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Humanized Exposures of a β-Lactam-β-Lactamase Inhibitor, Tazobactam, versus Non-β-Lactam-β-Lactamase Inhibitor, Avibactam, with or without Colistin, against *Acinetobacter baumannii* in Murine Thigh and Lung Infection Models

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Tazobactam · Colistin · *Acinetobacter baumannii*

Abstract
β-lactam-β-lactamase inhibitors (BLIs) have previously demonstrated antimicrobial activity against *Acinetobacter baumannii* (AB). Colistin retains the highest susceptibility rate against *A. baumannii*, and has demonstrated synergy with other antimicrobials, including β-lactam-BLIs. Therefore, we assessed the potential individual activity and synergistic combinations in vivo against carbapenem-susceptible (CS) and multidrug-resistant (MDR) *A. baumannii* isolates in neutropenic thigh and lung infection models. In vitro, colistin and tazobactam MICs were 1 and 16 μg/mL against AB 25–49 (CS) and 1 and 128 μg/mL against AB 5075 (MDR) respectively. In the lung model, tazobactam alone and in combination with colistin achieved a 1-log reduction in CFU, while colistin alone was not active against AB 25–49. No activity was observed against AB 5075. In the thigh model, tazobactam with and without colistin was bacteriostatic against AB 25–49 but did not demonstrate any activity against AB 5075. Avibactam and colistin alone and in combination were not active against either isolate. No synergy was observed; however, we found tazobactam activity against *A. baumannii*. This activity was not observed for the non-β-lactam-BLI, avibactam. This suggests that binding to penicillin-binding proteins of the β-lactam molecule is required for tazobactam activity against *A. baumannii*. These data point to an added role of β-lactam-BLIs beyond their primary purpose of β-lactamase inhibition in the treatment of MDR *A. baumannii* infections by enhancing the activity of peptide antibiotics, a property that is not shared by the novel non-β-lactam-BLIs. Future studies are needed to define tazobactam and colistin activity in an *A. baumannii* infection model.

Introduction

Multidrug resistant (MDR) *Acinetobacter baumannii* (AB) has evolved into a global concern due to its resistant phenotype to common antimicrobials and association with high mortality rates in nosocomial infections [1–3].

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Furthermore, the incidence of infections associated with MDR *A. baumannii* continues to rise as effective therapeutic options decline [2, 4]. Given the deficit of effective antimicrobials and lack of novel therapies in the antibiotic pipeline, clinicians have resorted to old, repurposed antibiotics and/or combination therapies for the treatment of MDR *A. baumannii* infections.

Historically, β-lactam-β-lactamase inhibitors (BLIs), such as tazobactam, have primarily been used for the protection of antibiotics from inactivating bacterial enzymes. However, these inhibitors have demonstrated the ability to bind to penicillin-binding proteins (PBPs) of *Acinetobacter* spp., suggesting a novel therapeutic role against MDR *A. baumannii* [5–9]. Moreover, colistin, a long-standing polymyxin that is regaining relevance due to its in vitro potency against MDR organisms, is another antibacterial of interest for the treatment of MDR *A. baumannii* [10]. In previous in vivo murine thigh and lung models, colistin alone and in combination with other antimicrobials against MDR *A. baumannii* demonstrated efficacy, although inconsistent, requiring further investigation [9, 11–13]. Most recently, we have found that β-lactam-BLIs can potentiate not only the activity of colistin against some strains of *A. baumannii* but also the activity of daptomycin against MRSA, further expanding upon the β-lactam-peptide antibiotic see-saw effect [14].

In order to examine the recently introduced non-β-lactam-BLIs on the activity of peptide antibiotics, we assessed the potential individual activity and synergistic combinations of tazobactam, avibactam (a non-β-lactam-BLI), and colistin in vivo against carbapenem-susceptible (CS) and MDR *A. baumannii* isolates in neutropenic thigh and lung infection models.

