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EXPERIMENTAL THERAPEUTICS

Efficacy of Apramycin against Multidrug-Resistant *Acinetobacter baumannii* in the Murine Neutropenic Thigh Model

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ABSTRACT Apramycin, an aminocyclitol aminoglycoside, was rapidly bactericidal against *Acinetobacter baumannii*. In a neutropenic murine thigh infection model, treatment-associated *A. baumannii* CFU reductions of >4 $\log_{10}$ per thigh were observed for all exposures for which area under the curve (AUC)/MIC ratio was >50 and maximum concentration of drug in serum (C_{max})/MIC was ≈10 or higher. Based on these findings, we suggest that apramycin deserves further preclinical exploration as a repurposed therapeutic for multidrug-resistant Gram-negative pathogens, including *A. baumannii*.

KEYWORDS antimicrobial, apramycin, maximum tolerated dose, mouse thigh model, pharmacodynamics, pharmacokinetics, resistance, time-kill

There is a pressing need for new antimicrobials that target multidrug-resistant Gram-negative pathogens, including *Acinetobacter baumannii* (1). Apramycin is an aminocyclitol aminoglycoside used in veterinary medicine. It differs from 16S rRNA decoding A-site aminoglycosides approved for use in human therapy (e.g., gentamicin, tobramycin, amikacin) in several respects. First, at a molecular level, apramycin is believed to have only a minor effect on amino acid coding fidelity (2), yet it still demonstrates bactericidal activity for *Escherichia coli* (3). Second, apramycin appears to be neither ototoxic nor nephrotoxic (3–5), potentially based in part on greater selectivity for bacterial over mitochondrial ribosomes (3). Third, apramycin has a broad activity spectrum against multidrug-resistant human clinical isolates of *A. baumannii*, *Pseudomonas aeruginosa*, and carbapenem-resistant *Enterobacteriaceae* (6, 7). For multidrug- and extensively drug-resistant *A. baumannii* in particular, the apramycin MIC_{50}/MIC_{90} (8/32 μg ml^{-1}) was notably lower than that for gentamicin, tobramycin, and amikacin (≥64/>256 μg ml^{-1}). Remarkably, only 2% of apramycin MICs for this highly resistant *A. baumannii* strain set were above the epidemiological cutoff value of 64 μg ml^{-1} (6).

Interestingly, apramycin, in contrast to other aminoglycosides, including plazomicin, retains activity in the presence of *armA* and *rmtA-H* 16S rRNA methylases, which are widely found in strains expressing NDM-1 (8, 9) and OXA-48 (10–15) carbapenemases and in some aminoglycoside-resistant *A. baumannii* strains (10, 16, 17). Only the *npmA* ribosomal methylase, through modification of a distinct nucleotide in the 16S RNA

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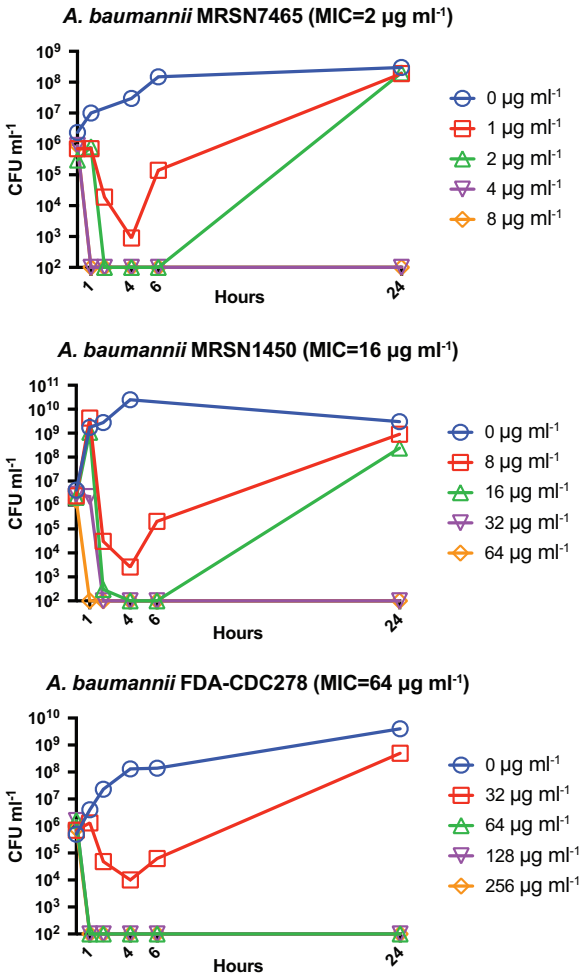

