EFFICACY OF REPEAT DOSING OF ORAL FOSFOMYCIN IN A DYNAMIC BLADDER INFECTION *IN VITRO* MODEL

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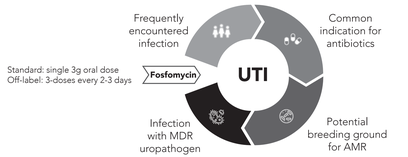
BACKGROUND

Oral fosfomycin, given as a single 3g dose, is indicated for an uncomplicated urinary tract infection (UTI), with maintained activity against multidrug-resistant (MDR) uropathogens.

Despite the lack of evidence, fosfomycin is also commonly prescribed off-label, giving 3 doses every 2-3 days.

We performed pharmacodynamic (PD) profiling using a dynamic bladder infection *in vitro* model to assess adequacy of repeat doses of fosfomycin.



METHODS

The *in vitro* model was constructed on a 1:16 scale to *in vivo*, enabling sixteen individual bladder compartments to be run in parallel, held within a water-bath at 36 – 38°C.

Test isolates were added to each bladder compartment, at an inoculum of 10^7 cfu, providing an equivalent total number of bacteria expected in human infections (i.e. 10^5 cfu/mL in an average 250 mL void).

Mueller-Hinton broth (MHB) with 25mg/L glucose-6-phosphate was used. Pathogen response and resistance was assessed by quantitative cultures on both drug-free and fosfomycin-containing Mueller-Hinton agar (MHA +64mg/L, +512mg/L). Fosfomycin exposures were validated by LC-MS/MS measurements.

Fosfomycin ('Fomicyt', InfectoPharm, Germany) was used to generate the urinary exposures after 3 doses were administered every 72, 48 and 24 hours. Results were compared to a single dose.

TEST ISOLATES

16 clinical Enterobacteriaceae were tested (8 *E. coli*, 4 *E. cloacae*, 4 *K. pneumoniae*; fosfomycin MIC by agar dilution: 0.25 – 64 mg/L). The final PD outcome was assessed 72-hours after the last dose of fosfomycin.

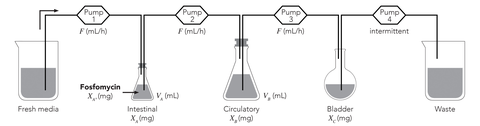
Baseline low-level resistant (LLR) and high-level resistant (HLR) subpopulation was determined after 24 hours incubation of the starting inoculum in MHB without exposure to fosfomycin. The proportion is the mean value from all experiments.

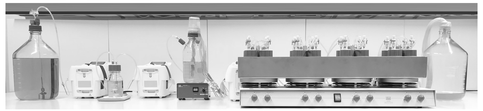
IN VITRO MODEL DESIGN

Applying drug distribution pharmacokinetic (PK) equations, mathematical simulation instructed the fosfomycin dose, compartment volumes and flow rates to obtain the dynamic changes in fosfomycin concentrations required.

$$X_A = X_{A^-} \cdot e^{-(F/V_A)t} \qquad X_B = \frac{(F/V_A) \cdot X_{A^-}}{F/V_B - F/V_A} \cdot \left(e^{-(F/V_A)t} - e^{-(F/V_B)t}\right) \qquad X_C = X_{A^-} - X_A - X_B$$

Normal urodynamics was simulated, with a urine output of 60 mL/h, six voids each day, and a post-void residual volume < 50 mL. Average urinary PK parameters following administration of a 3 g of oral fosfomycin tromethamine were targeted (Wijma RA *et al.*).





X_{A^-} initial amount of fosfomycin (mg); X_1 fosfomycin in GI tract (mg); X_2 fosfomycin in systemic circulation (mg); X_3 fosfomycin in bladder (mg); *t* time (h); *V* volume (mL); *F* flow rate (mL/h)

