ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1994, p. 2346–2350
0066-4804/94/$04.00+0

Vol. 38, No. 10

# Effectiveness of Rifabutin Alone or in Combination with Isoniazid in Preventive Therapy of Mouse Tuberculosis

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Received 29 March 1994/Returned for modification 31 May 1994/Accepted 1 July 1994

**The ever-increasing incidence of tuberculosis calls for the implementation of control measures, including new efficient, short-term preventive therapies to replace 6 to 12 months of isoniazid therapy. The efficacies of 12-week regimens of rifabutin or isoniazid given daily and the combination of the two drugs administered intermittently were evaluated in mice infected with *Mycobacterium tuberculosis* after vaccination with the bacillus Calmette-Guérin (BCG) to imitate some features of the natural infection in humans with a low number of persisting bacteria. Rifabutin at 10 mg/kg of body weight per day was highly effective as early as the eighth week of treatment: all spleens were sterilized and the number of bacteria was drastically reduced in the lungs (mean ± standard deviation log CFU, 0.2 ± 0.3, compared with 5.9 ± 0.6 for untreated controls). No bacilli were found in the spleens or lungs of any of the animals treated for 12 weeks. The combination of rifabutin at 10 mg/kg plus isoniazid at 25 mg/kg twice weekly was almost as effective as rifabutin daily: after 8 weeks of treatment only two of six mice harbored a small number of mycobacteria in their spleens and lungs; at week 12, all spleens were sterilized and a total of eight colonies were isolated from the lungs of two of six mice. Daily isoniazid and once-weekly rifabutin plus isoniazid therapies were less effective. Colonies randomly isolated from the spleens and lungs of mice from different experimental groups were also tested for their susceptibilities to the two drugs. The three surviving colonies from rifabutin-treated mice and all colonies from those administered rifabutin plus isoniazid remained fully susceptible to either drug. In contrast, 2 (18%) of the 11 colonies randomly selected from isoniazid-treated mice became resistant to isoniazid (MIC, > 2 μg/ml), although they were still susceptible to rifabutin. Three months of treatment with rifabutin, either daily alone or twice a week combined with isoniazid, proved to be a valid candidate for tuberculosis preventive therapy.**

Efficient preventive therapies for tuberculosis are much needed in the face of the ever-increasing incidence of tuberculosis worldwide (2), mostly owing to demographic factors and, to a lesser extent, to the increased risk of active tuberculosis in subjects coinfected with the human immunodeficiency virus (HIV) (14). The major drawbacks of daily therapy with isoniazid, the mainstay of tuberculosis prevention thus far, are that treatment is cumbersome, needing 6 to 12 months of daily therapy (thus often resulting in nonadherence), the drug is potentially toxic, and the drug's efficacy is limited by organism resistance (1, 3, 7, 11, 12). This has prompted investigations into alternative, shorter, and more efficient regimens. In one experimental model (9) mice were vaccinated with the bacillus Calmette-Guérin (BCG) before infection with a virulent strain of *Mycobacterium tuberculosis* to obtain an infection with a low number of persisting bacteria to imitate some features of the natural infection in humans. Under those experimental conditions, the results of a recently published study showed that rifabutin, a spiropiperidylrifamycin, is more effective than rifampin in preventing tuberculosis and compares well with rifapentine. In fact, rifabutin showed sterilizing activity when administered at 10 mg/kg of body weight per day for 12 weeks and holds potential for use in intermittent regimens (8).

Rifabutin is an intrinsically more potent antimycobacterial agent than rifampin because of its higher intracellular levels (3a, 12c) its higher levels in tissue (4), and its superior bactericidal activity (6).

