Research Article

Ceftaroline Fosamil in the Treatment of Experimental Meningitis Caused by Methicillin-Resistant *Staphylococcus aureus* - 🔓

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ABSTRACT

Introduction: Meningitis caused by methicillin resistant *Staphylococcus aureus* (MRSA) is rare and often fatal. The recommended treatment for MRSA meningitis, vancomycin, is limited by poor cerebrospinal fluid (CSF) penetration. Ceftaroline fosamil is a novel fifth-generation cephalosporin with potent antibacterial activity *in vitro* against MRSA. Several case reports in humans suggest that ceftaroline might be a potential treatment option for MRSA meningitis. Our primary objective was to compare the efficacy of ceftaroline and vancomycin for the treatment of MRSA meningitis using a rabbit meningitis model. We then characterized CSF drug concentrations over time.

Methods: Ninety rabbits received a direct intracisternal injection of 5×10^5 CFU *S. aureus* (MRSA 252). Following a 16-hour incubation period, approximately 0.5ml of CSF was withdrawn via intracisternal aspiration immediately prior to treatment for quantitative bacterial counts. Rabbits then received (by 1:1:1 random assignment) either no treatment (control group), vancomycin 20 mg/kg at 0 and 12hrs, or ceftaroline 40mg/kg at 0 and 4 hrs via marginal ear vein. CSF collection was repeated every 12 hours for up to 40 hours. All animals were humanely euthanized at 40 hours post inoculation. The primary endpoint (difference in bacterial load [expressed as CFU/mL] for each animal between the initial (T1) and terminal (T3) taps were characterized and compared between groups using the Kruskal-Wallis test. CSF drug concentrations were determined using liquid chromatography with tandem mass spectrometry (LC/MS/MS) assay.

Results: Among the forty-three evaluable animals with established MRSA meningitis, there was no statistical difference in median CFU observed between the groups in pretreatment CFU counts ($p = 0.16$). Reductions in bacterial load from baseline were 88%, 79% and 75% in the control, ceftaroline and vancomycin-treated groups, respectively. There was no statistical difference observed between vancomycin and either control or ceftaroline at any time point. While animals treated with ceftaroline exhibited a significant increase in clearance rate (log Δ) between T1 and T2 when compared to control ($p = 0.019$), these differences were no longer statistically significant by T3 ($p = 0.43$) as CSF ceftaroline concentrations were undetectable by that time point.

Conclusions: While ceftaroline may be a reasonable alternative to vancomycin for MRSA meningitis, additional animal and clinical studies are required to determine optimal dosing to establish its effectiveness.

Keywords: *Staphylococcus aureus*; MRSA; Meningitis; Ceftaroline; Vancomycin

ABBREVIATIONS

MRSA: methicillin resistant *Staphylococcus aureus;* CSF: cerebrospinal fluid; CFU: colony forming units; LC/MS/MS: liquid chromatography–mass spectrometry; T1: time point one (16 hours post inoculation); T2: time point 2 (28 hours post inoculation); T3: time point 3 (40 hours post inoculation)

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) is a common and often devastating cause of nosocomial bacterial meningitis [1]. Currently, the standard treatment for MRSA meningitis is vancomycin [2], but its penetration into the cerebral spinal fluid (CSF) is inconsistent and (at times) poor [3]. Adjunctive treatments, such as intrathecal administration of vancomycin, are of unproven inefficacy and can confer significant risks to the patient. As a result, alternative treatments for MRSA meningitis are greatly needed.

Cephalosporins have a rich history in the optimal management of bacterial meningitis in humans [4]. Ceftaroline fosamil (Teflaro®-Allergan, Madison, NJ, USA) is a fifth-generation cephalosporin approved for treatment of community-acquired bacterial pneumonia and acute skin and soft tissue infections [5]. Recent animal studies suggest that ceftaroline can penetrate the blood-brain barrier in rabbits [6,7], and therefore might play a role in the treatment of MRSA meningitis in humans. While a few cases have reported some promise with ceftaroline for the treatment of *S. aureus* and *Streptococcus pneumoniae* meningitis [8-10], the efficacy of ceftaroline against MRSA meningitis and its efficacy relative to vancomycin has yet to be comparatively tested experimentally. Our purpose was to evaluate the efficacy of ceftaroline in the treatment of MRSA meningitis using a rabbit meningitis model [11]. Our primary objective was to compare ceftaroline- and vancomycin-treated animals for the difference in bacterial load (expressed as CFU/mL) between the initial and terminal taps. Our secondary objective was to characterize CSF drug concentrations over time.

