Efficacy of Short-Course Ceftriaxone Therapy for *Borrelia burgdorferi* Infection in C3H Mice

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Ceftriaxone is highly effective clinically in patients with Lyme disease. We studied a representative invasive human isolate of *Borrelia burgdorferi* for which the MBC of ceftriaxone was 0.050 μg/ml. A once-per-day dosage regimen of ceftriaxone (50 mg/kg/dose) administered intramuscularly for 5 days was 100% effective in sterilizing tissue samples of C3H mice infected with this strain of *B. burgdorferi*, regardless of whether the mice were being treated concomitantly with a corticosteroid. Administration of the same five doses of ceftriaxone at 6-h intervals over just 24 h was also 100% effective. These experiments suggest that shorter courses of antibiotics than those currently recommended should be considered for study in patients with early uncomplicated Lyme disease.

Lyme disease caused by the spirochete *Borrelia burgdorferi* is the most common vector-borne infection in North America. In vitro, *B. burgdorferi* is susceptible to the lethal action of several different antibiotic classes. For example, the MBC of the expanded-spectrum cephalosporin ceftriaxone is usually ≤0.4 μg/ml and this agent is also effective clinically (2, 6). While most authoritative sources recommend treatment regimens for patients with Lyme disease similar to that employed for other spirochetal diseases, duration of therapy is nonetheless a topic of controversy (20, 21). The treatment durations recommended in the Infectious Diseases Society of America Practice Guidelines on the Treatment of Lyme Disease range from 14 to 28 days, which is considerably longer than those used successfully to treat patients with infection due to relapsing-fever borrelia (one dose to 10 days of therapy) (4, 19).

Unlike most other spirochetal microorganisms, *B. burgdorferi* can be readily cultured in vitro and a variety of rodents and other laboratory animals are suitable for experimental studies. In animal experiments, 5 to 10 days of treatment of rodents (7–9) and 30 days of therapy of dogs (14) using the appropriate dosage and type of antibiotic cure the infection on the basis of the inability to recover *B. burgdorferi* by culture of postmortem tissue samples. Shorter courses of treatment, however, have not been systematically studied.

The purpose of this study was to determine if ceftriaxone therapy of mice could be shortened to a time period estimated to achieve a drug level in serum in excess of the MBC for *B. burgdorferi* for a time period shorter than that which would follow a single parenteral dose given to humans. The second objective was to determine if immunosuppressive doses of corticosteroids affect the activity of short-course therapy with ceftriaxone in vivo.

**MATERIALS AND METHODS**

**Animals.** Female C3H mice (4 to 5 weeks old) were obtained from Charles River Laboratories (Wilmington, Mass.). All mice were housed in a filtered-air environment maintained at 20 ± 2°C. Antibiotic-treated and non-antibiotic-treated (control) mice were kept in separate cages for the infectivity experiments.

**Bacteria and bacterial cultures.** A strain of *B. burgdorferi* designated BL206 was used for these experiments. BL206 was isolated from the blood of a patient who presented with erythema migrans. This organism was maintained by passing in Barbour-Stoenner-Kelly (BSK) medium and used for the infectivity experiments (see below) after appropriate dilutions were made in BSK medium. The organism had been passaged between five and eight times.

The MIC and MBC of ceftriaxone for BL206 were determined by a modified microplate dilution assay (13). Triplicate wells contained 5 × 10^3 *B. burgdorferi* bacteria in BSK medium with and without diluted ceftriaxone (Roche, Nutley, N.J.). After incubation for 48 h, wells were examined by dark-field microscopy and surviving *Borrelia* bacteria were enumerated as previously described (12).

**Induction of immunosuppression.** At intervals designated to coincide with antibiotic treatment (described below), one group of mice was given cortisone acetate (Sigma, St. Louis, Mo.) once daily at a dose of 100 mg/kg of body weight for 5 days. The cortisone acetate was suspended and diluted in sterile saline (Abbott Laboratories, North Chicago, Ill.) and injected subcutaneously in a volume of 0.1 ml. The dosage and treatment regimens used here were similar to those used previously by others (3, 22) to immunocompromise mice during experimental *Chlamydia* or *Legionella* infection.