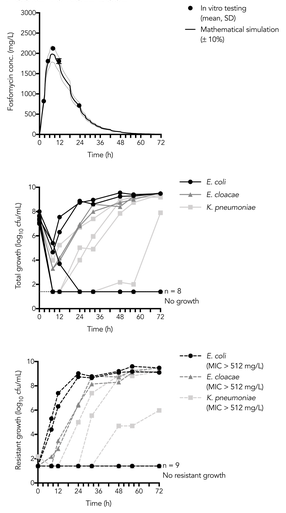
TABLE I. BASELINE BACTERIAL STRAIN CHARACTERISTICS AND DOSE-RESPONSE

Species	Strain no.	Source	ESBL	MIC (mg/L)	Baseline resistant subpopulation		PD outcome, cfu/mL (HLR proportion)			
							Single dose	Three doses		
					LLR (%)	HLR (%)		72-hourly	48-hourly	24-hourly
E. coli	41	urine	yes	0.25	-	-	killed	killed	killed	killed
	11	urine	yes	0.5	-	-	killed	killed	killed	killed
	39	urine	yes	0.5	-	-	killed	killed	killed	killed
	12620	rectal	yes	2	1×10^{-5}	4×10^{-6}	killed	killed	killed	killed
	1016	urine	yes	16	6×10^{-6}	2×10^{-6}	$9.5\ \log_{10}$ (HLR +++)	$9.6\ \log_{10}$ (HLR +++)	$9.5\ \log_{10}$ (HLR +++)	$9.6\ \log_{10}$ (HLR +++)
	1231	urine	yes	16	1×10^{-5}	2×10^{-6}	$9.5\ \log_{10}$ (HLR +++)	$9.5\ \log_{10}$ (HLR +++)	$9.3\ \log_{10}$ (HLR +++)	$9.3\ \log_{10}$ (HLR +++)
	4807	rectal	yes	32	4×10^{-5}	-	killed	killed	killed	killed
	4757	rectal	yes	64	9×10^{-5}	-	killed	killed	$7.5\ \log_{10}$ (HLR +)	killed
E. cloacae	35166	blood	no	0.5	-	-	killed	killed	killed	killed
	94	n.a.	yes	1	-	-	killed	killed	killed	killed
	21	rectal	yes	8	5×10^{-6}	1×10^{-5}	$9.5\ \log_{10}$ (HLR +++)	$8.9\ \log_{10}$ (HLR +++)	$8.6\ \log_{10}$ (HLR ++)	$6.6\ \log_{10}$ (no HLR)
	32	n.a.	yes	32	7×10^{-5}	3×10^{-6}	$9.5\ \log_{10}$ (HLR +++)	$9.7\ \log_{10}$ (HLR +++)	$9.5\ \log_{10}$ (HLR +++)	$9.4\ \log_{10}$ (HLR +++)
K. pneumoniae	34672	blood	no	1	3×10^{-6}	1×10^{-5}	$9.3\ \log_{10}$ (HLR +++)	$9.5\ \log_{10}$ (HLR +++)	$8.8\ \log_{10}$ (HLR ++)	killed
	31865	blood	no	2	5×10^{-6}	2×10^{-6}	$9.4\ \log_{10}$ (HLR +++)	killed	$6.8\ \log_{10}$ (no HLR)	$5.9\ \log_{10}$ (no HLR)
	55	sputum	yes	4	1×10^{-5}	1×10^{-6}	$6.9\ \log_{10}$ (no HLR)	$9.4\ \log_{10}$ (HLR +++)	$9.5\ \log_{10}$ (HLR +++)	$9.5\ \log_{10}$ (HLR +)
	52	urine	yes	16	2×10^{-5}	5×10^{-6}	$9.2\ \log_{10}$ (HLR +++)	$9.6\ \log_{10}$ (HLR ++)	$5.2\ \log_{10}$ (no HLR)	$9.2\ \log_{10}$ (HLR ++)

ESBL extended-spectrum beta-lactamase; +++ greater than 1%; ++ from 0.01 to 1%; + less than 0.01%

RESULTS

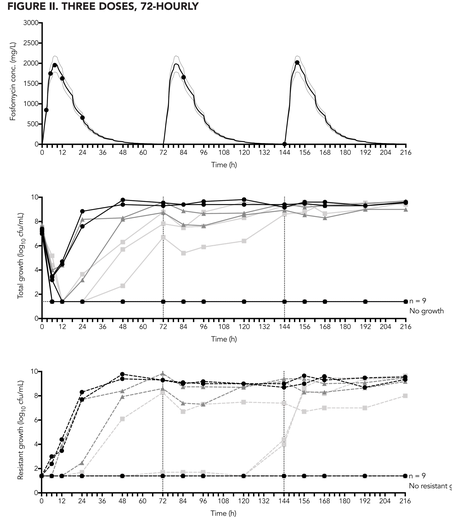
FIGURE I. SINGLE DOSE



Single dose: 8 out of 16 isolates re-grew.
(2/8 *E. coli*, 2/4 *E. cloacae*, 4/4 *K. pneumoniae*)

Complete population replacement with a HLR population occurred to 6 out of 8 isolates. One *K. pneumoniae* re-grew without a resistant population, and another re-grew with the resistant population in a lower proportion.