The present study was undertaken to further investigate the potential for rifabutin for use in the preventive chemotherapy for tuberculosis and to investigate four areas in particular. First, we wanted to evaluate combined intermittent treatment with isoniazid and rifabutin. In the study of Ji et al. (8) three rifamycins (i.e., rifampin, rifapentine, and rifabutin) with different pharmacokinetic prophiles and intrinsic activities were compared for their efficacies in preventing tuberculosis. These drugs were administered alone, either daily or intermittently. Because the results obtained with rifabutin indicated a potential for its use as intermittent therapy, the present study includes intermittent schedules with rifabutin plus isoniazid to improve treatment efficacy and minimize the number of doses. Second, we wanted to measure the minimum duration of therapy required for the drug to be sterilizing. In a prior study (8) assessments were made only at weeks 6 and 12; rifabutin given daily at 10 mg/kg for 6 days per week induced a dramatic reduction in the number of bacteria in the spleens on week 6, while complete sterilization was documented after 12 weeks. The aim of the present study was to check an intermediate time point (8 weeks) to verify whether the duration of therapy could be reduced further. Third, we wanted to include assessment of the bacterial load in the lung in addition to that in the spleen as an outcome measure. The lung is the target organ for tuberculosis; although the mouse model and the disease in humans might differ to some extent, the eradication of bacilli from their natural location, although more difficult to obtain, appears to be more predictive of clinical outcome. Fourth, we wanted to study the susceptibilities of bacilli after exposure to

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TABLE 1. Treatment schedule for preventive therapy in mice vaccinated with BCG and infected with *M. tuberculosis* H37Rv

| Treatment | Oral dose (mg/kg) | Schedule (days/wk) |
|---|---|---|
| Rifabutin | 10 | 6 |
| Isoniazid | 25 | 6 |
| Rifabutin + isoniazid | 10 + 25 | 2 |
| Rifabutin + isoniazid | 10 + 25 | 1 |

the drug treatments. The selection of resistant mutants following single-agent or combined (preventive) therapy for tuberculosis is a critical factor for choosing treatment regimens and schedules.

(Part of the study was presented in abstract form at the Conference on Multidrug Resistant *Mycobacterium tuberculosis*, Washington, D.C., 8 to 10 September 1993.)

## MATERIALS AND METHODS

In the present work the experimental model of Ji et al. (8) and Lecoeur et al. (9) was adopted, with minor modifications.

**Animals.** CD1 albino mice (females; ages, 28 days) were purchased from Charles River Italia.

**Drugs.** Rifabutin (batch 1019I155; Farmitalia Carlo Erba) and isoniazid (batch 315655/11292; Fluka Chemika) were suspended in 0.05% agar (in distilled water). Solutions were prepared once weekly and were kept at 4°C.

**BCG vaccination.** Mice were inoculated intravenously with 0.5 ml of a suspension containing $4 \times 10^3$ CFU of BCG (Institut Pasteur, Paris, France).

**Infection.** The inoculum of *M. tuberculosis* H37Rv (the strain was kindly provided by B. Ji, Faculté de Médecine Pitié-Salpêtrière, Paris, France) in Dubos broth was prepared starting from a Lowenstein-Jensen slant, and the organism was incubated at 37°C in 5% $CO_2$ for 14 days. Twenty-eight days after BCG vaccination, all mice were infected by intravenous injection of 0.5 ml of the diluted Dubos broth culture containing $2.6 \times 10^4$ CFU of *M. tuberculosis*.

**Treatment.** Sixteen days after infection with *M. tuberculosis*, mice were randomly allocated to groups of eight mice each. Groups of eight mice each were used, even though only six animals were planned for sacrifice, in case there were some deaths during the experiment. Oral treatment by gavage with rifabutin at 10 mg/kg, isoniazid at 25 mg/kg, or a combination of the two drugs was scheduled as described in Table 1.

**Bacterial count.** After 8 and 12 weeks of treatment six of the eight mice in each group were sacrificed, and their spleens and lungs were removed aseptically and were homogenized in 2 ml of saline. Two 0.5-ml portions of the suspension were plated on Lowenstein-Jensen flasks prepared in our laboratory (50 ml of medium in 75-$cm^2$ tissue culture flasks; Corning) (Fig. 1). A portion of 0.5 ml was used for further dilutions (two replicates of 0.5 ml for each dilution were plated). The number of CFU was determined after 6 weeks of incubation at 37°C in 5% $CO_2$.

