Examining the Use of Ceftaroline in the Treatment of *Streptococcus pneumoniae* Meningitis with Reference to Human Cathelicidin LL-37

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Five cases of bacterial meningitis treated with ceftaroline (4 *Streptococcus pneumoniae* and 1 *Staphylococcus aureus*) are summarized here. The pharmacodynamics of human cathelicidin LL-37 and ceftaroline were evaluated against *S. pneumoniae*. Patients who received ceftaroline 600 mg every 8 h (q8h) (1 *S. aureus* and 3 *S. pneumoniae*) were successfully treated; treatment failed in 1 patient with *S. pneumoniae* who received 600 mg q12h. Ceftaroline increased the negative surface charge and sensitized *S. pneumoniae* to killing by LL-37, a peptide implicated in blood-brain barrier defense.

Ceftaroline was approved in 2010 for the treatment of community-acquired pneumonia and complicated skin and soft structure infections (1, 2). In pneumonia, ceftaroline has been shown to perform significantly better than does the comparator ceftriaxone in cases caused by *Streptococcus pneumoniae* (2). Furthermore, ceftaroline frequently retains activity against penicillin-resistant strains of *S. pneumoniae* (3). In a previous study, ceftaroline was 8-fold more potent than ceftriaxone and 16-fold more potent than penicillin against multidrug-resistant *S. pneumoniae* isolates. Against a subset of 106 penicillin-nonsusceptible *S. pneumoniae* isolates, 4% were susceptible, 52% were intermediate, and 44% were resistant to ceftriaxone, but 100% were susceptible to ceftaroline (4).

Although undocumented, ceftaroline is expected to perform well in the treatment of pneumococcal meningitis due to its spectrum, potency of activity, and cerebrospinal fluid (CSF) penetration into inflamed meninges shown in rabbit models (5, 6). However, questions surrounding ideal dosing, lack of documentation of CSF penetration in humans, and the severity of illness in patients with meningitis have limited the clinical use of ceftaroline in this disorder. This study provides the first assessment of ceftaroline use for the treatment of Gram-positive bacterial meningitis and presents a laboratory evaluation of antimicrobial therapy from the perspective of pharmacodynamic interactions with cathelicidin, a cationic host defense peptide present in CSF.

In this study, ceftaroline was used to treat Gram-positive bacterial meningitis in 5 adult cases at two U.S. tertiary hospitals (Portland, OR, and San Diego, CA) between 2011 and 2013. The infecting pathogens were reported to be susceptible, according to a ceftaroline clinical microbiology laboratory, at ≤0.25 mg/liter for *S. pneumoniae* and ≤0.5 mg/liter for *Staphylococcus aureus*. The clinical data and outcomes were collected retrospectively and summarized for this analysis. This was not a clinical trial but rather a series of individual patient cases for which ceftaroline was chosen largely due to concerns of vancomycin nephrotoxicity seen increasingly at our hospitals, particularly with more aggressive dosing strategies, such as those employed to treat bacterial meningitis. For example, ceftaroline was used in 1 case as the initial therapy in the emergency room because of a high risk of nephrotoxicity from obesity, diabetes, and renal failure on presentation.

A subsequent change to standard penicillin-resistant *S. pneumoniae* therapy with vancomycin at day 4 was accompanied by increased systemic leukocytosis and fever that resolved with the reinitiation of ceftaroline. This experience, in addition to the risks of vancomycin and burden of therapeutic drug monitoring, has resulted in ceftaroline being increasingly used by infectious diseases physicians treating bacterial meningitis. In these cases, ceftaroline was initiated by infectious disease physicians within 24 h of diagnosis and, unless otherwise specified (e.g., in the patient who required alternative therapy), was continued until the end of therapy.

Penicillin-susceptible D39, a well-characterized *S. pneumoniae* strain (7), and the previously studied penicillin-resistant *S. pneumoniae* strains SP90 and SP1466 were used in the laboratory component of this study (3).

For the LL-37 killing assays, bacteria were grown in antibiotic-free THY (Todd-Hewitt with 0.5% yeast extract) broth or THY broth containing $0.25\times$ the MIC of vancomycin, ceftriaxone, linezolid, or ceftaroline to an optical density at 600 nm (OD_{600}) of 0.3 to 0.5, washed in phosphate-buffered saline (PBS), and subjected to 1 μM LL-37 (American Peptide Company) killing assays at a bacterial density of approximately 2×10^{3} in RPMI medium (Invitrogen) supplemented with 10% THY. After 1 or 2 h, 10-μl aliquots were plated on sheep blood agar plates (Hardy Diagnostics, Santa Maria, CA) and colonies enumerated after 24 h. The percent survival was calculated as the CFU at 1 or 2 h/CFU at time zero.