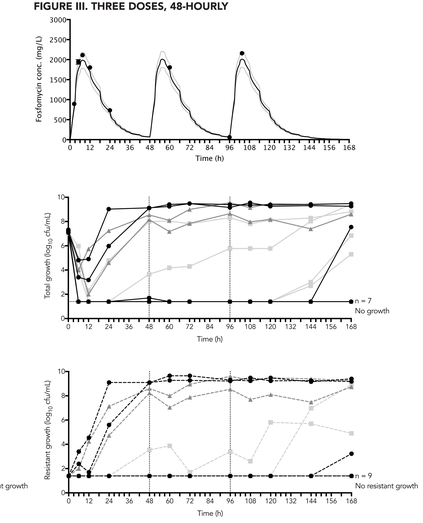
FIGURE II. THREE DOSES, 72-HOURLY



3 doses every 72 hours: 7 out of 16 isolates re-grew.
(2/8 *E. coli*, 2/4 *E. cloacae*, 3/4 *K. pneumoniae*)

An extra *K. pneumoniae* isolate was killed compared to single dose. Near complete population replacement with a HLR population occurred in all isolates that re-grew; HLR median proportion: 65.7%, IQR 41.7-100%.

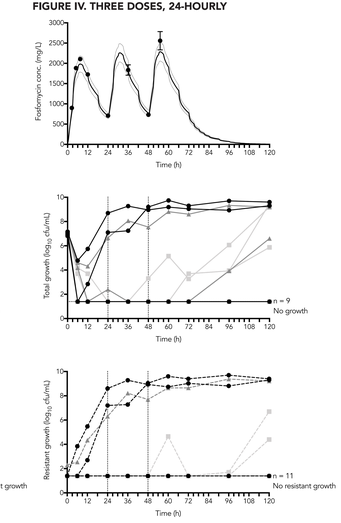
FIGURE III. THREE DOSES, 48-HOURLY



3 doses every 48 hours: 9 out of 16 isolates re-grew.
(3/8 *E. coli*, 2/4 *E. cloacae*, 4/4 *K. pneumoniae*)

An additional *E. coli* isolate re-grew, with minimal resistance, compared to single dose. Population replacement with HLR occured in 5-isolates (2 *E. coli*, 2 *E cloacae*, 1 *K.pneumoniae*); HLR median proportion: 32.0%, IQR 0.005-83.3%.

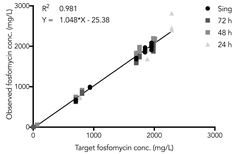
FIGURE IV. THREE DOSES, 24-HOURLY



3 doses every 24 hours: 7 out of 16 isolates re-grew.
(2/8 *E. coli*, 2/4 *E. cloacae*, 3/4 *K. pneumoniae*)

An extra *K. pneumoniae* isolate was killed compared to single dose. No resistant re-growth noted in 2-isolates (1 *K.pneumoniae*, 1 *E. cloacae*). HLR population selection was reduced; HLR median proportion 0.3%, IQR 0.0004-81.3%.

FIGURE V. OBSERVED AND TARGETED FOSFOMYCIN CONCENTRATIONS



Pharmacokinetics: Fosfomycin exposure following single and multiple doses were accurately reproduced compared to the target values (mean deviation from target 5.0% ±3.4%, max 11.8%) with minimal variability (mean relative SD 2.7% ±1.7%, max 8.8%).

CONCLUSION

Dynamic *in vitro* modelling of multiple doses of oral fosfomycin fails to additionally suppress regrowth in the majority of isolates compared to single dose therapy.

Baseline high-level heteroresistance and bacterial species are important predictors for regrowth, independent of the fosfomycin MIC value.

These results suggest that multi-dose, off-label use of fosfomycin may not necessarily be better than standard single dose therapy.

Reducing the time interval between repeat doses, however, may minimize the amplification of the HLR subpopulation and the emergence of fosfomycin resistance.

References

Rowe EL et al. J Pharm Sci 1969: 58(11); 1375-78.; Wijma RA et al. Clin Microb Infect 2018: 24(5); 528-532; Abbott IJ et al. J Antimicrob Chemo 2018: 73(3); 709-719.

Acknowledgements

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