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Macrolide therapy of chronic Lyme Disease

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Summary

Background:

Macrolide antibiotics are highly active in vitro against *B.burgdorferi*, but have limited efficacy in the treatment of patients with Lyme Disease. As macrolides are less active at a low pH, their poor clinical activity might be due to localization of borrelia to an acidic endosome, and their activity improved by alkalization of that compartment with hydroxychloroquine.

Material/Methods:

235 patients with a multi-symptom complex typical of chronic Lyme disease, ie fatigue, musculoskeletal pain, and neurocognitive dysfunction and with serologic reactivity against *B.burgdorferi* were treated with a macrolide antibiotic (eg clarithromycin) and hydroxychloroquine.

Results:

Eighty % of patients had self-reported improvement of 50% or more at the end of 3 months. After 2 months of treatment, 20% of patients felt markedly improved (75–100% of normal); after 3 months of treatment, 45% were markedly improved. Improvement frequently did not begin until after several weeks of therapy. There were no differences among the three macrolide antibiotics used. Patients who had been on hydroxychloroquine or macrolide antibiotic alone had experienced little or no improvement. Compared to patients ill for less than 3 years, the onset of improvement was slower, and the failure rate higher in patients who were ill for longer time periods.

Conclusions:

These results support the hypothesis that the Lyme borrelia reside in an acidic endosome and that the use of a lysosomotropic agent augments the clinical activity of macrolide antibiotics in the treatment of patients with chronic Lyme Disease. In contrast, the efficacy of tetracycline in such patients is not affected by hydroxychloroquine.

key words:

Lyme Disease • macrolide • hydroxychloroquine

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BACKGROUND

Infection of humans by *Borrelia burgdorferi* results in a spectrum of clinical illnesses. The earliest symptoms may include a typical (i.e. erythema migrans) or atypical rash, followed by a flu-like illness [1,2]. As the disease progresses, other neurologic and musculoskeletal symptoms and signs may develop [3,4]. The pathophysiology of the chronic symptoms is not well understood, with hypotheses ranging from persisting infection to autoimmunity, or a combination of the two [5,6].

The diagnosis of the chronic disease has been made difficult because of several factors. First, the multisymptom complex consisting of fatigue, musculoskeletal pains, and neurocognitive dysfunction that is typical of patients with chronic Lyme Disease cannot be distinguished from disorders that have been termed fibromyalgia, chronic fatigue, and Gulf War Veterans' Illness [7–10]. Second, laboratory testing has not been reliable, including cultures, antibody studies (ELISA, Western Blots), and PCR-DNA tests [11–13].

The appropriate treatment of patients with chronic Lyme Disease has been an equally controversial clinical issue. Recommendations have been made for both shorter and longer courses of treatment [14,15]; controlled clinical trials will be needed to test these opinions. Many physicians believe that a longer duration of treatment is needed to achieve significant improvement or cure [16], and the results of our previous studies with tetracycline [15] supports that view.

Borrelia burgdorferi is sensitive to several different antibiotics *in vitro* [17]. The location of the organisms *in vivo* is unknown, but is likely to be intracellular if they are to persist over long periods of time. Increasing numbers of microbes with potential for reactivation are being found to be located in lysosomes and other acidic endosomes. In studies of 277 patients with chronic Lyme Disease between 1988 and 1995 [15], treatment with tetracycline over a period of a several months resulted in marked improvement in most patients. Anecdotal observations using macrolide antibiotics have yielded mixed results, despite their excellent *in vitro* activity against *B.burgdorferi* and their excellent intracellular penetration [18,19]. As macrolide antibiotics have limited activity at an acid pH, the hypothesis was generated that the use of lysosomotropic agents, which can alter the pH of acidic intracellular compartments such as the lysosome [20], in conjunction with macrolide antibiotics would improve the activity of the macrolide antibiotic. Experiments in tissue culture have demonstrated improved killing by doxycycline of organisms residing in acidic endosomes using the lysosomotropic agents NH_4Cl , amantadine, and chloroquine [20]; as macrolide antibiotics have even greater restriction of antimicrobial activity at a low pH, it seemed a reasonable hypothesis that this observation might apply to the treatment of patients with chronic Lyme Disease using macrolide antibiotics. This report details the experience with 235 patients who were treated with the combination of a macrolide antibiotic and hydroxychloroquine.

