mg favipiravir every 12 h (6 × 200 mg tablets in the morning and evening) + 650 mg acetaminophen once (2 × 325 mg tablets in the morning)

Days 3–5: 800 mg favipiravir every 12 h (4 × 200 mg in the morning and evening)

Day 6: 800 mg favipiravir once (4 × 200 mg in the morning) + 650 mg acetaminophen once (2 × 325 mg tablets in the morning)

For each of the three acetaminophen trials (day 1, day 2 and day 6), blood samples were obtained by venipuncture before the dose, and at the following post-dose times: 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12 h on day 1. On day 2 and day 6, additional samples were drawn at 13 and 24 h. Urine samples were collected in intervals of 0–4 h, 4–8 h, 8–12 h and 12–24 h for all three trials.

Blood samples were collected in heparinized glass tubes and centrifuged immediately. Plasma specimens were transferred to glass scintillation vials, which were stored at –20°C until assayed. Urine samples were also frozen at –20°C for analysis.

Analysis of drug concentrations in plasma and urine

Acetaminophen, acetaminophen glucuronide sodium salt, acetaminophen sulfate potassium salt, and 3-acetamidophenol (3-AAP) were purchased from Sigma (St

Louis, MO, USA). Perchloric acid, potassium phosphate, methanol, and acetonitrile were supplied by Fisher Scientific Co., Waltham, MA, USA.

Concentrations of acetaminophen, acetaminophen glucuronide and acetaminophen sulfate in plasma and urine were analyzed by high performance liquid chromatography (Agilent HPLC 1100) with 3-AAP as the internal standard. Plasma samples were pretreated by adding perchloric acid and acetonitrile, vortexed for 1 min and frozen for 20 min at –20°C, and centrifuged for 10 min at 15 000 rev min⁻¹ before injecting onto the HPLC. Urine samples were diluted five-fold, then vortexed and centrifuged before injecting onto the HPLC. The separation was achieved by a Waters μ Bondapak C18 (3.9 × 300 mm, 10 μ m, 125A) column protected by a Phenomenex® guard cartridge. The mobile phase consisted of 20 mM potassium phosphate buffer (pH = 2.2) with a gradient of 3.5% methanol from 0–13 min, then 16% methanol at 14–21 min. The ultraviolet absorbance of acetaminophen and metabolites at 254 nm was used for detection. The retention times of acetaminophen, acetaminophen glucuronide, acetaminophen sulfate, and 3-AAP were 15.4, 11.8, 13.7, and 19.3 min, respectively.

For analysis of plasma samples, eight calibration standards were run with each analytical batch at 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 15 μ g ml⁻¹ for acetaminophen and its metabolites. The limit of quantification (LOQ) for acetaminophen and its two metabolites was 0.1 μ g ml⁻¹. The coefficient of variation (CV) of quality control samples across all analytical runs were 5.7%, 15.0% and 14.3% for acetaminophen, acetaminophen glucuronide and acetaminophen sulfate at lower concentrations (0.5 μ g ml⁻¹), and 5.1%, 7.5% and 5.7% at higher concentration (5 μ g ml⁻¹).

Six calibration standards were run for each analytical batch for urine samples, as follows: 0, 5, 10, 20, 30, 40, 50 μ g ml⁻¹ for acetaminophen, 0, 84.2, 168, 337, 505, 674, 842 μ g ml⁻¹ for acetaminophen glucuronide, and 0, 10, 20, 40, 60, 80, 100 μ g ml⁻¹ for acetaminophen sulfate. The LOQs were 5 μ g ml⁻¹ for acetaminophen, 84.2 μ g ml⁻¹ for acetaminophen glucuronide, and 10 μ g ml⁻¹ for acetaminophen sulfate. CVs were 7.5%, 7.0% and 14.6% at lower concentrations (10, 168 and 20 μ g ml⁻¹ for acetaminophen, acetaminophen glucuronide and acetaminophen sulfate) and 9.7%, 8.8% and 8.3% at higher concentrations (40, 674 and 80 μ g ml⁻¹).

