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Effectiveness of Antimicrobial Treatment against *Borrelia burgdorferi* Infection in Mice

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Although antimicrobial agents are effective therapy for early Lyme disease, optimal treatment schedules have not been conclusively established. The efficacy of various dosages of eight antibiotics used for borreliosis treatment was evaluated for C3H/HeNCrlBR mice, which reproducibly develop persistent infection, arthritis, and carditis when inoculated with *Borrelia burgdorferi*. Amoxicillin-clavulanic acid, ceftriaxone, and high-dose penicillin G effectively eliminated infection and disease. Oxytetracycline, doxycycline, chloramphenicol, erythromycin, and azithromycin failed to cure infected mice. There was a correlation between peak serum antibiotic concentrations in mice, as determined by agar well diffusion bioassays, and therapeutic levels in humans. When experimentally inoculated mice were treated at 1 week postinfection with ceftriaxone (16 mg/kg of body weight twice daily for 5 days) and monitored for up to 90 days, all treated mice were free of spirochetes and had no gross or histologic lesions. This antibiotic regimen at days 7 to 11 postinoculation eliminated the spirochetes so that there were no relapses during the 90-day observation period. For experimentally inoculated mice treated with ceftriaxone at 7 or 14 days postinfection, arthritis, carditis, and infection were eliminated. When treatment began at 30 and 90 days after inoculation, infection and active cardiac and arthritic lesions were eradicated; however, residual mild synovitis and vasculitis persisted in some mice. In comparison with inoculated, untreated mice, ceftriaxone therapy at 7, 14, 30, and 90 days postinfection abrogated the development of antibody titers against *B. burgdorferi*. Having the potential to determine the presence of the spirochete through culture and histologic lesions makes the *B. burgdorferi*-inoculated C3H mouse model a valuable adjunct in evaluating chemotherapeutic options for Lyme disease.

Caused by the tick-borne spirochete, *Borrelia burgdorferi*, Lyme disease elicits a wide array of clinical disorders in humans and domestic animals worldwide. Initially presenting with a characteristic rash (erythema migrans), untreated patients with Lyme borreliosis may subsequently develop neurologic disease, cardiac conduction abnormalities, or arthritis (50).

Although antibiotics are known to be effective in treating early Lyme disease, optimal treatment regimens have not been definitively established. Treatment recommendations are often based on stage of disease at presentation. Currently, the most commonly prescribed oral antibiotics for early Lyme disease include doxycycline, amoxicillin, and erythromycin (26, 43, 44). Late Lyme disease can be refractory to oral antimicrobial therapy; patients often receive parenteral penicillin or ceftriaxone (13, 26, 43, 44). Clinical Lyme disease is often characterized by a pattern of relapses and remissions; reinfection has also been described (36). This clinical picture in conjunction with the difficulties in confirming a diagnosis of Lyme disease also means that it is impossible to ascertain whether antimicrobial treatment has been truly successful.

To evaluate the effectiveness of antimicrobial therapy in a controlled environment, several laboratory animal models of Lyme disease have been studied. Hamsters (22–24) and gerbils (33, 41) have been used to assess the in vivo effectiveness of selected antibiotics against *B. burgdorferi*. In both situations, once-daily dosage schedules were used and culture results alone were used to determine clearance of *B. burgdorferi*. We have developed a mouse model for Lyme borreliosis in which C3H/HeNCrlBR (C3H) mice reproducibly develop infection and disease (arthritis and carditis) following intradermal (i.d.) inoculation with *B. burgdorferi* (3, 5). This study was undertaken to evaluate the effectiveness of eight antibiotic treatment regimens for eradicating *B. burgdorferi* infection and disease in infected mice. To evaluate systemic antibiotic absorption in treated mice, serum antibiotic concentrations were determined by an agar well diffusion bioassay. The second objective was to determine if the most effective antibiotic regimen was truly curative, as determined by examination for infection and lesions at various intervals posttreatment. The final goal was to evaluate the effectiveness of antimicrobial treatment in arresting lesion progression when the treatment was initiated at extended intervals postinfection.

