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Isoniazid Inhibition of the Synthesis of Monounsaturated Long-Chain Fatty Acids in *Mycobacterium tuberculosis* H37Ra

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Isoniazid inhibited C_{24} and C_{26} monounsaturated fatty acid synthesis in *Mycobacterium tuberculosis* H37Ra. Time courses of this inhibition and that of mycolic acid synthesis were similar.

The antimycobacterial drug isoniazid inhibits mycolic acid synthesis in *Mycobacterium tuberculosis* BCG (10) and H37Ra (5, 9). Mycolic acids, which are α-alkyl β-hydroxy fatty acids containing up to 90 carbon atoms, are important mycobacterial wall components (1, 2). Isoniazid also inhibits the production of very long-chain (greater than C_{26}) fatty acids other than mycolic acids (8). On the basis of structural analyses (4, 7), it was suggested that these very long-chain fatty acids may be precursors of mycolic acids and that the first reaction specific to mycolic acid synthesis may be the desaturation of tetracosanoic (C_{24}) and perhaps also hexacosanoic acid (C_{26}). We now report that isoniazid inhibits this reaction.

M. tuberculosis H37Ra was grown in enriched Middlebrook 7H9 medium (9). Isoniazid was added to 100-ml cultures in early exponential phase (absorbance at 650 nm of 0.1 to 0.2) to give concentrations of 0.5 μg/ml. Portions (10 ml each) were removed from the cultures immediately before and at various times after the addition of isoniazid. Control cultures had no isoniazid added. Mycolic and nonmycolic fatty acid synthesis was assayed in each portion as described previously (6), except that incubations were for 5 min with 50 μCi of sodium [1-^{14}C]-acetate (57.7 μCi/μmol, Amersham/Searle), and bacilli were harvested by filtration through a 0.45-μm filter (Millipore Corp.) and saponified in 2.5 ml of 10% KOH in ethanol-water (1:1, vol/vol) at 90°C for 16 h. The fatty acids were fractionated into mycolic acids and short-chain (C_{16} to C_{19}) saturated, long-chain (C_{24} to C_{26}) saturated, C_{16} to C_{19} monounsaturated, and C_{24} to C_{26} monounsaturated components by thin-layer chromatography, and then the radioactivity in each fraction was determined (3, 6, 9).

Isoniazid, at 0.5 μg/ml, rapidly inhibited the synthesis of C_{24} to C_{26} monounsaturated fatty acids, but the synthesis of C_{24} to C_{26} saturated fatty acids increased, reaching its maximum at 50 min (Fig. 1). The synthesis of C_{16} to C_{19} saturated fatty acids also increased; however, the synthesis of C_{16} to C_{19} monounsaturated fatty acids was unaffected by isoniazid (data not shown). The time courses of the inhibition of C_{24} to C_{26} monounsaturated fatty acid synthesis

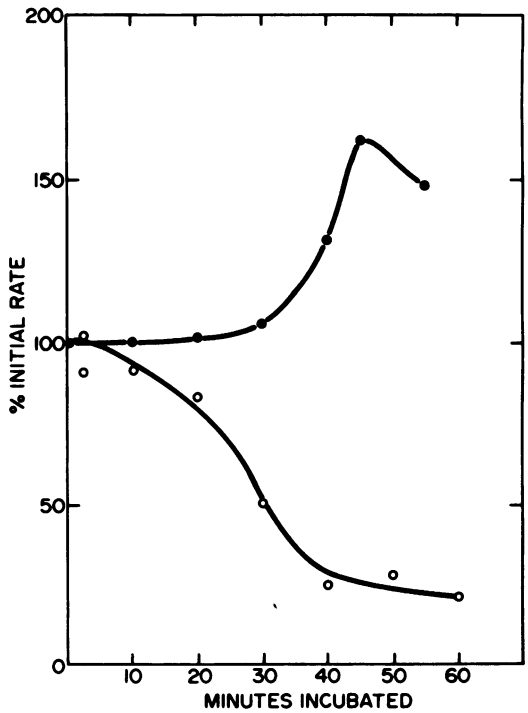

FIG. 1. *Effect of isoniazid (0.5 μg/ml) on the synthesis of C_{24} to C_{26} saturated (●) and C_{24} to C_{26} monounsaturated (○) fatty acids in M. tuberculosis H37Ra. The rate of synthesis of each fatty acid before addition of the drug was taken as 100%.*

(Fig. 1) and mycolic acid synthesis (Fig. 2) were similar.

Monounsaturated fatty acids from control and isoniazid-treated (0.5 μg/ml for 60 min) cultures were analyzed by gas-liquid chromatography on a Dexsil 300 column (Applied Sciences Laboratory, Inc.) in a Packard model 419 gas chromatograph. Peaks revealed by the thermal conductivity detector were recovered and analyzed for radioactivity (8). These analyses showed that the synthesis of monounsaturated fatty acids of sizes C_{24} and greater was 65 to 80% lower in the isoniazid-treated samples than in control samples; synthesis of C_{24} to C_{26} saturated fatty acids was almost 100% higher in the treated samples.

Inhibition of C_{24} to C_{26} monounsaturated fatty acid synthesis by isoniazid with a concomitant increase in C_{24} to C_{26} saturated fatty acids indicates that the drug may be inhibiting a desaturase which acts on these saturated fatty acids. The similarity of the time courses for the inhibition of C_{24} to C_{26} monounsaturated fatty acid and mycolic acid synthesis supports the theory that C_{24} to C_{26} monounsaturated fatty acids are precursors of mycolic acids (7). If this is true, then inhibition of the desaturase producing C_{24} to C_{26} monounsaturated fatty acids from C_{24} to C_{26} saturated fatty acids may be the means by which isoniazid inhibits mycolic acid synthesis. The relationship between the inhibition of mycolic acid synthesis and the lethal action of isoniazid remains unclear.

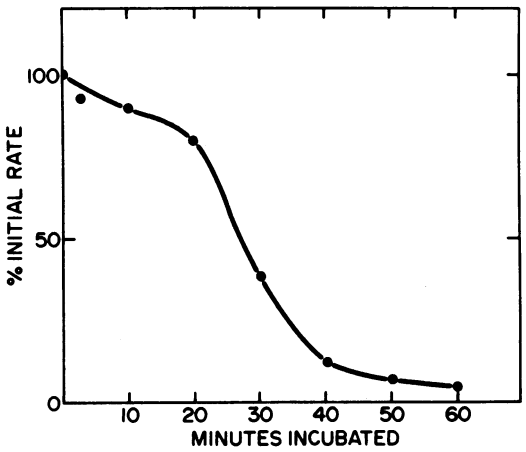

FIG. 2. *Inhibition of mycolic acid synthesis in M. tuberculosis H37Ra by 0.5 μg of isoniazid per ml. The rate of mycolic acid synthesis before addition of the drug was taken as 100%.*

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