Intra- and inter-day accuracy was between 89% to 104%. The linearity for both plasma and urine were around 0.99 (correlation coefficient).

Pharmacokinetic calculations

Plasma acetaminophen and metabolites. A logarithmic plot of plasma concentration vs. time was constructed for each analyte for each subject on each study day. The terminal log-linear phase of the plasma concentration

curve was identified visually. The beginning and ending time points were designated as the regression interval. The slope (β) of the terminal phase over the designated regression interval was calculated by log-linear regression. This was used to calculate the elimination half-life as follows: $t_{1/2} = (\ln 2)/\beta$.

The truncated area under the plasma concentration curve (AUC) was calculated using the linear trapezoidal method. The initial point was the time zero concentration value (C_0). The final point (C_{last}) was either the 12 h concentration, or the last non-zero concentration, whichever occurred first. Although sampling continued to 13 h on day 2 and 24 h on day 6, the truncated AUC calculation was brought only to 12 h to assure comparability among the three trials.

In a number of instances (particularly on day 2), the C_0 values were non-zero. Accordingly, it was necessary to correct the calculation of total AUC by subtracting the area contribution attributed to C_0 . This contribution is estimated as $(C_0)/\beta$. Therefore calculation of total AUC (extrapolated to infinity) was done as follows:

$$\text{Total AUC} = \text{Truncated AUC} + (C_{last})/\beta - (C_0)/\beta$$

Urinary excretion of acetaminophen and its metabolites For each urine collection interval, the average excretion rate was calculated as the quantity excreted divided by the length of the interval. Because of the small number of data points and individual variation, it was not possible to proceed with excretion rate calculations for each subject individually. Instead, calculations were based on aggregate excretion rates across subjects on each study day. A logarithmic plot of average excretion rate (aggregated across subjects) vs. the midpoint of the collection interval was constructed for each analyte on each study day. The terminal log-linear phase of the curve was identified visually, and the slope (β) determined by log-linear regression. This was used to calculate the excretion half-life. The estimated quantity remaining to be excreted from the termination of the actual collection to infinity was calculated as the estimated instantaneous excretion rate at the completion of collection divided by β . The net cumulative excretion from time zero to infinity was calculated as the actual cumulative excretion during the collection period plus the estimated quantity remaining to be excreted.

Urinary excretion data was also expressed as fraction of the administered dose of acetaminophen (650 mg). Molecular weights of the three entities are acetaminophen 151.2, acetaminophen glucuronide (free acid) 327, and acetaminophen sulfate (free acid) 231.32.

For acetaminophen, the quantity excreted as a fraction of the administered dose was calculated as (quantity excreted)/650. For acetaminophen glucuronide, the fraction was calculated as (quantity excreted) \times (151.2/327)/

650. For acetaminophen sulfate, the fraction was calculated as (quantity excreted) \times (151.2/231.32)/650.

Statistical analysis

For plasma acetaminophen and its metabolites, descriptive statistics were calculated for C_{max} , t_{max} , $t_{1/2}$, truncated AUC, total AUC, and percent extrapolated area. Geometric means and 90% confidence intervals were also calculated for C_{max} , $t_{1/2}$, truncated AUC, and total AUC. Analysis of variance (ANOVA) for repeated measures was used to compare the 3 study days. This was done using the untransformed values, as well as following rank transformation (non-parametric analysis). Dunnett's test was used to compare day 6 and day 2 each vs. the day 1 control.