MATERIALS AND METHODS

Mice. Three-week-old, specific-pathogen-free C3H mice were purchased from Charles River Laboratories (Raleigh, N.C.), shipped in filtered crates, and housed in Micro-Isolator cages (Lab Products, Maywood, N.J.). Autoclaved food (Agway, Syracuse, N.Y.) and water were provided ad libitum. Experimental protocols were initiated within 10 days after arrival. At the end of each experiment, the mice were killed with carbon dioxide and exsanguinated by cardiocentesis.

B. burgdorferi. The N40 isolate of *B. burgdorferi* was used. It is a low-passage (P2) tick isolate from Westchester County, N.Y., with proven pathogenicity in laboratory rodents and rabbits (7, 31, 32). The isolate was grown in modified Barbour-Stoenner-Kelly (BSK II) medium (2) at 34°C. Spirochetes were grown to a concentration of $\sim 10^5$ viable organisms per ml for inoculation.

For spirochete inoculation, mice were injected i.d. with 10^4 *B. burgdorferi* (N40) cells under methoxyflurane anesthesia

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(Metofane; Pitman-Moore, Inc., Mundelein, Ill.). This spirochete dose was previously shown to elicit infection and disease consistently in C3H mice (3). At necropsy, ear punch biopsies and 0.5 ml of 10% (wt/vol) homogenate of spleen from each mouse were cultured in 7 ml of BSK II medium (2). After incubation at 35°C for 14 days, cultures were examined for spirochetes by dark-field microscopy. We had previously determined that these two culture sites are adequate to determine the presence of spirochetes in infected mice when monitored for up to 1 year (6). Brain tissue is inconsistently culture positive and only in the earliest stages of infection, even in severe combined immunodeficient mice (6, 8).

Antimicrobial agents. The antimicrobial agents tested were penicillin G (Squibb-Marsam, Inc., Cherry Hill, N.J.), amoxicillin-clavulanic acid (Clavamox; SmithKline Beecham Animal Health, Exton, Pa.), chloramphenicol (Chloromycetin palmitate; Parke-Davis Division of Warner-Lambert Co., Morris Plains, N.J.), ceftriaxone (Rocephin; Hoffmann-La Roche Inc., Nutley, N.J.), oxytetracycline (Biomycin C; Bio-Ceutic, St. Joseph, Mo.), doxycycline (Elkins-Sinn, Inc., subsidiary of A. H. Robins Co., Cherry Hill, N.J.), erythromycin (Elkins-Sinn, Inc., subsidiary of A. H. Robins Co.), and azithromycin (Pfizer, Inc., New York, N.Y.). Antibiotics were reconstituted in the diluents recommended by their manufacturers.

Histopathology. At necropsy, heart and hind limb joints (knee, tarsus, metatarsus, and phalanges) were immersion fixed in 10% neutral buffered formalin (pH 7.2). Joints were demineralized in decalcifying solution (S/P decalcifying solution; Baxter Health Care Corp., McGaw Park, Ill.). Tissues were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin.

Characteristic arthritic lesions seen in untreated spirochete-inoculated mice included exudation of fibrin and neutrophils into joint spaces with synovial cell exfoliation, hypertrophy, and proliferation resulting in synovial thickening. Cardiac lesions in untreated inoculated mice consisted of mononuclear leukocyte, plasma cell, and neutrophil infiltration into connective tissue at the base of the heart and aortic adventitia, with inflammatory foci in the myocardium and pericardium (5, 31). In the present study, mouse arthritic lesions were graded on the basis of the degree of leukocyte and fibrin exudation and synovial proliferation. Cardiac lesions were assessed on the basis of the relative degree of inflammatory cell infiltration in the heart base tissues.

Serology. Serum immunoglobulin M (IgM) and IgG antibody titers to *B. burgdorferi* were determined with an enzyme-linked immunosorbent assay using *B. burgdorferi* N40 spirochetes as antigen (32).

Bioassay for serum antibiotic levels. For each antibiotic used, eight mice were treated with the highest experimental dose. Two mice were killed with carbon dioxide gas and exsanguinated at 15, 30, 60, and 120 min posttreatment. Sera were frozen at −70°C for later analysis.

