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An Open-Label, Single-Dose, Human Mass Balance Study of Amenamevir in Healthy Male Adults

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Abstract

Amenamevir is an inhibitor of the helicase-primase enzyme complex developed for the treatment of varicella zoster virus. This mass balance study investigated the absorption, metabolism, and excretion of a single dose (200 mg) of ^{14}C-labeled amenamevir in healthy male volunteers. Blood, urine, and feces samples were collected for up to 8 days after the dose. Safety and tolerability were assessed through voluntary reporting of adverse events, physical examination, and clinical laboratory testing. Amenamevir was rapidly absorbed, with a median time to peak drug concentration of 1.0 to 1.5 hours and a plasma half-life of 8 to 9 hours. Overall, 95.3% of the administered dose was recovered, with the majority of radiolabeled drug excreted in feces (74.6%) followed by urine (20.6%). The major route of elimination was fecal, with around 70% of the dose excreted as metabolites and <0.1% as the unchanged drug. Metabolic profiling revealed that predominantly radiolabeled amenamevir (80%) and its hydroxyl metabolite R5 (up to 7.1%) were present in plasma. Single-dose amenamevir was well tolerated; 3 transient and mild adverse events were reported in 3 subjects. Overall, >95% of a single 200-mg dose of amenamevir was eliminated by 168 hours after the dose, with the major route of elimination being fecal.

Amenamevir is a nonnucleoside antiherpes virus compound for the treatment of infections caused by varicella zoster virus (VZV).[1] Amenamevir is an inhibitor of the viral helicase-primase complex,[2] which is responsible for multiple enzymatic activities essential for viral DNA replication and growth.[3] Preclinical studies have demonstrated that amenamevir has activity against VZV that is more potent than acyclovir and valacyclovir.[1,4,5] The pharmacokinetic-pharmacodynamic relationships of amenamevir in mice, guinea pigs, and humans have been studied extensively.[6–8] Phase I studies have shown that amenamevir has less than dose proportional pharmacokinetics in healthy volunteers and is safe and well tolerated at single doses of 5 to 2400 mg or when administered at 300 to 600 mg/day for 7 days.[9] Additionally, amenamevir does not need dose adjustment in patients with renal or moderate hepatic impairment.[10] Amenamevir is both a substrate and inducer of cytochrome P450 (CYP) 3A.[11,12] Drug-drug interaction studies have shown that dose adjustment may be needed when amenamevir is coadministered with CYP3A substrates,

inhibitors, or inducers, while dose adjustment is unlikely to be required when coadministered with substrates of CYP2C8 or CYP2B6.[11–13] Amenamevir was launched in 2017 in Japan for the treatment of herpes zoster at a dose of 400 mg daily.[14] The product is being marketed by Maruho Co., Ltd.

A mass balance study in humans uses the administration of a radiolabeled drug to gather pharmacokinetic data, such as elimination routes and the extent of

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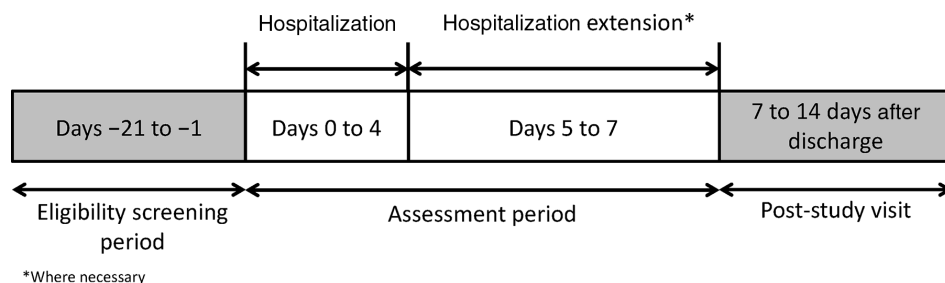(e-mail: kota.kato@astellas.com)

Figure 1. Study design.

elimination, the time course of elimination, identification and quantification of metabolites, and the elucidation of major metabolic pathways.[15] These data can be used to inform further investigations into drug interactions, the effect of hepatic or renal dysfunction, and potential drug accumulation.[15]

This article reports the results of an open-label mass balance study of single dose amenamevir in healthy volunteers, with particular focus on metabolism and excretion; metabolite profiling was also conducted in plasma, urine, and feces. The safety and tolerability of single-dose amenamevir was also assessed.

