A Novel Oxazolidinone, Contezolid (MRX-I), Expresses Anti-Mycobacterium abscessus Activity In Vitro

Qi Guo, Liyun Xu, Fusheng Tan, Junjie Zhang, Junsheng Fan, Xinghai Wang, Zhemin Zhang, Bing Li, Haiqing Chu

Department of Respiratory Medicine, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China

Tongji University School of Medicine, Shanghai, China

MicuRx Pharmaceuticals, Inc., Shanghai, China

Shanghai Key Laboratory of Tuberculosis, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China

Qi Guo, Liyun Xu, and Fusheng Tan contributed equally to this work. The order of names was decided in accordance with the contribution to this study.

ABSTRACT

An evaluation of the anti-Mycobacterium abscessus activity expressed by a novel oxazolidinone, contezolid (MRX-I), toward 12 reference strains and 194 clinical isolates was conducted. Contezolid was active against M. abscessus in vitro, with effects comparable to the anti-M. abscessus effects of linezolid both extracellularly and intracellularly. Contezolid did not antagonize the most frequently used anti-M. abscessus drugs, and preexposure to contezolid did not induce drug resistance. These results provide a novel approach to treating M. abscessus infections.

KEYWORDS

Mycobacterium abscessus, in vitro, intracellular, oxazolidinone, contezolid (MRX-I)

Therapeutic options for treating Mycobacterium abscessus infections are extremely limited (1). Oxazolidinones, e.g., linezolid, are recommended by the latest guidelines for treating M. abscessus pulmonary disease (2). However, a high rate of adverse, drug-related reactions (e.g., cytopenia, peripheral neuropathy, and optic neuritis) is a major health concern (3–5).

Contezolid (MRX-I), \( \text{(S)}\)-5-\((\text{isoxazol-3-ylamino})\text{methyl}\)-3-(2,3,5-trifluoro-4-(4-oxo-3,4-dihydropyridin-1(2\(\text{H}\))yl)phenyl)oxazolidin-2-one, is a novel oxazolidinone that exhibits antimicrobial effects similar to those of linezolid, i.e., a broad anti-Gram-positive bacterial spectrum when administered orally, and effectiveness in treating methicillin-resistant Staphylococcus aureus, penicillin-resistant Streptococcus pneumoniae, and vancomycin-resistant enterococci (6, 7). It exhibits an improved safety profile, compared to linezolid, and minimal effects with respect to myelosuppression and monoamine oxidase inhibition, two independent adverse events associated with linezolid therapy (6). Importantly, contezolid exhibits anti-Mycobacterium tuberculosis activity both in vitro and in vivo (8). Therefore, it has potential value for use in long-term combination therapy to treat M. abscessus infections, although supporting data are limited. In the present study, a detailed evaluation of the anti-M. abscessus activity of contezolid was undertaken to determine its potency in treating M. abscessus infections.

Contezolid is active against M. abscessus. Antimicrobial susceptibility testing was performed with 12 nontuberculous Mycobacterium reference strains and 194 clinical M. abscessus isolates collected from 182 different patients, according to the Clinical and Laboratory Standards Institute guidelines using the microdilution method (9). Contezezolid was active against most nontuberculous Mycobacterium reference strains with the exceptions of Mycobacterium avium and Mycobacterium intracellulare (Table 1). An additional 11 M. intracellulare and 8 M. avium clinical isolates selected at random from～200...
isolates were also tested and were found to be contezolid insensitive (see Table S1 in the supplemental material). Previous studies conducted by other investigators similarly reported that the majority of *M. abscessus* isolates were sensitive to linezolid, while 80% of *M. avium* and *M. intracellulare* isolates were insensitive (10–15).

Contezolid exhibited anti-*M. abscessus* activity toward extracellular *M. abscessus* in culture that was comparable to that of linezolid. The MICs ranged from 0.25 to 64 mg/liter; the MIC$_{50}$ was 16 mg/liter and the MIC$_{90}$ was 32 mg/liter for *M. abscessus subsp. abscessus*, and the MIC$_{50}$ was 8 mg/liter and the MIC$_{90}$ was 32 mg/liter for *M. abscessus subsp. massiliense* (Table 2). The detailed MIC distribution for all clinical isolates is shown in Table S2. Notably, while linezolid and tedizolid exhibited normal MIC distributions, the distribution for contezolid appeared biphasic. A lack of diversity could potentially contribute to this finding, since all the isolates were obtained at a single center. Genotypic and phylogenetic analyses were performed to exclude this possibility, and no duplicate clones were found (see Fig. S1). Therefore, the isolates were genetically diverse, and the biphasic response to contezolid remains to be clarified.

