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In vitro activity of tigecycline against multiple strains of *Borrelia burgdorferi*

Xiaohua Yang, Andrew Nguyen, Dan Qiu and Benjamin J. Luft*

Division of Infectious Diseases, Department of Medicine, Stony Brook University, Stony Brook, NY 11794, USA

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***Objectives*: To compare the antimicrobial activity of tigecycline and doxycycline against multiple isolates of *Borrelia burgdorferi*.**

***Methods*: *In vitro* antimicrobial assays were carried out using a microdilution assay. The time needed to inhibit, immobilize and kill the B31 strain of *B. burgdorferi* was determined. The MIC, MBC and concentration needed to immobilize the organism were determined for each antimicrobial for various strains of *B. burgdorferi*.**

***Results*: Tigecycline inhibited the growth of and killed the organism more rapidly than doxycycline. Tigecycline was able to kill *B. burgdorferi* within 24 h at clinically achievable concentrations (<1 mg/L). In contrast, doxycycline was bacteriostatic and required 48–72 h to achieve its maximal inhibitory effect. The anti-*Borrelia* activity of the antibiotics was tested against 20 different isolates from three species. Tigecycline was 16- to 1000-fold more active than doxycycline at immobilizing *Borrelia* for the 20 isolates tested.**

***Conclusions*: We demonstrate that the *in vitro* activity of tigecycline against *B. burgdorferi* is superior to that of doxycycline. Tigecycline acted more rapidly and was bactericidal, whereas doxycycline was bacteriostatic and required a more prolonged co-incubation to achieve its maximal inhibitory effect.**

Keywords: antibiotics, susceptibility, Lyme disease

Introduction

Lyme disease is a multisystem tick-borne infectious disease caused by the slowly dividing spirochete, *Borrelia burgdorferi*. The organism is able to evade host immunity and persist as a latent infection only to recrudesce, giving rise to a chronic disease.[1] Theoretically, it would appear that the ideal antibiotic for the treatment of Lyme disease would be one that is both highly active and rapidly bactericidal.

It has been noted that *B. burgdorferi* has a putative efflux system with significant homology to the RND-type efflux system (TolC and AcrAB).[2] Tigecycline, a new glycylcycline, with excellent activity against most Gram-positive and many Gram-negative bacteria, is a structural analogue of minocycline that avoids tetracycline resistance mediated by bacterial efflux pumps and ribosomal protection.[3] In this study, we wanted to determine whether tigecycline offered any specific benefit compared with doxycycline.

Materials and methods

Medium, antibiotics and spirochete strains

Twenty strains of *Borrelia* were used for the experiments. These included isolates belonging to *B. burgdorferi* (14 isolates), *Borrelia garinii* (3 isolates) and *Borrelia afzelii* (3 isolates). Of the *B. burgdorferi* isolates, six were laboratory strains and eight were strains recently isolated from ticks in Spring Mount, NY, USA. Both the laboratory-adapted B31 strain and the B31 isolate that had been passaged five times (B31-p5) were used for studies.

All *Borrelia* strains were grown in 5% CO_2 at 34°C in Barbour–Stoener–Kelly H (BSK H) medium supplemented with 6% rabbit

*Corresponding author. Tel: +1-631-444-8055; Fax: +1-631-444-6284; E-mail: bluft@notes.cc.sunysb.edu

serum (Sigma, St Louis, MO, USA) to mid-logarithmic stage (2×10^7 cells/mL) as we previously reported.[4]

Determination of antimicrobial activity of doxycycline and tigecycline

Antimicrobial activity was assessed for doxycycline (Sigma) and tigecycline (Wyeth Pharmaceuticals, Pearl River, NY, USA). MICs and immobilization assays (the loss of motility) were determined using the microdilution method (96-well plate) with minor modifications.[4,5] In preliminary studies, the minimal immobilization concentration correlated with killing of 95% of the organisms. Duplicate wells containing BSK H medium with and without the appropriately diluted antimicrobial agents were inoculated with a final density of 5×10^6 cells/mL of the test organism. The ranges of antibiotic concentrations tested were as follows: doxycycline, 0.024–25.0 mg/L; and tigecycline, 0.006–6.25 mg/L. After incubation at 34°C for 3 days, 10 μL aliquots were extracted from each well and live (motile) *Borrelia* were examined by dark-field microscopy. The MIC was the lowest concentration of antibiotic at which the number of cells after incubation did not exceed the initial number of cells. The loss of motility was determined as the minimum concentration of antibiotic that eliminated the characteristic motility of all spirochetes observed by dark-field microscopy (100 cells counted). *In vitro* activities of tigecycline and doxycycline were compared in time with immobilization studies of *B. burgdorferi*.