Serum antibiotic levels were determined by the method described by Bennett et al. (9) with the following modifications: stock cultures of *Staphylococcus aureus* (ATCC 29213) in tryptic soy agar (Difco, Detroit, Mich.) were used for determinations of penicillin, amoxicillin, tetracycline, erythromycin, doxycycline, and azithromycin levels. *Klebsiella pneumoniae* (ATCC 13883) in Mueller-Hinton agar (Difco) was used to determine serum antibiotic concentrations of chloramphenicol and ceftriaxone. Antibiotic stock solutions were reconstituted as 1-mg/ml concentrations in 0.1 M phosphate buffer, except penicillin, which was diluted to 100 U/ml. Wells were punched into bioassay plates with a 4-mm punch. Duplicate wells for antibiotic standards and mouse sera were filled with 20 μl each of appropriate samples and standards and then incubated overnight at 37°C. Several antibiotics with very low serum concentrations (erythromycin, azithromycin, and chloramphenicol) required doubling of both plate thickness and sample volume in each well. Zones of inhibition around each well were measured with calipers by using the mean value for analysis. Zone size was plotted against the natural log of drug concentration on three-cycle semilog paper with the best-fit line determined. The intersection of the serum zone size with the best-fit line was the serum antibiotic concentration for each time point.

TABLE 1. Evaluation of antibiotic therapy in C3H mice experimentally inoculated with 10^4 *B. burgdorferi* (N40) cells

Antibiotic	Dose (mg/kg)	Route[a]	Culture[b]	Histology[c]	
				Arthritis	Carditis
Penicillin G	10^5[d]	S.C. BID	3/5	3/5	1/5
	3.5×10^5[d]	S.C. BID	0/5	0/5	0/5
	3.5×10^6[d]	S.C. BID	0/5	0/5	0/5
Amoxicillin-clavulanic acid	25	P.O. BID	0/10	0/10	0/10
	50	P.O. BID	0/10	0/10	0/10
Chloramphenicol	50	P.O. QID	2/5	3/5	2/5
	100	P.O. QID	1/5	2/5	2/5
Ceftriaxone	16	S.C. BID	0/9	0/9	0/9
	50	S.C. BID	0/9	0/9	0/9
Oxytetracycline	50	P.O. QID	3/4	4/5	4/5
	100	P.O. QID	6/6	6/6	6/6
Doxycycline	1.3	P.O. BID	7/7	7/7	7/7
	13	P.O. BID	7/7	7/7	7/7
	1.3[e]	P.O. BID	5/5	5/5	5/5
	13[e]	P.O. BID	3/5	5/5	5/5
Erythromycin	50	P.O. QID	7/7	7/7	7/7
	100	P.O. QID	7/7	7/7	7/7
Azithromycin	1	S.C. SID	5/5	5/5	5/5
	10	S.C. SID	5/5	5/5	3/5
	20	S.C. SID	5/6	6/6	6/6
	100	S.C. SID	5/7	6/7	7/7
Controls					
Media + antibiotics			0/47	0/47	0/47
N40 only			18/20	20/20	20/20

[a] BID, every 12 h; QID, every 6 h; SID, once daily; P.O., orally; S.C., subcutaneously.
[b] Number of mice with positive cultures/number of mice tested.
[c] Number of mice with disease/number of mice tested.
[d] Value is in international units per kilogram.
[e] 14-day treatment regimen (all others were of 5-day duration).

RESULTS

To evaluate in vivo susceptibility of *B. burgdorferi*, various dosage regimens of antibiotics in experimentally inoculated mice were evaluated. For each antibiotic, 8 to 10 mice were inoculated i.d. with 10^4 *B. burgdorferi* (N40) cells. One week after inoculation, mice were treated with various antibiotic regimens (Table 1) on the basis of current treatment recommendations for human Lyme disease patients (42–44, 49). Where indicated, oral antibiotics were administered to the mice by gavage. Two inoculated mice per group were not treated (positive controls). Two mice in each experimental group received antibiotic only (negative controls). Fourteen

