In Vitro and *In Vivo* Activity of First Generation Cephalosporins Against *Leptospira*

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Abstract. Third generation cephalosporins are commonly used in the treatment of leptospirosis. The efficacy of first generation cephalosporins has been less well-studied. Susceptibility testing of 13 *Leptospira* strains (11 serovars) to cefazolin and cephalixin was conducted using broth microdilution. Median minimal inhibitory concentration (MIC) for cefazolin and cephalixin ranged from < 0.016 to 2 µg/mL (MIC₉₀ = 0.5 µg/mL) and from 1 to 8 µg/mL (MIC₉₀ = 8 µg/mL), respectively. Efficacy of cefazolin and cephalixin in an acute lethal hamster model of leptospirosis was studied. Survival rates for cefazolin were 80%, 100%, and 100%, and survival rates for cephalixin were 50%, 80%, and 100% (treated with 5, 25, and 50 mg/kg per day for 5 days, respectively). Each treatment group showed improved survival compared with no treatment ($P < 0.01$), and none of the therapies, regardless of dose, was statistically significantly different than doxycycline. These results support a potential role for first generation cephalosporins as alternative therapies for leptospirosis.

INTRODUCTION

Leptospirosis is an acute febrile illness caused by spirochetes of the genus *Leptospira*. In humans, leptospirosis typically manifests as a self-limiting acute febrile syndrome, but it may progress to life-threatening disease. Leptospirosis has emerged as a growing threat to human health, with disease increasing in incidence in rural and urban environments in both undeveloped and developed countries.¹ Outbreaks of leptospirosis occur after flood or natural disasters but also in association with increasing rat burden in the slums of large cities.^{1–4}

Leptospirosis has been traditionally treated with intravenous penicillin in severe cases and oral doxycycline in milder presentations.⁵ Guidelines produced by the International Leptospirosis Society and World Health Organization in 2003 recommended high-dose intravenous penicillin for severe disease and oral amoxicillin, ampicillin, doxycycline, or erythromycin for less severe disease.⁶ Subsequent clinical trials from Thailand support the use of ceftriaxone, cefotaxime, and azithromycin as well in this zoonotic infection.^{7–9}

Intravenous third generation cephalosporins have become the preferred agents for severe leptospirosis based on their proven efficacy, ease of use, availability, and ability to cover other etiologies of acute febrile illness when the diagnosis is in doubt. The potential efficacy of other cephalosporins in leptospirosis is less clear. Work published in 1966 using cephalothin, a first generation cephalosporin no longer in common use, showed that high doses of the drug could effectively protect the animals against experimental leptospirosis.¹⁰ However, when this work was repeated 20 years later by Alexander and Rule,¹¹ first generation cephalosporins (cefadroxil and cephalixin), even in high doses, were not shown to be effective against leptospirosis in their hamster model. Their work did show a survival benefit for second (cefamandole) and third (cefoperazone, cefotaxime, and moxalactam) generation cephalosporins. Employing the broth microdilution susceptibility testing technique developed in our laboratory, we have

previously shown that most second, third, and fourth generation cephalosporins produce low minimal inhibitory concentrations (MICs) against a wide range of *Leptospira* serovars (Table 1).^{12–14}

In this study, we evaluate the activity of two commonly used first generation cephalosporins, cefazolin and cephalixin, both *in vitro* in our broth microdilution susceptibility testing system and *in vivo* in an acute hamster model of leptospirosis.

MATERIALS AND METHODS

Antimicrobial agents. Parenteral formulations of doxycycline (Bedford Laboratories, Bedford, OH) and cefazolin (West-Ward Pharmaceutical Corporation, Eatontown, NJ) were purchased from their manufacturers for use in animal studies. Research-grade cephalixin (for use in animals and *in vitro*), cefazolin, and doxycycline were purchased in powder form from Sigma-Aldrich (St. Louis, MO). All drugs for animal studies were diluted to appropriate concentrations to allow for administration of each dose in a volume of 0.5 mL (5 mg/kg doxycycline or 5, 25, or 50 mg/kg cephalixin or cefazolin based on mean animal weight per group). All reconstituted drug solutions were stored at 4°C in the dark between uses, and they were allowed to come to room temperature before administration.

