Novel Approach To Optimize Synergistic Carbapenem-Aminoglycoside Combinations against Carbapenem-Resistant Acinetobacter baumannii

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Acinetobacter baumannii is among the most dangerous pathogens and emergence of resistance is highly problematic. Our objective was to identify and rationally optimize β-lactam-plus-aminoglycoside combinations via novel mechanism-based modeling that synergistically kill and prevent resistance of carbapenem-resistant A. baumannii. We studied combinations of 10 β-lactams and three aminoglycosides against four A. baumannii strains, including two imipenem-intermediate (MIC, 4 mg/liter) and one imipenem-resistant (MIC, 32 mg/liter) clinical isolate, using high-inoculum static-concentration time-kill studies. We present the first application of mechanism-based modeling for killing and resistance of A. baumannii using Monte Carlo simulations of human pharmacokinetics to rationally optimize combination dosage regimens for immunocompromised, critically ill patients. All monotherapies achieved limited killing (≤2.3 log10) of A. baumannii ATCC 19606 followed by extensive regrowth for aminoglycosides. Against this strain, imipenem-plus-aminoglycoside combinations yielded more rapid and extensive killing than other β-lactam-plus-aminoglycoside combinations. Imipenem at 8 mg/liter combined with an aminoglycoside yielded synergistic killing (>5 log10) and prevented regrowth of all four strains. Modeling demonstrated that imipenem likely killed the aminoglycoside-resistant population and vice versa and that aminoglycosides enhanced the target site penetration of imipenem. Against carbapenem-resistant A. baumannii (MIC, 32 mg/liter), optimized combination regimens (imipenem at 4 g/day as a continuous infusion plus tobramycin at 7 mg/kg of body weight every 24 h) were predicted to achieve >5 log10 killing without regrowth in 98.2% of patients. Bacterial killing and suppression of regrowth were best achieved for combination regimens with unbound imipenem steady-state concentrations of at least 8 mg/liter. Imipenem-plus-aminoglycoside combination regimens are highly promising and warrant further evaluation.

Antimicrobial resistance in Gram-negative bacteria is one of the three greatest threats to human health (1–3). Acinetobacter baumannii is one of the three most challenging Gram-negative pathogens, especially in intensive care units. In approximately 14,000 critically ill patients, A. baumannii infections were highly associated (P < 0.001) with increased mortality (4). A. baumannii often causes bloodstream, respiratory tract (including ventilator-associated pneumonia), and wound infections (including burns and combat wounds); these are associated with high morbidity (1, 2, 5) and up to 87% mortality (6). Multidrug-resistant A. baumannii strains have caused major outbreaks in the United States and worldwide (7, 8).

In the past, β-lactams and aminoglycosides were successfully used to treat susceptible A. baumannii (9), but unfortunately, strains have emerged that are resistant to virtually all antibiotics in monotherapy (10, 11). While carbapenems were hitherto considered the treatment of choice against severe A. baumannii infections, carbapenem-resistant A. baumannii isolates are rapidly increasing (11). Aminoglycoside monotherapy can cause significant killing of A. baumannii but is followed by rapid and extensive resistance emergence in vitro and in patients (12–14). The high rates of A. baumannii resistance highlight the urgent need for alternative treatment options, such as rationally optimized combination therapies.

A small number of in vitro and animal infection model studies assessed β-lactam-plus-aminoglycoside combinations and usually studied only one β-lactam and/or one aminoglycoside (12, 15, 16). We are aware neither of a systematic evaluation of monotherapies and combinations for a series of β-lactams and aminoglycosides in A. baumannii nor of any study which used time course modeling to optimize monotherapies or combinations against A. baumannii.

β-Lactam antibiotics are widely used and very safe, and clinicians worldwide are well trained in the safe use of aminoglycosides (17). Aminoglycoside and β-lactam antibiotics have different mechanisms of action and resistance; there is no efflux pump which affects both of these antibiotic classes in A. baumannii (18). This suggests that β-lactams may kill aminoglycoside-resistant bacteria and vice versa (subpopulation synergy [19, 20]). Additionally, disruption of the outer membrane by an aminoglycoside may enhance the target site penetration of β-lactams, since the outer membrane of A. baumannii is approximately 2- to 7-fold thicker than that of other Gram-negative species.
less permeable than that of Pseudomonas aeruginosa and approximately 50-fold less permeable than that of Escherichia coli (21, 22).

The first objective of this study was to identify synergistic bacterial killing and prevention of resistance for combinations of a β-lactam with an aminoglycoside against A. baumannii. The second objective was to quantify the extent, time course, and potential mechanisms of synergy via novel mechanism-based modeling of antibiotic combinations. Our final objective was to rationally optimize combination dosage regimens for immunocompromised, critically ill patients with bacteremia caused by carbapenem-resistant A. baumannii. These Monte Carlo simulations utilized novel mechanism-based models for bacterial killing and resistance and human population pharmacokinetic models to prospectively optimize combination dosage regimens for future studies in animal infection models and ultimately humans. (Part of this work was presented as an oral presentation [23] at the 53rd Interscience Conference on Antimicrob Agents and Chemotherapy, Denver, CO, 10 to 13 September 2013; as a poster presentation at the 2014 Annual Scientific Meeting of the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists, Melbourne, Australia, 7 to 11 December 2014; and as an oral presentation at the 17th annual meeting of the Population Approach Group in Australia and New Zealand, Melbourne, Australia, 28 to 30 January 2015.)