TABLE 2. Comparison of total antibiotic doses between treated mice and recommended treatment regimens for human Lyme disease patients

Antibiotic	Total human dose (mg/kg)	Reference(s)	Total mouse dose (mg/kg)
Penicillin G	2.9×10^6–7.2×10^6[a]	26, 42–44	1×10^6[a]
			3.5×10^6[a]
			3.5×10^7[a]
Amoxicillin-clavulanic acid	215–800	42–44, 49	250
			500
Chloramphenicol	571–1,765	16, 42–44	1,000
			2,000
Ceftriaxone	400–860	42–44	160
			500
Oxytetracycline	300–800	49	1,000
			2,000
Doxycycline	30–80	49	13
			36
			130
			364
Erythromycin	143–600	43	1,000
			2,000
Azithromycin	21–50	30, 47, 54, 55	5
			50
			100
			500

[a] Value is in units per kilogram.

days after the final antibiotic treatment, mice were killed and evaluated by culture and histology. A successful treatment regimen was defined as negative cultures at necropsy and the absence of arthritic and cardiac lesions histologically. Among the antibiotics evaluated, amoxicillin-clavulanic acid, ceftriaxone, and penicillin G at higher doses eliminated infection as well as arthritis and carditis in the experimentally inoculated mice (Tables 1 and 2). Erythromycin, oxytetracycline, and doxycycline all failed to eliminate infection and disease. Both chloramphenicol and azithromycin failed to cure infected mice; however, histologic lesion development for both therapies was milder at the higher dosage schedules. We chose ceftriaxone for later stages of this study to avoid potentially inconsistent absorption associated with oral amoxicillin-clavulanic acid administration.

The agar well diffusion bioassay was used to determine serum antibiotic concentrations in mice at 15, 30, 60, and 120 min posttreatment with the highest dose of each antibiotic (Table 3). Serum antibiotic concentrations met or exceeded reported peak serum concentration in humans for penicillin G, amoxicillin, ceftriaxone, doxycycline, erythromycin, and azithromycin, indicating that systemic absorption of these drugs had occurred in treated mice.

We next sought to determine if recrudescence of infection, arthritis, or carditis occurred in *B. burgdorferi*-infected mice at given intervals posttreatment. For each of three experimental groups, 10 mice were inoculated with 10^4 *B. burgdorferi* cells i.d.; two uninoculated mice were retained as negative controls. From 7 to 11 days postinoculation (PID), eight inoculated mice were treated with the most effective antibiotic as determined in the first experiment (ceftriaxone, 16 mg/kg of body weight subcutaneously [S.C.] twice daily [BID] for 5 days). The two remaining inoculated mice were not treated (positive controls). At 14, 30, and 90 days posttreatment, mice were killed and evaluated by culture and histology. For up to 90 days posttreatment, all mice inoculated with *B. burgdorferi* and treated with ceftriaxone were free of infection (as determined by negative culture) and had normal tissues on gross and histologic examination (Table 4). Ceftriaxone administered early in the course of *B. burgdorferi* infection apparently eliminated the spirochetes in that there was no spontaneous remission of infection or disease up to 90 days after the end of treatment.

Since antibiotic treatment is known to be most effective in early *B. burgdorferi* infections in humans, we sought to determine the effectiveness of ceftriaxone in eliminating spirochetal infection and abrogating the development of arthritis and carditis in mice when treatment was initiated at intervals up to 90 days after infection. For each experimental group, 10 mice were inoculated i.d. with 10^4 *B. burgdorferi* cells; two uninoculated mice were kept for negative controls (antibiotic treatment only). Twenty-four hours prior to treatment, mice were anesthetized with methoxyflurane and bled by retroorbital puncture to obtain samples for serology. At each of the treatment intervals, beginning at PID 7, 14, 30, and 90, eight inoculated mice and the two uninoculated mice were treated with 16 mg of ceftriaxone S.C. BID for 5 days. Two weeks posttreatment, the mice were killed. Ceftriaxone therapy initiated at up to 90 days postinfection was uniformly effective in eliminating infection from all treated mice (Table 5). At the earlier treatment intervals (PID 7 and 14), arthritis and carditis lesions were also eliminated. When treatment was initiated at later intervals (PID 30 and 90), active cardiac and arthritic lesions were eradicated; however, residual lesions consisting of mild tendonitis, bursitis or synovitis, and vasculitis characterized by focal accumulations of mononuclear inflammatory cells in the walls of the great vessels at the base of the heart persisted in a small number of mice. Antibiotic treatment caused a marked decrease in IgG titers compared with those of untreated *B. burgdorferi*-inoculated mice (Table 6). IgM titers

