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ORIGINAL RESEARCH

Study on Pharmacokinetic Interactions Between HS-10234 and Emtricitabine in Healthy Subjects: An Open-Label, Two-Sequence, Self-Controlled Phase I Trial

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ABSTRACT

Introduction: HS-10234, a novel prodrug of tenofovir (TFV), functions by inhibiting nucleotide reverse transcriptase against retroviral infections including hepatitis B virus and human immunodeficiency virus (HIV). As it is a possible substitute for TFV co-administration with emtricitabine, determining the drug-drug interactions (DDI) between HS-10234 and emtricitabine therapy will be helpful for researchers to design and conduct future phase II/III studies and merits careful examination in the era of evolving new combination antiretroviral therapy regimens.

Methods: We conducted an open-label, two-sequence, two-period, self-controlled phase I trial that enrolled 36 healthy volunteers randomized into two groups (group 1 and group 2). Eighteen subjects in group 1 were orally administered HS-10234 at a 25-mg daily dose for 7 days during period 1 (D1–D7) followed by co-administration of emtricitabine at a 200-mg dose once daily (QD) for 7 additional days during period 2 (D8–D14). Participants in group 2 were orally administered emtricitabine 200 mg QD for 7 days during period 1 (D1–D7) and then co-administered HS-10234 25 mg QD for 7 additional days during period 2 (D8–D14). Pharmacokinetics (PK) of HS-10234 and emtricitabine were characterized when administered alone and in combination. The concentrations of HS-10234 and its metabolites TFV and emtricitabine were determined using high performance liquid chromatography-mass spectrometry (HPLC-MS)/MS. Peripheral blood monocyte cells (PBMCs) were isolated for detection of intracellular concentrations of HS-

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10234's active metabolite, intracellular tenofovir diphosphate (TFV-DP) pre-dose and 2, 4, 8, 12 and 24 h post-dose on D7 and D14 in group 1. WinNonlin software was used to calculate PK parameters.

Results: After multiple-dose administration of HS-10234 with emtricitabine, the AUC_{0-tau} of HS-10234 and TFV-DP was 1.327- and 1.403-fold higher than that with HS-10234 administration alone. The C_{max} and AUC_{0-tau} were increased 1.120- and 1.077-fold compared to emtricitabine administration alone. Co-administration of HS-10234 with oral emtricitabine was well tolerated. No serious adverse events were observed.

Conclusions: Although a slightly increased steady-state PK exposure of HS-10234 and TFV-DP was observed with co-administration of oral HS-10234 with emtricitabine, these changes were not considered clinically relevant. Thus, dose adjustments are not recommended for HS-10234 combination with emtricitabine.

Trial Registration: NCT04477096, July 20, 2020.

Keywords: HS-10234; Emtricitabine; Drug-drug interaction; HIV; Pharmacokinetics

Key Summary Points
HS-10234 25 mg QD does not have a significant influence on the PK exposure of emtricitabine
Emtricitabine 200 mg QD slightly increases the steady-state PK of HS10234 and TFV-DP in healthy volunteers under fasted condition
Subjects tolerated HS-10234 co-administered with emtricitabine well

INTRODUCTION

Acquired immune deficiency syndrome (AIDS) is a severe infectious disease caused by HIV infection. WHO's 2019 report highlighted the grim situation of AIDS, which is a significant global public health issue; ~ 38 million people are living with AIDS. Furthermore, HIV/AIDS has claimed almost 33 million lives so far [1, 2]. Mechanically, AIDS is initiated by HIV infection, which specifically targets the human immune system (especially $CD4^+$ T cells), initiating the infectious cycle [3]. It destroys the $CD4^+$ T lymphocytes and causes the human body to lose its immune function. Strong evidence proves that higher HIV RNA levels in plasma are correlated with lower baseline $CD4^+$ cell count in plasma as well as more rapid $CD4^+$ cell decline and disease progression [4]. At present, several kinds of drugs, e.g., nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), are being developed and approved for treatment of HIV patients. These drugs rapidly reduce plasma HIV RNA levels, maintaining the plasma HIV RNA levels below 10,000/ml in the early phase of HIV infection, resulting in the reduction of HIV progression to AIDS [4, 5]. Maximizing the safety and tolerability of combination antiretroviral therapy (cART) is a high priority because current antiretroviral therapy regimens for HIV-1 cannot eradicate HIV, and treatment of HIV infection requires life-long therapy [6].