The MBC was the lowest antibiotic concentration from which spirochetes could not be cultured after 72 h of co-incubation with antibiotics. Following 72 h of incubation with the antibiotic, an aliquot (20 μL) from each test well was transferred to 5 mL of fresh BSK H medium and subcultures were assessed for the presence of motile spirochetes at 21 days.

Time–kill studies were evaluated for the B31 strain. The same microdilution method as described above was used for time–kill assays. The spirochetes were counted at 0, 16, 24, 48 and 72 h. In these time–kill experiments, the MIC and loss of motility were measured by dark-field microscopy.

Results

Time inhibition/killing studies

In our initial experiments, we defined the kinetics of antimicrobial activity by performing time inhibition experiments on the B31 strain of *B. burgdorferi*. We found that effective inhibition was quickly achieved with tigecycline by 24 h (MIC 0.048 mg/L). After 48 h, tigecycline was able to inhibit the growth of the organism at extremely low concentrations (MIC 0.012 mg/L). In contrast, doxycycline was able to inhibit the growth of *Borrelia*, but its action was much slower and clearly time-dependent. At 24 and 48 h, 1.5 and 0.78 mg/L was needed, respectively, to effect inhibition. Optimal inhibition was only achieved at 72 h of co-incubation (0.39 mg/L).

In a similar manner, time to immobilization experiments were carried out. Our results showed that ~0.1 mg/L tigecycline was able to effectively immobilize the organism at 24 h. Doxycycline was ineffective at 24 h (>25 mg/L). After 48 h, tigecycline effectively immobilized the organism at 0.0048 mg/L and doxycycline was active at 3.1 mg/L. After 72 h, doxycycline effectively immobilized the organism at a concentration of 1.5 mg/L.

Immobilization of B. burgdorferi

We next wanted to determine whether tigecycline was able to kill a wide array of both laboratory and recent tick isolates of *B. burgdorferi*. As can be seen in Table 1, doxycycline was unable to uniformly immobilize *B. burgdorferi*, with the concentrations of drug needed varying between 1.5 and 25 mg/L. In contrast, all of the isolates were susceptible to tigecycline with full immobilization occurring between 0.012 and 0.39 mg/L.

MIC and MBC studies

In these studies, we determined the MICs and MBCs for seven strains of *Borrelia* from the three genospecies of *B. burgdorferi*

Table 1. Loss of motility of *Borrelia* strains after 72 h of incubation with tigecycline or doxycycline

Strains	Loss of motility (mg/L)	
	tigecycline[a]	doxycycline[a]
B. burgdorferi		
B31	0.024	1.5
B31-p5	0.024	1.5
N40	0.024	6.2
SV1	0.190	6.2
GI71	0.390	6.2
Bo12	0.024	1.5
range	0.024–0.39	1.5–6.2
average	0.113	3.9
SD	0.151	2.6
Tick culture isolates		
A (1104)	0.012	6.2
B (1012)	0.048	25
E (1007)	0.024	25
E (1011)	0.048	25
H (1014)	0.048	25
K (1112)	0.024	1.5
T (1010)	0.048	12.5
G (1013)	0.048	25
range	0.012–0.048	1.5–25
average	0.038	18.2
SD	0.015	9.9
B. afzelii		
Pgau	0.048	6.2
VS185	0.097	12.5
ACA1	0.097	6.2
range	0.048–0.097	6.2–12.5
average	0.081	8.3
SD	0.028	3.6
B. garinii		
PBi	0.097	25
VSDA	0.024	1.5
DK29	0.048	6.2
range	0.024–0.097	1.5–25
average	0.056	10.9
SD	0.037	12.4

SD, standard deviation.
[a]The concentration needed to achieve 100% loss of motility.

