In Vitro Antimycobacterial Activity of 5-Chloropyrazinamide

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5-Chloropyrazinamide and 5-chloropyrazinoic acid were evaluated for in vitro activity against Mycobacterium tuberculosis, Mycobacterium bovis, and several nontuberculous mycobacteria by a broth dilution method. 5-Chloropyrazinamide was more active than pyrazinamide against all organisms tested. It is likely that this agent has a different mechanism of action than pyrazinamide.

Pyrazinamide (PZA) is a first-line agent for the treatment of tuberculosis (1, 4) and an essential element of experimental preventive therapy regimens (6, 9). PZA appears to function as a prodrug of pyrazinoic acid (PA) and is converted to PA intracellularly. The biochemical basis for the antituberculosis activity of PA has not been established (7).

It is known that the majority of Mycobacterium tuberculosis isolates resistant to PZA in vitro have low levels of pyrazinamidase activity, as do Mycobacterium bovis isolates (8, 10–12). PZA-susceptible and -resistant isolates are generally susceptible to PA in vitro, but PA is not active in vivo (5). A series of esters of PA and 5-substituted PA have been found to have enhanced in vitro activity against both PZA-susceptible and -resistant M. tuberculosis as well as against PZA-resistant M. bovis, Mycobacterium kansasii, and Mycobacterium avium isolates (2, 3). The aim of this study was to evaluate the in vitro activity of 5-chloro-PZA (5-Cl PZA) and 5-Cl PA against various mycobacterial isolates, including PZA-resistant M. tuberculosis.

PZA was obtained from Sigma Chemical Company, St. Louis, Mo. PA was obtained from Aldrich Chemical Company, Milwaukee, Wis. 5-Cl PZA and 5-Cl PA were synthesized from 5-chloropyrazinoyl chloride. 5-Cl PZA was obtained as follows: to 30 ml of NH₄OH, 3.55 g (20 mmol) of 5-Cl-pyrazinoyl chloride in 25 ml of dry tetrahydrofuran was added at 0°C over a 30-min period. After the addition was complete, the reaction mixture was stirred for another 30 min. The reaction mixture was diluted with 30 ml of ether, and the formed precipitate was filtered. The filtercake was washed with 30 ml of ether, and the filtrate was separated. The aqueous layer was extracted twice with 20 ml of ether each time, and the combined organic layer was filtered. The filtrate was separated. The crude product was recrystallized from EtOH. The yield was 78.6%. The melting point was 206 to 210°C, infrared 3,400, 3,436, 1,700 cm⁻¹, ¹H NMR (CDCl₃) 6.916 [J = 1.6 Hz, d, 1H], 8.53 [J = 1.6 Hz, d], 7.5 [br, 1H], 5.82 [br, 2H]. 5-Cl PZA and 5-Cl PA were ≥95% pure.

Stock solutions were prepared by dissolving each compound in modified 7H10 broth (concentrations ranged from 2,048 to 0.5 μg/ml). Stock solutions were sterilized by passage through a 0.22-μm-pore-size membrane filter. Stock solutions of PA and 5-Cl PA were adjusted to pH 5.8 with 1 N KOH prior to sterilization. Serial twofold dilutions of each compound were made in modified 7H10 broth (concentrations ranged from 2,048 to 0.5 μg/ml).

Strains of M. tuberculosis (ATCC 27294, ATCC 35801, and ATCC 35828), M. bovis (ATCC 35720 and ATCC 27289), Mycobacterium smegmatis (ATCC 19420), and Mycobacterium fortuitum (ATCC 49403) were obtained from the American Type Culture Collection, Rockville, Md. Isolates of PZA-resistant M. tuberculosis were kindly provided by Salman Siddiqi (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.). M. avium strain 101 (serotype 1) was provided by Lowell Young (Kuzell Institute for Arthritis and Infectious Diseases, California Pacific Medical Center Research Institute, San Francisco, Calif.). M. avium ATCC 49601 (serotype 1) is a clinical isolate from a patient with AIDS at State University of New York Health Science Center, Syracuse, N.Y. M. kansasii strain S was a clinical isolate from a patient at the Veterans Affairs Medical Center, Syracuse, N.Y.

