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PHARMACOLOGY

Combating Carbapenem-Resistant *Acinetobacter baumannii* by an Optimized Imipenem-plus-Tobramycin Dosage Regimen: Prospective Validation via Hollow-Fiber Infection and Mathematical Modeling

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ABSTRACT We aimed to prospectively validate an optimized combination dosage regimen against a clinical carbapenem-resistant *Acinetobacter baumannii* (CRAB) isolate (imipenem MIC, 32 mg/liter; tobramycin MIC, 2 mg/liter). Imipenem at constant concentrations (7.6, 13.4, and 23.3 mg/liter, reflecting a range of clearances) was simulated in a 7-day hollow-fiber infection model (inoculum, $\sim 10^{7.2}$ CFU/ml) with and without tobramycin (7 mg/kg q24h, 0.5-h infusions). While monotherapies achieved no killing or failed by 24 h, this rationally optimized combination achieved >5 $\log_{10}$ bacterial killing and suppressed resistance.

KEYWORDS *Acinetobacter baumannii*, dynamic hollow-fiber infection model, synergy, mathematical modeling, pharmacokinetics, pharmacodynamics, carbapenem resistance, mechanism based, mechanistic

Acinetobacter baumannii has an exceptional propensity for emergence of resistance against commonly used antibiotics (1, 2); current treatment options are extremely limited (3, 4). While carbapenems may combat infections by susceptible *A. baumannii*, carbapenem-resistant *A. baumannii* (CRAB) isolates now comprise more than half of the isolates in the United States and elsewhere (3, 5, 6). Aminoglycosides in monotherapy can achieve substantial bacterial killing; however, this is followed by rapid and extensive regrowth with emergence of resistance (7, 8). Overall, combination therapy holds promise to treat serious infections by CRAB but needs to be optimized.

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We previously designed an imipenem-plus-tobramycin combination dosage regimen against a clinical CRAB isolate based on static concentration time-kill experiments (SCTK), mechanism-based modeling (MBM), and Monte Carlo simulations (9). The optimized regimen was imipenem 4 g/day continuous infusion with a 1-g loading dose combined with tobramycin 7 mg/kg q24h as 0.5-h infusions. Monte Carlo simulations

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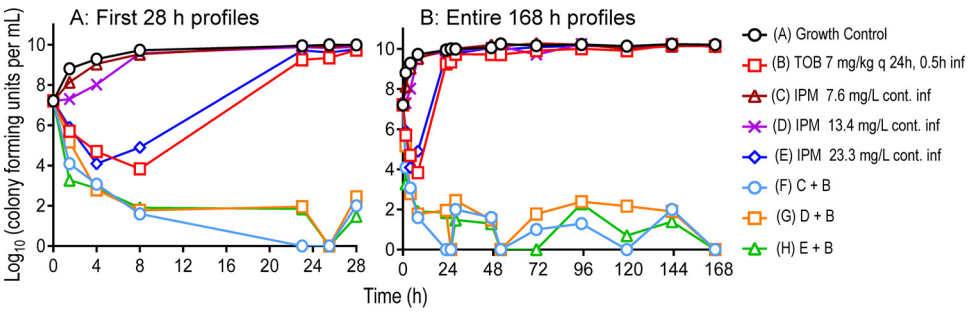

FIG 1 Observed viable count profiles (0 to 28 and 0 to 168 h) for the optimized imipenem-plus-tobramycin combination dosage regimen against isolate FADDI-AB034. Imipenem 7.6 (low, 5th percentile), 13.4 (intermediate, median), and 23.3 (high, 95th percentile) mg/liter are 3 clinically relevant imipenem profiles arising from a 4-g/day continuous infusion (with a 1-g loading dose). Observed viable counts below the limit of counting (i.e., <1.0 $\log_{10}$ CFU/ml) were plotted as zero.

predicted that this combination regimen would achieve >5 $\log_{10}$ killing without regrowth over 7 days in 98.2% of critically ill patients (9).

Very few studies have evaluated β-lactam-plus-aminoglycoside combination regimens against CRAB isolates in the dynamic *in vitro* hollow-fiber infection model (HFIM) or in *in vivo* models (10–12). Montero et al. (11) assessed an empirical, nonoptimized imipenem-plus-tobramycin combination in a murine pneumonia model against CRAB. However, no prior study evaluated bacterial killing and resistance suppression of a CRAB isolate for optimized combination dosage regimens in the HFIM.

Our primary objective was to simulate human pharmacokinetics (PK) and evaluate bacterial killing and resistance suppression by a rationally optimized combination dosage regimen against a clinical CRAB isolate. The secondary objective was to assess via MBM the translation from SCTK to the HFIM.