Contezolid inhibits the intracellular replication of *M. abscessus*. Killing assays were performed according to methods described previously to assess and compare the effects of contezolid and linezolid on the intracellular survival of two reference strains, i.e., ATCC 19977 (*M. abscessus subsp. abscessus*) and CIP108297 (*M. abscessus subsp. massiliense*), and two clinical isolates, i.e., A243 (*M. abscessus subsp. abscessus*) and G71 (*M. abscessus subsp. abscessus*), in primary mouse peritoneal macrophages (16). The cells of both the experimental and control groups were washed three times with warm phosphate-buffered saline to remove the extracellular organisms. Serial dilutions of the supernatants collected after the final wash were cultured on agar plates, and the CFU were counted to ensure that the number of residual extracellular bacteria was negligible.

Both contezolid and linezolid inhibited the intracellular growth of *M. abscessus*, relative to the untreated control, for all tested strains; inhibition was dose dependent (Fig. 1). There was no difference in the effects of contezolid and linezolid, indicating comparable intracellular anti-*M. abscessus* activity. Notably, the structural change in contezolid that results in lower toxicity did not weaken its ability to penetrate cells in our study. Contezolid was equivalent to linezolid and effective in inhibiting both the intracellular and extracellular growth of *M. abscessus in vitro* at the same concentration.

Contezolid is compatible with drugs most frequently used to treat *M. abscessus* infections. *M. abscessus* infections generally require treatment with multidrug combinations (2, 4). The compatibility between contezolid and eight antimycobacterial drugs that are frequently used therapeutically (i.e., clarithromycin, azithromycin, amikacin,

---

### Table 1

| Reference strain | MIC (mg/liter) of a: MRX-I | LZD | TZD |
|------------------|--------------------------|-----|-----|
| *M. abscessus subsp. abscessus* (ATCC 19977) | 16 | 8 | 2 |
| *M. abscessus subsp. massiliense* (CIP108297) | 16 | 8 | 1 |
| *Mycobacterium fortuitum* (ATCC 6841) | 8 | 4 | 2 |
| *Mycobacterium smegmatis* (ATCC 19420) | 1 | 1 | 1 |
| *Mycobacterium peregrinum* (ATCC 700686) | 1 | 1 | 1 |

| Slowly growing mycobacteria |
|-----------------------------|
| *M. avium* (ATCC 25291)    |
| *M. intracellulare* (ATCC 13950) |
| *Mycobacterium kansasii* (ATCC 12478) |
| *Mycobacterium gordonae* (ATCC 14470) |
| *Mycobacterium scrofulaceum* (ATCC 19981) |
| *Mycobacterium marinum* (ATCC 927) |
| *Mycobacterium xenopi* (ATCC 19250) |

| Reference strain               | MIC (mg/liter) of a: MRX-I | LZD | TZD |
|-------------------------------|--------------------------|-----|-----|
| *M. avium*                   | 32 | 16 | 16 |
| *M. intracellulare*           | 64 | 16 | 32 |
| *Mycobacterium kansasii*      | 1 | 1 | 0.125 |
| *Mycobacterium gordonae*      | 2 | 1 | 0.06 |
| *Mycobacterium scrofulaceum*  | 1 | 0.5 | 0.125 |
| *Mycobacterium marinum*       | 4 | 2 | 0.5 |
| *Mycobacterium xenopi*        | 1 | 1 | 0.06 |

aMRX-I, contezolid; LZD, linezolid; TZD, tedizolid.
imipenem, cefoxitin, tigecycline, bedaquiline, and moxifloxacin) was assessed in vitro using the broth microdilution chequerboard titration technique and five randomly selected clinical M. abscessus isolates. No antagonism between contezolid and the aforementioned antimycobacterial drugs was evident (see Table S3).

**TABLE 2** MICs of contezolid, linezolid, and tedizolid for 194 clinical M. abscessus isolates

| Antimicrobial agent and species (n = 194) | MIC<sub>50</sub> (mg/liter)<sup>a</sup> | MIC<sub>90</sub> (mg/liter)<sup>a</sup> | MIC range (mg/liter) | Linezolid susceptibility (%)<sup>c</sup> |
|-----------------------------------------|----------------------------------|----------------------------------|---------------------|----------------------------------------|
| MRX-I (M. abscessus subsp. abscessus (n = 148)) | 16 | 32 | 0.5–64 | NA |
| MRX-I (M. abscessus subsp. massiliense (n = 46)) | 8 | 32 | 0.25–64 | NA |
| LZD (M. abscessus subsp. abscessus (n = 148)) | 8 | 32 | 1–64 | 54.7<sup>c</sup> |
| LZD (M. abscessus subsp. massiliense (n = 46)) | 8 | 32 | 0.5–64 | 60.8 |
| TZD (M. abscessus subsp. abscessus (n = 148)) | 1 | 4 | 0.125–8 | NA |
| TZD (M. abscessus subsp. massiliense (n = 46)) | 1 | 4 | 0.125–8 | NA |

<sup>a</sup>MRX-I, contezolid; LZD, linezolid; TZD, tedizolid.