**Control of vaccination and infection.** Fourteen days after infection five control vaccinated and infected mice were sacrificed, and their spleens and lungs were removed, homogenized in saline as described above, and inoculated in plain Lowenstein-Jensen plates or Lowenstein-Jensen plates supplemented with cycloserine, which allows selective growth of BCG but not that of *M. tuberculosis*.

**MIC determination.** The antimycobacterial activities of rifabutin and isoniazid against *M. tuberculosis* were determined by the agar dilution method on Middlebrook 7H11 medium.

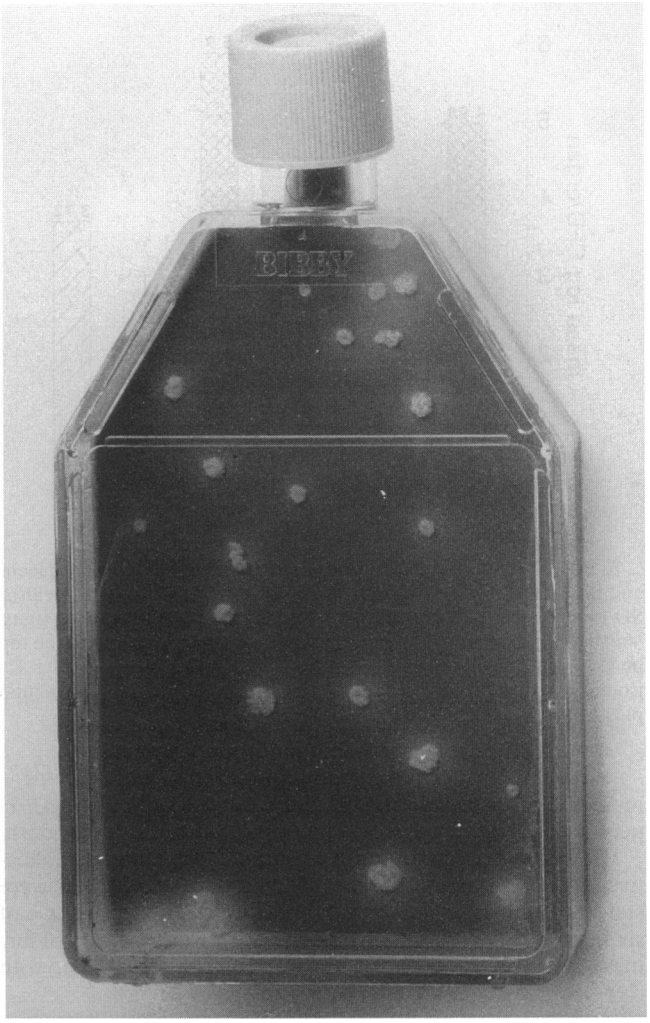

FIG. 1. Lowenstein-Jensen tissue culture flasks (75 $cm^2$) used for CFU determination.

Inocula were prepared in Middlebrook 7H9 medium and were diluted to an optical density corresponding to a 0.5 McFarland standard. MICs were recorded after 2 weeks of incubation at 37°C in 5% $CO_2$. MICs were also determined on colonies isolated from the spleens and lungs of mice in each experimental group; colonies were picked up and suspended in 0.5 ml of OADC supplemented Middlebrook 7H9 medium, and the suspensions were used for MIC determinations.

