Sensitivity of *Mycobacterium leprae* to Telacebec

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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:aa₃ terminal oxidase (also known as QcrB inhibitors) against *M. ulcers* 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:aa₃ terminal oxidase against *M. ulcers* 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. ulcers*, *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:aa₃ terminal oxidase for respiration, Scherr et al. hypothesized that telacebec and related QcrB inhibitors are effective against *M. leprae*.

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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 CO₂ 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 × 10⁷ 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

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**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. ¹⁴C, 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.

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