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ORIGINAL RESEARCH—CLINICAL

Safety, Tolerability, and Pharmacokinetics of a Novel Human Hepatitis B Virus Capsid Assembly Modulator Canocapavir: A Randomized First-in-Human Study

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BACKGROUND AND AIMS: Canocapavir (ZM-H1505R) is a small-molecule hepatitis B virus capsid assembly modulator with a novel pyrazole structure. This is the first-in-human study to investigate its safety, tolerability, and pharmacokinetics (PK) following oral administration in healthy subjects. **METHODS:** This was a randomized, double-blind, placebo-controlled study including single ascending dose (SAD) study with an additional crossover food-effect arm, and multiple ascending dose study. In SAD, 40 subjects, 8 in each cohort, were randomized in a 3:1 ratio to receive a single dose of 25, 75, 150, 300, and 450 mg of Canocapavir or placebo in fasted state. For food-effect study, subjects in the 150 mg cohort of SAD received a second dose (150 mg) of Canocapavir in the fed state after a 7-day washout period. In multiple ascending dose, 24 subjects, 8 in each cohort, were randomized in a 3:1 ratio to receive 75, 150, and 300 mg of Canocapavir or placebo once daily for 14 days. The safety and tolerability were assessed using vital signs, physical evaluation, electrocardiogram, laboratory investigations, and adverse events (AEs). Plasma PK parameters measured included area under the curves, C_{max}, C_{min}, T_{max}, and $T_{1/2}$. **RESULTS:** Oral administration of single doses (25–450 mg) and multiple doses (75–300 mg) of Canocapavir was well tolerated. The most common AE seen was increased alanine aminotransferase. No dose dependency was observed in incidence and intensity of AEs. Mean plasma area under the curve and C_{max} of Canocapavir increased dose-proportionally. A significant margin was observed between plasma exposure of Canocapavir and its in vitro anti-hepatitis B virus activity. Food had an effect on its absorption. **CONCLUSION:** The safety and PK profile of Canocapavir support its further evaluation in chronic hepatitis B patients. The study was registered on ClinicalTrial.gov with the number NCT04220801.

Keywords: Canocapavir; HBV; Capsid assembly modulator; First-in-human

See editorial on page 556.

Introduction

The global population of hepatitis B virus (HBV)-infected individuals are currently about 250 million, with an annual death toll of about 0.8 million, mostly from HBV infection-related cirrhosis and liver cancer.[1] Therefore, viral hepatitis B infection is still one of the significant threats facing global public health. Current anti-HBV drugs mainly include nucleos(t)ide analogs (NAs) and peg-interferon.[2] Although these drugs can effectively inhibit the replication of the virus, they have obvious limitations. For instance, NAs do not possess a mechanism to eradicate the virus; therefore, patients cannot stop taking the drugs, yet prolonged dosing could lead to resistance. On the other hand, peg-interferons are effective for only 30% of HBV patients with a cure rate of only around 7%–8%.[3] Since current therapeutic approaches are inadequate to address the clinical need for HBV therapeutics, new anti-HBV drugs (such as capsid assembly modulators [CAMs] and small interfering RNA), immunomodulators, and therapeutic vaccines are all currently being explored for achieving the goal of curing chronic hepatitis B.[4]

HBV core protein plays multiple roles in the replication and persistence of the virus, such as viral nucleocapsid disassembly, transcription and assembly. CAMs can interfere with these steps.[5] By shutting down the production of virus, CAMs can prevent new infection of liver cells, driving the number of infected cells to a declining direction, thus raising the cure rates.[6]

Canocapavir is an investigational HBV CAM with a novel chemical structure that is being developed for treating HBV infection by Shanghai Zhimeng Biopharma, Inc. Different from the reported Class I (heteroaryldihydropyrimidines) and Class II (sulfamoylbenzamides) CAMs, Canocapavir is a

Abbreviations used in this paper: **AE, adverse event; ALT, alanine aminotransferase; AUC, area under the curve; BMI, body mass index; CAM, capsid assembly modulator; CLr, renal clearance; CTCAE, Common Terminology Criteria for Adverse Events; FE, food effect; HBV, hepatitis B virus; MAD, multiple ascending dose; NA, nucleos(t)ide analog; PK, pharmacokinetics; SAD, single ascending dose; SAE, serious adverse event; SD, Sprague Dawley; TEAE, treatment-emergent adverse event; ULN, upper limit of normal.**

