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Efficacy of Clarithromycin for Treatment of Experimental Lyme Disease In Vivo

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Clarithromycin provided effective therapy against arthritis induced by *Borrelia burgdorferi* infection in the hamster. In vitro, clarithromycin was at least 1 log more potent than tetracycline against two isolates of *B. burgdorferi* from human sources, as measured by MICs and 50% inhibitory concentrations. Clarithromycin was effective in preventing the onset of *B. burgdorferi*-induced arthritis, as determined by several parameters of paw swelling. When administered after the onset of arthritis, clarithromycin therapy reduced the degree of swelling and decreased recovery time. These results suggest that clarithromycin has potential as an effective therapy for Lyme disease.

Lyme borreliosis is the most commonly reported tick-borne disease in Europe and the United States (4). In the first half of 1992, the Centers for Disease Control reported over 2,000 confirmed cases of Lyme borreliosis (5). However, because of difficulties in diagnosis (17, 19), the actual incidence of the disease is unknown. *Borrelia burgdorferi* is the causative agent of Lyme disease.

Lyme disease presents a wide variety of clinical manifestations, which are often divided into stages. Lyme disease is initially characterized by a flu-like illness and a migrating rash termed erythema migrans. Cardiac or neurologic involvement may also occur. Arthritis is the most common serious and chronic manifestation of Lyme disease (8, 10, 23). The knee joint is a frequent target of this arthritis, which may appear several months after erythema migrans. The progression and appearance of clinical signs may vary considerably among individuals. Treatment efficacy appears to vary with the severity of the arthritis and progression of the disease (3).

Commonly used oral antimicrobial agents for the treatment of Lyme disease include tetracycline, doxycycline, erythromycin, and penicillin V. Oral antimicrobial agents have appeared to be relatively ineffective in treating Lyme disease arthritis (4, 6, 7, 22). This situation, along with the long period required for antibiotics to kill *B. burgdorferi* in vitro (1, 16) and the chronic nature of the arthritis, suggests a need for effective oral antibiotics which are safe to administer on a long-term basis. The limited clinical trials support the need to investigate additional antimicrobial agents for their efficacy and safety in treatment of Lyme disease.

Several different animal models are used to simulate Lyme disease. Some laboratory animals do not manifest Lyme disease as arthritic joint swelling but instead suffer infections of various internal organs (17). Recovery of the pathogen from internal organs can be difficult and is usually judged on a plus-or-minus basis rather than on a quantitative scale. The hamster model allows assessment of arthritic paw swelling as a measure of pathology (21). The paw enlargement is easily quantitated and may be monitored over several weeks to produce a time course analysis of infection and response to antibiotic treatment. *Borrelia*-induced paw swelling in the hamster has been characterized histologically and includes many features of arthritis. There is destructive degradation of cartilage and an infusion of monocytes and lymphocytes (21). Spirochetes are also present within the damaged joints of infected hamsters (21). In addition, hamsters produce significant and measurable titers of antibody to *B. burgdorferi*.

This investigation demonstrates that clarithromycin has significant anti-*B. burgdorferi* activity in the hamster model of Lyme arthritis. This activity was measured on the basis of protection from joint swelling and in vitro potency.

MATERIALS AND METHODS

Origin and cultivation of *Borrelia* isolates. Two human Lyme disease *B. burgdorferi* isolates were used in this study. One isolate, *B. burgdorferi* 297, was obtained from human cerebrospinal fluid (24). This hamster-virulent isolate was subcultured once or twice before use. A second isolate, *B. burgdorferi* P/Bi, was obtained from a German human skin specimen (obtained from Ronald Schell, University of Wisconsin) and was not virulent in hamsters. All cultures were grown at 33°C in Barbour-Stoenner-Kelly (BSK II) medium (2).

Antimicrobial agents. The antimicrobial agents tested were tetracycline hydrochloride (Squibb, Princeton, N.J.), doxycycline (Geneva, Broomfield, Colo.), and clarithromycin and 14-hydroxy-clarithromycin (Abbott Laboratories, Abbott Park, Ill.).