MATERIALS AND METHODS

Bacterial isolates, media, and susceptibility testing. The A. baumannii strain ATCC 19606 and three clinical A. baumannii isolates (FADDI-AB014, FADDI-AB016, and FADDI-AB034) from the collection at Monash University were used for all experiments. All susceptibility testing and static-concentration time-kill experiments (SCTK) were performed in cation-adjusted Mueller-Hinton broth (CAMHB; BBL, BD, Sparks, MD). Viable counting was conducted on cation-adjusted Mueller-Hinton II agar (CAMHA; Medium Preparation Unit, The University of Melbourne). Stock solutions of imipenem (Merck Sharp & Dohme Pty, New South Wales, Australia), isepamicin (Waterstone Technology, LLC, Carmel, IN), amikacin (Sigma-Aldrich, St. Louis, MO), and tobramycin (AK Scientific, Inc., Union City, CA) were prepared in sterile distilled water and filter sterilized with a Millex-GV 0.22-μm polyvinylidene difluoride (PVDF) syringe filter (Merck Millipore Ltd., Cork, Ireland). The MICs were determined in triplicate according to the Clinical and Laboratory Standards Institute guidelines (24), and EUCAST breakpoints were used to define carbapenem resistance (25).

Static-concentration time-kill experiments. All A. baumannii strains were evaluated via in vitro SCTK experiments over 2 days. We studied monotherapy and combination therapies of 10 β-lactams and three aminoglycosides in A. baumannii ATCC 19606. Subsequent SCTK experiments assessed imipenem plus two aminoglycosides (tobramycin and isepamicin) in three imipenem-intermediate or imipenem-resistant clinical isolates. The antibiotic concentrations studied included the highest clinically achievable average unbound plasma concentrations at steady state. A tobramycin concentration of 4 mg/liter and isepamicin concentrations of 8 to 16 mg/liter represent the average unbound plasma concentrations in humans over a 24-h dosing interval for tobramycin at 7 mg/kg of body weight and 15 or 25 mg/kg isepamicin given once daily (17, 26). Additional experimental arms with 12 mg/liter tobramycin and 64 mg/liter isepamicin represent the average free plasma concentration of aminoglycosides during the first 6 h. For the highest clinically approved imipenem doses of 4 g per day, our Monte Carlo simulations predicted the unbound average steady-state concentrations to range from 7.61 to 22.6 mg/liter in adult critically ill patients (27).

The SCTK experiments were performed at a high initial inoculum using previously described methods (20, 28, 29). Serial broth samples were taken before dosing and at multiple time points over 2 days. At 24 h, the bacterial suspension was centrifuged, supernatant was removed, and bacteria were resuspended in fresh, prewarmed broth with the targeted antibiotic concentration. To further offset the thermal degradation of carbapenems, a carbapenem amount of 50% of the original dose was supplemented at 6 and 30 h. Our liquid chromatography-tandem mass spectrometry (LC-MS/MS) data (30) showed approximately 54% degradation of imipenem at 1 and 100 mg/liter in CAMHB after 24 h of incubation at 35°C. This supplementation procedure ensured that the targeted antibiotic concentration was achieved every 24 h and that the average carbapenem concentration was maintained throughout the experiment. Bacteria in all broth samples were washed twice by centrifugation and resuspension in fresh sterile saline. Viable counts were determined by manual plating of 100 μl of an undiluted or appropriately diluted suspension in saline onto CAMHA plates (20, 28, 29).

Mechanism-based modeling of antibiotic combinations. To characterize the extent, time course, and potential mechanisms of synergy for a β-lactam plus an aminoglycoside, mechanism-based pharmacodynamic (PD) modeling was performed. These mechanism-based models of bacterial killing and resistance are ideally suited to be used with human population pharmacokinetic models to predict and rationally optimize combination dosage regimens in humans.

Life cycle growth model. A life cycle growth model (Fig. 1A) was used to describe the underlying biology of bacterial replication (31, 32). Bacterial replication for each population was defined via two states. Bacteria which are growing and preparing for replication reside in state 1, and bacteria which are immediately before replication reside in state 2. The transition from state 1 to state 2 was governed by a first-order growth rate constant (k1) which determines the mean generation time (MGT), since replication was assumed to be fast (k1 was fixed at 50 h−1) (31). We considered models with a longer mean generation time for the resistant populations.

Antibiotic combinations. The model for the combinations of imipenem and an aminoglycoside contained three pre-existing populations; including a double-susceptible (SS), an imipenem-resistant aminoglycoside-intermediate (RI), and an imipenem-intermediate aminoglycoside-resistant (IR) bacterial population (Fig. 1B). As each population was described by two states (i.e., compartments) in the life cycle growth models, the full model contained six compartments. The total concentration of all viable bacteria (CFUSS) was described as follows:

\[
\text{CFU}_{\text{SS}} = \text{CFU}_{\text{SS1}} + \text{CFU}_{\text{SS2}} + \text{CFU}_{\text{RI1}} + \text{CFU}_{\text{RI2}} + \text{CFU}_{\text{IR1}} + \text{CFU}_{\text{IR2}}
\]

(1)

Each CFUSS term represents the concentration of viable bacteria for population NN in state x. The rate of bacterial growth was assumed to be inhibited at high concentrations of hypothetical signal molecules (CSig) as described by the term \(\text{Inh}_{\text{SS1}}\) (31).

\[
\text{Inh}_{\text{SS1}} = \left( \frac{I_{\text{max,SS1}} \cdot C_{\text{Sig}}}{C_{\text{Sig}} + I_{\text{comp,SS1}}} \right)
\]

(2)

\(I_{\text{max,SS1}}\) is the maximum extent of inhibition of the rate of replication at high \(C_{\text{Sig}}\) and \(I_{\text{comp,SS1}}\) is the signal molecule concentration associated with 50% of \(I_{\text{max,SS1}}\).