Materials and Methods

Antimicrobial Agents

Commercially available vials of colistin were acquired from Cardinal Health (Dublin, OH, USA) and reconstituted as described in the prescribing information and diluted as appropriate to achieve the desired concentrations. Analytical grade tazobactam sodium and avibactam sodium powders were obtained from Tecoland Corporation (Irvine, CA, USA). A pharmacokinetic study was conducted to confirm a tazobactam dosing regimen that would provide in vivo murine drug exposure similar to that of 0.5 g every 6 h (q6h) in humans, quantified by the free time above MIC from 0 to 24 h (fT > MIC) [15–16]. Furthermore, colistin was prepared to produce an exposure previously shown to result in bacterial stasis in a murine thigh infection model [13]. Avibactam was prepared to simulate the humanized exposure of a 0.5 g q8h dose [17]. Carbapenems, such as meropenem, were not used in this study, as previous pharmacodynamic murine models have described the expected bacterial reduction against *A. baumannii* isolates with similar MICs [18].

Animals

Specific pathogen-free, female ICR (CD-1) mice weighing 20–22 grams were obtained from Envigo RMS, Inc. (Indianapolis, IN, USA). The animals were allowed to acclimate for a minimum of 48 h before the experiment commenced and were provided food and water ad libitum. The protocol was reviewed and approved by the Institutional Animal Care and Use Committee at Hartford Hospital, Hartford, CT. ICR mice were rendered transiently neutropenic by intraperitoneal (IP) injections (0.2 mL in normal saline) of cyclophosphamide (Sigma-Aldrich., St. Louis, MO, USA).

Bacterial Isolates

Two clinical isolates of *A. baumannii*, 1 CS *A. baumannii* (AB 25–49) and 1 MDR *A. baumannii* (AB 5075), were selected for this study from the Center for Anti-infective Research and Development culture collection or isolated from a patient in the US military health care system respectively [19]. Colistin, tazobactam, and avibactam minimum inhibitor concentrations (MICs) were determined in triplicate via broth microdilution, and the modal MICs were reported [20]. Isolates were stored in skim milk (BD BioSciences, Sparks, MD, USA) at –80 °C and were subcultured twice onto trypticase soy agar with 5% sheep blood (TSA II™, BD BioSciences, Sparks, MD, USA) within 48 h prior to use for MIC studies.

For lung and thigh inoculation, bacterial colonies of a fresh subculture of each isolate were suspended in sterile normal saline to produce a suspension of approximately 10^7 CFU/mL. Final inoculum concentrations were confirmed by plating serial dilutions on Trypticase soy agar with 5% sheep blood (BD Biosciences, Sparks, MD, USA) and incubating at approximately 37 °C in ambient air overnight.

Neutropenic Lung Infection Model

Mice were anesthetized with isoflurane and inoculated with 0.05 mL of the infecting *A. baumannii* isolate into the nares. Groups of 6 mice were inoculated with AB 25–49 (CS) or AB 5075 (MDR). Four hours post-infection, tazobactam and colistin (10 mg/kg q24h) alone and in combination were administered via the subcutaneous or IP route respectively. Tazobactam dose was equivalent to a humanized exposure of 0.5 g q6h. Control animals received 0.2 mL of 0.9% normal saline solution subcutaneously (SC) in a frequency identical to the most frequently dosed drug regimen. At 24 h post-initiation of antimicrobial therapy, a group of 6 animals from each treatment arm, as well as from control groups, were euthanized by exposing them to CO_2 followed by cervical dislocation. After sacrifice, the lungs were removed and individually homogenized via Mini-beadbeater (Biospec Products, Inc. Bartlesville, OK, USA) in 0.9% normal saline solution. Serial dilutions were plated on TSA II™ plates for CFU determination. Antibacterial activity was measured as the change in lung bacterial density (Log_{10} CFU) relative to the starting inoculum (0 h). For the combination regimens, synergy was defined as ≥2 log_{10} CFU reduction in bacterial density compared with the most active agent [21].

Table 1. In vitro potency of tazobactam, avibactam, colistin, and carbapenems against each *A. baumannii* isolate

Isolate	MIC, μg/mL					
	TZB	AVI	CST	MEM	IMP	ETP
AB 25–49	16	>512	1	2	0.125	8
AB 5075	128	>512	1	32	32	≥128

MIC, minimum inhibitor concentration; AB, *Acinetobacter baumannii*; TZB, tazobactam; AVI, avibactam; CST, colistin; MEM, meropenem; IMP, imipenem; ETP, ertapenem.