**Infectivity experiments.** Mice infected with BL206 were treated with (i) ceftriaxone (Roche) over a 5-day period, (ii) ceftriaxone over a 24-h period, or (iii) both cortisone acetate and ceftriaxone for 5 days. Control animals included mice infected with BL206 that were treated with only sterile saline or cortisone acetate.

Mice were infected by injection with a tuberculin syringe intradermally in the abdominal area with 100,000 *B. burgdorferi* bacteria in a volume of 0.1 ml of BSK medium. At 14 to 21 days later, the mice were given a single daily dose of either saline, ceftriaxone (50 mg/kg/dose), cortisone acetate (100 mg/kg/dose), or ceftriaxone (50 mg/kg/dose) plus cortisone acetate (100 mg/kg/dose) for 5 days. The saline and ceftriaxone injections were given in a volume of 0.1 ml by the intramuscular route. In a separate experiment, one group of infected mice received the five doses of ceftriaxone (50 mg/kg/dose) over a 24-h period, with each dose given once every 6 h (i.e., at 0, 6, 12, 18, and 24 h). Treatment was initiated at a time point at which infection with BL206 is known to be widely disseminated in C3H mice on the basis of the results of our prior investigations (17).

Five to 7 days after completion of the saline, ceftriaxone, or cortisone acetate treatment, each mouse was humanely euthanatized and exsanguinated and urinary bladder and ear tissue samples (about 7 by 10 mm) were taken to ascertain the infection status of each of the infected and treated mice (13). Separate extracts of excised urinary bladders and ears (collected aseptically) were pre-
pared by mincing the tissues finely with scissors and forceps and suspending them in a small volume (0.3 to 0.4 ml) of BSK medium. After the heavy tissue particles settled out, each of the extract suspensions was added to separate snap-cap tubes containing 4 ml of BSK medium. Tubes were incubated at 33°C, and the cultures were examined microscopically (by dark-field or phase-contrast microscopy) at 2- and 4-week intervals for the presence of live, motile spirochetes.

**CEFTRIAXONE MEASUREMENTS IN MOUSE PLASMA AND PHARMACOKINETIC ANALYSIS**

Separate groups of age- and sex-matched C3H mice were evaluated for levels of ceftriaxone in plasma at various times after administration of the 24-h ceftriaxone treatment regimen. Whole blood was collected via the ventral tail vein or by cardiac puncture using a tuberculin syringe and placed into heparinized microcentrifuge tubes at 1, 2, 3, 4, 5, 6, and 7 h after administration of the last of the five intramuscular injections of ceftriaxone. About 0.5 to 0.7 ml of plasma per mouse was transferred to a screw-cap 2.0-ml Micro tube (Sarstedt, Newton, N.C.) after centrifugation of the whole blood at 8,000 × g for 5 min. The nonpooled plasma samples were frozen immediately at −70°C until tested. At least three plasma samples were collected at each time point. Ceftriaxone levels were determined by a high-performance liquid chromatographic procedure (1) done by a commercial laboratory (Department of Laboratories, Barnes-Jewish Hospital, St. Louis, Mo.). The lower limit of detection by the assay was 0.2 μg/ml.

**RESULTS**

According to a microplate dilution assay, the MIC and MBC of ceftriaxone for the *B. burgdorferi* BL 206 isolate studied were 0.025 and 0.050 μg/ml, respectively. The first set of in vivo experiments was designed to determine whether a 5-day course of ceftriaxone is efficacious in mice with early-stage disseminated *B. burgdorferi* infection and whether coadministration of cortisone acetate would interfere with the antimicrobial effects of ceftriaxone therapy. As shown in Table 1, 5 days of therapy with ceftriaxone was highly effective in eliminating borrelial infection due to the invasive human isolate BL 206 and was significantly more effective than saline treatment (*P < 0.001* [Fisher’s exact test, two tailed]). Concomitant administration of cortisone acetate did not impair antibiotic efficacy. Live spirochetes could not be recovered from the urinary bladders or ears of any of the steroid-treated mice that received ceftriaxone.

