In Vitro Susceptibility of Atypical Mycobacteria To Rifampin

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Atypical mycobacteria (209 strains) were examined for susceptibility to rifampin by the proportion method by using Middlebrook 7H-10 agar. All strains of Mycobacterium kansasii and tap-water scotochromogens were inhibited by 0.25 to 1 μg of the drug per ml. Seventy-six per cent of M. scrofulaceum and 61% of M. intracellulare strains were susceptible to 4 μg/ml or less; 5% of the former and 8% of the latter were resistant to 16 μg/ml. All strains of M. gastri and M. triviale and most strains of M. terrae were sensitive to 1 to 4 μg/ml. Two strains of M. borstelense were both inhibited by 8 μg/ml. Nearly all strains of M. fortuitum were resistant to the drug. The results of this study suggest that rifampin may be a valuable agent for the treatment of many atypical mycobacterial infections.

Rifampin is a semisynthetic orally administered derivative of rifamycin SV. In addition to its activity against gram-positive cocci (with the exception of enterococcus), Neisseria meningitidis and N. gonorrhoeae (1, 4), it is highly effective in vitro (3, 8-10, 12, 14) and in vivo (5, 7, 13, 15) against Mycobacterium tuberculosis. However, in vitro determinations of the sensitivity of atypical mycobacteria to this drug have yielded variable and somewhat conflicting results (3, 8-10, 12). This disparity may have been related, in some instances, to the small number of strains examined and to differences in methodology. The present investigation was undertaken for two purposes: (i) to reexamine the effects of rifampin on atypical mycobacteria by the proportion method of Canetti et al. (2) and (ii) to determine whether study of a large number of strains would produce sufficiently consistent results to allow predictions regarding the potential clinical usefulness of this antibiotic.

MATERIALS AND METHODS

Atypical mycobacteria (209 strains) studied were as follows: 40 M. kansasii, 63 M. scrofulaceum, 5 tap-water scotochromogens, 71 M. intracellulare, 7 M. gastri, 8 M. terrae, 6 M. triviale, 7 M. fortuitum, and 2 M. borstelense. Rifampin was supplied by Ciba Pharmaceutical Products, Inc., Summit, N. J.

The inhibitory concentrations of rifampin for atypical mycobacteria were determined in Middlebrook 7H-10 agar (Difco) by the proportion method of Canetti et al. (2). Rifampin was dissolved in dimethylsulfoxide to a concentration of 8 mg/ml; further dilutions were made in sterile distilled water. Varying quantities of the drug were incorporated into the agar to yield final concentrations of 16, 8, 4, 2, 1, 0.5, and 0.25 μg/ml. Media containing rifampin and the drug-free medium (control) were dispensed in 5-ml amounts into quadrants of sectioned, plastic petri dishes. The control medium contained 0.2% dimethylsulfoxide, equivalent to its highest concentration in drug-containing media.

All of the mycobacteria were cultured on Löwenstein medium for 4 to 6 weeks. Subcultures of each strain were then made in 7H-9 broth containing Tween 80; all were shaken daily for 1 min. After 7 to 10 days of incubation at 37 C (3 to 5 days for “rapid growers”), each culture was diluted with sterile water to a turbidity approximating the McFarland 1 standard (1 mg of bacterial mass per ml) and then diluted 1:100 and 1:10,000 in sterile water. Three drops (Pasteur pipette) of each dilution were spread on quadrants of agar containing different concentrations of rifampin and on control quadrants that were drug-free. The plates were sealed in polyethylene bags and incubated at 37 C for 2 to 4 weeks (7 to 10 days for “rapid growers”); at this time, heavy growth of the organisms was present on the agar to which no antibiotic had been added.

The number of viable units in the inoculum was indicated by the number of colonies that developed on drug-free media. A strain was considered resistant to a particular concentration of rifampin when the number of colonies detected was equal to or greater than 1% of the viable units present in the inoculum.

