Pharmacokinetics of fruquintinib in humans

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INTRODUCTION

Fruquintinib is a potent and highly selective oral small molecule tyrosine kinase inhibitor targeting VEGFR and demonstrates promising activities against a broad-spectrum cancer types with a favorable pharmacokinetic/ pharmacodynamic (PK/PD) and safety profile in nonclinical studies, which warrant further investigation, and a series of human trials have been initiated. VEGFR plays a pivotal role in tumor-related angiogenesis.[1-7]

The efficacy and safety of fruquintinib in the treatment of colorectal cancer (CRC) has been evidenced in a phase III registration trial (FRESCO NCT02314819). 5 mg fruquintinib oral once daily, on a 3-week on/1-week off cycle, significantly improved overall survival (OS) in patients with metastatic CRC comparing to placebo with good tolerability.[8] In addition, fruquintinib has been extensively studied in several other phase I, II and III clinical trials for solid tumors, including two ongoing registration trials for lung cancer[9] and gastric cancer[10].

Favorable pharmacokinetic (PK) properties of fruquintinib, high oral absorption and low clearance, were observed in nonclinical PK studies.[2] Following on studies in healthy volunteers were conducted to investigate the PK of fruquintinib in humans, including the effect of food on fruquintinib PK and the PK of the circulating metabolites.

METHODS

Clinical trials included

One food effect trial (n=20) was carried out to test the effect of high-fat food on the pharmacokinetics at a single oral dose of 4 mg and to identify circulating metabolites in humans. A bioequivalence (BE) study (n=24) for two oral capsule-formulations of fruquintinib was conducted to quantify fruquintinib and its metabolites in plasma at a single oral dose of 5 mg. All male healthy volunteers were ethnical Han Chinese aged 19 – 40 years. A written informed consent was signed before screening. Both trials adopted a cross-over design with a washout period of 14 days. The blood samples were collected at 0 (pre-dose), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 (food effect study only), 24, 36 (food effect study only), 48, 72, 96 and 120 hours post dose.

Bio-analytical method for metabolite identification

The plasma was protein-precipitated by methanol/acetonitrile (1:3, v/v). The supernatant was diluted with methanol/water (1:1, v/v) and then analyzed by the LC-MS^n system (Agilent LC instrument coupled to Thermo Fisher LTQ-XL ion trap mass spectrometer). The mass spectrometer was set to be positive mode. Full ion scan and MS^n scan were applied to finding metabolites and to proposed structures.

Bio-analytical method for quantitation

The concentrations of fruquintinib, HM5024093 (M9) and HM5025423 (M11) in plasma were determined by a validated LC-MS/MS method. The ion transitions for multiple reaction monitoring in the positive mode are as following: *m/z* 394 → 363 for fruquintinib, *m/z* 381 → 365 for M9, *m/z* 380 → 364 for M11, and *m/z* 398 → 367 for CMB (internal standard). For each analyte, the calibration curve ranged from 1.00 to 750 ng/mL. An additional LC-MS/MS method was validated for the determination of HM5029127 (M10) concentration. The ion transitions for multiple reaction monitoring in the positive mode are as following: *m/z* 410 → 380 for M10, and *m/z* 398 → 367 for CMB (internal standard). The calibration curve ranged from 1.00 to 800 ng/mL.

Human liver microsomal incubation

Fruquintinib (10 μmol/L) was incubated in 1 mg/mL human liver microsomes at 37°C for 1 hour in the presence of NADPH. Reactions in the incubation system were quenched by cold acetonitrile.

Pharmacokinetic analysis

PK parameters were calculated using a non-compartmental method (Pharsight WinNonlin). The bioequivalence assessment (for the food effect study) was performed using a linear mixed model (Pharsight WinNonlin). For the AUC (or C_{max}) comparison between fed condition and fasting condition, the point estimation and 90% confidence interval (CI) of the geometric means ratio were calculated.

RESULTS

Validation of quantitative bio-analytical methods

A LC-MS/MS method was developed and validated for the determination of fruquintinib and its metabolites M9 and M11 in human plasma. The acceptable accuracy and precision demonstrated that this bioanalytical method was reliable for the quantitation of the analytes. Fruquintinib, M9 and M11 in human plasma were stable during sample processing and storage as indicated by the results of stability tests under the following conditions: 25 hours at room temperature, 5 cycles of freeze-thaw at -10 to -30°C and -60 to -80°C, 270 days at -10 to-30°C and 329 days at -60 to-80°C.

Food effect

Table 1. Pharmacokinetic parameters for fruquintinib in healthy volunteers (n=20) following a single oral dose of 4 mg fruquintinib under fasting and fed conditions

Condition	C_{max} (ng/mL)[a]	T_{max} (h)[b]	AUC_{0-t} (h×ng/mL)[a]	$AUC_{0-\infty}$ (h×ng/mL)[a]	$t_{1/2}$ (h)[c]
Fed	106 (24.3)	4.0 (4.0, 24.0)	4204 (16.6)	4426 (17.6)	26.1 (4.7)
Fasting	127 (17.1)	3.0 (1.0, 4.0)	4347 (19.5)	4551 (20.9)	25.7 (4.3)

[a]: geometric mean (CV%); [b]: median (min, max); [c]: mean (SD)

Fruquintinib metabolites in plasma

Table 2. LC-MS^n data for fruquintinib, metabolites and metabolite reference compounds

