Intracellular Pharmacokinetics of Favipiravir-5'-Ribofuranosyl Triphosphate in COVID-19 Patients

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BACKGROUND

- Favipiravir (FVP) is metabolised intracellularly to the active favipiravir ribofuranosyl-5'-triphosphate (F-RTP)[1]. Due to its broad antiviral activity[2], FVP is a potential treatment for coronavirus disease-19 (COVID-19) as well as viral haemorrhagic fevers. However, oral FVP dosing is limited by a high pill burden.
- Pharmacokinetic (PK) data on F-RTP are lacking in humans, resulting in difficulties in selecting the appropriate dose of FVP for viral inhibition. PBPK models suggest higher doses of FVP may be required to reach therapeutic F-RTP concentrations[3] which could potentially be achieved with intravenous (IV) administration.
- AGILE CST-6 (NCT04746183), a phase Ib dose escalation study, aimed to characterise the PK of FVP[4] and its active metabolite, F-RTP, in COVID-19 patients receiving escalating doses of IV FVP.
- We report intracellular PBMC concentrations of F-RTP in patients enrolled on AGILE CST-6. Additionally, plasma separation cards [HemaSep; H-DBS], that separate cells (H-C) and plasma (H-PL) upon contact (Figure 1) were collected for quantification of F-RTP (H-C) and FVP (H-PL) and compared to the 'gold standard' liquid methods.



Figure 1. HemaSep™ Plasma Filtration Card. FVP was determined in the outer plasma ring (H-PL) and F-RTP from the inner cellular spot (H-C). H-PL [FVP] were quantified as ng/sample (sample = 50µL plasma) and subsequently normalised to ng/mL.

METHODS

- Samples were collected from patients receiving IV FVP (600, 1200, 1800, or 2400 mg BD) administered as an infusion over 1 hour (h), every 12 h for 7 days. Paired liquid plasma (L-PL) and H-DBS were collected on Day (d) 1, 3 and 5 over 0 h, 0-1, 2-4 and 6-12 h post-completion of infusion. PBMC were collected at 6-12 h only.
- FVP [L-PL and H-PL] and F-RTP [H-C and PBMC] were quantified using validated LC-MS methods[5,6]. F-RTP concentrations were expressed as pmol/sample (12mm punch, H-C) and pmol/10^6 cells (PBMC). PK parameters were calculated using WinNonlin..

- F-RTP levels in PBMC and H-DBS were in the pmol range (20.8 – 141.4 pmol/10^6 cells|12.0 -187.7 pmol/sample
- F-RTP exposures were dose-dependent and accumulate over administration (d1-d5) in H-DBS, but not in PBMC
- H-DBS offer a practical alternative to PK sampling in remote field settings, particularly for high-consequence infectious diseases

Day	Dose (mg, BD)	T_{max} (h)	$C_{0/trough}$ (pmol/sample)#	C_{max} (pmol/sample)	T_{last} (h)	C_{last} (pmol/sample)	$AUC_{0\text{-}last}$ (h*pmol/sample)
Day 1	600, N = 4	0.25	0.00	12.00*	5.08	12.00*	59.38
		(0.17, 0.38)	-	(12.00, 12.00)	(2.92, 8.85)	(12.00, 12.00)	(33.56, 105.05)
	1200, N = 4	0.39	0.00	12.00*	6.51	12.00*	75.75
		(0.25, 0.59)	-	(12.00, 12.00)	(6.05, 7.01)	(12.00, 12.00)	(69.92, 82.07)
	1800, N =4	0.24	0.00	12.00*	6.28	12.00*	73.62
		(0.08, 0.73)	-	(12.00, 12.00)	(6.06, 6.51)	(12.00, 12.00)	(69.92, 77.52)
	2400, N = 4	0.22	0.00	12.00*	6.27	12.00*	73.64
		(0.07, 0.06)	-	(12.00, 12.00)	(5.84, 6.73)	(12.00, 12.00)	(67.50, 80.35)
Day 3	600, N = 4	0.00	12.00*	12.00*	6.24	12.00*	74.92
		-	(12.00, 12.00)	(12.00, 12.00)	(5.95, 6.55)	(12.00, 12.00)	(71.38, 78.64)
	1200, N = 4	-	16.70	31.19	6.46	31.19	167.36
			(5.84, 47.77)	(5.39, 180.52)	(6.05, 6.89)	(5.39, 180.52)	(42.30, 662.17)
	1800, N = 4	-	22.33	33.50	6.19	32.25	187.63
			(6.42, 77.75)	(10.77, 104.20)	(5.88, 6.51)	(10.60, 98.12)	(65.84, 534.73)
	2400, N = 4	1.91	57.78	88.97	6.29	61.96	427.06
		(0.32, 11.52)	(8.69, 384.18)	(20.70, 382.36)	(5.90, 6.71)	(8.77, 437.96)	(90.94, 2005.58)

Table 1. Summary of F-RTP H-C PK characteristics, presented as geometric mean (95% confidence interval) for day 1 and day 3. C_0:sample prior to the first dose of the day;. AUC_{last}:area under the curve up to last quantifiable time point; T_{max}: time of maximum concentration; C_{max}: maximum concentration; T_{last}: time of the last measurable concentration; C_{last}: last measurable concentration * samples below the LLQ and imputed as ½ LLQ

RESULTS

PBMC

- Of 42 PBMC samples, 25 (60%) had quantifiable F-RTP. 25% (d1), 38% (d3) and 25% (d5) samples were <LLQ
- F-RTP ranged from **20.8 - 141.4 pmol/10^6 cells** (Figure 2) with high interindividual variability noted
- Limited evidence for accumulation of F-RTP from d1-d5; sampling issues: 26% haemolysed, 10% low cell count (<1x10^5/mL).
- *No significant correlation* observed between F-RTP [PBMC] and FVP L-PL (r^2=0.23, p=0.06).

