Optimizing pharmacokinetics/pharmacodynamics of β -lactam/ β -lactamase inhibitor combinations against high inocula of ESBL-producing bacteria

Vincent H. Tam ^{1,2,3*}, Henrietta Abodakpi^{2†}, Weiqun Wang¹, Kimberly R. Ledesma¹, Paul R. Merlau¹, Katrina Chan², Rachel Altman³, Truc T. Tran^{4,5}, Michael Nikolaou³ and Amelia K. Sofjan¹

¹Department of Pharmacy Practice and Translational Research, University of Houston College of Pharmacy, Houston, TX, USA;

²Department of Pharmacological and Pharmaceutical Sciences, University of Houston College of Pharmacy, Houston, TX, USA;

³Department of Chemical and Biomolecular Engineering, University of Houston Cullen College of Engineering, Houston, TX, USA;

⁴Center for Antimicrobial Resistance and Microbial Genomics, University of Texas McGovern Medical School, Houston, TX, USA; ⁵Division of Infectious Diseases, University of Texas McGovern Medical School, Houston, TX, USA

*Corresponding author. E-mail: vtam@uh.edu

†Present address: Office of Clinical Pharmacology, U.S. Food and Drug Administration, Silver Spring, MD, USA.

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Objectives: Reduced *in vitro* β -lactam activity against a dense bacterial population is well recognized. It is commonly attributed to the presence of β -lactamase(s) and it is unknown whether the inoculum effect could be diminished by a β -lactamase inhibitor. We evaluated different β -lactam/ β -lactamase inhibitor combinations in suppressing a high inoculum of ESBL-producing bacteria.

Methods: Three clinical isolates expressing representative ESBLs (CTX-M-15 and SHV-12) were examined. The impact of escalating β -lactamase inhibitor (tazobactam or avibactam) concentrations on β -lactam (piperacillin or ceftazidime) MIC reduction was characterized by an inhibitory sigmoid $E_{\max}$ model. The effect of various dosing regimens of β -lactam/ β -lactamase inhibitor combinations was predicted using $\%T_{>MIC}$ and selected exposures were experimentally validated in a hollow-fibre infection model over 120 h. The threshold exposure to suppress bacterial regrowth was identified using recursive partitioning.

Results: A concentration-dependent reduction in β -lactam MIC was observed ($r^2 \geq 0.93$). Regrowth could be suppressed in all six experiments using $\%T_{>MIC} \geq 73.6\%$, but only one out of six experiments below the threshold ($P=0.015$). The exposures to suppress regrowth might be attained using the clinical dose of avibactam, but a much higher dose than the standard dose would be needed for tazobactam.

Conclusions: A dense population of ESBL-producing bacteria could be suppressed by an optimized dosing regimen of selected β -lactam/ β -lactamase inhibitor combinations. The reversibility of enzyme inhibition could play an important role in diminishing the inoculum effect. *In vivo* investigations to validate these findings are warranted.

Introduction

The estimated prevalence of ESBL-producing Enterobacteriaceae in the inpatient setting in the USA is 12.6% (approximately 290 220 ESBL isolates annually), which is associated with at least 1700 deaths annually.¹ Carbapenems are considered the treatment of choice for serious infections caused by ESBL-producing organisms, but continued widespread use of carbapenems may worsen carbapenem resistance in Gram-negative organisms.² As a result, carbapenem-sparing therapy such as a β -lactam/ β -lactamase inhibitor combination is an attractive alternative since many ESBL-producing Enterobacteriaceae are susceptible to β -lactam/ β -lactamase inhibitor combinations *in vitro*. However, clinical studies

that compared β -lactam/ β -lactamase inhibitor combinations (specifically amoxicillin/clavulanate or piperacillin/tazobactam) versus carbapenems for bacteraemia due to ESBL-producing Enterobacteriaceae have yielded mixed efficacy results.³ One potential reason for suboptimal outcomes with β -lactam/ β -lactamase inhibitor combinations for serious infections due to ESBL organisms is the inoculum effect.