Analyte	Retention time (min)	Ionization mode	MS^n	Product ions (relative intensity)
Fruquintinib	56.0	ESI, positive	MS^2: m/z 394 →	363 (100%), 379 (10%)
		ESI, positive	MS^3: m/z 394 → 363 →	335 (100%), 348 (5%)
HM5024093	63.4	ESI, positive	MS^2: m/z 381 →	366 (100%), 365 (95%), 337 (17%)
(M9 reference)		ESI, positive	MS^3: m/z 381 → 366 →	337 (100%), 320 (33%), 347 (27%), 164 (17%), 202 (10%)
M9	63.9	ESI, positive	MS^2: m/z 381 →	366 (100%), 365 (90%), 337 (20%)
		ESI, positive	MS^3: m/z 381 → 366 →	337 (100%), 320 (50%), 347 (25%), 164 (15%), 202 (10%)
HM5029127	50.0	ESI, positive	MS^2: m/z 410 →	380
(M10 reference)		ESI, positive	MS^3: m/z 410 → 380 →	363 (100%), 335 (10%), 320 (4%)
M10	49.8	ESI, positive	MS^2: m/z 410 →	380
		ESI, positive	MS^3: m/z 410 → 380 →	363 (100%), 335 (9%), 320 (4%)
HM5025423	52.1	ESI, positive	MS^2: m/z 380 →	363 (100%), 335 (13%), 320 (5%)
(M11 reference)		ESI, positive	MS^3: m/z 380 → 363 →	335 (100%), 347 (40%), 319 (8%)
M11	52.5	ESI, positive	MS^2: m/z 380 →	363 (100%), 335 (15%), 320 (5%)
		ESI, positive	MS^3: m/z 380 → 363 →	335 (100%), 347 (32%), 319 (8%)

Metabolism for M11 formation

Fruquintinib was incubated with human liver. As opposed to the finding in human plasma, neither M9 nor M11 was the major metabolite in the microsomal system. The most abundant metabolite in liver microsomes was M10. The structure of M10 was confirmed by the synthesized reference compound HM5029127 (Table 2). The mixture of microsomal incubation supernatant and human plasma was incubated at 37°C overnight.

The analysis results showed that the amount of M10 decreased by 60%, and M11 increased by over 69-fold. The amount of fruquintinib reminded unchanged. The stability tests indicated that M10 was stable in the following scenarios: blood on ice for 3 hours, plasma on ice for 4 hours, and plasma at -80°C for 30 days. It was proposed that M10 was converted to M11 in the body but not during the blood collection/processing and the period of plasma storage.

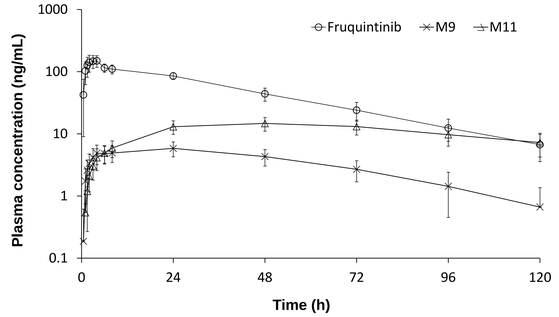
Pharmacokinetics of fruquintinib and metabolites

Table 3. Pharmacokinetic parameters for fruquintinib, M9 and M11 in healthy volunteers (n=24) following a single oral dose of 5 mg fruquintinib

Analyte	C_{max} (ng/mL)[a]	T_{max} (h)[b]	AUC_{0-t} (h×ng/mL)[a]	$AUC_{0-\infty}$ (h×ng/mL)[a]	$t_{1/2}$ (h)[c]	$AUC_{0-\infty}$ ratio (M/P)[c]
Fruquintinib	155 (21.3)	3.0 (1.5, 24.0)	5370 (15.6)	5620 (17.0)	25.5 (4.5)	
HM5024093 (M9)	5.73 (26.3)	24.0 (4.0, 24.0)	362 (36.6)	462 (25.4)	35.9 (7.5)	0.08 (0.02)
HM5025423 (M11)	14.9 (22.4)	48.0 (24.0, 72.0)	1280 (25.8)	1910 (44.2)	57.9 (22.8)	0.36 (0.13)

[a]: geometric mean (CV%); [b]: median (min, max); [c]: mean (SD); M/P: metabolite/parent

Figure 1. Profiles of concentrations of fruquintinib, M9 and M11 in plasma versus time in healthy volunteers following a single oral dose of 5 mg fruquintinib (n=24)



CONCLUSIONS

- **Fruquintinib showed rapid absorption, high exposure and long half-life in humans.**
- **No significant food effect was observed on the absorption extent of fruquintinib.**
- **Two major circulating metabolites M9 and M11 were derived from the amide bond hydrolysis and the N-demethylation of the parent fruquintinib, respectively.**

References: **1**, Q.L. Sun et al, *Cancer Biol Ther* 2014 15(12) 1635-45; **2**, Y. Gu et al, *Cancer Chemother Pharmacol* 2014 74(1) 95-115; **3**, J.N. Cao et al, *Cancer Chemother Pharmacol* 2016 78(2) 259-69; **4**, R.H. Xu et al, J Hematol Oncol 2017 10(1) 22; **5**, S. Lu et al, *WCLC 2016 Annual Meeting*, #4571; **6**, R.H. Xu et al, *ASCO-GI 2017* Annual Meeting, #128; **7**, S. Lu et al, *WCLC 2017* Annual Meeting, #10907; **8**, J. Li et al, *ASCO 2017* Annual Meeting, #3508; **9**, FALUCA NCT02691299; **10**, FRUTIGA NCT03223376