Activity of the Fluoroquinolone DC-159a in the Initial and Continuation Phases of Treatment of Murine Tuberculosis

Zahoor Ahmad,1† Austin Minkowski,1 Charles A. Peloquin,2 Kathy N. Williams,1 Khisimuzi E. Mdluli,3 Jacques H. Grosset,1 and Eric L. Nuermberger1,4*

Center for Tuberculosis Research, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland; College of Pharmacy, University of Florida, Gainesville, Florida; Global Alliance for TB Drug Development, New York, New York; and Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland.

Received 1 November 2010/Returned for modification 6 December 2010/Accepted 25 January 2011

DC-159a is a new fluoroquinolone with more potent in vitro activity than available fluoroquinolones against both drug-susceptible and fluoroquinolone-resistant Mycobacterium tuberculosis. Here, we report that DC-159a displays pharmacokinetics similar to those of moxifloxacin yet is more active than moxifloxacin during both the initial and continuation phases of treatment in a murine model. These results warrant further preclinical evaluation of DC-159a in selected drug combinations against drug-susceptible and fluoroquinolone-resistant tuberculosis.

Fluoroquinolones are cornerstone agents in the treatment of multidrug-resistant (MDR) tuberculosis (TB). The most potent members of the class, moxifloxacin (MXF) and gatifloxacin, may be capable of shortening the treatment of drug-susceptible as well as MDR TB (1, 2, 11). However, the emergence of fluoroquinolone resistance in Mycobacterium tuberculosis threatens to undermine any such advances (4). Moreover, selection of fluoroquinolone resistance in other bacterial pathogens as a result of prolonged TB treatment is an increasing concern (13).

DC-159a is a new 8-methoxyfluoroquinolone with an MIC90 of 0.06 μg/ml against M. tuberculosis, which is 4 and 8 times lower than that of MXF and levofloxacin (LVFX), respectively (3). With an MIC90 of 0.5 μg/ml against clinical MDR TB isolates which are resistant to other fluoroquinolones (e.g., MXF and LVFX MIC90 of 4 and 16 μg/ml, respectively) (3), DC-159a may retain more activity than other fluoroquinolones against such MDR and extensively drug-resistant (XDR) TB isolates. Moreover, there is evidence that DC-159a is less likely than other fluoroquinolones to select for drug resistance in common bacterial pathogens other than mycobacteria (5, 7).

Based on these promising in vitro data, we sought to compare the dose-ranging activity of DC-159a with that of MXF in a murine model of TB. Because fluoroquinolones may differ in their bactericidal activity against nonmultiplying or slowly multiplying M. tuberculosis in a fashion that may not be predicted from their activity against actively multiplying bacilli (6, 9), we compared the activities of MXF and DC-159a during the continuation phase as well as the initial phase of treatment. Because it measures a drug’s activity against a population of “persisters” bacilli surviving 2 months of treatment with rifampin, isoniazid, and pyrazinamide (RHZ), the continuation phase assessment may provide greater insight into a drug’s treatment-shortening potential (8, 12).

These studies were performed as part of a strategic initiative by the Global Alliance for TB Drug Development to evaluate new drug combinations (http://www.tballiance.org/new/strategic.php). DC-159a was kindly provided by Daiichi-Sankyo Co., Ltd. (Tokyo, Japan). Other drugs and 6-week-old female BALB/c mice were obtained as previously described (12). All procedures involving animals were approved by the Institutional Animal Care and Use Committee.

Serum samples were collected from uninfected mice for up to 8 h after a single, oral dose of 25, 50, or 100 mg/kg of DC-159a or 100 mg/kg of MXF administered by gavage. DC-159a and MXF concentrations were determined by a validated high-performance liquid chromatography (HPLC) assay and analyzed by standard noncompartmental techniques using WinNonlin (version 5.2.1; Pharsight, Mountain View, CA). Dose proportional increases in maximal serum concentration and the area under the concentration-time curve were observed between the 25- and 100-mg/kg doses of DC-159a (Fig. 1). At 100 mg/kg, the DC-159a exposure was comparable to that observed for MXF (Table 1).

