In Vitro and In Vivo Susceptibility of the Lyme Disease Spirochete, *Borrelia burgdorferi*, to Four Antimicrobial Agents

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The antimicrobial susceptibility of *Borrelia burgdorferi* isolated from human spinal fluid was determined in vitro and in vivo. A broth dilution technique was used to determine the MBCs of four antimicrobial agents. The Lyme disease spirochete was most susceptible to ceftriaxone (MBC, 0.04 µg/ml) and erythromycin (MBC, 0.05 µg/ml), then tetracycline (MBC, 0.8 µg/ml), and finally penicillin G (MBC, 6.4 µg/ml). Syrian hamsters were used to determine the 50% curative doses (CD₅₀s) of the four antimicrobial agents. Ceftriaxone and tetracycline had the highest activities, with CD₅₀s of 240 and 287 mg/kg, respectively. Both erythromycin and penicillin G possessed low activities. The CD₅₀ of erythromycin was 2,353 mg/kg, and the CD₅₀ of penicillin G was >1,975 mg/kg.

Lyme disease and related disorders (Lyme borreliosis) are caused by the spirochete *Borrelia burgdorferi* (6, 9, 10, 15, 22). The major vectors of these illnesses are *Ixodes* species of ticks (2, 8-10). Lyme disease frequently begins with the characteristic skin lesion erythema chronicum migrans, which may be followed by carditis, neuritis, or arthritis (24). The neurological abnormalities and arthritis may be chronic (24; A. R. Pachner and A. C. Steere, Zentralbl. Bakteriologie, Mikrobiol. Hyg. Ser. A, in press). Lyme disease has now been reported from at least 24 states and is the most commonly reported tick-borne disease in the United States (11). In 1983, Steere et al. (23) reported on the effectiveness of penicillin, tetracycline, and erythromycin for resolving erythema chronicum migrans and its associated symptoms as well as preventing the development of major late complications. They concluded that for patients with early stages of Lyme disease, tetracycline appears to be the most effective drug, then penicillin, and finally erythromycin. Subsequent studies on the treatment of the complications of late stages of Lyme disease indicate that the neurological abnormalities could be successfully treated with high-dose intravenous penicillin (25). For established arthritis, benzathine penicillin (7.2×10^6 U) or intravenous penicillin G (20×10^6 U/day in divided doses for 10 days) seemed to be curative in 35% of the patients given the former treatment and 55% of those given the latter treatment (21). Neither of the regimens tested was uniformly effective, and further experiments were considered necessary to determine the optimal course of therapy (21).

In vitro antimicrobial susceptibility studies of several human Lyme disease spirochete isolates have been reported (7, 16). The Lyme disease spirochetes were most susceptible to erythromycin but were also susceptible to minocycline, penicillin G, ampicillin, doxycycline, and tetracycline hydrochloride. The spirochetes were resistant to rifampin (16).

We report the in vitro and in vivo susceptibilities of the Lyme disease spirochete *B. burgdorferi* to four antimicrobial agents, i.e., penicillin G, ceftriaxone, tetracycline hydrochloride, and erythromycin.

MATERIALS AND METHODS

Origin and cultivation of *B. burgdorferi*. The *B. burgdorferi* strain used in this study was isolated from human spinal fluid and was obtained from Allen Steere, Yale University, New Haven, Conn. (22). The isolate was passaged from hamster to hamster 11 times. This isolate was not subcultured in media more than three times after isolation from the hamster spleen or kidney before use in the in vitro or in vivo antibiotic studies. The spirochetes were cultured in Barbour-Stoenner-Kelly (BSK) medium (5) at 30°C. Medium for the isolation of spirochetes from animals was prepared by the addition of 0.15% agarose (SeaKem LE; FMC Corp., Marine Colloids Div., Rockland, Maine) to the BSK medium.