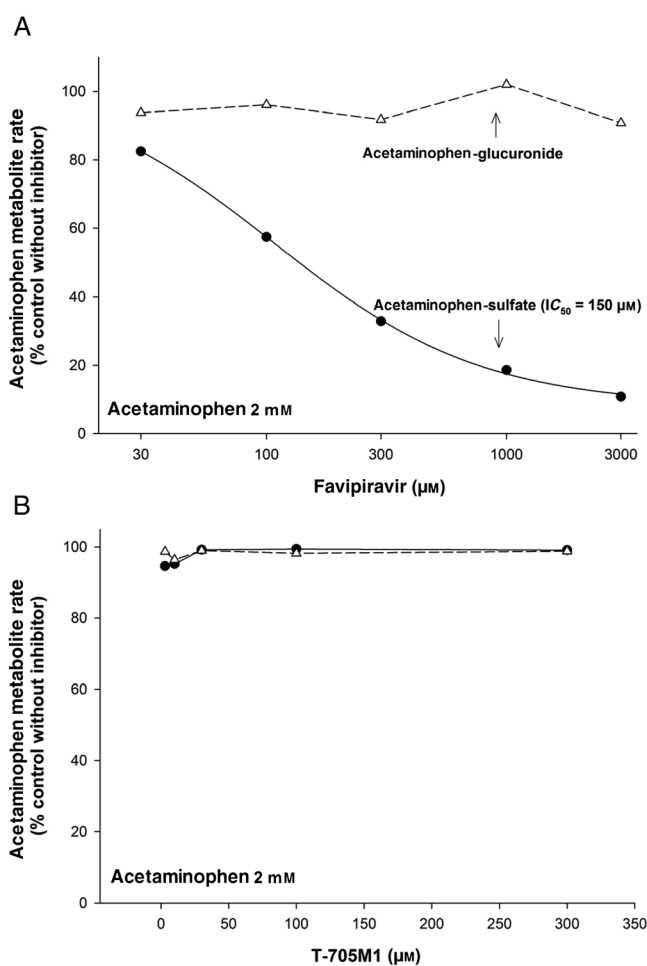


Figure 1

Inhibition curve for favipiravir vs. acetaminophen metabolite formation. Acetaminophen (2mM) and favipiravir were incubated with pooled human hepatic S9 fractions for 15 min. The reaction was stopped by adding acetonitrile. Acetaminophen and its metabolites were detected by LC/MS with electrospray ionization (ESI) negative and multiple reaction monitoring (MRM) mode. The 50% inhibition concentration (IC_{50}) vs. acetaminophen sulfate formation was determined by non-linear regression. \bullet —, Acetaminophen-sulfate; \triangle —, Acetaminophen-glucuronide

For each analyte for each subject, individual ratios were calculated as follows:

$$R2 = (\text{day 2 value}) / (\text{day 1 value})$$

$$R6 = (\text{day 6 value}) / (\text{day 1 value}).$$

This was done for C_{max} , truncated AUC, and total AUC.

Descriptive statistics were calculated for R2 and R6 values, as well as the geometric mean and 90% confidence interval. The 90% CI was evaluated in relation to the default regulatory boundaries of 0.80 and 1.25.

Projected total urinary excretion of acetaminophen and metabolites was evaluated using ANOVA for repeated measures and Dunnett's test as described above.

Results

In vitro study

Favipiravir inhibited acetaminophen sulfation, with a 50% inhibitory concentration (IC_{50}) of 150 μM (Figure 1). Favipiravir (up to 3000 μM concentration) did not inhibit