Mice were aerosol infected with 4.01 ± 0.12 log10 CFU of M. tuberculosis H37Rv as described previously (12). Mice were randomized to treatment groups (4 mice/group) and treated 5 days/week, by gavage, as shown in Table 2. For the initial phase, treatment began 14 days after infection (day 0), when the mean lung CFU count was 6.85 ± 0.16 log10. For the continuation phase, mice that received 2 months of RHZ received 2 additional months of the regimens indicated in Table 2. After 1, 2, and 4 months of treatment, mice were sacrificed.
and lung CFU counts were determined as described previously (12). Group means for each MXF-treated group were compared to those of each DC-159a-treated group by one-way analysis of variance (ANOVA) with Bonferroni’s post-test (GraphPad Prism, version 4; GraphPad Software, La Jolla, CA).

All untreated mice died within 1 month of infection. No deaths were observed in treated mice. During the initial 2 months, isoniazid alone and RHZ reduced lung CFU counts by nearly 2 log_{10} and nearly 4 log_{10} respectively. Both MXF and DC-159a exhibited dose-dependent bactericidal activity. At 100 mg/kg, DC-159a was equivalent to isoniazid. Other monotherapy regimens were inferior to isoniazid. DC-159a at 25 mg/kg was superior to MXF at 25 mg/kg (P < 0.05) but inferior to MXF at 100 mg/kg (P < 0.001). DC-159a at 50 mg/kg was superior to MXF at 25 mg/kg (P < 0.001) but not significantly different from MXF at 50 or 100 mg/kg, and DC-159a at 100 mg/kg was superior to all MXF doses (P < 0.001). Therefore, we conclude that DC-159a is superior to MXF on a milligram/kilogram basis and may yield the same bactericidal activity we conclude that DC-159a is superior to MXF on a milligram/kg basis and may yield the same bactericidal activity.

Both MXF and DC-159a exhibited dose-dependent activity which was primarily bacteriostatic at doses of 25 to 50 mg/kg and bactericidal at 100 mg/kg. No statistically significant differences in effect size were identified when comparing the activity of DC-159a with that of MXF at the 25- and 50-mg/kg dose sizes, although the design of the experiment provided limited power to detect differences of less than 0.6 log_{10}. At the 100-mg/kg dose level, DC-159a was equivalent to isoniazid and statistically superior to MXF at 100 mg/kg (P < 0.05), even after adjustment for up to 6 pairwise comparisons. The superiority of isoniazid over MXF at 100 mg/kg has been observed previously in this model and implies that multiplication continues to some degree during the continuation phase and/or that isoniazid kills by mechanisms other than inhibition of cell wall synthesis (12).

The principal finding of this study is that the new 8-methoxyfluoroquinolone DC-159a has dose-dependent bactericidal activity which is clearly superior to MXF during the initial phase and may be superior in the continuation phase as well. These results are consistent with the findings that the MIC of DC-159a is 2 to 4 times lower than that of MXF (3) and that similar drug exposures are achieved after the same dose. Therefore, we conclude that DC-159a may be useful for shortening the duration of treatment for drug-susceptible and MDR TB. The greater in vitro potency of DC-159a against fluoroquinolone-resistant, MDR M. tuberculosis isolates with common gyrA mutations suggests that it should retain some activity against XDR TB (3, 10).

Therefore, we believe DC-159a warrants further comparisons to MXF in selected combination regimens containing both new and existing drugs against drug-susceptible and fluoroquinolone-resistant strains to determine whether it may lend superior treatment-shortening potential.

We acknowledge Norio Doi for fruitful discussions related to the experimental design. The study was funded by the Global Alliance for TB Drug Development.
REFERENCES


