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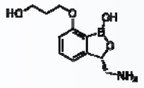

The Effect of Urine, Lung Surfactant and Testing Parameter Variations on the *In Vitro* Activity of GSK2251052 and Meropenem

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Abstract

Background: GSK2251052 (GSK052), a novel boron-containing leucyl-tRNA synthetase inhibitor in development for the treatment of serious Gram-negative bacterial infections, is active *in vitro* against multidrug-resistant Gram-negative bacilli. This study was undertaken to determine the effect of various testing parameters, including the presence of urine and lung surfactant, on the *in vitro* activity of GSK052 and a comparator agent, meropenem (MER). **Method**: Ten isolates each of *E. coli, K. pneumoniae, Proteus* spp., *P. aeruginosa* and 6 QC strains were tested. Fifteen testing variables (temperature, incubation time, atmospheric conditions, inoculum, pH, calcium, magnesium, zinc, potassium, thymidine, polysorbate 80, albumin, serum, urine and lung surfactant) were studied using CLSI broth microdilution (BMD). **Results**: There was good correlation of MICs using methods with alterations to testing conditions in comparison to reference method MICs. With the exception of the variables noted (in bold font) in the table, GSK052 and MER MICs were within ± 1 dilution compared to reference MICs.

Mean dilution difference of GSK052 and MER MICs compared to reference BMD MICs for key variables:

Variable	*Enterobacteriaceae* (n=30)		*P. aeruginosa* (n=10)	
	GSK052	MER	GSK052	MER
Zinc (2, 5, 10 µg/mL))	-0.1, -0.1, -0.03	0.1, 0.8, 0.9	0, 0.2, -0.2	**2.0, 1.8, 2.0**
Calcium (100 µg/mL)	0.8	-0.2	**1.1**	-0.25
CO_2 (5, 10%)	-0.1, 0.1	-0.1, 0.1	0, 0.2	0, 0.3
Inoculum (10^5 cfu/mL)	**2.2**	**2.4**	1.0	0.3
pH (5.5, 6.5, 8.5)	-0.6, -0.03, 0.03	0, 0.2, 0.1	-0.1, -0.1, -0.1	-0.5, -0.3, 0
Serum (25, 50%)	-0.7, -0.97	0.2, -0.2	**-1.3, -1.7**	0.8, 1.0
Lung surfactant (1, 5, 10%)	-0.1, -0.1, -0.1	-0.1, 0.04, 0.6	-0.5, -0.9, -0.9	0, 0.5, 0.8
Urine (25, 50, 100%)	**2.1, 1.9, 2.7**	-0.8, -0.1, -0.8	**1.7, 1.7, 2.6**	-0.5, 0, -1.0

(bolded values are >±1 dilution)

Conclusion: When performing susceptibility testing with GSK052 and MER it is important to control the inoculum concentration according to standard methods. GSK052 or MER MICs were not significantly affected by the pH levels tested or up to 10% lung surfactant. A high concentration of calcium (100 µg/mL) and the addition of serum affected GSK052 MICs against *P. aeruginosa* and the presence of urine also affected GSK052 MICs against *Enterobacteriaceae* and *P. aeruginosa* by up to 2.7 dilutions.

Introduction

- GSK2251052 (GSK052), a novel boron-containing leucyl-tRNA synthetase inhibitor in development for the treatment of serious Gram-negative bacterial infections, is active *in vitro* against multidrug-resistant Gram-negative bacilli
- This study was undertaken to determine the influence of various factors, including urine and lung surfactant on the *in vitro* activity of GSK2251502 against *Enterobacteriacae* and *P. aeruginosa*

Methods

Study Strains

- 40 Gram-negative bacilli including:
  - 11 *Escherichia coli* (2 carbapenem-resistant)
  - 11 *Klebsiella pneumoniae* (4 carbapenem-resistant)
  - 7 *Proteus* spp.
  - 11 *Pseudomonas aeruginosa* (7 carbapenem-resistant)
- 6 quality control strains including: *E. coli* ATCC 25922, *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 700603, *K. pneumoniae* BAA-1705, *K. pneumoniae* BAA-1706

Methods cont.

Broth Microdilution

- Eleven concentrations of GSK2251052 in doubling dilutions (0.06-64 mcg/mL) and meropenem (0.008-8 mcg/mL) were prepared in cation adjusted Mueller Hinton broth (CAMHB) and 100 mcL of each antimicrobial dilution was added to the appropriate well in the 96 well microtiter trays.
- Organism suspensions equivalent to a 0.5 McFarland) were prepared in 5 mL tubes of sterile water and transferred to 11 mL CAMHB tubes as based on initial colony counts (450 mcL for *Enterobacteriaceae* and 700 mcL for *P. aeruginosa)* and 10 mcL was added to each well of the microtitre tray.
- Plates were incubated under ambient conditions at 35oC for 16-20 hours.
- The MIC was recorded as the lowest concentration showing no growth.