MATERIALS AND METHODS

Ethics

All animal procedures were reviewed and approved by the Duke Institutional Animal Care and Use Committee prior to institution of any study-related procedures (Duke IACUC #A006-15-01).

Test organism

An isolated colony of *S. aureus* MRSA 252 [12], a well characterized and widely used MRSA isolate, was incubated overnight in tryptic soy broth at 37°C/220 RPM. The following day, a subculture was permitted to grow until the bacteria reached the log-phase of growth (approximately 2 hrs.). Cells were then washed twice in phosphate buffered saline (PBS) and re-suspended in PBS containing 20% glycerol at a concentration of ~10^6 cfu/μL. The suspension was divided into 1 mL glycerol stock aliquots, and stored at -80°C. Bacterial inoculations were derived from frozen glycerol stock, and re-suspended at 5×10^5 cfu in 300 μL sterile PBS.

Animal model

The overall experimental design is illustrated in figure 1. Ninety 6-8-week-old male New Zealand white rabbits (~2.5 kg) were acquired from Robinson Services Inc. (Mocksville, NC, USA). Following a
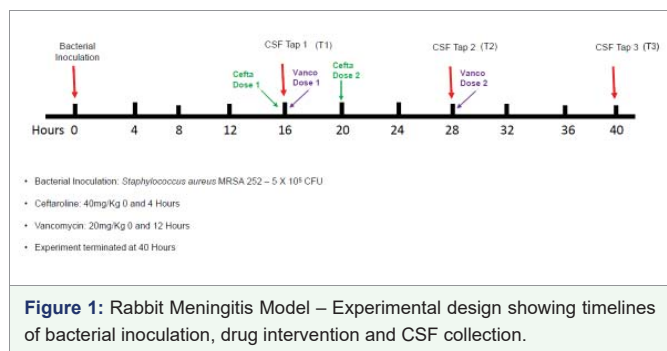
Figure 1: Rabbit Meningitis Model – Experimental design showing timelines of bacterial inoculation, drug intervention and CSF collection.

week of habituation, animals were anesthetized with an intramuscular injection of ketamine (38 mg/kg of body weight) and xylazine (5 mg/kg of body weight) prior to each intracisternal procedure. Animals were assigned to treatment groups 1:1:1 using a random number generator in Microsoft Excel (Microsoft Corp., Redmond, WA) at the beginning of the experiment. Each animal received an intracisternal inoculation of MRSA 252 at 0 hours, following the method of Perfect and Durack [11]. At 16 Hours Post-Inoculation (HPI), we collected the first CSF sample followed immediately by antibiotic administration (timepoint 1[T1]). We chose an inoculation dose of 5 X 10^5 cfu in order to attain sufficient bacterial load without resulting in unacceptable mortality, which meant that most animals (regardless of treatment) cleared infection by the terminal time point. Dosing regimens for ceftaroline and vancomycin were based on the published literature [6], and our experience in clinical practice in human beings, respectively. Commercial vials of ceftaroline fosamil for injection for human use (Teflaro®-Allergan, Madison, NJ, USA) 600 mg/vial was reconstituted with sterile water for injection, USP to a final volume of 100 mg/ml. Vancomycin hydrochloride USP (Sigma-Aldrich, St. Louis, MO, USA) was reconstituted in sterile water for injection, at a final concentration of 50 mg/ml. Ceftaroline (40 mg/kg dose repeated at 0 and 4 hours) or vancomycin (20 mg/kg dose repeated at 0 and 12 hours) were administered via the marginal ear vein. Control animals received no treatment. Additional CSF samples were similarly collected at 28 HPI (timepoint 2 [T2]), and at the terminal time point of 40 hours HPI (timepoint 3 [T3]). Blood samples were taken at T1 and T3 to identify potential secondary blood infection. At T3, animals were humanely euthanized using an intravenous injection of 1 mL Euthasol.

Quantification of bacterial burden

All CSF samples were plated following a serial dilution (50 μL). Bacterial load was determined by manual counting after 24 hours, and expressed as colony forming units (CFU) /ml. Blood samples were serially plated for culture.

CSF antibiotic concentrations

CSF samples (approximately 50 μl) were analyzed by the Duke Cancer Institute's Pharmacokinetic/Pharmacodyamic Core Laboratory at various timepoints (T1 as negative controls as well as T2 and T3) to determine ceftaroline and vancomycin concentrations using LC/MS/MS Assay. Briefly, ceftaroline T2 samples were diluted 1/50 and vancomycin T2 and T3 samples 1/10 with artificial CSF. A 20 μL aliquot of the sample was transferred to a polypropylene autosampler vial containing a 20 μL aliquot of aminopterin (internal standard), vortexed, centrifuged at 6000 g for 5 minutes, and placed in the refrigerated autosampler for analysis.