Most current article

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novel pyrazole compound. Preclinical virology studies of Canocapavir demonstrated that it is a potent HBV CAM that effectively inhibited HBV replication in both in vitro and in vivo models.[7] Canocapavir showed potent activity against HBV replication with an EC_{50} of 10 nM and 12 nM in the HepG2.2.15 cells culture model and primary human hepatocytes infection assay, respectively. It is highly active against all major HBV genotypes tested and some HBV variants that are resistant to reported Class I and Class II CAMs. In the animal toxicity study, oral administration of Canocapavir to the Sprague Dawley (SD) rats and Beagle dogs at 100, 300, and 1000 mg/kg was well tolerated and did not result in any treatment-related mortality or adverse effects. The no observed adverse effect level was considered to be 300 in Beagle dogs and 1000 mg/kg/d in SD rats. Here, we report the safety, tolerability, and pharmacokinetics of Canocapavir following single and multiple ascending oral doses in healthy subjects, from a randomized, double-blind, placebo-controlled first-in-human study. An extended crossover cohort in the fed state was also conducted to assess food effect on the pharmacokinetics of Canocapavir.

The study complied with Food and Drug Administration's regulations: Title 21, *Code of Federal Regulations*, and International Council for Harmonisation guideline: International Council for Harmonisation E6, R2 *Good Clinical Practice*. It was registered on ClinicalTrail.gov with the number NCT04220801. The study was conducted in the facility of Frontage Clinical Services, Inc, US. The study protocol, informed consent forms, and relevant safety and study-related correspondence were submitted and approved by the IntegReview Ethics Review Board from Austin, Texas, USA.

Methods

Study Design

This was a Phase I, randomized, double-blind, placebo-controlled, and dose-escalation study to evaluate the safety, tolerability, and pharmacokinetics (PK) of Canocapavir following single and multiple ascending oral dose administration. The study consisted of 2 parts: Part 1, single ascending dose (SAD) with an additional crossover food-effect (FE) arm, and Part 2, multiple ascending dose (MAD). In Part 1, 40 healthy subjects were enrolled and randomized in a 3:1 ratio to receive Canocapavir or placebo. Six subjects per cohort received a single oral dose of 25, 75, 150, 300, and 450 mg of Canocapavir, and 2 subjects in each of the 5 cohorts received the placebo in the fasted state. After a 7-day washout, subjects in SAD cohort 3 (150 mg cohort) received another 150 mg of Canocapavir following a high-fat breakfast. In Part 2, 24 healthy subjects were enrolled and randomized in a 3:1 ratio to receive Canocapavir or placebo for 14 consecutive days. Six subjects per cohort received once-daily oral doses of Canocapavir at 75, 150, and 300 mg, 2 subjects per cohort received placebo. All doses were administered under fasted conditions in MAD.

Study Population

For both parts of the study, major inclusion criteria were: 1) healthy male or nonpregnant female subjects between 18 and 55 years of age, inclusive; 2) with a body mass index (BMI) within 18.0–32.0 kg/m^2, and body weight not less than 50 kg; 3) with normal liver and renal function, evidenced by alanine aminotransferase (ALT) and aspartate aminotransferase $<1.2\times$ upper limit of normal (ULN) and estimated glomerular filtration rate >70 mL/min/1.73 m^2. Moreover, major exclusion criteria were: 1) having clinically significant history of medical disorders; 2) reported or suspected malignancy; 3) unexplained syncope; 4) symptomatic hypotension or hypoglycemia; 5) a family history of prolonged QTc syndrome; and 6) positive blood screen result for human immunodeficiency virus, hepatitis B surface antigen, or hepatitis C antibody. In addition, subjects with a history of alcohol or drug abuse, or use of prescription medications within 14 days, must be excluded. Any over-the-counter medications or herbal supplements within 7 days of first dosing were strictly prohibited.

Blood and Urine Sampling

In Part 1 SAD and FE study, plasma samples were collected at pre-dose and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, and 48 hours after dosing. In Part 2 MAD study, plasma samples were collected on days 1 and 14, at pre-dose and 0.5, 1, 2, 4, 6, 8, 12, and 24 hours post-dose; on days 5, 8, 10 and 13, at pre-dose.

Urine samples were only collected in the SAD study part at the following time intervals: −2 to 0 hours, 0–4 hours, 4–8 hours, 8–12 hours, 12–24 hours, and 24–48 hours after dosing.

