In vitro activity of omadacycline and levofloxacin against *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus saprophyticus* in human urine supplemented with calcium and magnesium

Paul Pagano, Andrea Marra*, Dean Shinabarger and Chris Pillar

Micromyx, Inc., Kalamazoo, MI, USA

*Corresponding author. E-mail: amarra@micromyx.com

Background: Omadacycline, an aminomethylcycline, was approved in 2018 for the treatment of acute bacterial skin and skin structure infections and community-acquired bacterial pneumonia. In a Phase Ib study, around 34% of the absorbed dose of omadacycline was shown to be excreted in urine—an important property for urinary tract infection (UTI) treatment. Therefore, omadacycline has been studied in two Phase II trials for the treatment of uncomplicated UTIs and acute pyelonephritis. The activity of omadacycline against UTI pathogens in human urine is important to understand in this context.

Objectives: To study the *in vitro* activity of omadacycline against UTI pathogens in human urine supplemented with calcium and magnesium.

Methods: Omadacycline activity was compared with that of levofloxacin against the urinary pathogens *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus saprophyticus* in standard medium, pooled normal human urine and neutral pH-adjusted pooled normal human urine spiked with calcium or magnesium at concentrations consistent with hypercalcaemia and hypermagnesaemia.

Results: The activities of omadacycline and levofloxacin against these urinary pathogens were lower in urine relative to standard medium; addition of Mg^{2+} to broth and urine had a further negative impact on omadacycline activity, whereas the addition of Ca^{2+} had less of an impact. Levofloxacin activity was not substantially reduced in either broth or urine by the addition of divalent cations.

Conclusions: The activity of omadacycline against UTI organisms was lower in urine relative to standard medium and was negatively impacted by magnesium. Omadacycline displayed slightly reduced activity when excess calcium was present, but, overall, the differences were ≤2-fold. These observations should be considered along with the pharmacokinetics of the agent for clinical context.

Introduction

Urinary tract infections (UTIs) are among the most common infections in the USA, accounting for nearly 10 million outpatient visits and 1 million emergency room visits annually, posing a substantial healthcare burden on the community, long-term care facilities and hospitals.[1] *Escherichia coli* causes most UTIs; however, other species are associated with recurring and hospital-acquired UTIs.[2] The rise of organisms containing ESBLs has prioritized the need for new agents to treat these infections.[3]

The aminomethylcycline omadacycline has completed clinical evaluation for the treatment of uncomplicated UTI and acute

pyelonephritis;[4] results are expected in late 2020.[5] Omadacycline is active *in vitro* against UTI-causing Enterobacterales with MIC_{50} and MIC_{90} values of 1 and 2 mg/L, respectively, for *E. coli* and 2 and 4 mg/L, respectively, for *Klebsiella* spp.[6] when tested under standard broth microdilution conditions (pH 7.2–7.4; 20–25 mg/L Ca^{2+} and 10–12.5 mg/L Mg^{2+}). Both pH and concentration of divalent cations are known to impact the activity of antibiotics during *in vitro* testing and are important variables to consider when evaluating an antibiotic for activity in urine. The pH of urine can range from 5.1 to 6.8[7] and during infection by certain organisms, notably *Proteus* spp., *Klebsiella pneumoniae* and *Staphylococcus saprophyticus*, bacterial production of urease increases urinary pH.[8] In

addition, both magnesium and calcium cations play crucial roles in human physiology and their serum concentrations are tightly controlled.[9,10] Patients with abnormally high levels of these cations (due to decreased renal function or diets high in magnesium, in the case of hypermagnesaemia,[9] or an overactive parathyroid gland, in the case of hypercalcaemia[10,11]) can excrete elevated levels of magnesium and calcium, respectively, in their urine. Whereas approximately 150 mg of magnesium[12] and 100–300 mg of calcium[10,11] are normally excreted daily in the urine, excretion of higher levels of these cations can be indicative of these disorders.[12,13] Hypercalcaemia is defined as a serum calcium level exceeding 3.5 mmol/L,[11] whereas hypermagnesaemia is defined as a serum magnesium level of greater than 1.05 mmol/L.[14]