TABLE 1 Clinical summary of ceftaroline for Gram-positive meningitis

Patient no.	Age range (yr)	Organism[a]	Comorbidities/complications	Dose (mg)	Duration (days)	Success
1	51–60	*S. pneumoniae* PEN[r]	Diabetes mellitus, obesity, remote mastoid surgery, bacteremia, pneumonia, ICU, respiratory failure	600 q8h	14	Yes
2	41–50	*S. pneumoniae*	Remote history of skull fracture, right otitis media and mastoiditis, bacteremia	600 q8h	21	Yes
3	51–60	*S. aureus* (MSSA)	Traumatic skull fracture, PEN allergy	600 q8h	21	Yes
4	60–70	*S. pneumoniae*	Acute myelogenous leukemia, diabetes mellitus, bacteremia, ICU	600 q8h	10	Yes
5	21–30	*S. pneumoniae*	i.v. drug abuse, pneumonia, bacteremia, ICU, respiratory failure	600 q12h	12	No

[a] PEN, penicillin; MSSA, methicillin-susceptible *S. aureus*.

For the LL-37 binding assays, *S. pneumoniae* D39 was labeled with 2 μM TAMRA (6-carboxytetramethylrhodamine) azide-tagged LL-37 (American Peptide Company) for 45 min in 50% phenol-free RPMI medium–50% THY broth, washed, and visualized microscopically as previously described (8). Note that TAMRA-tagged LL-37 demonstrated approximately a 2-fold reduction in potency, as measured by its MIC, compared to that of unlabeled LL-37, resulting in double the concentration being used in the binding studies compared to that in the killing assays.

Cytochrome *c* binding assays were performed by growing *S. pneumoniae* D39 to an OD_{600} of 0.3 to 0.4, washing in 30 mM MOPS (morpholinepropanesulfonic acid) buffer (pH 7), and resuspending to an OD_{600} of 0.5 in MOPS buffer. Cytochrome *c* was added to a final concentration of 1 mg/ml, incubated at room temperature for 60 min, and the supernatant at OD_{540} was measured to quantify the concentration of cytochrome *c* using control standards.

Statistical evaluations of the differences in LL-37 survival, LL-37 binding, and cytochrome *c* binding were performed via Mann-Whitney U test (GraphPad Prism 5.0; GraphPad Software, Inc., San Diego, CA). *P* values of <0.05 were considered statistically significant.

The clinical summary of the 5 patients with Gram-positive bacterial meningitis is provided in Table 1. Four cases were due to *S. pneumoniae*, and one was due to *S. aureus*. The patient age range was 29 to 63 years. Three of the five patients were admitted to the intensive care unit, and two required mechanical ventilation. The duration of therapy was 10 to 21 days. All 4 cases treated with every 8 h (q8h) dosing were successfully treated, whereas one with q12h dosing failed, requiring alternative therapy for complete resolution. Success was defined as the completion of ceftaroline therapy as initially prescribed without the need to discontinue due to treatment failure or an adverse event.

Figure 1A shows the percentages of survival in LL-37 killing assays of *S. pneumoniae* D39 after growth in antibiotic-free medium versus those in 0.25× the MIC of the respective antibiotics. Ceftaroline significantly increased susceptibility to LL-37 killing, whereas linezolid and vancomycin rendered the organism more resistant to killing. The results of LL-37 killing assays for penicillin- and ceftriaxone-resistant *S. pneumoniae* strains SP90 and SP1466 after growth in the specified antibiotics are shown in Fig. 1B and C, respectively. Growth in ceftaroline or ceftriaxone rendered the bacteria more vulnerable to LL-37 killing, whereas growth in vancomycin did not. Figure 1D shows *S. pneumoniae*

D39 after labeling with TAMRA azide-tagged LL-37. Bacteria grown in ceftaroline showed significantly more LL-37 binding than bacteria grown in vancomycin ($P < 0.05$). In addition, the ceftaroline-exposed bacteria showed marked increases in elongation. While there was a trend toward ceftriaxone inducing increased LL-37 binding against D39, the difference was not statistically significant.

S. pneumoniae D39 was subjected to cytochrome *c* binding assays, an established method for determining surface charge changes. A decrease in net surface charge would be expected to result in increased cytochrome *c* binding in this assay and therefore increased susceptibility to cationic antimicrobial peptide killing. D39 showed significantly more cytochrome *c* binding after growth in ceftaroline than that with the control or growth in vancomycin, ceftriaxone, or linezolid (Fig. 1E), paralleling the LL-37 killing (Fig.1A) and LL-37 binding (Fig. 1D) observed against this strain.