The maximum rate of killing by imipenem or an aminoglycoside in the present data set was higher than the maximum rate of growth. Therefore, we specified killing by either antibiotic via a direct killing process described by a Hill function. The differential equation for the concentration of bacteria belonging to the double-susceptible population in state 1 (CFUSS1) comprised killing by imipenem and the aminoglycoside (initial conditions, model parameters, and variables described below):
where $C_{IPM}$ is the imipenem concentration and $C_{AGS}$ the aminoglycoside concentration (i.e., tobramycin, isepamicin, or amikacin) in broth.

2. This yields the differential equation for state 2 of the double-susceptible population ($CFU_{SS2}$):

$$\frac{d(CFU_{SS2})}{dt} = -k_{21} \cdot CFU_{SS2} - k_{12SS} \cdot (1 - \text{Inh}_{12}) \cdot CFU_{SS1} - \left( \frac{K_{max,IPM} \cdot C_{IPM}}{C_{IPM} + K_{SO,IPM} \cdot C_{IPM} + K_{SS,IPM} \cdot C_{IPM}} \right) \cdot CFU_{SS1} - \left( \frac{K_{max,SS,AGS} \cdot C_{AGS}}{C_{AGS} + K_{SO,AGS} \cdot C_{AGS} + K_{SS,AGS} \cdot C_{AGS}} \right) \cdot CFU_{SS1} (3)$$

The differential equations for the two resistant populations (i.e., IR and RI) contained the same terms as those for $CFU_{SS1}$ and $CFU_{SS2}$ but contained different estimates for $K_{max}$, $K_{C50}$, and $k_{12}$ compared to the double-susceptible population.

**Mechanism-based modeling of synergy.** We considered and evaluated subpopulation synergy (i.e., antibiotic A killing the bacteria resistant to antibiotic B and vice versa) and mechanistic synergy (i.e., antibiotic A enhancing the killing by antibiotic B of one or multiple bacterial populations) as previously described (20). Mechanistic synergy was implemented by assuming that the aminoglycoside could enhance the target site penetration of imipenem due to disruption of the outer membrane (33, 34). This was implemented in the model by estimating a lower $K_{SO,IPM}$ in

![FIG 1](A) Life cycle growth model utilized for each of the three populations to describe bacterial replication. (B) Subpopulation model with three bacterial populations that was applied for each of the four studied *A. baumannii* strains. The IPM/AGS population is susceptible to imipenem and the aminoglycoside, the IPM'/AGS population is imipenem resistant and has intermediate susceptibility to the aminoglycoside, and the IPM'/AGS' population is aminoglycoside resistant and has intermediate susceptibility to imipenem. The maximum killing rate constants ($K_{max}$) and the antibiotic concentrations ($K_{C50}$) causing 50% of $K_{max}$ are explained in Table 3. Subpopulation synergy (i.e., imipenem killing the aminoglycoside-resistant population and vice versa) was present for all strains. Mechanistic synergy (i.e., the aminoglycoside enhancing the target site penetration of imipenem) was additionally present for strains ATCC 19606 and FADDI-AB034.

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the presence of a certain aminoglycoside concentration (e.g., ≥4 mg/liter tobramycin). This was implemented in the model code via an IF condition which used the KC_{90/IPM} for combination therapy if the aminoglycoside concentration was at least 4 mg/liter and the KC_{50/IPM} for monotherapy for lower aminoglycoside concentrations.

Initial conditions. We estimated the total inoculum (log CFU) and the log_{10} mutation frequency for each of the two resistant populations. The initial condition of the double-susceptible population was calculated as the difference between CFU and the initial conditions of the two less-susceptible populations. All bacteria were initialized in state 1, and the initial conditions for CFU_{S2}, CFU_{R2}, and CFU_{R2} were set at 0 (31).

Observation model. The log_{10} viable counts were fitted using an additive residual error model on a log_{10} scale. For observations below 100 CFU/ml (equivalent to fewer than 10 colonies per plate), the number of colonies per plate was directly fitted using a previously developed residual error model (28). Viable counts below the limit of counting (i.e., below 1 log_{10} CFU/ml) and model-predicted viable counts less than 0 log_{10} CFU/ml were plotted as 0.

Estimation. All PD model parameters were simultaneously estimated using all viable-count data from the respective strain via the importance sampling algorithm (method = 4) in parallelized S-ADAPT (version 1.57). The analysis was facilitated by the SADAPT-TRAN tool (35, 36). The between-curve variability of the PD parameters was fixed to a coefficient of variation of 10% during the end of the estimation (28). Competing models were assessed by the objective function (−1×log likelihood), plausibility of the parameter estimates, standard diagnostic plots, and visual predictive checks (37, 38).