The inoculum effect is a laboratory phenomenon in which the MIC of an antibiotic increases when the antibiotic is exposed to a high density of organisms (e.g. $>10^7$ cfu/mL versus 10^5 cfu/mL). The inoculum effect has been observed *in vitro* and *in vivo* with piperacillin/tazobactam against ESBL-producing Enterobacteriaceae, while

carbapenems have been more resilient to the inoculum effect.^{4,5} Treatment efficacy could be compromised in serious infections associated with high inocula (e.g. intra-abdominal infections) as a result. However, it is largely unknown whether the inoculum effect could be diminished in the presence of newer β -lactamase inhibitors (e.g. avibactam, vaborbactam).

The objective of this study was to evaluate the pharmacokinetics and pharmacodynamics of different β -lactam/ β -lactamase inhibitor combinations in suppressing a high inoculum of ESBL-producing bacteria. A special emphasis was placed on the impact of the mode (reversibility) of β -lactamase inhibition on outcomes.

Materials and methods

Antimicrobial agents and bacteria

Piperacillin was purchased from Sigma-Aldrich (St Louis, MO, USA). Ceftazidime and tazobactam were obtained from Chem-Impex International (Wood Dale, IL, USA). Avibactam was acquired from BOC Sciences, Inc. (Shirley, NY, USA). Stock solutions were prepared in sterile water, aliquotted, stored at -80°C and thawed immediately before use. Three clinical isolates expressing representative ESBLs were examined; their genotypic and phenotypic characterizations were described previously.⁶ The isolates were stored at -80°C and sub-cultured twice overnight at 35°C before use.

Susceptibility studies and effect predictions

Piperacillin MIC was determined by a broth dilution method with escalating concentrations of tazobactam (up to 256 mg/L) and avibactam (up to 64 mg/L). Similarly, ceftazidime MIC was also determined with escalating concentrations of tazobactam and avibactam. The impacts of tazobactam and avibactam concentrations on MIC reduction were characterized by an inhibitory sigmoid E_{max} model. Subsequently, the pharmacokinetic/pharmacodynamic exposures of various dosing regimens of β -lactam/ β -lactamase inhibitor combinations were predicted using $\%T_{>\text{MICi}}$ (a novel index reflecting dynamic susceptibility over time) as described previously.⁶

Hollow-fibre infection model and experimental validations

The schematics and set-up of the infection model have been described previously.⁷ The reference (population mean) pharmacokinetics to be simulated in the models are shown in Table 1. The unbound maximum concentrations (but not elimination half-lives) were adjusted proportionally for different doses to be given. If the agents to be examined have different elimination half-lives, the experimental set-ups were revised as shown previously.⁸⁻¹⁰

For each isolate, several discrete colonies from an overnight culture were grown to late log phase. The bacterial suspension (20 mL) was

adjusted to approximately 1×10^8 cfu/mL. The agents were given every 8 h (each dose administered over 30 min) for up to 120 h. Serial samples were obtained from the internal circulating loop to verify the simulated concentration-time profiles. To validate the anticipated antibacterial effect, serial samples (500 μL) were also obtained from the bioreactor cartridges in duplicate. Samples were centrifuged at 10 000 g at 4°C for 15 min, reconstituted with sterile saline to minimize drug carry-over and plated quantitatively on Mueller-Hinton agar plates. Additionally, drug-supplemented agar plates (using $3 \times$ the baseline respective MIC with 4 mg/L of β -lactamase inhibitor) were used to detect regrowth associated with the development of resistance over time (for susceptible isolates at baseline only).

A range of simulated dosing regimens of β -lactam/ β -lactamase inhibitor combinations were screened initially. Since we have previously demonstrated $\%T_{>\text{MICi}} \geq 55.1\%$ was associated with suppression of a standard bacterial inoculum,⁶ experimental validation primarily focused on combination dosing regimens associated with $\%T_{>\text{MICi}} > 60\%$.

Drug assays and pharmacokinetic modelling

Drug concentrations were assayed using a validated LC-MS/MS method, as described previously.^{10,11} A one-compartment model with zero-order infusion input was fit to the observed drug concentration-time profiles using ADAPT 5.¹² The pharmacokinetic simulations were deemed acceptable if the best-fit maximum concentrations and elimination half-lives were both within 20% of target values.