Dynamic hollow-fiber *in vitro* infection model. An HFIM (13, 14) was used to simulate the time course of antibiotic concentrations as expected in critically ill patients for the proposed dosage regimen. For imipenem, the median (13.4 mg/liter), 5th percentile (7.6 mg/liter), and 95th percentile (23.3 mg/liter) of the unbound concentrations at steady state during continuous infusion of 4 g/day were simulated. These concentrations were based on Monte Carlo simulations using published population PK models (9, 15, 16). We simulated the two-compartment behavior of tobramycin by changing the pump flow rate at 5 h each day. Our target inoculum of $10^{7.2}$ CFU/ml was chosen to mirror bacterial densities in severe infections in patients (17–19). Serial viable counts of the total and of resistant populations (on 1.75× and 3× MIC agar plates for imipenem and 3× and 5× MIC plates for tobramycin) were determined over 7 days, and drug concentrations were measured by liquid chromatography-tandem mass spectrometry. We developed MBM in S-ADAPT (20–22) and evaluated competing models as described previously (23, 24). Further methodological details are provided in the supplemental material.

Observed PK and viable count profiles. The observed tobramycin concentration-time profiles adequately matched the targeted profiles following administration of 7 mg/kg tobramycin as 0.5-h infusions (see Fig. S1 in the supplemental material); unbound peak concentrations for tobramycin were 12.3 mg/liter at 1.2 h, and unbound trough concentrations were 1.37 mg/liter at 23 h. Constant imipenem concentrations of 7.6 (low, 5th percentile) and 13.4 (intermediate, median) mg/liter in monotherapy failed to achieve any killing against isolate FADDI-AB-034 (MIC, 32 mg/liter) at an inoculum of $10^{7.2}$ CFU/ml. Although 23.3 mg/liter (high, 95th percentile) resulted in 3.1 $\log_{10}$ of killing at 4 h, it was followed by extensive regrowth to ~10 $\log_{10}$ CFU/ml at 24 h (Fig. 1A). Tobramycin monotherapy (MIC, 2 mg/liter) yielded 3.4 $\log_{10}$ of killing at 8 h followed by extensive regrowth. The combinations of tobramycin with each of the three imipenem concentrations were synergistic, provided near-complete bacterial