Monte Carlo simulations. We simulated 10,000 adult critically ill patients with normal renal function for each dosage regimen via Monte Carlo simulations using Berkeley Madonna (version 8.3.18). These patients completely lacked any effect of the immune system and were assumed to have bacteremia caused by the most difficult-to-treat, carbapenem-resistant isolate, FADDI-AB034. This isolate had an imipenem MIC of 32 mg/liter, which represents the 97th percentile of the MIC distribution for resistant isolate, FADDI-AB034. This isolate had an imipenem MIC of 32 mg/liter. Against ATCC 19606 at an initial inoculum of 10^7.0 CFU/ml, 8 mg/liter imipenem was the most active monotherapy with 2.3 log_{10} killing at 50 h. Meropenem achieved 2 log_{10} killing, but initial killing was slower than that by imipenem (Table 1). Killing by all noncarbapenem β-lactams was substantially slower than killing by carbapenems. Cefepime, aztreonam, and cefusulodin monotherapy achieved less than 0.8 log_{10} killing, whereas 32 mg/liter ceftriaxime or ceftazidime yielded 1.6 to 2.0 log_{10} killing at 24 and 50 h. Initial killing by tobramycin or amikacin (<1.4 log_{10}) was followed by extensive (re)growth (Table 1).

Imipenem at 8 mg/liter plus tobramycin or amikacin provided the most extensive killing (Table 1) and achieved the highest extent of synergy. Killing by imipenem at 8 mg/liter plus tobramycin was more rapid than that of imipenem plus amikacin. All combinations of a noncarbapenem β-lactam plus an aminoglycoside yielded less than 1.8 log_{10} killing at 24 and 50 h with exception of the cefepime plus amikacin combination at 50 h (Table 1). Meropenem (4 mg/liter)-plus-aminoglycoside combinations achieved less killing than imipenem at 8 mg/liter plus an aminoglycoside (Table 1). Against ATCC 19606 at an initial inoculum of 10^7.0 CFU/ml, imipenem and biapenem monotherapy at 8 mg/liter achieved approximately 1 to 1.5 log_{10} more killing than doripenem and meropenem at 8 mg/liter (Table 2). Imipenem plus tobramycin was the only combination that yielded eradication (confirmed by a sterility check at 50 h; i.e., plating of the entire broth suspension). Thus, subsequent studies assessed imipenem plus aminoglycoside combinations against carbapenem-intermediate and –resistant clinical isolates.

Monotherapy and combinations against clinical isolates. Against high inocula (range, 10^6.6 to 10^6.9 CFU/ml) of the clinical isolates, imipenem at 8, 16, or 32 mg/liter in monotherapy was required to achieve >0.5 log_{10} killing at any time and to prevent regrowth (Fig. 2). Isepamicin at 8 to 16 mg/liter and tobramycin at 4 mg/liter in monotherapy achieved up to 3 log_{10} killing at 5 h, followed by rapid and extensive regrowth by 24 h. Isepamicin at 64 mg/liter or tobramycin at 12 mg/liter in monotherapy was required to prevent regrowth of aminoglycoside-resistant bacteria. Against the four tested strains, clinically achievable concentrations of 2 to 8 mg/liter imipenem combined with 8 to 16 mg/liter isepamicin, 4 mg/liter tobramycin, or 4 mg/liter amikacin yielded at least 5 log_{10} killing. Importantly, these combinations effectively prevented regrowth over 48 h.

Mechanism-based modeling. Our novel mechanism-based model required a susceptible population, an imipenem-resistant population, and an aminoglycoside-resistant population (Fig. 1B).
The imipenem-resistant population displayed stasis or was only slowly killed by imipenem monotherapy, and the aminoglycoside-resistant population caused regrowth during aminoglycoside monotherapy (results not shown).

Subpopulation synergy. Against all four strains, imipenem killed the aminoglycoside-resistant population and aminoglycosides killed the imipenem-resistant population (i.e., subpopulation synergy was present). The imipenem-resistant population
slowly replicated and required high imipenem concentrations to be killed. The imipenem concentration resulting in half-maximal killing was considerably higher for the imipenem-resistant population (KC50,RI,IPM) than the susceptible population (KC50,SS,IPM) (Table 3). Also, higher aminoglycoside concentrations resulting in half-maximal killing by the aminoglycoside were required for the imipenem-resistant population (KC50,RI,ISE/TOB/AMK) than for the susceptible population (KC50,SS,ISE/TOB/AMK) (Table 3). The aminoglycoside-resistant populations of isolates FADDI-AB016 and FADDI-AB014 were less susceptible to imipenem, as shown by the higher imipenem concentration yielding half-maximal killing of the aminoglycoside-resistant population (KC50,IR,IPM) (Table 3) compared to the susceptible population (KC50,SS,IPM). For these two isolates, subpopulation synergy alone was not sufficient to describe the extensive and synergistic killing by the combinations. Population predictions for the treatment arms containing 4 mg/liter aminoglycoside were considerably better for models with mechanistic synergy than for models without this feature. A thorough modeling analysis identified tobramycin enhancing the...
target site penetration of imipenem as the most likely additional synergy mechanism. This was expressed as smaller KC\textsubscript{50,IR,IPM} in the presence of at least 4 mg/liter aminoglycoside (Fig. 1B). The imipenem concentration yielding half-maximal killing of the aminoglycoside-resistant population (KC\textsubscript{50,IR,IPM}) decreased approximately 4-fold in the presence of the absence of at least 4 mg/liter aminoglycoside (P < 0.0001 for FADDI-AB034 and P = 0.015 for ATCC 19606; likelihood ratio test) (Table 3). Additionally, the aminoglycoside (Fig. 1B) reduced the imipenem concentration, yielding half-maximal killing of the imipenem-resistant population (KC\textsubscript{50,IR,IPM}) from 16.5 to 13.2 mg/liter (P = 0.015) for strain ATCC 19606 (Table 3); this contributed considerably to killing of this population by imipenem. Models with subpopulation and mechanistic synergy yielded unbiased and precise curve fits for strains ATCC 19606 and FADDI-AB034 (Fig. 2 and 3).