Antimicrobial agents. The antimicrobial agents tested were erythromycin and tetracycline hydrochloride (Sigma Chemical Co., St. Louis, Mo.), ceftriaxone (Rocephin; Hoffmann-La Roche Inc., Nutley, N.J.), and penicillin G (E. R. Squibb & Sons, Princeton, N.J.). The antimicrobial solutions were prepared and stored as recommended by Anhalt and Washington (3).

In vitro antimicrobial susceptibility procedures. The broth dilution method was used to determine MBCs. The antimicrobial agents evaluated are listed in Table 1. The concentrations of antimicrobial agents tested ranged from 0.01 to 25.0 µg/ml. Triplicate tubes containing BSK medium with the appropriately diluted antimicrobial agents and control tubes (BSK medium without antimicrobial agents) were inoculated to a final density of 10^5 cells per ml from an actively growing culture. Cell numbers were determined with the Petroff-Hausser counting chamber. For 6 weeks, the assay tubes were examined weekly by dark-field microscopy for the presence of spirochetes. Three separate determinations were made for each antimicrobial agent. The MBC was the lowest concentration of antimicrobial agent in which spirochetes could not be detected. Spirochetes could not be subcultured from BSK medium containing these concentrations of antimicrobial agents to BSK medium without antimicrobial agents by using a 10% (vol/vol) inoculum. The suitability of BSK medium for in vitro antimicrobial susceptibility testing was previously established (16).

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TABLE 1. MBCs of four antimicrobial agents for *B. burgdorferi*

Drug	MBC ($\mu\text{g/ml}$) ^a
Penicillin G.....	6.5
Ceftriaxone.....	0.04
Tetracycline/hydrochloride.....	0.8
Erythromycin.....	0.05

^a Final concentration of inoculum, 10^5 cells. Incubated at 30°C for 6 weeks.

In vivo antimicrobial susceptibility procedure. Syrian hamsters weighing between 80 and 100 g were injected intraperitoneally with 1,000 50% infective doses of *B. burgdorferi* isolated from human spinal fluid. The 50% infective dose of this isolate for the hamster was previously determined to be 10,000 cells (13). Fourteen days later, when the spirochete was present in as many as six organs (14), antimicrobial treatment was initiated. The treatment schedule consisted of five equal daily subcutaneous injections of the test antimicrobial agent. The total dosages of the antimicrobial agents tested are given in Table 2. Control hamsters received saline injections. Fourteen days after the final treatment, the hamsters were sacrificed, and the kidneys and spleen were cultured as previously described (14). Cultures were examined for spirochetes by dark-field microscopy after 3 weeks of incubation at 30°C. An animal was considered infected if one or more organs were culture positive. At least five animals were tested per antimicrobial dose. A number of animals could not be evaluated because of contamination of the isolation medium. The 50% curative dose (CD_{50}) was calculated by the method of Reed and Muench (20).

RESULTS

The MBCs of penicillin G, ceftriaxone (Rocephin), tetracycline hydrochloride, and erythromycin were determined

TABLE 2. Therapeutic evaluation of hamsters experimentally infected with *B. burgdorferi* and then treated with four antimicrobial agents^a

Drug	Total dose (mg/kg)	No. of hamsters treated	No. of culture-positive hamsters	% Protection
Penicillin G	1,975	10	9	10
	988	10	8	20
	494	10	10	0
	247	9	9	0
	99	8	7	12
	9	7	6	14
Ceftriaxone (Rocephin)	1,815	8	0	100
	908	15	1	93
	454	10	4	60
	182	10	6	40
	91	10	6	40
Tetracycline hydrochloride	18	7	7	0
	1,323	5	1	80
	661	4	0	100
	331	5	2	60
	133	5	4	20
Erythromycin	13	5	5	0
	4,037	5	2	60
	2,019	5	2	60
	1,009	5	5	0
	202	1	1	0
None (control)	20	4	4	0
		19	17	

^a Treatment consisted of five equally divided daily subcutaneous injections of the test antimicrobial agent.