Safety Assessment

Safety and tolerability were assessed by measurements of vital signs, physical evaluation findings, electrocardiogram, and laboratory safety tests and adverse events (AEs). AEs were evaluated by severity, seriousness, and relationship to study drug and coded by using the Medical Dictionary for Regulatory Activities, Version 22.1 for the preferred term and system organ class and listed by subject. The severity and toxicity of each AE were graded by the investigator by using a 4-grade scale: Mild (Grade 1), Moderate (Grade 2), Severe (Grade 3), and Potentially Life Threating (Grade 4). Meanwhile, for some clinical and laboratory abnormalities, a composite Toxicity Grading Guidance Tables incorporating *Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials* and *Common Terminology Criteria for Adverse Events (CTCAEs)*, Version 5.0, local laboratory reference values, baselines, and principle investigator's confirmation was used.

Pharmacokinetic Assessment

Plasma and urine samples were analyzed using validated methods of liquid chromatographic separation with tandem mass spectrometric detection (LC-MS/MS). The primary PK parameters of interest for SAD were C_{max}, T_{max}, $T_{1/2}$, area under the curve ($AUC_{0\text{-}t}$), and $AUC_{0\text{-}\infty}$ for plasma and the amount of parent drug excreted in the urine. The primary PK parameters of interest for MAD were C_{max}, T_{max}, and $AUC_{0\text{-}\tau}$ (τ = 24 hours) for plasma on day 1; C_{max}, T_{max}, $T_{1/2}$, and $AUC_{0\text{-}\tau}$ for plasma on day 14. Urine PK parameters measured include the amount of unchanged drug excreted into urine from time 0 to 48 hours post-dose ($Ae_{(0\text{–}48\ h)}$) and renal clearance (CLr). The CLr was determined as $Ae_{0\text{–}48}/AUC_{0\text{–}48}$ based on the same time period.

Statistical Methods

Since this was an early development study, no statistical considerations were given in the sample size determination. It was expected that the sample size of 8 subjects (6 subjects receiving active drug and 2 subjects receiving placebo) in each cohort should be adequate for evaluating tolerability and PK parameters in this SAD and MAD study. Subject treatment assignment was based on a computer-generated randomization scheme with a ratio of 3:1 (active: placebo) for Part 1 and Part 2. The study treatment assignment was blinded. Neither the investigational staff nor the subjects were aware of the study drug assignments. The clinical site pharmacist or designee prepared the study drug according to the randomization scheme, maintained the drug packaging and labeling log, and kept the blinding for treatment assignment per the randomization scheme.

Data collected in this study were presented using summary tables and subject data listings. Statistical calculations were done using SAS software (Version 9.4 SAS Institute, Cary, North Carolina, USA). Plasma concentration-time data for Canocapavir was analyzed using non-compartmental methods with the calculation done on Phoenix WinNonlin (Version 8.1, Certara, Princeton, USA) using actual PK elapsed sampling times.

All authors had access to the study data and reviewed and approved the final manuscript.

Results

Demographic Characters

In Part 1, SAD and FE study, 40 healthy adult subjects were enrolled and randomized, including 30 subjects receiving Canocapavir and 10 subjects receiving placebo. There were 31 males and 9 females (77.5%/22.5%), with ages ranging from 20 to 55 years with a mean (SD) of 40.3 (10.07) years. Mean (SD) BMI was 27.10 (2.860) kg/m^2, and the racial composition was 26 Black or African American (65.0%), 13 White (32.5%), and 1 Asian (2.5%). All 40 subjects completed the study treatment per protocol (Table 1).

In Part 2, the MAD study, a total of 24 healthy adult subjects were enrolled and randomized, including 18 subjects receiving Canocapavir and 6 subjects receiving placebo. There were 15 males and 9 females (62.5%/37.5%), ranging from 18 to 54 years of age with a mean (SD) of 38.5 (10.38) years. Mean (SD) BMI was 26.76 (3.035) kg/m^2, and the racial composition was 13 Black or African American (54.2%), 8 White (33.3%), 2 American Indian or Alaskan Native (8.3%), and 1 Asian (4.2%). Twenty-three subjects completed the study treatment, while one subject in the 300 mg cohort (*47* Y, F) discontinued prematurely from the study due to AEs of lab abnormalities. Demographics were generally comparable across the dose cohorts, with minor differences likely due to the relatively small cohort sizes (Table 1).

