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In Vitro Activity of New Tetracycline Analogs Omadacycline and Eravacycline against Drug-Resistant Clinical Isolates of *Mycobacterium abscessus*

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ABSTRACT Tigecycline is used in multidrug regimens for salvage therapy of *Mycobacterium abscessus* infections but is often poorly tolerated and has no oral formulation. Here, we report similar *in vitro* activity of two newly approved tetracycline analogs, omadacycline and eravacycline, against 28 drug-resistant clinical isolates of *M. abscessus* complex. Since omadacycline and eravacycline appear to be better tolerated than tigecycline and since omadacycline is also formulated for oral dosing, these tetracycline analogs may represent new treatment options for *M. abscessus* infections.

KEYWORDS *Mycobacterium abscessus*, omadacycline, drug susceptibility assay, eravacycline, tetracyclines, tigecycline

Mycobacterium abscessus complex, consisting of the subspecies *abscessus*, *massiliense*, and *bolletii*, is a group of rapidly growing, nontuberculous mycobacteria (NTM) known for its extensive intrinsic and acquired drug resistance (1). It can cause treatment-refractory lung infections (especially among cystic fibrosis patients), as well as other serious infections (2). Increasing prevalence of pulmonary NTM infections over the last several decades has been reported from several parts of the world, including the United States and Europe (3–6). *M. abscessus* complex is now the most common rapid-growing NTM causing lung infection and the second most common among all NTM after *Mycobacterium avium* complex. It is also the most difficult-to-treat NTM lung infection (3–6). A typical multidrug treatment regimen for cystic fibrosis patients with *M. abscessus* infection consists of an oral macrolide, intravenous amikacin, along with one or more additional intravenous antibiotics, such as cefoxitin, imipenem, or tigecycline (7). Tigecycline (a glycylcycline of the tetracycline class) is active *in vitro* against most clinical isolates of *M. abscessus* and has been used clinically for *M. abscessus* lung infections with some success, but nausea and vomiting are frequent, often treatment-limiting, adverse effects (8, 9). In addition, tigecycline's intravenous mode of administration is undesirable for a disease that is often treated for more than a year (7, 9). Therefore, new antibiotics with similar or better efficacy, fewer adverse effects, preferably with oral bioavailability, are desperately needed to improve the treatment of *M. abscessus* infections.

Omadacycline (an aminomethylcycline) is a new tetracycline analog, approved for the treatment of acute bacterial skin and skin-structure infections (ABSSSI) and community-acquired bacterial pneumonia (CABP). It is available in both intravenous and oral formulations (10, 11). Eravacycline (a fluorocycline) is a new tetracycline analog approved for the treatment of complicated intraabdominal infections in an intravenous formulation (12). In the present study, we evaluated the activity

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of omadacycline and eravacycline against a panel of drug-resistant *M. abscessus* complex organisms.

Omadacycline, eravacycline, and tigecycline were purchased from MedChem Express, Monmouth Junction, NJ (purity, >95%). All antimicrobials were received in powdered form, stored at −20°C, and dissolved in dimethyl sulfoxide or deionized water in accordance with the manufacturer's recommendations. *M. abscessus* strain ATCC 19977 was purchased from the American Type Culture Collection (Manassas, VA) and used as a reference strain. Twenty-eight unique clinical isolates of *M. abscessus* complex were obtained from the Johns Hopkins Hospital Clinical Microbiology Laboratory from 2005 to 2015, as described previously (13, 14). Isolates were identifies to the subspecies level based on the length of *erm*(41), which is truncated in *M. abscessus* subsp. *massiliense*, and the *rpoB* sequence (15–17). Reference genomes for each subspecies were as follows: *abscessus* strain ATCC 19977 (NCBI accession NC_010397), *massiliense* strain GO 06 (NCBI accession NC_018150), and *bolletii* strain CIP 198541 (NCBI accession NZ_JRMF00000000). These isolates are resistant to nearly all drugs used to treat *M. abscessus* infection (amikacin, clarithromycin, imipenem, sulfamethoxazole/trimethoprim, linezolid, and moxifloxacin). The MICs were determined using the broth microdilution method in cation-adjusted Mueller-Hinton broth (CAMHB) in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (18). In brief, CAMHB (100 μl/well) was added in each well of 96-well, U-bottom, polystyrene plates (Corning, Inc., Corning, NY). Serial 2-fold dilutions of compounds were prepared. *M. abscessus* strains were grown to the mid-log phase. An inoculum adjusted to 1×10^4 to 5×10^4 CFU in a 0.1-ml volume was added in each well except the medium control. Plates were sealed and incubated at 30°C for 3 days. Plates were incubated up to 5 days if the pellet size in control wells without drug was small on days 3 and 4. MICs were determined on the basis of presence or absence of pellet with unaided eyes (13). Drug susceptibility assays were repeated to confirm the MICs.

Against *M. abscessus* strain ATCC 19977, the MIC of omadacycline was similar to that of tigecycline (1 μg/ml), whereas the eravacycline MIC was 2-fold lower (Table 1). Likewise, omadacycline and tigecycline had the same MIC_{50} and MIC_{90} against 28 drug-resistant clinical isolates (2 μg/ml), while the MIC_{50} and MIC_{90} of eravacycline were 2-fold lower. Interestingly, while the present study was under review, a newly published study reported similar MICs for tigecycline and omadacycline against *M. abscessus* complex clinical isolates (19).

