Sklodowska-Curie grant no. 845479 to C.A.), the National Institutes of Health (grant no. 1 S10 RR023735 [Zeiss LSM 510 Laser Scanning Microscope] to M.G.J.), and the Programa de Apoyo a la Investigación Científica y Tecnológica of the Universidad Autonoma de Nuevo León (grant no. SA-1900-21).

About the Author

Dr. Vera-Cabrera is a professor of dermatology at the Faculty of Medicine in University of Nuevo León, Monterrey, Mexico. His research interests include immune response against intracellular infectious agents and pathogenic mechanisms of bacteria of the genus *Mycobacterium* and *Nocardia*.

References

1. Truman RW, Singh P, Sharma R, Busso P, Rougemont J, Paniz-Mondolfi A, et al. Probable zoonotic leprosy in the southern United States. N Engl J Med. 2011;364:1626–33. https://doi.org/10.1056/NEJMoa1010536
2. Sharma R, Singh P, Loughry WJ, Lockhart JM, Inman WB, Duthie MS, et al. Zoonotic leprosy in the southeastern United States. Emerg Infect Dis. 2015;21:2127–34. https://doi.org/10.3201/eid2112.150501
3. World Health Organization. Global leprosy (Hansen disease) update, 2019: time to step-up prevention initiatives. Wkly Epidemiol Rec. 2020;36:417–40.
4. Ploemacher T, Faber WR, Menke H, Rutten V, Pieters T. Reservoirs and transmission routes of leprosy; a systematic review. PLoS Negl Trop Dis. 2020;14:e0008276. https://doi.org/10.1371/journal.pntd.0008276
5. Braet S, Vandelannoote K, Meehan CJ, Brum Fontes AN, Hasker E, Rosa PS, et al. The repetitive element RLEP is a highly specific target for detection of *Mycobacterium leprae*. J Clin Microbiol. 2018;56:e01924–17. https://doi.org/10.1128/JCM.01924-17
6. Benjak A, Avanzi C, Singh P, Loiseau C, Girma S, Busso P, et al. Phylogenomics and antimicrobial resistance of the leprosy bacillus *Mycobacterium leprae*. Nat Commun. 2018;9:352. https://doi.org/10.1038/s41467-017-02576-z
7. Feng X, Papeş M. Ecological niche modelling confirms potential north-east range expansion of the nine-banded armadillo (*Dasypus novemcinctus*) in the USA. J Biogeogr. 2015;42:803–7. https://doi.org/10.1111/jbi.12427
8. Taulman JF, Robbins LW. Range expansion and distributional limits of the nine-banded armadillo in the United States: an update of Taulman & Robbins (1996). J Biogeogr. 2014;41:1626–30. https://doi.org/10.1111/jbi.12319

Address for correspondence: Charlotte Avanzi, Department of Microbiology, Immunology and Pathology, Mycobacteria Research Laboratories, Colorado State University, 1682 Campus Delivery, Fort Collins, CO 80523-1682, USA; email: charlotte.avanzi@colostate.edu; Lucio Vera-Cabrera, Servicio de Dermatologia, Hospital Universitario, UANL, Madero y Gonzalitos s/n, Colonia Mitras Centro, Monterrey, Nuevo León, Mexico CP64460; email: luvera_99@yahoo.com

Sensitivity of *Mycobacterium leprae* to Telacebec

Ramanuj Lahiri, Linda B. Adams, Sangeeta Susan Thomas, Kevin Pethe

Author affiliations: US Department of Health and Human Services, Health Resources and Services Administration, Health Systems Bureau, National Hansen's Disease Program, Baton Rouge, Louisiana, USA (R. Lahiri, L.B. Adams); Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore (S.S. Thomas, K. Pethe)

DOI: https://doi.org/10.3201/eid2803.210394

The treatment of leprosy is long and complex, benefiting from the development of sterilizing, rapidly-acting drugs. Reductive evolution made *Mycobacterium leprae* exquisitely sensitive to Telacebec, a phase 2 drug candidate for tuberculosis. The unprecedented potency of Telacebec against *M. leprae* warrants further validation in clinical trials.