TABLE 3. The CD_{50} s of four antimicrobial agents for hamsters experimentally infected with *B. burgdorferi*

Drug	CD_{50} (mg/kg)
Penicillin G.....	>1,975
Ceftriaxone.....	240
Tetracycline hydrochloride.....	287
Erythromycin.....	2,352

by the broth dilution technique during a 6-week test period. The MBC was the lowest concentration of the antimicrobial agent in which viable *B. burgdorferi* isolates from human spinal fluid could not be observed by dark-field microscopy. During the test period, the MBCs of some of the antimicrobial agents changed. The greatest change occurred with penicillin G. After week 1 of the assay, the MBC of penicillin G was 0.05 $\mu\text{g/ml}$, and from weeks 4 to 6, the MBC remained constant at 6.4 $\mu\text{g/ml}$. The spirochetes growing at the higher concentration of penicillin G did not possess greater resistance to penicillin than the parent strain did. When these organisms were subcultured and retested, they displayed the same level of susceptibility as the parent strain. The MBC of ceftriaxone was 0.02 $\mu\text{g/ml}$ for weeks 1 and 2 and 0.04 $\mu\text{g/ml}$ thereafter. The MBC of tetracycline was 0.4 $\mu\text{g/ml}$ at week 1 and 0.8 $\mu\text{g/ml}$ thereafter. The MBC of erythromycin was 0.05 $\mu\text{g/ml}$ throughout the test period. The MBCs (in micrograms per milliliter) of the test antimicrobial agents at the end of the 6-week observation period were 0.04 (ceftriaxone), 0.05 (erythromycin), 0.8 (tetracycline hydrochloride), and 6.4 (penicillin G) (Table 1).

The in vivo antimicrobial susceptibility of *B. burgdorferi* to the four test drugs was evaluated by using the Syrian hamster as the experimental animal. The total concentrations of antimicrobial agents tested varied from 20 to 4,037 mg/kg, and the agents were administered in five daily equally divided doses (Table 2). The antimicrobial agents with the greatest in vivo anti-*B. burgdorferi* activity were ceftriaxone and tetracycline. The higher doses of these antimicrobial agents resulted in the elimination of the Lyme disease spirochete from all of the animals receiving this course of treatment (Table 2). Erythromycin, which possessed high in vitro activity against *B. burgdorferi*, was not effective in vivo. Only 60% of the animals receiving the highest concentration of erythromycin tested, 4,037 mg/kg, were cured of the infection (Table 2). Penicillin G, the antimicrobial agent that had the lowest anti-*B. burgdorferi* in vitro activity, also had poor therapeutic activity in the hamsters. At the highest level of penicillin G tested, 1,925 mg/kg, only 10% of the infected animals were cured.

The CD_{50} s of the test antimicrobial agents are shown in Table 3. Ceftriaxone and tetracycline had high in vitro activities and similar levels of therapeutic activity. The CD_{50} s of ceftriaxone and tetracycline were 240 and 287 mg/kg, respectively. The CD_{50} of erythromycin was 2,352 mg/kg, markedly less than that of ceftriaxone or tetracycline. The results obtained for penicillin G did not permit the determination of the CD_{50} . However, it would be >1,975 mg/kg (Table 3).

DISCUSSION

Lyme disease is an illness of increasing public health importance. It is a complex disease that can be divided into three stages (21). The early stage of the disease, stage 1, occurs approximately 1 week after the bite of an infected

vector. The primary vector, endemic to Connecticut, Massachusetts, New York, New Jersey, Rhode Island, Wisconsin, and Minnesota, is the nymphal (immature) stage of the northern deer tick, *Ixodes dammini*. Because of the smallness of the nymphal form and its painless bite, only 30% of patients who develop Lyme disease are aware of a preceding tick bite (22). The hallmark of this stage of the disease is the presence of a characteristic skin rash, erythema migrans chronicum, that appears first at the site of the tick bite. The other symptoms associated with stage 1 are influenzalike. After about 1 month, stage 1 of Lyme disease is followed by stage 2, which lasts for 1 to 4 months. During stage 2, cardiac and neurological abnormalities as well as arthritis may be present. Stage 3 of Lyme disease occurs 4 months to several years after the initial infection. It is characterized by arthritis (24) and chronic neurological abnormalities (19).

