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Dalbavancin and Azithromycin Synergy/Antagonism Study by Checkerboard MIC

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Abstract

Background: This study utilizing a MIC checkerboard method against 2 primary community acquired pneumonia (CAP) pathogens, *S. pneumoniae* and *H. influenzae*, is being performed to determine if there is any synergistic or antagonistic effect to the in vitro efficacy of azithromycin with the addition of dalbavancin. **Methods:** Synergy testing by checkerboard broth microdilution was setup against 9 *S. pneumoniae*, 7 *H. influenzae* and 5 quality control strains (*S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247, *H. influenzae* ATCC 49766, *H. influenzae* NCTC 8468 and *S. aureus* ATCC 29213). The fractional inhibitory concentrations (FICs) were calculated as the MIC of the agent alone divided by the MIC of the agent when tested in combination. The FIC index was obtained by adding the FICs. FIC indices were interpreted as synergistic when values were ≤0.5, additive when values were >0.5 – 1.0, indifferent when values were >1.0-≤4.0 and antagonistic when values were >4. When MIC was off-scale at > highest concentration tested, the highest concentration + 1 was used. When DAL combination MIC was off-scale at less than the lowest concentration in the combination well, the lowest concentration tested was used for the FIC calculation. **Results:** Based on evaluation of FIC indices, there was no synergy or antagonism detected. All results were considered either additive or indifferent (see table). In most cases the combination of dalbavancin and azithromycin had a slight increase in activity against *S. pneumoniae* (dalbavancin and azithromycin MICs decreased by 1 doubling dilution). The combination of dalbavancin and azithromycin against *H. influenzae* had little to no effect on the activity of azithromycin. MIC results for all QC strains were within established CLSI ranges.

Isolate	MIC (µg/mL)				FIC Index	FIC Interp.
	DAL (Alone)	AZI (Alone)	DAL (in Combo)	AZI (in Combo)		
1SP	0.03	0.12	0.015	0.03[1]	≤0.7500	AD
2SP	0.015	0.06	0.004[1]	0.03[1]	≤0.7500	AD
3SP	0.015	0.06	0.015	0.06	2	IN
4SP	0.015	0.06	0.015	0.03[1]	≤1.5000	IN
5SP	0.015	0.06	0.004[1]	0.03[1]	≤0.7500	AD
6SP	0.015	4	0.008	2	1	IN
7SP	0.015	4	0.008	0.5	0.625	AD
8SP	0.015	8	0.008	4	1	IN
9SP	0.03	64[2]	0.015	0.03[1]	≤0.5003	AD
10HI	16[2]	0.5	0.12	1	2.0039	IN
11HI	16[2]	1	0.12	2	2.0039	IN
12HI	16[2]	1	0.12	2	2.0039	IN
13HI	16[2]	1	0.12	1	≤1.0039	IN
14HI	16[2]	1	0.12	1	≤1.0039	IN
15HI	16[2]	1	0.12	2	2.0039	IN
16HI	16[2]	1	0.12	2	2.0039	IN

[1]MIC is offscale at ≤ the lowest concentration, lowest concentration tested was used
[2]MIC is offscale at > highest concentration tested, highest concentration +1 was used
SP – *S. pneumoniae*, HI – *H. influenzae*, AD – additive, IN - indifferent

Conclusions: Overall, the combination of dalbavancin and azithromycin showed no significant increase or decrease in each agent's in vitro activity by checkerboard methodology based on the FIC index evaluation.

Introduction

- Dalbavancin was recently approved by the U.S. Food and Drug Administration for the treatment of adult patients with acute bacterial skin and skin structure infections (ABSSSI) caused by susceptible isolates of *Staphylococcus aureus* (including methicillin-susceptible and methicillin-resistant strains), *Streptococcus pyogenes*, *Streptococcus agalactiae*, and *Streptococcus anginosus* group (including *S. anginosus, S. intermedius, S. constellatus*).
- *S. pneumoniae* and *H. influenzae* are the two major pathogens contributing to the pathogenesis of ~~a~~ community acquired pneumonia.
- Dalbavancin is active against *S. pneumoniae in vitro* and, if administered with azithromycin for treatment of CAP, because of the long half life of both dalbavancin (8.5 days) and azithromycin, understanding the potential for any interaction between the two antimicrobial agents is relevant.
- The purpose of this study was to determine the *in vitro* susceptibility of *S. pneumoniae* and *H. influenzae* to dalbavancin with azithromycin using checkerboard MIC methodology (1-3)

