

# Antibiotics and Increased Temperature against *Borrelia burgdorferi* In Vitro

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In 1917, spirochaetal neurosyphilis was treated successfully with malariotherapy in combination with salvarsan or bismuth. Malariotherapy for spirochaetal Lyme disease has been discussed, but the mechanism of an antispirochaetal effect remains unclear. We cultured *Borrelia burgdorferi* at different temperatures, alone and in combination with antibiotics. Our data demonstrate that growth of the strains PKo and ATCC 35210 (B31) was impaired at temperatures of 37°C and inhibited at 39°C and 40°C, respectively. Strain ATCC 35211, however, grew well up to 39°C but did not multiply at 40°C. A bactericidal effect was seen at 41°C for the strains B31 and PKo and at 42°C for all strains. The susceptibility of all strains to penicillin and ceftriaxone was increased up to 16-fold by an elevation of temperature from 36°C to 38°C. These in vitro data suggest that elevated body temperature may be beneficial during antimicrobial treatment of Lyme disease. This may be particularly important in tissues where high concentrations of antibiotics are difficult to achieve.

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## INTRODUCTION

The persistence of late Lyme disease despite antibiotic treatment (1, 2) has spurred a search for other therapeutic means. *Treponema pallidum*, another spirochaete, causes syphilis, which has a similar spectrum of manifestations, including skin lesions and chronic multi-organ involvement (2). In the pre-penicillin era, benign tertian malaria alone (3) and in combination with salvarsan or bismuth was reported to cure neurosyphilis (4). Malariotherapy for Lyme disease has recently been discussed in this context (5). The mode of action of malariotherapy has not been elucidated so far; it has, however, been attributed to immunological mechanisms. The rapid improvement of clinical symptoms with ceftriaxone in 2 of our patients with febrile neuroborreliosis led to the initiation of the study presented. Here we show the effect of increased temperatures and antibiotics on *Borrelia burgdorferi* in vitro.

## MATERIALS AND METHODS

### *Culture technique*

*Borrelia burgdorferi* type strains ATCC 35210 (B31) (tick isolate from *Ixodes dammini*, kindly provided by Prof. G. Stanek, Vienna), ATCC 35211 (tick isolate from *Ixodes ricinus*, German Collection of Micro-organisms, Braunschweig, Germany) and strain PKo (skin isolate, kindly provided by Prof. V. Preac-Mursic, Munich) were cultured in modified Barbour–Stoenner–Kelly medium (BSK II) (6). All experiments were performed in 24-well tissue culture plates (Costar, Cambridge, MA). Before use, the wells of the tissue culture plates were equilibrated with sterilized 5% neopeptone (Difco, Detroit, USA) in water at 35°C for 24 h.

### *Antibiotic testing at different temperatures*

Benzylpenicillin and ceftriaxone (Sigma, Munich, Germany) were serially 2-fold diluted to obtain final concentrations of 0.002–8 and 0.001–1 µg/ml, respectively. BSK II medium (1800 µl), serial antibiotic dilutions (100 µl) and an actively growing *Borrelia*

culture (100 µl) were dispensed into the wells. The final concentration was  $5 \times 10^5$  cells/ml. The tissue culture plates were taped, sealed airtight and incubated for 4 days at 36°C or 38°C in an air incubator. The minimum inhibitory concentration (MIC) was defined as the lowest antimicrobial concentration with  $\leq 5 \times 10^5$  motile *Borrelia*/ml, as determined using a Petroff–Hausser counting chamber and darkfield microscopy.

Subcultures were performed (5% vol/vol) and incubated at 35°C for 3 weeks. The minimum bactericidal concentration (MBC) was defined as the lowest antimicrobial concentration with no spirochaetes detectable by dark-field microscopy. The experiments were performed on 3 separate occasions, using duplicates each time.

### *Effect of temperature without antimicrobials*

To investigate the effect of temperature alone, the growth of *Borrelia* was determined after 4 days at different temperatures (36–42°C). Subcultures were performed as described above and incubated at 35°C for 3 weeks.

## RESULTS

The strains B31 and PKo grown at 36°C had MIC values for penicillin of 0.06 µg/ml and for ceftriaxone of 0.015 µg/ml and 0.03 µg/ml, respectively. In the cultures incubated at 38°C, the MICs of penicillin for both strains decreased by 4 and 3 titre steps to 0.004 µg/ml and 0.008 µg/ml, respectively. At 38°C, the MICs of ceftriaxone for both strains decreased by 3 and 4 titre steps to 0.002 µg/ml (Fig. 1). Subcultures of these experiments with strains B31 and PKo were cultivated for 3 weeks at 35°C. The subcultures derived from the original 36°C cultures showed MBCs of 4 µg/ml and 0.06 µg/ml for penicillin and of 0.125 µg/ml and 0.06 µg/ml for ceftriaxone, respectively. In the subcultures derived from the original 38°C cultures the MBC of penicillin decreased by 4 and 2 titre steps to 0.25 µg/ml and 0.015 µg/ml, respectively; the MBC of ceftriaxone decreased by 3 and 2 titre steps to 0.015 µg/ml, respectively.