TABLE 3. Comparison of peak serum antibiotic concentrations in humans and C3H mice

Antibiotic	Peak serum concn (μg/ml) for humans	Reference(s)	Peak serum concn (μg/ml) in mice at the following times (min):			
			15	30	60	120
Penicillin G	0.5[a]	29	118[a]	90[a]	15[a]	1.2[a]
Amoxicillin-clavulanic acid	4.0	29	8.5	17	6.3	<3.1
Chloramphenicol	10–13	46	<0.35	<0.35	<0.35	<0.35
Ceftriaxone	62.1	29, 35	62	ND[b]	10.5	5.4
Oxytetracycline	2.0–2.5	46, 48	<0.7	<0.7	<0.7	<0.7
Doxycycline	3.0	46	7.2	1.75	1.65	1.2
Erythromycin	0.3–0.5	46	3.8	3.1	2.6	1.9
Azithromycin	0.4	18	6.5	<0.35	<0.35	<0.35

[a] Value is in units per milliliter.
[b] Not determined.

TABLE 4. The long-term course of *B. burgdorferi*-infected mice treated 1 week postinoculation with ceftriaxone

Days post-treatment	Inoculum	Antibiotic	Culture[a]	Histology[b]	
				Arthritis	Carditis
14	N40	Ceftriaxone	0/5	0/8	0/8
	None	Ceftriaxone	0/2	0/2	0/2
	N40	None	0/2	1/2	1/2
30	N40	Ceftriaxone	0/8	0/8	0/8
	None	Ceftriaxone	0/2	0/2	0/2
	N40	None	2/2	2/2	1/2
90	N40	Ceftriaxone	0/8	0/8	0/8
	None	Ceftriaxone	0/2	0/2	0/2
	N40	None	1/2	1/2	2/2

[a] Number of mice with positive cultures/number of mice tested.
[b] Number of mice with disease/number of mice tested.

TABLE 6. Geometric mean titers of *B. burgdorferi*-infected C3H mice treated at various intervals postinfection

Time of treatment initiation[a]	Treatment	Pretreatment mean titer		Posttreatment mean titer	
		IgM	IgG	IgM	IgG
7	N40 + Cef[b]	Negative	Negative	14	2,792
	N40 only			320	57,926
14	N40 + Cef	905	640	135	17,221
	N40 only			226	40,960
30	N40 + Cef	293	22,334	113	22,334
	N40 only			160	115,852
90	N40 + Cef	56	48,710	67	31,584
	N40 only			Negative	81,920

[a] Days postinfection.
[b] Cef, ceftriaxone.

of untreated mice peaked at PID 14 and then precipitously declined. IgG titers of untreated mice continued to rise for the duration of the experiment.

DISCUSSION

There are many difficulties inherent in the diagnosis and treatment of Lyme borreliosis in human patients which suggest that there is no "gold standard" for the diagnosis of Lyme disease and no test of cure after antimicrobial treatment (57). Optimal therapeutic modalities are, therefore, not established with certainty (43, 57). Oral tetracyclines, amoxicillin, erythromycin, and, recently, azithromycin are utilized for treatment of early Lyme disease, although treatment failures have been reported in up to 50% of patients (10, 12, 20, 30, 52, 53, 56). Amoxicillin-clavulanic acid has also been used in humans and gerbils with borreliosis; it was more effective than amoxicillin alone in gerbils, with essentially no superiority demonstrated in human patients (33, 56, 57). Treatment failure is even greater in chronic Lyme disease, in which parenteral ceftriaxone appears to be more effective than penicillin, with sporadic successes reported with chloramphenicol and tetracyclines (1, 16, 28). Since *B. burgdorferi* is killed slowly, it appears that prolonged elevated antimicrobial levels are required to eradicate infection (28, 34).