It is well established that highly active antiretroviral therapy (HAART) is effective in HIV post-exposure prophylaxis and initial therapy. This regimen often contains two NRTIs and another non-NRTI drug, among which tenofovir disoproxil fumarate (TDF) is the most recommended NRTI because it has a potent antiviral effect and is well tolerated [6, 7]. However, TDF treatment is associated with a higher risk of nephrotoxicity and reduced bone mineral density compared with other antiretrovirals [8, 9]. Tenofovir alafenamide (TAF) is a modified NRTI based on TFV with superior efficacy and improved properties compared to TDF. Developed as a prodrug of TFV, TAF first metabolizes to its active drug, TFV, by cathepsin A and carboxylesterase 1 in PBMCs and hepatic cells, followed by intracellular phosphorylation to generate the active metabolite TFV-DP, which is active against HIV [10, 11]. With higher stability, TAF usually

achieves about four-fold higher levels of TFV-DP in PBMCs compared to TDF, at approximately 1/10th of the dose [12].

Considering the higher safety and tolerability of NRITs for the treatment of HIV patients, a novel prodrug of TFV, named HS-10234, was recently developed by the Jiangsu Hansoh Pharmaceutical Group Co., Ltd. The antiretroviral activity of HS-10234 showed a slight improvement compared with TAF both in vitro [12] and in vivo. In hepatitis B virus patients, hepatitis B virus DNA reduction in plasma after HS-10234 treatment (− 2.70 to − 2.89 $\log_{10}$ IU/ml) at a dose of 10 mg and 25 mg was comparable to the reduction (− 2.19 to − 2.81 $\log_{10}$ IU/ml) after TAF treatment at a dose of 8–40 mg [13,14].

Since the combination of TDF/TAF with emtricitabine is the backbone of anti-HIV therapy [7], it is possible that HS-10234 co-administered with emtricitabine will provide superior efficacy and safety. Here, we performed a phase I clinical trial in 36 healthy volunteers to evaluate the DDI of HS-10234 and emtricitabine. The secondary objective was to evaluate the safety and tolerability of HS-10234 co-administered with emtricitabine.

METHODS

Study Design

This was a phase I, single-center, open-label, two-sequence, two-period self-controlled study to assess the DDI of HS-10234 and emtricitabine as well as the PK and safety profiles of HS-10234 and emtricitabine in healthy adults. This study consisted of a 14-day screening period. There was no washout time between two sequential treatment periods, but there was an 8-day follow-up visit after the last dose. Thirty-six subjects were recruited and randomized into two equal groups (group 1 and group 2). Participants in group 1 were orally administered HS-10234 at a 25-mg daily dose for 7 days during period 1 (day 1–7) followed by co-administered with emtricitabine at a 200-mg dose once daily (QD) for 7 additional days during period 2 (day 8–14). Participants in group 2 were orally administered emtricitabine 200 mg QD for 7 days during period 1 (day 1–7) and then co-administered HS-10234 25 mg QD for 7 additional days during period 2 (day 8–14). All subjects were fasted for at least 10 h before drug administration at each determined time point. All subjects were fasted for at least 2 h during D1–D6 and D8–D13 and 4 h on D7 and D14 after drug administration. Water was forbidden for 1 h before and after administration. The clinical progress is shown in Fig. 1.

Safety assessments included monitoring of adverse events (AEs), clinical laboratory tests, vital signs and electrocardiograms. Research fellows were responsible for detecting, recording and reporting AEs. Subjects who received at least one dose of HS-10234/emtricitabine were advised about the safety analyses. All AEs were graded using Common Terminology Criteria for AE standards.

Regulatory and ethics approvals were obtained before initiating the study (Third Xiangya Hospital of Central South University Ethics Committee), which was conducted in accordance with the principles of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Good Clinical Practice and the Declaration of Helsinki. All subjects signed written informed consents prior to any study-related procedures.