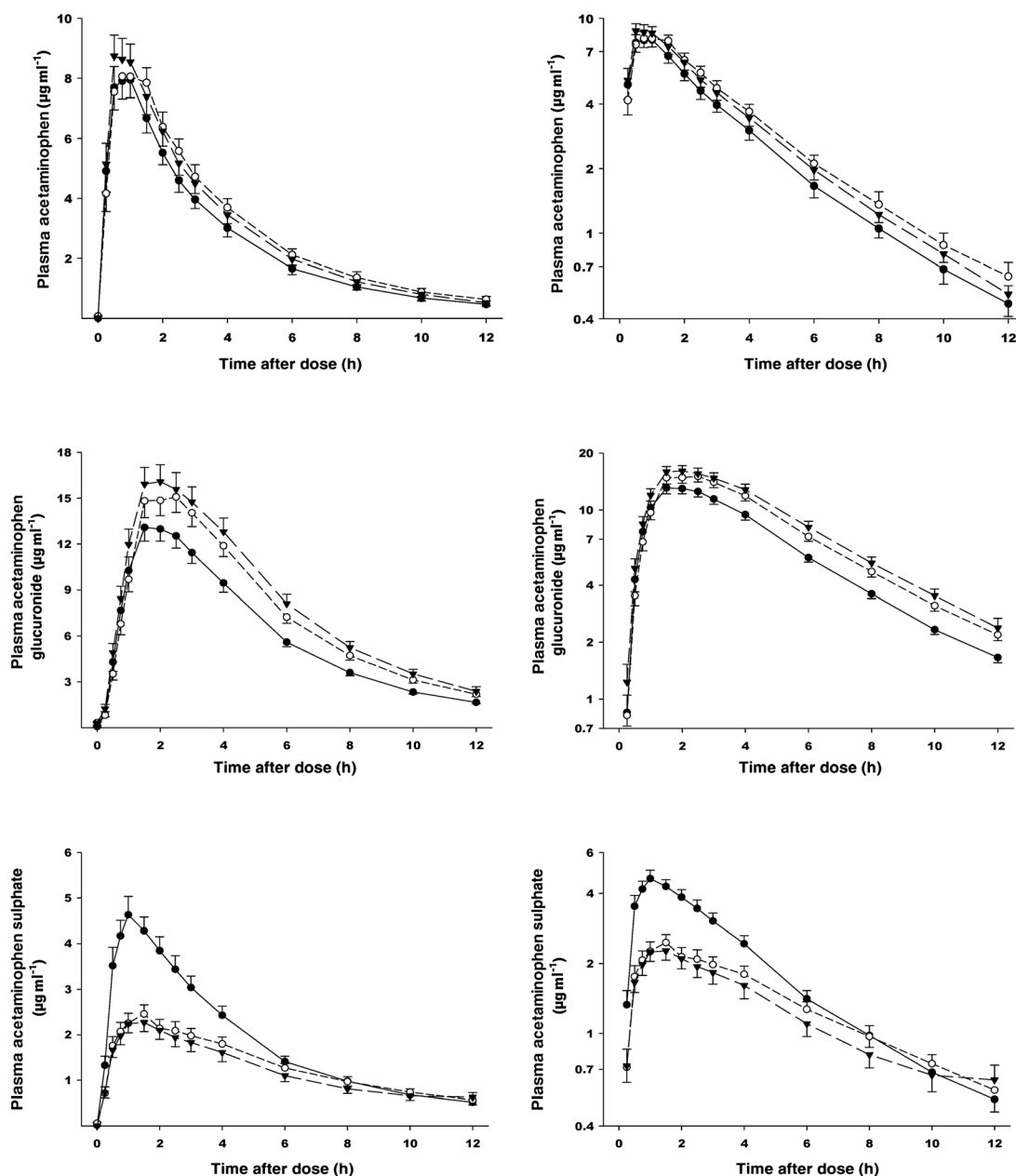


Figure 2

Mean (\pm SE) plasma concentration of acetaminophen and metabolites at corresponding times for the 3 study days. Linear concentration axes are on the left, and logarithmic concentration axes are on the —●—, day 1; ---○---, day 2; —▼—, day 6

acetaminophen glucuronide formation (Figure 1) and the principal metabolite of favipiravir (T-705M1) did not inhibit either glucuronide or sulfate formation.

Plasma concentrations of favipiravir observed in clinical trials are in the range of 110 to 541 $\mu\text{mol l}^{-1}$ (maximum plasma concentration following repeated doses of 800 mg twice a day). The C_{max}/IC_{50} ratio (1.95) for a principal metabolic pathway exceeds the usual lower regulatory limit (0.1), triggering suspicion of a possible clinical drug interaction [10].

Clinical study

All 28 subjects completed all the three trials of study. Four of the 28 (14.3%) subjects reported a single treatment-emergent adverse event (TEAE) during the study. Three of these TEAEs were considered unrelated to study drug, and the other event (headache) was considered possibly related to study drug. All TEAEs were mild, and all resolved by the end of the study. There were no deaths, serious TEAEs, or discontinuations from the study due to an adverse event.

The mean plasma concentration vs. time profiles of acetaminophen and its metabolites on each study day are presented in Figure 2. Mean urinary excretion of acetaminophen glucuronide and acetaminophen sulfate vs. the collection endpoint time are presented in Figure 3. Pharmacokinetic parameters and statistical data for each study day are summarized in Table 1. The analysis of ratios for day 2 and day 6 each vs. day 1 are provided in Table 2. The urinary excretion data are presented in Table 3.

