Omadacycline Is Highly Active In Vitro against Mycoplasma genitalium

Ken B. Waites,a Donna M. Crabb,a T. Prescott Atkinson,b William M. Geisler,c Li Xiao c

aDepartment of Pathology, University of Alabama at Birmingham, Birmingham, Alabama, USA
bDepartment of Pediatrics, University of Alabama at Birmingham, Birmingham, Alabama, USA
cDepartment of Medicine, University of Alabama at Birmingham, Birmingham, Alabama, USA

ABSTRACT

Here, we performed in vitro susceptibility testing on 10 Mycoplasma genitalium isolates against omadacycline, minocycline, tetracycline, doxycycline, moxifloxacin, levofloxacin, and azithromycin. Omadacycline was the most potent agent, with all MICs of ≤0.5 μg/mL. MICs were not affected by resistance to other agents, including resistance to other tetracycline class drugs. Omadacycline may be a potential treatment option for M. genitalium infection.

IMPORTANCE

There are very few clinical isolates of Mycoplasma genitalium available for in vitro susceptibility testing. We studied 10 isolates and determined that the new semisynthetic aminomethylcycline omadacycline is active against isolates that are resistant to tetracyclines, macrolides, and quinolones. These data suggest that clinical studies should be performed in order to see if omadacycline may be useful to treat urogenital infections caused by M. genitalium.

KEYWORDS

Mycoplasma genitalium, omadacycline, antimicrobial resistance, tetracyclines, macrolide, quinolone, antimicrobial treatment, cervicitis, pelvic inflammatory disease, urethritis

Mycoplasma genitalium causes urethritis and cervicitis and has been associated with reproductive sequelae (1). CDC-recommended treatments for M. genitalium include doxycycline, azithromycin, and/or moxifloxacin, depending on whether resistance has been documented and the clinical syndrome being treated (2). Increasing resistance to macrolides and fluoroquinolones and poor clinical efficacy with doxycycline underscore the urgent need for new treatments (3).

Omadacycline is a semisynthetic aminomethylcycline antibiotic that is active against a broad spectrum of Gram-positive and Gram-negative bacteria and some anaerobes, as well as atypical bacteria, including Legionella, Chlamydia, Mycoplasma pneumoniae, Mycoplasma hominis, and Ureaplasma species (4–8). The C-7 modification of the tetracycline D-ring circumvents the tetracycline efflux pump resistance mechanism (e.g., tetK), and the C-9 modification circumvents the ribosomal protection resistance mechanism (e.g., tetM) (9). Omadacycline is FDA cleared and indicated for community-acquired bacterial pneumonia and acute bacterial skin and soft tissue infection. The proportion of administered omadacycline dose excreted in the urine, its extensive tissue distribution, a cystitis study showing favorable outcomes with omadacycline treatment, and in vitro activity against other mycoplasmal species (6, 10) all suggest omadacycline may be beneficial in treating M. genitalium infections. Moreover, omadacycline is a derivative of minocycline, and minocycline has been shown to effectively treat some multidrug resistant M. genitalium infections (11). Our study determined MICs for omadacycline and other agents against M. genitalium reference strains and clinical isolates, including multidrug-resistant strains.

Antimicrobials were obtained in powdered form of known purity and dissolved per the manufacturer’s instructions. Stock solutions were prepared and used to make dilutions in
96-well microtiter plates. Agents tested included omadacycline, minocycline, tetracycline, doxycycline, moxifloxacin, levofloxacin, and azithromycin. Inoculum preparation, broth microdilution MIC assays, and quality-control procedures were performed by methods established for human mycoplasmas by the Clinical and Laboratory Standards Institute (CLSI) guideline M43-A (12). Some modifications were necessary due to the very slow growth of *M. genitalium*. All organisms were stored frozen at −80°C until thawed to room temperature for testing. *M. genitalium* ATCC reference strain 33530 (G37) was used for quality control. Additional quality-control MICs were determined using *M. hominis* ATCC 23114, which has reproducible MICs and designated MIC reference ranges for various drugs. Clinical isolates of *M. genitalium* are extremely rare due to difficulty in isolating them in culture from clinical specimens. Therefore, reference strains (n = 5), older stored clinical isolates (n = 2), and recent clinical isolates from patients undergoing testing at the University of Alabama at Birmingham (UAB) Diagnostic Mycoplasma Laboratory (n = 4) were used. There were three multidrug-resistant isolates obtained at different time points from an immunocompromised male who had failed multiple treatments that included doxycycline, azithromycin, and moxifloxacin and an additional multidrug-resistant isolate from another immunocompromised male who had received doxycycline. For these four drug-resistant *M. genitalium* isolates, mutations in 23S rRNA associated with macrolide resistance and mutations in the quinolone resistance-determining regions of *gyrA*, *gyrB*, *parC*, and *parE* were determined by Sanger sequencing of the corresponding genes (13). The 16S rRNA gene sequence was also tested for mutations (14), and the presence of the *tetM* element was assessed by PCR (15).