<sup>b</sup>MIC<sub>50</sub> and MIC<sub>90</sub> are defined as the concentrations at which 50% and 90% of the clinical isolates tested, respectively, were inhibited.

<sup>c</sup>Sensitivity (MIC of ≤8 mg/liter) and resistance (MIC of ≥32 mg/liter) to linezolid were classified according to Clinical and Laboratory Standards Institute document M24-A2 (9). NA, not applicable.

**FIG 1** Relative intracellular antimicrobial activities of contezolid and linezolid in vitro. (A) M. abscessus subsp. abscessus reference strain ATCC 19977; the MIC of linezolid is 8 mg/liter, and the MIC of contezolid is 16 mg/liter. (B) M. abscessus subsp. massiliense reference strain CIP108297; the MIC of linezolid is 8 mg/liter, and the MIC of contezolid is 16 mg/liter. (C) M. abscessus subsp. abscessus clinical isolate A243; the MICs of both linezolid and contezolid are 2 mg/liter. (D) M. abscessus subsp. abscessus clinical isolate G71; the MICs of both linezolid and contezolid are 4 mg/liter. Ctrl, control; MRX-I, contezolid; LZD, linezolid.
Preexposure to contezolid does not induce antibiotic resistance in *M. abscessus*.

The risk of resistance induced by contezolid exposure was determined by preexposing *M. abscessus* strains (ATCC 19977 and two randomly selected clinical *M. abscessus* isolates) to contezolid at one-fourth and one-half the MIC and then subsequently quantifying the MICs of contezolid and eight other antibiotics postexposure. The MIC values of contezolid, as well as those of the other eight drugs listed above, did not increase following contezolid preexposure (see Table S4). Huang and coworkers reported similar results, i.e., contezolid exhibited a lower potential than linezolid to induce mutations and resistance in *S. aureus* (17).

In conclusion, contezolid is active against *M. abscessus in vitro* and is compatible with antibiotics that are most frequently used to treat *M. abscessus* infections. Therefore, contezolid is a potential candidate to include in novel therapeutic anti-*M. abscessus* regimens.

ACKNOWLEDGMENTS

We sincerely thank Stephen H. Gregory (Providence, RI, USA) for his help writing and editing this manuscript.

This work was funded by grants provided by the National Natural Science Foundation of China (grants 81971973 and 81800003), the Natural Science Foundation of Shanghai Municipal Science and Technology Commission (grants 18ZR1431600, 19ZR1442800, and 20ZR1447200), the Medical Guide Program of the Shanghai Science and Technology Committee (grants 18411970600 and 19411969600), the Development Fund for Shanghai Talents (grant 2019112), the Shanghai Health and Family Planning Commission Excellent Talents Training Program (grant 2018YQ55), and the General Project of the Shanghai Health and Family Planning Commission (grant 201904229).

We have no conflicts of interest to declare.

