In Vitro Evaluation of the Peptide Deformylase Inhibitor LBM415 In Combination with Aztreonam to Detect Possible Synergistic Interactions

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ABSTRACT

Objective: To evaluate the interactions between LBM415 (LBM) and aztreonam (AZT) in vitro. LBM is a peptide deformylase inhibitor (PDI), a class of compounds with a novel mechanism of action against Gram-positive (GP) organisms and some respiratory tract Gram-negative (GN) fastidious pathogens that is currently in clinical development.

Methods: A checkerboard broth microdilution test method was used to test 108 clinical isolates: Enterobacteriaceae (ENT; 81), non-fermentative GN (NFGN; 15), *N. meningitidis* (NM; 2), staphylococci (included MRSA and MR-CoNS; 6) and *Enterococcus* spp. (4). Tests were performed using Mueller-Hinton broth (supplemented with lysed horse blood for NM). The following definitions were used in characterizing combination MIC results: antagonism (ANT), ≥ 4-fold increase of both agents; synergy (SYN), ≥ 4-fold decrease of both agents; partial SYN (PSYN), ≥ 4-fold decrease of 1 agent/2-fold decrease of the other; additive (ADD), 2-fold decrease of both agents; indifference (IND), no decrease or only a 2-fold decrease or increase for 1 agent.

Results: No ANT was observed for any isolate or tested combination. All 10 GP pathogens showed IND to the combination of LBM and AZT. The ENT showed a significant enhanced activity with 69-100% of each species giving additive or greater interaction results (exception, *S. marcescens*). Among *Klebsiella* spp. (KSP) isolates, 53% displayed SYN, *E. coli* (EC) and *Enterobacter* spp. (EBS) showed 56% PSYN or SYN and 80% of *P. mirabilis* (PM) showed PSYN. Among the NFGN, *Acinetobacter* (ASP) isolates showed 60% SYN and 40% PSYN, whereas only 27% of *P. aeruginosa* were PSYN or ADD. *S. maltophilia* (40%) and *B. cepacia* (20%) isolates displayed ADD effects; the rest were IND to the combination. One of the NM showed an ADD effect.

Conclusions: The combination of LBM and AZT provided predominant SYN or PSYN interactions against many GN organisms (ASP, KSP, EC, EBS and PM). Several strains of other GN species showed at least an ADD interaction effect. These preliminary studies demonstrate that PDIs may display enhanced activity when tested in combination with a GN active agent and a complete lack of ANT in either GP or GN pathogens, potentially expanding the spectrum of activity of both agents. The unique mechanism of action of PDIs warrants further investigation into their potential role in combination therapy.

INTRODUCTION

LBM415 (NVP-713) is one of the first inhibitor of the bacterial enzyme peptide deformylase (PDF) to be advanced into human clinical trials for the oral treatment of community-acquired respiratory tract and skin and skin structure infections. PDF is required in bacteria for protein maturation and is not found in eukaryotic cells, making it a unique antibacterial target. LBM415 has documented activity against the major pathogens of the respiratory tract and skin and skin structures, but is inactive against Enterobacteriaceae, *Pseudomonas aeruginosa* and most other non-fermentative bacilli. Fortunately, cross-resistance with other classes of antimicrobials has not been detected.

Aztreonam, while lacking Gram-positive activity, has pronounced activity against the commonly occurring Gram-negative pathogens, including the Enterobacteriaceae and non-fermentative Gram-negative bacilli, and is often used in combination with other agents to extend the spectrum of coverage in treatment of serious infections. This report assesses the contribution of aztreonam to LBM415 activity versus key Gram-negative and -positive pathogens causing nosocomial pneumonia, complicated skin and skin structure infections (SSSI) and bloodstream infections (BSI) when tested in combination.

MATERIALS AND METHODS

Susceptibility Testing Methods: A broth microdilution checkerboard configuration in a 96 well panel conforming to the NCCLS M7-A6 standard [2003] was utilized. Antimicrobial agents included LBM415 tested over the range of 0.06 - 64 mg/L and aztreonam over the range of 0.25 - 16 mg/L; both agents were tested alone and in combination. Mueller-Hinton cation-adjusted broth was used throughout, with the addition of 2 - 5% lysed horse blood added for testing of fastidious species. Interpretive criteria, where applicable, were those of the NCCLS M100-S15 document (2005).

Organism Collection: Organisms were those pathogens (resistant and susceptible strains, including QC strains) most frequently associated with bloodstream infections (*E. coli, Klebsiella* spp., *P. aeruginosa, Enterobacter* spp., *P. mirabilis*, oxacillin-susceptible *S. aureus*, oxacillin-resistant and -susceptible coagulase-negative staphylococci, *E. faecalis* and vancomycin-resistant *E. faecium*); nosocomial or community-acquired pneumonia (*Klebsiella* spp., *E. coli*, *P. aeruginosa, Enterobacter* spp., *S. marcescens, S. maltophilia, B. cepacia, Acinetobacter* spp., and oxacillin-resistant *S. aureus);* and skin and skin structure infections (*E. coli, Klebsiella* spp., *P. aeruginosa, Enterobacter* spp., *S. marcescens*, oxacillin-susceptible *S. aureus, E. faecalis* and *E. faecium;* Table 1). *N. meningitidis* was also tested (two strains). Quality control organisms used included *E. coli* ATCC 35922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213 and 25923, *E. faecalis* ATCC 29212 and *S. pneumoniae* ATCC 49619. These six strains were assessed during the study to determine the reproducibility of the checkerboard, synergy method for this combination.