## RESULTS

**Control of vaccination and infection.** The mean ± standard deviation (SD) log CFU per spleen determined 14 days after infection were 2.6 ± 0.75 and 5.88 ± 0.28 for BCG and *M. tuberculosis*, respectively. These data are in agreement with those reported in the literature (8, 9); 14 days after infection the number of BCG accounted for less than 1% of the total mycobacteria, and therefore, the results of CFU determinations on plain Lowenstein-Jensen slants essentially represent the number of *M. tuberculosis* organisms. The immunity evoked by BCG vaccination was capable of controlling bacterial multiplication; in fact at the time of sacrifice the number of viable mycobacteria in the spleens of untreated controls re-

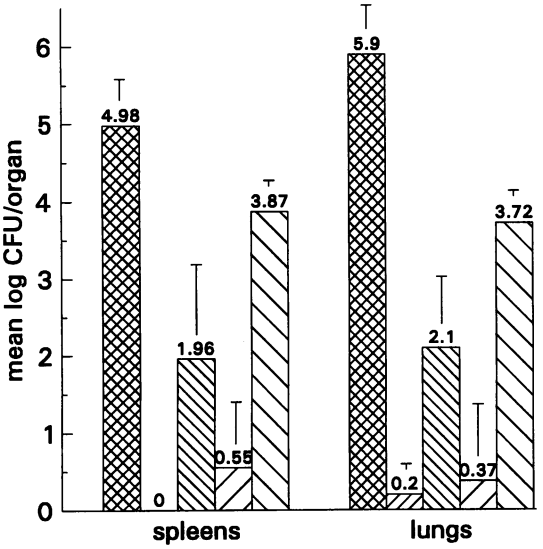


FIG. 2. Numbers of *M. tuberculosis* organisms isolated from spleens and lungs after 8 weeks of treatment. Values are expressed as mean ± SD log CFU per organ. A O CFU count was taken as O in log units. Values are means ± SDs for six animals per group. The bars at the top of the columns indicate SDs. ▩, control; ▨, rifabutin daily; ▧, isoniazid daily; ▨, rifabutin plus isoniazid twice weekly; ▧, rifabutin plus isoniazid once weekly.

mained almost unchanged (4.98 ± 0.49 and 4.73 ± 0.61 CFU per spleen after 8 and 12 weeks, respectively) (Fig. 2 and 3).

**Effectiveness of therapy.** Rifabutin was already highly effective after 8 weeks of treatment (Fig. 2). All spleens were sterilized, and a total of only eight colonies was found in two of six lungs. No bacilli were found in the spleens or lungs of any mice after 12 weeks of treatment (Fig. 3). Treatment with

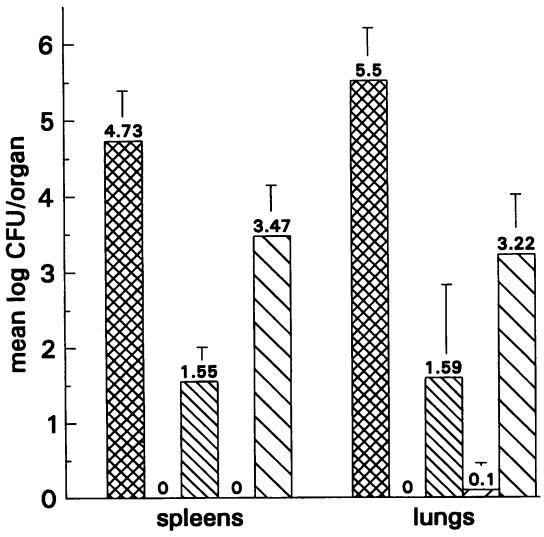


FIG. 3. Number of *M. tuberculosis* organisms isolated from spleens and lungs after 12 weeks of treatment. Values are expressed as mean log CFU per organ. A O CFU count was taken as O in log units. Values are means ± SDs for six animals per group. The bars at the top of the columns indicate SDs. ▩, control; ▨, rifabutin daily; ▧, isoniazid daily; ▨, rifabutin plus isoniazid twice weekly; ▧, rifabutin plus isoniazid once weekly.