Methods

Study Design

This open-label, single-dose, absorption, metabolism, and excretion study was conducted at a single center (PRA International, Zuidlaren, The Netherlands). The study consisted of an eligibility screening period, an assessment period, and a post-study visit (Figure 1). The study protocol was approved by an independent ethics committee (the Evaluation of the Ethics of Biomedical Research foundation, Assen, The Netherlands), and the study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines. Written informed consent was obtained from all study participants at the prestudy screening visit before any study procedures commenced.

Study Participants

The inclusion criteria for the study were healthy male subjects aged 18 to 65 years with a body weight of ≥ 60 kg and <100 kg and a body mass index of ≥ 18 and <30 kg/m^2. The exclusion criteria for the study are summarized in Table S1.

Assessments and Treatment

During the screening visit, informed consent was obtained, and demographic data, medical history and medication, and smoking and drug use history were collected, and subjects provided a blood and urine sample for assessment (Table S2).
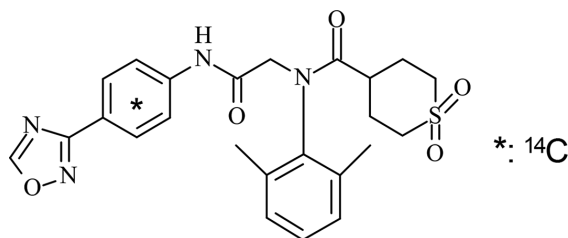

Figure 2. Structure of ^{14}C-amenamevir.

Subjects were admitted to the clinical phase of the study on day 0, when they were admitted to the clinical research unit, and assessments were repeated (Table S2). The occurrence of pretreatment adverse events (AEs) and concomitant medication use were recorded, and a predose feces sample was collected.

All participants received a single 200-mg oral dose of ^{14}C-labeled amenamevir (1.8 MBq; synthesized at Sekisui Medical Co., Ltd, Japan) (Figure 2) in 200 mL of water with 8% hydroxypropyl-β-cyclodextrin on day 1 of the study under fasting conditions, and were then hospitalized until at least day 4. The 200-mg dose was selected because it was expected to be included in further clinical development. Blood, plasma, urine, feces, and expired air samples were collected for the analysis of ^{14}C-radioactivity, amenamevir, and/or metabolic profiling. AEs were continuously monitored, as were other safety assessments (Table S2). Subjects were discharged on day 4 as long as they met the criteria outlined in the supporting information. Upon discharge, tests that were performed upon admittance to the clinical unit were repeated (Table S2), and the use of concomitant medications was checked and recorded.

Of the 14 subjects screened and enrolled in the study, 6 subjects were dosed. All dosed subjects completed the study and had their stay in the clinical unit extended past day 4, according to the discharge criteria (see supporting information). Subjects had a mean $\pm$ standard deviation (SD) age of 29.0 $\pm$ 15.0 years (median, 23.5 years; range, 19–59 years), were mostly Caucasian (n = 5; 83.3%) and had a mean $\pm$ SD body mass index of 23.3 $\pm$ 1.4 kg/m^2 (Table 1).

Table 1. Baseline demographics and characteristics

Demographic	N = 6
Age, years	29.0 ± 15.0
	23.5 (19–59)[a]
Male, n (%)	6 (100.0)
Race, n (%)	
White	5 (83.3)
Asian	1 (16.7)
Weight, kg	75.1 ± 5.4
Height, m	179.8 ± 8.4
BMI, kg/m^2	23.3 ± 1.4

All values are presented as mean ± standard deviation unless otherwise stated.
BMI, body mass index.
[a]Values are given as median (range).