Table 2. Comparison of %fT > MIC values achieved with tazobactam at each MIC in humans and in mice receiving the humanized regimen

Drug	Species	%fT > MIC for a MIC, μg/mL						
		4	8	16	32	64	128	256
Tazobactam	Mouse[a]	85.00	75.00	61.67	45.00	28.33	13.33	6.67
	Human[b]	83.33	70.00	56.67	43.33	28.33	13.33	0.00

[a] 62.5 mg/kg (0 h), 12.5 mg/kg (0.25 h), 25 mg/kg (2.5 h), 9.375 mg/kg (5 h) q6h.
[b] 0.5 g q6h, 30-min infusion.

Neutropenic Thigh Infection Model

In the neutropenic thigh model, groups of 3 mice were inoculated with *A. baumannii* isolates via intramuscular injection of 0.1 mL of the inoculum into each thigh (n = 2) of the mouse 2 h prior to the initiation of antimicrobial therapy. Colistin (10 mg/kg q24h) and humanized exposures of tazobactam (0.5 g q6h) and avibactam (0.5 g q8h) were administered independently and in combination with each other [17]. Colistin was administered IP. Tazobactam and avibactam were administered SC. Control animals received 0.2 mL of 0.9% normal saline solution SC in a frequency identical to the most frequently dosed drug regimen. After 24 h, animals were euthanized, thighs excised, and antibacterial activity was measured as the change in thigh Log_{10} CFU relative to the starting inoculum (0 h). Synergy was defined as above.

Results

In vitro Susceptibility

Table 1 shows tazobactam, avibactam, and colistin MICs for the isolates evaluated in the in vivo studies. Both isolates were susceptible to colistin. AB 25–49 demonstrated a lower tazobactam MIC (16 μg/mL) compared with AB 5075 (128 μg/mL). Avibactam did not demonstrate in vitro activity against either isolate.

Confirmatory Pharmacokinetic Study

Table 2 shows the comparison of %fT > MIC values achieved with tazobactam at each MIC in humans and in mice receiving the humanized regimen. At an MIC of 16, both human and murine exposures were similar, with %fT > MIC of 56.67 and 61.67 respectively.

Antibacterial Efficacy

Figure 1 illustrates the antibacterial efficacy of tazobactam and colistin alone and in combination against AB 25–49 and AB 5075 in the neutropenic lung infection model. In the lungs of control animals inoculated with AB 25–49, the bacterial density at 0 h was 7.25 log_{10} CFU and increased by 2.47 log_{10} CFU at 24 h. Tazobactam alone and tazobactam plus colistin regimens produced a cumulative log_{10} CFU reduction by 1.01 and 1.05 at 24 h respectively. The bacterial density increased by 2.40 log_{10} CFU in colistin-treated animals at 24 h. In the lungs of control animals inoculated with AB 5075, the bacterial density increased by 2.18 log_{10} CFU at 24 h. Tazobactam, colistin, and tazobactam plus colistin regimens resulted in increased bacterial densities of 2.23, 2.11, and 2.12 log_{10} CFU at 24 h respectively. Tazobactam regimens were most effective against AB 25–49, although no synergy was observed. None of

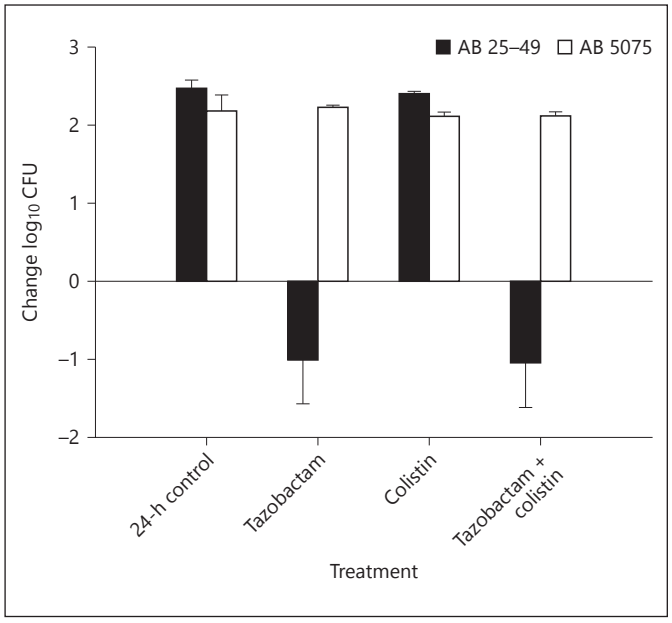

Fig. 1. Efficacy of tazobactam (human simulated regimen) and colistin alone and in combination against AB 25–49 and AB 5075 in a neutropenic murine lung infection model.