In vitro susceptibility procedures. The 50% inhibitory concentrations (IC_{50}s) of the study agents were determined by cultivating *B. burgdorferi* in BSK II medium both with and without the agents. Concentrations of the test antimicrobial agents ranged from 0.01 to 100 μg/ml. Tubes containing 1.0 ml of BSK II medium with the appropriately diluted antimicrobial agents and control tubes (no antimicrobial agent) were inoculated to a final density of 5×10^6 organisms per ml. The spirochetes were in the early log phase of growth. After incubation at 33°C for 48 h, the tubes were examined by dark-field microscopy for the presence of viable spirochetes. A total of 100 spirochetes from each tube were examined for the presence of motility. IC_{50}s were calculated by linear regression. MICs were calculated as the lowest dilution yielding less than 5% viability.

In vivo susceptibility procedures (12). Golden Syrian ham-

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sters, 5 to 6 weeks old and weighing 80 to 100 g, were immunosuppressed by a single intraperitoneal injection of cyclophosphamide (Sigma, St. Louis, Mo.) (100 mg/kg of body weight) 1 day prior to inoculation. Hamsters were then inoculated (day 0) by intradermal injection of 10^7 *B. burgdorferi* 297 organisms into each hind paw (0.1 ml of a culture containing 10^8 organisms per ml). The test animals received daily subcutaneous injections of clarithromycin, the 14-hydroxy human metabolite of clarithromycin, doxycycline, or tetracycline for 7 consecutive days (days 1 to 7). In addition, uninfected animals and infected but untreated animals were included as controls in each experiment. There were four hamsters per treatment group. In one trial, treatment was delayed until swelling became apparent (day 6). Efficacy was quantitated by measuring the degree of paw swelling, as determined by measuring the width and thickness of the section of hind paw between the proximal two toe pads with electronic digital calipers (Mitutoyo, Toyko, Japan). The mass of the section was calculated as length × width^2/2, and trapezoidal estimation was used to calculate the area under the curve (AUC) of paw mass versus time.

Antibody titers. Antibody titers were determined by using a fluorescent-antibody assay for hamster antibody to *B. burgdorferi*. Slides coated with *B. burgdorferi* (Wampole/Zeus, Cranbury, N.J.) were incubated with a 1:320 dilution of test hamster serum in a moist chamber at room temperature for 30 min. The slides were rinsed according to the manufacturer's instructions and incubated with a 1:100 dilution of goat anti-hamster fluorescent conjugate antiserum (Boehringer Mannheim, Indianapolis, Ind.) at room temperature for 30 min, as before. The slides were then rinsed and mounted in buffered glycerol and scored on a scale of 0 to 4+ according to the manufacturer's instructions (Wampole/Zeus). The fluorescence was scored as follows: 0, negative; 1+, weak; 2+, moderate; 3+, strong; and 4+, very strong.

Recovery of *B. burgdorferi* from organs. At the conclusions of trials, bladders, spleens, kidneys, and/or heart tissues were aseptically removed from the hamsters. The tissues were coarsely minced and individually incubated in 15 ml of BSK II medium per organ. The tubes were incubated at 33°C for up to 3 weeks. The cultures were examined by dark-field microscopy at weekly intervals for the presence of spirochetes.

Pharmacokinetics. The concentrations of clarithromycin, 14-hydroxy-clarithromycin, tetracycline, and doxycycline in hamster sera following a single 20-mg/kg subcutaneous dose was determined by bioassay (9). Sera were collected 0.5, 1, 2, 3, 6, 8, 12, 16, and 24 h following administration of the dose. There were four hamsters tested per time point.