TABLE 2. Susceptibilities of single colonies isolated from spleens and lungs of mice treated with rifabutin, isoniazid, or a combination of the two drugs

| Isolate source | MIC (μg/ml) of | |
|---|---|---|
| | Rifabutin | Isoniazid |
| Untreated control[a] | 0.01 | 0.125 |
| Rifabutin, 8 wk | 0.0037 | 0.062 |
| | 0.0037 | 0.125 |
| | 0.0037 | 0.062 |
| Isoniazid, 8 wk | 0.0037 | 0.125 |
| | 0.0037 | 0.125 |
| | 0.0037 | 0.125 |
| | 0.0037 | 0.125 |
| | 0.0037 | 0.125 |
| | 0.0037 | 0.125 |
| | 0.0037 | 0.125 |
| | 0.0037 | >2 |
| | 0.0075 | 0.125 |
| Isoniazid, 12 wk | 0.031 | >2 |
| | 0.031 | 0.125 |
| Rifabutin + isoniazid (2 to 8 wk) | 0.0075 | 0.5 |
| | 0.0037 | 0.125 |
| | 0.0075 | 0.125 |
| | 0.0037 | 0.25 |
| | 0.0075 | 0.25 |
| Rifabutin + isoniazid (1 to 8 wk) | 0.0037 | 0.125 |
| | 0.0037 | 0.125 |
| | 0.015 | 0.125 |
| | 0.031 | 0.125 |

[a] Values for untreated controls are expressed as geometric mean MICs for nine isolates.

isoniazid alone for 8 weeks reduced the number of bacilli by 3 log units in both the spleens and the lungs in comparison with the numbers in untreated controls, the mean ± SD log CFU per spleen being 1.96 ± 1.23, and the mean ± SD log CFU per lung being 2.1 ± 0.94 (Fig. 2). The continuation of isoniazid treatment up to the 12th week did not produce further improvement; the number of bacilli in the spleens and lungs was still approximately 3 log units lower than the numbers in the untreated controls (Fig. 3). The combination of rifabutin-isoniazid given at 2 days per week was almost as effective as rifabutin alone: after 8 weeks of treatment only two of six mice still harbored a small number of mycobacteria in their spleens and lungs (Fig. 2), while after 12 weeks of treatment all spleens were sterilized and a total of four colonies were present in the lungs of only two of six animals. When the frequency of administration was reduced to once a week, the therapeutic effectiveness was markedly reduced; the bacterial concentration was about 1 to 2 log units lower than those in untreated controls after 8 weeks of treatment, and suppression remained at the same level after an additional 3 weeks of treatment.

**MICs.** The MICs of rifabutin and isoniazid for *M. tuberculosis* H37Rv were 0.015 and 0.125 mg/liter, respectively. Colonies randomly isolated from the spleens and lungs of animals from different experimental groups were also tested for their susceptibilities to the two drugs, and the MICs are reported in Table 2. It is worth noting that two of the five colonies collected from rifabutin-treated mice and one colony from isoniazid-treated mice did not grow after suspension and distribution on the agar surface, probably because of a drug

carryover effect. The three surviving colonies from the rifabutin group maintained their susceptibilities to both drugs, as did all of the colonies from groups treated with the combination rifabutin-isoniazid. Among the 11 colonies randomly collected from the isoniazid group, 2 (18%) were resistant to isoniazid (MICs, greater than 2 mg/liter), but they were still susceptible to rifabutin.

## DISCUSSION

The experiment described here was primarily aimed at exploring further openings for short-term intermittent or daily single-agent chemoprophylaxis for tuberculosis with rifabutin.

A previous in vivo study conducted with a similar protocol and the same *M. tuberculosis* strain (8) showed that rifabutin and rifapentine had greater bactericidal effects than rifampin. The optimal dosage was set at 10 mg/kg/day for both rifabutin and rifapentine for 12 weeks; both schedules sterilized the spleens of infected mice. However, the significant bactericidal activity of rifabutin was retained when the drug was administered daily at 5 mg/kg/day or on alternate days at 10 mg/kg for 12 weeks. Moreover, culture results suggested that rifabutin at 10 mg/kg/day had significant activity as early as the sixth week of therapy, while its effect was comparable to that of rifampin administered at the same dosage for 12 weeks.

