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Colistin Methanesulfonate against Multidrug-Resistant *Acinetobacter baumannii* in an In Vitro Pharmacodynamic Model[▽]

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Using an in vitro pharmacodynamic model, a multidrug-resistant strain of *Acinetobacter baumannii* was exposed to colistin methanesulfonate alone and in combination with ceftazidime. Pre- and postexposure colistin sulfate MICs were determined. A single daily dose of colistin methanesulfonate combined with continuous-infusion ceftazidime prevented regrowth and postexposure MIC increases.

Acinetobacter baumannii is an opportunistic nosocomial pathogen associated with significant morbidity and mortality (3, 9). High rates of resistance to commonly used broad-spectrum antibiotics are frequently reported (2, 5). Current approaches to therapy include carbapenems and aminoglycosides, but resistance to these antibiotics is increasing, as well (4). Should these agents fail, colistin (polymyxin E) may represent the last line of defense.

The purpose of this study was to examine time-kill profiles of colistin methanesulfonate (CMS), the parenteral salt form of colistin, alone and combined with ceftazidime.

CMS (Fluka, Buchs, Switzerland) was prepared 1 day prior to use and stored at 4°C (6). A ceftazidime (GlaxoSmithKline, Research Triangle Park, NC) stock solution was prepared and stored at −80°C. Colistin sulfate (Sigma-Aldrich, St. Louis, MO) stock solutions were prepared for use in MIC trays and stored at 4°C.

A clinical isolate of *A. baumannii* (06-74; source unknown) was obtained from Regions Hospital (Saint Paul, MN), where identification and sensitivity testing were performed using the Vitek 2 (bioMérieux, Balmes-les-Grottes, France). The time-kill experiments used CMS alone or in combination with continuous-infusion ceftazidime. The previously described in vitro phamacodynamic model (10) contained cation-adjusted Mueller-Hinton broth (Becton Dickinson Microbiology Systems, Sparks, MD). The pump rate was set to create the desired 3-hour half-life of colistin sulfate (8).

Two sets of duplicate 24-hour experiments were performed. In set 1, four experiments simulating a range of peak CMS concentrations were performed to evaluate the time-kill profiles of *A. baumannii*. At time zero, a bolus dose was injected into the vessel. The desired CMS peak concentrations were 3, 6, 12, and 24 μg/ml. The peak concentrations and half-lives were not verified via assay, so the desired and actual values might have differed. Additionally, one experiment was performed to evaluate the effect of a second 24-μg/ml dose of CMS at 12 h.

Set 2 included three experiments utilizing a CMS bolus of 24 μg/ml administered along with continuous infusions of ceftazidime at 50 μg/ml. To simulate continuous infusions, ceftazidime was added to the media reservoir, and a loading dose was injected into the vessel. In the first experiment, CMS was injected into the vessel at time zero, and the continuous infusion of ceftazidime was initiated at 2 h. In the second experiment, the order was reversed; continuous-infusion ceftazidime was initiated at time zero, followed by a CMS bolus injection at 2 h. Finally, a third experiment was conducted with a continuous infusion of ceftazidime initiated at time zero.

An aliquot of *A. baumannii* was added to each vessel, resulting in an initial inoculum between 10^5 and 10^6 CFU/ml. The drug was introduced, and nine samples were collected between 0.5 and 24 h. Three additional samples were collected when a second dose was administered. Fifty microliters of each sample was deposited onto trypticase soy agar plates supplemented with 5% sheep blood (Becton Dickinson and Company, Sparks, MD) using the WASP 2 spiral plater. The lower limit of accuracy was 400 CFU/ml (20 colonies divided by 0.05 ml deposited on the plate) and was determined using the guidelines outlined in the WASP 2 manual (1). Following incubation, bacteria were enumerated using the aCOLyte automated colony counter (Synbiosis, Cambridge, United Kingdom). Concentration–time-kill curves were compared graphically by plotting $\log_{10}$ CFU/ml versus time.

Microdilution MIC tests for both colistin sulfate and CMS were performed on postexposure isolates present at 24 h using the Bio-Tek Precision 2000 pipetting system (Bio-Tek Instruments, Inc. Winooski, VT) following CLSI guidelines (7). *Pseudomonas aeruginosa* ATCC 27853 was used as the quality control strain. Ceftazidime MIC testing was performed on *A. baumannii* isolates from experiments combining ceftazidime and CMS. The resistance stabilities of postexposure isolates from experiments simulating CMS concentrations of 3, 6, 12, and 24 μg/ml were evaluated by subculturing them daily for 10 days and repeating the MIC tests.