TABLE 1. IC_{50}s and MICs[a] of therapeutic agents for two *B. burgdorferi* strains

Compound	297 strain		P/Bi strain	
	IC_{50}	MIC	IC_{50}	MIC
Clarithromycin	0.044	0.2	0.07	0.2
14-Hydroxy-clarithromycin	0.042	0.2	0.25	ND[b]
Tetracycline	1.960	8	4.05	20
Doxycycline	0.811	2	6.13	ND

[a] IC_{50}s and MICs both given in micrograms per milliliter.
[b] ND, not determined.

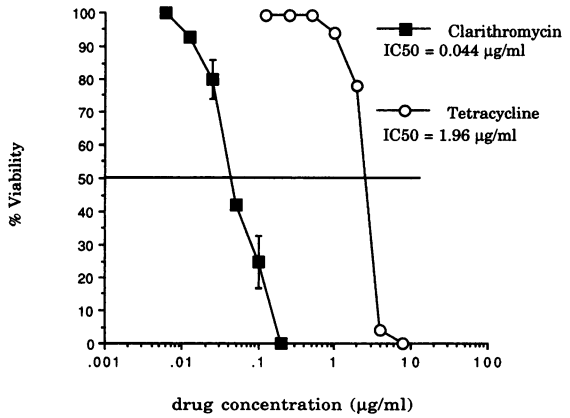

FIG. 1. Viability of *B. burgdorferi* 297 following 48 h of incubation with clarithromycin or tetracycline. IC_{50}s were calculated by linear regression analysis.

RESULTS

In vitro susceptibility of the *B. burgdorferi* isolates to antimicrobial agents. The IC_{50}s of clarithromycin (0.044 μg/ml) and the 14-hydroxy metabolite of clarithromycin (0.042 μg/ml) were lower in vitro than those of either tetracycline (1.960 μg/ml) or doxycycline (0.811 μg/ml) against the pathogenic 297 strain (Table 1). The IC_{50}s were based upon viability curves of *B. burgdorferi* exposed to known concentrations of drugs. These curves represent typical dose susceptibility profiles for in vitro exposure to bactericidal compounds (Fig. 1). MICs were approximately 1 log greater than the corresponding IC_{50}s. The German strain, which produces no pathology in the hamster, was also more susceptible to clarithromycin and the 14-hydroxy metabolite than to tetracycline or doxycycline. The German strain was more resistant to all four antibiotics and higher IC_{50}s were obtained for each compound.

Antimicrobial treatment of *B. burgdorferi*-infected hamsters. The effect of early therapy on the increase in paw mass of hamsters infected with *B. burgdorferi* 297 is illustrated in Fig. 2. The swelling peaked on day 7 in untreated hamsters and gradually declined over the next two weeks. By contrast, there was complete prevention of paw swelling in

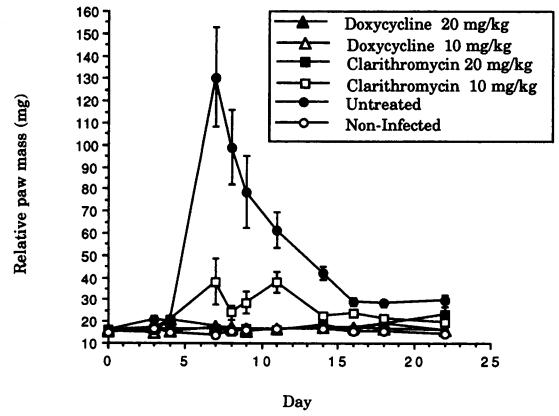

FIG. 2. Increase in paw mass of hamsters infected with *B. burgdorferi* 297 and treated with clarithromycin or doxycycline. Test drugs were administered intraperitoneally once a day on days 1 to 7.