MIC by Broth Microdilution with Addition of Urine

- The 40 strains were tested by broth microdilution (as described above) with the exception that urine was added to the broth
- One set of plates each was set up with CAMHB with 25% and 50% normal human pooled urine and 100% normal human pooled urine with final total well volume of 100 mcL
- The pH of the 25%, 50% and 100% urine plates were adjusted to pH 7.2-7.4

MIC by Broth Microdilution with Addition of Lung Surfactant

- The 40 strains were tested by broth microdilution (as described above) with the exception that lung surfactant (Survanta™) was added to the broth
- One set of plates each was set up with CAMHB with 1%, 5% and 10% Survanta™ with final total well volume of 100 mcL
- The pH of the 1%, 5% and 10% Survanta™ broth mixtures were adjusted to pH 7.2-7.4 prior to plate production

MIC by Broth Microdilution with Modification of Testing Variables

- The 40 strains were tested by broth microdilution (as described above) with the exception of variation of the following test factors: temperature, incubation time, inoculum, atmosphere, calcium, magnesium, potassium, zinc, thymidine, pH, serum, albumin and P80
- The specific differences studies are shown along with a summary of results in Table 1

Results

- Table 1 shows the mean dilution difference between comparative and reference condition MICs for GSK2251052 and meropenem against *Enterobacteriaceae* and *P. aeruginosa*
- Dilution differences were calculated for each MIC by subtracting the $\log_2$+10 test MIC from the $\log_2$+10 reference MIC and then mean dilution differences were determined for each method.
- Comparative and reference conditions were considered comparable if the mean comparative MIC was within plus or minus 1 dilution of the mean MIC for the reference condition.
- Figures 1-4 show the distribution of GSK2251502 and meropenem MICs against *Enterobacteriaceae* and *P. aeruginosa*, in the presence and absence of urine and lung surfactant

Acknowledgements

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Table 1. Mean dilution difference of GSK2251052 and Meropenem MICs compared to reference broth microdilution MICs

Reference (CLSI) Condition	Comparative Condition	GSK2251052		Meropenem	
		Enterobacteriaceae (n=29)	*P. aeruginosa* (n=11)	*Enterobacteriaceae* (n=23)	*P. aeruginosa* (n=4)
Temperature (35°C)	30°C	0.24	0.18	-0.31	0.00
	40°C	-0.41	-1.00	0.18	-0.25
Incubation Time (20 hours)	16 hours	-0.45	-0.45	-0.22	-0.25
	24 hours	0.09	0.09	0.09	0.50
	48 hours	0.64	0.64	0.39	0.75
Inoculum ($4.7x10^5$)	10^4 ($4.7x10^3$)	-0.66	-0.73	-0.92	-0.25
	10^6 ($9.6x10^5$)	0.14	0.18	0.66	0.00
	10^7 ($9.7x10^6$)	2.18[a]	1.00[b]	2.43[a]	0.33[b]
Atmosphere (ambient)	5% CO_2	-0.10	0.00	-0.05	0.00
	10% CO_2	0.07	0.18	-0.09	0.25
Calcium (25.96 mcg/mL)	10.61 mcg/mL	0.83	0.00	-0.22	-0.50
	55.93 mcg/mL	1.00	0.91	-0.12	-0.50
	97.32 mcg/mL	0.79	1.09	-0.22	-0.25
Magnesium (14.2 mcg/mL)	5. 6 mcg/mL	0.76	0.18	-0.22	0.00
	21.8 mcg/mL	0.24	0.00	0.00	0.25
	29.2 mcg/mL	0.07	-0.09	0.09	0.25
Potassium (none)	12.5 mmol/L	-0.10	0.45	0.04	-0.25
	25 mmol/L	0.00	0.36	0.00	-0.25
	50 mmol/L	0.07	0.00	0.04	0.00
Zinc (none)	2 mmol/L	-0.07	0.00	0.13	2.00
	5 mmol/L	-0.14	0.18	0.84	1.75
	10 mmol/L	-0.03	-0.18	0.93	2.00
Thymidine (none)	1 mcg/mL	0.10	0.09	0.09	-0.25
	5 mcg/mL	0.00	0.09	0.05	-0.25
pH (7.22)	5.43	-0.62	-0.09	0.00	-0.50
	6.51	-0.03	-0.09	0.18	-0.25
	8.56	0.03	-0.09	0.05	0.00
Serum (none)	25%	-0.66	-1.27	0.23[c]	0.75
	50%	-0.97	-1.73	-0.22[c]	1.00
Albumin (none)	4 mg/dL	0.14	0.18	0.18	0.25
P80 (none)	0.002% P80	0.00	0.45	0.00	-0.25
Urine (none)	25%	2.10	1.73	-0.80	-0.50
	50%	1.90	1.73	-0.09	0.00
	100%	2.69	2.55	-0.80	-1.00
Lung Surfactant (none)	1%	-0.07	-0.45	-0.09	0.00
	5%	-0.14	-0.91	0.04	0.50
	10%	-0.14	-0.91	0.57	0.75