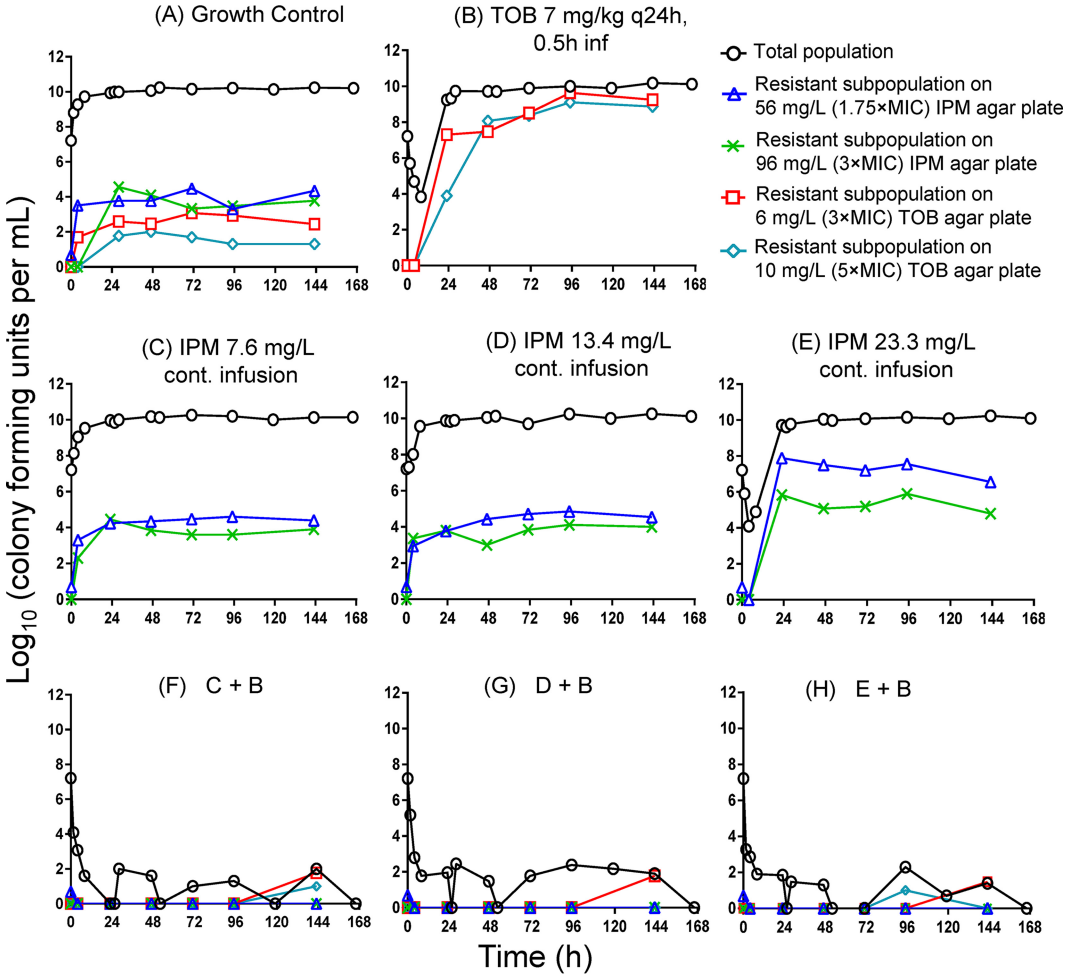

FIG 2 Effect of monotherapies (B to E) and combinations (F to H) on the total bacterial population and resistant populations (quantified on agar plates containing 1.75× or 3× the imipenem MIC and 3× or 5× the tobramycin MIC). While tobramycin monotherapy and imipenem monotherapy at 23.3 mg/liter led to extensive emergence of resistance, all combinations suppressed resistance over 3 days. All four types of antibiotic-containing agar plates were determined for the combinations; most counts were zero on these antibiotic-containing agar plates (F to H).

killing (>5 $\log_{10}$), and prevented regrowth (i.e., total viable counts remained at <2 $\log_{10}$) over 7 days (Fig. 1B).

Combinations but not monotherapies suppressed resistance. All three combinations of imipenem with tobramycin suppressed resistance over 7 days in the HFIM; only one or two colonies were observed on tobramycin-containing agar plates at 95 and 143 h (Fig. 2F, G, and H). In contrast, tobramycin monotherapy and the high-concentration (23.3-mg/liter) imipenem monotherapy failed, with substantial regrowth and amplification of resistance (Fig. 2B and E). For tobramycin monotherapy, the tobramycin-resistant population at 3× and 5× MIC almost completely replaced the susceptible population by ~47 h (see Table S1 in the supplemental material and Fig. 2B). Imipenem monotherapy at 23.3 mg/liter created sufficient drug pressure to increase the frequency of the highly resistant subpopulation by 2 to 4 $\log_{10}$ by 23 h (Table S1; Fig. 2E).

Mechanism-based modeling. MBM simultaneously described all treatments and the growth control (see Fig. S2 in the supplemental material). The coefficient of correlation for the observed versus individual (population) fitted $\log_{10}$ viable counts was 0.995 (0.968). Synergy due to imipenem killing the tobramycin-resistant population and vice versa (i.e., subpopulation synergy) was not sufficient to characterize the time course of bacterial killing and regrowth for the combinations. Inclusion of mechanistic

TABLE 1 Population mean parameter estimates for the imipenem-plus-tobramycin combination model against isolate FADDI-AB034

Parameter[a]	Symbol (unit)	Population mean value (relative SE, SE %)
Initial inoculum (log_{10} CFU/ml)	log_{CFU0}	7.29 (2.4)
Maximum population size (log_{10} CFU/ml)	$logCFU_{max}$	10.1 (1.1)
Replication rate constant (h^{-1})	k_{21}	50 (fixed)
Mean generation time (min)		
IPM^S/TOB^S	$k_{12,SS}^{-1}$	33.7 (9.9)[c]
IPM^R/TOB^I	$k_{12,RI}^{-1}$	63.2 (11.0)
IPM^I/TOB^R	$k_{12,IR}^{-1}$	33.7 (9.9)[c]
Log_{10} mutation frequencies at 0 h		
IPM	$log_{MUT,IPM}$[b]	−5.59 (4.9)
TOB	$log_{MUT,TOB}$	−3.22 (7.8)
Killing by IPM		
Maximum killing rate constant (h^{-1})	$K_{max,IPM}$	1.74 (19.0)
Imipenem concn causing 50% of $K_{max,IPM}$ (mg/liter)		
IPM^S/TOB^S	$KC_{50,SS,IPM}$	0.175 (29.4)
IPM^R/TOB^I	$KC_{50,RI,IPM}$	645 (17.8)
IPM^I/TOB^R	$KC_{50,IR,IPM}$	Monotherapy, 112 (17.9); combination (TOB ≥1.15 mg/liter), 1.60 (41.2)
Hill coefficient for imipenem	$HILL_{IPM}$	3.0 (fixed)[d]
Killing by TOB		
Maximum killing rate constant (h^{-1})		
IPM^S/TOB^S	$K_{max,TOB,SS}$	4.69 (14.7)[e]
IPM^R/TOB^I	$K_{max,TOB,RI}$	0.992 (9.5)
IPM^I/TOB^R	$K_{max,TOB,IR}$	4.69 (14.7)[e]
Tobramycin concn causing 50% of $K_{max,TOB}$ (mg/liter)		
IPM^S/TOB^S	$KC_{50,SS,TOB}$	0.156 (49.4)
IPM^R/TOB^I	$KC_{50,RI,TOB}$	0.316 (25.6)
IPM^I/TOB^R	$KC_{50,IR,TOB}$	27.7 (10.7)
Tobramycin concn required for mechanistic synergy (mg/liter)	TOB_{cut}	1.15 (20.5)
SD of residual error on log_{10} scale	SD_{CFU}	0.304 (10.7)