Leprosy, also known as Hansen disease, is a chronic infectious disease caused primarily by *Mycobacterium leprae* and to a lesser extent by *M. lepromatosis* bacteria. Both species have a strong tropism for the Schwann cells; infection causes peripheral neuropathy, which leads to the characteristic deformities and disabilities. Despite successful implementation of multidrug therapies for the treatment of leprosy, >200,000 new cases were reported globally in 2019. Drug-resistant *M. leprae* strains, although rare, are emerging in several parts of the world (*1*). Therefore, newer rapidly acting bactericidal, orally bioavailable drugs are required to shorten treatment time and reduce transmission.

The high potency of drugs targeting the cytochrome *bcc:aa*$_3$ terminal oxidase (also known as QcrB inhibitors) against *M. ulcerans* has been reported (*3*). Of particular importance is the finding that a single dose of the drug candidate, Telacebec (Q203) (*3*), eradicates infection in a mouse model of Buruli ulcer (*4*). The potency of drugs targeting the cytochrome *bcc:aa*$_3$ terminal oxidase against *M. ulcerans* is explained by the absence of a functional cytochrome *bd* oxidase, an alternate terminal oxidase that limits the potency of telacebec in *M. tuberculosis* (*5*,*6*). Like *M. ulcerans*, *M. leprae* has lost the genes encoding the cytochrome *bd* oxidase and any other alternate terminal electron acceptors (*7*). Because *M. leprae* relies exclusively on the cytochrome *bcc:aa*$_3$ terminal oxidase for respiration, Scherr et al. hypothesized that telacebec and related QcrB

inhibitors could represent a new class of bactericidal drugs for leprosy (*2*).

The potency of telacebec was initially tested against extracellular *M. leprae* using a radio-respirometry assay to determine bacterial β-oxidation rate. This assay is used to assess viability of noncultivable *M. leprae* and measures cumulative production of CO_2 by the bacilli when palmitic acid is the sole carbon source (*8*). Telacebec at a concentration of 0.2 nM inhibited ≈90% ($p<0.001$) and 2 nM inhibited ≈99.9% ($p<0.0001$) of *M. leprae* metabolic activity after 3 days of incubation (Figure, panel A). In comparison, rifampin used at 2.0 μM inhibited only ≈45% ($p = 0.020$) of the metabolic activity compared with untreated control in the same time frame (Figure, panel A). We observed a similar trend after 7 days of incubation (Figure, panel A); 0.2 nM of telacebec was significantly more potent than 2 μM of rifampin at all tested concentrations in this assay. Telacebec was also active against intracellular *M. leprae* maintained in murine bone marrow–derived macrophages (*9*). Telacebec at 2.0 nM inhibited ≈97% ($p<0.001$ vs. untreated) of the metabolic activity of intracellular *M. leprae* in 3 days. Telacebec was also marginally potent against intracellular *M. leprae* at 0.2 nM but required longer incubation; we observed a statistically nonsignificant reduction of ≈33% ($p = 0.069$) after 3 days' incubation and a significant reduction of ≈40% ($p = 0.034$) after 7 days. Under similar experimental conditions, rifampin at 2.0 μM inhibited metabolic activity of intracellular *M. leprae* by ≈44% ($p = 0.025$) at day 3 and ≈72% ($p<0.001$) at day 7 compared with the untreated control group (Figure, panel B). Telacebec at 2 or 20 nM was more potent than rifampin in this assay as well.

The high nanomolar potency of telacebec against both intracellular and extracellular *M. leprae* prompted us to evaluate its efficacy in a mouse foot pad model of infection. We inoculated groups of 5 athymic nude mice with 3×10^7 viable *M. leprae* in both hind foot pads. At 8 weeks postinfection, we administered telacebec (2 mg/kg) or rifampin (10 mg/kg) by gavage as 1 dose, 5 consecutive daily doses, or 20 doses (5 days × 4 weeks). We harvested foot pads 4 weeks after completion of the drug treatment. Because *M. leprae* is noncultivable, we measured mycobacterial load using an established molecular method (*10*). We determined *M. leprae hsp18* and *esxA* expression levels as a surrogate measure of viability (*10*). Bacterial *hsp18* and *esxA* expression were significantly lower in mice receiving 1 ($p<0.001$) or 5 ($p<0.001$) consecutive doses of telacebec compared with rifampin or to the vehicle-treated control group, indicating a faster in vivo bactericidal efficacy of telacebec (Figure, panels C, D). Although ≥5 consecutive doses of rifampin were needed to detect a bactericidal efficacy, 1 dose of telacebec at a low dose of 2 mg/kg was sufficient to reduce the bacterial viability substantially (Figure, panels C, D).