REFERENCES

1. Griffith DE. 2019. *Mycobacterium abscessus* and antibiotic resistance: same as it ever was. Clin Infect Dis 69:1687–1689. https://doi.org/10.1093/cid/cdy071.
2. Daley CL, laccarino JM, Lange C, Cambau E, Wallace RJ, Jr, Andrejak C, Böttger EC, Brozek J, Griffith DE, Guglielmetti L, Huitt GA, Knight SL, Leitman P, Marras TK, Olivieri KN, Santin M, Stout JE, Tortoli E, van Ingen J, Wagner D, Winthrop KL. 2020. Treatment of nontuberculous mycobacterial pulmonary disease: an official ATS/ERS/ESCMID/IDSA clinical practice guideline. Eur Respir J 56:2000535. https://doi.org/10.1183/13993003.00535-2020.
3. Chen J, Zhao L, Mao Y, Ye M, Guo Q, Zhang Y, Xu L, Zhang Z, Li B, Chu H. 2019. Clinical efficacy and adverse effects of antibiotics used to treat *Mycobacterium abscessus* pulmonary disease. Front Microbiol 10:1977. https://doi.org/10.3389/fmicb.2019.01977.
4. Daley CL, laccarino JM, Lange C, Cambau E, Wallace RJ, Andrejak C, Böttger EC, Brozek J, Griffith DE, Guglielmetti L, Huitt GA, Knight SL, Leitman P, Marras TK, Olivieri KN, Santin M, Stout JE, Tortoli E, van Ingen J, Wagner D, Winthrop KL. 2020. Treatment of nontuberculous mycobacterial pulmonary disease: an official ATS/ERS/ESCMID/IDSA clinical practice guideline: executive summary. Clin Infect Dis 71:e1–e36. https://doi.org/10.1093/cid/ciaa241.
5. Vính DC, Rubinstein E. 2009. Linezolid: a review of safety and tolerability. J Infect 59(Suppl 1):S59–S74. https://doi.org/10.1016/S0163-4453(09)60009-8.
6. Gordeev MF, Yuan ZY. 2014. New potent antibacterial oxazolidinone (MRX-I) with an improved class safety profile. J Med Chem 57:4487–4497. https://doi.org/10.1021/jm401931e.
7. Li CR, Zhai QQ, Wang XK, Hu XX, Li GQ, Zhang WX, Pang J, Lu X, Yuan H, Gordeev MF, Chen LT, Yang XY, You XF. 2014. In vivo antibacterial activity of MRX-I, a new oxazolidinone. Antimicrob Agents Chemother 58: 2418–2421. https://doi.org/10.1128/AAC.01526-13.
8. Shoen C, DeStefano M, Hafkin B, Cynamon M. 2018. In vitro and in vivo activities of contezolid (MRX-I) against *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 62:e00493-18. https://doi.org/10.1128/AAC.00493-18.
9. Clinical and Laboratory Standards Institute. 2011. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes; approved standard—second edition. Document M24-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
10. Zhang Z, Lu J, Song Y, Pang Y. 2018. In vitro activity between linezolid and other antimicrobial agents against *Mycobacterium abscessus* complex. Diagn Microbiol Infect Dis 90:31–34. https://doi.org/10.1016/j.diagmicrobio.2017.09.013.
11. Cho EH, Huh HJ, Song DJ, Lee SH, Kim CK, Shin SY, Ki CS, Jhun BW, Moon SM, Kwon OJ, Koh WJ, Lee NY. 2019. Drug susceptibility patterns of *Mycobacterium abscessus* and *Mycobacterium massiliense* isolated from respiratory specimens. Diagn Microbiol Infect Dis 93:107–111. https://doi.org/10.1016/j.diagmicrobio.2018.08.008.
12. Hatakeyama S, Ohama Y, Okazaki M, Nukui Y, Moriya K. 2017. Antimicrobial susceptibility testing of rapidly growing mycobacteria isolated in Japan. BMC Infect Dis 17:197. https://doi.org/10.1186/s12879-017-2298-8.
13. Ye M, Xu L, Zou Y, Li B, Guo Q, Zhang Y, Zhan M, Xu B, Yu F, Zhang Z, Chu H. 2019. Molecular analysis of linezolid-resistant clinical isolates of *Mycobacterium abscessus*. Antimicrob Agents Chemother 63:e01842-18. https://doi.org/10.1128/AAC.01842-18.
14. Brown-Elliott BA, Crist CJ, Mann LB, Wilson RW, Wallace RJ, Jr. 2003. In vitro activity of linezolid against slowly growing nontuberculous mycobacteria. Antimicrob Agents Chemother 47:1736–1738. https://doi.org/10.1128/AAC.47.5.1736-1738.2003.
15. Schön T, Chrysanthou E. 2011. Minimum inhibitory concentration distributions for *Mycobacterium avium* complex-towards evidence-based susceptibility breakpoints, Int J Infect Dis 55:122–124. https://doi.org/10.1016/j.ijid.2016.12.027.
16. Zhang S, Zou Y, Guo Q, Chen J, Xu L, Wan X, Zhang Z, Li B, Chu H. 2020. AR-12 exhibits direct and host-targeted antibacterial activity toward *Mycobacterium abscessus*. Antimicrob Agents Chemother 64:e00236-20. https://doi.org/10.1128/AAC.00236-20.
17. Huang Y, Xu Y, Liu S, Wang H, Xu G, Wu B, Gordeev MF, Wang W, Yuan Z, Wang M. 2014. Selection and characterisation of *Staphylococcus aureus* mutants with reduced susceptibility to the investigational oxazolidinone MRX-I. Int J Antimicrob Agents 43:418–422. https://doi.org/10.1016/j.ijantimicag.2014.02.008.