[a]IPM, imipenem; TOB, tobramycin; S, susceptible; R, resistant; I, intermediate.
[b]MUT, mutant.
[c]Mean generation time (transition from state 1 to state 2) was assumed to be the same for the SS and IR populations. Estimation as separate values yielded no benefit.
[d]It was beneficial to include a Hill coefficient for imipenem. Following a sensitivity analysis involving different Hill coefficient values, the Hill coefficient was fixed at 3.0.
[e]The maximum rate of bacterial killing by tobramycin ($K_{max,TOB}$) was assumed to be the same for the SS and IR population. Estimation as separate values yielded no benefit.

synergy with tobramycin enhancing the target site concentration of imipenem was greatly beneficial ($P < 0.0001$, likelihood-ratio test) (Fig. S2). Tobramycin was modeled to permeabilize the outer bacterial membrane toward imipenem against the imipenem-intermediate and tobramycin-resistant population (i.e., population 3 in Fig. S4 in the supplemental material). Mechanistic synergy was expressed as a 70-fold decrease ($P < 0.0001$) of the imipenem concentration resulting in half-maximal killing of population 3 ($KC_{50,IR,IPM}$) in the presence of at least 1.15 mg/liter (estimated) tobramycin (Table 1); the $KC_{50,IR,IPM}$ was 112 mg/liter in the absence of and 1.60 mg/liter in the presence of at least 1.15 mg/liter tobramycin (Table 1). For populations 1 and 2 in Fig. S4, mechanistic synergy was estimated to be small or absent.

Validation of novel combination dosing strategies. This study is the first to demonstrate that a rationally optimized imipenem-plus-tobramycin combination dosage regimen can achieve extensive (>5 log_{10}) synergistic bacterial killing and suppress resistance of a CRAB isolate in the HFIM over 7 days. When combined with tobramycin, synergy was achieved by imipenem concentrations as low as 7.6 mg/liter (equivalent to 24% of the MIC of 32 mg/liter) in the dynamic *in vitro* HFIM system. This was in complete absence of an immune system. We further showed that translational predictions from MBM Monte Carlo simulations based on SCTK data were successfully validated in the HFIM (9). This MBM was subsequently refined to characterize the HFIM data.

Both tobramycin and the highest-studied imipenem concentration (23.3 mg/liter, 95th percentile) in monotherapy achieved slightly more than 3 $\log_{10}$ bacterial killing; however, this was followed by rapid and extensive emergence of high-level resistance in our CRAB isolate (Fig. 2). The proposed optimized combination dosage regimen achieved rapid and extensive bacterial killing with near-complete suppression of resistance (Fig. 2).

This combination regimen proved robust when we used the HFIM to simulate the median, 5th, and 95th percentiles of concentrations expected to occur in critically ill patients; the Monte Carlo simulations were performed for a continuous infusion of imipenem 4 g/day with a 1-g loading dose. In combination with tobramycin 7 mg/kg q24h, all three imipenem concentration-time profiles yielded synergistic killing and resistance suppression over 7 days even for patients with the 5th percentile of the imipenem (7.6 mg/liter) concentration.

For tobramycin 7 mg/kg q24h (as a 0.5-h infusion) against this CRAB isolate, the area under the unbound concentration-time curve over 24 h divided by the MIC (*f*AUC/MIC) was 51.5, and the free maximum unbound concentration over MIC (fC_{max}/MIC) was 8.9. Note that the unbound imipenem concentrations were below the MIC during the entire therapy (i.e., $fT_{>MIC}$, 0%), even for the 95th percentile of imipenem concentrations arising from an imipenem 4-g continuous infusion with a 1-g loading dose. For this rationally optimized dosage regimen, the imipenem concentrations were 24% (7.6 mg/liter), 42% (13.4 mg/liter), and 73% (23.3 mg/liter) of the MIC (32 mg/liter); these concentrations achieved enhanced killing and resistance suppression in combination with tobramycin against this CRAB isolate.