Favipiravir produced small but statistically significant overall changes (ANOVA, $P < 0.01$) in AUC of acetaminophen and its metabolites (Table 1). AUC values for acetaminophen and acetaminophen glucuronide were significantly increased by about 20% for acetaminophen ($P < 0.001$) and 23%–34% for acetaminophen glucuronide ($P < 0.001$) on study day 2 and day 6 compared to day 1. In contrast, mean AUC values for acetaminophen sulfate were significantly decreased by 29%–35% ($P < 0.001$) with acute (day 2) and extended administration of favipiravir (day 6).

Evaluation of geometric mean ratios yielded similar findings (Table 2). For intact acetaminophen AUC, the upper boundary of the 90% CI fell just outside the default limit (1.25). This was also true of C_{max} and AUC for acetaminophen glucuronide. For acetaminophen sulfate, one or both boundaries of the 90% CI fell below the lower default limit (0.8).

Urinary excretion data showed that the projected total excretion of acetaminophen and acetaminophen glucuronide were significantly increased ($P < 0.05$), and that of acetaminophen sulfate was significantly decreased ($P < 0.05$), due to co-administration of favipiravir (days 2 and 6) compared to control (day 1)

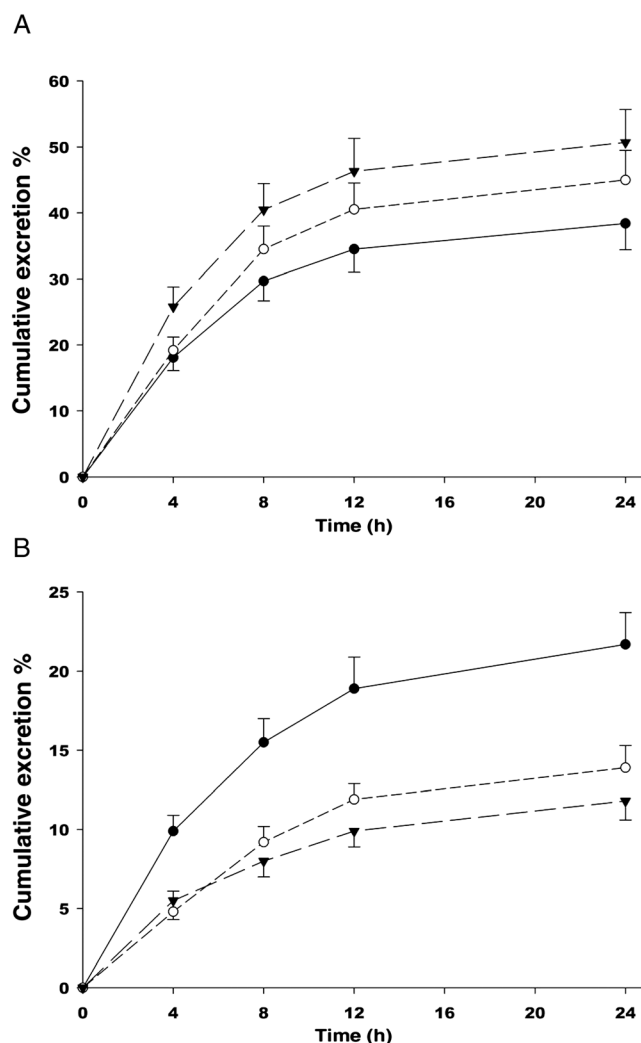


Figure 3

Mean (\pm SE) urinary excretion of acetaminophen glucuronide (A) and acetaminophen sulfate (B). Excretion values are expressed as percent of the administered dose. —●—, day 1; ---○---, day 2; ---▼---, day 6

(Table 3, Figure 3). A small fraction of the dose was excreted as intact acetaminophen compared with acetaminophen metabolites (Table 3).

The study design did not allow the evaluation of whether acetaminophen had an effect on the pharmacokinetics of favipiravir. This is a limitation of the study.