Statistical analysis

Outcomes observed in the infection model were classified as suppression versus regrowth (i.e. viable bacterial burden towards the end of experiment was considerably higher than that from a sample obtained earlier in the treatment course). The breakpoint threshold of targeted $\%T_{>\text{MICi}}$ to prevent bacterial regrowth was identified by classification and regression tree ('CART') analysis. Subsequently, the relationship between drug exposures (above and below the breakpoint threshold) and regrowth was compared using Fisher's exact test. A P value of <0.05 was considered statistically significant.

Results

Susceptibility studies and effect predictions

The known mechanisms of resistance of the isolates examined and their susceptibility to different β -lactam/ β -lactamase inhibitor combinations are shown in Table 2. For all three isolates, a non-linear reduction in β -lactam MIC was observed with escalating inhibitor concentrations. In all cases, the relationship between β -lactam susceptibility and inhibitor concentrations was reasonably well characterized ($r^2 \geq 0.93$) (data not shown). The projected $\%T_{>\text{MICi}}$ values associated with different dosing regimens of β -lactam/ β -lactamase inhibitor combinations are shown in Table 3.

Experimental validations

The simulated pharmacokinetic profiles in the infection model were acceptable (all $r^2 \geq 0.96$ and within the target ranges) (data not shown). Viable bacterial burden was considerably reduced in the beginning of all active (i.e. non-placebo) experiments, but regrowth was observed in 5 out of 12 experiments. Representative killing profiles are shown in Figure 1. In addition, selective amplification of bacterial sub-population(s) with reduced susceptibility over time was associated with regrowth (data not shown).

Table 1. Reference exposures simulated for various β -lactams and β -lactamase inhibitors

Agent	Dosing (g)	C_{max} (mg/L)	Elimination half-life (h)
Piperacillin	4.0	240	1.0
Ceftazidime	2.0	120	2.5
Tazobactam	0.5	30	1.0
Avibactam	0.5	15	2.5

C_{max} , maximum concentration.

Table 2. Characteristics and susceptibility (MIC) of clinical isolates examined

Bacteria	ESBL gene	MIC (mg/L)			
		PIP/TZB	PIP/AVI	CAZ/TZB	CAZ/AVI
K. pneumoniae (Kp3A)	CTX-M-15	256/4	8/4	16/4	1/4
K. pneumoniae (KpK91A)	CTX-M-15	>256/4	32/4	64/4	2/4
E. coli (EcF65)	SHV-12	4/4	2/4	64/4	0.5/4

PIP, piperacillin; TZB, tazobactam; AVI, avibactam; CAZ, ceftazidime.

Tazobactam MICs for all isolates were >256 mg/L and avibactam MICs for all isolates were >64 mg/L.

Bold font denotes resistant phenotype.

Table 3. Projections of % $T_{>MIC_i}$ for selected dosing regimens

Bacteria	β -Lactam (C_{max})	β -Lactamase inhibitor (C_{max})	% $T_{>MIC_i}$
Kp3A	PIP (240)	TZB (30)	25.2
	PIP (480)	TZB (120)	45.6
	PIP (480)	TZB (240)	52.7
	PIP (240)	AVI (15)	75.2 ^a
	CAZ (120)	TZB (30)	48.5
	CAZ (120)	TZB (60)	62.1 ^a
	CAZ (240)	TZB (120)	75.4 ^a
	CAZ (240)	TZB (240)	85.4
	CAZ (120)	AVI (15)	100.0 ^a
	CAZ (120)	AVI (30)	100.0 ^a
KpK91A	PIP (240)	TZB (30)	10.2
	PIP (480)	TZB (120)	35.2
	PIP (480)	TZB (240)	45.2
	PIP (240)	AVI (15)	57.2
	PIP (240)	AVI (30)	65.0 ^a
	PIP (480)	AVI (15)	66.3
	PIP (480)	AVI (30)	75.5 ^a
	CAZ (120)	TZB (30)	38.4 ^a
	CAZ (120)	TZB (120)	55.6
	CAZ (240)	TZB (120)	65.7 ^a
EcF65	CAZ (240)	TZB (240)	73.6 ^a
	CAZ (120)	AVI (15)	100.0
	PIP (240)	TZB (30)	43.8
	PIP (240)	TZB (60)	60.0 ^a
	PIP (240)	TZB (120)	69.9 ^a
	PIP (240)	AVI (15)	76.0 ^a
	CAZ (120)	TZB (30)	41.3
	CAZ (120)	AVI (15)	100.0

C_{max} , maximum concentration; PIP, piperacillin; TZB, tazobactam; AVI, avibactam; CAZ, ceftazidime.

