EFFICACY OF SINGLE AND MULTIPLE ORAL FOSFOMYCIN DOSING FOR *PSEUDOMONAS* URINARY TRACT INFECTIONS

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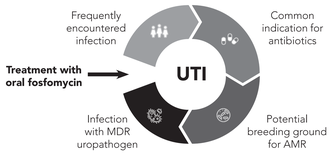
BACKGROUND

Oral fosfomycin is indicated for uncomplicated urinary tract infections, with activity against MDR-uropathogens.

Limited data are available to guide dosing in antibiotic-resistant UTIs.

We use a dynamic *in vitro* bladder model with synthetic human urine (SHU) to demonstrate drug exposures that can effectively kill, or select for resistance, among *Pseudomonas aeruginosa* urinary isolates.



METHODS

Sixteen *P. aeruginosa* isolates were selected based on their baseline fosfomycin MIC value. *P. aeruginosa* ATCC 27853 underwent susceptiblity testing, but was not run in the bladder infection model.

1. Susceptiblity testing

— Agar dilution: Reference susceptiblity MIC testing method using 10^4 cfu/spot of each isolate inoculated on Mueller–Hinton II agar plates (MHA; BD Diagnostics, USA) containing 25 mg/L glucose-6-phosphate (G6P; Sigma, Germany) and fosfomycin (InfectoPharm, Germany) following CLSI recommendations, in a concentration range of 0.25 – 1024 mg/L. Isolates were tested in triplicate.

— Disc diffusion: using FOT 200 disc (Oxoid Ltd/Thermo Fisher Scientific, UK) containing 200 µg fosfomycin + 50 µg G6P.

— Automated testing: Vitek-2 (BioMérieux, France) using the AST-N344 card.

— Broth microdilution (BMD): MIC determined in MHB alone, MHB with G6P and SHU. Isolates were tested in triplicate.

2. Dynamic bladder infection *in vitro* model

— Urinary fosfomycin concentrations after absorption of a 3g oral dose were simulated following a single dose, and 7 doses given daily.

— 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).

— Fosfomycin ('Fomicyt', InfectoPharm, Germany) was used to generate average urinary PK exposures (C_{max} 1984 mg/L, T_{max} 7.5h, $AUC_{0\text{-}24}$ 30938 mg.h/L),[1] with *in vitro* concentrations validated by LC-MS/MS.

— Pathogen kill/resistance was assessed over 72-hours following a single dose, and 216-hours following 7 daily doses, by quantitative cultures on drug-free and fosfomycin-containing Mueller-Hinton agar (64 mg/L, 512 mg/L).

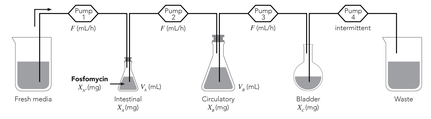
— Growth capacity in SHU and baseline fosfomycin heteroresistance was determined following an 18-hour drug-free dynamic incubation within the bladder infection model.

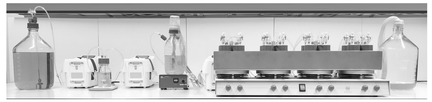
BLADDER INFECTION *IN VITRO* MODEL DESIGN

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

Normal urodynamics was simulated, with a urine output of 60 mL/h, six voids each day, and a post-void residual volume < 50 mL.

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 37°C ±1°C.

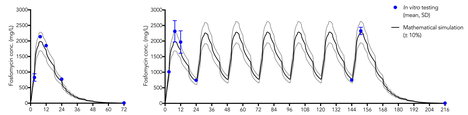




X_0: initial amount of fosfomycin (mg); X_1 fosfomycin in GI tract (mg); X_2 fosfomycin in systemic circulation (mg); X_3 fosfomycin in bladder (mg); *t* time (h); *V* volume (mL); *F* flow rate (mL/h)

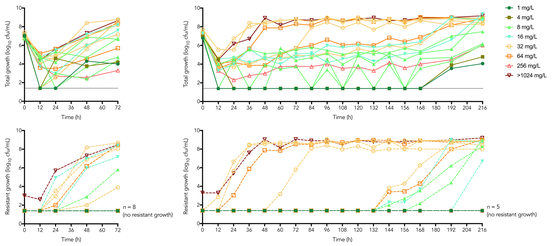
PHARMACOKINETIC / PHARMACODYNAMIC OUTCOMES

FOSFOMYCIN PHARMACOKINETICS



Fosfomycin concentration-time curves. Solid line represents the expected concentration time-curve from the mathematical simulation (dotted line represents ±15%). Solid dots represent the average observed values (mean ±SD).