The post-study visit included safety and clinical assessments (Table S2) along with assessment of concomitant medications.

Pharmacokinetic and Metabolic Profiling Sampling Schedules

To assess the pharmacokinetics of amenamevir in plasma and the level of radioactivity in plasma and whole blood, the sampling times were before the dose and 15 minutes, 30 minutes, and 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 24, 36, 48, 60, and 72 hours after the dose. Blood samples for plasma metabolic profiling were taken before the dose and 30 minutes and 1, 2, 4, 6, 8, 10, and 12 hours after the dose.

To assess the pharmacokinetics and metabolic profile of amenamevir and the level of radioactivity, urine collection took place at the following intervals: before the dose and 0 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, 144 to 168, 168 to 192, 192 to 216, 216 to 240, 240 to 264, 264 to 288, 288 to 312, and 312 to 336 hours after the dose. For radioactivity levels and metabolic profiling in feces, the feces collection intervals were the same as urine collection intervals.

Expired air was sampled for the assessment of $^{14}CO_2$ expiration before the dose, and 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 hours after the dose.

Blood samples were kept on ice until centrifugation, which was undertaken within 30 minutes of collection. For analysis, blood samples were centrifuged at 1500g for 10 minutes at 4°C, and the plasma was transferred into 2 tubes that were stored at −20°C or −70°C before analysis. Whole blood was stored at −20°C before analysis. Urine samples were homogenized by high-speed stirring for 5 minutes before being aliquoted into sample tubes for the various tests and stored at −20°C or −70°C before analysis. Feces samples were homogenized with water and transferred into sample tubes for analysis. For metabolic profiling in feces, homogenized

samples were stored at −70°C before analysis. Expired air was collected into 4 mL of a trapping solution containing 2 mL of hyamine hydroxide (1 N) and 2 mL of a solution of 0.02% phenolphthalein in 95% ethanol as an indicator. Samples were stored at 4°C before analysis.

Radioactivity in plasma, urine, and fecal samples was detected using liquid scintillation counting. Analysis of the metabolites of ^{14}C-amenamevir was undertaken using high-performance liquid chromatography for plasma, urine, and feces, and liquid chromatography/tandem mass spectrometry (LC-MS/MS) for urine and faeces. Bioanalysis of amenamevir in plasma and urine was conducted using LC-MS/MS by the Bioanalysis section of the Exploratory Development Department at APEB, Leiderdorp, The Netherlands. Metabolic profiling was completed by an independent laboratory (Nemoto Science Co., Ltd, Ibaraki, Japan). The methods for the bioanalysis and metabolic profiling are summarized in the supporting information.

Safety Assessments

Safety assessments included AEs, clinical laboratory parameters, vital signs, electrocardiogram and physical examination (Table S2). Subjects were regularly asked via non-leading questions about any AEs. These and spontaneously reported AEs were recorded. AEs were coded using the system organ class and preferred terms in the Medical Dictionary for Regulatory Activities (version 10.0). The severity of the AEs and their relationship to study treatment were also determined. Vital signs recorded were blood pressure and heart rate. Clinical laboratory parameters recorded included electrolytes, renal and liver parameters, albumin and lipid levels, hematology, urinalysis, serology (hepatitis and HIV), and urine drug and alcohol screen.

Statistical Analyses

No sample size calculation was performed for this study, as 6 subjects is a commonly accepted number for mass balance studies. Two populations for the analysis were defined: the safety analysis set and the pharmacokinetic analysis set. The safety analysis set included all subjects who received the study medication, while the pharmacokinetic analysis set included all subjects who received the study drug and had sufficient plasma concentration data available to facilitate the derivation of at least 1 pharmacokinetic parameter. Descriptive statistics were calculated for continuous variables when there were quantifiable data for at least 2 subjects. These included number of subjects, mean, SD, median, minimum, and maximum. Frequencies and percentages were calculated for categorical data. Values below the lower limit of quantitation were set to zero for calculations. Pharmacokinetic parameters were calculated