CMS peak concentrations of 3, 6, 12, and 24 μg/ml all re-

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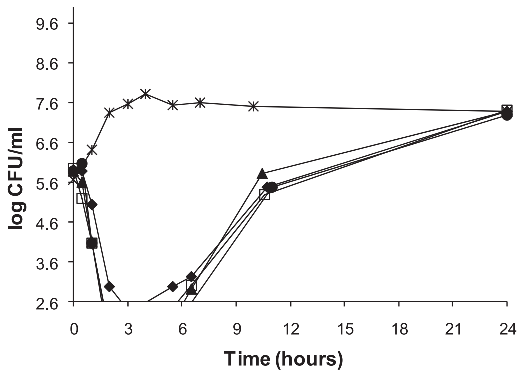

FIG. 1. Time-kill curves of CMS against *A. baumannii* at maximum serum drug concentrations of 3 μg/ml (◆), 6 μg/ml (●), 12 μg/ml (▲), and 24 μg/ml (□). The growth control (×) is also depicted.

duced the bacterial load by 3 $\log_{10}$ CFU/ml within 2 to 3 h. Regrowth was observed beginning at 3 h for all single-dose CMS experiments and continued through 24 h, reaching approximately 7 $\log_{10}$ CFU/ml (Fig. 1). The addition of a second dose of CMS (24 μg/ml) at 12 h was ineffective in further reducing the bacterial burden or preventing regrowth of *A. baumannii*.

Continuous-infusion ceftazidime administered alone produced a 3 $\log_{10}$ CFU/ml reduction in the bacterial burden within 3 to 4 h, but at 24 h, regrowth to approximately 6.5 $\log_{10}$ CFU/ml had occurred. When a bolus CMS dose was combined with continuous-infusion ceftazidime, a 3 $\log_{10}$ CFU/ml reduction was quickly achieved—within 2 to 3 h—and regrowth was subsequently suppressed (Fig. 2).

The preexposure MICs were 0.5 and 2 μg/ml for colistin sulfate and CMS, respectively. All postexposure isolates from time-kill experiments using CMS alone had CMS MICs of >2,048 μg/ml. In order to obtain definitive endpoints, further MIC testing was performed using colistin sulfate only. Colistin sulfate MICs increased from 0.5 μg/ml preexposure to 32 to >128 μg/ml postexposure for experiments using CMS alone. The addition of continuous-infusion ceftazidime along with CMS prevented resistance to colistin

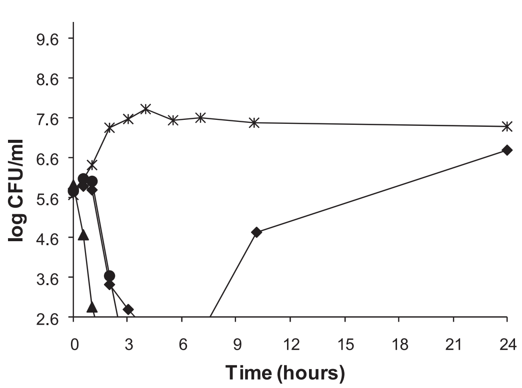

FIG. 2. Time-kill curves of CMS given at time zero plus the addition of continuous-infusion ceftazidime started at 2 h (▲) and continuous-infusion ceftazidime started at time zero plus a CMS bolus given at 2 h (●) against *A. baumannii*. Continuous-infusion ceftazidime given alone (◆) and the growth control (×) are also depicted.

TABLE 1. Colistin sulfate and ceftazidime MIC results

Expt	MIC[a] (μg/ml)	
	Colistin sulfate	Ceftazidime
A. baumannii preexposure	0.5	≥64
A. baumannii postexposure		
3 μg/ml CMS	64–128	
6 μg/ml CMS	64–>128	
12 μg/ml CMS	128	
24 μg/ml CMS	32–>128	
24 μg/ml CMS (bolus dose at 0 and 12 h)	≥128	
24 μg/ml CMS and 50 μg/ml ceftazidime[b]	0.5	32–64
50 μg/ml ceftazidime and 24 μg/ml CMS[c]	0.06	32–64

[a] Vitek 2 MIC data (μg/ml) for *A. baumannii* 06-74 are as follows: ampicillin, ≥32; ceftazidime, ≥64; ciprofloxacin, ≥4; levofloxacin, ≥8; gentamicin, ≥16; aztreonam, ≥64; nitrofurantion, ≥512; ampicillin-sulbactam, ≤2/4; amikacin, ≤2; imipenem, ≤1; meropenem, 1; and piperacillin-tazobactam, 32/4.

[b] CMS given as a bolus dose at time zero, followed by ceftazidime administered as a continuous infusion at 2 h.

[c] Ceftazidime administered as a continuous infusion at time zero, followed by CMS given as a bolus dose at 2 h.

sulfate, and ceftazidime MICs remained unchanged (Table 1). Colistin sulfate MICs were stable at 128 to 256 μg/ml after a 10-day serial passage.

The ability to prevent the MIC increases when CMS was combined with ceftazidime and the apparent benefit when the drugs were used in combination were unexpected findings. Likewise, the antibacterial effect of continuous-infusion ceftazidime given alone proved to be an interesting observation, considering that *A. baumannii* was intrinsically resistant to ceftazidime (MIC ≥ 64 μg/ml).

The order and frequency of antibiotic administration may factor into an optimal dosing strategy for the treatment of *A. baumannii* infections. The use of CMS first results in a rapid and significant reduction of the bacterial burden. Furthermore, the small numbers of survivors, if now resistant to CMS, may succumb to a second antibiotic, e.g., ceftazidime given as a continuous infusion.

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