Discussion

Favipiravir (T-705) is not metabolized by human liver microsomes when incubations include dihydronicotinamide-adenine dinucleotide phosphate (NADPH) (unpublished data), but is metabolized to T-705M1 in human liver cytosol by aldehyde oxidase without NADPH. The active metabolite (T-705RTP) is formed in human peripheral blood mononuclear cells. The possible inhibitory effect of this active metabolite (T-705RTP) on

Table 1

Pharmacokinetics variables for acetaminophen and metabolites in the control condition (day 1), and during co-administration of favipiravir (day 2 and day 6)

	Mean (\pm SE) values ($n = 28$)			Values of F from ANOVA	Dunnnett's test	
	Day 1	Day 2	Day 6		Day 2 vs. 1	Day 6 vs. 1
Acetaminophen						
C_{\max} ($\mu\text{g ml}^{-1}$)	9.3 (\pm 0.6)	9.3 (\pm 0.5)	9.9 (\pm 0.7)	0.59 (NS)		
t_{\max} (h)*	0.78 (\pm 0.06)	0.84 (\pm 0.08)	0.74 (\pm 0.08)	1.03 (NS)		
Truncated AUC ($\mu\text{g ml}^{-1}$ h)	31.2 (\pm 2.1)	36.5 (\pm 2.6)	35.3 (\pm 2.4)	4.86 ($P < 0.02$)	$P < 0.05$	$P < 0.05$
Total AUC ($\mu\text{g ml}^{-1}$ h)	33.3 (\pm 2.3)	39.3 (\pm 3.1)	37.4 (\pm 2.5)	5.56 ($P < 0.01$)	$P < 0.05$	$P < 0.05$
$t_{1/2}$ (h)	2.8 (\pm 0.1)	3.0 (\pm 0.1)	2.8 (\pm 0.1)	0.35 (NS)		
Acetaminophen glucuronide						
C_{\max} ($\mu\text{g ml}^{-1}$)	13.6 (\pm 0.9)	16.0 (\pm 1.0)	16.9 (\pm 1.2)	15.1 ($P < 0.001$)	$P < 0.05$	$P < 0.05$
t_{\max} (h)*	2.0 (\pm 0.1)	2.2 (\pm 0.1)	2.1 (\pm 0.1)	2.07 (NS)		
Truncated AUC ($\mu\text{g ml}^{-1}$ h)	73.9 (\pm 3.9)	89.5 (\pm 4.8)	98.3 (\pm 6.6)	31.5 ($P < 0.001$)	$P < 0.05$	$P < 0.05$
Total AUC ($\mu\text{g ml}^{-1}$ h)	81.6 (\pm 4.1)	99.3 (\pm 5.1)	109.0 (\pm 7.1)	34.1 ($P < 0.001$)	$P < 0.05$	$P < 0.05$
$t_{1/2}$ (h)	3.3 (\pm 0.1)	3.5 (\pm 0.2)	3.5 (\pm 0.2)	4.04 ($P < 0.03$)		$P < 0.05$
Acetaminophen sulfate						
C_{\max} ($\mu\text{g ml}^{-1}$)	4.8 (\pm 0.4)	2.6 (\pm 0.2)	2.4 (\pm 0.2)	77.0 ($P < 0.001$)	$P < 0.05$	$P < 0.05$
t_{\max} (h)*	1.00 (\pm 0.33)	1.51 (\pm 0.1)	1.27 (\pm 0.09)	9.86 ($P < 0.001$)	$P < 0.05$	$P < 0.05$
Truncated AUC ($\mu\text{g ml}^{-1}$ h)	22.4 (\pm 1.7)	16.0 (\pm 1.3)	14.6 (\pm 1.6)	47.8 ($P < 0.001$)	$P < 0.05$	$P < 0.05$
Total AUC ($\mu\text{g ml}^{-1}$ h)	25.1 (\pm 1.9)	21.2 (\pm 2.1)	20.1 (\pm 2.3)	12.8 ($P < 0.001$)	$P < 0.05$	$P < 0.05$
$t_{1/2}$ (h)	3.4 (\pm 0.1)	5.5 (\pm 0.6)	5.3 (\pm 0.4)	63.9 ($P < 0.001$)	$P < 0.05$	$P < 0.05$

*Median and range

acetaminophen metabolite formation was not evaluated in this study.