Table 2. Pharmacokinetic parameters in plasma and whole blood after a single oral administration of 200 mg of ^{14}C-amenamevir (n = 6)

Parameter measured	AUC_{last} ng · h/mL	AUC_{inf} ng · h/mL	C_{max} ng/mL	t_{max} hours[a]	$t_{1/2}$ hours	t_{lag} hours
^{14}C-radioactivity						
In plasma	10,076 ± 2912	10,997 ± 2530	1041 ± 324	1.00 (1.00–3.02)	8.03 ± 0.81	0.08 ± 0.13
In whole blood	8530 ± 2263	10,425 ± 2265	919 ± 284	1.00 (1.00–2.00)	9.19 ± 2.13	0.13 ± 0.14
Whole blood:plasma ratio	0.852	0.951	0.885	–	–	–
Amenamevir						
In plasma	8691 ± 2492	8817 ± 2508	1018 ± 404	1.50 (1.00–3.00)	7.65 ± 0.49	0

All values are presented as mean ± standard deviation unless otherwise stated.
AUC_{inf}, area under the plasma concentration versus time curve from zero to infinity; AUC_{last}, area under the plasma concentration versus time curve from zero to last time point with concentrations above the quantifiable concentration; C_{max}, peak drug concentration; $t_{1/2}$, terminal half-life; t_{lag}, lag-time observed from dosing to the time point prior to that of the first quantifiable concentration; t_{max}, time to peak drug concentration.
[a]Values are given as median (range).

by the noncompartmental method using WinNonLin Professional (Version 5.0.1). The renal clearance (CL_R) was calculated as: $CL_R = Ae/AUC$, where Ae (amount of drug excreted in urine) and AUC (area under the plasma concentration-time curve) were taken over the largest common interval in which plasma and urine concentrations were both quantifiable. All other statistics were calculated using SAS version 9.1.3.

Results

Pharmacokinetics

After a single 200-mg dose of ^{14}C-amenamevir, amenamevir was rapidly absorbed, with a median time to peak drug concentration ranging from 1.0 to 1.5 hours for ^{14}C-radioactivity in plasma and whole blood, and for amenamevir in plasma (Table 2). The mean AUC_{last} and AUC_{inf} for ^{14}C-radioactivity in plasma were approximately 16% and 25% higher than the mean AUC_{last} and AUC_{inf} for amenamevir in plasma (Table 2). At all time points measured, the arithmetic mean concentration-time profiles of ^{14}C-radioactivity in plasma and whole blood, and amenamevir concentrations in plasma ran in parallel, with ^{14}C-concentrations in plasma being the highest (Figure 3). The mean whole blood-to-plasma ratios for ^{14}C-radioactivity concentrations ranged between 0.78 and 1.06 over the 24-hour postdose period. The elimination of plasma amenamevir and ^{14}C-radioactivity from plasma and the elimination of ^{14}C-radioactivity from blood proceeded with similar mean half-life values of approximately 8 to 9 hours.

Over the 8 days of sampling, a mean of 95.3% of the administered dose was recovered, with 74.6% of ^{14}C-radioactivity excreted in feces and 20.6% in urine (Table 3, Figure 4). No ^{14}C-radioactivity was detected in expired air. A mean of 10.5% of the dose (determined by LC-MS/MS) was excreted as unchanged amenamevir in urine (Table 3).
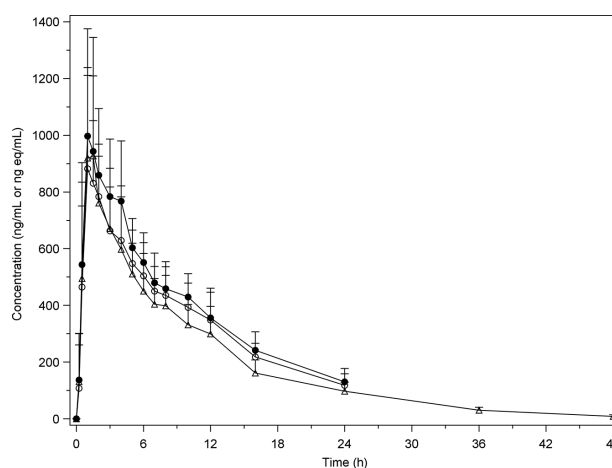