MATERIAL AND METHODS

Patients were seen at the University of Connecticut's Lyme Disease Clinic between 1992 and 1993, and at Boston University Medical Center from 1993–1997. The patients reported here are those whose symptoms existed for 3 months or more, with or without a prior history of tick bite (29% with known bite) or rash (29% with known rash). Inclusion criteria were a combination of at least two of three major symptoms, i.e. fatigue, neurological (e.g. paresthesias, cognitive dysfunction, radicular pains), and musculoskeletal (e.g. arthralgias, myalgias, stiffness); many patients had other symptoms as well, such as visual and auditory disturbances, palpitations, gastrointestinal disturbances, and genitourinary dysfunction (This combination of symptoms has also been noted in other studies [15,21,22]). All patients were evaluated for alternative diagnoses such as rheumatoid arthritis, systemic lupus erythematosus, and other neurologic disorders. Some patients had been given alternative diagnoses of fibromyalgia and/or chronic fatigue; if these patients met the clinical inclusion criteria, they were offered a trial of macrolide and hydroxychloroquine therapy.

Serologic evaluations from 1992–1993 included ELISA and Western Blot tests conducted at the University of Connecticut Health Center; those from 1993–1997 included IgM Capture Assays and Western Blots (IgM, IgG) conducted by BBI/North American Laboratories. Both laboratories have rigid quality control programs and have participated in the evaluation of laboratory tests for Lyme Disease [11]. Western Blots were considered to be positive if there were one or more reactions to proteins specific to *Borrelia burgdorferi*, i.e. 23kd, 31kd, 34kd, 37kd, 39kd, 83kd; most patients had reactions to several specific proteins, and all had reactions to at least two proteins (e.g. 23kd, 41kd).

Patients were treated with hydroxychloroquine, 200 mg twice daily, and a macrolide antibiotic (clarithromycin 500 mg twice daily, azithromycin 250–500 mg daily, or erythromycin 500 mg three times daily). The treatment was continued until patients' symptoms were resolved or improved. In those whose symptoms completely resolved, treatment was generally continued for 1 more month. In those whose symptoms were improved, but not resolved, treatment was generally continued for 1–2 more months before stopping or changing therapies. In those who failed treatment, the therapy was continued for a minimum of 3 months prior to being judged a treatment failure. A treatment cure was defined as the absence of symptoms for 1 or more years following cessation of therapy; marked improvement was defined as having recovered 75% or more of previous normal function, as assessed by the patient. Patients whose symptoms resolved completely by the end of treatment but who subsequently had relapsing symptoms after therapy was discontinued were considered markedly improved, but not cured; these patients usually relapsed within 2–6 weeks following therapy.

Table 1. Western blot vs EIA in the diagnosis of chronic LYME disease.

EIA	Western blot			
	M+ G+	M+ G-	M- G+	M- G-
Positive [49]	28 (57%)*	7 (14%)*	11 (22%)*	3 (6%)*
Negative [142]	28 (20%)	49 (35%)	18 (12%)	47 (33%)

* Number of patients (% of total) with positive (+) or negative (-) serologic tests by EIA (Enzyme Immunoassay) or Western Blot (M=IgM, G=IgG)

Table 2. Western blot vs EIA in the diagnosis of chronic LYME disease.

EIA	Cure	Improvement	Failure
+	4 (8%)*	40 (78%)	7 (14%)
-	4 (3%)	107 (76%)	29 (21%)
Western blot			
M+ G+	1 (2%)	52 (82%)	10 (16%)
M+ G-	4 (6%)	55 (76%)	13 (18%)
M- G+	3 (9%)	23 (64%)	10 (27%)
M- G-	3 (6%)	41 (74%)	11 (20%)

* Number of patients (% of total) with positive (+) or negative (-) serologic tests by EIA (Enzyme Immunoassay) or WB (Western Blot, M=IgM, G=IgG) who were cured, improved, or who failed treatment

Table 3. Gender vs outcome in the treatment of chronic LYME disease.

	Cure	Improvement	Failure
Male	3 (5%)*	48 (80%)	10 (16%)
Female	8 (5%)	126 (75%)	34 (20%)

* Number of patients (% of total) who were cured, improved, or failed treatment

Table 4. Prior symptom duration vs treatment outcome in chronic LYME disease.