Mycobacteria were grown in modified 7H10 broth, pH 6.6, with 10% OADC enrichment and 0.05% Tween 80 (13). Cell suspensions were diluted in modified 7H10 broth, pH 5.8, to yield 1 Klett unit of M. tuberculosis, M. bovis, and M. smegmatis per ml and 0.1 Klett unit of M. avium, M. kansasii, and M. fortuitum per ml (Klett-Summerson colorimeter; Klett Manufacturing, Brooklyn, N.Y.) or approximately 5 × 10⁵ CFU/ml. A 0.1-ml volume of culture suspension was added to each tube containing mycobacterial broth (1.9 ml of modified 7H10 broth, pH 5.8, 5.8 μg/ml), yielding a final inoculum of approximately 2.5 × 10⁴ CFU/ml. Susceptibility testing was performed with modified 7H10 broth, pH 5.8, because some isolates of M. tuberculosis grow poorly at pH 5.6, the standard pH used for susceptibility testing in agar. Inoculum size was determined by titration and counting from duplicate 7H10 agar plates (BBL Microbiology Systems, Cockeysville, Md.). A tube without drug was included for each isolate as a positive control. Tubes were incubated on a rotary shaker (190 rpm) at 37°C for 24 h to 2 weeks. The MIC was defined as the lowest concentration of drug that yielded no visible turbidity.

The broth dilution MICs of PZA, 5-Cl PZA, PA, and 5-Cl PA for the M. tuberculosis isolates (n = 7) are shown in Table 1. The MIC ranges of PZA and 5-Cl PZA were from 32 to >2,048 μg/ml and from 8 to 32 μg/ml, respectively. The MIC ranges of PA and 5-Cl PA were from 16 to 64 μg/ml and from 64 to 256 μg/ml, respectively. The MICs of 5-Cl PZA and PA for M. tuberculosis are more favorable than those of PZA and 5-Cl PA. PZA-resistant isolates retain susceptibility in vitro to 5-Cl PZA, PA, and 5-Cl PA, suggesting that 5-Cl PZA can circumvent the requirement for activation by mycobacte-
TABLE 1. MICs of pyrazinamide analogs for various mycobacteria

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC (µg/ml) of:</th>
<th>PZA</th>
<th>5-Cl PZA</th>
<th>PA</th>
<th>5-Cl PA</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. tuberculosis</em> strain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATCC 27294</td>
<td>64</td>
<td>16</td>
<td>32</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>ATCC 35801</td>
<td>32</td>
<td>16</td>
<td>32</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>ATCC 35828</td>
<td>&gt;2,048</td>
<td>32</td>
<td>32</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>VA 205</td>
<td>&gt;2,048</td>
<td>32</td>
<td>32</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>BDDIS 20</td>
<td>&gt;2,048</td>
<td>32</td>
<td>64</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>DHMH 4319</td>
<td>2,048</td>
<td>8</td>
<td>16</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>CDC-BP-98</td>
<td>2,048</td>
<td>16</td>
<td>32</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td><em>M. bovis</em> strain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATCC 35720</td>
<td>&gt;2,048</td>
<td>8</td>
<td>32</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>ATCC 27289</td>
<td>&gt;2,048</td>
<td>8</td>
<td>64</td>
<td>256</td>
<td></td>
</tr>
</tbody>
</table>

*Nontuberculous mycobacteria*  
*M. kansasii* S  
*M. smegmatis* 19420  
*M. fortuitum* 49403  
*M. avium* 49601  

The MICs of 5-Cl PZA for nontuberculous mycobacteria are lower than those of 5-Cl PA, PZA, or PA. The activity against *M. avium* is noteworthy, particularly in light of the poor activity of 5-Cl PA.

The presumption that PZA is a prodrug for PA is supported by previous studies (3, 8). The lower MICs of PA relative to PZA are consistent with this hypothesis. While the mechanism of action of PA remains to be defined, assumptions based upon the effect of PA increasing intracellular pH are confounded by the observation that 5-Cl PA is significantly less effective than PA against *M. tuberculosis*. The largest difference, an eightfold increase in the MIC of 5-Cl PA relative to that of PA, is found with organisms such as ATCC 35828, which are resistant to PZA and deficient in amidase.

When the activity of PZA relative to 5-Cl PZA is considered, these organisms are more susceptible to the substituted compound. If PZA is activated by hydrolysis to PA, inhibition is not likely to be based upon acidification by PA acting as a proton donor. According to the Hammet relationship, 5-Cl PA should be a stronger acid and therefore a more potent inhibitor than PA. It is unclear whether 5-Cl PZA has a different mechanism of action than PZA or whether it functions as a prodrug with an alternative method of activation. The hypothesis that 5-Cl PZA has an alternative activation pathway is not consistent with the observation that 5-Cl PA is less effective than PA against the same organisms.

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