RESULTS

The inhibitory concentrations of rifampin and the cumulative percentages of all of the mycobac-
TABLE 1. Inhibitory concentrations of rifampin for 209 strains of atypical mycobacteria and the cumulative percentage of strains inhibited

| Group | Species                  | No. of strains | No. of strains inhibited with various concn of rifampin |
|-------|--------------------------|----------------|--------------------------------------------------------|
|       |                          |                | 0.25 (µg/ml)   | 0.5 (µg/ml) | 1 (µg/ml) | 2 (µg/ml) | 4 (µg/ml) | 8 (µg/ml) | 16 (µg/ml) | >16 (µg/ml) |
| I     | Mycobacterium kansasii   | 40             | 12 (30)%       | 18 (75)       | 10 (100)%   |           |           |           |            |            |
| II    | M. scrofulaceum          | 63             | 11 (33)        | 6 (60)        | 6 (67)      | 6 (76)     | 6 (86)    | 6 (95)    | 3           |
|       | Tap-water scotochromogens | 5              | 1 (20)         | 2 (60)        |            |           |           |           |             |
| III   | M. intracellulare        | 71             | 4 (6)          | 16 (28)       | 13 (46)     | 10 (61)    | 15 (82)   | 7 (92)    | 6           |
|       | M. gastri                | 7              | 3 (43)         | 2 (71)        | 2 (100)     |           |           |           |             |
|       | M. terrae                | 8              | 1 (12)         | 2 (37)        | 2 (75)      | 1 (87)     | 1 (100)   |           |             |
|       | M. triviale              | 6              | 3 (50)         | 2 (83)        | 1 (100)     |           |           |           |             |
| IV    | M. fortuitum             | 7              |               | 1 (14)        |           |           |           |           | 6           |
|       | M. borstelense           | 2              |               | 2 (100)       |           |           |           |           |             |

* Cumulative percentage.

† Values in parentheses are expressed as percentages.

Fig. 1. Cumulative percentage of strains of three major species of atypical mycobacteria susceptible to rifampin.

M. kansasii was quite susceptible to the drug. Forty strains were examined; 30 (75%) were inhibited by 0.25 to 0.5 µg/ml and 10 (25%) were suppressed by 1 µg/ml. Sixty-three strains of M. scrofulaceum were studied; 32 (51%) were sensitive to 0.25 to 0.5 µg/ml and 16 (25%) were susceptible to 1 to 4 µg/ml. Of the remaining 15 strains, all but 3 were inhibited by 8 to 16 µg/ml. All tap-water scotochromogens were sensitive to 0.25 to 1 µg/ml.

Compared to photo- and scotochromogens, M. intracellulare was less susceptible to rifampin. Seventy-one strains were examined, 20 (28%) were inhibited by 0.5 to 1 µg/ml and 23 (32%) were suppressed by 2 to 4 µg/ml. Of the remaining 28 strains, all but 6 were susceptible to 8 to 16 µg/ml. The inhibitory concentrations of rifampin for all M. gastri and M. triviale and most M. terrae strains ranged from 1 to 4 µg/ml.

Seven strains of M. fortuitum were studied. One was sensitive to 4 µg/ml; the other six were resistant to 16 µg/ml. Only two strains of M. borstelense were examined; both were inhibited by 8 µg/ml.

DISCUSSION

Rifampin is the 3-(4-methyl-1-piperazinyl-iminomethyl) derivative of rifamycin SV. It is well absorbed when given orally; a single dose of 600 mg taken on an empty stomach produces peak blood levels of 7 to 10 µg per ml in 0.5 to 2 hr
The drug is excreted primarily in the bile; it has a relatively long half-life.

Rifampin is highly active against *M. tuberculosis*. All strains of this organism, whether sensitive or resistant to other tuberculostatic agents, are inhibited in vitro by low concentrations of this antibiotic (3, 8–10, 14). The rate of spontaneously occurring resistant mutants is about 1 in 10⁷ organisms (10, 14). This drug has proved very effective in the therapy of advanced or multidrug-resistant pulmonary tuberculosis, or both, when used together with other tuberculostatic agents, especially ethambutol (5, 7, 13, 15). Resistance to rifampin may develop in a single-step fashion during treatment; this usually occurs when it is used without a satisfactory companion drug (5, 13).

Determination of the sensitivity of atypical mycobacteria to rifampin in vitro has yielded variable and somewhat conflicting results (3, 8–10, 12). For example, strains of *M. kansasii* were reported by Clark and Wallace (3) to be resistant and by others (8–10, 12) to be sensitive to this agent. Reasons for this discrepancy include: (i) the small number of strains examined by some investigators, (ii) failure to speciate organisms within Runyon groups II to IV in certain instances, (iii) differences in media (liquid versus solid) used for determination of drug susceptibility, (iv) the use of inocula of varying size, and (v) differences in the criterion of resistance employed by investigators. In the present investigation, a large number of strains identified by species were studied. Susceptibility determinations were performed by the proportion method, which eliminates problems related to the inoculum size.