Studies have shown that the pH of urine can affect antibiotic efficacy, with tetracyclines displaying improved activity at lower pH (pH 5.0) compared with higher pH (pH 8.0) and with levofloxacin showing improved activity at higher pH compared with lower pH.[15] Previous results for omadacycline indicated that MIC values of this drug were several-fold higher at pH 5.0 relative to standard pH when tested by broth microdilution in CAMHB medium for *Staphylococcus aureus*, *Enterococcus faecalis* and *E. coli*, but were unaffected at higher pH.[16] In contrast, there was little impact observed on the activity of omadacycline (MIC values within 2-fold) against *E. coli* and *S. saprophyticus* when the medium was supplemented with up to twice the calcium and magnesium relative to standard medium [25 mg/L (0.62 mmol) Ca^{2+} and 12.5 mg/L (0.51 mmol) Mg^{2+}].[16] It is also known that tetracyclines can form complexes with divalent cations,[17] which inhibits their activity against bacteria;[17,18] supplementation of the medium with 50 mg/L calcium and 25 mg/L magnesium increased tetracycline MIC values by 2- to 32-fold for *E. coli*, *Acinetobacter* spp. and *Pseudomonas* spp.[19]

As pH and divalent cations have been shown to affect the activity of omadacycline in a medium, it is important to understand how these parameters can impact omadacycline in urine, as these variables are known to fluctuate even in healthy individuals.[20] For example, it has been observed that in women, urine pH decreases with age—as does Mg^{2+} excretion in urine—and calcium excretion increases, whereas these trends are much less evident in men.[20] In this study, the inhibitory activities of omadacycline and levofloxacin were evaluated in CAMHB medium, urine and pH-adjusted urine, both with and without cation supplementation at concentrations consistent with hypercalcaemia and hypermagnesaemia.

Materials and methods

Clinical isolates were collected between 2010 and 2012 from adult patients (≥18 years of age) with UTIs. The isolates were selected to reflect a range of resistance phenotypes. The activities of omadacycline and levofloxacin were evaluated against non-duplicate, non-consecutive clinical isolates of *E. coli* (n = 3), *K. pneumoniae* (n = 3) and *S. saprophyticus* (n = 3) from the Micromyx repository, the National Collection of Type Cultures (NCTC; Salisbury, UK) and ATCC (Manassas, VA), as shown in Table 1. In addition to *E. coli* ATCC 25922, *S. aureus* ATCC 29213 was included for quality control purposes during the testing of *S. saprophyticus*. Omadacycline powder was provided by Paratek; levofloxacin was obtained from Sigma. Working stocks of omadacycline and levofloxacin were made in water at 40× the highest test concentration.[21] Concentration ranges used spanned relevant quality control ranges and breakpoints as established for each test compound.[21,22]

Broth microdilution assays were performed per CLSI guidelines[21,22] in CAMHB as standard medium (pH 7.3) or using pooled normal human urine (pH 6.2; Innovative Research, Lot No. IR100007P-22502; pooled from two or more donors) and pH-adjusted (pH 7.2) pooled urine with and without added calcium (4 mmol/L Ca^{2+} or 443.92 mg/L $CaCl_2$) or magnesium (4 mmol/L Mg^{2+} or 813.2 mg/L $MgCl_2$:6H_2O) as the medium. MIC values were within CLSI quality control ranges under standard conditions.[21] MIC values were read as the lowest concentration where the button of bacterial growth at the bottom of the test well was no longer visible.

Results and discussion

The MIC values of omadacycline and levofloxacin in standard medium, urine and pH-adjusted urine, with and without supplemental calcium and magnesium, are shown in Table 1. Also shown is the fold-change in MIC value of each drug for each condition.

