Evaluation of a 2-Pyridone, KRQ-10018, against Mycobacterium tuberculosis In Vitro and In Vivo

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Quinolones are a family of broad-spectrum antibiotics whose activity inhibits mycobacterial DNA gyrase. They possess many desirable attributes for a first-line therapeutic agent against Mycobacterium tuberculosis, including potent bactericidal activity against both replicating and nonreplicating Mycobacterium tuberculosis (4, 6), favorable long-term safety indicators, and good pharmacokinetics. Moxifloxacin (MXF), a licensed antimicrobial developed by BayerHealthcare AG, has already demonstrated its ability to shorten therapy length in mouse tuberculosis models (10, 11) and is currently being tested in clinical trials. In 2003 the Global Alliance for TB Drug Development initiated a lead identification and optimization project with the goal of identifying a new generation of quinolones against M. tuberculosis. A series of novel subclass of quinolones, named the 2-pyridones (9), had the greatest activity against M. tuberculosis. KRQ-10018, an early lead in the 2-pyridone series, is highly active against a combination of both gram-positive and gram-negative bacteria (M. tuberculosis, Staphylococcus aureus, Streptococcus pneumoniae, Salmonella enterica serovar Typhimurium, Escherichia coli, and Pseudomonas aeruginosa). Activity was also demonstrated intracellularly within macrophages, and low cell toxicity was shown by testing the compounds on Vero cells (12). The chemical structure of KRQ-10018 is shown in Fig. 1. In this report, KRQ-10018 was tested using a series of in vitro and in vivo assays. The oral bioavailability of KRQ-10018 was evaluated in a bioassay using M. tuberculosis, as previously described (3), and results were compared to those of MXF and INH. Mice were given a single oral dose of KRQ-10018 (300 mg/kg), MXF (300 mg/kg), or INH (25 mg/kg) and bled via the tail vein after 30 min and 2.5 h. The collected serum samples were assayed in a bioassay to determine approximate bioactive compound levels. For both INH and KRQ-10018, the oral bioavailability was

Against M. tuberculosis, the MIC of KRQ-10018 was <0.025 to 0.041 µg/ml, compared to 0.1 to 0.12 µg/ml for MXF.

The activity of KRQ-10018 was also tested against nonreplicating bacteria using a modified method by Wayne et al. (14), as described earlier (7). M. tuberculosis H37Rv culture was grown under gradual oxygen depletion for 24 days, and drugs were injected through sterile septa under continuous anaerobic conditions. KRQ-10018 and the following control compounds were evaluated. Isoniazid (INH), rifampin, metronidazole (MET), pyrazinamide, and streptomycin were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO). Rifapentine (RFP) was obtained from Marion Merrell Dow Pharmaceuticals (Cincinnati, OH). The fluoroquinolones (MXF, gatifloxacin, ciprofloxacin, and levofloxacin) were extracted from tablets by R. Reynolds (SRI, Birmingham, AL) as described before (7). The results, shown in Table 1, show that INH had little activity against the nonreplicating bacteria, which was as expected as it is mainly active against actively replicating bacilli (14). In contrast, rifampin, RFP, and MXF were highly active against nonreplicating bacilli (14). MET, which is used against anaerobic infections in the clinic and is known to have some activity solely against nonreplicating M. tuberculosis (14), also showed activity. Of significance, KRQ-10018 showed strong activity (1.7% regrowth of bacteria), similar to that of RFP and MXF (both 2.5% regrowth) (Table 1).

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TABLE 1. Activities of KRQ-10018 and other tuberculosis drugs in the Wayne model against M. tuberculosis H37Rv cultures following 24 days of oxygen depletion

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc (mg/kg)</th>
<th>% Growth vs untreated controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>KRQ-10018</td>
<td>10</td>
<td>1.7</td>
</tr>
<tr>
<td>INH</td>
<td>10</td>
<td>70.0</td>
</tr>
<tr>
<td>Rifampin</td>
<td>10</td>
<td>5.6</td>
</tr>
<tr>
<td>MET</td>
<td>10</td>
<td>36.0</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>10</td>
<td>4.2</td>
</tr>
<tr>
<td>RFP</td>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td>Ciproflaxacin</td>
<td>10</td>
<td>83.0</td>
</tr>
<tr>
<td>Gatiflaxacin</td>
<td>10</td>
<td>41.0</td>
</tr>
<tr>
<td>MXF</td>
<td>10</td>
<td>2.5</td>
</tr>
</tbody>
</table>

a For details of the Wayne model, see references 8 and 14.

by a one-way analysis of variance followed by a multiple-comparison analysis of variance by a one-way Tukey test (SigmaStat software program). Differences were considered significant at the 95% level of confidence.

KRQ-10018 showed significant activity in the GKO model at both concentrations used versus untreated controls (P < 0.01), in a dose-dependent manner (Table 3). In the lungs, KRQ-10018 at 100 mg/kg showed a 1.5-log10 reduction in bacterial load versus the untreated control group (P < 0.05), while at 300 mg/kg a reduction of more than 2.5-log10 was observed (P < 0.01). KRQ-10018 was slightly less effective in the spleens, resulting in a ~0.7-log10 reduction versus untreated controls when dosed at 100 mg/kg (P < 0.01) and an ~1.7-log10 reduction at 300 mg/kg versus the untreated controls (P < 0.01) (Table 3). When compared to the efficacy of INH and MXF in lungs, the activity of KRQ-10018 at 300 mg/kg was statistically similar to that of INH at 25 mg/kg (P > 0.05) (Table 3) and was less active versus MXF at 300 mg/kg (P < 0.001). In spleens, KRQ-10018 at 300 mg/kg was less effective than INH at 25 mg/kg and MXF at 300 mg/kg (P < 0.01) (Table 3).

Overall KRQ-10018 showed potent activity against actively replicating and nonreplicating bacilli. Oral bioavailability was higher than that of MXF, although the drug appeared to be cleared somewhat faster. The efficacy of KRQ-10018 in our rapid tuberculosis mouse model was similar to that of INH but less than that of MXF. Broad-spectrum activity against a diverse panel of bacterial species makes this quinolone series attractive for further optimization. The considerable activity against M. tuberculosis in a potentially latent state and in our in vivo infection model shows the potential of this new quinolone series and gives initial information to optimize the initial lead for treatment of tuberculosis.

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REFERENCES