BLADDER INFECTION MODEL GROWTH CURVES



Total growth (solid lines) and resistant growth (dashed lines). Resistant growth determined by quantitative growth on Mueller-hinton agar with 512 mg/L fosfomycin. Dotted line represents the limit of detection (1.4 log_{10} cfu/mL).

In vitro flow rates were accurately reproduced to ensure fosfomycin exposure matched the simulation, measurements confirmed by LC-MS/MS.

Fosfomycin exposure following single and multiple doses were accurately reproduced compared to the target values (mean deviation from target 8.9% ± 4.8%) with minimal variability (mean relative SD 6.5% ± 6.1%).

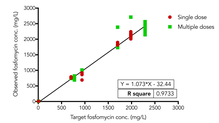
All isolates had detectable re-growth both dosing schedules. Overall, fosfomycin had limited activity against isolates with baseline MIC > 8 mg/L.

Following a single dose:
— Half of the isolates regrew with emergence of high-level resistance (HLR, growth on MHA + 512 mg/L fosfomycin)
— MIC of the re-growth of 6-isolates was > 1024 mg/L.
— The remaining isolates did not demonstrate an MIC rise.

Following 7-doses given daily:
— 11 out of 16 isolates re-grew with HLR, of which 10 isolates had a rise in the total population MIC to > 1024 mg/L.
— The remaining isolates did not have an MIC rise.

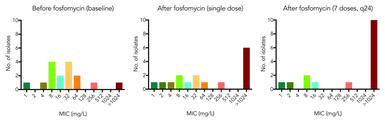
Where there was emergence of HLR without a rise in the total population MIC result, the MIC of the HLR subpopulation still found to be > 1024 mg/L.

OBSERVED AND TARGETED FOSFOMYCIN CONCENTRATIONS



Relationship between observed and targeted free-drug fosfomycin concentrations across all studies. Target fosfomycin concentrations were determined from the mathematical simulation.

CHANGE IN FOSFOMYCIN MIC POST EXPOSURE



Each colour respresent isolates with a specific MIC value. All MIC results were determined by agar dilution.

Compared to the baseline MIC value (by agar dilution), following exposure to fosfomycin, the MIC of the re-growth total population increased > 1024 mg/L in 5-isolates following a single fosfomycin dose, and in 9-isolates following 7 doses given daily.

FOSFOMYCIN SUSCEPTIBILITY

BASELINE ISOLATE CHARACTERISTICS

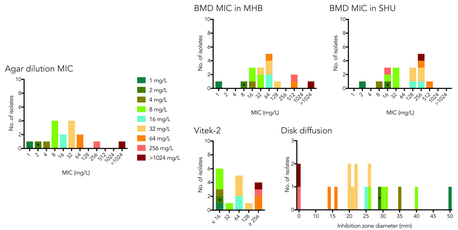
Species	Strain no.	Source	Baseline fosfomycin susceptibility testing					
			Agar dilution median MIC (mg/L)[a]	Broth microdilution median MIC (mg/L)[b]		Vitek-2 MIC (mg/L)	Disc diffusion (mm)	Resistant subpopulation[c] (%)
				MHB[c]	Synthetic human urine			
P. aeruginosa	A934	n.a.	1	1	2	≤ 16	50	—
	A24354	n.a.	4	16	8	≤ 16	35	—
	R006	urine	8	16	16	≤ 16	40	—
	D019	urine	8	16	32	≤ 16	31	1E-04
	H004	urine	8	32	32	≤ 16	30	2E-04
	D001[d]	urine	8	32	32	32	26	—
	F020	urine	16	64	256	64	25	2E-04
	H002	urine	16	64	128	64	22	5E-05
	I002	urine	32	32	128	64	26	8E-05
	A019	urine	32	128	256	128	20	4E-05
	F002	urine	32	64	128	64	20	7E-05
	I005	urine	32	64	256	64	21	2E-05
	H008	urine	64	64	256	≥ 256	16	1E-04
	D004	urine	64	512	512	≥ 256	14	4E-05
	R005	urine	256	512	16	≥ 256	0	—
	H011	urine	>1024	>1024	256	≥ 256	0	6E-02
P. aeruginosa	ATCC 27853		2	8	16	≤ 16	29	—

MHB, Mueller-hinton broth; [a] agar dilution MIC testing performed in triplicate on MHA supplemented with 25 mg/L glucose-6-phosphate; [b] broth microdilution MIC testing performed in triplicate; [c] results from testing in MHB were concordant with testing in MHB supplemented with 25 mg/L glucose-6-phosphate; [c] determined after an 18-hour dynamic incubation in the bladder infection model in SHU without fosfomycin, with the percentage of the resistant bacterial population determined from comparative quantitative cultures on drug free MHA and MHA containing 512 mg/L fosfomycin; [d] isolate D001 demonstrated restricted growth in drug-free SHU (starting inoculum of 6.9 log_{10} cfu/mL to an 18-hour bacterial density of 6.8 log_{10} cfu/mL); n.a., not available.