TABLE 2. Efficacy of clarithromycin and doxycycline in treatment of *B. burgdorferi* 297 infection in hamsters

Group, drug, and dose (mg/kg)[a]	AUC_{0-18} (mg · days)[b]	Paw mass (mg)[c]	T_{max}[d]
Infected			
Clarithromycin			
20	298 (1.06)	19.2 (1.14)	18
10	466 (1.65)	38.1 (2.26)	7
Doxycycline			
20	298 (1.06)	17.2 (1.02)	3
10	310 (1.10)	21.0 (1.25)	4
None	928 (3.29)	130.5 (7.76)	7
Noninfected	282	16.8	

[a] All treatments were once a day on days 1 to 7. There were four animals per group.
[b] AUC values were calculated for days 0 to 18 of the trial. Test/control (noninfected) ratios are given in parentheses.
[c] Maximum mean paw mass during days 0 to 18. Test/control (noninfected) ratios are given in parentheses. Measurements of paw mass were taken on days 0, 3, 4, 7 to 9, 11, 14, 16, and 18 postinfection.
[d] T_{max}, time in days to maximum mean paw mass.

TABLE 4. Efficacy of clarithromycin and doxycycline on a b.i.d. schedule in treatment of *B. burgdorferi* 297 infection in hamsters

Group, drug, and dose (mg/kg)[a]	AUC_{0-24} (mg · days)[b]	Paw mass (mg)[c]	T_{max}[d]
Infected			
Clarithromycin			
25	411 (1.00)	22.4 (1.20)	18
12.5	492 (1.19)	34.6 (1.86)	13
Doxycycline			
25	410 (0.99)	22.7 (1.22)	13
12.5	509 (1.23)	30.3 (1.63)	14
None	1,866 (4.52)	150.4 (8.09)	6
Noninfected	413	18.6	

[a] All treatments were b.i.d. on days 1 to 7 (total daily dose, 50 or 25 mg/kg). There were eight hamsters per treatment group.
[b] AUC values were calculated for days 0 to 24 of the trial. Test/control (noninfected) ratios are given in parentheses.
[c] Maximum mean paw mass during days 0 to 24. Test/control (noninfected) ratios are given in parentheses. Measurements of paw mass were taken on days 0, 6, 10 to 14, 17, 18, 20, and 24 postinfection.
[d] T_{max}, time in days to maximum mean paw mass.

hamsters treated on days 1 to 7 with clarithromycin or doxycycline at 20 mg/kg. The numerical results of this trial are presented in Table 2. The AUC values, calculated as milligrams · days, indicated that clarithromycin or doxycycline at 20 mg/kg daily effectively prevented paw swelling in infected hamsters. Over the course of the trial, the AUC values for untreated infected hamsters were approximately three times greater than those for infected hamsters treated with clarithromycin at 20 mg/kg. Hamsters treated with clarithromycin or doxycycline at 10 mg/kg daily suffered a slight and transient swelling of the hind paws. The onset of this minimal swelling was not delayed, but there was a more rapid recovery (return to baseline values for paw mass) in the treated hamsters.

Clarithromycin and 14-hydroxy-clarithromycin yielded similar efficacies against *B. burgdorferi* 297 infection (Table 3). There was some transient swelling in both groups of treated hamsters. There was a delay in time to maximal paw swelling from 7 days in untreated hamsters to 15 to 17 days in treated hamsters. Untreated hamsters had a mean paw mass (AUC) nearly five times that of hamsters treated with clarithromycin at 25 mg/kg. By day 20, the mean paw masses of all treated hamsters had returned to baseline (noninfected) values. Untreated hamsters had a mean paw mass that stabilized at approximately twice that of noninfected hamsters.

Clarithromycin was also effective when given on a twice-daily (b.i.d.) schedule (Table 4). At 25 mg/kg b.i.d. (50-mg/kg total daily dose), clarithromycin prevented the onset of swelling due to *B. burgdorferi* infection. Similar efficacy was obtained with doxycycline. Untreated hamsters had a mean AUC value over four times that of noninfected hamsters. By the conclusion of the trial, the paw masses of all treated hamsters had returned to baseline values.

Clarithromycin therapy was also effective in reducing the severity of swelling and decreasing recovery time when given after the onset of swelling (Fig. 3 and Table 5).

