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Research note

Pharmacodynamic modelling of β-lactam/β-lactamase inhibitor checkerboard data: illustration with aztreonam–avibactam

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ABSTRACT

Objectives: Checkerboard experiments followed by fractional inhibitory concentration (FIC) index determinations are commonly used to assess *in vitro* pharmacodynamic interactions between combined antibiotics, but FIC index cannot be determined in case of antibiotic/non-active compound combinations. The aim of this study was to use a simple modelling approach to quantify the *in vitro* activity of aztreonam-avibactam, a new β-lactam–β-lactamase inhibitor combination.

Methods: MIC checkerboard experiments were performed with 12 Enterobacteriaceae with diverse β-lactamases profiles. Aztreonam MICs in the absence and presence of avibactam at different concentrations (ranging from 0.0625 to 4 mg/L) were determined. Aztreonam MIC versus avibactam concentrations were fitted by an inhibitory E_{max} model with a baseline effect parameter.

Results: A concentration-dependent relationship was observed with a steep initial reduction of aztreonam MIC at low avibactam concentrations and reaching a maximum at higher avibactam concentrations that was adequately fitted by the model. Maximum avibactam effect was characterized by the ratio of aztreonam MICs in the absence of avibactam (MIC_0) and when avibactam concentration tends toward infinity (MIC_∞), and this ratio ranged between 90 and 10 068 depending on the strain. Avibactam potency was characterized by avibactam concentrations corresponding to 50% of the maximum effect (IC_{50} values between 0.00022 and 0.053 mg/L).

Conclusions: An inhibitory E_{max} model with a baseline effect could quantify maximum avibactam effect and potency among various strains. This simple modelling approach can be used to compare the activity of other combinations of antibiotics with non-antibiotic drugs when FIC index is inappropriate. **A. Chauzy, Clin Microbiol Infect 2019;25:515.e1–515.e4**

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Introduction

Infections caused by carbapenem-resistant Enterobacteriaceae (CRE) represent a major threat to human health worldwide [1]. These pathogens are resistant to most prescribed antibiotics because of the production of various types of β-lactamases and are extremely hard to eradicate. Combining a β-lactam antibiotic with a β-lactamase inhibitor to inactivate β-lactamase activity constitutes an interesting option for treating CRE infections [2]. Aztreonam–avibactam is one of the new β-lactam–β-lactamase inhibitor combinations under development. Aztreonam is a monobactam antibiotic that is not hydrolysed by metallo-β-lactamases (MBLs), which inactivate most β-lactam antibiotics [3]. Yet it is hydrolysed by some serine β-lactamases, such as extended-spectrum β-lactamases (ESBLs) and serine carbapenemases including KPC [3]. However, these serine β-lactamases are efficiently inhibited by the β-lactamase inhibitor avibactam [4]. Therefore, combining aztreonam with avibactam is expected to restore the clinical utility of aztreonam against CRE.

Pharmacodynamic interaction between antibiotics used in combination is usually investigated by performing checkerboard experiments followed by fractional inhibitory concentration (FIC) index determinations [5]. This is based on the comparison of the MIC of each antibiotic alone (MIC_A and MIC_B for antibiotics A and B,

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respectively) with the combination-derived MICs ($MIC_{A/B}$ and $MIC_{B/A}$) (Eq. 1).

$$FIC\ index = \frac{MIC_{A/B}}{MIC_A} + \frac{MIC_{B/A}}{MIC_B} \quad (1)$$

It is a simple approach that can be used for rapid screening of antibiotic combinations in the presence of various strains. However, if an antibiotic is combined with a non-antibiotic compound, such as a β-lactamase inhibitor, the FIC index cannot be determined since the non-antibiotic drug has *a priori* no antimicrobial activity and cannot be characterized by an MIC value. Antimicrobial activity has been reported for non-antibiotic compounds, but with MIC values much higher than concentrations clinically achievable [6,7]. Accordingly, antibiotic/non-antibiotic combinations have been defined as synergistic (27th European Congress of Clinical Microbiology and Infectious Diseases, abstract P0655) but for non-antibiotic drug concentrations corresponding to toxic concentrations [8].

The objective of the present study was to use a simple but meaningful modelling approach for the analysis of checkerboard-type data obtained with β-lactam–β-lactamase inhibitor combinations, or any combination of an active antibiotic with a non-active compound, based on the E_{max} model previously used in that context [9] or to describe the pro-convulsant effect of biphenyl acetic acid on fluoroquinolones [10].