DBS

- F-RTP was quantifiable in 49/153 (32%) of H-C samples (Figure 3).
- 100% (d1), 7% (d3) and 1% (d5) were < LLQ (Table 1).
- Accumulation observed from d3 to d5 [$C_{6\text{-}12}$ GMR (95% CI) 1.71(1.07,2.74)]; d3 exposures were dose linear (r^2= 0.83).
- F-RTP d3 exposures were correlated with L-PL and H-PL (Figure 4).
- The ratio of peak-to-trough concentrations (P:T) on d3 was 1.0-5.1 for FVP (H-PL) and 1.0-2.2 for F-RTP (H-C).

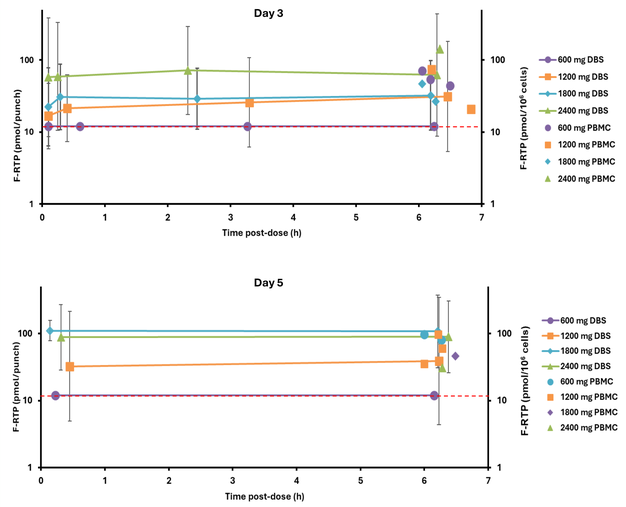


Figure 3. Geometric mean (95% CI) F-RTP concentrations in H-C and PBMC on d3 (top) and d5 (bottom) in patients receiving FVP twice daily for 1 h at 600, 1200, 1800 and 2400 mg. Dashed lines indicate the assay limit of quantification (LLQ) for H-C (24 pmol/punch) and PBMC (24 pmol/sample).

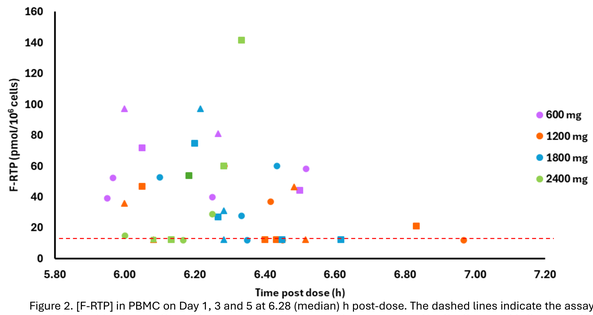


Figure 2. [F-RTP] in PBMC on Day 1, 3 and 5 at 6.28 (median) h post-dose. The dashed lines indicate the assay limit of quantification (LLQ) of 24 pmol/sample. Values <LLQ expressed as ½LLQ. Circles: D1; Squares: D3: Triangles: D5

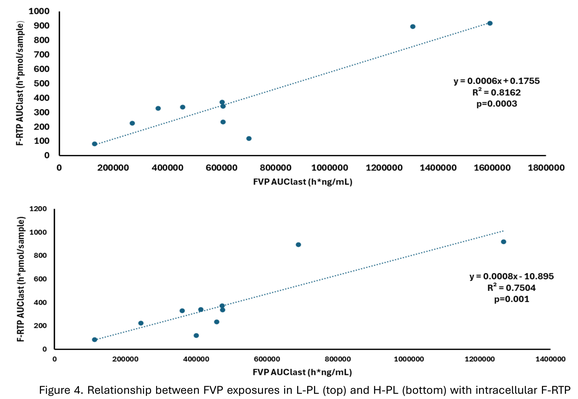


Figure 4. Relationship between FVP exposures in L-PL (top) and H-PL (bottom) with intracellular F-RTP exposures in H-C on day 3 [D3] (n=10)

CONCLUSIONS

- Dose-dependent increases in F-RTP were seen using H-DBS sampling, which was more robust than PBMC collection. The latter was confounded by sampling and processing issues.
- Large interindividual variability appears to be a hallmark of FVP and F-RTP PK.
- Despite PK sampling not extending beyond 7 h post dose, the low P:T ratio of F-RTP indicates an extended intracellular half-life compared to FVP.
- DBS may be more robust than PBMC for quantitation of F-RTP. Moreover, plasma separation cards are a pragmatic alternative to liquid blood in remote field settings.

ADDITIONAL KEY INFORMATION

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