Figure 3. Mean (SD) concentration-time profiles of radioactivity in plasma and blood, and unchanged amenamevir in plasma after a single oral administration of 200 mg ^{14}C-labeled amenamevir to healthy subjects. Open circles = ^{14}C in blood; closed circles = ^{14}C in plasma; open triangles = amenamevir in plasma.

Table 3. Cumulative recovery of ^{14}C-radioactivity and amenamevir urine pharmacokinetics after a single oral administration of 200 mg of ^{14}C-amenamevir

	N = 6
Cumulative recovery of ^{14}C-radioactivity	
Ae urine, %	20.6 ± 4.0
Ae feces, %	74.6 ± 3.0[a]
Ae expired air, %	0
Ae total, %	95.3 ± 1.8[a]
Urine amenamevir pharmacokinetics	
Cumulative Ae_{last}, mg	20.9 ± 5.5
Cumulative Ae_{last}, %	10.5 ± 2.8
CL_R, L/hour	2.44 ± 0.35

All values are presented as mean ± standard deviation.
Ae_{last}, cumulative amount excreted into the urine at the last sampling interval; CL_R, renal clearance.
[a]n = 5 for Ae feces and Ae total, as 1 subject was excluded from the descriptive statistics due to low recovery in feces.
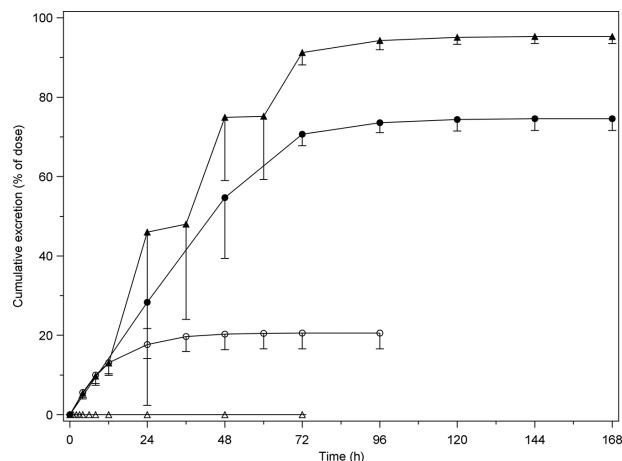
Figure 4. Mean (SD) cumulative radioactivity excretion in urine and feces after a single oral administration of 200 mg of ^{14}C-labeled amenamevir to healthy subjects. Open circles = urine excretion; closed circles = feces excretion; open triangles = expired air; closed triangles = total recovery.

Representative radiochromatograms of amenamevir and its metabolites in plasma, urine, and feces are presented in Figure 5. In plasma samples collected 0.5 to 12 hours after the dose, approximately 80% of the total radioactivity was amenamevir, and most of the remaining radioactivity was from the metabolite R5, which is formed by hydroxylation of a methyl group (Figure 6). Metabolite R5 accounted for 3.4% to 7.1% of the total radioactivity, and no other metabolite peaks could be detected. In urine (0–48 hours after the dose), approximately 55% of the total radioactivity excreted was amenamevir and approximately 37% was R5; these accounted for approximately 11.2% and 7.5% of the administered dose (by liquid scintillation counting), respectively. Other metabolites were excreted in urine, but the amounts were negligible (<0.5% each). In feces, samples taken 0 to 72 hours after the dose, 2 unknown metabolites, UK-F1 and UK-F2, were the main components, accounting for approximately 21.0% and 49.1% of the administered dose, respectively. Less than 0.1% of the dose was recovered as unchanged in the feces.