Prior Sx duration	Cure	Improvement	Failure
<1 yr	3 (9%)*	30 (85%)	2 (6%)
1-3 yr	4 (6%)	51 (79%)	10 (15%)
>3 yr	4 (3%)	93 (72%)	33 (25%)

* Number of patients (% of total) who were cured, improved, or failed treatment

RESULTS

Serological reactivities and outcomes

A total of 235 patients were treated with a macrolide antibiotic for one or more months. Table 1 shows a comparison of their enzyme immunoassays (EIA) and Western Immunoblots. Overall, 75% were positive by EIA or Western Blot. The EIA was positive in 26% of patients, and the Western Blot was positive in 74%. Whereas the EIA was positive in 6% of patients with a negative Western Blot, the EIA was negative in 67% of patients whose Western Blot was positive. Of positive Western Blots, IgM reactivity was found in 71% of EIA-positive patients and 55% of of EIA-negative patients; in

contrast, IgG reactions were noted in 79% of EIA-positive patients and 32% of EIA-negative patients.

A comparison of treatment outcomes according to serologic reactivity is shown in Table 2. There were no significant differences in treatment outcomes between those whose Western Blots or EIAs were positive compared to those who were seronegative.

Age and gender and outcomes

Patients ranged in age from 15 to 78 (median of 41); 73% were females. There were no differences in treatment outcomes according to age (data not shown) or gender (Table 3).

Prior symptom duration and outcome

Patients whose symptoms had been present for more than one year had more failures (15-25% vs 6%) than patients with symptoms for less than one year (Table 4). Patients with symptoms longer than 3 years fared poorer than those with symptoms for either 1-3 years or less than 1 year.

The prior duration of symptoms was also directly correlated with the time to onset of any improvement, i.e. the longer the prior duration of symptoms, the longer the interval of time before any signs of improvement were noted (Table 5). Compared to patients whose symptoms were present for less than one year, more than 50% of patients with symptoms of longer duration noted no improvement until at least 4 weeks of therapy had elapsed. Almost 30% of patients with symptoms longer than three years had no improvement until 6 weeks or more into treatment.

Figure 1 shows the degree of improvement at 2 months and 3 months following the initiation of therapy. By 2 months, the mean improvement was 50-75%, with only one-fifth of patients feeling markedly improved (75-100%); by 3 months, 42% of patients were markedly improved.

Type of macrolide and outcome

There were no differences in outcome among the three different macrolide antibiotics used (Table 6). The failure rate ranged from 12% to 21%, and the cure rate 4% to 12%.

Duration of treatment and outcome

Patients were treated for as little as 1 month or as long as 18 months, with a median of 6 months. Most patients were treated for between 4 and 8 months. The duration of treatment required to produce sustained improvement was correlated with the prior duration of symptoms (data not shown). Some patients had difficulty tolerating hydroxychloroquine, either because of diarrhea or skin hypersensitivity reactions. No one had any optic complications of hydroxychloroquine therapy.

Table 5. Prior symptom duration vs onset of improvement in the treatment of chronic LYME disease.

Prior Sx duration	Patients*	Time to first improvement (weeks)					
		2	3	4	6	8	12
<1 yr	25	68**	4	20	8		
1-3 yr	39	33	6	44	7	10	
>3 yr	92	26	6	37	12	17	2

* number of patients;

** percent of patients

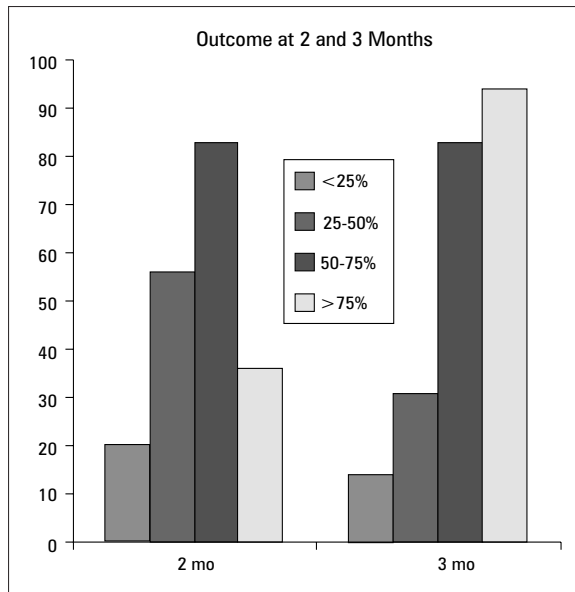


Figure 1. Number of patients improved at 2 months and 3 months post-onset of macrolide and hydroxychloroquine therapy. The degree of improvement is indicated by the bars: 0-25%, 25-50%, 50-75%, and 75-100%.