Material and methods

Chemicals

Aztreonam (Sigma-Aldrich, St Quentin Fallavier, France) and avibactam (provided as a dry powder by AstraZeneca, Macclesfield, UK) were used to prepare stock solutions of 50 mg/mL of aztreonam in methanol/dimethyl sulphoxide (50/50, v/v) and 1 mg/mL of avibactam in sterile water. Aztreonam and avibactam stock solutions were further diluted in cation-adjusted Mueller–Hinton broth (CAMHB).

Bacterial strains

Twelve Enterobacteriaceae strains, resistant to aztreonam, including four *Klebsiella pneumoniae*, two *Escherichia coli*, two *Citrobacter freundii*, three *Enterobacter cloacae*, and one *Enterobacter aerogenes* were studied (Table 1). The β-lactamase content of each strain was characterized by the IHMA laboratory (International Health Management Associates, Inc., Schaumburg, IL, USA) using PCR.

In vitro susceptibility testing

For each bacterial strain, MICs of aztreonam and avibactam alone were determined according to the EUCAST guidelines for broth microdilutions [11]. Aztreonam MICs in the presence of avibactam were also determined using checkerboard method. Checkerboards were set up with twofold dilutions of aztreonam (0.0078 to 512 mg/L) and avibactam (0.0625 to 4 mg/L) in such a way that different combinations of aztreonam and avibactam concentrations were obtained in each well. After drug dilution, 96-well plates were inoculated with each organism to yield the appropriate density (0.5×10^6 CFU/mL) and incubated for 16–20 h at 37°C. The MIC was recorded as the lowest concentration of antimicrobial agent that completely inhibited visible growth of the organism in microdilution wells. Negative growth controls were performed in wells containing only CAMHB. All experiments were performed in duplicate; the second replicate was used to validate the first one. Only the first replicate was used for data analysis.

Data analysis

The antibacterial effect of aztreonam in combination with avibactam was investigated by modelling the interaction between both compounds, using WinNonlin software (version 6.2, Certara, Princeton, NJ, USA). Aztreonam MICs versus avibactam concentrations were fitted according to an inhibitory E_{max} model with a baseline effect parameter (Eq. 2). A Gauss–Newton minimization method with the Levenberg and Hartley modification was used for data fitting. Data were not log-transformed before analysis and we used the weighted least squares method for minimization based on observations ($Yobs_i$), i.e. the weight applied to each observation (w_i) was $w_i = 1/(Yobs_i)^2$.

$$MIC_{CAVI} = MIC_0 - \frac{(MIC_0 - MIC_\infty) \times C_{AVI}}{(C_{AVI} + IC_{50})} \quad (2)$$

Table 1
Parameter estimates of the inhibitory E_{max} model with baseline effect for 12 resistant Enterobacteriaceae isolates

Organism	IHMA no.	β-lactamases	ATM MIC (mg/L)	ATM-AVI MIC[a] (mg/L)	Parameter estimates (RSE%)				
					MIC0 (mg/L)	MIC∞ (mg/L)	MIC0/MIC∞	AVI IC50 (mg/L)	AVI IC90 (mg/L)
Escherichia coli	1266865	TEM-OSBL(b), CMY-42, NDM-5	32	4	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
	1275629	CTX-M-15, VIM-23	128	0.0625	128 (22)	0.056 (22)	2262	0.00040 (27)	0.0036
Klebsiella pneumoniae	1277372	SHV-12(e), TEM-OSBL(b), CTX-M-15, NDM-7	128	0.0625	128 (20)	0.044 (22)	2894	0.00047 (24)	0.0043
	1289268	SHV-OSBL(b), TEM-OSBL(b), CTX-M-15, VIM-4	128	0.0625	127 (32)	0.033 (50)	3909	0.00064 (38)	0.0058
	1251604	SHV-5(e), VIM-26	256	0.125	256 (33)	0.12 (32)	2178	0.00053 (39)	0.0048
	947566	SHV-12(2be), VIM-42	64	0.125	64 (29)	0.064 (41)	995	0.0018 (36)	0.016
Citrobacter freundii	974673	SHV-12(2be), TEM-OSBL(2b), CTX-M-3, CMY-34, NDM-1	512	0.125	511 (32)	0.051 (83)	10068	0.00053 (38)	0.0048
	1080008	VIM-23	128	1	133 (38)	0.23 (166)	580	0.015 (49)	0.14
Enterobacter aerogenes	1286221	TEM-OSBL(b), CTX-M-15, NDM-1	128	0.03125	127 (46)	0.019 (64)	6808	0.00026 (58)	0.0023
Enterobacter cloacae	1285905	CTX-M-15, NDM-1	64	0.25	64 (30)	0.21 (32)	299	0.0037 (38)	0.034
	1318536	CTX-M-15, NDM-1	512	0.125	510 (35)	0.083 (41)	6182	0.00022 (43)	0.0020
	1251704	TEM-OSBL(b), VIM-1	32	1	33 (30)	0.37 (84)	90	0.053 (47)	0.55

