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Roxithromycin in Lyme Borreliosis: Discrepant Results of an *In vitro* and *In vivo* Animal Susceptibility Study and a Clinical Trial in Patients with Erythema Migrans*

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A new semisynthetic macrolide roxithromycin was evaluated for its potential use in the treatment of Lyme borreliosis. Using a macro-dilution broth technique, *Borrelia burgdorferi* was shown to be susceptible to roxithromycin with a minimal bactericidal concentration (MBC) of 0.06–0.25 μg/ml. A systemic *B. burgdorferi* infection was established in gerbils; a dosage of ≥ 25 mg/kg/day roxithromycin for 10 days eliminated the infection. A single blind, randomized multicenter study was performed to evaluate the efficacy of roxithromycin 150 mg b.i.d. versus phenoxymethyl-penicillin 1 g b.i.d. for 10 days in patients with uncomplicated erythema migrans. The study was interrupted when 19 patients had enrolled because of five treatment failures. All 5 patients had received roxithromycin; three patients had persisting or recurrent erythema migrans, one developed a secondary erythema migrans-like lesion and severe arthralgia and one developed neuroborreliosis. *B. burgdorferi* was isolated from skin biopsies after roxithromycin therapy from two patients with persistent erythema migrans and both isolates were still highly susceptible to roxithromycin (MBC = 0.03 μg/ml). No treatment failures were seen in 10 patients treated with phenoxymethyl-penicillin. Roxithromycin is thus not recommended for treatment of Lyme borrreliosis.

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Lyme borreliosis (LB) is a tick-borne multisystemic spirochetosis caused by *Borrelia burgdorferi*. It is now the most frequent vector-transmitted human infectious disease in Europe and USA. Penicillin and tetracycline were early shown to be effective (1, 2) and are currently the most widely used antimicrobial agents for Lyme borreliosis. Whereas *B. burgdorferi* is highly susceptible to erythromycin in vitro (3, 4), it is a common experience that treatment failures may occur when erythromycin is used in patients with Lyme borreliosis (1). The reason for this discrepancy is unknown. We speculated whether the low therapeutic efficiency of erythromycin might be due to insufficient dosage and low bio-availability after oral administration and whether a new semisynthetic macrolide roxithromycin could be more effective. Compared to erythromycin, roxithromycin has a significantly higher bio-availability, tissue penetration and potency (5). The question is relevant because we still lack an alternative oral therapy in children and pregnant women allergic to penicillin.

The aim of this study was first to investigate the in vitro and in vivo animal susceptibility of *B. Burgdorferi* to roxithromycin and, if favourable, to perform a comparative clinical trial of roxithromycin versus penicillin in patients with uncomplicated erythema migrans.

MATERIALS AND METHODS

In vitro study

We used 8 *B. burgdorferi* strains as listed in Table I; except DK6 which was isolated from the CSF of a patient with neuroborreliosis all other strains were isolated from skin biopsies from patients with erythema migrans (n = 2) and acrodermatitis chronica atrophicans (n = 5). The antimicrobial agents tested were: penicillin, erythromycin, roxithromycin, doxycycline and ceftriaxone. The antibiotic susceptibility of *B. burgdorferi* was studied by the macrodilution broth technique in BSK medium to determine minimal bactericidal concentrations (MBCs) – the lowest antimicrobial concentration to completely inhibit growth of *B. Burgdorferi*. The concentrations of the agents tested ranged from 0.03 μg/ml to 32 μg/ml. Duplicate tubes containing 7 ml BSK medium with antimicrobial agents and control tubes without antimicrobial agents were inoculated to a final density of 10^5 log phase *B. burgdorferi*. After 7 days of incubation at 32°C 0.1 ml of the medium was transferred to 7 ml BSK medium without antimicrobials and incubated for a further 7 days. The spirochete cell count of the last culture was then read by darkfield microscopy.

In vivo study

A systemic *B. burgdorferi* infection was established in gerbils (weight 70 g) by intraperitoneal inoculation of 10^8 low passage spirochetes, either strain DK1 or DK7. Before inoculation the spirochetes were washed once and resuspended in PBS. One week after inoculation roxithromycin was given twice daily for 10 days subcutaneously at 5 mg/kg/day (4 animals), 25 mg/kg/day (8 animals) and 125 mg/kg/day (6 animals). Twenty-five gerbils inoculated with *B. burgdorferi* at the same time did not receive antibiotic treatment and served as controls. One week after the last roxithromycin dose all animals were killed and a culture for *B. burgdorferi* in BSK medium was initiated from spleen, kidney and urinary bladder tissue. Triplicate culture tubes from every organ were incubated at 32°C and examined by darkfield microscopy weekly for 1 month as previously described (6). The in vivo susceptibility was expressed as the lowest roxithromycin dose which could eradicate the *B. burgdorferi* infection.