The second set of experiments was designed to determine whether short-term treatment with ceftriaxone (five doses administered over a 24-h period) is as effective as the above-described 5-day treatment regimen in eradicating viable *B. burgdorferi* in mice at an early stage of infection. As shown in Table 2, ceftriaxone administered over 24 h was significantly more effective than saline in eliminating viable *B. burgdorferi* (*P = 0.002* [Fisher’s exact test, two tailed]). There was no difference in efficacy between the 24-h and 5-day treatment courses; 100% of the evaluate mice in both groups had negative bladder and ear tissue cultures.

Ceftriaxone was detectable in plasma at all of the time points studied, and the levels are plotted in Fig. 1. The data were best fitted by a two-compartment model. A least-squares analysis was carried out with a total of 26 datum points; the mean ceftriaxone level in plasma and the standard error for each time point are presented. The half-time of elimination for the first phase of distribution and elimination (t_{1/2b}) was 29 min. The terminal elimination half-time (t_{1/2t}) was 200 min.

**DISCUSSION**

This study has demonstrated that five doses of ceftriaxone given over either 24 h or 5 days cures *B. burgdorferi* infection in C3H mice, as defined by the absence of positive cultures in two key tissue sites 5 to 7 days after completion of therapy. Five days of therapy was effective regardless of whether the mice were immunosuppressed with corticosteroids. Unlike most prior studies on the effects of antibiotics on laboratory animals, our study involved an invasive human strain of *B. burgdorferi* initially recovered from the blood of a patient with erythema...
migrants. The MIC (0.025 µg/ml) and MBC (0.050 µg/ml) of ceftriaxone for this isolate, however, are similar to those reported for other strains of *B. burgdorferi* (10).

The lack of a detrimental effect of corticosteroid treatment on the success of a 5-day course of therapy was consistent with the findings reported by Kazragis et al. (7), who showed that a 9-day course of ceftriaxone reliably eradicated *B. burgdorferi* infection in mice with severe combined immunodeficiency (SCID mice).

The every-6-h dosage regimen was evaluated because the *t*₁/₂ of ceftriaxone in mice was believed to be ≤1 h on the basis of several reports (5, 11, 15, 16). Our data, however, suggest that the pharmacokinetics of ceftriaxone in C3H mice may be more complicated than previously believed and are consistent with a two-compartment model in which the *t*₁/₂ of the first phase is short (29 min) but in which there is a more prolonged terminal *t*₁/₂ of approximately 200 min. Some caution may be warranted in the interpretation of these results since, despite the relatively good fit of the data by least-squares analysis, certain of the individual-animal datum points are close to the sensitivity limit of the analytical method used. Supporting evidence for two-compartment kinetics exists for mice treated with other cephalosporins. In a study by van Ogtrop et al. (15) in which drug concentrations in plasma were measured for a 4-h period after dosing, cefoperazone, ceftazidime, and cefepime showed evidence of two-compartment elimination. Ceftriaxone, also evaluated in the Ogtrop study (15), did not show evidence of two-compartment elimination over the 4-h period, but our data demonstrate that the slower elimination phase could be missed unless datum points are extended beyond 4 h. On the basis of our analysis, administration of five doses of ceftriaxone over a 24-h period should result in levels of ceftriaxone in mouse plasma in excess of the MBC for the *B. burgdorferi* strain used in this study for just under 60 h, which is a shorter time period than would be expected for intramuscular administration of a single 125-mg dose of ceftriaxone to humans (data not shown). This is attributable to the approximately 7-h *t*₁/₂ of ceftriaxone in adult humans (11).

A limitation of this study is that only a single strain of *B. burgdorferi* was studied and therefore, before generalizing conclusions can be drawn, additional strains should be investigated. In addition, only a single class of antibiotic and only mice with recent *B. burgdorferi* infection were studied. In prior reports, however, a 5-day course of ceftriaxone eradicated *B. burgdorferi* infection in C3H mice independently of when the infection occurred and for as long as 90 days after the onset of infection (8).

Extrapolation from studies with laboratory animals to humans should be done with caution. However, it is interesting that Weber et al. (18) successfully treated a group of German patients with erythema migrans with single daily 1-g doses of ceftriaxone administered for just 5 days. Rigorous clinical trials are needed to determine more precisely the appropriate duration of antibiotic therapy for patients with early uncomplicated Lyme disease.

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REFERENCES