ATM, aztreonam; AVI, avibactam; RSE, relative standard error; ND, not determined; OSBL, Original-spectrum β-lactamase; TEM, Temoneira; CMY, cephamycins; NDM, New Delhi metallo-β-lactamase; CTX-M, cefotaxime-hydrolysing β-lactamase; VIM, Verona integrin-encoded metallo-β-lactamase; SHV, sulphydryl variable.
[a] Aztreonam MIC determined in the presence of 4 mg/L avibactam.
[b] The model could not be fitted to the data obtained from this isolate.

Where, MIC_0 is the MIC of aztreonam in the absence of avibactam and MIC_∞ the asymptotic value of MIC when avibactam concentration (C_{AVI}) tends toward infinity. MIC_0/MIC_∞ ratio characterizes avibactam maximum effect, traditionally referred as efficacy in the E_{max} model and expressed here as a percentage, while IC_{50}, the avibactam concentration corresponding to 50% of the maximum inhibitory effect, characterizes the potency of avibactam. Then, avibactam IC_{90}, corresponding to the avibactam concentration which produces 90% of the maximum effect, was derived from the model for the different strains studied.

Results

The inhibitory E_{max} model with a baseline effect parameter adequately described the steep initial decay of aztreonam MIC in the presence of very low avibactam concentrations before reaching a plateau at higher avibactam concentrations, as shown in the individual plots (Fig. 1). Weighted residuals were evenly distributed around 0. However, for *E. coli* 1266865, a slightly different profile was observed, with a much shallower decrease of aztreonam MIC in the presence of increasing avibactam concentrations and no plateau within this range of avibactam concentrations. For the other strains, estimated MIC_0 were close to measured values, and the maximum avibactam effect (MIC_0/MIC_∞) ranged between roughly 100 and 10 000, and potency (IC_{50}) between 0.00022 and 0.053 mg/L (Table 1).

Discussion

An inhibitory E_{max} model with a baseline effect parameter successfully described the effect of avibactam on aztreonam MIC in all but one strain. The presence of avibactam dramatically reduced aztreonam MIC, up to roughly 100–10 000-fold and starting at low concentrations (most IC_{50} values <<0.1 mg/L). Because avibactam possesses some intrinsic antibacterial activity at relatively high levels (MIC ≥8 mg/L) [12], the maximum concentration of avibactam used in these checkerboard experiments was 4 mg/L in order to restrict this investigation to the effect of avibactam on aztreonam MIC, presumably because inhibition of β-lactamases. Maximum effect and potency of avibactam were highly variable between strains, reflecting the wide range of β-lactamases produced by the different strains and the difference of affinity of β-lactamases for avibactam [13]. However, model-derived aztreonam MIC in the absence of avibactam was higher for *E. cloacae* 1318536 (MIC = 510 mg/L) than for *E. cloacae* 1285905 (MIC = 64 mg/L) although both strains express exactly the same β-lactamases, suggesting differences in efflux or outer membrane permeability and in expression levels of enzymes between the two strains.