Monte Carlo simulations. For 7 mg/kg tobramycin given as a 0.5-h infusion, the predicted median concentrations (5th to 95th percentiles) were 17.7 (12.8 to 23.8) mg/liter at 0.5 h, 15.0 (11.5 to 19.1) mg/liter at 1.0 h, and 1.43 (0.583 to 2.76) mg/liter at 24 h. A continuous infusion of 4 g/day imipenem yielded an unbound steady-state concentration of 13.4 (7.61 to 22.6) mg/liter (Fig. 4A). The imipenem concentration yielding half-maximal killing of the aminoglycoside-resistant population (KC\textsubscript{50,IR,IPM}) from 16.5 to 13.2 mg/liter (P = 0.015) for strain ATCC 19606 (Table 3); this contributed considerably to killing of this population by imipenem. Models with subpopulation and mechanistic synergy yielded unbiased and precise curve fits for strains ATCC 19606 and FADDI-AB034 (Fig. 2 and 3).

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**DISCUSSION**

The present study demonstrates that _A. baumannii_ is extremely difficult to kill by monotherapy with any β-lactam or aminoglycoside. In vitro time-kill studies showed that at the highest clinically relevant unbound steady-state concentration, all tested monotherapies achieved limited killing against strain ATCC 19606 at a high inoculum (Table 1). Imipenem was the most active monotherapy, whereas noncarbapenem β-lactams and aminoglycosides achieved much less killing, which was followed, for aminoglycosides, by extensive regrowth. This highlights the need to rationally optimize combinations.

Imipenem combinations provided the most extensive killing and were most synergistic in comparison to all other tested β-lactam–plus-aminoglycoside combinations against ATCC 19606 (Table 1 and 2). Therefore, imipenem-plus-aminoglycoside combinations were progressed to further studies with three clinical isolates. Imipenem plus an aminoglycoside at clinically relevant

| Table 2 Log\textsubscript{10} change in viable counts compared to 0-h counts for _A. baumannii_ ATCC 19606 in static-concentration time-kill studies comparing four carbapenems with or without tobramycin at an initial inoculum of 10\textsuperscript{6.6} CFU/ml\textsuperscript{a} |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|
| Treatment                        | 1.5 h          | 4.0 h          | 9.25 h         | 24 h           | 50 h           |
| Growth control                   | 0.23           | 0.83           | 1.26           | 1.89           | 1.53           |
| Tobramycin (4 mg/liter)          | -1.69          | -2.04          | -2.10          | 1.21           | 1.20           |
| Imipenem (8 mg/liter)            | -2.61          | -3.96          | -4.58          | -4.29          | -4.73          |
| Biapenem (8 mg/liter)            | -2.15          | -3.84          | -4.32          | -4.40          | -4.50          |
| Doripenem (8 mg/liter)           | -1.93          | -2.77          | -3.95          | -3.47          | -2.95          |
| Meropenem (8 mg/liter)           | -1.36          | -2.64          | -2.99          | -2.66          | -3.70          |
| Imipenem (8 mg/liter) + tobramycin (4 mg/liter) | -3.29          | -4.28          | ≤ -6.80\textsuperscript{a} | ≤ -6.80\textsuperscript{a} | -7.02\textsuperscript{a} |
| Biapenem (8 mg/liter) + tobramycin (4 mg/liter) | -3.10          | -3.92          | -5.32          | -4.42          | -5.57          |
| Doripenem (8 mg/liter) + tobramycin (4 mg/liter) | -2.81          | -3.78          | -5.42          | -5.01          | -4.75          |
| Meropenem (8 mg/liter) + tobramycin (4 mg/liter) | -2.68          | -3.33          | -4.19          | -2.80          | -4.75          |

\textsuperscript{a}To achieve a limit of counting of 0.22 log\textsubscript{10} CFU/ml (equivalent to one colony over 6 agar plates), in total 600 μl of this bacterial suspension was plated on six agar plates (100 μl per plate).

\textsuperscript{b}A sterility check was performed by plating the entire volume of the bacterial suspension at the end of the experiment. The sterility check demonstrated that all bacteria were killed (i.e., zero colonies observed after plating the entire bacterial suspension).

\textsuperscript{c}Green shading indicates > 2 log\textsubscript{10} more killing than that achieved with the most active monotherapy (i.e., values meet the empirical definition of synergy), blue shading indicates 1.0 to 2.0 log\textsubscript{10} more killing than the most active monotherapy, and purple shading indicates 0.5 to 1.0 log\textsubscript{10} more killing than the most active monotherapy.