Values in orange are >±1 dilution

[a]One isolate was offscale for GSK2251052 and six isolates were offscale for meropenem. Therefore, the GSK2251052 n=28 and the meropenem n=17

[b]One isolate was offscale for GSK2251052 and meropenem (GSK2251052 n=10 and the meropenem n=3)

[c]One isolate was offscale for meropenem. Therefore, the meropenem n=22

Figure 1. Distribution of GSK2251052 MICs in CAMHB, 25%, 50% and 100% urine

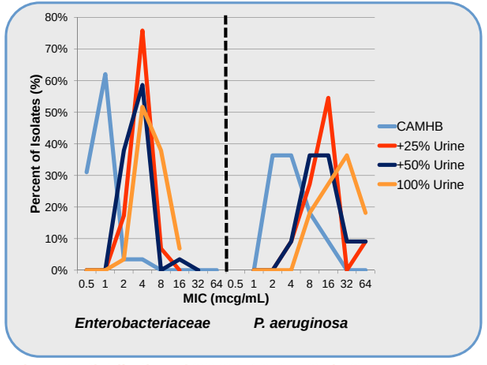


Figure 2. Distribution of Meropenem MICs in CAMHB, 25%, 50% and 100% urine

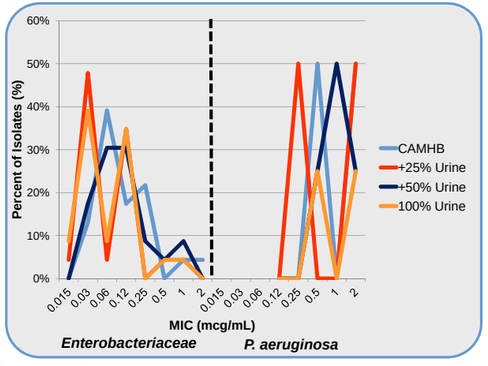


Figure 3. Distribution of GSK2251052 MICs in CAMHB, 1%, 5% and 10% Lung Surfactant

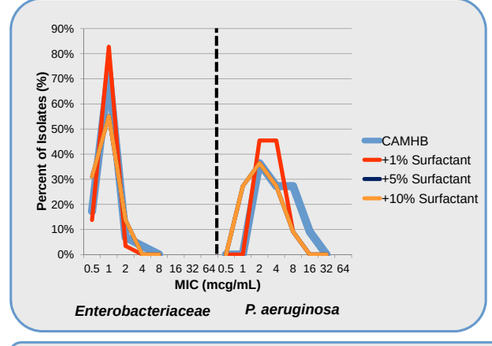
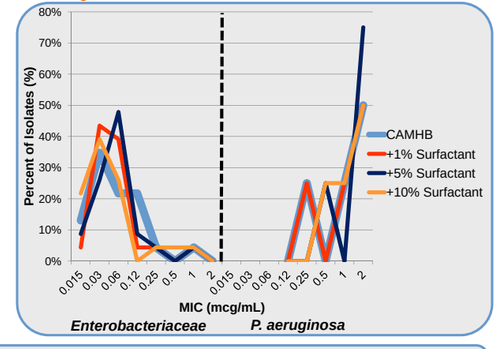


Figure 5. Distribution of Meropenem MICs in CAMHB, 1%, 5% and 10% Lung Surfactant



Conclusions

- A high inoculum (10^7 CFU/mL) increased GSK2251052 and meropenem MICs against *Enterobacteriaceae* by >2 dilutions
- GSK2251052 or meropenem MICs were not significantly affected by the pH levels tested or up to 10% lung surfactant
- A high concentration of calcium (100 µg/mL) increased GSK2251052 MICs by 1.1 dilutions, while the addition of serum decreased GSK2251052 MICs by up to 1.7 dilutions against *P. aeruginosa*
- The presence of urine increased GSK2251052 MICs (which in CAMHB alone were 0.5-4 mcg/mL and 2-16 against *Enterobacteriaceae* and *P. aeruginosa*, respectively) by up to 2.7 dilutions resulting in MICs of 2-16 mcg/mL and 4-64 mcg/mL against *Enterobacteriaceae* and *P. aeruginosa*, respectively