MIC data are shown individually in Table 1 and are summarized in Table 2. Omadacycline was potent against all *M. genitalium* strains, with MICs of ≤0.5 μg/mL (Table 2). Four *M. genitalium* isolates, of which three were recovered from a single patient at different time points, had mutations conferring resistance to azithromycin (MICs, 2 to 16 μg/mL), levofloxacin (MICs, 4 to 8 μg/mL), and moxifloxacin (MICs, 2 to 4 μg/mL). Tetracycline MICs (8 to 16 μg/mL) for these isolates were also 2- to 4-fold higher than the highest MIC for the isolates that were susceptible to macrolides and fluoroquinolones (4 μg/mL). Omadacycline MICs (0.063 to 0.125 μg/mL) were unaffected by resistance phenotype. Minocycline MICs (0.25 μg/mL) for two of the four multidrug-resistant isolates were similar to those obtained for the other six isolates, but the other two multidrug-resistant isolates had minocycline MICs of 0.5 to 1 μg/mL, which were 2- to 4-fold higher than the highest minocycline MIC for the isolates that were susceptible to macrolides and fluoroquinolones (0.25 μg/mL). Doxycycline MICs were 0.125 to 0.25 μg/mL for isolates for which tetracycline MICs were ≤4 μg/mL and were 2- to 8-fold higher (0.5 to 2 μg/mL) in four isolates that exhibited very high MICs for tetracycline (8 to 16 μg/mL).

Our study is the first to our knowledge to determine MICs of omadacycline for *M. genitalium*. We found that omadacycline had potent in vitro activity against *M. genitalium*, with very low MIC values against all strains tested, including multidrug-resistant strains for which omadacycline MICs were severalfold lower than other drugs in the tetracycline class. Most clinical experience and research to date using a tetracycline class antibiotic for treatment of *M. genitalium* have been with doxycycline, which has been found to have poor clinical and microbiological efficacy despite relatively low MICs (3, 16). However, limited case reports show minocycline can sometimes be an effective *M. genitalium* treatment in the setting of doxycycline failure (11, 17). Minocycline is also known to be more efficacious than doxycycline against some other bacteria, such as methicillin-resistant *Staphylococcus aureus* and *Acinetobacter*, despite having similar MIC values (18). Differences in antibacterial efficacy between minocycline and doxycycline could possibly relate to differing pharmacokinetic/pharmacodynamic parameters and tissue penetration.