Methods

- Synergy testing was performed by checkerboard broth microdilution procedure (1-3)
- A set of 21 isolates were tested: 10 *S. pneumoniae* (including ATCC 49619, 2 penicillin-susceptible, 2 penicillin-intermediate, 5 penicillin-resistant [including 3 macrolide-resistant] isolates, 10 *H. influenzae* (including ATCC 49247, ATCC 49766, NCTC 8468, 3 β-lactamase negative, 4 β-lactamase positive isolates) and 1 *S. aureus* ATCC 29213.
- All study isolates were tested initially using broth microdilution MIC methods to determine dalbavancin and azithromycin MIC results. This initial BMD testing for *H. influenzae* was performed in Haemophilus Test Media (HTM), HTM+0.002% P80 and cation adjusted Mueller Hinton (CAMHB) +5% LHB+20 mg/L β-NAD to determine optimal media for the checkerboard study.

Media Tested:

Cation Adjusted Mueller Hinton Broth (CAMHB) + 5% Lysed Horse Blood (LHB) + 20 mg/L β-nicotinamide adenine dinucleotide (NAD)*
*Preferred media for both species; initial dalbavancin MIC results for this media were comparable to HTM+P80 and endpoints easier to read compared to HTM.

Antimicrobials – Concentrations Tested (µg/mL):

Dalbavancin – 0.004 – 0.25 (SP Plate Format 1)
Dalbavancin – 0.12 – 8 (SP Plate Format 2 and *H. influenzae*)
Azithromycin – 0.03 – 32 (*S. pneumoniae*)
Azithromycin – 0.12 – 128 (*H. influenzae*)

Data Analysis:

The fractional inhibitory concentrations (FICs) were calculated as the MIC of the agent alone divided by the MIC of the agent when tested in combination. The FIC index was obtained by adding the FICs. FIC indices were interpreted as synergistic when values were ≤0.5, additive when values were >0.5 – 1.0, indifferent when values were >1.0-≤4.0 and antagonistic when values were >4. When MIC was off-scale at > highest concentration tested, the highest concentration + 1 doubling dilution was used. When DAL combination MIC was off-scale at less than the lowest concentration in the combination well, the lowest concentration tested was used for the FIC calculation.(3)

Results

- Based on evaluation of FIC indices, there was no synergy or antagonism detected (Tables 1-3)
- All results were considered either additive or indifferent. In most cases the combination of dalbavancin and azithromycin had a slight increase (dalbavancin and azithromycin MICs decreased by 1 doubling dilution) in activity against *S. pneumoniae*
- The combination of dalbavancin and azithromycin against *H. influenzae* had little to no effect on the activity of azithromycin

Table 1: *S. pneumoniae* Results (Plate Format 1)

Isolate	MIC (µg/mL)				FIC DAL	FIC AZI	FIC Index	FIC Interp.	Colony Count CFU/mL
	DAL (Alone)	AZI (Alone)	DAL (in Combination)	AZI (in Combination)					
1SP	0.03	0.12	0.015	0.03[1]	0.50	≤0.25	≤0.7500	AD	
2SP	0.015	0.06	0.004[1]	0.03[1]	≤0.25	≤0.50	≤0.7500	AD	5.65E+05
3SP	0.015	0.06	0.015	0.06	1.00	1.00	2.0000	IN	
4SP	0.015	0.06	0.015	0.03[1]	1.00	≤0.50	≤1.5000	IN	
5SP	0.015	0.06	0.004[1]	0.03[1]	≤0.25	≤0.50	≤0.7500	AD	4.20E+05
6SP	0.015	4	0.008	2	0.50	0.50	1.0000	IN	
7SP	0.015	4	0.008	0.5	0.50	0.13	0.6250	AD	4.55E+05
8SP	0.015	8	0.008	4	0.50	0.50	1.0000	IN	
9SP	0.03	64[2]	0.015	0.03[1]	0.50	≤0.0003	≤0.5003	AD	
SP49619QC	0.015	0.12	0.004[1]	0.03[1]	≤0.25	≤0.25	≤0.5000	AD	5.30E+05
SA29213QC	0.12	0.5	0.06	0.25	0.50	0.50	1.0000	AD	4.80E+05

[1]MIC is offscale at ≤ the lowest concentration, lowest concentration tested was used for analysis — Average CFU/mL: 4.90E+05
[2]MIC is offscale at >32 µg/mL, 64 µg/mL (highest concentration +1 dilution) was used for analysis
AD – additive, IN – indifferent

Results (cont.)