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### TABLE 3 Population parameter estimates for imipenem plus aminoglycoside combination models against four *A. baumannii* strains

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol (unit)</th>
<th>Mean value (SE%) for strain with drugs&lt;sup&gt;a&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Log&lt;sub&gt;10&lt;/sub&gt; initial inoculum</td>
<td>Log&lt;sub&gt;10&lt;/sub&gt;IPM</td>
<td>ATCC 19606; IPM + AGS (TOB, ISE, and AMK)</td>
</tr>
<tr>
<td>Log&lt;sub&gt;10&lt;/sub&gt; maximum population size</td>
<td>CFU&lt;sub&gt;max&lt;/sub&gt;</td>
<td>FADDI-AB016; IPM + AGS (ISE)</td>
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<tr>
<td>Replication rate constant</td>
<td>k&lt;sub&gt;12.1&lt;/sub&gt; (h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>FADDI-AB014; IPM + AGS (ISE)</td>
</tr>
<tr>
<td>Mean generation time</td>
<td></td>
<td>FADDI-AB034; IPM + AGS (TOB)</td>
</tr>
<tr>
<td>IPM&lt;sup&gt;d&lt;/sup&gt; AGS&lt;sup&gt;e&lt;/sup&gt;</td>
<td>k&lt;sub&gt;12.1&lt;/sub&gt; (min)</td>
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</tr>
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<td>IPM&lt;sup&gt;d&lt;/sup&gt; AGS&lt;sup&gt;e&lt;/sup&gt;</td>
<td>k&lt;sub&gt;12.1&lt;/sub&gt; (min)</td>
<td>1,010 (4.0)</td>
</tr>
<tr>
<td>IPM&lt;sup&gt;d&lt;/sup&gt; AGS&lt;sup&gt;e&lt;/sup&gt;</td>
<td>k&lt;sub&gt;12.1&lt;/sub&gt; (min)</td>
<td>96.4 (11.2)</td>
</tr>
<tr>
<td>IPM&lt;sup&gt;d&lt;/sup&gt; AGS&lt;sup&gt;e&lt;/sup&gt;</td>
<td>k&lt;sub&gt;12.1&lt;/sub&gt; (min)</td>
<td>50 (fixed)</td>
</tr>
<tr>
<td>Mean mutation frequency</td>
<td></td>
<td>50 (fixed)</td>
</tr>
<tr>
<td>IPM</td>
<td>Log&lt;sub&gt;MUT&lt;/sub&gt;IPM</td>
<td>96.4 (11.2)</td>
</tr>
<tr>
<td>AGS</td>
<td>Log&lt;sub&gt;MUT&lt;/sub&gt;AGS</td>
<td>96.4 (11.2)</td>
</tr>
<tr>
<td>Killing by imipenem</td>
<td></td>
<td>50 (fixed)</td>
</tr>
<tr>
<td>Maximum killing rate constant</td>
<td>K&lt;sub&gt;max&lt;/sub&gt;IPM (h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>2.48 (12.4)</td>
</tr>
<tr>
<td>IPM&lt;sup&gt;d&lt;/sup&gt; AGS&lt;sup&gt;e&lt;/sup&gt;</td>
<td>K&lt;sub&gt;max&lt;/sub&gt;SS IPM (mg/liter)</td>
<td>5.42 (13.7)</td>
</tr>
<tr>
<td>IPM&lt;sup&gt;d&lt;/sup&gt; AGS&lt;sup&gt;e&lt;/sup&gt;</td>
<td>K&lt;sub&gt;max&lt;/sub&gt;RI IPM (mg/liter)</td>
<td>1.38 (19.8)</td>
</tr>
<tr>
<td>IPM&lt;sup&gt;d&lt;/sup&gt; AGS&lt;sup&gt;e&lt;/sup&gt;</td>
<td>K&lt;sub&gt;max&lt;/sub&gt;IR IPM (mg/liter)</td>
<td>5.42 (13.7)</td>
</tr>
<tr>
<td>Aminoglycoside concn causing 50% of K&lt;sub&gt;max&lt;/sub&gt;AGS</td>
<td></td>
<td>5.42 (13.7)</td>
</tr>
<tr>
<td>IPM&lt;sup&gt;d&lt;/sup&gt; AGS&lt;sup&gt;e&lt;/sup&gt;</td>
<td>K&lt;sub&gt;max&lt;/sub&gt;SS AGS (mg/liter)</td>
<td>22.1 (44.2)</td>
</tr>
<tr>
<td>IPM&lt;sup&gt;d&lt;/sup&gt; AGS&lt;sup&gt;e&lt;/sup&gt;</td>
<td>K&lt;sub&gt;max&lt;/sub&gt;RI AGS (mg/liter)</td>
<td>52.5 (6.8)</td>
</tr>
<tr>
<td>IPM&lt;sup&gt;d&lt;/sup&gt; AGS&lt;sup&gt;e&lt;/sup&gt;</td>
<td>K&lt;sub&gt;max&lt;/sub&gt;IR AGS (mg/liter)</td>
<td>147 (8.9)</td>
</tr>
<tr>
<td>Mean turnover time for hypothetical signal molecules</td>
<td>MTT (h)</td>
<td>0.942 (14.1)</td>
</tr>
<tr>
<td>Maximum inhibition by hypothetical signal molecules</td>
<td>I&lt;sub&gt;max&lt;/sub&gt;sig12</td>
<td>0.992 (9.64)</td>
</tr>
<tr>
<td>Log&lt;sub&gt;10&lt;/sub&gt; of hypothetical signal molecule concn at 50% of max effect</td>
<td>Log&lt;sub&gt;10&lt;/sub&gt;IC50&lt;sub&gt;sig&lt;/sub&gt;</td>
<td>7.61 (1.5)</td>
</tr>
<tr>
<td>Hill coefficient</td>
<td></td>
<td>7.61 (1.5)</td>
</tr>
<tr>
<td>IPM</td>
<td>Hill&lt;sub&gt;IPM&lt;/sub&gt;</td>
<td>5.0 (fixed)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>AGS</td>
<td>Hill&lt;sub&gt;AGS&lt;/sub&gt;</td>
<td>2.71 (12.6)</td>
</tr>
<tr>
<td>SD of residual error on log&lt;sub&gt;10&lt;/sub&gt; scale</td>
<td>SD&lt;sub&gt;CFU&lt;/sub&gt;</td>
<td>0.401 (7.3)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Initial inocula from two different experiments. All other model parameter estimates were assumed to be the same at both initial inocula.