Based on our clinical experience, there are also patients with *M. genitalium* infection who experience treatment failure with minocycline; it is possible in some that the *M. genitalium* strains may be tetracycline-resistant like the strains we tested here (Table 1). However, in most cases of *M. genitalium* treatment failure, it is unknown whether the strain is tetracycline resistant because the strain cannot be grown in culture for susceptibility testing, and there is no genotypic marker currently available for detecting a tetracycline-resistant strain.
| Strain                  | Yr  | Body site | MICs (µg/mL) of: | Genetic alterations\(^b\) of: |
|------------------------|-----|-----------|------------------|-----------------------------|
|                        |     |           | Oma  | Min  | Tet  | Dox  | Azi  | Lev  | Mox  | 16S rRNA | 23S rRNA | gyrA | gyrB | parC | parE |
| ATCC 33530-G37 control | 1980| Urethra   | 0.125| 0.125| 0.5  | 0.125| ≤0.004| 1    | 0.125|          |          |      |      |      |      |
| JB                     | 1980| Urethra   | 0.5  | 0.25 | 0.5  | 0.125| ≤0.004| 1    | 0.063|          |          |      |      |      |      |
| ATCC 19896-TW10        | 1974–1975| Throat | 0.25 | 0.25 | 1    | 0.25 | ≤0.004| 2    | 0.125|          |          |      |      |      |      |
| ATCC 49897-R32G        | 1974–1975| Throat | 0.25 | 0.125| 0.5  | 0.125| ≤0.004| 2    | 0.063|          |          |      |      |      |      |
| ATCC 49898-TW48-5G     | 1974–1975| Throat | 0.125| 0.25 | 0.5  | 0.25 | ≤0.004| 1    | 0.125|          |          |      |      |      |      |
| ATCC 49895-M30         | 1980 | Urethra   | 0.063| 0.063| 0.5  | 0.125| ≤0.004| 2    | 0.063|          |          |      |      |      |      |
| M2341                  | 1991| Urethra   | 0.125| 0.25 | 4    | 0.25 | ≤0.004| 0.25 | 0.032|          |          |      |      |      |      |
| UAB-73697\(^c\)        | 2018| Urine     | 0.125| 1    | 8    | 0.5  | 4    | 8    | 2    | C1440T   | A2072G   | WT   |      |      |      |
| UAB-75956\(^c\)        | 2019| Urine     | 0.125| 0.5  | 16   | 2    | 16   | 8    | 4    | C1440T   | A2072G   | WT   |      |      |      |
| UAB-84535\(^c\)        | 2021| Urine     | 0.063| 0.25 | 16   | 1    | 2    | 4    | 2    | C1440T   | A2072G   | WT   |      |      |      |
| UAB-84211              | 2020| Urine     | 0.063| 0.25 | 8    | 0.5  | 4    | 8    | 4    | WT       | A2071G   | G295T(D99Y)| A1483G(I495V)| G248T(S83I)| WT   |

\(\text{MIC}=10\); Oma, omadacycline; Min, minocycline; Dox, doxycycline; Azi, azithromycin; Lev, levofloxacin; Mox, moxifloxacin; WT, wild type, no mutations; ATCC, American Type Culture Collection.

\(\text{Sequences were compared to strain ATCC 33530-G37.}\)

\(\text{Isolates obtained at different time points from the same patient.}\)
TABLE 2 MIC summary data for antimicrobial agents tested against *Mycoplasma genitalium*

| Parameter | Omadacycline | Minocycline | Tetracycline | Doxycycline | Azithromycin | Levofloxacin | Moxifloxacin |
|-----------|--------------|-------------|--------------|-------------|--------------|--------------|--------------|
| **Range** | 0.063 to 0.5 | 0.063 to 1  | 0.5 to 16    | 0.125 to 2  | ≤0.004 to 16 | 0.25 to 8    | 0.032 to 4   |
| MIC₅₀     | 0.125        | 0.25        | 1            | 0.25        | ≤0.004       | 2            | 0.125        |
| MIC₉₀     | 0.25         | 0.5         | 16           | 1           | 4            | 8            | 4            |

We did not detect the tetM sequence in the tetracycline-resistant isolates we studied, and we are currently investigating other tetracycline resistance mechanisms to determine a genotypic marker(s) for tetracycline resistance. Our data suggest that omadacycline may be a potential treatment alternative for infections with multidrug-resistant *M. genitalium* infections that do not respond to other agents, and our results justify clinical studies to investigate this possibility.

**ACKNOWLEDGMENT**

Omadacycline powder was provided by Paratek Pharmaceuticals, Boston, USA.