The unknown metabolites seen in the radiochromatograms of urine and feces samples were elucidated by LC-MS/MS. Of the unknown metabolites in urine (Figure 5), UK-U1 was not identified, while UK-U2 was identified as R3 (formed by glucuronidation of R5), UK-U3 as R8 (formylation of R5), and UK-U4 as a monoxide of amenamevir, of which the oxidation position was different from R5 and R6 (hydroxylation of a 1,2,3-trisubstituted benzene ring) (Figure 6). Of the unknown metabolites in feces (Figure 5), UK-F1 and UK-F2 were identified as R10 (formed by the breakdown of an oxadiazole ring; Figure 6) and a mixture of monoxides of R10 with different oxidation positions.

Safety

Three subjects experienced treatment-emergent AEs: One subject had an arthropod bite on day 5; 1 subject experienced dizziness approximately 1.5 hours after the dose, which lasted 13 hours; and 1 subject had diarrhea approximately 5.5 hours after the dose, which lasted 3 days and 6 hours. Both the dizziness and diarrhea were considered possibly related to study medication. All 3 AEs were mild in severity, were transient, and resolved without sequelae. No deaths, serious AEs, or AEs resulting in discontinuation were reported. No clinically important abnormalities in clinical laboratory evaluations, vital signs, or electrocardiogram recordings were reported.

Discussion

This mass balance study showed that a single oral dose of ^{14}C-labeled amenamevir was rapidly absorbed, after which it was abundantly present in plasma and was mainly excreted in feces. The mean whole blood to plasma ratios of ^{14}C-radioactivity concentrations were relatively constant and concentrations of ^{14}C-radioactivity in whole blood were distributed more or less equally between red blood cells and plasma. Amenamevir was metabolized into several compounds, and the proportion of metabolites differed among plasma, urine, and feces. The major component in plasma was unchanged amenamevir, while unchanged amenamevir and the R5 metabolite were the main amenamevir-related components excreted in urine, and the R10 metabolite and its monoxides were excreted in faeces. R5 is not an active metabolite.

Single-dose amenamevir was well tolerated, with AEs being mild in severity, transient, and resolving without sequelae. The safety and tolerability of amenamevir reported in this study is similar to that in another single-dose amenamevir study in healthy volunteers[9] and in clinical studies in patients with genital herpes or herpes zoster.[16,17]