In CAMHB, omadacycline had MIC values of 0.5–2 and 0.25–0.5 mg/L for *E. coli*/*K. pneumoniae* and *S. saprophyticus*, respectively. MIC values of omadacycline in both urine and pH-adjusted urine were 2- to >8-fold higher than in CAMHB for *E. coli*/*K. pneumoniae*, but were similar for *S. saprophyticus*. When CAMHB was supplemented with magnesium, MIC values of omadacycline were 4- to 8-fold higher for *E. coli*/*K. pneumoniae* and 2- to 4-fold higher for *S. saprophyticus* compared with standard CAMHB. MIC values in calcium-supplemented CAMHB were within 2-fold of those observed in standard CAMHB. When omadacycline activity was evaluated in either urine preparation supplemented with magnesium, MIC values were up to 4-fold higher for *E. coli*/*K. pneumoniae*. In pH-adjusted urine supplemented with magnesium, omadacycline MIC values were 8- to 16-fold higher for *S. saprophyticus*. Calcium modestly impacted omadacycline activity in both urine preparations, with MIC values identical or within 2-fold of those observed in unsupplemented urine. Given that FDA-approved breakpoints of omadacycline for Enterobacterales (*K. pneumoniae* and *Enterobacter cloacae* only) are ≤4 mg/L for susceptibility, it is likely that omadacycline would largely lack activity against many of these organisms in urine.

In CAMHB, levofloxacin demonstrated MIC values of ≤0.03–0.5 mg/L for all susceptible organisms and MIC values of 8–32 mg/L for carbapenem- or fluoroquinolone-resistant strains. Levofloxacin MIC values for *E. coli*/*K. pneumoniae* were higher in urine and pH-adjusted urine than in CAMHB (≤0.03–1 mg/L for susceptible isolates and ≥32 mg/L for carbapenem- or fluoroquinolone-resistant strains). Fluoroquinolone-susceptible isolates remained susceptible under the various testing conditions in this study, likely a factor in their efficacy in treating infections caused by these organisms. When medium or urine was supplemented with magnesium or calcium, these values were typically within 2-fold of those for unsupplemented medium or urine.

In summary, the *in vitro* activity of omadacycline and levofloxacin against UTI organisms was lower in urine (including pH-adjusted urine) relative to standard medium. The addition of 4 mmol/L magnesium commonly had a negative impact on the activity of omadacycline, though the degree of this impact was typically 2- to 4-fold for omadacycline and ≤2-fold for levofloxacin. Omadacycline and levofloxacin displayed slightly reduced activity in the presence of excess calcium, but, overall, the observed differences were ≤2-fold. These results indicate there may be a threshold of magnesium concentration to impact activity, as a previous

Table 1. MIC values (mg/L) and fold-changes in MIC values of omadacycline and levofloxacin for urinary pathogens in broth and urine supplemented with high concentrations (4 mmol/L) of magnesium and calcium