Despite promising *in vitro* and clinical data demonstrating the potent activity of ceftaroline against *S. pneumoniae*, including penicillin-resistant strains, there are no clinical data describing ceftaroline use in bacterial meningitis in humans. This small case series demonstrates that ceftaroline may be a viable option for the treatment of bacterial meningitis due to *S. pneumoniae*, including penicillin-resistant strains, and *S. aureus*. In the first case, the patient appeared to respond adversely (leukocytosis and low-grade fever) when changed to vancomycin, with improvement observed after switching back to ceftaroline. Interestingly, the only patient who did not have a satisfactory clinical response was administered 600 mg intravenous (i.v.) q12h, the standard approved dose for soft tissue infection and pneumonia, whereas all 4 patients who received q8h dosing had an excellent response. While we did not measure the concentrations of ceftaroline in the serum and CSF from these patients, this experience suggests that ceftaroline dosing may need to be increased above the approved doses for meningitis. Despite the higher doses, none of the patients had any adverse effects.

Currently, CSF penetration is the only pharmacological metric of anticipated antibiotic activity and clinical efficacy in meningitis. For ceftaroline, a rabbit model of meningitis demonstrated 15% penetration into the CSF in inflamed meninges and 3% in uninflamed meninges (5, 6), translating into concentrations measured in the CSF of approximately 5 and 1 mg/liter, respectively. In human subjects who were administered the approved 600 mg q12h dosing, the serum maximum concentration of drug (C_{max})
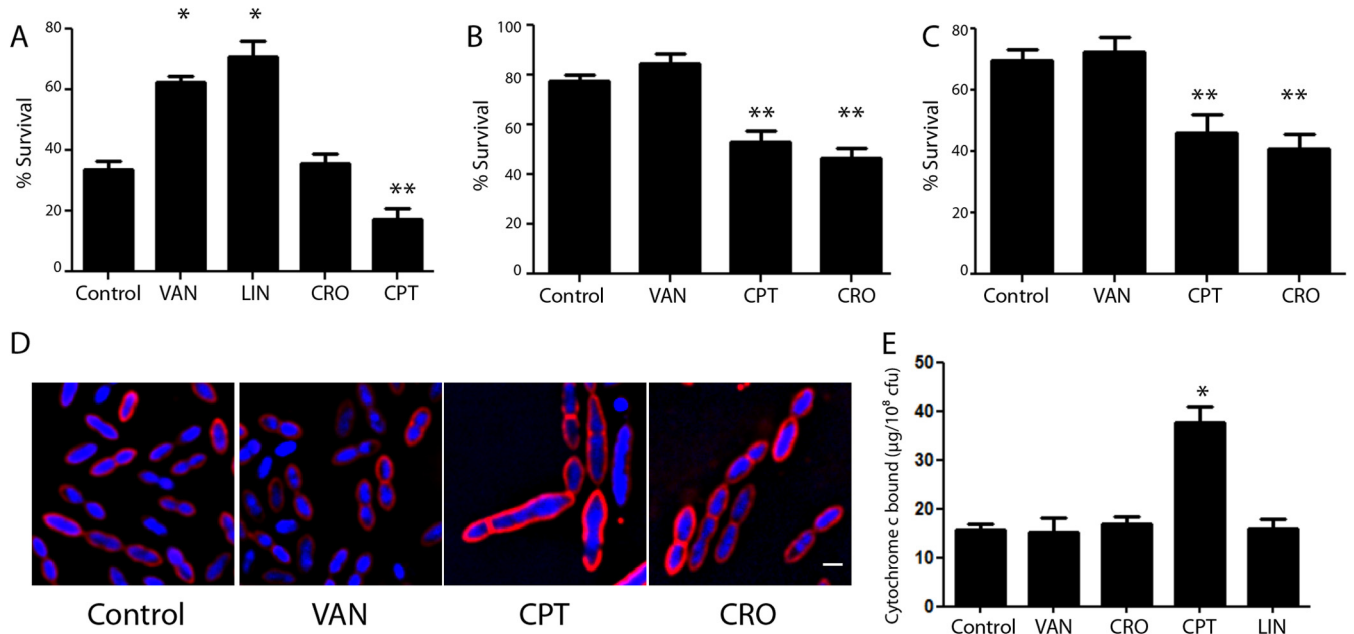
FIG 1 (A) *S. pneumoniae* D39 subjected to LL37 killing assays (at 1 mM for 2 h) after growth in 0.25× the MIC of vancomycin (VAN) (0.125 mg/liter), linezolid (LIN) (0.25 mg/liter), ceftriaxone (CRO) (0.005 mg/liter), or ceftaroline (CPT) (0.005 mg/liter) versus antibiotic-free medium (control [CON]). (B and C) Percent survival in LL-37 killing assays (at 1 mM for 1 h) of penicillin-resistant *S. pneumoniae* SP90 (B) and SP1466 (C) after growth in 0.25 mg/liter VAN, 0.025 mg/liter CPT, and 0.25 mg/liter CRO (approximately 0.25× the MIC). *, $P < 0.05$ for increase versus control; **, $P < 0.05$ for decrease versus control. (D) TAMRA-labeled LL-37 (at 2 mM for 1 h) binding assays of *S. pneumoniae* strain D39 after growth in the specified antibiotics as per panel A. Binding was significantly increased after growth in CPT versus VAN. The MIC of TAMRA-LL-37 is approximately 2× that of unlabeled LL-37 against the tested strains. (E) Relative cytochrome *c* binding of *S. pneumoniae* D39 after growth in THY broth containing no antibiotic (control) compared to growth in 0.25× the MIC of vancomycin (VAN), linezolid (LIN), ceftriaxone (CRO), or ceftaroline (CPT). *, $P < 0.05$ versus control. Data for panels A to C and E are expressed as means and standard deviations.