Several phase I studies of amenamevir have been conducted; of these, 2 pharmacokinetic studies of single-dose amenamevir have been completed: both were dose-escalating studies investigating the safety and pharmacokinetics of 5 to 600 mg of amenamevir (15L-CL-001)[9] and 5 to 2400 mg of amenamevir (15L-CL-002)[9,18] in healthy subjects. Amenamevir has less than dose proportional pharmacokinetic characteristics (most likely due to its absorption profile),[9] which may explain why the pharmacokinetics of the 200-mg dose (solution) in the current study were similar to the 300-mg dose in these other studies. For example, subjects who received a 300-mg dose (capsule) had a mean ± SD peak concentration of drug in blood plasma, AUC$_{inf}$, and half-life of 1040 ± 264 ng/mL, 11 700 ± 1920 ng · h/mL, and 6.88 ± 0.56 hours
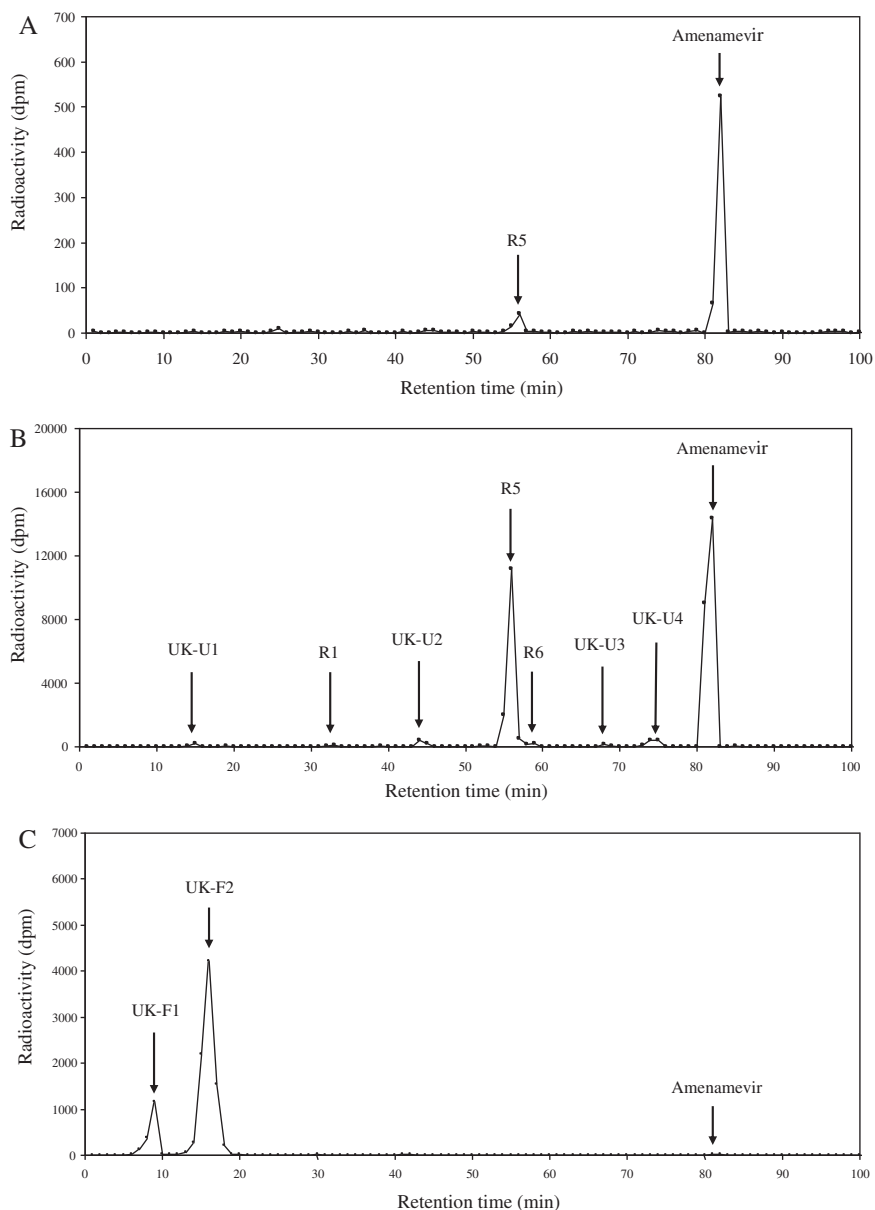
Figure 5. Representative high-performance liquid chromatography radiochromatograms of amenamevir and its metabolites after a single oral administration of 200 mg ^{14}C-labeled amenamevir in healthy subjects. (A) Plasma (1 hour); (B) urine (0–4 hours); (C) feces (0–24 hours).

(15L-CL-001)[9] and 1093 ± 201 ng/mL, 12 077 ± 2718 ng · h/mL, and 7.85 ± 1.62 hours (15L-CL-002),[18] respectively. Urine pharmacokinetic data for the 200-mg dose in our study were broadly similar to those of a single 300-mg (capsule) dose in a multiple-dose study of amenamevir in healthy subjects (15L-CL-003), that is, on day 1 of administration, with 11.07% of the drug excreted in the urine and a renal clearance of 2.45 L/hr.[18]

The influence of renal and hepatic impairment on the pharmacokinetics of amenamevir has also been investigated in phase I studies, which suggested that amenamevir does not require dose adjustments in patients with moderate hepatic impairment or renal impairment.[10] The results of these studies showed that although amenamevir is metabolized by the liver, its pharmacokinetic profile was not significantly altered in subjects with moderate hepatic impairment. Also, despite reduced renal clearance of amenamevir in subjects with severe renal impairment, treatment was considered safe, with no treatment-emergent severe AEs.[10]