The *in vitro* study showed that favipiravir inhibited acetaminophen sulfate formation with an IC_{50} value of 150 μM , but had no effect on acetaminophen glucuronide formation. The principal metabolite (T-705M1) of favipiravir had no effect on either pathway. Based on the IC_{50} value relative to anticipated *in vivo* exposure to favipiravir, the clinical study was performed to evaluate the possibility of a pharmacokinetic interaction involving acetaminophen and favipiravir.

The pharmacokinetic profile of acetaminophen and its metabolites in the present study is consistent with previous studies from our laboratory [11–20], and as reported in the medical literature [21–27]. Acetaminophen was biotransformed principally to the glucuronide metabolite, with parallel conversion in smaller quantities to the sulfate metabolite. On average, less than 10% of the dose was excreted in urine as intact acetaminophen.

Acute and extended exposure to favipiravir (days 2 and 6, respectively) had no significant effect on acetaminophen C_{\max} , and the 90% confidence interval for the geometric mean ratios [(day 2)/(day 1) and (day 6)/(day 1)] fell entirely within the 0.80–1.25 default range for bioequivalence or 'no interaction'.

Net systemic exposure to acetaminophen, measured either by truncated (12 h) AUC or total AUC, was increased by approximately 15–20% (compared to day 1) due to acute or extended exposure to favipiravir. The increase,

though small in magnitude, was statistically significant. The upper boundary of the 90% confidence interval around the geometric mean ratio was 1.26, which slightly exceeds the 1.25 upper default limit. The findings suggest that, based on net systemic exposure, favipiravir causes weak or negligible inhibition of acetaminophen clearance. This interaction is unlikely to be of clinical importance, inasmuch as there is no significant effect on C_{\max} . Nonetheless, a conservative clinical recommendation would be that total daily dosage of acetaminophen be limited to 3.0 g or less in patients concurrently taking favipiravir.

It is of interest that systemic exposure to acetaminophen glucuronide was increased by co-administration of favipiravir, whereas exposure to acetaminophen sulfate was significantly reduced. This was confirmed by the urinary excretion data, demonstrating a small but statistically significant increase in excretion of acetaminophen glucuronide, and a substantial and significant reduction in acetaminophen sulfate excretion. The mechanism of these changes is not fully explained by the study, since AUC for the metabolites represents a balance between formation via clearance of the parent drug precursor and metabolite clearance via renal excretion. We are not aware of data to suggest that favipiravir has the capacity to induce glucuronide formation. In any case, the findings do suggest that the small increase in acetaminophen AUC caused by favipiravir is explained mainly by inhibition of the sulfate formation pathway. This is consistent with findings from *in vitro* models.

Table 2

Analysis of ratios for pharmacokinetic variables for acetaminophen and its metabolites during favipiravir co-administration (day 2 and day 6) divided by the value in the control condition (day 1)

	C_{max} ratios		Truncated AUC ratios		Total AUC ratios	
	(Day2) / (Day1)	(Day6) / (Day1)	(Day2) / (Day1)	(Day6) / (Day1)	(Day2) / (Day1)	(Day6) / (Day1)
Acetaminophen						
Arithmetic						
mean	1.07	1.17	1.19*	1.19*	1.20*	1.19*
SD	0.32	0.54	0.24	0.33	0.24	0.32
SE	0.06	0.1	0.05	0.06	0.05	0.06
Geometric						
mean	1.03	1.08	1.17	1.14	1.17	1.14
90% CI	0.93, 1.14	0.96, 1.22	1.08, 1.26	1.04, 1.26	1.09, 1.26	1.04, 1.26
Acetaminophen glucuronide						
Arithmetic						
mean	1.19*	1.26*	1.23*	1.34*	1.23*	1.35*
SD	0.19	0.33	0.18	0.32	0.19	0.31
SE	0.04	0.06	0.03	0.06	0.04	0.06
Geometric						
mean	1.18	1.22	1.21	1.31	1.22	1.32
90% CI	1.12, 1.24	1.12, 1.32	1.16, 1.27	1.22, 1.41	1.16, 1.28	1.23, 1.41
Acetaminophen sulfate						
Arithmetic						
mean	0.52*	0.52*	0.71*	0.65*	0.84*	0.81
SD	0.13	0.29	0.15	0.33	0.32	0.52
SE	0.02	0.06	0.03	0.06	0.06	0.1
Geometric						
mean	0.51	0.47	0.7	0.6	0.8	0.72
90% CI	0.48, 0.56	0.42, 0.54	0.66, 0.75	0.53, 0.68	0.72, 0.88	0.63, 0.83