Safety

In the SAD and FE study, single oral doses of Canocapavir, ranging from 25 to 450 mg, were safe and well-tolerated when administered to healthy subjects in this study. As listed in Table A1, 10 of 40 subjects (25.0%) reported a total of 11 treatment-emergent adverse events (TEAEs), including 8 of 30 (26.7%) subjects who received Canocapavir and 2 of 10 subjects (20.0%) who received placebo. TEAEs in subjects who received Canocapavir include elevated ALT, increased amylase (reaching 166 U/L, 1.7 $\times$ ULN, Grade 2 by CTCAE V5.0), hypoglycemia, pain in extremity, abdominal pain, and nausea. The most common type of TEAEs are ALT increased (2 subjects, reaching 84 U/L and 63 U/L, respectively; both $<$2.5 $\times$ ULN, Grade 1 as assessed by CTCAE V5.0), hypoglycemia (2 subjects, 1 mild and 1 severe) and pain in extremity (2 subjects, both mild). All subjects recovered, and all AEs were resolved without any intervention. No severe AE or serious adverse event (SAE) was observed in any cohorts, and no AE leading to drug discontinuation was reported.

In the MAD study, Canocapavir was shown to be safe and well tolerated following multiple oral administrations of 75, 150, or 300 mg once daily for 14 consecutive days. Twelve of 24 subjects (50.0%) reported a total of 19 TEAEs, including 11 of 18 subjects (61.1%) who received Canocapavir and 1 of 6 subjects (16.7%) who received placebo (Table A2). TEAEs in subjects who received Canocapavir include abdominal pain, diarrhea, flatulence, oral paresthesia, increased ALT (one subject, reaching 49 U/L at Day 14), increased amylase, increased blood alkaline phosphatase, increased gamma-glutamyl transferase, increased lipase, headache, anemia, tachycardia, conjunctival hyperemia, mouth injury, and back pain. The most common type of TEAEs are gastrointestinal disorders, reported in 4 of 18 subjects (22.2%) receiving Canocapavir and 1 of 6 subjects (16.7%) receiving placebo. The cases of abdominal pain, constipation, diarrhea, and flatulence were deemed mild and not related to the study drug, while the cases of oral paresthesia were assessed as probably related to the study drug. The subjects recovered, and AEs were resolved without any intervention. There were no deaths or SAEs in the MAD study.

One of the subjects in the 300 mg MAD cohort discontinued study participation due to increase in amylase, reaching 229 U/L (2.3 $\times$ ULN, Grade 3 as assessed by CTCAE V5.0 and probably related to the study drug) and increase in lipase (reaching 6.8 $\times$ ULN, Grade 3 as assessed by CTCAE V5.0 and related to study drug). Despite these AEs, this subject's ALT had not increased throughout, see Table A3. The subject suffered a mild diarrhea (mild and not related to study drug), and eventually all AEs were resolved after drug discontinuation without any interventions.

Overall, single oral doses of Canocapavir ranging from 25 to 450 mg and multiple daily oral doses of Canocapavir at 75, 150, and 300 mg QD for 14 days were well tolerated in healthy adult subjects. The most common AE seen in subjects receiving Canocapavir was increased ALT, seen in 2 subjects in SAD ($<$2.5 $\times$ ULN) and one in MAD (1.5 $\times$ ULN). No dose dependency was observed in the incidence and intensity of AEs in the study. Overall, there were no clinically significant changes in clinical safety laboratory parameters, vital signs, electrocardiogram parameters, or physical examination findings attributable to Canocapavir.