of ceftaroline was 20 mg/liter (1). The extrapolated C_{max} values in CSF would be 3 mg/liter and 0.6 mg/liter in inflamed and uninflamed meninges, respectively (5, 6). While these ranges exceed the MICs for susceptibility to *S. pneumoniae*, at this time, they are only extrapolations, and data are lacking. A recent letter showed that in 2 cases of ventriculoperitoneal shunt infections, ceftaroline achieved approximately 4% CSF penetration, with peak concentrations of about 0.5 mg/liter (9). We anticipate that ceftaroline CSF pharmacokinetics will be more thoroughly investigated and published by other groups and/or the drug manufacturer.

We explored a novel avenue of the study in the interaction between antibiotics and the critical innate host defense peptide present in CSF, human cathelicidin LL-37. In uninflamed human CSF, concentrations of cathelicidin LL-37 have been measured at 0.01 to 0.7 μM (10), with much higher concentrations anticipated in inflamed meninges due to neutrophil recruitment and reduced blood-brain barrier integrity. A recent study in a model of *S. pneumoniae* meningitis in cathelicidin-related antimicrobial peptide (CRAMP)-deficient mice demonstrated that the deficiency of the murine homolog to LL-37 was associated with (i) a higher mortality rate, (ii) increased bacterial titers in the cerebellum and blood, (iii) decreased meningeal neutrophil infiltration, and (iv) a higher degree of glial cell activation with a more proinflammatory response (11).

Little is known about how antibiotics influence bacterial pathogen susceptibilities to cationic host defense peptides and the clinical relevance of these effects. We anticipate that these interactions may play a critical role in some cases in which the concentrations of antibiotics in CSF are at or even below the MIC of the pathogen. For example, we described a case in which tigecycline was used to successfully treat nosocomial meningitis in a neurosurgical patient by a carbapenem-resistant *Klebsiella pneumoniae* organism, and the measured concentrations of tigecycline in CSF fell below the MIC of the pathogen (12). We recently demonstrated that β-lactams, which have no activity under standard susceptibility testing conditions against resistant Gram-positive organisms, such as methicillin-resistant *S. aureus* (MRSA) or vancomycin-resistant *Enterococcus faecium*, render these pathogens extremely vulnerable to killing by innate host defense cationic antimicrobial peptides, such as cathelicidin (8, 13, 14). The of β-lactam antibiotics against β-lactam-resistant pathogens as adjunctive therapy has allowed for the clearance of recalcitrant bloodstream infections. Along similar lines of reasoning, we believe that the investigation of antimicrobials, including ceftaroline, for the treatment of bacterial meningitis should involve an assessment of whether they influence susceptibility to host defense factors, such as cathelicidin. Antibiotics appear to vary greatly in their ability to sensitize *S. pneumoniae* to killing by LL-37. Consistent with our prior studies with *S. aureus* (13), β-lactams, such as ceftaroline and ceftriaxone, may increase the vulnerability of *S. pneumoniae* to killing by cathelicidin, whereas linezolid and vancomycin do not.

The limitations of our study include a small sample size, retrospective design, and the fact that it was noncomparative to other therapies. However, we believe this study represents the initial steps toward the study of ceftaroline in meningitis and toward the

examination of antimicrobials in the treatment of central nervous system (CNS) infections in reference to their pharmacodynamic interactions with cathelicidin LL-37 and possibly other cationic host antimicrobial peptides produced by the innate immune system.

This study demonstrates that ceftaroline administered 600 mg i.v. q8h may be a viable therapeutic option for patients with meningitis caused by *S. pneumoniae*, including penicillin-resistant strains, and *S. aureus*. Ceftaroline, unlike vancomycin, appears to assist the ability of the critical host defense peptide cathelicidin LL-37 to kill *S. pneumoniae*. Further studies are needed to evaluate ceftaroline for the treatment of bacterial meningitis.

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