***In vitro* broth microdilution susceptibility assay.** Susceptibility testing was performed as previously described using serial twofold dilutions of antibiotics (32.0–0.016 µg/mL) in Ellinghausen–McCullough–Johnson–Harris (EMJH) medium (Becton Dickinson, Sparks, MD).^{12–14} The following 13 strains of *Leptospira* were tested against each antimicrobial agent in triplicate: *L. borgpetersenii* serovar Ballum (strain S102), *L. interrogans* serovars Australis (strain Ballico), Autumnalis (strain Akiyami A), Bataviae (strain BAMC 141), Canicola/Portlandvere (strain CA-12-029/CDC Ni1808), Copenhageni (strain M20), Icterohaemorrhagiae (strains RGA, BAMC 132, and BAMC 215), new unnamed (strain BAMC 118), *L. kirschneri* serovar Grippotyphosa (strain BAMC 230), *L. noguchii* serovar Fortbragg (strain Fort Bragg), and *L. weilii* serovar Celledoni (strain Celledoni). Strains BAMC 118 (Thailand), 132 (Egypt), 141 (Egypt), 230 (Egypt), and 215 (Hawaii) are recently obtained clinical (blood) isolates.

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TABLE 1

Susceptibility of *Leptospira* strains to second, third, fourth, and fifth generation cephalosporins by broth microdilution testing

Drug (generation)	MIC ₅₀ (MIC range)	Strains (serovars) tested	Reference
Cefoxitin (2nd)	0.1 (<0.01–0.2)	26 (26)	13
Cefdinir (3rd)	3.13 (0.39–6.25)	26 (26)	13
Cefotaxime (3rd)	0.1 (<0.01–0.2)	12 (12)	12
Cefotaxime (3rd)	0.1 (<0.01–0.1)	26 (26)	13
Cefotaxime (3rd)	0.03 (<0.016–0.03)	13 (10)	14
Ceftriaxone (3rd)	0.39 (<0.01–0.78)	26 (26)	13
Ceftriaxone (3rd)	0.06 (<0.016–0.06)	13 (10)	14
Ceftazidime (3rd)	8 (2–8)	11 (10)	Unpublished data
Cefepime (4th)	<0.01 (<0.01–0.02)	26 (26)	13
Cefepime (4th)	<0.016 (all <0.016)	13 (10)	14
Ceftaroline (5th)	<0.016 (<0.016–0.03)	11 (10)	Unpublished data

MIC values are given in micrograms per milliliter.

TABLE 2

Susceptibility of 13 *Leptospira* strains to first generation cephalosporins by broth microdilution testing

Species	Serovar	Median MIC (µg/mL)	
		Cefazolin	Cephalexin
<i>L. borgpetersenii</i>	Ballum	0.25	4
<i>L. interrogans</i>	Australis	0.5	2
<i>L. interrogans</i>	Autumnalis	0.25	2
<i>L. interrogans</i>	Bataviae*	0.25	8
<i>L. interrogans</i>	Canicola/Portlandvere†	2	8
<i>L. interrogans</i>	Copenhageni	0.25	8
<i>L. interrogans</i>	Icterohaemorrhagiae	0.25	2
<i>L. interrogans</i>	Icterohaemorrhagiae*	0.25	2
<i>L. interrogans</i>	Icterohaemorrhagiae*	0.0625	1
<i>L. interrogans</i>	Unknown*	0.25	2
<i>L. kirschneri</i>	Grippotyphosa*	0.25	2
<i>L. noguchii</i>	Fortbragg	0.125	2
<i>L. weilii</i>	Celledoni	<0.016	2

MIC₅₀/MIC₉₀ was 0.25/0.5 for cefazolin and 2/8 for cephalexin.

* Recent human clinical (blood) isolates.

† Animal model infecting strain.

Animal model. Using our previously developed model, 74 female golden Syrian hamsters (*Mesocricetus auratus*) weighing 80–110 g (Harlan, Indianapolis, IN) were inoculated intraperitoneally (IP) with 0.5 mL EMJH medium containing 10⁵ leptospires (*L. interrogans* serovar Canicola/Portlandvere strain CA-12-029/CDC Ni 1808).^{15–17} On study days 2–6, groups of 10 animals were treated by daily IP injection with cefazolin or cephalexin in concentrations of 5, 25, or 50 mg/kg. A group of five hamsters was left untreated as a negative control, and a group of nine hamsters was treated with doxycycline (5 mg/kg, IP, daily) as positive control.¹⁶ All hamsters were observed multiple times a day during the 21-day study period, and any study animal showing significant pain or distress or characteristics of a moribund state was humanely euthanized. Blood cultures were obtained from each hamster at the time of death. Study was conducted as approved by our Institutional Animal Care and Use Committee and monitored by the facility veterinarian to ensure compliance with ethical standards of animal care.