Therefore, the original protocol of Ji et al. (8) was modified as follows. (i) In addition to the use of untreated controls and rifabutin at 10 mg/kg/day, intermittent schedules of rifabutin at 10 mg/kg combined with isoniazid at 25 mg/kg either twice or once a week were included; daily isoniazid at 25 mg/kg was also included as a comparison, and (ii) an assessment was introduced at week 8 to seek evidence of early responses and to compare the results with those of the study by Lecoeur et al. (9). A further modification, involving a technical aspect of the protocol, was the introduction of 75-$cm^2$ flasks for Lowenstein-Jensen culture. Although the in-house preparation of the medium can be time-consuming, this procedure offers several advantages. First, it allows the distribution of each organ homogenate in only two flasks instead of 10 to 15 commercially available Lowenstein-Jensen tubes, as described in the original protocol (8). Moreover, the wider plating surface greatly facilitates sample distribution and colony counting. The use of tissue culture flasks streamlined and shortened the long and cumbersome procedure of plating, thus allowing an increase in the number of animals per experimental group and at the same time reducing the risk of error inherent to handling large numbers of tubes.

As expected, a 3-month course of isoniazid was inadequate for preventing tuberculosis. The drop in CFU counts on isoniazid observed in the present study is consistent with that reported previously (9). The most significant information obtained from the isoniazid arm of the study is the high rate of selection of resistant mutants resulting from the use of this drug alone. It is worth noting that 2 of the 11 colonies randomly selected were resistant to concentrations of isoniazid greater than 2 mg/liter, despite regular intake for only 12 weeks. The ease with which isoniazid prophylaxis encourages resistance is conceivably greater in the clinical setting because of the long period of possible irregular drug exposure because of patient nonadherence.

Consistent with earlier data (3b, 12a, 12b), the use of the single agent rifabutin did not lead to the selection of resistant *M. tuberculosis* mutants.

Infection of the lung, which was not evaluated in the previous study (8), proved more difficult to eradicate in comparison with infection of the spleen.

Rifabutin plus isoniazid once a week was the least effective schedule and compares with rifabutin alone at 10 mg/kg twice a week for 6 to 12 weeks and at 5 mg/kg/day for 6 weeks (8).

Rifabutin was highly effective as a single-agent daily treatment as early as week 8 and as early as week 12 when combined with isoniazid twice weekly. Evidence of the bactericidal activity of rifabutin has been obtained from in vitro studies in which the ratio between inhibiting and cidal concentrations (MIC:MBC ratio) was 1:4 for rifampicin-susceptible *M. tuberculosis* strains; the geometric mean MBC was 0.125 mg/ml for rifabutin, whereas it was 0.250 mg/ml for rifampin (6). Rifabutin appears to be more effective on stationary-phase than log-phase cultures in vitro (5). Its superior activity over rifampin, as shown by Ji et al. (8), should be attributed to its basic physicochemical characteristics which primarily control drug disposition and, ultimately, clinical use and response to treatment (10). In fact, rifabutin is characterized by high lipophilicity, tissue uptake, and tissue/plasma ratio and intracellular concentrations (9a, 12c, 13). The results of the present study substantiate the rapid bactericidal effect of rifabutin and support its use as either single-agent short-term (2 or 3 months) or intermittent combination (twice weekly for 3 months) preventive therapy for tuberculosis. With all of these regimens good compliance and the potential for tuberculosis control can be predicted. In particular, the choice of regimen might be tailored to the background epidemiological situation in the target group or area, e.g., a single short-term treatment in which the risk of subsequent reinfection is low or lifelong or repeat courses in areas with high levels of transmission. Complete clearance and lifelong treatment or repeat courses might be required in special patient groups, such as those also infected with human immunodeficiency virus, because the few surviving organisms may grow after the discontinuation of drug pressure (9).

## ACKNOWLEDGMENTS

We thank M. Raviglione and R. O'Brien, Tuberculosis Unit, World Health Organization, Geneva, Switzerland, for helpful advice, and B. Ji and J. Grosset for providing the *M. tuberculosis* strain and for kind assistance in setting up the study.

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