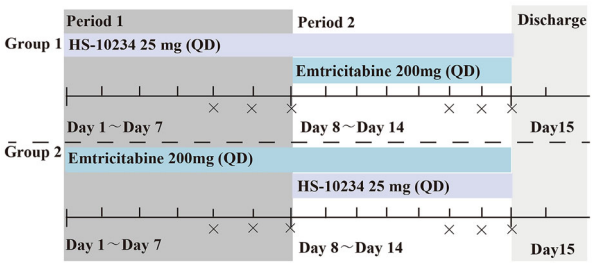

Fig. 1 Trial process. Day 7 and 14: Intensive PK samples collected at ≤ 60 min pre-dose, 10 min, 20 min, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24 post-dose. Day 5, Day 6, Day 12 and Day 13: Serial samples collected ≤ 60 min pre-dose

Study Population

Eligible volunteers were recruited according to the listed criteria: healthy adults, both men and women, aged between 18 and 55 years (inclusive); male weight $\geq$ 50.0 kg, female weight $\geq$ 45.0 kg, with a BMI between 19 and 26 $kg{\cdot}m^{-2}$ (inclusive); female subjects of childbearing potential were sexually inactive (abstinent or following contraceptive methods) for 3 months after the last dose. Exclusion criteria were related to medical history (e.g., history of liver, gastrointestinal, cardiac or psychiatric disorders), laboratory screening tests (including prolonged QT interval, alanine aminotransferase, aspartate transaminase, alkaline phosphatase and bilirubin $\geq 1.2 \times$ upper limit of normal; hepatitis B and hepatitis C positive, positive results on syphilis serological tests and HIV positive) and abnormal vital signs. Subjects with a history of allergy to drugs, food or other substances were excluded as were those who had taken any prescription or non-prescription drugs (including Chinese herbal medicines) possibly affecting the PK of the study drug within 14 days before study initiation. Other exclusion criteria included being pregnant or breastfeeding and having a history of alcohol abuse, tea consumption, coffee consumption, tobacco smoking or drug dependence.

Study Drug

Jiangsu Hansoh Pharmaceuticals produced and supplied HS-10234 tablets (specification: 25 mg/tablet; lot: 01200201). Emtricitabine capsules (specification: 200 mg/capsule, lot: 018324) were also provided by Jiangsu Hansoh Pharmaceuticals.

Pharmacokinetic Assessments and Bioanalysis

Four milliliters of venous blood was collected into heparinized Vacutainer™ tubes for analysis of the PK of HS-10234, TFV and emtricitabine; 8 ml venous blood was collected in BD Vacutainer®CPT preparation tubes for analysis of the intracellular PK of TFV-DP. Group 1 blood samples were collected on D5, D6, D12 and D13 within 60 min before administration for analysis of the PK of HS-10234, TFV and emtricitabine. Intensive PK blood samples were collected on D7 and D14 within 60 min before administration and at 10 min, 20 min, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 h after dosing for analysis of the PK of HS-10234, TFV and FTC. PBMC blood samples were collected at pre-dose and 2, 4, 8, 12 and 24 h post-dose on D7 and D14 for analysis of the intracellular PK of TFV-DP. Group 2 blood samples were collected on D5, D6, D12 and D13 within 60 min before administration; on D7 and D14 within 60 min before administration; and at 10 min, 20 min, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 h after dosing.

After that, PBMCs were isolated following the published method [15]. Plasma was separated by centrifugation (1500*g* at 4 for 10 min) within 1 h of sample collection and then stored at − 80 °C before analyses. Urine samples of both groups were collected to determine concentrations of TFV and emtricitabine at 0–4 h, 4–8 h, 8–12 h and 12–24 h after drug administration on D7 and D14.

Concentrations of related drugs and metabolites were determined using LC-MS/MS by Frontage Laboratories, Inc. (Shanghai, China). Sample processing was performed by solid-phase extraction (TFV) or protein precipitation (HS-10234, emtricitabine). Tenofovir-d_7, HS-10234-d_7 and emtricitabine-13C,$15N_2$ served as internal standards for the bioanalytical assay. Separation of TFV was carried out using a Waters Xbridge C18 4.6 × 50-mm, 3.5-μm column, and analytes were detected on a Triple Quad 6500 + mass analyzer. HS-10234 was separated using a Waters Xbridge C18 4.6 × 50-mm, 3.5-μm column and quantified using an API4000 mass spectrometer. Emtricitabine was separated using an Agilent Poroshell HPH-C18 2.1 × 50-mm, 2.7-μm column and quantified using an API4000 mass spectrometer. The assay was linear over the range of 0.3–300 ng/ml for TFV, 1–1000 ng/ml for HS-10234 and 5–5000 ng/ml for emtricitabine. The precision and accuracy of the analysis method were acceptable for all analytes ($\leq$ 15% [20% at the lower limit of quantification]). Recovery

rate was 88.5% for TFV, 109.2% for HS-10234 and 103.2% for emtricitabine. All assays were validated in accordance with recommendations of the NMPA, FDA, Guidance for Industry and Bioanalytical Method Validation guidelines [16].