Statistical analysis. SPSS version 18.0 was used to create Kaplan–Meier plots for each study group. Survival differences between study groups were compared by the log rank test. *P* values < 0.05 were considered significant.

RESULTS

Broth microdilution susceptibility testing revealed consistently lower MICs with cefazolin compared with cephalexin (Table 2). Median cephalexin MICs were 1–8 µg/mL for the strains tested. Both cefazolin and cephalexin improved survival in hamsters with acute leptospirosis compared with untreated controls (*P* < 0.01) (Figures 1 and 2). The overall survival for each of the cefazolin and cephalexin groups was not significantly different from the survival of the doxycycline control group (*P* > 0.05). As expected, all untreated animals died by day 8 after infection, whereas 89% (8/9) of doxycycline controls survived to day 21. On the final day of the study, the remaining animals in the doxycycline control group were noted to be well and without evidence of disease. Through the observation period, these remaining animals maintained or gained weight appropriately. Blood from all untreated hamsters grew *Leptospira* in culture, but blood from the doxycycline-treated controls did not. This group included the doxycycline group animal that was euthanized on day 13 of the study. Animals

in the cefazolin treatment groups remained healthy until halfway through the treatment period, at which time two animals in the 5-mg/kg group required euthanasia. Blood cultures from one of two animals that did not survive to day 21 grew *Leptospira*. Although five animals in the low-dose (5 mg/kg) cephalexin treatment group did require euthanasia because of their moribund appearance by day 13, the animals in this treatment group that survived until the end of the study had recovered enough to seem healthy and behaved appropriately. At the time that the study was terminated, all 8 surviving animals in the cephalexin 25-mg group and 10 animals in the 50-mg group appeared healthy, had maintained weights, and were appropriately interactive. Blood cultures of three hamsters that did not survive to day 21 grew *Leptospira* (two in the 5-mg/kg treatment group and one in the 25-mg/kg treatment group).

DISCUSSION

Based on recent clinical trials, the third generation cephalosporins, cefotaxime and ceftriaxone, have emerged as preferred drugs for the treatment of severe human leptospirosis. The usefulness of other cephalosporins is not as clear. Ceftiofur, a third generation drug used in veterinary medicine, has been shown to be effective in experimental disease in cattle, but this drug was unable to clear chronic infection in swine.^{18,19} We have previously shown many of these antimicrobials to produce low MICs *in vitro* (Table 1), and in this study, we show that the first generation cephalosporins, cefazolin and cephalexin, have low MICs as well (Table 2). These MICs seem to translate to efficacy for both these agents in our animal model of acute leptospirosis. For cefazolin, these data are quite supportive of probable efficacy in human disease. The MICs produced are many dilutions below the levels of cefazolin achievable with intravenous dosing²⁰ and well below established breakpoints for cefazolin against the Enterobacteriaceae and *Staphylococcus* species (susceptibility MIC ≤ 8 µg/mL).²¹ The clinical applicability for cephalexin is more difficult to ascertain with these results. The MICs noted are much higher for cephalexin than seen for cefazolin, and achievable levels of this typically oral antimicrobial are much lower than those levels seen with parenterally dosed cefazolin.²² The use of intraperitoneal dosing

FIGURE 1. Survival of hamsters treated for 5 days with cefazolin (5, 25, or 50 mg/kg one time daily). Controls include untreated animals and those animals treated with doxycycline (5 mg/kg one time daily) for 5 days.

in our animal model may have unrealistically improved the efficacy of cephalixin. Animals treated in the study by Alexander and Rule¹¹ with single oral doses of cephalixin (10, 40, or 160 mg/kg) did not survive. Lack of survival with the 160-mg/kg dose was attributed to colitis, but clearly, the

10- and 40-mg/kg doses did not rescue animals; however, lack of survival might be attributed to the fact that only a single dose was given in their study.

The proper role of first generation cephalosporins in the treatment of leptospirosis is not clear. Cefazolin could be

FIGURE 2. Survival of hamsters treated for 5 days with cephalixin (5, 25, or 50 mg/kg one time daily). Controls include untreated animals and those animals treated with doxycycline (5 mg/kg one time daily) for 5 days.

used in penicillin-allergic patients who tolerate cephalosporin, especially in circumstances where third generation cephalosporins are unavailable. Patients who receive cefazolin for other indications before suspicion of leptospirosis can be assumed to have been effectively treated with this drug. When and whether to use oral cephalexin is much less clear. In the absence of other agents with known efficacy, one might treat patients with the drug, but based on our results, we do not recommend cephalexin for the therapy of leptospirosis when other agents are available.

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