Organism	MIC (and fold-change in MIC) of omadacycline in cation-supplemented media									MIC (and fold-change in MIC) of levofloxacin in cation-supplemented media								
	CAMHB (pH 7.3)			urine (pH 6.2)			pH-adjusted urine[a] (pH 7.2)			CAMHB (pH 7.3)			urine (pH 6.2)			pH-adjusted urine[a] (pH 7.2)		
	cation supplements			cation supplements			cation supplements			cation supplements			cation supplements			cation supplements		
	0	Mg	Ca[b]	0	Mg	Ca[b]	0	Mg	Ca[b]	0	Mg	Ca[b]	0	Mg	Ca[b]	0	Mg	Ca[b]
E. coli ATCC 25922	0.5	4 (8)	1 (2)	4	16 (4)	8 (2)	1	2 (2)	2 (2)	≤0.03	≤0.03 (1)	≤0.03 (1)	0.25	0.25 (1)	0.25 (1)	≤0.03	0.12 (≥4)	0.06 (≥2)
E. coli NCTC 13351 (ESBL)	0.5	2 (4)	1 (2)	2	8 (4)	4 (2)	ND	ND	ND	≤0.03	≤0.03 (1)	≤0.03 (1)	0.25	0.5 (2)	0.25 (1)	ND	ND	ND
E. coli MMX 6100 (fluoroquinolone resistant)	2	16 (8)	4 (2)	>16	>16 (NA)	>16 (NA)	8	16 (2)	8 (1)	32	>32 (>1)	32 (1)	>32	>32 (NA)	>32 (NA)	>32	>32 (NA)	>32 (NA)
K. pneumoniae MMX 6268 (carbapenem resistant)	1	4 (4)	1 (1)	8	16 (2)	8 (1)	4	8 (2)	4 (1)	8	16 (2)	16 (2)	>32	>32 (NA)	>32 (NA)	32	>32 (>1)	>32 (>1)
K. pneumoniae MMX 6119	2	8 (4)	4 (2)	>16	>16 (NA)	>16 (NA)	16	>16 (NA)	8 (0.5)	0.06	0.12 (2)	0.12 (2)	1	1 (1)	1 (1)	0.25	0.5 (2)	0.5 (2)
K. pneumoniae MMX 6272 (fluoroquinolone resistant)	2	8 (4)	2 (1)	16	>16 (>1)	>16 (>1)	8	>16 (>2)	8 (1)	16	32 (2)	16 (1)	>32	>32 (1)	>32 (1)	32	>32 (>1)	>32 (>1)
S. saprophyticus MMX 6512	0.25	1 (4)	0.12 (0.5)	0.5	1 (2)	0.5 (1)	0.12	2 (16)	ND	0.5	1 (2)	0.5 (1)	2	2 (1)	2 (1)	2	2 (1)	ND
S. saprophyticus MMX 6198	0.25	1 (4)	0.25 (1)	0.5	1 (2)	1 (2)	0.25	2 (8)	0.25 (1)	0.25	0.5 (2)	0.25 (1)	1	2 (2)	2 (2)	2	4 (2)	2 (1)
S. saprophyticus MMX 6617	0.5	1 (2)	0.25 (0.5)	1	1 (1)	1 (1)	0.25	2 (8)	0.5 (2)	0.5	1 (2)	0.5 (1)	2	2 (1)	2 (1)	2	4 (2)	2 (1)
S. aureus ATCC 29213	0.25 (0.12–1)[c]	1 (4)	0.25 (1)	0.5	1 (2)	1 (2)	ND	ND (ND)	ND (ND)	0.12 (0.06–0.5)[c]	0.5 (4)	0.25 (2)	0.5	1 (2)	0.5 (1)	ND	ND (ND)	ND (ND)

MMX, Micromyx; NA, not applicable (fold-change could not be determined due to lack of a defined MIC with unsupplemented broth or urine); ND, not determined (growth of test organism could not be determined due to cloudiness of medium and lack of a button of bacterial growth).

[a] pH-adjusted urine became cloudy post-incubation; MIC values were read as the lowest concentration where the button of bacterial growth at the bottom of the test well was no longer visible.

[b] Test medium became cloudy after supplementation with calcium, but was clear after subsequent filter sterilization prior to testing.

[c] CLSI QC ranges are shown in parentheses.

study[16] showed that concentrations of magnesium and calcium within 2-fold of standard medium concentrations had little effect on omadacycline activity. In the present study, at much higher magnesium and calcium concentrations, there were observable impacts. The clinical relevance of these findings should be considered in the context of the pharmacokinetics of the agent. In a Phase Ib study of omadacycline in female patients with cystitis, where omadacycline was administered 200 mg IV or 300–400 mg orally q12h on Day 1 followed by 300–450 mg orally q24h, the steady-state urine concentrations compared favourably with MIC values of omadacycline for UTI organisms. The mean steady-state urine concentrations (measured over 24 h on Day 5) across the dosing regimens ranged from 17.94 to 48.12 mg/L.[4] These urine concentrations exceed the MIC_{90} for most UTI pathogens;[6] however, the MIC increases in urine for omadacycline observed here are consistent with the findings of the two Phase II studies of this drug in UTI, where microbiological responses were generally lower than the comparators despite comparable levels of clinical success.[5]

Acknowledgements

Funding

Transparency declarations

P.P. was an employee of Micromyx, A.M. is an employee of Micromyx, D.S. is a consultant at Micromyx and C.P. is an employee of Micromyx.

References

1 Cortes-Penfield NW, Trautner BW, Jump R. Urinary tract infection and asymptomatic bacteriuria in older adults. *Infect Dis Clin North Am* 2017; **31**: 673–88.