In the past, low-inoculum checkerboard and SCTK studies showed synergy for imipenem plus tobramycin against *A. baumannii* isolates (25, 26); however, no study assessed this combination in the HFIM. Cefepime plus amikacin was studied in HFIM and murine models against CRAB (10, 12), but extensive regrowth occurred in the HFIM. Empirical, nonoptimized imipenem-plus-aminoglycoside combinations were studied at a single time point (i.e., 24 or 48 h) against CRAB in murine and guinea pig pneumonia models (11, 27, 28). The present prospective HFIM validation study evaluated bacterial killing, regrowth, and resistance in a CRAB isolate over 7 days.

A limitation of this study was the use of a single CRAB isolate that was assessed by translational MBM. We only studied one replicate of each dosage regimen, although the results were robust and consistent for our combinations, which used three imipenem concentrations. Our observed tobramycin concentrations fell within the range of concentrations found in critically ill patients (15). While it is a limitation of this study that imipenem concentrations were not measured, we precisely achieved the targeted imipenem concentrations after continuous infusions in previous HFIM studies. Tobramycin monotherapy failed, with rapid and extensive resistance at the studied high inoculum (Fig. 2B). Although we found extensive synergy against a tobramycin-susceptible CRAB isolate, synergy may be less pronounced or absent in tobramycin-resistant CRAB isolates. However, we previously showed in SCTK and a mouse thigh infection model that rationally optimized imipenem-plus-tobramycin combinations yielded extensive synergistic killing without resistance emergence in a *Pseudomonas aeruginosa* isolate resistant to both imipenem (MIC, 16 mg/liter) and tobramycin (MIC, 32 mg/liter) (29, 30).

Moreover, the present study was not designed to identify the PK/pharmacodynamic index for tobramycin that best predicts outer membrane permeabilization. Future studies are required to investigate this question. The synergy mechanism proposed by our MBM is in agreement with electron micrographs of ultrastructural damage and loss of cytosolic green fluorescent protein from a *P. aeruginosa* strain (31); tobramycin 0.25 mg/liter disrupted the outer membrane of this *P. aeruginosa* strain, which was in the range of the estimate (1.15 mg/liter) in the present study.

We employed the latest MBM to describe the antibacterial effects of the imipenem and tobramycin concentration-time profiles in monotherapy and combination; the final model contained three preexisting bacterial populations of different susceptibility (Fig. S4; Table 1). Both subpopulation synergy and mechanistic synergy (i.e., tobramycin

enhancing the outer membrane penetration of imipenem) were needed to adequately describe the HFIM data (32).

The outer membrane of *A. baumannii* and *P. aeruginosa* isolates presents a formidable penetration barrier (33–36), and its disruption may enhance the target site penetration of imipenem (9, 37–39). Mechanistic synergy in our model is supported by studies that demonstrated disruption of the outer membrane of *P. aeruginosa* by albumin-conjugated aminoglycosides (40, 41) and the outer membrane permeabilizing effect of aminoglycoside hybrid antibiotics (42, 43).

In our previous SCTK studies, imipenem 8 mg/liter achieved synergistic killing and resistance suppression in combination with tobramycin against the studied CRAB isolate, as observed in the HFIM (Fig. 2F) (9). Imipenem 4 g/day continuous infusion was predicted to achieve an unbound steady-state concentration of at least 7.6 mg/liter in 95% of Monte Carlo-simulated critically ill patients (9). For patients with low or intermediate imipenem clearance, slightly lower doses should be sufficient to achieve the desired imipenem exposure, especially if therapeutic drug management is employed (44, 45).

In summary, the proposed rationally optimized imipenem-plus-tobramycin combination dosage regimen demonstrated synergistic killing and suppressed resistance at clinically relevant exposure profiles of both antibiotics. This is the first study to report the prospective evaluation of an optimized combination regimen against a CRAB isolate in the HFIM over 7 days. Synergy was explained by tobramycin disrupting and thereby permeabilizing the outer membrane toward imipenem. Future animal infection models and ultimately clinical studies are warranted to evaluate this highly promising combination regimen, which was rationally optimized by translational mechanism-based modeling.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02053-17.

SUPPLEMENTAL FILE 1, PDF file, 0.5 MB.

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We have no conflicts of interest to declare.

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