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Susceptibility of Clinical *Mycobacterium tuberculosis* Isolates to a Potentially Less Toxic Derivate of Linezolid, PNU-100480[▽]

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Susceptibility of clinical *Mycobacterium tuberculosis* isolates to PNU-100480 and linezolid was evaluated by the MGIT 960 system. The isolates had various susceptibilities to isoniazid (INH), rifampin, ethambutol, and streptomycin. The mean MIC for PNU-100480 was 3.2 times lower than that for linezolid. Therefore, PNU-100480 is a promising candidate to be developed further as an adjunct in the treatment of multidrug- and extensively drug-resistant tuberculosis (MDR/XDR-TB).

The number of drugs available for the treatment of multidrug- and extensively drug-resistant tuberculosis (MDR/XDR-TB) is limited. Moreover, (injectable) second- and third-line drugs are generally less effective and more toxic than first-line drugs. Adjusting the treatment according to the *in vitro* susceptibility profile of MDR/XDR *Mycobacterium tuberculosis* may be helpful to optimize the treatment (6, 10, 21, 23). Linezolid has a high antibacterial activity (MIC of 0.125 to 0.5 mg/liter) against *M. tuberculosis* (1, 5, 20) and has therefore been used at several TB centers to treat complicated cases of TB. Treatment-limiting adverse events associated with linezolid, like anemia (11) and peripheral neuropathy (4), which is likely to be caused by inhibition of mitochondrial protein synthesis (17), resulted in lower dosing schemes (2, 9, 13, 16, 18, 19). However, despite reduction of the dose, adverse effects still occur. PNU-100480, a structure analogue of linezolid, is a potential new candidate that entered phase I/II studies for the treatment of MDR- and XDR-TB. Its structure differs from that of linezolid by a sulfur atom instead of the oxygen atom in the ring structure. Structure modification of oxazolidinones influences both activity and toxicity; whether this result is relevant *in vivo* depends on the penetration of the respective analogue into the mitochondria (15). Although the *in vitro* and animal model data are limited and PNU-100480 has not yet been applied in clinical practice, data from studies of PNU-100480 in healthy volunteers look promising (24, 25).

Moreover, in a murine model of tuberculosis, it became clear that PNU-100480 is more active against TB than linezolid (27) and that the efficacy was similar to that of isoniazid (INH) and/or rifampin (7). When added to a first-line regimen in a murine model, PNU-100480 had a synergistic bactericidal effect, while linezolid had an antagonistic effect (26). However, these data for PNU-100480 were obtained from testing drug-susceptible isolates. Although PNU-100480 is a candidate to treat drug-susceptible isolates (26), treatment of resistant bacteria is much more urgent, as the arsenal of drugs to treat MDR-TB is limited. Unfortunately, data on PNU-100480 *in vitro* activity have been obtained exclusively from testing drug-susceptible isolates. The objective of this study was to explore the activity of PNU-100480 against clinical isolates from MDR-TB patients with a known level of susceptibility to linezolid.

Strains. We selected 23 *M. tuberculosis* strains from the strain collection of the Tuberculosis Reference Laboratory of the National Institute for Public Health and the Environment (RIVM) with various susceptibilities to INH, rifampin, ethambutol, and streptomycin.

Absolute-concentration method. The susceptibilities to INH, rifampin, ethambutol, and streptomycin were determined by an absolute-concentration method as described earlier (18).

MGIT 960 system with EpiCenter TB eXiST software. Each isolate was inoculated into tubes of the MGIT 960 system using a 2-fold dilution series from 0.25 to 1 mg/liter linezolid (Pfizer, NY) and 0.0625 to 1 mg/liter PNU-100480 (Pfizer, NY). To each MGIT tube, 0.8 ml of Bactec MGIT drug susceptibility testing (DST) supplement was added, as was 100 μl of the appropriate drug solution of linezolid or PNU-100480. The tubes were inoculated with 0.5 ml of suspensions of bacterial strains that had been grown in plain MGIT medium to obtain equal concentrations of bacteria in each test tube, mandatory for a valid growth test. All suspensions were used within 1 to 3 days after they were found positive in the MGIT incubator. The growth control (GC) tube was inoculated with 0.5 ml of 1:100-diluted bacterial suspension (0.1 ml of the test inoculum into 10 ml of sterile saline) and also incubated in the MGIT 960 instrument. The growth of the bacteria in the tubes was continuously monitored using EpiCenter TB eXiST software. The DST was considered "complete" when the growth control

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TABLE 1. MICs of linezolid and PNU-100480 and susceptibility to INH, rifampin, ethambutol, and streptomycin for 23 isolates of *Mycobacterium tuberculosis*

Isolate no.	Resistance/susceptibility profile[a] for:				MIC (mg/liter) of:	
	Isoniazid	Rifampin	Ethambutol	Streptomycin	Linezolid	PNU-100480
1	R	R	R	R	≤0.25	≤0.0625
2	R	R	S	R	≤0.25	0.125
3	R	R	R	R	≤0.25	≤0.0625
4	R	R	R	R	≤0.25	0.25
5	R	R	S	R	0.5	0.25
6	R	R	R	R	0.5	0.125
7	R	R	S	R	0.5	0.125
8	R	R	S	R	1	0.125
9	R	R	R	R	1	0.25
10	R	R	R	R	1	0.25
11	S	R	R	R	>1	0.5
12	S	S	S	R	1	0.125
13	R	R	R	S	≤0.25	0.125
14	R	R	R	S	≤0.25	0.125
15	R	R	S	S	0.5	0.25
16	R	R	R	S	0.5	0.125
17	R	R	S	S	0.5	≤0.0625
18	R	S	R	S	0.5	0.25
19	S	S	S	S	0.5	0.25
20	S	S	S	S	1	0.25
21	S	S	S	S	1	0.5
22	S	S	S	S	1	0.25
23	S	S	S	S	1	0.25

[a] R, resistant; S, susceptible.

reached a growth unit (GU) value of 400. The lowest concentration with no growth was recorded as the MIC (15).