Table 1. Demographics and Baseline Characteristics

	Part 1, SAD and food effect							Part 2, MAD				
Parameter	Placebo N = 10	Canocapavir 25 mg N = 6	Canocapavir 75 mg N = 6	Canocapavir 150 mg N = 6	Canocapavir 300 mg N = 6	Canocapavir 450 mg N = 6	All subjects N = 40	Placebo N = 6	Canocapavir 75 mg N = 6	Canocapavir 150 mg N = 6	Canocapavir 300 mg N = 6	All subjects N = 24
Age, mean(min, max)	42.9 (23, 55)	32.7 (20, 47)	39.7 (28, 51)	38.5 (29, 51)	41.8 (30, 53)	44.5 (31, 55)	40.3 (20, 55)	40.3 (29, 54)	34.8 (20, 54)	35.7 (18, 46)	43.3 (37, 54)	38.5 (18, 54)
Gender, male/female n (%)	8/2 (80.0/20.0)	6/0 (100.0/0.0)	4/2 (66.7/33.3)	4/2 (66.7/33.3)	4/2 (66.7/33.3)	5/1 (83.3/16.7)	31/9 (77.5/22.5)	3/3 (50.0/50.0)	4/2 (66.7/33/3)	5/1 (83.3/16.7)	3/3 (50.0/50.0)	15/9 (62.5/37.5)
Race, n (%)												
American Indian or Alaskan Native								1 (16.7)	0	0	1 (16.7)	2 (8.3)
Asian	0	0	1 (16.7)	1 (16.7)	0	0	1 (2.5)	0	0	0	1 (16.7)	1 (4.2)
Black or African American	6 (60.0)	4 (66.7)	3 (50.0)	3 (50.0)	5 (83.3)	5 (83.3)	26 (65.0)	5 (83.3)	4 (66.7)	3 (50.0)	1 (16.7)	13 (54.2)
White	4 (40.0)	2 (33.3)	2 (33.3)	2 (33.3)	1 (16.7)	1 (16.7)	13 (32.5)	0	2 (33.3)	3 (50.0)	3 (50.0)	8 (33.3)
Weight (kg), mean	79.71	80.40	75.88	80.61	84.03	75.80	79.44	74.15	74.57	77.50	79.54	76.44
Height (cm), mean	174.30	171.77	167.33	171.42	174.30	170.07	171.81	165.95	168.00	170.88	171.63	169.12
BMI (kg/m^2), mean(min, max)	26.14 (22.7, 29.2)	27.27 (21.7, 30.7)	27.27 (21.4, 30.3)	28.30 (25.5, 30.6)	27.70 (21.5, 30.0)	26.53 (22.2, 30.7)	27.10 (21.4, 30.7)	26.82 (25.0, 28.7)	26.38 (21.3, 30.3)	26.20 (22.1, 30.1)	27.65 (19.1, 31.1)	26.76 (19.1, 31.1)

Table 2. Summary of Canocapavir Plasma Pharmacokinetic Parameters by Dose–Part 1, SAD and Food Effect

Parameter (unit)	Statistic	25 mg (N = 6)	75 mg (N = 6)	150 mg (N = 6)	150 mg fed (N = 6)	300 mg (N = 6)	450 mg (N = 6)
$AUC_{0\text{-}t}$ (h*ng/mL)	Mean (SD)	6710 (2033)	28,700 (8849)	50,400 (24,258)	29,700 (14,589)	90,000 (44,534)	168,000 (55,954)
$AUC_{0\text{-}inf}$ (h*ng/mL)	Mean (SD)	7070 (2247)	30,600 (10,000)	45,500 (15,004)	33,500 (18,780)	80,700 (27,200)	161,000 (33,729)
$AUC_{0\text{–}24}$ (h*ng/mL)	Mean (SD)	5590 (1575)	23,100 (6347)	38,300 (16,327)	22,200 (9418)	68,300 (28,841)	113,000 (31,807)
$AUC_{0\text{–}48}$ (h*ng/mL)	Mean (SD)	6710 (2033)	28,700 (8849)	50,400 (24,223)	29,700 (14,589)	90,000 (44,534)	168,000 (55,954)
C_{max} (ng/mL)	Mean (SD)	688 (187)	2430 (454)	3780 (1282)	1950 (605)	6460 (2119)	8490 (1702)
T_{max} (h)	Median (min-max)	3.00 (2.00–4.00)	3.00 (2.00–4.10)	3.00 (2.00–4.00)	3.00 (3.00–4.00)	3.00 (2.00–3.00)	3.00 (2.00–3.00)
$T_{1/2}$ (h)	Mean (SD)	11.3 (1.89)	12.0 (1.79)	13.8 (1.58)	14.4 (2.72)	13.0 (3.75)	18.4 (1.56)
V_z/F (L)	Mean (SD)	61.1 (15.0)	46.0 (16.1)	76.8 (43.7)	108 (41.2)	72.9 (25.5)	76.5 (15.6)
CL/F (L/h)	Mean (SD)	3.81 (1.02)	2.72 (1.02)	3.79 (1.93)	5.44 (2.37)	4.07 (1.33)	2.91 (0.697)
MRT (h)	Mean (SD)	12.3 (1.51)	13.4 (1.44)	14.9 (1.36)	15.5 (1.81)	14.8 (2.40)	18.1 (1.20)
K_{el} (1/h)	Mean (SD)	0.0626 (0.0101)	0.0591 (0.00832)	0.0507 (0.00589)	0.0494 (0.00784)	0.0591 (0.0259)	0.0380 (0.00310)

The 150 mg fed arm for food effect was presented next to SAD cohort 3 150 mg fasted for comparison.
Max, maximum; Mean, arithmetic mean; Min, minimum; MRT, mean residence time; N, number of subjects in the specified study population under each treatment; SD, standard deviation.