PK and Statistical Methods

PK parameters were calculated using Phoenix WinNonlin. Statistical analyses were carried out using SAS v9.4 (SAS Institute, Cary, NC, USA). Based on the PK parameter analysis set, the treatment group (single drug and combination drug) was divided to list and describe the PK parameters of the subjects. A mixed effect model was used to statistically analyze the AUC and C_{max} of combined drugs and single drug after natural logarithmic transformation. The treatment group was treated as a fixed effect and the participants as random effects. Based on this model, the corrected least-square mean difference and 90% confidence interval (CI) between administration of a single drug (emtricitabine/HS-10234) alone and combination drug (co-administration of emtricitabine and HS-10234) were obtained. The adjusted geometric mean ratios (GMRs) (single drug and combination drug) and 90% CIs were obtained by taking the antilog of the corrected least-square mean difference and its 90% CI.

RESULTS

Subject Demographics

Thirty-six subjects were enrolled in the study, which was equally randomized into two groups. Thirty-five subjects completed the study; one subject in group 1 withdrew before co-administration because of an unexpected heart rate increase. Demographic information is shown in Table 1.

Table 1 Demographics and baseline characteristics (mean ± SD)

Characteristic, unit	Group 1 (n = 18)	Group 2 (n = 18)
Age, years	25.40 ± 8.38	24.20 ± 5.87
Male, n (%)	13 (72.2)	14 (77.8)
Female, n (%)	5 (27.8)	4 (22.2)
BMI, $kg{\cdot}m^{-2}$	22.02 ± 1.70	22.20 ± 1.67
Height, cm	164.72 ± 9.25	163.89 ± 7.92
Weight, kg	59.97 ± 8.77	59.64 ± 6.20
Ethnicity, n (%)		
Han	18 (100.0%)	17 (94.4%)
Other	0	1 (5.6%)

Pharmacokinetics

Mean plasma concentration-time profiles for HS-10234, TFV and TFV-DP after multiple-dose administration of HS-10234 with and without emtricitabine are presented in Figs. 2a, b and 3, respectively. The mean plasma concentration-time profiles for emtricitabine after multiple-dose administration of emtricitabine with and without HS-10234 are presented in Fig. 2c. In group 1, plasma concentrations of HS-10234, TFV and TFV-DP were increased during period 2 compared with period 1. In group 2, only a minor increase was observed for emtricitabine during period 2 compared with period 1.

PK parameters of plasma HS-10234, TFV and TFV-DP measured at each visit are illustrated in Table 2 and emtricitabine in Table 3. The GMR (90% CI) of main PK parameters for HS-10234, TFV, TFV-DP and emtricitabine are presented in Fig. 4. Co-administration of HS-10234 with emtricitabine in group 1 caused 1.293- and 1.327-fold increases in HS-10234 C_{max} and AUC_{0-tau}, 1.086- and 1.144-fold in TFV C_{max} and AUC_{0-tau} and 1.259- and 1.403-fold in TFV-DP C_{max} and AUC_{0-tau}, respectively. In group 2, co-administration of HS-10234 with emtricitabine increased the C_{max} and AUC_{0-tau} of emtricitabine by 1.120- and 1.077-fold, respectively.

To sum up, upon co-administered with emtricitabine in group 1, the mean C_{max} of HS-10234 and TFV-DP increased by approximately

