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Polymyxin B-Resistant *Acinetobacter baumannii* Clinical Isolate Susceptible to Recombinant BPI_{21} and Cecropin P1

Many strains of *Acinetobacter baumannii* have become resistant to a variety of clinically available antibacterial agents by both intrinsic and extrinsic mechanisms (4, 15). Several investigators have documented multidrug-resistant *A. baumannii* causing nosocomial infections and have demonstrated the in vitro and in vivo activities of imipenem, sulbactam, and the polymyxins (2, 9, 11, 14, 16). Increasing resistance to antibacterials, including sulbactam and the carbapenems, has prompted the use of polymyxin B and colistin as therapeutic agents, and within the last several years, the polymyxins have been used with increasing frequency to treat patients infected with multidrug-resistant, gram-negative bacteria, including *Acinetobacter* (9, 14, 16). Although the literature, both clinical and microbiological, has shown that *A. baumannii* has retained susceptibility to the polymyxins despite resistance to all other antibacterial agents (2, 15), we document the isolation of a polymyxin B-resistant strain of *A. baumannii* from a patient who was given polymyxin B for treatment of a multidrug-resistant, polymyxin-susceptible strain of *A. baumannii*. The minimal inhibitory concentrations for the polymyxin-resistant strain (L1) were 48 μg/ml (polymyxin B) and 128 μg/ml (colistin) as determined by E-test methodology (AB Biodisk North America Inc., Piscataway, N.J.). More importantly, we have found that this strain is susceptible in vitro to $rBPI_{21}$ (Neuprex; XOMA Corporation, Berkeley, Calif.) (provided by S. Carroll), a recombinant form of the N-terminal domain of the human bactericidal/permeability-increasing protein (Table 1). This isolate was also susceptible to cecropin P1 (Sigma, St. Louis, Mo.), an antibacterial peptide from pig intestine (3). The antibacterial effects of $rBPI_{21}$ and cecropin P1 were manifest both in conventional MIC and minimal bactericidal concentration (MBC) assays with Mueller-Hinton broth and in bactericidal assays with nutrient broth. In the later type of assay, the antibacterial potency of $rBPI_{21}$, but not of cecropin P1, toward *A. baumannii* was further increased nearly 100-fold in the presence of sublethal amounts of serum (data not shown).

TABLE 1. Polymyxin B-resistant isolate of *Acinetobacter baumannii* is sensitive to $rBPI_{21}$ and to cecropin P1[a]

Antibacterial agent	MIC and MBC (μM)		LD_{90} (μM)		
	L1	D41	L1	D41	A1
Polymyxin B	>10	0.5	>10	0.1	0.1
$rBPI_{21}$	3.0	1.0	0.3	0.3	0.2
Cecropin P1	2.0	1.0	0.5	0.3	NT[b]

[a] The MIC and MBC of polymyxin B, $rBPI_{21}$, and cecropin P1 for *Acinetobacter baumannii* were determined by incubation of bacteria (5×10^5 bacteria/ml) overnight at 37°C in Mueller-Hinton broth containing increasing concentrations of the antibacterial (poly)peptides. Under all conditions, the MIC and MBC were the same. The bactericidal activities of these agents for *Acinetobacter baumannii* were also measured by incubation with bacteria (10^6 bacteria/ml) in phosphate-buffered (pH 7.4) nutrient broth–0.9% sodium chloride for 2 h at 37°C. All results shown are the means of at least three independent determinations. LD_{90}, 90% lethal dose.

[b] NT, not tested.

The bactericidal and antiendotoxin properties of cationic membrane active (poly)peptides are well known (5–7, 10, 12, 13). The superior in vitro activity of $rBPI_{21}$, the results of extensive preclinical testing in animal models, and the protein's apparent lack of immunogenicity and toxicity for human recipients have encouraged therapeutic trials in settings where conventional antibiotics are unable to control infection by gram-negative bacteria and/or proinflammatory effects of endotoxin (1, 6, 8, 10). Sublethal alterations of the gram-negative bacterial outer membrane in combination with the use of antibiotics that, because of resistance, are now ineffective alone may further extend therapeutic opportunities (1, 7, 8). The activity of $rBPI_{21}$ toward gram-negative bacteria with high levels of resistance to polymyxin B, documented here for *A. baumannii* and previously observed experimentally with several other species of gram-negative bacteria (5, 7), illustrates additional important attributes that may support its use as a therapeutic agent. Investigations of the molecular bases of polymyxin B resistance in *Acinetobacter* and other gram-negative bacteria and the activities of BPI and its derivatives against these multidrug-resistant organisms are under way.

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