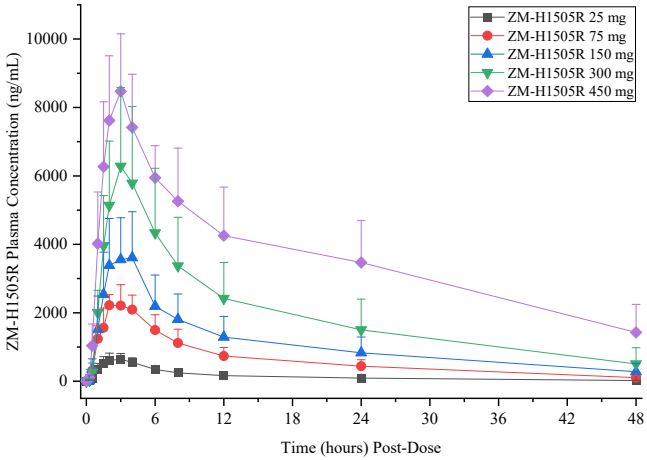

Figure 1. Mean Canocapavir (ZM-H1505R) plasma concentration (ng/mL) vs time curve when subjects were dosed 25, 75, 150, 300, and 450 mg in SAD, displaying plasma drug concentration within 48 hours after drug administration under linear time scale. Error bars represent standard deviation within the dosing cohorts. A preliminary dose-proportional increase in Cmax and AUC can be observed from the plots.

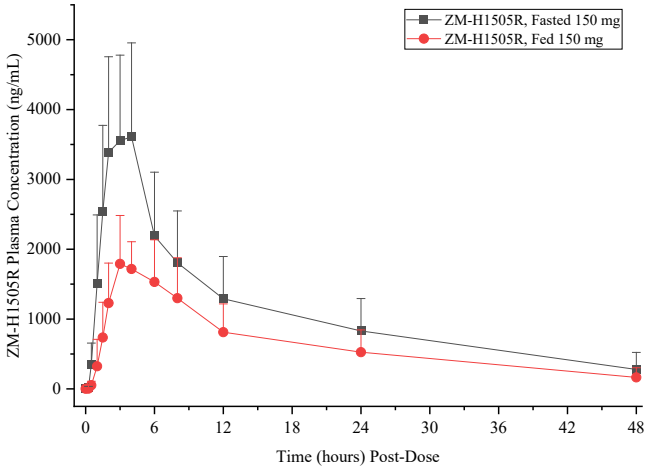

Figure 2. Mean Canocapavir (ZM-H1505R) plasma concentration (ng/mL) vs time curve when subjects were given a single dose of 150 mg in food effect study, displaying plasma drug concentration within 48 hours after drug administration under linear time scale. Error bars represent standard deviation within the dosing group. A negative food effect can be observed when Canocapavir was administered following a high-fat, high-calories meal.

Pharmacokinetics

SAD and FE. Pharmacokinetic parameters derived from Canocapavir plasma concentration data in Part 1 SAD and FE are summarized in Table 2, and the plasma PK profile is presented in Figure 1 & Figure A1.

As shown in Figure 1, the absorption of Canocapavir occurred rapidly after single-dose administration to healthy subjects in SAD cohorts. Plasma concentrations peaked at a median of 3.0 hours (2.0–4.1) under fasted conditions. From 25 mg to 450 mg, mean AUC and C_{max} increased approximately dose-proportionally (Figure 1 and Table 2). The average elimination half-life ($T_{1/2}$) ranged from 11.3 to 18.4 hours, and the mean residence time ranged from 12.3 to 18.1 hours. Corresponding to 25, 75, 150, 300, and 450 mg dosing of Canocapavir, the mean plasma trough concentration (C_{trough}) of the drug was 0.7-, 3.3-, 6.2-, 11.1-, and 25.7-fold of its protein-binding adjusted HBV DNA EC_{50} (12 nM), respectively. Meanwhile, Canocapavir exhibited relatively low total body clearance (ranging from 2.72 to 4.07 L/h on average) and was widely distributed throughout the body and tissues, with an apparent volume of distribution ranging from 46.0 to 76.8 L on average.