Treatment outcome and serologic reactivities

No systematic study was made of changes in seroreactivity with treatment. In general, IgM seropositivity reverted to negative with successful therapy, results similar to those previously observed with tetracycline treatment [15].

Retreatment and outcomes

One hundred and twenty patients who were improved at the end of treatment but who had relapsing symptoms were retreated with antibiotics (Table 7). Thirty-three patients were given another course of a macrolide and hydroxychloroquine, and all but one improved. Tetracycline was given to 74 patients, and 73% improved on this regimen; the other 27% failed this therapy. Of 23 patients treated with IV ceftriaxone, nine improved, but 14 failed treatment.

DISCUSSION

The entity of chronic Lyme Disease has been the subject of much controversy [23,24]. That it exists is supported by published reports of epidemiologic studies in endemic areas such as Nantucket (MA) [21] and Westchester

Table 6. Type of macrolide vs treatment outcome in chronic LYME disease.

Macrolide	Cure	Improvement	Failure
Azithromycin	2 (11%)*	13 (68%)	2 (21%)
Clarithromycin	7 (4%)	149 (77%)	38 (20%)
Erythromycin	2 (12%)	12 (75%)	2 (12%)

* Number of patients (% of total) who were cured, improved, or failed treatment

Table 7. Retreatment of chronic LYME Disease and outcome.

Rx type	Cure	Improvement	Failure
Tetracycline	0*	54	20
Macrolide + Hydroxychloroquine	2	30	1
IV Cephalosporin	0	9	14

* Number of patients (% of total) who were cured, improved, or failed treatment

County (NY) [22]. One of the major clinical issues has been the absence of objective criteria that are sometimes seen in late Lyme Disease, such as actual arthritis. Laboratory data have not been very reliable indicators for the existence or absence of Lyme Disease. Many physicians who see patients with the typical multiple symptoms of chronic Lyme Disease make a diagnosis of fibromyalgia, chronic fatigue, or psychosomatic illness by default. Currently, there are no reliable means by which to separate the various multisymptom disorders, the most recent of which is Gulf War Illness.

Whether chronic Lyme Disease represents continuing infection, or is a 'post-Lyme' disorder, is currently unknown. There is limited information regarding the utility of extended antibiotic treatments for this disorder, and whether one class of antibiotics is any more effective than others. With the possibility that the chronic disease is due to persistent infection, the approach was taken to begin to assess the effects of various antibiotic treatments on the course of disease. One of the earliest observations was that patients appeared to respond better to certain antibiotics [15,25], leading to the hypothesis that chronic Lyme Disease is due to a persistent, intracellular, infection. The previously published report on the use of tetracycline in the treatment of chronic Lyme Disease further supports that hypothesis [15]. Many microbial pathogens are now known to have an intracellular reservoir (e.g. chlamydia, Legionella, toxoplasma, histoplasma, leishmania, rickettsiae, cryptococci, M. tuberculosis) [26-33], some-

times remodeling existing endosomes, and frequently occupying an existing endosome such as the lysosome. If this was true for *B.burgdorferi*, then it could explain the variable and generally poor in vivo activity of the macrolide antibiotics, which have markedly diminished antimicrobial activity at a lower pH [34]. With this assumption, then, patients were treated with a combination of macrolide antibiotic, generally clarithromycin, 500 mg twice daily, and the lysosomotropic agent hydroxychloroquine, 200 mg twice daily. The results of this experience with over 200 patients supports the hypothesis that combination therapy is effective. Controlled clinical trials are now needed to test this hypothesis. It is possible that other macrolides, eg dirithromycin, or newer ketolides such as telithromycin, which have excellent in vitro activity against *B.burgdorferi* and which may be more acid-stable than the earlier macrolide antibiotics, may be able to be clinically effective without the adjunctive need for lysosomotropic agents. Controlled clinical trials would also be needed to evaluate this hypothesis.