FIG 1 Time-kill studies. Apramycin demonstrates rapid bactericidal activity against representative *A. baumannii* strains. Data points plotted at 10^2 CFU correspond to the detection limit of the assay. Results shown are representative of two independent experiments.

decoding A-site, undermines apramycin activity. However, at present, there is only one report of *npmA* in a clinical isolate (18, 19).

Despite these intriguing attributes, there is a paucity of toxicological, pharmacokinetic, and pharmacodynamic data for apramycin in the peer-reviewed literature. Therefore, we further characterized activity of apramycin against *A. baumannii* in *in vitro* time-kill assays and in the neutropenic mouse thigh infection model.

To evaluate *in vitro* bactericidal activity of apramycin, we selected three strains of *A. baumannii* that had representative apramycin MICs of 2, 16, and 64 μg ml^{-1}, within the previously determined epidemiological cutoff value of 64 μg ml^{-1} (6), and were virulent in neutropenic CD-1 mice (see Table S1 in the supplemental material). Time-kill studies were performed according to CLSI guidelines (20), with CFU quantified using the drop-plate method (21). In time-kill analyses, apramycin demonstrated rapid bactericidal activity (99.9% killing) within 1 to 2 h of antibiotic exposure at 1× to 4× the broth microdilution MIC (Fig. 1).

To identify the single maximum tolerated dose (MTD) of apramycin, CD-1 mice (Charles River Laboratories, Inc., Kingston, NY), weighing 25 to 30 g, were injected intraperitoneally (i.p.) with ascending doses of apramycin. Over the next 72 h, no signs of distress were observed with doses up to 1,500 mg kg^{-1}. Two of three mice from the 3,000-mg kg^{-1} group died ~24 h postinjection. Thus, the single-dose MTD was 1,500 mg kg^{-1}.

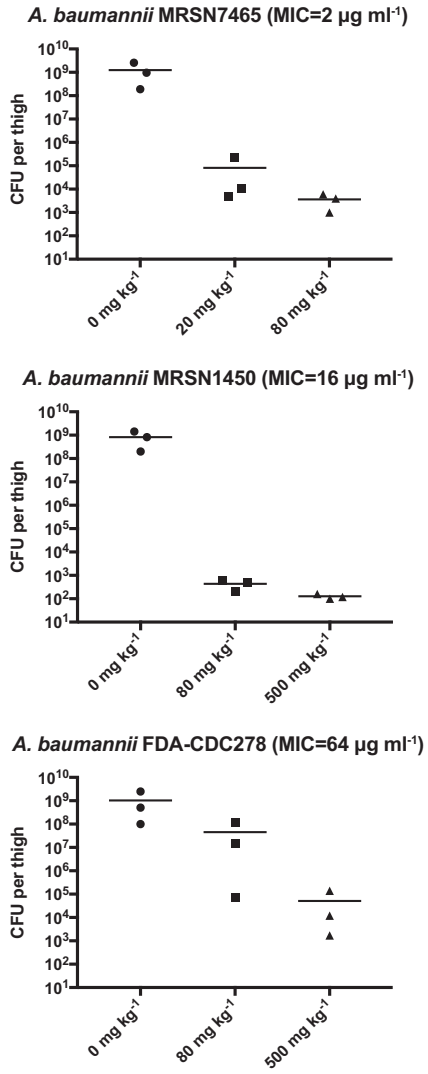

FIG 2 Apramycin demonstrates substantial treatment effect in the murine thigh model. Mice were infected with *A. baumannii* strains and dosed with apramycin 2 h later. CFU were enumerated 24 h postinfection. There were no CFU recovered from a single mouse infected with strain MRSN 7465 treated with 80 mg kg^{-1} apramycin and a single mouse infected with strain MRSN 1450 and treated with 500 mg kg^{-1} apramycin. Data points are plotted at the 10^3 CFU and 10^2 CFU assay limit of detection in these respective experiments.