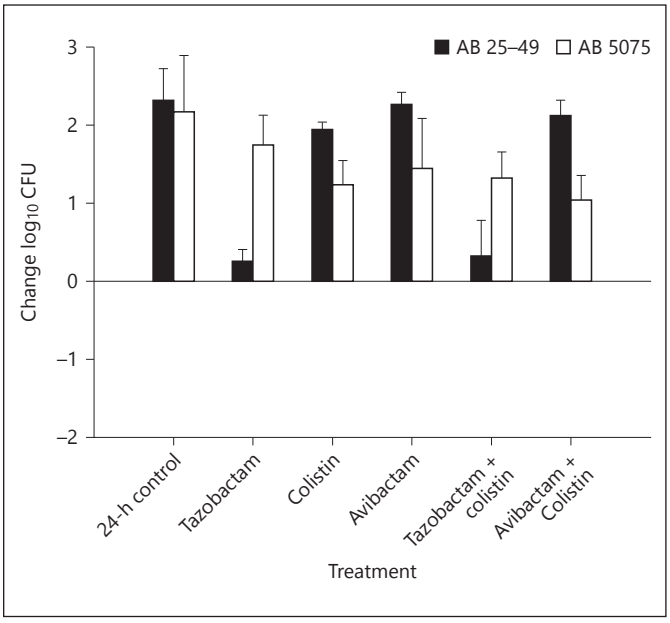

Fig. 2. Efficacy of tazobactam (0.5 g q6h), avibactam (0.5 g q8h), and colistin alone and in combination against AB 25–49 and AB 5075 in a neutropenic murine thigh infection model.

the regimens were effective or synergistic against AB 5075.

Figure 2 illustrates the antibacterial efficacy of tazobactam, avibactam, and colistin alone and in combination against AB 25–49 and AB 5075 in the neutropenic murine thigh infection model. In thighs inoculated with AB 25–49, the bacterial density increased from 5.86 at 0 h by 2.32 $\log_{10}$ CFU in control animals at 24 h. Against AB 25–49, tazobactam alone and tazobactam plus colistin regimens resulted in bacterial growth of 0.25 and 0.32 $\log_{10}$ CFU at 24 h, respectively. The bacterial density increased by 1.94, 2.27, and 2.12 $\log_{10}$ CFU in colistin, avibactam, avibactam plus colistin-treated animals at 24 h respectively. In thighs inoculated with AB 5075, the bacterial density at 0 h was 6.25 $\log_{10}$ CFU and increased by 2.17 $\log_{10}$ CFU at 24 h. Tazobactam, colistin, avibactam, tazobactam plus colistin, and avibactam plus colistin regimens resulted in increased bacterial densities of 1.75, 1.24, 1.45, 1.32, and 1.04 $\log_{10}$ CFU at 24 h respectively. Tazobactam regimens were most effective against AB 25–49, although no reduction in bacterial density or synergy was observed. None of the regimens were effective (>1 $\log_{10}$ CFU reduction in bacterial density) or synergistic against AB 5075.

Discussion

In recent years, the increasing prevalence of powerful bacterial β-lactamases among resistant Gram-negative pathogens that can hydrolyze all available β-lactam drugs has driven a need for development of novel BLIs that can counter them in order to protect primary β-lactam antibiotics. For the first time, many of the novel BLIs are deviating from the classic β-lactam structure. The first of these, avibactam, has been combined with ceftazidime, a cephalosporin that has been available for 3 decades, into a β-lactam-BLI drug to provide some of the broadest Gram-negative activity available.

We had previously shown that β-lactam-BLIs provide additional collateral antibacterial activity by enhancing the activities of administered peptide antibiotics such as daptomycin against MRSA and colistin against *A. baumannii*, and even endogenous peptide antibiotics such as cathelicidin LL37 that are produced by the innate immune system against these pathogens [14]. While the activity of sulbactam, another β-lactam-BLI, alone has been characterized against some strains of *A. baumannii*, tazobactam has been less extensively studied against *A. baumannii*. We found that tazobactam reduced bacterial growth compared with the control against *A. baumannii* in both models, although further enhanced in the lung model. This

