Specific Inhibition of Spermidine Synthesis in *Mycobacteria* spp. by the Dextro Isomer of Ethambutol

LARS G. PAULIN,1 ELIAS E. BRANDER,2 AND HANNU J. PÖSÖ1*

Department of Pharmacology and Toxicology, College of Veterinary Medicine, SF-00551, Helsinki 55,1 and Central Public Health Laboratory, SF-00280, Helsinki 28,2 Finland

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Only the dextro isomer of ethambutol inhibited mycobacterial spermidine synthase and spermidine biosynthesis. Inhibition of mycobacterial spermidine synthase appeared to be specific. Spermidine synthase from *Mycobacterium fortuitum*, which was resistant to ethambutol in vitro, required a higher concentration of ethambutol for its inhibition than the enzyme of susceptible *Mycobacterium bovis*.

Bacteria usually contain putrescine and spermidine as their polyamines (4, 11, 17). Numerous studies have shown that these compounds are essential for the normal growth of bacteria (1, 6, 10, 18). We have shown earlier (13) that ethambutol, which is an effective antituberculosis drug (16, 19), specifically inhibited spermidine synthase from *Mycobacteria* spp. but not from other bacteria. This suggested that the effects of ethambutol on the growth of *Mycobacteria* spp. are mediated at least partly via polyamine biosynthesis.

Since the dextro form of ethambutol is effective only against *Mycobacteria* spp. (5, 16, 19) but not against other bacteria and microorganisms (5), and its effects are reversed by spermidine (2), we decided to test the effect of the dextro ethambutol on spermidine biosynthesis by *Mycobacterium bovis* both in vivo and in vitro. We show that the drug specifically inhibits spermidine synthesis in growing *Mycobacteria* spp.

The bacterial strains used included *Pseudomonas aeruginosa* ATCC 27559, *Escherichia coli* ATCC 11303, *M. bovis* BCG (Glaxo Pharmaceuticals, Ltd.), *Mycobacterium fortuitum* biovariant *fortuitum* (isolated from a patient in 1982), and *Mycobacterium flavescens* (isolated from a patient in 1978). *P. aeruginosa* and *E. coli* was grown in LB medium (7), the BCG organisms were grown in Löwenstein-Jensen medium at 37°C for 3 weeks, and *M. fortuitum* and *M. flavescens* were grown for 1 week.

To obtain cell extracts for spermidine synthase assays, bacteria were treated exactly as described previously (6, 13). Spermidine synthase (putrescine aminopropyltransferase) activity was measured in the dialyzed supernatants as described earlier (8, 15).

[Propylamine-1-14C]-decarboxylated-S-adenosyl-L-methionine for the assay of spermidine synthase was synthesized by the method of Pöösö et al. (12).

The dextro ethambutol was a generous gift from Oriola Oy, Helsinki, Finland, and the levo ethambutol was kindly provided by American Cyanamid Co., Pearl River, N.Y. S-Adenosyl-L-methionine (62 Ci/mmol) for the synthesis of radioactive decarboxylated adenosylmethionine and [3H]putrescine (30 Ci/mmol) were purchased from New England Nuclear Co., Dreieichenhain, Federal Republic of Germany. All other biochemicals were obtained from Sigma Chemical Co., St. Louis, Mo.

The incorporation of [3H]putrescine into spermidine was followed by adding 100 μCi of putrescine to each culture (about 50 μg [wet weight] of *M. bovis*) for 48 h. After the cells were washed with ice-cold 0.9% NaCl containing 25 mM unlabeled putrescine (to remove all radioactive putrescine bound to the outer membrane of the bacteria), the polyamines were extracted and separated by paper electrophoresis as described earlier (14). The DNA content of the cells was measured by the method of Giles and Myers (3).

Inhibition of mycobacterial spermidine synthase by dextro ethambutol. Only the dextro form of ethambutol (at micromolar concentrations) inhibited spermidine synthase in extracts of *M. bovis* (Fig. 1). However, the corresponding enzyme activity in extracts from *P. aeruginosa* or from *E. coli* was not inhibited by the dextro form of the drug. Levo ethambutol, even at a concentration of 1 mM, did not inhibit mycobacterial spermidine synthase (Fig. 1).

