ICAAC 2013
53rd ICAAC | SEPT 10-13 | Denver, CO

A1021

Activity and Pharmacodynamic (PD) Evaluation of Ceftazidime-Avibactam (CAZ-AVI) against Extracellular and Intracellular Forms of CAZ-susceptible and CAZ-resistant *Pseudomonas aeruginosa* (PA)

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LDRI Louvain Drug Research Institute

Abstract (edited)

Background

AVI reverses CAZ resistance in PA over-expressing AmpC and several ESBLs when tested in broth (Mushtaq *et al.* JAC 2010; 65:2376-81). We have examined whether such reversion is also observed for intracellular PA.

Methods

The strains used are shown in Table 1. MICs were determined by microdilution (CA-MHB; CLSI method). Extracellular and intracellular activities were evaluated after 24 h of incubation in MHB and in infected human monocytes (THP-1), respectively, using CAZ concentrations ranging from 0.01 to 200 mg/L with or without AVI (4 mg/L) (see Buyck et al. AAC 2013; 57:2310-8). Potency (C_s [static concentr.; mg/L]) and maximal relative efficacy (E_{max}; change in $\log_{10}$ CFU) were calculated using the Hill equation (sigm. funct.: slope fact.=1).

Results

Data: See the Tables 1 and 2 in the Results.

Description: AVI reduced the MIC of CAZ-resistant strains to a susceptible range. For both extracellular and intracellular bacteria, C_s was close to the MIC. For CAZ-resistant strains, C_s of CAZ was brought to a value similar to that of the CAZ-susceptible strains when combining it with AVI. E_{max} was beyond the limit of detection for the extracellular forms of all strains but only between -0.3 and -2.9 log10 CFU for intracellular forms. AVI made CAZ-resistant strains behaving intracellularly as CAZ-susceptible strains.

Conclusions

AVI restored CAZ potency (Cs) towards intracellular CAZ-resistant strains to the same extent as to their extracellular forms but did not modify the antibiotic intracellular maximal relative efficacy. The data suggest that AVI reaches intracellular bacteria and cooperates therein with CAZ. This might prove useful in the treatment of persistent or recurrent infections in which intracellular bacteria are thought to play a major role.

References

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Funding

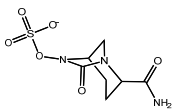
This work was supported by a grant-in-aid from AstraZeneca Pharmaceuticals, Waltham, MA and Forest/Cerexa, Oakland, CA. J.M.B. was supported by the Belgian *Région Wallonne* though the program "*Aides à la Recherche et à l'Innovation technologique*". F.V.B. is Senior Research Associate of the Belgian *Fonds de la Recherche Scientifique* (F.R.S.-FNRS). K.M.K. and W.W.N are employees of Cerexa Inc. and AstraZeneca Pharmaceuticals, respectively. C.L. and P.M.T. were unpaid.

Background and Aims

Pseudomonas aeruginosa, a major cause of nosocomial infections in immunocompromised or debilitated patients, is of concern to clinicians because of

- its high level of resistance in contemporary isolates through constitutive and inducible expression of β-lactamases (including ESBL) and of efflux pumps, as well as the low permeability of its outer-membrane;
- its ability to enter and survive in eucaryotic cells where the efficacy of antibiotics is considerably reduced (1).

Avibactam (see structure and biophysical properties hereunder) is a novel β-lactamase inhibitor with activity against class A ESBLs, most class C enzymes, and class A carbapenemases (2).



Avibactam

- **pKa = -1.98**
- **main species at pH 5 to 8: negative**
- **log P = -1.76**
- **log D pH 7.4 and pH 5.4 = -4.14**

calculated with Reaxys [Reed/Elsevier] (http://www.reaxys.com)

In broth, avibactam fully reverses AmpC- and ESBL PER-1-mediated ceftazidime resistance in *P. aeruginosa* (3), which shows that it penetrates at least into the periplasm and, therefore, crosses the outer membrane of *P. aeruginosa*.

Our aim was to examine whether avibactam is also able to restore ceftazidime activity against intracellular forms of AmpC-producing *P. aeruginosa*. To this effect, we used a pharmacodynamic model originally developed in our laboratory for the study of intracellular *S. aureus* (4) and recently expanded to the study of intracellular *P. aeruginosa* (1).

Methods

Bacterial strains

- ATCC 27853 (fully susceptible to CAZ) and PAO1 (reference strain),
- clinical strains (nosocomial pneumonia [5]);
- engineered linked parent-daughter (isogenic) pairs [6] procured by Dr D. Livermore (HPA, UK) as
  - basal non-inducible AmpC (M1405 def and 2297 def; low MIC of ceftazidime)
  - corresponding spontaneous mutants with stably-derepressed AmpC (M1405 CON and 2297 CON; high MIC for ceftazidime)

Susceptibility testing

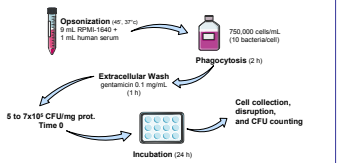
- MIC measured according to the recommendations of CLSI in CA-MHB.