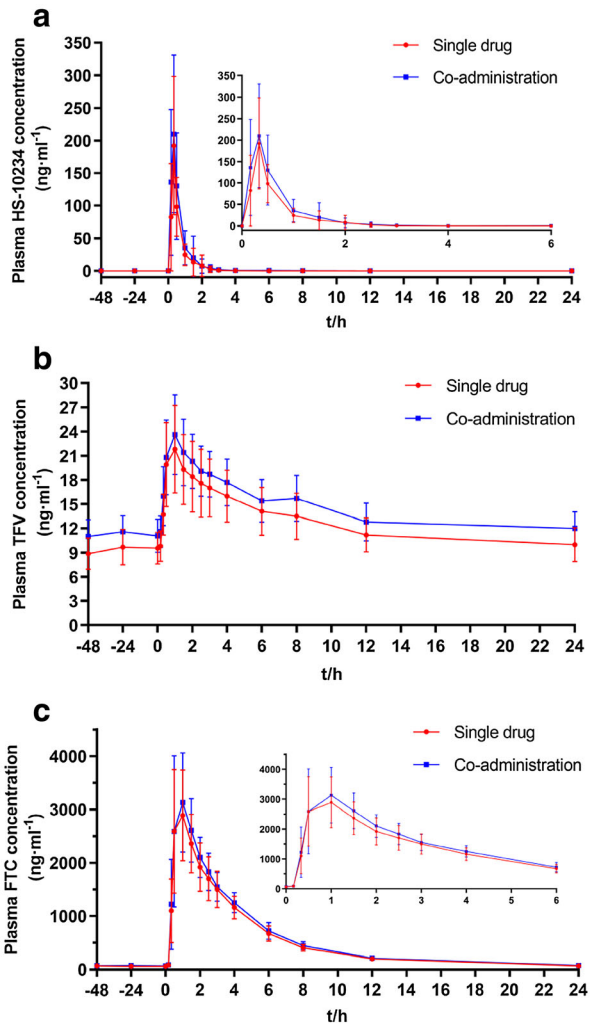

Fig. 2 **a** Mean ± SD (standard deviation) plasma HS-10234 (n = 17) concentration-time curve of subjects after multiple administration of HS-10234 under single drug and co-administration with emtricitabine (FTC). **b** Mean ± SD (standard deviation) plasma TFV (n = 17) concentration-time curve of subjects after multiple administration of HS-10234 under single drug and co-administration with emtricitabine (FTC). **c** Mean ± SD plasma FTC (n = 18) concentration-time curve of subjects after multiple administration of FTC under single drug and co-administration with HS-10234

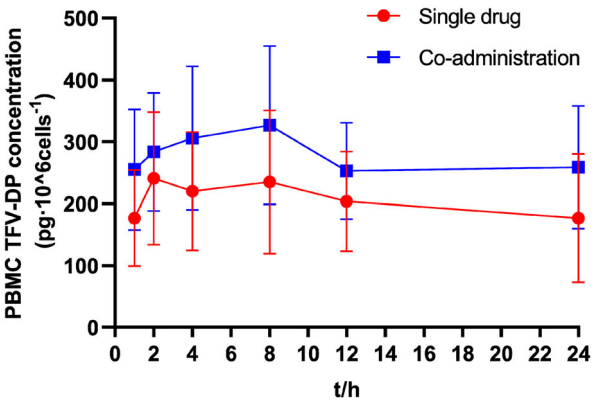

Fig. 3 Mean ± SD PBMC (TFV-DP) concentration-time curve of subjects after multiple administration of HS-10234 under single drug and co-administration with emtricitabine (FTC)

29% and 26%, and the mean AUC_{0-tau} of HS-10234 and TFV-DP increased by approximately 33% and 44%, while the mean TFV C_{max} and AUC_{0-tau} were just increased by 8% and 14%, respectively. In group 2, co-administration with HS-10234 resulted in slightly increased exposure of emtricitabine in plasma, and emtricitabine's C_{max} and AUC_{0-tau} were only 12% and 8% higher than with administration of emtricitabine alone.

Safety

All AEs are summarized in Table 4. In group 1, the proportions of subjects who reported at least one treatment-emergent adverse event (TEAE) were 2/18 (11.11%) after oral HS-10234 25 mg QD on D1–7 and 7/17 (41.17%) after co-administration of emtricitabine 200 mg QD on D8–14. Seven subjects had TEAEs (bilirubin elevations, urine red blood cell positive, urinary leukocyte esterase positive, decreased systolic blood pressure, bacteria test positive and upper respiratory tract infection) that were possibly related to HS-10234. Six subjects had TEAEs (bilirubin elevations, urine red blood cell positive, bacteria test positive and upper respiratory tract infection) that were possibly related to emtricitabine.