TABLE 5. Effectiveness of antibiotic treatment of *B. burgdorferi*-infected C3H mice at various intervals postinfection

Treatment started[a]	Inoculum	Antibiotic	Positive culture[b]	Histology result[c]	
				Arthritis	Carditis
7	N40	Ceftriaxone	0/8	0/8	0/8
	None	Ceftriaxone	0/2	0/2	0/2
	N40	None	2/2	1/2	1/2
14	N40	Ceftriaxone	0/8	0/8	0/8
	None	Ceftriaxone	0/2	0/2	0/2
	N40	None	2/2	2/2	2/2
30	N40	Ceftriaxone	0/8	2/8[d]	3/8[e]
	None	Ceftriaxone	0/2	0/2	0/2
	N40	None	1/1	2/2	2/2
90	N40	Ceftriaxone	0/8	1/8[d]	2/8
	None	Ceftriaxone	0/0	0/2	0/2
	N40	None	2/2	1/2	2/2

[a] Days postinfection.
[b] Number of mice with positive cultures/number of mice tested.
[c] Number of mice with disease/number of mice tested.
[d] Residual arthritis.
[e] Residual carditis and/or vasculitis.

In vitro susceptibility studies indicate that erythromycin, amoxicillin, amoxicillin-clavulanic acid, tetracyclines, ceftriaxone, and azithromycin have high activity against *B. burgdorferi*, with generally low activity reported for penicillin G (15, 23–25, 33, 40, 41, 45). However, in vitro results have no proven correlation with antimicrobial clinical effectiveness in vivo (49, 57), since the relationship of MICs or MBCs of drugs against slowly dividing organisms such as *B. burgdorferi* and in vivo efficacy has not yet been established (26). In addition, in vitro antibiotic susceptibility testing is not standardized, and for at least one antibiotic (penicillin), poor in vitro performance is a result of the drug's instability in the spirochete medium rather than low activity against the parasite (14).

Previous animal model studies have indicated that amoxicillin-clavulanic acid, ceftriaxone, tetracycline, and azithromycin are more active than penicillin in eliminating spirochetal infection from gerbils and hamsters (22–24, 33, 39, 41). Many of these studies have limited applicability, since culture alone was used as the criterion for spirochetal eradication. Histopathologic analyses were not performed with the hamsters or gerbils, since these species do not develop significant lesions when infected with *B. burgdorferi* (17, 38). A further disadvantage of these studies is that once-daily dosing schedules were used, regardless of the pharmacokinetics of each antibiotic.

In the present study, the beta-lactam antibiotics (amoxicillin-clavulanic acid, ceftriaxone, and high-dose penicillin G) were most effective at eliminating infection, carditis, and arthritis in experimentally inoculated mice. All three of these antibiotics achieved peak serum concentrations greater than or equivalent to human treatment values (29, 35). Once-daily penicillin therapy for 5 or 7 days failed to eliminate infection in experimentally infected hamsters and gerbils (22, 24, 33); however, similar doses given four times daily were effective in the mice of this study and in gerbils treated three times daily for 10 days (21), indicating that frequency of administration can be a significant variable in animal studies.

Although the tetracyclines are commonly prescribed for human Lyme disease patients, their efficacy in infected mice was poor. Treatment for up to 14 days with doxycycline resulted in abatement of gross joint enlargement; however, there was no significant effect in abrogating development of histologic arthritis or carditis. For oxytetracycline, peak serum

concentrations were well below that of humans. In rats, low serum tetracycline concentrations were also reported following oral administration. It has been suggested that there is limited gastrointestinal absorption of this antibiotic in rats (and possibly mice), which may limit systemic efficacy (37). Mice treated with doxycycline had serum antibiotic concentrations comparable to those of humans at 15 min. This expanded-spectrum tetracycline has greater lipid solubility than other tetracyclines, which may account for more-efficient absorption after oral administration (46, 48). Although we demonstrated systemic absorption of doxycycline, clearance of infection and disease in inoculated mice did not occur.