**REFERENCES**

1. Taylor-Robinson D, Jensen JS. 2011. *Mycoplasma genitalium*: from chrysalis to multicellular butterfly. Clin Microbiol Rev 24:498–514. https://doi.org/10.1128/CMR.00006-11.
2. Workovský K, Bachman L, Chan P, Johnston C, Muzny C, Park I, Reno H, Zenilman JM, Bolan G. 2021. Sexually transmitted infections treatment guidelines. Morbidity and Mortality Wkly Rep 70:1–187. https://doi.org/10.15585/mmwr.r.7004a1.
3. Bradshaw CS, Jensen JS, Waites KB. 2017. New horizons in *Mycoplasma genitalium* treatment. J Infect Dis 216:S412–S419. https://doi.org/10.1093/infdis/jix132.
4. Macone AB, Caruso BK, Leahy RG, Donatelli J, Trieber CA, Tanaka SK, Levy SB. 2014. In vitro and in vivo antibacterial activities of omadacycline, a novel aminomethylcycline. Antimicrob Agents Chemother 58:1127–1135. https://doi.org/10.1128/AAC.01242-13.
5. Villano S, Steenbergen J, Loh E. 2016. Omadacycline: development of a novel aminomethylcycline antibiotic for treating drug-resistant bacterial infections. Future Microbiol 11:1421–1434. https://doi.org/10.2217/fmb-2016-0100.
6. Waites KB, Crabbb DM, Liu Y, Duffy LB. 2016. In vitro activities of omadacycline (PTK 0796) and other antimicrobial agents against human mycoplasmas and ureaplasmas. Antimicrob Agents Chemother 60:7502–7504. https://doi.org/10.1128/AAC.01734-16.
7. Gallagher JC. 2019. Omadacycline: a modernized tetracycline. Clin Infect Dis 69:51–55. https://doi.org/10.1093/cid/czr394.
8. Zhanle GG, Esquivel J, Zelenitsky S, Lawrence CK, Adam HJ, Golden A, Hink R, Berry L, Schweizer F, Zhanel MA, Bay D, Lagace-Wiens PRS, Walkty AJ, Lynch JP, III, Karlowsky JA. 2020. Omadacycline: a novel oral and intravenous aminomethylcycline antibiotic agent. Drugs 80:285–313. https://doi.org/10.1007/s40265-020-01257-4.
9. Draper MP, Weir S, Macone A, Donatelli J, Triebel CA, Tanaka SK, Levy SB. 2014. Mechanism of action of the novel aminomethylcycline antibiotic omadacycline. Antimicrob Agents Chemother 58:1279–1283. https://doi.org/10.1128/AAC.01066-13.
10. Overcah JS, Bhiwandi P, Garrity-Ryan L, Steenbergen J, Bai S, Chitra S, Manley A, Tzianis E. 2019. Pharmacokinetics, safety, and clinical outcomes of omadacycline in women with cystitis: results from a phase 1b Study. Antimicrob Agents Chemother 63:e02083-18. https://doi.org/10.1128/AAC.02083-18.
11. Glaser AM, Geisler WM, Ratiff AE, Xiao L, Waites KB, Gaisa M. 2019. Two cases of multidrug-resistant genitourinary *Mycoplasma genitalium* infection successfully eradicated with minocycline. Int J STD AIDS 30:512–514. https://doi.org/10.1177/0956462418816757.
12. CLSI. 2011. Methods for antimicrobial susceptibility testing of human mycoplasmas; approved guideline CLSI document M4-A3. CLSI, Wayne, PA.
13. Xiao L, Waites KB, Van Der Pol B, Aaron KJ, Hook EW, III, Geisler WM. 2019. *Mycoplasma genitalium* infections with macrolide and fluoroquinolone resistance-associated mutations in heterosexual African American couples in Alabama. Sex Transm Dis 46:18–24. https://doi.org/10.1097/OLQ.0000000000000891.
14. Weisburg WG, Barns SM, Pelletier DA, Lani DJ. 1991. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697–703. https://doi.org/10.1128/JB.173.2.697-703.1991.
15. Blanchard A, Crabbb DM, Dybwig K, Duffy LB, Cassell GH. 1992. Rapid detection of tetM in *Mycoplasma hominis* and *Ureaplasma urealyticum* by PCR: tetM confers resistance to tetracycline but not necessarily to doxycycline. FEMS Microbiol Lett 95:277–281. https://doi.org/10.1007/s00268-9929200379x.
16. Wood GE, Jensen NL, Astete S, Jensen JS, Kenny GE, Khoropou CM, Gillespie CW, Manhart LE, Totten PA. 2021. Azithromycin and doxycycline resistance profile sequences of U.S. *Mycoplasma genitalium* strains and their association with treatment outcomes. J Clin Microbiol 59:e0081921. https://doi.org/10.1128/JCM.00819-21.
17. Deguchi T, Ito S, Yusauda M, Kondo H, Yamada Y, Nakane K, Mizutani K, Tsuchiya T, Yokai S, Nakano M. 2017. Emergence of *Mycoplasma genitalium* with clinically significant fluoroquinolone resistance conferred by amino acid changes both in GyrA and ParC in Japan. J Infect Chemother 23:648–650. https://doi.org/10.1016/j.jiac.2017.03.008.
18. Cunha BA, Baron J, Cunha CB. 2018. Similarities and differences between doxycycline and minocycline: clinical and antimicrobial stewardship considerations. Eur J Clin Microbiol Infect Dis 37:15–20. https://doi.org/10.1007/s10096-017-3081-x.