In group 2, the proportions of subjects who reported at least one TEAE were 1/18 (5.6%) after oral emtricitabine 200 mg QD on D1–7 and 7/18 (38.89%) after co-administration of HS-10234 25 mg QD on D8–14. Seven subjects had TEAEs (bacteria test positive, urine white blood cell positive, urine red blood cell positive, triglyceride elevation, upper respiratory tract

Table 2 Summary of HS-10234, TFV and TFV-DP's pharmacokinetic parameters (n = 17)

PK parameters	HS-10234 (median[a], SD)			HS-10234 + emtricitabine (median, SD)		
	HS-10234	TFV	TFV-DP (PBMC)[b]	HS-10234	TFV	TFV-DP (PBMC)[b]
Plasma						
T_{max}[a], h	0.33 (0.17, 1.50)	1.00 (0.50, 2.50)	4.00 (2.00, 23.90)	0.33 (0.17, 1.50)	1.00 (0.50, 2.00)	8.00 (2.00, 24.00)
C_{max}, ng/ml	198 (1.59)	22.60 (1.22)	258 (1.53)	256 (1.42)	24.5 (1.16)	324 (1.48)
AUC_{0-t}, h·ng/ml	91.6 (1.34)	298 (1.21)	4540 (1.47)	121 (1.39)	343 (1.19)	6280 (1.40)
$AUC_{0\ -tau}$, h·ng/ml	92.4 (1.33)	299 (1.21)	4350 (1.52)	123 (1.39)	342 (1.19)	6270 (1.40)
$t_{1/2}$, h	0.34 (1.31)	30.20 (1.25)		0.477 (1.84)	32 (1.23)	
λ_z, 1/h	2.04 (1.31)	0.0229 (1.25)	0.0182 (2.85)	1.45 (1.84)	0.0217 (1.23)	0.0103 (2.43)
Cl_{ss}/F, l/h	271 (1.33)	83.50 (1.21)		204 (1.39)	73 (1.19)	
V_z/F, l	133 (1.37)	3640 (1.40)		140 (1.67)	3370 (1.28)	
Urine						
Ae, ng	114,000 (1.32)	3,350,000 (1.29)		110,000 (2.07)	3,430,000 (1.43)	
Fe, %	0.455 (1.32)	13.4 (1.29)		0.442 (2.07)	13.7 (1.43)	
CLr, ml/h	1230 (1.32)	11,200 (1.38)		901 (1.95)	10,000 (1.47)	
$AURC_{0-t}$, ng	52,200 (1.71)	2,460,000 (1.32)		57,500 (2.02)	2,470,000 (1.54)	

TFV tenofovir, *FTC* emtricitabine, *TFV-DP* tenofovir diphosphate

[a]Median (min–max)

[b]Units of TFV-DP C_{max}, AUC_{0-t} and AUC_{0-tau} are pg/10^6 cells, h·pg/10^6 cells and h·pg/10^6 cells, respectively

infection, gastroenteritis, vomiting) that were possibly related to emtricitabine and HS-10234.

Overall, single drug (HS-10234/emtricitabine) and combination drug (HS-10234 / emtricitabine) were well tolerated, but the AE incidence rates were significantly increased for combination compared to single drug. One subject discontinued and withdrew from the study because of elevated heart rate after 5 days of HS-10234 administration. All TEAEs were mild in severity. No deaths or other serious adverse events were observed.

DISCUSSION

HS-10234 is a novel therapy for the treatment of HIV-1 infection. To our knowledge, this is the first study to evaluate the PK interactions between HS-10234 and emtricitabine in healthy subjects. Because there are no available PK/PD data on HS-10234 in HIV patients, we inferred that the effective dose of HS-10234 is 25 mg depending on the effective dose of TAF and the concentration of its active metabolite, TFV-DP, in PBMC [12, 13, 17]. We found that after co-administration with emtricitabine, the C_{max}

Table 3 Summary of emtricitabine's pharmacokinetic parameters (n = 18)

PK parameters	FTC (median, SD)	HS-10234 + FTC (median, SD)
Plasma		
T_{max}[a], h	1.00 (0.50, 1.50)	1.00 (0.50, 2.50)
C_{max}, ng/ml	2890 (1.37)	3240 (1.32)
AUC_{0-t}, h·ng/ml	12,500 (1.17)	13,500 (1.15)
AUC_{tau}, h·ng/ml	12,500 (1.17)	13,500 (1.15)
$t_{1/2}$, h	5.98 (1.21)	5.61 (1.25)
λ_z, 1/h	0.116 (1.21)	0.124 (1.25)
Cl_{ss}/F, l/h	16.0 (1.17)	14.8 (1.15)
V_z/F, l	138 (1.32)	120 (1.33)
Urine		
Ae, μg	147,000 (1.19)	128,000 (1.19)
Fe, %	73.3 (1.19)	63.8 (1.19)
CLr, l/h	11.7 (1.15)	9.46 (1.21)
$AURC_{0-t}$, μg	117,000 (1.21)	101,000 (1.23)