Table I. Number of motile *Borrelia*<sup>a</sup> after incubation at different temperatures for 4 days

Strain	Incubation temperature (°C)						
	36	37	38	39	40	41	42
ATCC 35210	>100 (+)	75 (+)	26 (+)	10 (+)	0 (+)	0 (-)	0 (-)
PKo	>100 (+)	59 (+)	9 (+)	6 (+)	2 (-)	2 (-)	0 (-)
ATCC 35211	>100 (+)	>100 (+)	>100 (+)	>100 (+)	1 (+)	1 (+)	0 (-)

<sup>a</sup> Mean of 4 experiments  $\times 10^5$  borrelia/ml. (+) growth in the subcultures; (-) no growth in the subcultures.

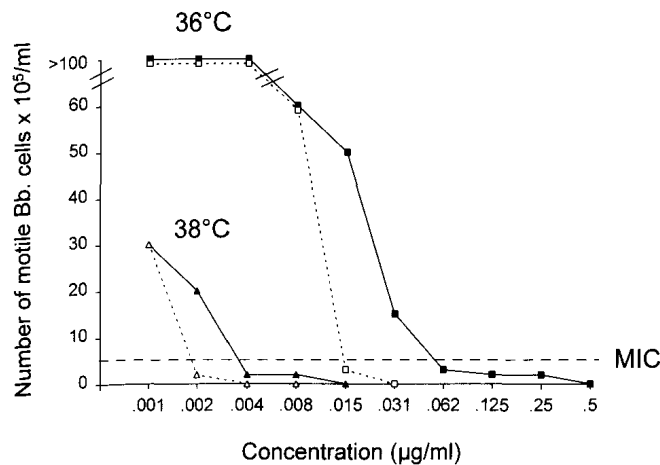


Fig. 1. Type strain ATCC 35210 (B31): MICs for penicillin at 36°C (■) and 38°C (▲) and ceftriaxone at 36°C (□) and 38°C (△).

Type strain ATCC 35211 grown at 36°C had MIC values for penicillin of 0.06 µg/ml and for ceftriaxone of 0.03 µg/ml. At 38°C the MICs of penicillin and ceftriaxone decreased by 1 titre step to 0.03 µg/ml and 0.015 µg/ml, respectively. Subcultures of these experiments with type strain ATCC 35211 derived from the original 36°C and 38°C cultures showed a decrease of the MBC for penicillin from 2 µg/ml to 1 µg/ml and for ceftriaxone from 0.5 µg/ml to 0.25 µg/ml.

When *B. burgdorferi* spirochaetes were incubated at different temperatures in the absence of antibiotics, growth was unaffected at 36°C after 4 days of incubation. At 37°C the cells of strain B31 elongated into filaments with reduced motility. At 38°C, the growth of the strains PKo and B31 was reduced and finally inhibited at 39°C and 40°C, respectively. The growth of strain ATCC 35211 was unaffected up to 39°C and inhibited at 40°C. A bactericidal effect was shown at 41°C for the strains B31 and PKo and at 42°C for all strains tested (Table I).

## DISCUSSION

Raising the temperature affects the growth, morphology, motility, metabolism and virulence of various bacteria (7, 8) and treponema species (9). Barbour (10) observed that the growth of Lyme disease spirochaetes is delayed at 39°C and

does not occur at 40°C and above. Our results show that the growth of strains B31 and PKo was impaired at 37°C and above, while strain ATCC 35211 grew well up to 39°C. These data could suggest that different strains may affect different parts of the body according to their temperature. Thus heat-stable strains would preferentially affect warm regions of the body (e.g. neuroborreliosis), while heat-sensitive strains would show tropism for body regions with lower temperature (e.g. skin).

Our data show that raising the temperature from 36°C to 38°C resulted in a decrease in MICs and MBCs up to 4 titre steps for both *B. burgdorferi* strains (B31 and PKo), which is equivalent to a 16-fold increase in antibiotic activity. However, with strain ATCC 35211 the combination of temperature and antibiotics had a minimal effect.

In vitro experiments do not always reflect the in vivo situation. However, reduced growth of certain strains of *B. burgdorferi* with increasing temperature in vitro suggests that increased body temperature may be beneficial during antimicrobial treatment of Lyme borreliosis. This may be particularly important for infections in tissues and compartments where high concentrations of antibiotics are difficult to achieve. Clinically, a variety of exogenous or endogenous pyrogens other than benign tertian malaria could be used to induce factitious fever. Controlled clinical trials thus seem justified to establish the efficacy of fever alone or as an adjunct measure during antibiotic treatment in late Lyme disease refractory to common antibiotic treatment.

## ACKNOWLEDGEMENTS

We thank Prof. G. Stanek, Vienna, for his help in establishing the *B. burgdorferi* culture technique in our laboratory, and Dr K. Tamussino for reviewing the manuscript.

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*Submitted June 13, 1995; accepted December 12, 1995*