In conclusion, the results of this study showed that over 95% of an amenamevir dose of 200 mg was eliminated by 168 hours after the dose. Several metabolites of amenamevir were detected in urine and feces. The major route of elimination for amenamevir was
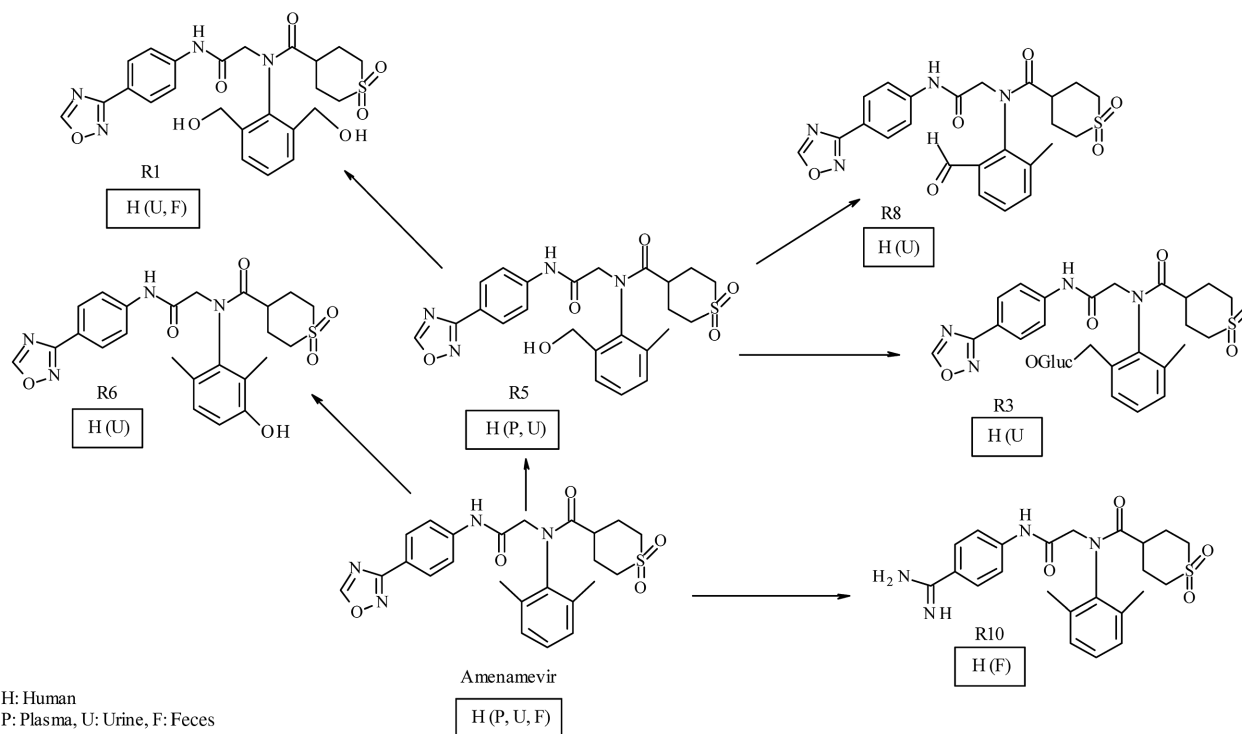
Figure 6. Postulated metabolic pathways of amenamevir in humans.

fecal, considering 2 major metabolites of amenamevir, accounting for around 70% of the dose detected in feces. Amenamevir 200 mg was safe and well tolerated.

Acknowledgments

Author Contributions

K.K. contributed to the study design and metabolic profiling, and read and approved all manuscript drafts. D.G.v.d.M. contributed to the study design and pharmacokinetic data analysis, and read and approved the manuscript drafts. M.d.A. and M.K. contributed to the study design and pharmacokinetic data analysis, and read and approved the manuscript drafts. A.T. contributed to the study design and pharmacokinetic data analysis, and read and approved the manuscript drafts.

Declaration of Conflicting Interests

All authors are employees of Astellas Pharma and have no other conflict of interest to declare.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.