If Lyme disease is adequately treated with the appropriate antimicrobial agents during stage 1, the later, more serious manifestations of the disease can be drastically reduced in frequency (23). Lyme meningitis responds well to intravenous penicillin (25), but certain stage 2 manifestations such as Bell's palsy and peripheral radiculoneuritis and stage 3 arthritis may be unresponsive to penicillin therapy (21, 23). The therapy of carditis (stage 2) has not been examined in detail.

The Syrian hamster provides a useful model for evaluating the anti-*B. burgdorferi* activity of antimicrobial agents, since a generalized persistent experimental infection can be readily established in this animal (14). The antimicrobial agents chosen for this study were those that are used to treat Lyme disease, namely, penicillin G, tetracycline, and erythromycin. Ceftriaxone was included in the study because we previously demonstrated that it was highly effective (CD₅₀, 0.96 mg/kg) for the treatment of experimentally induced syphilis in rabbits (12).

In vitro antimicrobial susceptibility studies have been reported for *B. burgdorferi* isolated from blood and skin (7, 16). These human isolates were susceptible to erythromycin, tetracycline, minocycline, and penicillin G. Erythromycin possessed the highest in vitro activity to *B. burgdorferi* (7, 16). Our in vitro antimicrobial studies with the human spinal fluid isolate of *B. burgdorferi* also showed that this spirochete is susceptible to erythromycin, tetracycline, and penicillin G and that erythromycin possessed the highest level of antispirochetal activity. Our MBC for penicillin, 6.4 µg/ml, was somewhat higher than the range of MBCs, 0.08 to 2.5 µg/ml, for the six skin isolates reported by Berger et al. (7).

Steere et al. (23) reported that for patients with early stages of Lyme disease, tetracycline appears to be the most effective drug, then penicillin, and finally erythromycin. Major late complications (meningoencephalitis, myocarditis, or recurrent attacks of arthritis) did not occur in the 39 patients treated with tetracycline but did occur in 3 of 40 patients given penicillin and 4 of 29 patients given erythromycin (23). These results correlate with the results of our in vivo studies. Of the antimicrobial agents used, tetracycline was most effective, with a CD₅₀ of 287 mg/kg. Neither penicillin G (CD₅₀, >1,975 mg/kg) nor erythromycin (CD₅₀, 2,352 mg/kg) was effective in curing the experimentally infected hamsters of Lyme disease. Ceftriaxone possessed a high level of anti-*B. burgdorferi* activity both in vitro (MBC, 0.04 µg/ml) and in vivo (CD₅₀, 240 mg/kg). Ceftriaxone has the potential of being a highly effective antibiotic for the treatment of Lyme disease and related disorders. The CD₅₀ of ceftriaxone is similar to that of tetracycline, which appears to be the most effective drug for patients with early

stages of Lyme disease. In addition, because of its extended half-life of 6.5 h and the levels achieved in serum, ceftriaxone can be administered once or twice a day, depending on the type and severity of infection. Also, ceftriaxone is distributed to many body sites including interstitial and cerebrospinal fluids (1; E. Squires and R. Cleeland, *Microbiology and Pharmacokinetics of Parenteral Cephalosporins*, Hoffmann-La Roche, 1984). Recently, it was reported that ceftriaxone effectively penetrates into the synovial fluid of the inflamed joint, attaining concentrations of between 66 and 100% of the concomitant levels in serum (18). These observations are particularly important, since the chronic forms of Lyme disease and related disorders appear to be due to the persistence of *B. burgdorferi* in the affected sites (4, 17, 19, 26, 27).

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