C_{max} in mg/L.

All agents were given over 30 min every 8 h.

^aExperimentally validated.

Stratifying the exposures by outcomes, the most significant threshold to prevent regrowth over time was identified. Regardless of the specific β -lactam/ β -lactamase inhibitor used, regrowth was suppressed in all six experiments with % $T_{>MIC_i} \geq 73.6\%$, but only

Figure 1. Killing profiles against Kp3A over time. Data shown as mean $\pm$ SD. Ceftazidime/tazobactam % $T_{>MIC_i}$ = 62.1%. Piperacillin/avibactam % $T_{>MIC_i}$ = 75.2%. PIP, piperacillin; TZB, tazobactam; AVI, avibactam; CAZ, ceftazidime. **Figure 2.** Drug exposures (% $T_{>MIC_i}$) stratified by outcomes. Each data point represents a hollow-fibre infection model experiment. The most significant threshold (% $T_{>MIC_i}$ = 73.6%) is depicted by the horizontal broken line. Open triangles, ceftazidime/avibactam; open squares, piperacillin/avibactam; filled circles, ceftazidime/tazobactam; open inverted triangles, piperacillin/tazobactam.

one out of six experiments below the threshold ($P = 0.015$). A summary of the outcomes observed is shown in Figure 2. To achieve the optimal pharmacokinetic/pharmacodynamic exposure, it was noted that a considerably larger dose (4–8 $\times$ the clinical dose, up to 4 g) of tazobactam would be needed, whereas the standard dose (within the range of inter-subject variability of 0.5 g) of avibactam could suffice (Table 3).¹³

Discussion

Reduced *in vitro* activity against a dense bacterial population (i.e. the inoculum effect) is well recognized for β -lactams and commonly attributed to the presence of β -lactamase enzyme(s).¹⁴ The

in vivo relevance of the inoculum effect has been questioned previously.¹⁵ Hypothetically, the activity of the β -lactams could be restored in the presence of a β -lactamase inhibitor. However, conventional β -lactamase inhibitors (e.g. tazobactam, sulbactam) are β -lactam structural analogues. They bind to the β -lactamase active site irreversibly and are consumed in the process. In the presence of a dense bacterial population, it is recognized that the β -lactamase inhibitor could be overwhelmed by a high expression level of β -lactamase enzyme(s). In contrast, newer β -lactamase inhibitors (e.g. avibactam, vaborbactam) are not structurally related to the β -lactams. These agents inhibit β -lactamase reversibly and thus can be recycled in the process. Theoretically, the mode (i.e. reversibility) of enzyme inhibition could play a critical role in diminishing the inoculum effect. In order to ascertain the impact of the mode of β -lactamase inhibition, selected regimens with comparable $\%T_{>MIC}$ predicted were experimentally compared between tazobactam (a non-reversible β -lactamase inhibitor) and avibactam (a reversible β -lactamase inhibitor) in this study. An overall trend in regrowth (indicative of a lack of sustained suppression) was observed when the bacteria were exposed to decreasing exposures of β -lactam/ β -lactamase inhibitor combinations.

Instead of using time above a threshold concentration, we captured a fluctuating susceptibility profile over time in this study. Our approach is deemed to allow for more flexibility in the concentration–response relationship. In an extreme scenario when there is a drastic response (i.e. the sigmoid E_{max} model approaches that of a ‘step-like’ or ‘on-off’ function), both approaches would be very similar. We have previously demonstrated that $\%T_{>MIC}$ could be used as a surrogate index to rank pharmacokinetic/pharmacodynamic exposures of different β -lactam/ β -lactamase inhibitor combination dosing regimens. Our findings implied that a threshold exposure could be associated with suppression of a standard bacterial inoculum. In this study, we extended the concept to explore if a high density of bacteria could also be contained. Our findings provided several important insights to make inroads to optimizing β -lactam/ β -lactamase inhibitor combinations.