TABLE 3. Efficacy of clarithromycin, the 14-hydroxy-clarithromycin, and tetracycline in treatment of *B. burgdorferi* 297 infection in hamsters

Group, drug, and dose (mg/kg)[a]	AUC_{0-20} (mg · days)[b]	Paw mass (mg)[c]	T_{max}[d]
Infected			
Clarithromycin			
25	411 (1.19)	29.6 (1.63)	17
12.5	705 (2.04)	66.1 (3.65)	13
14-Hydroxy-clarithromycin			
25	470 (1.36)	44.4 (2.45)	15
12.5	750 (2.17)	62.8 (3.47)	13
Tetracycline, 25	354 (1.02)	19.4 (1.07)	20
None	1,508 (4.37)	202.7 (11.20)	7
Noninfected	345	18.1	

[a] All treatments were once a day on days 1 to 7. There were eight animals per group.
[b] AUC values were calculated for days 0 to 20 of the trial. Test/control (noninfected) ratios are given in parentheses.
[c] Maximum mean paw mass during days 0 to 20. Test/control (noninfected) ratios are given in parentheses. Measurements of paw mass were taken on days 0, 3, 6 to 10, 13 to 15, 17, and 20 postinfection. On day 20, the paw mass of untreated hamsters had stabilized at approximately twice that of noninfected hamsters.
[d] T_{max}, time in days to maximum mean paw mass.

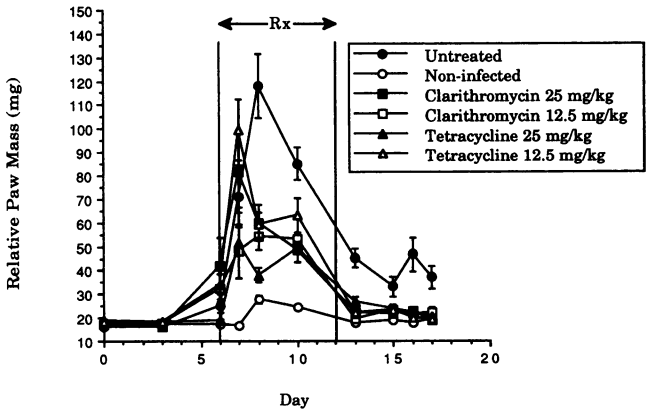

FIG. 3. Efficacies of clarithromycin and tetracycline therapies on a delayed schedule for treatment of *B. burgdorferi* infection in hamsters. Test drugs were administered intraperitoneally once a day on days 6 to 12. The treatment period is indicated (Rx).

TABLE 5. Efficacy of clarithromycin and tetracycline on a delayed-treatment schedule for therapy of *B. burgdorferi* 297 infection in hamsters

Group, drug, and dose (mg/kg)[a]	AUC_{0-6} (mg · days)[b]	AUC_{7-17} (mg · days)[b]	Paw mass (mg)[c]	T_{max}[d]
Infected				
Clarithromycin				
25	138	373 (1.75)	81.5 (2.92)	7
12.5	128	355 (1.67)	54.2 (1.94)	8
Tetracycline				
25	112	343 (1.61)	51.7 (1.85)	7
12.5	134	417 (1.96)	99.8 (3.57)	7
None	110	655 (3.08)	118.1 (4.23)	8
Noninfected	104	213	27.9	

[a] All treatments were once a day on days 6 to 12. There were four animals per group.
[b] AUC values were calculated for days 0 to 6 (before treatment) and days 7 to 17 (during and after treatment) of the trial. Test/control (noninfected) ratios are given in parentheses.
[c] Maximum mean paw mass during days 0 to 20. Test/control (noninfected) ratios are given in parentheses. Measurements were taken on days 0, 3, 6 to 8, 10, 13, and 15 to 17 postinfection.
[d] T_{max}, time in days to maximum mean paw mass.