This study demonstrates the exquisite sensitivity of *M. leprae* to telacebec and the potential of a shorter treatment regimen. Dose-finding studies in animals will help to determine an optimum dosing regimen for rapid bacterial eradication. Combination

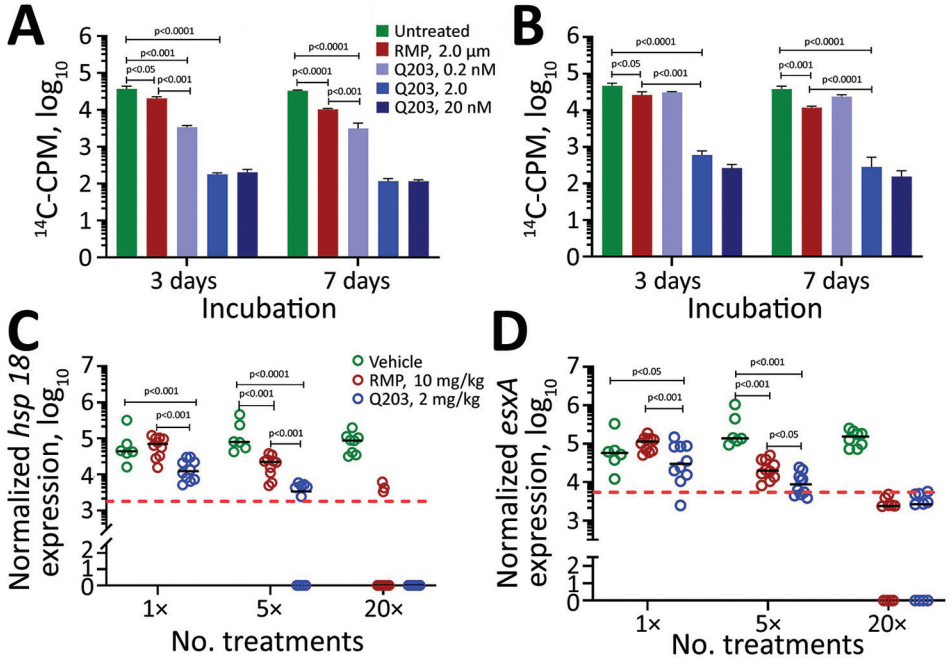

Figure. Efficacy of telacebec against *Mycobacterium leprae* bacteria in axenic culture (A), in murine bone marrow–derived macrophages (B), and in athymic nude mouse foot pad model (C, D). *M. leprae hsp18* (C) and *esxA* (D) expression levels were used as a surrogate measure of viability. For panels A and B, the assays were performed in triplicate for each condition. For panels C and D, each foot pad is taken as a data point, and the red dotted lines indicate ≈99% *M. leprae* kill. Significance was determined by 2-tailed unpaired Student *t*-test. ^{14}C, carbon 14; CPM, counts per minute; Q203, telacebec; RMP, rifampin.

therapies between telacebec and first- or second-line drugs such as rifampin, clofazimine, or minocycline should be evaluated in preclinical animal models to guide the development of a potent, fast-acting, sterilizing drug combination for humans that has a low propensity for resistance development for humans. The curative promise of telacebec or other advanced QcrB inhibitors should be validated in human clinical trials.

Acknowledgments

This work was supported in part by the Lee Kong Chian School of Medicine, Nanyang Technological University Start-Up Grant (K.P.), the National Research Foundation, Singapore, under its Investigatorship Programme (grant no. NRF-NRFI06-2020-0004), and the New York Community Trust Heiser Program (grant no. P18-000248). US National Institute of Allergy and Infectious Diseases funded the provision of viable *M. leprae* through an interagency agreement with Health Resources and Services Administration, Healthcare Systems Bureau, National Hansen's Disease Program (no. AAI20009-001-00000).