Agilent 1200 series LC system interfaced with Applied Biosystems/SCIEX API 5500 QTrap hybrid triple quadrupole-linear trap MS/MS spectrometer equipped with electrospray ionization (ESI) source was used for analysis. Analyst (version 1.6.2) software was used for mass tuning, data acquisition, and quantification. The assay compounds were individually infused as 100 nM solutions in 50%A/50%B at 10 μL/min flow rate and internal ion path parameters optimized to provide maximal ion count for "parent" and collision-produced ("daughter") MS/MS ions. Samples were prepared by adding pure standard of the measured compound to artificial CSF. Lower limit of quantification (LLOQ) at 80% accuracy limit for both vancomycin and ceftaroline was 0.81 ng/mL from 20 μL CSF sample. The response of the peak area standard/internal standard to nominal concentration was linear with r^2 = 0.998 or better.

Data analyses

Only animals with CSF samples meeting the minimum criteria of 10^3 CFU/mL at T1 who successfully underwent CSF collection at T3 were included in the final analyses. For the primary endpoint, the difference in bacterial load for each animal between T1 and T3 were characterized and compared between groups. Absolute bacterial loads as well as proportional changes between T1 and T2 and between T2 and T3 were examined. Descriptive statistics were performed for both bacterial loads, changes in these loads and drug concentrations. Differences in these measures by treatment group and specific time points were assessed for statistical differences using the Kruskal-Wallis test. These tests were performed for all three treatment groups together and for pairwise comparisons of the treatment groups. Drug concentrations were characterized at two time points (T2 and T3) using descriptive statistics.

RESULTS

Of the ninety animals used in this experiment, forty-three met our minimum criteria for inclusion, with fourteen animals analyzed in each of the control and ceftaroline groups and fifteen in the vancomycin group. CSF bacterial loads at T1, T2 and T3 were comparable among all three treatment groups (T1: $p = 0.16$, T2: $p = 0.17$ and T3: $p = 0.47$; Table 1). Reductions in bacterial load from baseline were 88%, 79%, and 75% in the control, ceftaroline and vancomycin-treated groups, respectively (Table 1). No statistically significant differences occurred at any time point between controls or those receiving either vancomycin or ceftaroline (T1: $p = 0.16$, T2: $p = 0.17$ and T3: $p = 0.47$; Table 1). The rate of bacterial clearance among the three groups approached significance between T_1 and T_2 ($p = 0.063$) with ceftaroline clearing at a much faster rate than control ($p = 0.019$), finally reaching statistically not significant difference by T3 ($p = 0.43$). The bacterial clearance by ceftaroline was reversed

Table 1: Change in CFU at time points T1, T2 and T3 for all three treatments (vancomycin, ceftaroline and control).

Treatment (N)	Median T_1 (log_{10})	Median %Δ T_2-T_1	Median %Δ T_3-T_2	% Reduction in Median CFU From Initial to Final
Control (14)	3.84	1.23	- 0.80	88
Ceftaroline (14)	4.20	- 0.93*	0.98	79
Vancomycin (15)	3.68	- 0.37	- 0.61	75

Unless otherwise noted, comparisons with and between groups are not statistically significant
*Ceftaroline cleared significantly more than Control (p = 0.019).

by end of T_2-T_3, period with CSF bacterial counts increasing in the ceftaroline group, while the control group continued to clear bacteria ($p = 0.0039$). This result was partly explained by a notable drop in ceftaroline concentrations from T_2 to T_3, while the vancomycin group maintained relatively high drug concentrations (Figure 2).
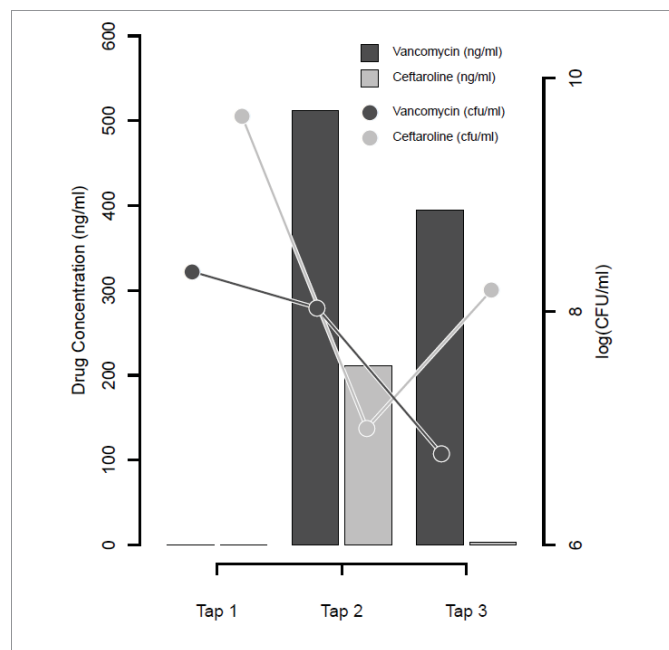