First, although it is commonly accepted that the *in vitro* activity of β -lactams is compromised against a dense bacterial population, a recent study provided contradicting data refuting this connotation.¹⁶ With cefepime and meropenem, a change in inoculum was found to be positively correlated with a change in MIC; the inoculum effect was deemed sufficiently pronounced to affect categorical interpretations during standard laboratory testing. On the other hand, ceftazidime/avibactam MIC was not appreciably affected by the inoculum. To the best of our knowledge, our study is the first to date to directly compare two β -lactamase inhibitors with different modes of action in the hollow-fibre model. For the most relevant assessment, we adjusted both the partnering β -lactams and dosing regimens for similar pharmacokinetic/pharmacodynamic exposures. Our results implied that additional pharmacological properties such as inhibitor affinity (e.g. k_{cat}/k_{inact} ratio) and/or the mode of enzyme inhibition might also be important to consider.

Second, there could be multiple sub-populations with different drug susceptibilities (or enzyme expression levels) in a dense bacterial population. We previously attributed the development of resistance over time to selective amplification of resistant sub-population(s) present at baseline.¹⁷ In this study we showed that a

dense bacterial population of ESBL-producing Enterobacteriaceae could be suppressed by an optimized dosing regimen of a β -lactam/ β -lactamase inhibitor combination. Compared with a standard inoculum, a higher drug exposure ($\%T_{>MIC}$ 73.6% versus 55.5%) was necessary to suppress a dense population. With these preliminary findings and a novel pharmacokinetic/pharmacodynamic index ($\%T_{>MIC}$) as the foundational framework, future investigations could examine dosing regimens of β -lactam/ β -lactamase inhibitor combinations to counter-select resistance development over time, as we and others have demonstrated.^{11,18}

Finally, our findings highlighted that fixed (dose) combinations of β -lactam/ β -lactamase inhibitor may not be optimal for all clinical scenarios. In addition to (matched) pharmacokinetics of the agents in a combination, other factors such as initial bacterial burden, specific enzyme(s) present, the level(s) of expression, susceptibility of the backbone β -lactam and the affinity of the β -lactamase inhibitor could also contribute to the optimal combination selection. While ceftazidime/avibactam at standard clinical doses achieved the most optimal exposure (i.e. $\%T_{>MIC} = 100\%$) in this study, it is not our intent to suggest that this specific drug combination is most effective in suppressing a dense population of all ESBL-producing organisms. Rather, we sought to establish a framework that can be utilized to evaluate various β -lactam/ β -lactamase inhibitor combinations in a more robust fashion. The ‘one size fits all’ approach might be overly optimistic. If a robust susceptibility testing method is available to guide clinicians in timely selection of agents, a case could be made for β -lactamase inhibitors to be made clinically available as standalone agents.

There were several limitations with this study. Only a handful of ESBL-producing Enterobacteriaceae isolates were examined; a wider range of ESBL (and variant)-producing pathogens would have enhanced the generalizability of the study. In addition, not all β -lactam/ β -lactamase inhibitor combinations were comprehensively evaluated. We also did not restrict the investigations to only commercially available combinations and clinically attainable concentration ranges. The tolerance of some of the doses examined may not have been established. Instead, we focused on only several dosing regimens as a proof-of-concept study. Nonetheless, we believe our insights are clinically relevant and of value to readers who are clinicians. We hope our findings will stimulate dialogue among colleagues for better approaches to advance patient care.

In summary, a dense population of ESBL-producing Enterobacteriaceae could be suppressed by an optimized dosing regimen of selected β -lactam/ β -lactamase inhibitor combinations. After adjusting for similar pharmacokinetic/pharmacodynamic exposures, avibactam was found to be more potent than tazobactam in restoring β -lactam activity, possibly due to reversibility of β -lactamase inhibition. *In vivo* investigations to validate these findings are warranted.

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Disclaimer

The opinions expressed in this article are those of the authors and should not be construed to represent the Food and Drug Administration's views or policies.

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