Hamsters treated with clarithromycin beginning on day 6 (the first day on which swelling was apparent) had a mean AUC value during and after therapy (AUC from days 7 to 17 [AUC_{7-17}]) approximately 50% that of untreated hamsters. Prior to the start of therapy (AUC_{0-6}), the paw masses of all groups of infected hamsters were similar. There was similar efficacy in reducing peak paw swelling and overall mean paw mass with 25 and 12.5 mg of clarithromycin per kg daily. By the conclusion of the trial, the paw masses of treated hamsters had returned to values similar to those for noninfected hamsters, while untreated hamsters had a mean paw mass approximately twice that of noninfected hamsters (Fig. 3).

Recovery of borreliae from treated hamsters. The recovery and cultivation of borreliae from hamster bladders, kidneys, hearts, or spleens at the conclusion of the trials (days 20 to 24 after inoculation) was not a useful indicator of drug efficacy. Untreated hamsters often did not yield detectable numbers of spirochetes. The recovery of *B. burgdorferi* was not used as an indicator of drug efficacy.

Titers of antibody to *B. burgdorferi* in hamster sera. Hamster sera collected at the conclusion of the trial were assayed for anti-*Borrelia* antibody by using an immunofluorescent-antibody test. There was no apparent difference between treated and untreated hamsters in the amount of anti-*Borrelia* antibody present at the trial's conclusion. Both drug-treated and untreated infected hamsters yielded immunofluorescent-antibody mean scores of 3.0 or greater, while noninfected hamsters yielded a mean score of 0.

Pharmacokinetic evaluation of anti-*Borrelia* compounds. A single 20-mg/kg dose of clarithromycin in hamsters yielded a maximum concentration of drug in serum (C_{max}) value of 2.098 μg/ml (Table 6), which was well in excess of the concentration required to kill *B. burgdorferi* in vitro (IC_{50} = 0.044 μg/ml). The C_{max}, AUC_{0-24}, and half-life ($t_{1/2}$) values were similar to those achieved clinically in humans (9). A 20-mg/kg dose of the 14-hydroxy metabolite of clarithromycin, which is not produced in rodents, yielded C_{max}, AUC_{0-24}, and $t_{1/2}$ values less than those for clarithromycin at equivalent doses. The C_{max} of doxycycline was near the in vitro IC_{50} and less than the MIC of the drug.

TABLE 6. Pharmacokinetic evaluation of anti-*Borrelia* drugs in hamsters[a]

Compound	$t_{1/2}$ (h)	C_{max} (μg/ml)	T_{max} (h)[b]	AUC_{0-24} (μg · h/ml)
Clarithromycin	4.11	2.098	2.0	14.37
14-Hydroxy-clarithromycin	1.69	1.466	3.0	7.41
Tetracycline	0.77	0.753	1.0	2.26
Doxycycline	11.5	0.99	3.0	12.70

[a] A single 20-mg/kg subcutaneous dose was given. There were 12 hamsters per treatment group. Drug concentrations in serum were determined 0, 0.5, 1 to 3, 6, 8, 12, 16, and 24 h after dosing.
[b] T_{max}, time to maximum concentration of drug in serum.

DISCUSSION

There is a need for systematic preclinical testing of potential anti-*Borrelia* compounds. In vitro, *B. burgdorferi* is susceptible to a number of agents, including macrolides, tetracyclines, and β-lactam antibiotics (11, 13, 16–18, 20). However, comparison of in vitro potencies from different studies is difficult because of the lack of standard protocols for inoculum size, growth phase of *B. burgdorferi*, or time of incubation. A long period of antibiotic exposure is required to kill *B. burgdorferi*, which complicates the correlation of in vitro potency to potential in vivo efficacy (1, 16). Treatment failures with both tetracyclines and β-lactam antibiotics have been reported, especially in treatment of arthritis associated with late-stage Lyme disease (15, 16, 25). Macrolides are not yet in widespread use against *B. burgdorferi*. Other macrolides with in vitro activities have not shown promise in preclinical development tests (12, 14).