<sup>b</sup> The final model for isolate FADDI-AB034 used the same parameter estimate for K<sub>50,SS,IPM</sub> and K<sub>50,RI,IPM</sub> with and without the aminoglycoside, since allowing for different parameter estimates yielded no improvement. The model benefitted significantly from using different estimates for K<sub>50,IR,IPM</sub> for monotherapy and combination therapies.

<sup>c</sup> We used only three imipenem concentrations in each of the static-concentration time-kill studies. Therefore, precise estimation of the Hill coefficients was difficult. It was, however, beneficial to include a Hill coefficient for imipenem. After evaluation of different Hill coefficient values, the Hill coefficients were fixed at the values shown here.

<sup>d</sup> SE%, relative standard error, in percent; mono, monotherapy; combo, combination therapy.
concentrations achieved extensive killing (>5 \log_{10}) and completely prevented regrowth against a high inoculum of three carbapenem-intermediate or carbapenem-resistant A. baumannii isolates (Fig. 2). Overall, imipenem combinations eradicated three of four strains. We did not quantify resistant bacteria on antibiotic-containing agar plates and did not characterize the potential emergence of a new resistance mechanism(s) during antibiotic exposure. However, eradication suggested that imipenem-plus-aminoglycoside combinations killed resistant bacteria and prevented emergence of resistance.

Our results considerably extend those from the relatively few published studies on \(\beta\)-lactam-plus-aminoglycoside combinations in A. baumannii. Checkerboard studies at low inocula showed promising results for these combinations (16, 39–41), and SCKT experiments showed extensive killing (12, 15, 42–45) but also revealed regrowth of A. baumannii by several \log_{10} for some combinations (12, 45). Cefepime plus amikacin was synergistic in SCKT experiments and yielded approximately 1 \log_{10} more killing than cefepime monotherapy but was followed by approximately 6 \log_{10} regrowth at 48 h in a hollow-fiber infection model (12). This combination was effective in a mouse pneumonia model (cefepime given every 8 h plus amikacin every 24 h; monotherapies not studied) (46). An empirical, nonoptimized combination regimen with imipenem plus tobramycin (both given every 6 h in mice) yielded 5.5 \log_{10} killing of two carbapenem-resistant A. baumannii isolates in a mouse pneumonia model (47). All but one of the published SCKT studies (15) assessed monotherapies and combinations for only one \(\beta\)-lactam or one aminoglycoside, and no study on A. baumannii applied time course modeling (12, 15, 42–47). We identified synergistic combinations of 10 clinically important \(\beta\)-lactams and three aminoglycosides and used novel mechanism-based modeling and Monte Carlo simulations to rationally optimize combination dosage regimens for future studies in animals and humans.

To quantify the extent, time course, and potential mechanisms of synergy, we developed new mechanism-based models for four A. baumannii strains. The proposed model structure and parameter estimates were consistent across all strains (Fig. 1B; Table 3). Each model contained a susceptible population, an imipenem-resistant population which was slowly replicating (resembling persisters), and an aminoglycoside-resistant population. The aminoglycoside-resistant population (Fig. 1B) was killed by imipenem, whereas aminoglycoside-related killing of the imipenem-resistant population was relatively slow (as shown by \(K_{\text{max,RL,AGS}}\)) (Table 3) potentially due to a lower rate of protein synthesis. Future studies (for example, using a green-fluorescent-protein-labeled A. baumannii strain) would be required to experimentally prove the presence of persisters. Models with subpopulation synergy (i.e., imipenem killing the aminoglycoside-resistant population and vice versa) excellently described the in vitro viable-count profiles for strains FADDI-AB014 and FADDI-AB016 (Fig. 2 and 3).

To describe the extensive synergistic killing of strains ATCC 19606 and FADDI-AB034 by imipenem-plus-aminoglycoside combinations, an additional synergy mechanism was required. Modeling suggested that the aminoglycoside most likely enhanced the target site concentrations of imipenem. This mechanism is in agreement with studies using an albumin-conjugated aminoglycoside that disrupts the outer membrane of P. aeruginosa (33, 34). The outer membrane presents a major penetration barrier, particularly in A. baumannii (21, 22), and its disruption likely yields higher imipenem concentrations at the periplasmic target site and thus increased killing by imipenem.

The presence of 4 mg/liter aminoglycoside was modeled to significantly enhance the target site penetration of imipenem for the imipenem-resistant and the aminoglycoside-resistant populations (Fig. 1B; Table 3). This is a plausible mechanism, since it is the extracellular aminoglycoside concentration that likely causes disruption of the outer membrane. Mechanism-based models with subpopulation and mechanistic synergy excellently described...
FIG 4 Simulated plasma concentrations and viable-count profiles of the imipenem-resistant (MIC, 32 mg/liter) isolate *A. baumannii* FADDI-AB034 for monotherapy and combination dosage regimens. These Monte Carlo simulations predicted bacteremia in critically ill patients with normal renal function who completely lacked any effect of the immune system. The success rate ($P_{\text{success}}$ defined as $\leq$6 log$_{10}$ CFU/ml at 168 h) is indicated for each simulated dosage regimen. All imipenem infusions were given over 1 h (except for the continuous infusion). Tobramycin was infused over 0.5 h. The initial inoculum was $10^6$ CFU/ml.
all viable-count profiles for strains ATCC 19606 and FADDI-AB034 (Fig. 2 and 3).