Figure 2: Median CSF bacterial loads (CFU/mL) and antibiotic concentration in animals treated with Vancomycin (Black) and ceftaroline (Gray). Drug concentrations (ng/mL) are represented by bars, and bacterial loads are represented by lines represent (CFU/mL).

DISCUSSION

Nosocomial bacterial meningitis caused by MRSA is a devastating disease [1], and the standard treatment is vancomycin [2]. Vancomycin is associated with inconsistent and poor penetration into the cerebral spinal fluid (CSF), is inconsistent and (at times) poor [3]. In this study, we attempted to explore alternative treatments for MRSA meningitis using ceftaroline and rabbit meningitis model. We compared CSF bacterial clearance over different time points among the rabbits treated with ceftaroline, vancomycin and control groups. In consistence with the previous studies, we demonstrated a very rapid bacterial clearance from the CSF in rabbits [13], suggesting that shorter time points would potentially help to properly assess bactericidal potential of newer antibacterial agents. We noticed a much faster bacterial clearance in ceftaroline treated rabbits compared to control between T_1 and T_2 ($p = 0.019$) however, it reached statistically not significant difference by T3 ($p = 0.43$). This result was partly explained by a notable drop in ceftaroline concentrations from T_2 to T_3, while the vancomycin group maintained relatively high drug concentrations (Figure 2). Others have demonstrated a similarly short half-life of ceftaroline in rabbit CSF compared to humans [14], suggesting that additional or higher doses of ceftaroline are necessary for persistent necessary drug exposure in the rabbit model.

This study has several limitations. First, as about fifty percent of the rabbits included didn't meet the inclusion criteria, we didn't have substantially enough rabbits for a robust statistical comparison among the treatment groups. Second, the lack of prior information

about the natural clearance of *Staphylococcus aureus* by rabbits complicated the study. Despite these limitations, using a very challenging rabbit meningitis model, this study is able to provide some useful information about the potential use of ceftaroline in selected MRSA meningitis patients unable to take vancomycin, or for patients in whom vancomycin has failed [8,9]. Future work could consider developing a swine meningitis model, as pigs are physiologically similar to humans. Additionally, implantation of a CSF reservoir would be easier in the swine model, allowing for increased sampling.

We observed comparable early central nervous system action against MRSA meningitis between ceftaroline and vancomycin in our animal model. Further work is needed to define optimal ceftaroline dosing in both the animal model of CSF infection as well as for use in selected MRSA meningitis patients unable to take vancomycin, or for patients in whom vancomycin has failed [8,9].

CONFLICT OF INTERESTS

V.G.F. served as Chair of the V710 Scientific Advisory Committee (Merck); has received grant support from Cerexa/Actavis/Allergan, Pfizer, Advanced Liquid Logics, National Institutes of Health (NIH), MedImmune, Basilea Pharmaceutica, Karius, ContraFect, Regeneron Pharmaceuticals and Genentech; has been a consultant for Achaogen, AmpliPhi Biosciences, Astellas Pharma, Arsanis, Affinergy, Basilea Pharmaceutica, Bayer, Cerexa, ContraFect, Cubist, Debiopharm, Destiny Pharmaceuticals, Durata Therapeutics, Grifols, Genentech, MedImmune, Merck, The Medicines Company, Pfizer, Novartis, NovaDigm Therapeutics, Theravance Biopharma, XBiotech, Integrated BioTherapeutics, and C3J; and has received honoraria from Theravance Biopharma and Green Cross; and has a patent pending in sepsis diagnostics.

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BKH, BW and BKSK carried out the experiments. BKSK, VGF and RHD conceptualized and designed the experiments. DLT, CG and JRP provided trainings to develop the rabbit meningitis model. LPP carried out the statistical analysis. BKH, BKSK, RHD and VGF wrote the manuscript.

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