The results of these and previous studies also emphasize the need for better diagnostic tools, as well as therapeutic approaches. The existing recommendation of a two-tiered approach for the diagnosis of Lyme Disease, beginning with a screening EIA, followed by a Western Blot [35], has been challenged, and accumulating evidence supports the view that this approach falls short of the desired goal of a reliable test to support or refute the clinical diagnosis. Western Blot testing is not an ideal test for clinical laboratories, and there have been substantive criticisms of what have been the recommended criteria for a positive blot. For example, a blot may be positive when there are two reactions by IgM, but the same two reactions by IgG are considered negative unless accompanied by three more reactions. Apart from this illogical discrepancy is the issue of specificity. If a reaction is thought to be highly specific, then the need for additional reactions is not evident. The results of this current study and prior studies also support the increasing evidence that IgM reactivity is common in chronic, active, disease. It is known that IgM reactivity may represent reactivation of latent disease or persistent infection in other chronic infections (e.g. toxoplasmosis, cytomegalovirus), and is likely the case with Lyme Disease. Kalish et al. reported persistence of IgM reactivity for 10–20 years in 10–15% of patients who had Lyme Disease [36]. Seronegativity also occurs in Lyme Disease, both early and later in the disease [1,15,37–39 and Kezler K, Tilton RC, Manak M, and Donta ST. Detection of *Borrelia burgdorferi* DNA by PCR in patients with seronegative Lyme disease (unpublished observations)], and the results of this and prior studies demonstrate that a third of patients with similar clinical symptoms who are seronegative have responses to antibiotic treatment that are indistinguishable from those of seropositive patients (including those who meet the current CDC criteria). These findings suggest that antibody responses are not the best correlates of disease presence or activity, consistent with what might be expected in intracellular infection.

The hypothesis that Lyme Disease is an intracellular infection draws support from what is known about the

treatment of other chronic infections, ie that beta-lactam antibiotics are not as effective as are other classes of antibiotics in eradicating infection. Data from tissue-culture models of *B.burgdorferi* infection in which ceftriaxone and penicillin were ineffective against intracellular organisms [40,41], but in which doxycycline and erythromycin were effective [41], provide additional support for this hypothesis.

Treatment over several months appears to be required to achieve significant improvement in most patients with chronic Lyme Disease. The most important determinant of treatment duration and outcome appears to be the duration of symptoms prior to therapy (Tables 4,5). Improvement frequently did not even begin until after several weeks of therapy, especially in patients who had symptoms for more than a few years. This slow rate of improvement might be related to the slow rates of metabolism and multiplication (if any) of *B.burgdorferi* [42]. The actual duration of treatment needed to effect cure or maximum improvement has yet to be determined, but in our observations thus far appear to be closer to 12–18 months of therapy. This duration of treatment should not be surprising when considering the long durations of therapy needed to control other chronic infections such as tuberculosis, some fungal diseases, leprosy, and Q fever [43].

As with the prior results that showed that tetracycline was effective in resolving symptoms associated with central nervous system function (i.e. cognitive, emotional) [15], the combination therapy of macrolide and hydroxychloroquine was also associated in improvement in cognitive and emotional function. Brain SPECT scan abnormalities commonly seen in patients with chronic Lyme Disease also improved with these therapies (data not shown). Therefore, it would not seem imperative that agents which are thought to better traverse blood-brain barriers be used in the treatment of Lyme Disease. Although no studies comparing either tetracycline or the combination of a macrolide with lysosomotropic agent with intravenous ceftriaxone have as yet been conducted, the results obtained thus far appear to show a greater efficacy of the intracellular-type antibiotics over the beta-lactams, and at lower costs and inconveniences compared to intravenous therapies.

The theoretical possibility exists that the hydroxychloroquine itself could be ameliorating some of the symptomatology of patients with chronic Lyme Disease. This seems unlikely, as most patients with chronic Lyme Disease have little or no relief of their symptoms with the use of non-steroidal anti-inflammatory drugs, supporting the view that most of the arthralgias and myalgias are neuropathic, and not arthropathic, in origin. Furthermore, most patients who have been on hydroxychloroquine for presumed seronegative rheumatoid arthritis, but were subsequently shown to have chronic Lyme Disease, derived no benefit from its use.

CONCLUSIONS

Although the results with both tetracycline and macrolide therapies suggest that patients with chronic Lyme

Disease have marked improvement with these treatments, and that these approaches are relatively simple and inexpensive, controlled clinical trials comparing longer-term with short-term therapy are needed to validate or refute these observations. Recently, a controlled trial comparing one month of IV ceftriaxone followed by two months of oral doxycycline treatment with placebo treatments failed to show obvious differences between the two groups [44]. These results are consistent with both the failure of ceftriaxone to adequately penetrate intracellular reservoirs of organisms and with either too small a dose or too short a duration of doxycycline treatment, and/or high protein binding of doxycycline compared to tetracycline; these issues have also been previously addressed [15]. Additional treatment trials, taking into account pharmacologic considerations of different antibiotics and observations made in this and prior studies, should be helpful in answering questions about which antibiotic regimens are the most effective in the treatment of chronic Lyme Disease.

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