In the FE arm, following a high-fat, high-calories meal, mean C_{max} was 1950 ng/mL (C_{max} was 3780 ng/mL in fasted condition), mean AUC_{0-t} was 29,700 h*ng/mL (AUC_{0-t} was 50,400 h*ng/mL in fasted condition), and the median T_{max} (3.00 hours) was the same for both fasted and fed groups. For C_{max}, the percent geometric mean ratio (90% CI) was 53.3 (39.2, 72.4), which is not contained within the 80.00%–125.00% boundaries. The same was found for AUC_{0-24} and AUC_{0-inf}, which were 59.4 (42.2, 83.5) and 68.4 (39.6, 118), respectively. These results indicate an effect of high-calorie food on the bioavailability of Canocapavir, with an approximate 50% decrease in exposure when dosed in fed conditions. The plasma PK profiles in fasted and fed conditions are displayed in Figure 2.

Urinary excretion of Canocapavir was low (<1.5% of the dose, on average). Mean CLr was similar among the dose levels, ranging from 0.0266 to 0.0364 L/h, as shown in Table A4.

MAD. Canocapavir plasma PK parameters for subjects in MAD on day 1 and day 14 are shown in Table 3. Following oral administration of MADs ranging from 75 to 300 mg, mean AUC and C_{max} increased approximately dose-proportionally on day 1 and day 14. Steady-state was achieved by day 8. Corresponding to 75, 150, and 300 mg dosing of Canocapavir, the mean plasma trough concentration (C_{trough}) of the drug was 6.3-, 18.2-, and 37.5-fold of its protein-binding adjusted HBV DNA EC_{50} (12 nM), respectively. A modest accumulation effect was observed with an approximately 2-fold increase comparing day 14 to day 1 AUC values. The Canocapavir plasma PK profiles in Part 2 MAD are displayed in Figure 3 and Figure A2.

Discussion

This is the first-in-human study to evaluate the safety, tolerability, pharmacokinetic features, and food effect of Canocapavir, a modulator of HBV capsid assembly, following oral administration in healthy subjects.

Single oral doses of Canocapavir ranging from 25 to 450 mg and multiple daily oral doses of 75, 150, and 300 mg QD for 14 days, were well tolerated in healthy adult subjects. No deaths or SAEs occurred during either part of the study. Only one subject in the MAD 300 mg treatment group was

Table 3. Summary of Plasma Canocapavir Pharmacokinetic Parameters by Dose–Part 2, MAD

Parameter (unit)	Statistic	75 mg (N = 6)	150 mg (N = 6)	300 mg (N = 6)
Day 1				
AUC_{0-24} (h*ng/mL)	Mean (SD)	20,600 (9530)	43,600 (14,777)	88,200 (30,834)
C_{max} (ng /mL)	Mean (SD)	2430 (1268)	4110 (1113)	7280 (2281)
T_{max} (h)	Median (min, max)	2.00 (2.00–6.00)	3.00 (2.00–4.00)	3.00 (2.00–4.00)
Day 14				
AUCτ (h*ng/mL)	Mean (SD)	43,700 (19,365)	89,000 (21,235)	170,000 (38,688)
C_{max} (ng/mL)	Mean (SD)	3940 (1580)	6400 (950)	10,700 (1375)
T_{max} (h)	Median (min, max)	2.00 (2.00–4.00)	2.00 (2.00–4.00)	4.00 (2.00–4.00)
$T_{1/2}$ (h)	Mean (SD)	12.2 (1.87)	17.3 (2.19)	17.5 (1.94)
$AR(C_{max})$	Mean (SD)	1.82 (0.841)	1.61 (0.301)	1.71 (0.715)
$AR(AUC)_{0-24}$	Mean (SD)	2.18 (0.545)	2.14 (0.538)	2.35 (1.18)

MAD, multiple ascending doses; Max, maximum; Min, minimum; Mean, arithmetic mean; N, number of subjects in the specified study population under each treatment; SD, standard deviation.

discontinued from the study due to increased lipase and amylase, which needs to be closely monitored in future trials. The most common AE seen was increased ALT (3 cases in total). As the phenomenon of ALT increase occurred without hyperbilirubinemia and with no symptoms, it is considered as an adaptation[8] of the body to the drug. As we can see from the trend of the ALT alteration (see Figure A3), most of the data points are within the 2 × ULN (normal range 4–36 U/L) range. Therefore, no risk of idiosyncratic drug-induced liver injury is shown, only a presence of fluctuation of ALT levels. Furthermore, no leukocyte infiltration or abnormal hepatocellular injury was observed in the pathological sectioning in our preclinical animal study of Canocapavir. Nonetheless, the cause of ALT elevation will be further studied in the Phase II study, when a wider variety of populations is included.