Compared with FIC index that only concludes to synergism, additivity or antagonism, the E_{max} model can quantify efficacy and potency among various strains or β-lactam–β-lactamase inhibitor combinations and is therefore more informative. This simple E_{max} model was previously used to characterize the activity of an

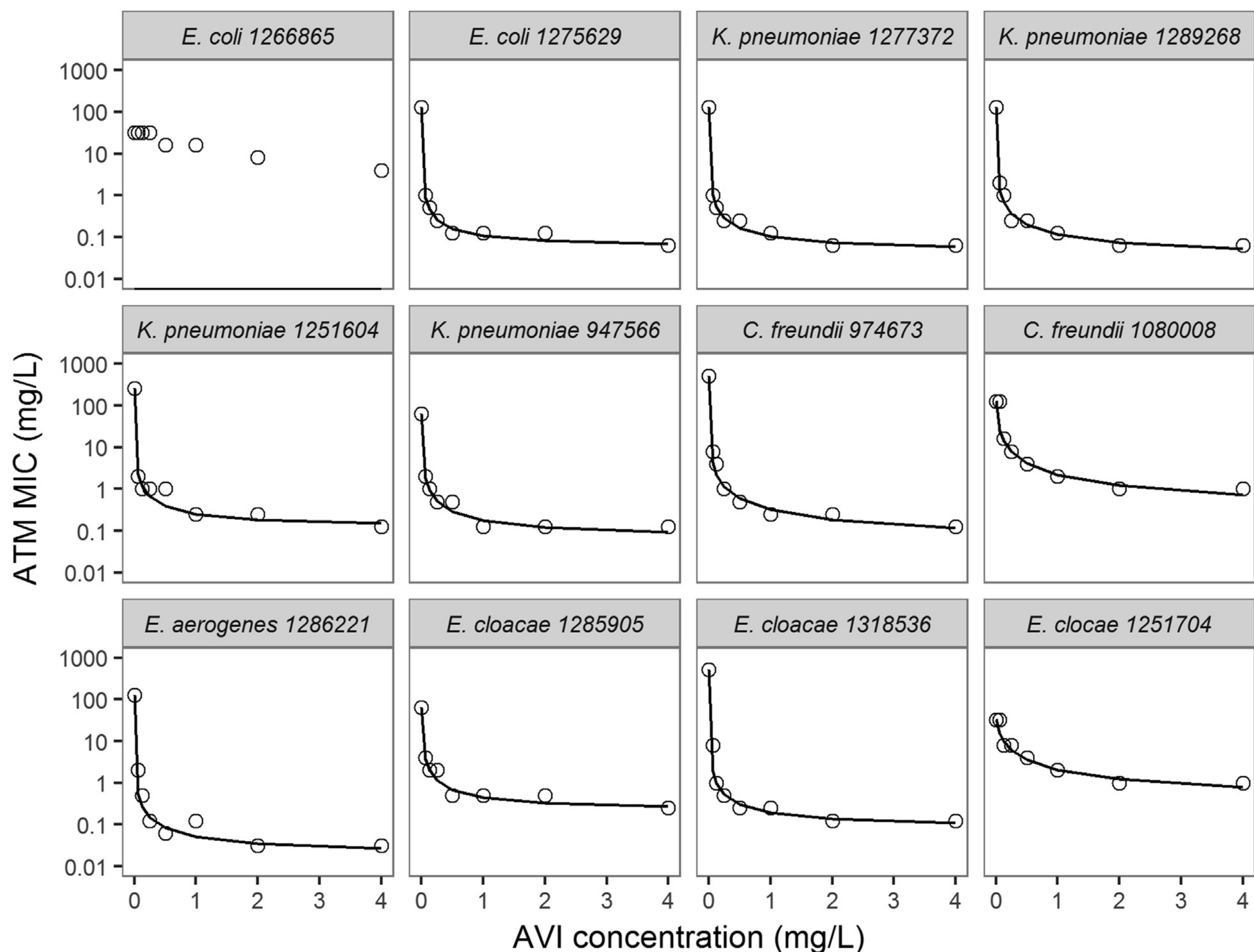

Fig. 1. Aztreonam MICs versus avibactam concentrations (mg/L) for 12 Enterobacteriaceae isolates. The circles represent the aztreonam MICs determined during one checkerboard experiment and the solid lines the individual aztreonam MICs predicted by the E_{max} model with baseline effect.

experimental β-lactamase inhibitor in combination with imipenem for selecting optimal dosing strategies of the combination [9]. However, this objective may fall beyond the limits of these checkerboard experiments. Alternative approaches such as time-kill experiments combined with semi-mechanistic pharmacokinetic/pharmacodynamics (PK/PD) modelling to characterize the aztreonam–avibactam combination are better suited for that [14].

Yet simple checkerboard experiments analysed with an E_{max} model seem appropriate for comparing β-lactam-β-lactamase inhibitor combinations efficacy and potency.

Transparency declaration

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