*Increased resistance of* *M. fortuitum* towards ethambutol leads to spermidine synthase activity insensitive to ethambutol.* Table 1 gives more evidence for a possible reason for the

* Corresponding author.

FIG. 1. Effect of dextro and levo ethambutol on mycobacterial spermidine synthase activity. Spermidine synthase from different bacteria was assayed as described in the footnotes to Table 1 in the presence of dextro or levo ethambutol. Results are expressed as the percentage of the activity in the absence of any inhibitor. Symbols: ▲, spermidine synthase from *M. bovis* with dextro ethambutol; ○, spermidine synthase from *M. bovis* with levo ethambutol; ●, spermidine synthase from *E. coli* with dextro ethambutol; ■, spermidine synthase from *P. aeruginosa* with dextro ethambutol.
TABLE 1. Inhibition of spermidine synthase from various *Mycobacteria* spp. by the dextro isomer of ethambutol<sup>a</sup>

<table>
<thead>
<tr>
<th>Conc of ethambutol (µM)</th>
<th>Spermidine synthase activity (pmol of methylthioadenosine formed per 60 min per mg of protein) in&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>M. bovis</em></td>
</tr>
<tr>
<td>0</td>
<td>315 (100)</td>
</tr>
<tr>
<td>10</td>
<td>267 (85)</td>
</tr>
<tr>
<td>20</td>
<td>236 (75)</td>
</tr>
<tr>
<td>30</td>
<td>161 (51)</td>
</tr>
<tr>
<td>50</td>
<td>69 (22)</td>
</tr>
<tr>
<td>80</td>
<td>16 (5)</td>
</tr>
<tr>
<td>100</td>
<td>6 (2)</td>
</tr>
<tr>
<td>500</td>
<td>3 (1)</td>
</tr>
</tbody>
</table>

<sup>a</sup> The assay medium contained about 0.25 mg of protein from dialyzed 42,000 × g supernatant (13) and 0.5 mM putrescine in a 0.2-M solution containing 20 µmol of sodium phosphate (pH 8.2) and 25 nmol of decarboxylated [methyl-<sup>14</sup>C]adenosymethionine. After 60 min of incubation, the reaction was stopped with 0.5 ml of 25 mM HCl, and [<sup>14</sup>C]ethiomethyladenosine was isolated from 0.5 ml of the mixture as described earlier (15). Results are the means of duplicate experiments.

<sup>b</sup> The activities remaining (percent of uninhibited control) in the presence of ethambutol are shown in parentheses.

The effect of ethambutol on the growth of *Mycobacteria* spp. *M. fortuitum* (requiring 8 µg of ethambutol per ml for growth inhibition instead of 1 µg/ml as in the case of *M. bovis* and *M. flavescent*) was more resistant to ethambutol than other *Mycobacteria* spp. and had a spermidine synthase activity which required 80 µM dextro ethambutol for 50% inhibition, whereas both *M. bovis* and *M. flavescent* possessed spermidine synthase activity requiring only 30 µM ethambutol to obtain the same degree of inhibition (Table 1).

**Inhibition of cellular spermidine biosynthesis by ethambutol.** To confirm the inhibitory effect of ethambutol on spermidine biosynthesis, its effect was measured when *Mycobacteria* spp. were grown in culture. A concentration of ethambutol (0.5 µg/ml) which does not inhibit the growth of *M. bovis* (19) did not inhibit the incorporation of [<sup>3</sup>H]putrescine into spermidine, whereas 8 µg/ml (a concentration causing the death of *Mycobacteria* spp.; reference 19) of the drug clearly inhibited the synthesis of spermidine by 64% and caused an accumulation of [<sup>3</sup>H]putrescine by the cells (Table 2). This can be taken as an indication that ethambutol did not inhibit the synthesis of spermidine simply by preventing the cellular uptake of putrescine.

Since no radioactivity was found (in the experiments described in Table 2) where spermine migrated in paper electrophoresis, it can be concluded that there is no spermine biosynthesis in *M. bovis* which, with one known exception (9), is characteristic of bacteria (17).

Taken together, our results suggest that the effect of ethambutol on the growth of *Mycobacteria* spp. may be, at least partially, attributable to a disturbance of polyamine biosynthesis in these bacteria.

We are grateful for the gifts of different isomers of ethambutol. We thank M. Sandholm for the initiation of these experiments.

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**LITERATURE CITED**


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