Clinical trial

We performed a, for the investigator, single blind, randomized multicenter study with parallel groups of patients receiving either roxithromycin 150 mg b.i.d. or phenoxymethyl-penicillin 1 g b.i.d. for 10 days. Because of the high rate of spontaneous cure of erythema migrans it was estimated that 100 patients were necessary, 50 patients in each treatment group, for a significant difference to be detectable. Patients were recruited from five Danish and three Swedish dermatological

*The results of this study were presented at the 4th International Conference on Lyme Borreliosis, Stockholm 18.–21. June 1990.

Table I. *MBC (μg/ml) of 5 antimicrobials against 8 strains of B. burgdorferi*

Borrelia burgdorferi strains	Penicillin	Roxithromycin	Erythromycin	Ceftriaxone	Doxycycline
DK1	4.0	0.12	0.03	0.03	0.50
DK2	2.0	0.12	0.50	0.03	2.0
DK3	0.5	0.12	0.12	0.03	0.50
DK4	0.5	0.06	0.06	0.03	2.0
DK5	0.5	0.12	0.06	0.06	0.50
DK6	4.0	0.25	n.e.	n.e.	n.e.
DK7	1.0	0.25	n.e.	n.e.	n.e.
S ACA 1	4.0	0.25	0.25	0.06	4.0
MBC range	0.50–4.0	0.06–0.25	0.03–0.50	0.03–0.06	0.50–4.0
median	2.0	0.12	0.12	0.03	2.0

n.e. not examined

centers during the summer 1989. Only otherwise healthy patients > 17 years old and with a uncomplicated erythema migrans based on clinical evidence were included. At the pretreatment visit a physical routine laboratory and borrelia serological examination of the patients were performed. In two patients with pronounced constitutional symptoms the cerebrospinal fluid was examined and normal findings excluded neuroborreliosis. During and after treatment the patients were requested to record the course of the erythema, general symptoms and side effects on a special report form. Patients were followed up 3–6 weeks and 6 months after therapy. In some patients skin biopsies were taken for histopathology and spirochetal cultivation. The evaluation of drug efficacy was based on the clinical outcome. The study was approved by the Danish (ref. no. 1989-1-53) and Swedish ethics committees (ref. no. 88-59).

RESULTS

The results of the in vivo susceptibility study are summarized in Table I. All *B. burgdorferi* strains were susceptible to roxithromycin in vitro with an MBC of 0.06–0.25 μg/ml. Comparable values were obtained for erythromycin. MBCs for penicillin were considerably higher and revealed a pronounced variation between strains 0.5–4.0 μg/ml. Ceftriaxone showed the lowest MBC.

The results of the in vivo animal susceptibility study are shown in Table II. In 20 of 25 untreated infected gerbils a systemic *B. burgdorferi* infection could be demonstrated by a positive organ culture. Roxithromycin, 5 mg/kg was ineffective, whereas all organ cultures were negative in 14 animals receiving ≥ 25 mg/kg/day roxithromycin for 10 days.

The clinical trial was interrupted within three months and blindness was broken because of five treatment failures among the 19 patients who had by that time entered the study. These 19 patients consisted of 9 males, 10 females with a median age of 54 years (26–71 years); the median duration of their erythema migrans at the time of diagnosis and treatment start was 8 days (1–60 days). Serological examination for anti-*B. burgdorferi* antibodies revealed that before therapy one patient was IgG seropositive and 2 patients were IgM seropositive; a seroconversion 3–6 weeks after therapy was found in two patients regarding IgG and in 5 patients regarding IgM antibodies to *B. burgdorferi*. All 5 treatment failures occurred among the nine patients who had received roxithromycin. The treatment failures occurred at four different participating centers. The 5 patients did not differ in age, disease duration or severity from the remaining patients. Three patients showed persistent or recurrent erythema migrans. In 2 of them *B. burgdorferi* was isolated from a skin biopsy after roxithromycin therapy. One patient with an erythema migrans on the left leg developed a secondary erythema migrans-like lesion one week after start of roxithromycin treatment. The skin lesions disappeared a few days after the treatment was completed. However, 2 weeks later the patient complained of severe arthralgia in the left hip and knee but no joint swelling was found. The fifth patient developed a severe back pain 7 days after the last roxithromycin dose. On admission to hospital a lumbar puncture revealed lymphocytic pleocytosis (190 cells/μl) but no borrelia specific antibody synthesis in CSF. The findings were consistent with early neuroborreliosis and the patient recovered completely on a 10-day-course of high dose intravenous penicillin G.

Table II. *In vivo animal study of B. burgdorferi susceptibility to roxithromycin*

Roxithromycin dose (mg/kg/day)	*B. burgdorferi* strain used for infection	Number of gerbils		
		Infected	Culture positive	
0	DK1	9	5/9	20/25
0	DK7	16	15/16	
5	DK1	4	4/4	
25	DK1	3	0/3	0/8
25	DK7	5	0/5	
125	DK1	3	0/3	0/6
125	DK7	3	0/3	

After the blindness of the study was broken all patients treated with roxithromycin were, regardless of persisting symptoms, retreated with phenoxymethyl-penicillin. During the follow-up period of 5 to 10 months none of the 19 patients developed further symptoms or serological evidence of persistent infection.