Table 2. MICs and MBCs of tigecycline and doxycycline for different *Borrelia* strains

Strains	Concentration (mg/L)			
	TGC MIC	TGC MBC	DOX MIC	DOX MBC
B. burgdorferi				
B31	0.006	0.195	0.390	25.0
B31-p5	0.006	0.095	0.390	12.5
Bo12	0.012	0.195	0.780	12.5
B. afzelii				
ACA1	0.012	0.095	0.195	12.5
Pgau	0.024	0.195	0.780	25.0
B. garinii				
PBi	0.048	0.195	0.390	25.0
VSDA	0.012	0.195	0.780	25.0

TGC, tigecycline; DOX, doxycycline.

that cause Lyme disease, *B. burgdorferi*, *B. afzelii* and *B. garinii* (Table 2). As can be seen in Table 2, the MIC of tigecycline was 8–65 times lower than that of doxycycline and the MBC of tigecycline was between 64 and 132 times lower than that of doxycycline. Furthermore, the concentration needed for the killing of *B. burgdorferi* by tigecycline was clearly clinically achievable; it was not possible with doxycycline.

Discussion

Although a number of antibiotics, including penicillin, amoxicillin, ceftriaxone, doxycycline and various macrolides, have proved to be useful in the treatment of Lyme disease, they do so with varying degrees of success.[1] Furthermore, animal experiments indicate that *Borrelia* may remain viable even after antibiotic administration.[6] Whether this is associated with clinical human disease is a source of considerable consternation and controversy. Antimicrobial susceptibility studies of *B. burgdorferi* should be able to provide valuable information to shape new therapies.[5]

A number of animal studies have demonstrated that although antibiotics are effective in ameliorating disease, the infection may persist even after seemingly effective therapy.[6] In studies by Moody *et al.*,[7] treatment with oxytetracycline, erythromycin or doxycycline in mice failed to eradicate acute *Borrelia* infection or ameliorate the disease. Chloramphenicol and azithromycin failed to eradicate the organism but ameliorated the disease. In contrast, ceftriaxone, amoxicillin and high-dose penicillin eradicated the disease and cured the infection.[7] In a dog model of infection, Straubinger *et al.*[6] showed that antibiotic-treated dogs continued to have persistent *Borrelia*-specific DNA in their tissue albeit at lower levels than observed in untreated animals. In some instances, this was accompanied by disease.[8]

Although ceftriaxone appeared to be among the most active agents at eradicating the infection in animal models, studies indicate that the organism may persist even in mice that appear to be receiving adequate therapy.[9,10] Bockenstedt *et al.*[9] demonstrated that genetically altered non-cultivatable *B. burgdorferi* could be isolated from mice treated with ceftriaxone. Hodzic *et al.*[10] also found that following antibiotic (ceftriaxone) treatment, mice remained infected with non-dividing, but infectious, spirochetes, particularly when antibiotic treatment was commenced during the chronic stage of infection. In all of these experimental models, the mechanisms involved in microbial persistence after antimicrobial therapy have not been elucidated.

In our time–kill experiments, we showed that tigecycline acted both rapidly and at easily achievable concentrations to immobilize and kill *B. burgdorferi* within 24 h. In previous experiments, we and others noted that in order to maximize the efficacy of an antibiotic, prolonged (72 h) co-incubation of *Borrelia* and the drug was necessary. In this study, tigecycline was found to be remarkably active after 24 h of co-incubation. This rapid level of activity at such an early timepoint is unique to tigecycline.

The prolonged time needed to kill *B. burgdorferi* has been the rationale for using drugs with a longer $t_{1/2}$ as therapeutic agents, as well as using these drugs for a more prolonged period of time. In other microbial systems, the *in vitro* time–kill curve of the pathogen has been shown to have the best correlation to cure in animal models of infection. It will be important to determine whether tigecycline, with its rapid action, is more effective in radically curing an animal than antibiotics that act more slowly. Effective treatment of the latent infection has not been characterized; however, an antibiotic that is rapidly bactericidal and active against the dividing and stationary organism may be of significant benefit. Further animal studies are needed to determine the optimal way to use tigecycline alone and in combination with other drugs that act upon the cell wall, such as amoxicillin and ceftriaxone. However, tigecycline may be an important alternative for individuals who fail to respond to current therapy.

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Transparency declarations

None to declare.

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