The long exposure period required for antibiotics to kill *B. burgdorferi* in vitro suggests that drugs with long $t_{1/2}$ in serum may be effective therapeutic agents. The recommended tetracycline therapy of 250 mg four times daily (17a) produces serum drug concentrations that are less than the MICs reported by several laboratories (15). Clarithromycin, with both superior in vitro potency against *B. burgdorferi* and promising in vivo pharmacokinetic parameters, including a biologically active metabolite in humans, merited preclinical testing.

Clarithromycin and the 14-hydroxy metabolite of clarithromycin were over 1 log more potent in vitro than either tetracycline or doxycycline against *B. burgdorferi* 297. The use of IC_{50}s rather than traditional MICs is appropriate for comparing the in vitro potencies of anti-*B. burgdorferi* compounds. The in vitro assay depends upon microscopic observation for the determination of spirochete viability. The most accurate calculation of drug potency occurs at the 50% point of the viability curve (Fig. 1). The high potency of clarithromycin and the 14-hydroxy metabolite of clarithromycin suggests that effective serum drug concentrations could be maintained for longer periods in humans, since hamsters do not produce the metabolite.

Early treatment with clarithromycin or doxycycline prevented arthritic paw swelling (Fig. 2). This effect was measured as both a decrease in maximal paw swelling and a reduction in the paw swelling over the course of the trial (Table 2).

Therapy with 14-hydroxy-clarithromycin, a major metabolite in humans but not in hamsters, showed in vivo efficacy similar to that of clarithromycin. Additionally, compared with untreated controls, there was a 6- to 10-day delay in the time required to produce maximal paw swelling (Table 3).

The activity of 14-hydroxy-clarithromycin was tested separately, since this metabolite is not produced in rodents.

Clarithromycin efficacy increased with b.i.d. dosing (Table 4). Hamsters treated with clarithromycin at 12.5 mg/kg b.i.d. suffered less maximal and overall paw swelling than hamsters treated on a once-daily schedule (Tables 2 and 3). Clarithromycin had a $t_{1/2}$ in serum of 4.11 h and a C_{max} of 2.098 μg/ml in hamsters (Table 6). Thus, b.i.d. dosing increased the duration of clarithromycin concentrations in excess of the MIC in plasma. In humans, clarithromycin is usually administered on a b.i.d. schedule, resulting in prolonged concentrations of the parent drug and 14-hydroxy-clarithromycin in serum (9).

Clarithromycin therapy initiated after the onset of arthritis rapidly reduced both the maximal swelling and the duration of arthritis (Fig. 3 and Table 5). After initiation of therapy, animals treated with clarithromycin experienced paw swelling only 50% as great as that of untreated, infected hamsters. This result demonstrated the potential utility of clarithromycin in treatment of established arthritic Lyme disease.

Treated and untreated hamsters produced similar titers of antibody against *B. burgdorferi*. This result may be due to the prolonged exposure time required for antibiotics to kill *B. burgdorferi*, which has a low growth rate. Without rapid killing of the pathogen, the hamster immune system has an opportunity for activation and production of specific antibody. The high antibody titers produced by treated animals indicated that infection was not prevented. The efficacy of the drug treatments was based upon treatment of infection rather than prevention of infection.

The hamster model of Lyme arthritis allows treatment efficacy to be judged by at least three criteria: maximal paw swelling, time to maximal paw swelling, and AUC value for paw swelling (milligrams · days). Clarithromycin, especially on a b.i.d. schedule, was effective treatment for *B. burgdorferi* infection by all three criteria. When initiated after the onset of arthritic paw swelling, clarithromycin therapy was associated with a reduction in maximal paw swelling and a shorter recovery time. The concentrations in serum produced by these doses of clarithromycin in hamsters are similar to those achieved in humans. The successful therapy with clarithromycin and the 14-hydroxy metabolite of clarithromycin in this model suggests that efficacy against established or incubating arthritic Lyme disease may be achievable in humans.

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