Dose-kill curve studies in extracellular medium

- 24 h incubation in 2 mL of CA-MHB (initial inoculum: ~10^6 CFU/mL)

Dose-kill curve studies in intracellular medium

- Human THP-1 monocytes infected and treated as described in [1] and pictorially shown here:



Drug concentrations: CAZ: 0.01 to 200 mg/L with or without avibactam (4 mg/L)

Presentation of results and Modeling

- Change of CFU (in $\log_{10}$ units) at 24 h from initial (broth) or post-phagocytosis/ extracellular wash (time 0 h)
- Fitting of a Hill-Langmuir function (sigmoidal equation with slope factor = 1) to calculate
  - E_{min} (change of CFU for an infinitely low antibiotic concentration),
  - E_{max} (change of CFU for an infinitely large antibiotic concentration),
  - EC_{50} (antibiotic extracellular concentration yielding a change of bacterial counts half-way between E_{min} and E_{max}) and
  - C_s (concentration for which there is no apparent change in bacterial counts from the original inoculum).

Conclusions

- AVI restores the potency of CAZ to several strains producing a β-lactamase acting on CAZ, making them almost indistinguishable from their susceptible counterparts;
- This restoration of activity is observed to a similar extent for **both** extracellular and intracellular bacteria, which implies that avibactam has sufficient access to the bacteria at their site of location in THP-1 monocytes.

Results

Table 1: Susceptibility testing

Strains	MIC (mg/L)	
	CAZ	CAZ+AVI
ATCC 27853	2	2
PAO1	8	2
Clinical strains (resistant to CAZ) [a]		
PA59	64	4
PA104	64	4
PA115	64	4
PA152	128	4
PA156	128	4
PA185	64	8
PA299	256	8
PA315	128	4
PA331	256	8
PA340	128	4
PA362	64	4
Engineered strains		
2297 def [b]	2	2
2297 CON [c]	128	8
M1405 def [b]	4	4
M1405 CON [c]	128	8

[a] strains from patients with clinically confirmed nosocomial pneumonia
[b] basal non-inducible AmpC
[c] stable overexpressing AmpC (isogenic)

Strains highlighted in yellow have been used in pharmacodynamic studies illustrated in Figures 1, 2, 3 (see numeric data in Table 2)

Main observations

1. AVI restores potency of CAZ to all strains (MIC [Table 1 [CLSI method] and C_s [pharmacodynamic extracellular and intracellular model, see Figs 1 and 2 and Table 2]);
2. E_{max} of ceftazidime (pharmaco-dynamic model) is always much weaker (less negative) against intracellular than extracellular forms (Figs 1 and 2 and Table 2) as previously reported (1), and is not influenced by AVI;
3. When changes in CFU (pharmaco-dynamic model) are expressed as multiples of MIC, all strains behave alike (Fig. 3), with indistinguishable extracellular or intracellular pharmacodynamic parameters.

Figure 1: Concentration-response curves of CAZ and CAZ+AVI for ATCC 27853, PA152 and PA315

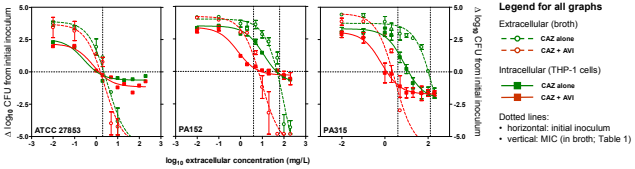


Figure 2: Concentration-response curves of CAZ and CAZ+AVI for 2297 (def/CON) and M1405 (def/CON)

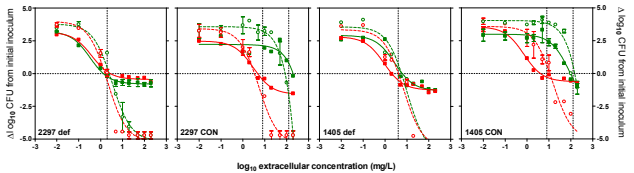


Table 2: Pharmacodynamic parameters (Figs 1 and 2)

Strains [a]	Extracellular		Intracellular			
	C_s (mg/L) [b,e]		C_s (mg/L) [b]		E_{max} (Δ $\log_{10}$ CFU) [c]	
	- AVI	+ AVI	- AVI	+ AVI	- AVI	+ AVI
ATCC27853	2.3	1.9	1.1	1.4	-0.6 ± 0.1	-1.2 ± 0.1
PA152	47.6	6.6	44.3	8.4	-0.9 ± 0.2	-0.3 ± 0.1
PA315	104	2.3	12.6	1.2	-2.1 ± 0.3	-1.7 ± 0.2
2297 def	2.5	1.6	1.3	2.7	-0.8 ± 0.1	-0.5 ± 0.1
2297 CON	85.4	3.4	196.0	4.7	[d]	-1.6 ± 0.2
M1405 def	5.6	4.1	6.1	2	-1.3 ± 0.3	-1.3 ± 0.1
M1405 CON	150	10.8	95.4	4	-2.9 ± 1.4	-0.6 ± 0.1

[a] see Table 1 for strain description
[b] apparent static concentration (no change from the original inoculum (time 0 h) as determined by graphical interpolation (Hill equation; R^2 = 0.67 to 0.99);
[c] decrease in $\log_{10}$ CFU as extrapolated for an infinitely large CAZ concentration (Hill equation); limit of detection: -5 $\log_{10}$ CFU (compared to the initial inoculum (10^6 CFU/mL in broth; 5-7 x10^8 CFU/mg protein in cells);
[d] plateau not reached at the highest CAZ concentration tested;
[e] E_{max} was < 5 $\log_{10}$ CFU for the extracellular form of all strains and below the lowest limit of detection.

Figure 3: Concentration-response curves of CAZ and CAZ+AVI for all strains as a function of multiples of MIC of CAZ