2 Foxman B. Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. *Infect Dis Clin North Am* 2014; **28**: 1–13.

3 Zilberberg MD, Shorr AF. Secular trends in Gram-negative resistance among urinary tract infection hospitalizations in the United States, 2000–2009. *Infect Control Hosp Epidemiol* 2013; **34**: 940–6.

4 Overcash JS, Bhiwandi P, Garrity-Ryan L *et al*. Pharmacokinetics, safety, and clinical outcomes of omadacycline in women with cystitis: results from a phase 1b study. *Antimicrob Agents Chemother* 2019; **63**: e02083–18.

5 Paratek. Paratek Announces Top Line Results of Phase 2 Clinical Studies of Omadacycline in Urinary Tract Infections. 2019. https://investor.paratek pharma.com/news-releases/news-release-details/paratek-announces-top-line-results-phase-2-clinical-studies.

6 Pfaller MA, Rhomberg PR, Huband MD *et al*. Activity of omadacycline tested against Enterobacteriaceae causing urinary tract infections from a global surveillance program (2014). *Diag Microbiol Infect Dis* 2018; **91**: 179–83.

7 Carlsson S, Wiklund NP, Engstrand L *et al*. Effects of pH, nitrite and ascorbic acid on nonenzymatic nitric oxide generation and bacterial growth in urine. *Nitric Oxide* 2001; **5**: 580–6.

8 Ronald A. The etiology of urinary tract infection: traditional and emerging pathogens. *Dis Mon* 2003; **49**: 71–82.

9 van Laecke S. Hypomagnesemia and hypermagnesemia. *Acta Clin Belg* 2019; **74**: 41–7.

10 Foley KF, Boccuzzi L. Urine calcium: laboratory measurement and clinical utility. *Lab Med* 2010; **41**: 683–6.

11 Chen K, Xie Y, Zhao L *et al*. Hyperthyroidism-associated hypercalcemic crisis: a case report and review of the literature. *Medicine (Baltimore)* 2017; **96**: e6017.

12 Dirks JH. The kidney and magnesium regulation. *Kidney Int* 1983; **23**: 771–7.

13 Wilhelm SM, Wang TS, Ruan DT. The American Association of Endocrine Surgeons guidelines for definitive management of primary hyperparathyroidism. *JAMA Surg* 2016; **151**: 959–68.

14 Haider DG, Lindner G, Ahmad SS *et al*. Hypermagnesemia is a strong independent risk factor for mortality in critically ill patients: results from a cross-sectional study. *Eur J Int Med* 2015; **26**: 504–7.

15 Yang L, Wang K, Li H *et al*. The influence of urinary pH on antibiotic efficacy against bacterial uropathogens. *Urology* 2014; **84**: 731.e1–7.

16 Thwaites M, Shinabarger D, Pillar C, The impact of non-standard test conditions on the *in vitro* activity of omadacycline by broth microdilution. *Twenty-Seventh European Congress of Clinical Microbiology and Infectious Diseases, Vienna, Austria, 2017*. Abstract P-1263.

17 Weinberg ED. The mutual effects of antimicrobial compounds and metallic cations. *Bacteriol Rev* 1957; **21**: 46–68.

18 Fass RJ, Barnishan J. Effect of divalent cation concentrations on the antibiotic susceptibilities of nonfermenters other than *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1979; **16**: 434–8.

19 D'Amato RF, Thornsberry C, Baker CN *et al*. Effect of calcium and magnesium ions on the susceptibility of *Pseudomonas* species to tetracycline, gentamicin, polymyxin B, and carbenicillin. *Antimicrob Agents Chemother* 1975; **7**: 596–600.

20 Hesse A, Classen A, Knoll M *et al*. Dependence of urine composition on the age and sex of healthy subjects. *Clin Chim Acta* 1986; **160**: 79–86.

21 CLSI. *Performance Standards for Antimicrobial Susceptibility Testing—Twenty-Sixth Edition: M100*. 2016.

22 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Tenth Edition: M07*. 2015.20