FTC emtricitabine
[a]Median (min–max)

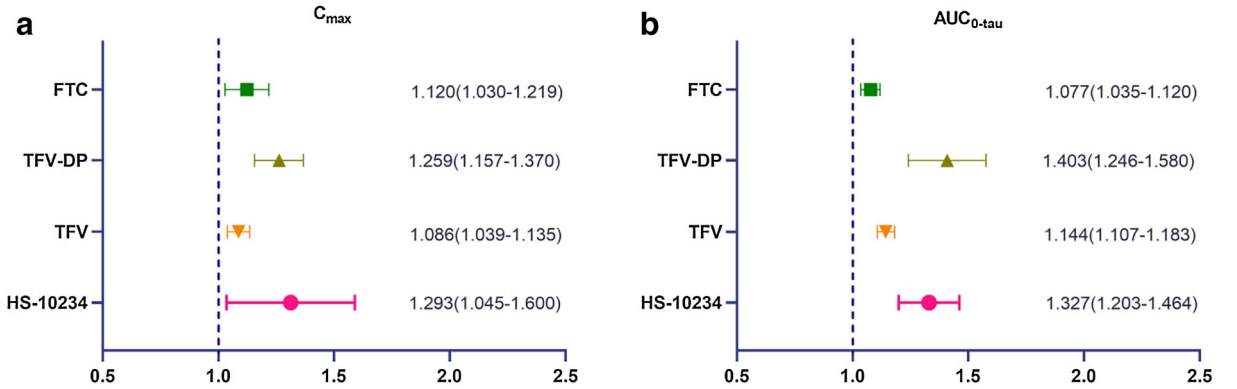

Fig. 4 Forest plot of geometric least square mean ratios and 90% confidence intervals showing the effects of co-administration on exposure to HS-10234, tenofovir (TFV), tenofovir diphosphate (TFV-DP) and emtricitabine (FTC). Transformed values on log scale (base 2). **a** C_{max}, maximum observed concentration; **b** AUC_{0-tau}, area under the concentration-time curve from time zero to steady-state dose interval (tau)

and AUC_{0-tau} of intracellular TFV-DP and HS-10234 were much higher than those of HS-10234 alone, indicating that HS-10234 and TFV-DP exposure was significantly increased. However, TFV exposure was comparable after administration of HS-10234 with or without

Table 4 Summary of adverse events (AEs)

	Group 1		Group 2	
	HS-10234 (*n* = 18), *n* (%)	**HS-10234+ FTC (*n* = 17), *n* (%)**	**FTC (*n* = 18), *n* (%)**	**FTC + HS-10234 (*n* = 18), *n* (%)**
All adverse events	2 (11.1)	8 (47.1)	1 (5.6)	7 (38.9)
Bilirubin elevations	0	3 (17.6)	0	0
Urine red blood cell positive	0	2 (11.8)	0	1 (5.6)
Urine white blood cell positive	0	0	0	1 (5.6)
Urinary leukocyte esterase positive	0	1 (5.9)	0	0
Triglycerides elevations	0	0	0	1 (5.6)
Decreased systolic blood pressure	1 (5.6)	0	0	0
Bacteria test positive	0	1 (5.9)	0	4 (22.2)
Increased heart rate	1 (5.6)	0	0	0
Upper respiratory tract infection	0	1 (5.9)	1 (5.6)	1 (5.6)
Gastroenteritis	0	0	0	1 (5.6)
Vomiting	0	0	0	2 (11.1)
HS-10234-related adverse events	1 (5.6)	7 (41.2)	–	6 (33.3)
Bilirubin elevations	0	3 (17.6)	–	0
Urine red blood cell positive	0	2 (11.8)	–	1 (5.6)
Urine white blood cell positive	0	0	–	1 (5.6)
Urinary leukocyte esterase positive	0	1 (5.9)	–	0
Triglycerides elevations	0	0	–	1 (5.6)
Decreased systolic blood pressure	1 (5.6)	0	–	0
Upper respiratory tract infection	0	1 (5.9)	–	1 (5.6)
Gastroenteritis	0	0	–	1 (5.6)
Vomiting	0	0	–	2 (11.1)

FTC emtricitabine

emtricitabine. Conversely, HS-10234 did not have a significant influence on the PK behavior of emtricitabine.