The majority of test isolates (14 out of 16) were from a urinary source.

Fosfomycin MIC, by agar dilution, ranged from 1 to >1024 mg/L, with 12 out of 16 isolates with a median MIC of 8 – 64 mg/L.

AGAR DILUTION MIC COMPARED WITH OTHER METHODS



Each colour respresent isolates with a specific agar dilution MIC result; BMD, broth microdilution; MHB, Mueller-Hinton broth; SHU, synthetic human urine; * indicates the result of the ATCC isolate.

Compared to agar dilution, MIC values by BMD (in MHB) were 1 to 2-fold higher in 12 of 17 isolates. 4-isolates returned the same value and 1-isolate (D004) returned an MIC of 512 mg/L (compared to 64 mg/L). There was no difference when tested with or without G6P.

BMD (in SHU) returned MIC values that were 2 to 3-fold higher than agar dilution in 12 of 17 isolates. 3-isolates had only a 1-fold higher MIC; and 2-isolates, noteably, had a reduction in their MIC (isolates R005 and H011).

Vitek-2 tended to overestimate the MIC compare to agar dilution. Disk diffusion appeared to adequately separate the isolates by agar dilution MIC.

PHARMACODYNAMIC OUTCOME POST FOSFOMYCIN EXPOSURE

Species	Strain no.	PD outcome post fosfomycin exposure					
		Single dose			Daily dosing (7 days)		
		cfu/mL	HLR %	MIC (mg/L)	cfu/mL	HLR %	MIC (mg/L)
P. aeruginosa	A934	4.0 log_{10}	—	1	4.1 log_{10}	—	1
	A24354	4.2 log_{10}	—	2	4.8 log_{10}	—	2
	R006	4.7 log_{10}	—	8	6.2 log_{10}	—	8
	D019	6.7 log_{10}	—	4	8.5 log_{10}	+++	>1024
	H004	6.7 log_{10}	+++	>1024	9.0 log_{10}	+++	>1024
	D001	4.4 log_{10}	—	8	6.1 log_{10}	—	8
	F020	8.2 log_{10}	+++	1024	8.7 log_{10}	+++	>1024
	H002	7.6 log_{10}	+++	16	9.2 log_{10}	++	16
	I002	7.2 log_{10}	++	32	8.4 log_{10}	+++	>1024
	A019	8.7 log_{10}	—	32	8.3 log_{10}	+++	>1024
	F002	8.8 log_{10}	+++	>1024	8.7 log_{10}	+++	>1024
	I005	8.2 log_{10}	+++	>1024	8.9 log_{10}	+++	>1024
	H008	8.4 log_{10}	+++	>1024	8.9 log_{10}	+++	>1024
	D004	5.7 log_{10}	—	64	8.9 log_{10}	+++	>1024
	R005	3.3 log_{10}	—	256	6.0 log_{10}	—	256
	H011	8.7 log_{10}	+++	>1024	9.2 log_{10}	+++	>1024

PD, pharmacodynamic; HLR, high level resistance; Proportion of HLR catagorised by +++ greater than 1%; ++ from 0.01 to 1%; + less than 0.01%; post exposure MIC testing performed by agar dilution on MHA with 25 mg/L glucose-6-phosphate

Baseline high-level heteroresistance was detected in 11 out of 16 isolates. This accurately predicted re-growth with emergence of HLR following exposure to 7 doses given daily.

Isolate R005 (MIC 256 mg/L) had limited re-growth without emergence of resistance following both dosing schedules (3.3 log_{10} and 6.0 log_{10} cfu/mL).
— Importantly, this isolate did not have a HLR subpopulation detected at baseline, and demonstrated a reduction in fosfomycin MIC when tested by BMD in SHU (MIC = 16 mg/L).

CONCLUSION

Pseudomonas aeruginosa urinary isolates are ineffectively killed after single or multiple oral fosfomycin doses.

Post exposure emergence of resistance was observed in the majority of isolates with a baseline MIC > 8 mg/L, significantly worsened following prolonged therapy (7-doses, given daily).

The presence of a baseline HLR subpopulation predicted re-growth with emergence of resistance after the multiple dosing schedule.

These results demonstrate that monotherapy with oral fosfomycin in our model is ineffective against *P. aeruginosa* urinary isolates, with the use of multiple doses not improving the outcome.

Acknowledgements

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1. Ipe *et al.* J Microbiol Methods (2016)
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