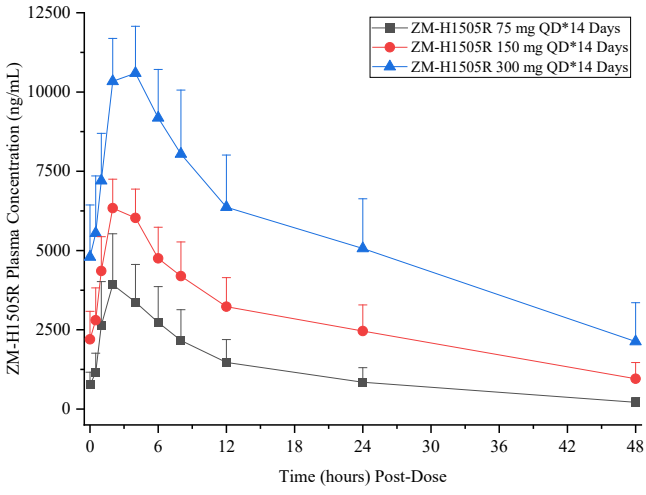

Figure 3. Mean Canocapavir (ZM-H1505R) plasma concentration (ng/mL) vs time curve when subjects were dosed 75, 150, and 300 mg in MAD on day 14, displaying plasma drug concentration within 48 hours after drug administration under linear time scale. Error bars represent standard deviation within the dosing cohorts. A preliminary dose-proportional increase in Cmax and AUC can be observed from the plots.

In the SAD study, Canocapavir showed an average elimination half-life ($T^1/_2$) of 11.3–18.4 hours and a mean residence time of 12.3–18.1 hours. When combined with the large therapeutic margins observed between C_{trough} of Canocapavir and its protein-binding adjusted HBV DNA EC_{50}, these data favorably support a once-daily dosing regimen. Nonetheless, we noticed that the mean $T_{1/2}$ in 450 mg cohort was a 1.4-fold increase compared with 300 mg cohort, as shown in Table 2. In addition, we also observed an approximately 2-fold accumulation of Canocapavir in the MAD by comparing its D1 and D14 exposure. Possible reasons for the prolonged half-life in the higher dose groups and the positive dose-related accumulation could be the saturation of the capacity of a metabolic route or the saturation of the transporter.

The plasma exposure of Canocapavir decreases significantly after a high-fat meal, reaching a 50% level. The negative food effect on plasma exposure of Canocapavir was probably caused by its decreased solubility and/or permeability in the upper intestinal tract in the fed state compared with that in the fasted state. Our in vitro permeability study showed that Canocapavir has a low permeability crossing through the Caco-2 cell monolayer.

As we observed in the Table A4, the urinary excretion of Canocapavir was low (<1.5% of the dose, on average), and the mean CLr was similar among the dose levels, ranging from 0.0266 to 0.0364 L/h. It is quite plausible that the excretion of Canocapavir after oral administration was mainly through feces. This hypothesis is supported by the mass balance study of Canocapavir in rats in which 76.0 ± 26.8% in male and 102 ± 44.9% in female SD rats were excreted through feces.

Conclusion

In conclusion, single oral dosing of up to 450 mg and multiple oral dosing of 75–300 mg of Canocapavir for 14 days in healthy subjects were safe and well tolerated. There is approximately a liner dose-exposure relationship observed in both the SAD and MAD dosing. A significant therapeutic margin existed between exposures obtained

from this study and the serum protein-binding adjusted HBV EC_{50}. The safety and PK profile of Canocapavir support its further evaluation in chronic hepatitis B patients.

Supplementary Materials

Material associated with this article can be found in the online version at https://doi.org/10.1016/j.gastha.2023.01.001.

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Authors' Contributions:
X. Jiang conceived and supervised the study, and analyzed the data. B. Hua, G. Liu, and T. Xia analyzed the data. G. Liu, Q. Jin, and B. Liang supported with supplying the study drugs. A. Deng, H. Lu, and R. Guo managed the study. Z. Wang wrote the manuscript. H. Chen conceived and supervised the study. Z. Zhang conceived and supervised the study, analyzed the data, reviewed and edited the manuscript. All authors approved the final version of the manuscript.

Conflicts of Interest:
These authors disclose the following: all authors are the employees of Shanghai Zhimeng Biopharma, Inc.

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Ethical Statement:
The corresponding author, on behalf of all authors, jointly and severally, certifies that their institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

Data Transparency Statement:
Data and analytic methods will be available to other researchers upon request. However, study materials will not be made available to other researchers.

Reporting Guidelines:
Helsinki Declaration, CONSORT, SAGER.