Analyses: Antimicrobial interactions were determined using the following categories: antagonism (four-fold or greater increase in the MIC values of either or both agents); synergy (four-fold or greater decrease in the MIC values of both agents); partial synergy (four-fold or greater decrease in the MIC value for one agent and a two-fold reduction in the MIC of the other); additive (two-fold decrease in MIC values of both tested agents); indifference (no decrease in the MIC values of either agent or only a two fold decrease or increase in the MIC of one agent); and indeterminant (results inconsistent with the described categories).

RESULTS

- All strains of staphylococci (6 strains) and enterococci (4 strains) showed indifference to the combination of LBM415 and aztreonam (Table 2).
- All QC results for *S. aureus* (14 results), *E. faecalis* (8 results) and *S. pneumoniae* (3 results) also displayed indifference.
- 44.9% of Gram-negative species tested displayed either synergistic (15.3%) or partially synergistic interactions (29.6%).
- 40.8% of interactions among Gram-negative species were indifferent.
- Synergy or partial synergy results were observed for *Acinetobacter* spp. (60% and 40%, respectively), *Klebsiella* spp. (53% and 18%), *E. coli* (19% and 37%), *Enterobacter* spp. (0% and 56%) and *P. mirabilis* (0% and 80%).
- 27% of *P. aeruginosa* displayed partial synergy or additive effects; synergistic interactions were absent for *B. cepacia* and *S. maltophilia* (additive or indifferent results only).
- Antagonism was NOT detected between the compounds with any organism evaluated.

Table 1. Distribution of tested organisms by infection site and species.

Organism	No. tested by infection site			
	BSI	Pneumonia	SSSI	Total
Gram-negative pathogens				
Klebsiella spp.	6	6	5	17
E. coli	5	6	5	16
P. aeruginosa	5	5	5	15
Enterobacter spp.	6	5	5	16
P. mirabilis	5	-	-	5
S. marcescens	-	6	6	12
S. maltophilia	-	5	-	5
B. cepacia	-	5	-	5
Acinetobacter spp.	-	5	-	5
Neisseria meningitidis	2	-	-	2
Gram-positive pathogens				
MRSA	-	2	-	2
MSSA	1	-	1	2
MR-CoNS	1	-	-	1
MS-CoNS	1	-	-	1
E. faecalis	1	-	1	2
E. faecium	1	-	1	2
Total	34	45	29	108

Table 2. Interactions of LBM415 with aztreonam tested against selected Gram-positive and -negative pathogens.

Organism	Synergy	Partial Synergy	Additive	Indifferent	Indeterminant	Antagonism	Total
Gram-positive							
MRSA	0	0	0	2	0	0	2
MSSA	0	0	0	2	0	0	2
MR-CoNS	0	0	0	1	0	0	1
MS-CoNS	0	0	0	1	0	0	1
E. faecalis	0	0	0	2	0	0	2
E. faecium	0	0	0	2	0	0	2
Total	0	0	0	10(100%)	0	0	10
Gram-negative							
Klebsiella spp.	9	3	1	4	0	0	17
E. coli	3	6	3	4	0	0	16
P. aeruginosa	0	3	1	10	1	0	15
Enterobacter spp.	0	9	2	4	1	0	16
P. mirabilis	0	4	1	0	0	0	5
S. marcescens	0	2	0	10	0	0	12
S. maltophilia	0	0	2	3	0	0	5
B. cepacia	0	0	1	4	0	0	5
Acinetobacter spp.	3	2	0	0	0	0	5
N. meningitidis	0	0	1	1	0	0	2
Total	15(15.3%)	29(29.6%)	12(12.2%)	40(40.8%)	2(2.0%)	0	98
Quality control							
E. coli ATCC 25922	0	0	0	0	8	0	8
P. aeruginosa ATCC 27853	0	0	0	8	0	0	8
S. aureus ATCC 29213	0	0	0	8	0	0	8
S. aureus ATCC 25923	0	0	0	6	0	0	6
E. faecalis ATCC 29212	0	0	0	8	0	0	8
S. pneumoniae ATCC 49619	0	0	0	3	0	0	3
Total	0	0	0	33(80.5%)	8(19.5%)	0	41
Combined							
Gram-negative	15(15.3%)	29(29.6%)	12(12.2%)	40(40.8%)	2(2.0%)	0	98
Gram-positive	0	0	0	10(100%)	0	0	10
Total	15(13.9%)	29(26.8%)	12(11.1%)	83(76.8%)	10(9.3%)	0	108

CONCLUSIONS

- Predominant synergistic or partially synergistic interactions were noted with *Acinetobacter* spp. (100%), *Klebsiella* spp. (71%), *E. coli* (56%), *Enterobacter* spp. (56%) and *P. mirabilis* (80%).
- All Gram-positive species tested demonstrated complete indifference to the combination of LBM415 and aztreonam.
- No antagonism was noted between LBM415 and aztreonam with any organism tested.
- These preliminary studies demonstrate that peptide deformylase inhibitors may display enhanced activity when combined with a Gram-negative active agent, but only against Gram-negative pathogens. In clinical trials, the aztreonam would not interfere (indifference) with the LBM415 activity evaluations against Gram-positive species.

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