Apramycin was then given daily at 500 mg kg^{-1} i.p. for 14 consecutive days. Treated animals showed no signs of distress or change in body weight in comparison to controls during the experiment (see Fig. S1A in the supplemental material). On day 15, the mice were euthanized. Terminal measurements of serum creatinine (Fig. S1B) and organ histology, i.e., kidney (Fig. S1C) and liver (data not shown), were unremarkable. The multidose MTD was therefore $\geq$500 mg kg^{-1}.

Pharmacokinetic and treatment studies were performed using CD-1 mice, rendered neutropenic with cyclophosphamide and mildly renal deficient with uranyl nitrate to more closely simulate human excretion kinetics (22). For pharmacokinetic studies, animals were injected subcutaneously (s.c.) with 20, 80, and 500 mg kg^{-1} apramycin ($n = 3$ per dose). Plasma apramycin concentrations were measured as described previously and are detailed in the supplemental material (23). Apramycin demonstrated first-order elimination kinetics (data not shown). Maximum concentrations of drugs in serum (C_{max}) were 29 ($\pm$16), 141 ($\pm$19), and 2,100 μg ml^{-1} ($\pm$1,200); and area under

the curve (AUC) values determined by the linear trapezoidal method were 138 (±97), 991 (±486), and 11,500 (±9,400) μg h ml^{-1}, respectively.

For mouse thigh infection studies, mice were inoculated with 10^6 CFU of *A. baumannii* strains MSRN7465 and MSRN 1450 or 10^7 CFU of *A. baumannii* strain FDA-CDC278 and subsequently treated with apramycin 2 h postinfection with single doses of 20, 80, or 500 mg kg^{-1} s.c. Tissue was harvested 24 h after infection, ground, and serially diluted for CFU determination. Notably, apramycin showed a dramatic treatment effect against all three strains (Fig. 2). There was at least a 4-log10 reduction in CFU for all dosing in which AUC/MIC ratio was >50 and C_{max}/MIC was ~10 or more (24, 25).

Previously, therapeutic effects of apramycin against single strains of *Staphylococcus aureus* and *Mycobacterium tuberculosis* in murine infection models were described (26). In these studies, the apramycin MIC for *S. aureus* was 4 to 8 μg ml^{-1}, and the therapeutic effect increased in an immunocompromised murine septicemia model in a stepwise fashion when dosed at 16, 32, or 80 mg kg^{-1}. The *M. tuberculosis* MIC was not noted; however, a significant reduction in lung CFU occurred after dosing at 200 mg kg^{-1} for 9 days. Here, we provide evidence for an *in vivo* activity spectrum that also includes *A. baumannii*.

Several limitations of the study should be noted. First, absence of pathologies in MTD studies with relatively high systemic exposure provides some support for low toxicity. However, mice are insensitive to nephrotoxic effects of aminoglycosides (27–29). Therefore, our findings do not rule out the potential for kidney toxicity, an area that deserves further investigation in more relevant models (30). Furthermore, large doses were needed to obtain a 4-log10 reduction for strains with high MIC values. It is unclear how dosing would scale in future potential human use and ultimately what fraction of strains may prove treatable. Despite the preliminary nature of our findings, we believe, based on *in vitro* and *in vivo* data, that apramycin deserves further consideration as a repurposed therapeutic and as a starting point for future medicinal chemistry efforts targeting MDR Gram-negative pathogens such as *A. baumannii*.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02585-17.

SUPPLEMENTAL FILE 1, PDF file, 1.2 MB.

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