Similar to TAF, HS-10234 is a phosphonate prodrug of TFV with a structure adding one methyl group to TAF (Vemlidy) [12]. HS-10234 can be effectively delivered to hepatocytes with reduced systemic TFV exposure, showing higher plasma stability than TDF [12]. HS-10234 primarily maintains the prototype until entering into hepatocytes and lymphoid cells, subsequently metabolizing to TFV by carboxylesterase and cathepsin A there [10, 11, 18]. Therefore, the concentration of TFV in PBMC of the HS-10234 and TAF administration group was several times that of the TDF administration group. This can be explained as the effect of HS-10234 being equivalent to or slightly better than that of TAF, and both were significantly better than TDF. In an in vitro study of the anti-HIV effect of HS-10234 in MT-4 cells and C8166 cells on experimental strains HIV-1RF, the EC50 of HS-10234 was comparable to that of TAF and significantly better than that of TDF. Besides, HS-10234 had a good inhibitory effect on HIV-1KM018, HIV-1TC-1 and HIV-1WAN subtype clinical HIV strains in PBMC cells, which was better than TAF and TDF.

Since in vitro studies demonstrated that HS-10234 or emtricitabine overdose did not affect most of CYP450 enzymes, and both TFV and emtricitabine were eliminated largely as prototypical drugs in urine, it is reasonable that they were less susceptible to metabolic drug interactions [19, 20]. This was verified in our finding that HS-10234 has no effect on the PK exposure of emtricitabine. However, we noticed that emtricitabine could slightly increase the PK exposure of HS-10234 as well. The possible explanation is that although the carboxylesterase and cathepsin A pathways are not commonly impacted by DDIs, TAF disposition was possibly affected by the induction of nuclear receptors, i.e., pregnane X receptor and constitutive androstane receptor, or by inhibitor-induced P-gp and BCRP transporter activation [21]. Therefore, we speculated that in our study the co-administration of emtricitabine increased HS-10234 exposure and intracellular TFV-DP concentration because of the inhibition or induction of several transporters, which can promote the absorption of HS-10234 in the gastrointestinal tract and the following lymphocyte entry.

In fact, compared with administration of HS-10234 alone, the increase in HS-10234 exposure and its active metabolite, TFV-DP, by co-administration with emtricitabine in Study 1 is not concluded to cause clinically relevant changes in the overall safety profile. Although there was an increasing trend in incidence of AEs in subjects administered HS-10234 and emtricitabine, this may be related to higher HS-10234 and TFV-DP exposure. All of the TEAEs were mild in severity. No deaths or other SAEs were observed. Since HS-10234 is a novel antiviral drug that may be frequently used in seropositive treatment in the future, we hope that the results here will help researchers design and conduct future phase II/III studies in the era of evolving new combination antiretroviral therapy regimens.

There are some limitations in this DDI study: we performed it in healthy volunteers instead of the intended population of HIV patients owing to ethical and feasibility considerations. In summary, the findings in our study support further evaluation of HS10234 in combination with emtricitabine in treatment-naive adults with HIV-1 infection.

CONCLUSION

HS-10234 25 mg QD does not have a significant influence on the PK exposures of emtricitabine. Emtricitabine 200 mg QD slightly increases the steady-state PK of HS10234 and TFV-DP in healthy volunteers under fasted condition. Subjects tolerated HS-10234 co-administered with emtricitabine well. Thus, no dose adjustments are needed when HS-10234 is co-administered with emtricitabine.

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Author Contributions. The principal investigator of this clinical trial was Guoping Yang. Qi Pei, Guoping Yang and Zhuo Li conceived and designed the study. Yeping Luo, Wenjing Chen, Chan Zou, Jie Huang, Yun Kuang, Kai Shen, Basheng Zhang, Shuang Yang and Hong Xiang implemented the clinical trials. Qi Pei assessed the biological samples. Yeping Luo, Wenjing Chen, Qi Pei, Chan Zou and Yun Kuang analyzed and interpreted the data. Yeping Luo, Wenjing Chen and Chan Zou wrote the paper.

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Compliance with Ethics Guidelines. Regulatory and ethics approvals were obtained before initiating the study (Third Xiangya Hospital of Central South University Ethics Committee). The study was conducted in accordance with the principles of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Good Clinical Practice, and the Declaration of Helsinki. All subjects signed written informed consents prior to initiating any study-related procedures.

Data Availability. All data generated or analyzed during this study are included in this published article/as supplementary information files.

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