These novel mechanism-based models were used together with population pharmacokinetic models for critically ill patients (17, 26, 27) to predict the time course of bacterial counts for infections in humans. Our simulations are based on in vitro time-kill studies with static antibiotic concentrations. In a previous study on ceftazidime against P. aeruginosa (31), we developed a mechanism-based model using static concentration time-kill data and showed that this model successfully predicted the time course of viable counts in dynamic in vitro infection models from eight published studies (31). Additional Monte Carlo simulations (19) showed that this model successfully predicted the targets for cephalosporins for stasis to 1 log_{10} killing (40% time of unbound drug concentrations above MIC [fT>MIC]) and near-maximal bacterial killing at 24 h (60 to 70% fT>MIC) in mice (48) and the targets for successful therapy in patients (49). Our in vitro experiments and mechanism-based models do not include the effect of the immune system (50, 51) and thus mirror immunocompromised patients. Acknowledging the uncertainty arising from these potential limitations, mechanism-based Monte Carlo simulations can predict and rationally optimize bacterial killing and prevention of resistance for combination dosage regimens (19, 31, 38, 52).

Our Monte Carlo simulations were performed using the carbapenem-resistant clinical isolate FADDI-AB034 at a high inoculum. This strain had an imipenem MIC of 32 mg/liter, which is the 97th percentile of the MIC distribution for A. baumannii by EUCAST and thus represents a near-worst-case scenario. The target population was comprised of critically ill patients with normal renal function; these patients were assumed to completely lack any effect of the immune system and to have bacteremia.

As imipenem and tobramycin clearance had coefficients of variation of 34% and 31% and imipenem had a large variability (81%) for volume of distribution of the central compartment in critically ill patients (26, 27), Monte Carlo simulations predicted a large between-patient variability in the unbound plasma concentrations and viable counts (Fig. 4). While all monotherapies were predicted to fail in >95% of patients (Fig. 4B), the extensive synergy between imipenem and tobramycin was highly beneficial for all combination regimens. Combinations with 7 mg/kg tobramycin (Fig. 4D) displayed more rapid killing than combinations with 5 mg/kg tobramycin (Fig. 4C).

Combinations with short-term imipenem infusions of 1 g every 6 or 8 h were predicted to fail with extensive regrowth of resistant bacteria in 9.3% to 39.1% of patients. For continuous infusion of 3 g/day imipenem (with a 1-g loading dose) combined with 5 or 7 mg/kg tobramycin, 6.8% to 16.2% of patients were predicted to fail with extensive regrowth (Fig. 4C and D). Excitingly, imipenem at 4 g/day as a continuous infusion (with a 1-g loading dose) plus tobramycin at 7 mg/kg every 24 h was predicted to yield extensive killing (>5 log_{10}) without regrowth in 98.2% of patients (i.e., regrowth in only 1.8% of patients) (Fig. 4D). This highlights the importance of evaluating the robustness of combination dosage regimens via Monte Carlo simulations in the presence of the large between-patient variability in pharmacokinetics (17, 19, 53, 54).

While imipenem at 4 g/day clearly benefitted the predicted success rate of therapy, this dose may slightly increase the risk of seizures compared to 2 or 3 g imipenem per day (55, 56). To obtain extensive synergy with tobramycin and prevent resistance, an unbound imipenem concentration of approximately 8 mg/liter was needed against our carbapenem-resistant isolates. Therapeutic drug monitoring may be valuable to achieve unbound steady-state concentrations of at least 8 mg/liter imipenem using 2 or 3 g imipenem per day.

In summary, bacterial killing by any β-lactam or aminoglycoside in monotherapy was limited against a high inoculum of wild-type A. baumannii ATCC 19606. Among all tested combinations, imipenem plus an aminoglycoside provided the most extensive killing without regrowth against high inocula of susceptible, carbapenem-intermediate, and carbapenem-resistant strains. Mechanism-based modeling identified both subpopulation synergy and mechanistic synergy for imipenem–plus-aminoglycoside combinations. This study presents the first application of Monte Carlo simulations for A. baumannii that were used to rationally optimize combination dosage regimens based on human population pharmacokinetics. Monte Carlo simulations predicted a 98.2% success rate for clinically relevant imipenem–plus-tobramycin combination dosage regimens against a carbapenem-resistant clinical A. baumannii isolate with an MIC of 32 mg/liter. This strongly suggests the future evaluation of these highly promising combination dosage regimens.

ACKNOWLEDGMENTS

We thank Jessica Shan, Yen Mei Chuah, and Kate Rogers for excellent support on generating parts of the experimental data presented in this work.

This work was supported by the Australian Research Council (ARC; DECRA fellowship number DE120103084 to J.B.B.) and the Australian National Health and Medical Research Council (NHMRC; Career Development fellowship number 1062509 to C.B.L.). This research was supported in part by the Australian National Health and Medical Research Council (NHMRC; project grant 1045105 to J.B.B.).

We have no conflicts of interest to declare.

REFERENCES