Table 2: *S. pneumoniae* Results (Plate Format 2)

Isolate	MIC (µg/mL)				FIC DAL	FIC AZI	FIC Index	FIC Interp.	Colony Count CFU/mL
	DAL (Alone)	AZI (Alone)	DAL (in Combination)	AZI (in Combination)					
1SP	0.12[1]	0.12	0.12[1]	0.03[1]	N/A	≤0.25	N/A	N/A	4.85E+05
2SP	0.5	0.06	0.12[1]	0.03[1]	≤0.25	≤0.50	≤0.750	AD	
3SP	0.12[1]	0.06	0.12[1]	0.03[1]	N/A	≤0.50	N/A	N/A	
4SP	0.12[1]	0.06	0.12[1]	0.03[1]	N/A	≤0.50	N/A	N/A	
5SP	0.12[1]	0.06	0.12[1]	0.03[1]	N/A	≤0.50	N/A	N/A	
6SP	0.12[1]	4	0.12[1]	0.03[1]	N/A	≤0.25	N/A	N/A	5.85E+05
7SP	0.12[1]	4	0.12[1]	0.03[1]	N/A	≤0.0078	N/A	N/A	
8SP	0.12[1]	8	0.12[1]	0.03[1]	N/A	≤0.0039	N/A	N/A	4.65E+05
9SP	0.12[1]	64[2]	0.12[1]	0.03[1]	N/A	≤0.0003	N/A	N/A	
SP49619QC	0.12[1]	0.12	0.12[1]	0.03[1]	N/A	≤0.25	N/A	N/A	4.80E+05
SA29213QC	0.12[1]	0.5	0.12[1]	0.03[1]	N/A	≤0.06	N/A	N/A	

[1]MIC is offscale at ≤ the lowest concentration, lowest concentration tested was used for analysis — Average CFU/mL: 5.04E+05
[2]MIC is offscale at >32 µg/mL, 64 µg/mL (highest concentration +1 dilution) was used for analysis
AD – additive
N/A –FIC not applicable because the dalbavancin MIC is below the lowest concentration tested for both the individual and combination wells

Table 3: *H. influenzae* Results

Isolate	MIC (µg/mL)				FIC DAL	FIC AZI	FIC Index	FIC Interp.	Colony Count CFU/mL
	DAL (Alone)	AZI (Alone)	DAL (in Combination)	AZI (in Combination)					
10HI	16[2]	0.5	0.12	1	≤0.0039	2.00	2.0039	IN	4.90E+05
11HI	16[2]	1	0.12	2	≤0.0039	2.00	2.0039	IN	
12HI	16[2]	1	0.12	2	≤0.0039	2.00	2.0039	IN	
13HI	16[2]	1	0.12	1	≤0.0039	1.00	≤1.0039	IN	5.60E+05
14HI	16[2]	1	0.12	1	≤0.0039	1.00	≤1.0039	IN	4.70E+05
15HI	16[2]	1	0.12	2	≤0.0039	2.00	2.0039	IN	
16HI	16[2]	1	0.12	2	≤0.0039	2.00	2.0039	IN	6.10E+05
HF49247QC	16[2]	0.5	1	1	≤0.03	2.00	2.0313	IN	4.80E+05
HF49766QC	16[2]	1	0.12	2	≤0.0039	2.00	2.0039	IN	4.20E+05
HF8468QC	16[2]	0.5	8	1	≤0.25	2.00	≤2.2500	IN	5.55E+05
SA29213QC	0.12[1]	0.5	0.12[1]	0.12[1]	N/A	≤0.25	N/A	N/A	

[1]MIC is offscale at ≤ the lowest concentration, lowest concentration tested was used for analysis — Average CFU/mL: 5.12E+05
[2]MIC is offscale at >8 µg/mL, 16 µg/mL (highest concentration +1 dilution) was used for analysis
IN - indifferent
*Plate was only readable after 48 hour incubation
N/A - FIC not applicable because the dalbavancin MIC is below the lowest concentration tested for both the individual and combination wells

Conclusion

- Overall, the combination of dalbavancin and azithromycin showed no significant increase or decrease in each agent's in vitro activity by checkerboard methodology based on the FIC index evaluation.

References

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