The criterion for resistance suggested by Canetti et al. (2) for *M. tuberculosis*, namely the growth of 1% or more of the viable units in the inoculum on a given drug concentration, was used in the present study. However, had 0.1% instead of 1% been selected as the dividing line between resistance and sensitivity, the levels of susceptibility of more than 90% of the organisms examined would have been the same. Whether this criterion employed for evaluation of resistance of the tubercle bacillus is applicable to atypical mycobacteria remains to be determined.

Many of the atypical mycobacteria responsible for an increasing number of infections are relatively insensitive to the commonly used tuberculostatic agents. The results of the present study indicate that all of *M. kansasii*, 76% of *M. scrofulaceum*, and 61% of *M. intracellulare* strains are susceptible to 4 μg of rifampin per ml, a level readily produced in serum by administration of conventional doses of the drug. Since this drug is given in a single, daily, oral dose and toxic effects are virtually nil (5, 7, 13, 15), it could prove a valuable adjunct to drug regimens used in the management of atypical mycobacterial infections due to susceptible strains.

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**LITERATURE CITED**

1. Atlas, E., and M. Turck. 1968. Laboratory and clinical evaluation of rifampicin. Amer. J. Med. Sci. 256:247–254.
2. Canetti, G., N. Rist, and J. Groset. 1963. Mésure de la sensibilité du bacille tuberculeux aux drogues antibactériennes par la méthode des proportions. Rev. Tuberc. Pneumol. 27:217–272.
3. Clark, J., and A. Wallace. 1967. The susceptibility of mycobacteria to rifamide and rifampicin. Tubercle 48:144–150.
4. Dans, P. E., R. F. McGehee, Jr., C. Wilcox, and M. Finland. 1970. Rifampicin: antibacterial activity in vitro and absorption and excretion in normal young men. Amer. J. Med. Sci. 259:120–132.
5. Engbaek, H. C., and B. Vergmann. 1970. Rifampicin in the treatment of drug-resistant tuberculosis. A report on 13 patients with pulmonary and one patient with renal disease. Scand. J. Resp. Dis. 51:11–27.
6. Furesz, S., R. Scotti, R. Pallanza, and E. Mapelli. 1967. Rifampicin: a new rifamycin. Arzneimittelforschung. 17:534–537.
7. Gyselen, A., L. Verbiest, J. Cosemans, L. M. Laquet, and E. Vandenberghen. 1968. Rifampin and ethambutol in the retreatment of advanced pulmonary tuberculosis. Amer. Rev. Resp. Dis. 98:933–943.
8. Hobby, G. L., T. F. Lenert, and J. Maier-Engallena. 1969. Experimental observations on the antituberculous activity of rifampicin. Proc. Soc. Exp. Biol. Med. 131:323–326.
9. Lorián, V., and M. Finland. 1969. In vitro effect of rifampin on mycobacteria. Appl. Microbiol. 17:202–207.
10. McClatchy, J. K., R. F. Waggoner, and W. Lester. 1969. In vitro susceptibility of mycobacteria to rifampin. Amer. Rev. Resp. Dis. 100:234–236.
11. Maggi, N., C. R. Pasqualucci, R. Ballotta, and P. Sensi. 1966. Rifampicin: a new orally active rifamycin. Chemotherapy (Basel) 11:285–292.
12. Sotommeier, K. D., G. P. Kubica, and C. L. Woodley. 1969. Antituberculous activity of rifampin under in vitro and simulated in vivo conditions. Appl. Microbiol. 17:861–865.
13. Val-Spinosa, A., W. Lester, T. Moulding, P. T. Davidson, and J. K. McClatchy. 1970. Rifampin in the treatment of drug-resistant Mycobacterium tuberculosis infections. N. Engl. J. Med. 283:616–621.
14. Verbiest, L., and A. Gyselen. 1968. Antituberculous activity of rifampin in vitro and in vivo and the concentrations attained in human blood. Amer. Rev. Resp. Dis. 98:923–932.
15. Wäire, M., O. Heinivaara, R. Elo, and E. Tala. 1969. Clinical experience of the treatment of drug-resistant pulmonary tuberculosis with rifampin combined with ethambutol and capreomycin. Scand. J. Resp. Dis. 50(Suppl. 69):59–63.