Asterisk (*) indicates that the arithmetic mean differs significantly from 1.0 based on Student's t-test ($P < 0.05$, two-tailed test).

Table 3

Projected total urinary excretion of acetaminophen and metabolites in the control condition (day 1) and during co-administration of favipiravir (day 2 and day 6)

	Mean (\pm SE) values ($n = 28$)			Values of F for ANOVA	Dunnett's test	
	Day 1	Day 2	Day 6		Day 2 vs. 1	Day 6 vs. 1
Acetaminophen						
mg	52 (\pm 6)	43 (\pm 4)	43 (\pm 4)	8.28 ($P < 0.001$)	$P < 0.05$	$P < 0.05$
% of dose	7.9 (\pm 0.9)	6.6 (\pm 0.7)	6.6 (\pm 0.7)			
Acetaminophen glucuronide						
mg	547 (\pm 43)	641 (\pm 53)	721 (\pm 59)	18.94 ($P < 0.001$)	$P < 0.05$	$P < 0.05$
% of dose	39 (\pm 3)	46 (\pm 4)	51 (\pm 4)			
Acetaminophen sulfate						
mg	223 (\pm 20)	142 (\pm 12)	123 (\pm 11)	39.92 ($P < 0.001$)	$P < 0.05$	$P < 0.05$
% of dose	22 (\pm 2)	14 (\pm 1)	12 (\pm 1)			

Experimental data has suggested that sulfotransferase activity may theoretically be impaired by certain nutrients and natural substances [28–30]. However there is minimal literature describing drug–drug interactions involving inhibition of a sulfation pathway. In any case, it

should be remembered that acetaminophen glucuronide and sulfate appear to be inactive from a therapeutic or toxicologic standpoint [21].

In summary, the pharmacokinetics and metabolism of single doses of acetaminophen were determined under

control conditions (day 1), and again during acute and extended co-administration of favipiravir (day 2 and day 6). Acetaminophen C_{\max} was not altered by favipiravir, based on bioequivalence guidelines. Acetaminophen AUC was increased by 15–20% with co-administration of favipiravir. The upper boundary of the 90% confidence interval for the geometric mean ratio of AUC (1.26) just exceeded the upper limit (1.25). The interaction of favipiravir with acetaminophen, though small or negligible in magnitude, was apparently due to relatively selective inhibition of acetaminophen sulfate formation, as demonstrated by the *in vitro* study. Overall, the acetaminophen–favipiravir interaction is unlikely to be clinically important. Based on the observed pharmacokinetic results, a conservative clinical recommendation would be to limit daily acetaminophen dosage to 3.0 g or less in patients taking favipiravir, as opposed to the usually recommended upper boundary of 4.0 g day⁻¹. However this recommendation is based on estimation as opposed to clinical evidence.

Competing Interests

All authors have completed the Unified Competing Interest form and declare YZ, JSH, MHC and DJG received no support from any organization for the submitted work. Tufts University received support from MediVector. CRH, and DG are employees of MediVector, Inc. YN, CK, TN and TK are employees of Toyama Chemical Co. YZ, JSH, MHC and DJG had no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years. CRH and DG are employees of MediVector, Inc. YN, CK, TN and TK are employees of Toyama Chemical Co. None of the authors has other relationships or activities that could appear to have influenced the submitted work.

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