While no formal susceptibility breakpoint has been established for tigecycline against *M. abscessus*, breakpoints ranging from 0.5 to 4 μg/ml have been proposed (8, 20). Clinical isolates of rapidly growing mycobacteria are susceptible to tigecycline concentrations of ≤2 μg/ml (21–24), which is the approved susceptibility breakpoint against *Enterobacteriaceae* (25). The MIC_{50} and MIC_{90} of omadacycline reported here are 4- and 2-fold lower, respectively, than the susceptibility breakpoint for *Enterobacteriaceae* (26). The MIC_{50} of eravacycline reported here matches its susceptibility breakpoint for *Enterobacteriaceae* and anaerobes (27). It is noteworthy that steady-state plasma concentrations equivalent to our observed MIC_{90} for drug-resistant *M. abscessus* clinical isolates are achievable with intravenous dosing of omadacycline and eravacycline (28–30). At an intravenous omadacycline dose of 100 mg/day (approved marketed dose for CABP and ABSSSI), the steady-state plasma C_{max} and AUC_{0-24} are 2.12 μg/ml and 12.14 μg · h/ml, respectively, compared to 0.87 μg/ml and 4.7 μg · h/ml for tigecycline at 50 mg twice daily (29, 31, 32). Oral omadacycline doses of 300 to 450 mg produced C_{max} values of 9.52 to 10.8 μg/ml and AUC_{0-24} values of 11.2 to 13.4 μg · h/ml, respectively (29). Eravacycline after intravenous dosing 1.0 mg/kg every 12 h produced a plasma C_{max} of 1.83 μg/ml and an AUC_{0-24} of at least 12.6 μg · h/ml (25). Although there is no marketed oral formulation of eravacycline, a single oral dose of 100 mg produced a C_{max} of 0.17 μg/ml and an $AUC_{0-\infty}$ of 2.25 μg · h/ml (33). In addition, omadacycline has a low protein binding (21%) compared to eravacycline (79 to 87%) and tigecycline (69 to 87%) (28).

The free drug AUC/MIC ratio was the pharmacokinetic/pharmacodynamic pa-

TABLE 1 MICs of tigecycline, omadacycline, and eravacycline against *Mycobacterium abscessus* ATCC 19977 and 28 drug-resistant *M. abscessus* complex clinical isolates in CAMHB

Isolate or MIC	*M. abscessus* subspecies	MIC (μg/ml)		
		Tigecycline	Omadacycline	Eravacycline
Isolates				
Strain ATCC 19977[a]	*abscessus*	1	1	0.5
1N	*abscessus*	1	1	0.5
2N	*massiliense-bolletii*[b]	1	1	0.25
3N	*abscessus*	2	2	1
4N	*massiliense*	1	1	0.25
5N	*massiliense*	1	0.5	0.25
6N	*abscessus*	2	4	1
11N	*abscessus*	1	2	2
12N	*abscessus*	1	0.5	0.25
13N	*massiliense-bolletii*	1	2	0.5
14N	*massiliense-bolletii*	2	2	1
19N	*abscessus*	1	0.5	0.25
201	*abscessus*	1	0.5	0.25
202	*abscessus*	1	2	0.5
203	*massiliense-bolletii*	1	2	0.5
204	*massiliense*	1	1	0.5
206	*massiliense*	0.5	0.5	0.125
208	*massiliense*	2	2	0.5
210	*abscessus*	2	2	0.5
211	*abscessus*	2	2	0.5
212	*massiliense-bolletii*	1	1	0.25
214	*massiliense*	1	1	0.5
215	*abscessus*	1	1	0.25
216	*massiliense*	1	1	0.25
218	*abscessus*	4	4	2
JHH2	*abscessus*	1	1	0.25
JHH4	*abscessus*	1	1	0.25
JHH9	*abscessus*	2	2	0.5
JHHKB	*abscessus*	2	2	0.5
MIC data				
MIC range		0.5–4	0.5–4	0.125–2
MIC_{50}		1	1	0.5
MIC_{90}		2	2	1

[a]*M. abscessus* strain ATCC 19977 is included as a reference strain, and the MIC values for this strain were not included when determining the MIC range, MIC_{50}, and MIC_{90}.
[b]Five isolates had truncated *erm*(41) genes, indicating subsp. *massiliense*, but had *rpoB* sequences matching subsp. *bolletii*.

rameter most closely correlated with tigecycline activity in an *in vitro* hollow fiber model of *M. abscessus* infection (8). Considering the steady-state AUC and protein binding data described above and the MICs obtained in our study against *M. abscessus*, the free drug AUC/MIC ratios for omadacycline and eravacycline given intravenously are expected to be approximately 8 to 10 times higher and 2 times higher, respectively, compared to tigecycline. This preliminary comparison suggests that eravacycline and, especially, omadacycline could be more efficacious clinically than tigecycline. These hypotheses should be evaluated further in nonclinical models of *M. abscessus* infection.

Despite tigecycline's appreciable activity as a component of multidrug regimen for *M. abscessus* infections, its clinical utility is limited by significant nausea and vomiting (7, 9), especially at the 200-mg daily dose identified as the optimal dose in the hollow fiber infection model (8). Omadacycline and eravacycline appear better tolerated. Omadacycline was associated with significantly less nausea and fewer treatment-emergent adverse events (TEAEs) compared to tigecycline in one study (31). Omadacycline also demonstrated similar safety and side effect profiles to linezolid (for treatment of ABSSI) and moxifloxacin (for CABP) in pivotal trials (10, 11). In IGNITE1 and IGNITE4 trials, eravacycline-treated patients experienced

only slightly more TEAEs compared to ertapenem- and meropenem-treated patients (12, 34).

In conclusion, omadacycline and eravacycline may represent new options for treatment of *M. abscessus* complex infections. The results presented here support further investigation of their efficacy and exposure-response profiles in animal models and clinical trials to better understand their potential clinical utility.

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