Chloramphenicol at the higher dose was moderately efficacious in eliminating infection and disease from over half of the inoculated mice. Peak serum concentrations, however, were well below those reported for humans.

Erythromycin was ineffective in eliminating infection or disease in *B. burgdorferi*-inoculated mice. Despite having high in vitro efficacy against the spirochete, it has not shown significant in vivo efficacy in hamsters or gerbils (22, 33). Erythromycin has also been less effective in humans with Lyme disease, although it is an alternative treatment for the penicillin- or tetracycline-allergic patient (42). Azithromycin is a new azalide antibiotic structurally related to erythromycin. It is effective in eliminating *B. burgdorferi* infection in hamsters (23) and gerbils (39, 41) and has shown promise because of its high tissue concentrations and once-daily dosing (18). Preliminary studies of patients with early Lyme disease indicate that azithromycin eradicates erythema migrans more quickly than doxycycline; however, *B. burgdorferi* persists in the skin for up to 3 months after treatment (55). Azithromycin has also been shown to be less effective than amoxicillin for erythema migrans treatment in one study (27); however, a comparison of azithromycin, doxycycline, or amoxicillin plus probenecid showed similar efficacy for the three regimens (30). A related azalide antibiotic, roxithromycin, was effective in eliminating infection from gerbils but failed in humans (20). In the present study, azithromycin at up to 10 times the total recommended human dose failed to eliminate infection or disease in experimentally inoculated mice, despite attainment of adequate therapeutic serum concentration at 15 min.

The MICs and MBCs of the 8 antimicrobial agents against the N40 strain of *B. burgdorferi* were not determined; however, other in vitro studies indicate that geographically disparate strains of *B. burgdorferi* are similar in their antimicrobial susceptibility patterns (40, 45). Although we have demonstrated in this study a correlation between peak serum concentrations of antibiotics in mice and therapeutic concentrations in humans, it should be noted that samples were obtained from uninfected mice. Infection with *B. burgdorferi* could be a complicating factor affecting the renal handling, pharmacokinetics, and resultant serum concentrations of these drugs.

Treatment failures are documented for all antibiotics used in Lyme disease patients. Reasons for these failures include sequestering of the organism in sites inaccessible to the antibiotic (19, 50), patient genetic predisposition to chronic Lyme disease (51), the presence of inflammatory mediators mimicking the signs of active Lyme disease, the placebo effect of antibiotic treatment (42), inadequate duration of antimicrobial treatment (11), and, perhaps most frequently, incorrect diagnosis (41, 50). In this study, we evaluated whether ceftriaxone could produce a microbiologic cure when administered early in the course of infection with a 90-day follow-up. Within the observation period, there was no evidence of recrudescence of infection or disease in any of the mice, suggesting that ceftriaxone effectively eliminated the spirochete. This finding has been useful in ongoing studies of reinfection in this model (4, 6).

When *B. burgdorferi*-infected mice were treated at intervals of up to 90 days postinfection, we found that early intervention with antibiotics eliminated infection and prevented the development of later lesions. When treatment was initiated later in the course of infection, mice became culture negative but were left with residual lesions. Serologically, antibiotic treatment abrogated the development of antibody response to the spirochete, a finding which has been reported for humans (39, 42). The 90-day interval may not reflect treatment in the chronic stages of Lyme disease, however, indicating that further studies of mice treated later in the course of infection could provide information on the course and duration of residual lesions and the associated immune response.

The potential to determine spirochete presence accurately through culture and histologic lesions makes the *B. burgdorferi*-inoculated C3H mouse a valuable research tool to evaluate chemotherapeutic options for Lyme disease. Amoxicillin-clavulanic acid, high-dose penicillin G, and ceftriaxone consistently eliminated spirochetal infection in *B. burgdorferi*-inoculated mice. Since ceftriaxone appears to effect a microbiologic cure in treated animals, this mouse model provides ample opportunity to explore other therapeutic dilemmas associated with Lyme disease, including reinfection with identical or dissimilar strains of *B. burgdorferi*.

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