We tested the in vitro susceptibility to roxithromycin of the two *B. burgdorferi* strains which were isolated from two recurrent erythema migrans lesions after roxithromycin therapy. Both isolates were still fully susceptible (MBC 0.03 μg/ml). Shortly before starting the blinded clinical trial a female with erythema migrans was treated with roxithromycin 150 mg

b.i.d. for 10 days at one of the participating centers. The erythema migrans faded but recurred shortly afterwards. *B. burgdorferi* was isolated from a skin biopsy in this patient before and after roxithromycin therapy. Both isolates were equally susceptible to roxithromycin (MBC 0.06 μg/ml; 0.12 μg/ml).

DISCUSSION

The therapeutic failure of roxithromycin 150 mg b.i.d. for 10 days in patients with erythema migrans was unexpected, considering the high in vitro susceptibility and its efficacy in the in vivo animal model. The in vitro susceptibility of *B. burgdorferi* to roxithromycin was comparable with previous reports (7). However, regarding the susceptibility in the animal model results were discrepant compared with a recent study (7), where roxithromycin was not effective in the gerbil. However, an important difference in the design of the two studies could explain the different results. In the other study, roxithromycin was given as a single daily dose for 7 days, while we administered the drug twice a day for 10 days. Considering the significantly higher drug clearance in small laboratory animals compared to humans (8), an antimicrobial agent should always be administered in divided doses and for at least 10 days, comparable to the therapy recommendations for human Lyme borreliosis (9). Equally inappropriate administration of i.e. penicillin to *B. burgdorferi* infected gerbils (4) and hamsters (3) is very likely the explanation for the often cited inefficacy of penicillin to eradicate *B. burgdorferi*. In both studies penicillin was given only once a day and only for 7 and 5 days respectively. In a previous study we found that penicillin given 3 times a day for 10 days eradicated a systemic *B. burgdorferi* infection in gerbils (10). Considering agents with very long half-lifes, i.e. ceftriaxone, the administration only once a day may not interfere with their high efficacy in the animal model (3, 4).

Similarly, results from in vitro susceptibility studies of *B. burgdorferi* do not always allow one to predict the clinical efficacy of the drug. Roxithromycin was despite of low MBCs not effective. On the other hand, penicillin has shown rather high MBC values (3, 4). Because of this the role of penicillin in the therapy of Lyme borreliosis (11, 12) has been questioned, although firmly verified treatment failures in documented cases of Lyme borreliosis treated appropriately with penicillin are very rare. An explanation for the low in vitro susceptibility of *B. burgdorferi* to penicillin may very likely be the instability of the compound (13) when it is incubated in the culture medium at 32–35°C for 8 days (4) or even 6 weeks (3).

The efficacy of penicillin obtained in the present clinical trial is in accordance with previous experiences of patients with erythema migrans (2) and patients with neuroborreliosis (14, 15). The mechanism for therapeutic failures of the two macrolides erythromycin and roxithromycin is obscure. MBCs of three strains obtained after roxithromycin therapy did not show any development of resistance. In analogy high rates of treatment failure have been reported in patients with primary and secondary syphilis treated with erythromycin (16) despite of a high in vitro activity. Erythromycin is thus no longer listed as a recommended equally effective alternative to penicillin or tetracyclin for the treatment of syphilis (17). Roxithromycin has shown a highly species-dependent protein binding ranging from 7% in rabbits, 30% in rats to 86% in humans (18). This fact may influence: (I) the in vitro determined MBCs because BSK medium contains rabbit serum and bovine serum albumin and (II) the different results obtained from infected humans and laboratory animals treated with roxithromycin. Another new semisynthetic macrolide azithromycin has recently been shown to be highly effective against *B. burgdorferi* in vitro and in infected gerbils and hamsters (7, 19). A clinical evaluation of this compound in Lyme borreliosis would be of great interest.

Recently two patients presumed to have Lyme borreliosis were reported to improve on combined treatment with roxithromycin and cotrimoxazole (trimethoprim/sulphamethoxazole) (20, 21). Both patients had been refractory to penicillin and one even to ceftriaxone therapy. These reports do not agree with the lack of roxithromycin efficacy in Lyme borreliosis demonstrated in this paper. Moreover it is surprising that co-trimoxazole, which is used to avoid contamination in *B. burgdorferi* cultures (22), was reported to be effective. Such cases should, like other presumed penicillin treatment failures that have been reported, lead to a critical reevaluation of the diagnosis. The outcome of this clinical trial furthermore confirmed previous estimates (2) of the incidence of neuroborreliosis (12%) in European patients with untreated erythema migrans.

We conclude that roxithromycin 150 mg b.i.d. is not a recommendable treatment in Lyme borreliosis and that antimicrobial susceptibility studies of *B. burgdorferi* in vitro and in animal models should be interpreted with caution.

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