humanized tazobactam regimen achieves substantial free drug concentrations above an MIC of 16, but not at an MIC of 128, consistent with its in vivo efficacy. Previous work with tazobactam and sulbactam demonstrated binding of these agents to PBPs, concluding that they behave both as inhibitors of hydrolyzing enzymes as well as some enzymatic steps in cell wall synthesis [6]. Moreover, the IC50s of tazobactam and sulbactam for PBPs, specifically PBP1a and PBP3, in *A. baumannii* and *Acinetobacter* sp. are relatively low suggesting adequate saturation of these targets with current clinical doses [7]. When compared with the lack of in vivo activity demonstrated by avibactam, a non-β-lactam-BLI, our findings further suggest that binding to the organism's PBPs is required for tazobactam activity against *A. baumannii.* This influence on PBP activity likely alters the bacterial surface, rendering it more susceptible to endogenous antimicrobial peptides like cathelicidins that are present at very high concentrations in sites of infection [14].

In addition to the use of β-lactam-BLIs as a potential solution to MDR *A. baumannii*, colistin remains to be one of the most frequently used alternative agents, as it retains the highest susceptibility rate, although resistance has emerged [2, 22–23]. In vitro, colistin has demonstrated synergy against *A. baumannii* with various antibiotics, such as rifampin, aztreonam, meropenem, vancomycin, and minocycline [24–26], albeit findings with colistin and sulbactam are mixed, thereby demonstrating minimal synergy against colistin-resistant *A. baumannii* and antagonism against MDR *A. baumannii* [14, 24, 27]. Conversely, a meta-analysis of 7 studies involving polymyxins combined with sulbactam or ampicillin/sulbactam yielded synergy rates of 56.0 and 54.1%, respectively, against 70 *A. baumannii* isolates [28]. Furthermore, a recent study found an additive effect ratio of 38.9% with colistin plus sulbactam against carbapenem-resistant *A. baumannii* [29]. It is important to point out that these in vitro studies fail to account for the endogenous antimicrobial peptides that are present in vivo, the activities of which could be enhanced to clinically relevant levels by β-lactam-BLIs.

To our knowledge, this is the first murine study assessing synergy between colistin and humanized exposures of tazobactam against *A. baumannii*. Colistin has previously been shown to be efficacious in vivo, although the data are inconsistent, especially in murine lung models [12–14]. Differences in colistin efficacy may be attributed to model variability, such as infection source (thigh vs. lung), mouse strain (ICR-swiss vs. BalbC), or functionality of immune system. In a previous murine thigh infection model, colistin as monotherapy against XDR *A. baumannii* was bactericidal after 48 h of treatment. Notably in the same study, sulbactam with colistin did not result in synergistic effects [9]. Our colistin regimen was not effective in either model against colistin-susceptible isolates using exposures previously shown to produce bacterial stasis in an *A. baumannii* thigh model [13]. Furthermore, we explored doses upwards of 8 times the dose used in the lung study without any signs of efficacy (data not shown). Similar to our findings with tazobactam, sulbactam has demonstrated efficacy in murine thigh and lung models, but not notable synergy [9, 12]. Given the variability in efficacy surrounding colistin monotherapy and limited in vivo data, further investigation is required to continue to define this relationship.

Conclusion

In summary, this study has provided greater insight into the role of β-lactam-BLI, specifically tazobactam, in the treatment of MDR *A. baumannii* infections. Tazobactam monotherapy is not recommended, but the use of tazobactam in combination with another agent may contribute activity or synergy against these infections, not just of colistin but of the endogenous peptides produced by the innate immune system of the host. Notably, the non-β-lactam-BLI avibactam did not potentiate the activity of colistin nor did demonstrate activity in vivo. We acknowledge there is conflicting data in the literature; therefore, future studies are required to further define colistin's role in the management of MDR *A. baumannii* infections. Given the considerable heterogeneity across the different strains, individual case-by-case assessments may be required. Nevertheless, we are slowly gaining a greater appreciation of the antibacterial properties of β-lactam molecules beyond what is detected by conventional susceptibility testing methods. The development of non-beta-lactam molecules with a broader spectrum of beta-lactamase enzyme inhibition comes at the price of losing these important adjunctive properties.

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β-Lactam-BLI, Tazobactam, vs.
Non-β-Lactam-BLI, Avibactam

Ethics Statement

The protocol was reviewed and approved by the Institutional Animal Care and Use Committee at Hartford Hospital, Hartford, CT, USA.

Disclosure Statement

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