Statistical analysis. We log transformed the MIC of each of the two oxazolidinones, linezolid and PNU-100480, and analyzed it as an interval-censored, normally distributed response variable. Growth inhibition in every concentration of the doubling dilution series was treated as left censored (MIC ≤ lowest concentration), whereas growth in all concentrations (MIC > highest concentration) was treated as right censored. Because linezolid and PNU-100480 were tested on the same isolates, the MICs of the two oxazolidinones were treated as paired. Each isolate was put into the model as a random effect, forcing a coupling between observations for the same isolate. This procedure is similar to the classical *t* test for paired values of a continuous noncensored response variable (9).

Each isolate had been tested for resistance or susceptibility to four different antibiotics: INH, rifampin, ethambutol, and streptomycin. Differences in the activities of linezolid and PNU-100480 in relation to the susceptibility to these four different antibiotics (resistant or susceptible) were determined by using the same modeling technique described above, expanded with extra terms for each antibiotic and its interaction with oxazolidinone. The data were processed using the statistical software package R (R Development Core Team, 2010). Modeling was done using WinBUGS (14).

The MICs of linezolid and PNU-100480 for each of the 23 isolates of *Mycobacterium tuberculosis* and the susceptibilities of the isolates to INH, rifampin, ethambutol, and streptomycin are shown in Table 1. The average MIC of PNU-100480 was 3.2-fold lower than the MIC of linezolid (95% confidence interval [95% CI], 2.4- to 4.1-fold). Remarkably, INH-susceptible isolates showed a statistically significant, 2.5-fold-higher MIC of PNU-100480 and linezolid (95% CI, 1.1- to 5.7-fold). Susceptibility to the other agents (rifampin, ethambutol, and streptomycin) did not have any significant effect on the MICs of the two oxazolidinones.

We showed that PNU-100480 was 3.2-fold more active against MDR clinical *M. tuberculosis* isolates than linezolid. Recently, it was shown that adding PNU-100480 to first-line regimens resulted in increased activity against TB (27). This might translate into possible shortening of duration of treatment, as PNU-100480 appeared more active than INH in potentiating the sterilizing activity of rifampin (26). What caused the difference in susceptibility to PNU-100480 between INH-susceptible and -resistant isolates remains unclear. Reduced fitness and growth of KatG mutants may be an explanation, but after sufficient time, the differences between susceptible and resistant mutants in terms of growth kinetics seem to fade away (8). Therefore, more effort is needed to understand the relationship between drug resistance and fitness of the TB bacilli (3). However, our observation is consistent with earlier data that showed altered PNU-100480 susceptibility in streptomycin-, ethambutol-, and pyrazinamide-resistant strains of *M. tuberculosis* (22). Our study adds important information on the susceptibility of multidrug-resistant isolates to PNU-100480, as previous studies tested only fully susceptible isolates. This seems important since, especially for the treatment of MDR- and XDR-TB, new, active drugs are badly needed. It is therefore important that PNU-100480 be considered a potential candidate for treatment of not only drug-susceptible but also drug-resistant TB. Besides, it is important to notice that the sulfoxide metabolite (PNU-101603) of PNU-100480 reaches concentrations of approximately four times that of the parent compound (25). Although the activity of this main metabolite is two times lower than that of PNU-100480 (12), testing the susceptibility of PNU-101603 against clinical isolates is relevant. As mitochondrial protein inhibition is an important driver of toxicity, the side effects from PNU-100480 are presumably less severe than those from linezolid, considering the fact that the concentrations of PNU-100480 and its metabolites are well below the IC_{50} for mitochondrial protein synthesis, in contrast with linezolid concentrations, which exceed this value (24). The clinical importance remains to be seen when phase III studies are performed.

The increasing knowledge on how to dose linezolid in MDR-TB patients could easily be applied to the clinical development of PNU-100480 for the treatment of MDR- and XDR-TB. The advantage of PNU-100480 over linezolid is that dose-finding studies can be targeted specifically at MDR-TB, in contrast to studies with linezolid, which was originally developed for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA). Therefore, labeling and marketing issues do not exist for PNU-100480. Results from a recent phase I study combined with the evaluation of bactericidal activity in a whole-blood assay showed promising results (25). The drug was well tolerated at a dose that reached therapeutic concentrations. A well-designed phase II study, followed by a phase III study, could give further insight into the potency of this

drug and the manner in which it should be dosed in MDR-TB patients.

We conclude that PNU-100480 is an important candidate to be developed further for the treatment of MDR/XDR-TB.

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