The views expressed in this article are solely the opinions of the authors and do not necessarily reflect the official policies of the U.S. Department of Health and Human Services or the Health Resources and Services Administration, nor does mention of the department or agency names imply endorsement by the US Government.

About the Author

Dr. Lahiri is a principal investigator at the Laboratory Research Branch, National Hansen's Disease Program, Baton Rouge, Louisiana, USA. His research focuses on understanding host pathogen relationship, developing animal models and evaluating novel therapeutic interventions in Hansen disease.

References

1. Mahajan NP, Lavania M, Singh I, Nashi S, Preethish-Kumar V, Vengalil S, et al. Evidence for *Mycobacterium leprae* drug resistance in a large cohort of leprous neuropathy patients from India. Am J Trop Med Hyg. 2020;102:547–52. https://doi.org/10.4269/ajtmh.19-0390
2. Pethe K, Bifani P, Jang J, Kang S, Park S, Ahn S, et al. Discovery of Q203, a potent clinical candidate for the treatment of tuberculosis. Nature Medicine. 2013;19:1157. https://doi.org/10.1038/nm.3262
3. Scherr N, Bieri R, Thomas SS, Chauffour A, Kalia NP, Schneide P, et al. Targeting the *Mycobacterium ulcerans* cytochrome *bc1:aa3* for the treatment of Buruli ulcer. Nat Commun. 2018;9:5370. https://doi.org/10.1038/s41467-018-07804-8
4. Thomas SS, Kalia NP, Ruf MT, Pluschke G, Pethe K. Toward a single-dose cure for Buruli ulcer. Antimicrob Agents Chemother. 2020;64:e00727-20. https://doi.org/10.1128/AAC.00727-20
5. Kalia NP, Hasenoehrl EJ, Ab Rahman NB, Koh VH, Ang MLT, Sajorda DR, et al. Exploiting the synthetic lethality between terminal respiratory oxidases to kill *Mycobacterium tuberculosis* and clear host infection. Proc Natl Acad Sci U S A. 2017;114:7426–31. https://doi.org/10.1073/pnas.1706139114
6. Moosa A, Lamprecht DA, Arora K, Barry CE III, Boshoff HIM, Ioerger TR, et al. Susceptibility of *Mycobacterium tuberculosis* cytochrome *bd* oxidase mutants to compounds targeting the terminal respiratory oxidase, cytochrome *c*. Antimicrob Agents Chemother. 2017;61:e01338-17. https://doi.org/10.1128/AAC.01338-17
7. Cole ST, Eiglmeier K, Parkhill J, James KD, Thomson NR, Wheeler PR, et al. Massive gene decay in the leprosy bacillus. Nature. 2001;409:1007–11. https://doi.org/10.1038/35059006
8. Franzblau SG, Hastings RC. Rapid in vitro metabolic screen for antileprosy compounds. Antimicrob Agents Chemother. 1987;31:780–3. https://doi.org/10.1128/AAC.31.5.780
9. Bailey MA, Na H, Duthie MS, Gillis TP, Lahiri R, Parish T. Nitazoxanide is active against *Mycobacterium leprae*. PLoS One. 2017;12:e0184107. https://doi.org/10.1371/journal.pone.0184107
10. Davis GL, Ray NA, Lahiri R, Gillis TP, Krahenbuhl JL, Williams DL, et al. Molecular assays for determining *Mycobacterium leprae* viability in tissues of experimentally infected mice. PLoS Negl Trop Dis. 2013;7:e2404. https://doi.org/10.1371/journal.pntd.0002404

Address for correspondence: Kevin Pethe, Lee Kong Chian School of Medicine, Experimental Medicine Building, 59 Nanyang Dr, 636921, Singapore; email: kevin.pethe@ntu.edu.sg; Ramanuj Lahiri, Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, National Hansen's Disease Program, 9181 Interline Ave, Baton Rouge, LA 70809, USA; email: rlahiri@hrsa.gov