Activity of rifapentine and its metabolite 25-O-desacetylirifapentine compared with rifampicin and rifabutin against Mycobacterium tuberculosis, Mycobacterium africanum, Mycobacterium bovis and M. bovis BCG

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Introduction

Rifapentine (DL473), a new rifamycin derivative, has considerably better pharmacokinetic properties than the reference compound, rifampicin.1–3 After oral administration of a single 600 mg oral dose, rifapentine is absorbed slowly, reaching a peak concentration in plasma (Cmax) of about 15 mg/L within 4–5 h. Its terminal elimination half-life, 13–14 h, is much longer than that of rifampicin (2–3 h), allowing extended dosing intervals in HIV-infected patients and healthy subjects undergoing treatment for tuberculosis.2 After oral administration of a single dose of rifapentine 600 mg, its main active metabolite, 25-O-desacetylirifapentine, forms slowly with a Cmax of 4–6 mg/L; the half-life of this metabolite is comparable to that of its parent compound.1 Rifapentine is active against Mycobacterium tuberculosis4,5 and Mycobacterium leprae,6 and its efficacy has been underlined in experimental models of infection and in controlled clinical trials,7–9 but its in vitro activity against other members of the M. tuberculosis complex (Mycobacterium africanum, Mycobacterium bovis and M. bovis BCG) has not been reported. The anti-mycobacterial activity of 25-O-desacetylirifapentine has not yet been investigated either. Consequently, we have extended the observations on the in vitro activity of rifapentine and 25-O-desacetylirifapentine, compared with the reference compounds rifampicin and
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MIC determination using Middlebrook 7H11 agar medium

For this purpose, the 1% proportional method\textsuperscript{11} was used.\textsuperscript{11} Briefly, bacteria were scraped from Löwenstein–Jensen slants and thoroughly homogenized in sterile distilled water with 2 mm glass beads. The suspension was allowed to stand for about 2 min to remove aggregates of organisms; the upper portion of the suspension was carefully removed and the optical density at 650 nm was adjusted to 0.15. Bacterial suspensions were diluted stepwise up to 10\textsuperscript{5}-fold dilutions, and 1 mL of 10\textsuperscript{5}, 10\textsuperscript{4}- and 10\textsuperscript{3}-fold dilutions were plated on to 7H11 agar medium containing the desired concentrations of the drugs to be tested. The plates were incubated at 37°C and the resulting bacterial counts were determined after 21 days of incubation. The MIC was defined as the lowest drug concentration that inhibited \(>99\%\) of bacterial colonies as compared with the counts for control untreated bacteria.\textsuperscript{11}

Drugs

Rifapentine, 25-\(O\)-desacylrlifapentine and rifampicin were provided by Hoechst-Marion-Roussel, Romainville, France, and rifabutin was obtained from Pharmacia, Guyancourt, France. All stock solutions were initially prepared in dimethylsulphoxide (DMSO) and serially diluted in sterile distilled water before use. Equivalent amounts of DMSO did not inhibit the growth of control bacteria treated likewise.

Results

The MICs of rifapentine, 25-\(O\)-desacylrlifapentine, rifampicin and rifabutin against a drug-susceptible (type strain H37Rv) and a rifampicin-resistant clinical isolate (strain 920492) of \textit{M. tuberculosis}, as determined by the Bactec method, are shown in Figure 1. The results for the H37Rv type strain show that the activity of rifapentine (Figure 1a) was comparable to that of rifabutin (Figure 1b), whereas the activity of 25-\(O\)-desacylrlifapentine (Figure 1c) was comparable to that of the reference compound rifampicin (Figure 1d). The corresponding radiometric MICs were 0.063 mg/L for rifapentine and rifabutin, and 0.25 mg/L for the 25-desacyetyl metabolite and rifampicin (Table). None of the drugs tested was active against the rifampicin-resistant clinical isolate 920492: 25-\(O\)-desacylrlifapentine (Figure 1g) and rifampicin (Figure 1h) were not inhibitory even at concentrations as high as 32 mg/L, rifapentine resulted in only a slight inhibition of growth at concentrations of 16 and 32 mg/L (Figure 1e) and a MIC of 16 mg/L was obtained for rifabutin (Figure 1f).

The Table compares the MICs obtained by the radiometric method with those obtained by the 1% proportional method on 7H11 agar. The radiometric MICs of rifapentine and its metabolite for drug-susceptible \textit{M. tuberculosis

Materials and methods

Bacteria and growth

We studied 25 isolates of the \textit{M. tuberculosis} complex (see Table), 10 of which were \textit{M. tuberculosis} (seven rifampicin-susceptible isolates and three rifampicin-resistant isolates, as determined by the 1% proportional method using 7H11 agar with a critical rifampicin concentration of 1 mg/L), five \textit{M. africanum}, five \textit{M. bovis} and five \textit{M. bovis} BCG. All strains were from our own culture collection and were freshly cultured on Löwenstein–Jensen medium before the experiments.

Bactec MIC and MBC determination

Bacterial growth was monitored in a confined atmosphere using the Bactec 460-TB apparatus (Becton Dickinson, Sparks, MD, USA); this determines the ability of bacteria to catabolize \(^{14}\text{C}\)palmitic acid in 7H12 broth by measuring the \(^{14}\text{CO}_2\) released. The growth of the bacteria is represented as a numerical value called growth index (GI), which ranges from 1 to 999. The initial bacterial inoculum was standardized as reported previously for \textit{M. tuberculosis}.\textsuperscript{5,10,11} Strains were first grown in a Bactec vial to a GI of 500, then drug-containing vials were inoculated with 0.1 mL of cultures. The change in daily growth index (\(\Delta\text{GI}\)) of the drug-containing vials was compared with that in a control vial which was initially inoculated with 100 times fewer bacteria in the absence of drug (1/100 control). Under these conditions, the MIC was interpreted once the GI in the 1/100 control reached a value of \(\geq 30\) and was defined as the lowest drug concentration for which the \(\Delta\text{GI}\) was less in the drug-containing sample than in the control.\textsuperscript{5,10,11}

In addition to MICs, MBCs were also determined for selected strains using a method reported previously for \textit{M. tuberculosis}.\textsuperscript{10} The MBC was defined as the lowest concentration of drug that effectively reduced the bacterial viable counts in the drug-containing sample as compared with the initial inoculum by \(\geq 99\%\). For this purpose, the cfu/mL for each strain was determined (i) at the time of inoculation of Bactec vials (time 0) and (ii) at the end of the experiment, as follows: 0.1 mL of culture from the Bactec vials was removed and serial 10-fold dilutions in sterile double-distilled water were prepared, giving 10\textsuperscript{-1}, 10\textsuperscript{-2}, \ldots, and 10\textsuperscript{-10}-fold dilutions and 0.1 mL of each of these dilutions was plated on to 7H11 agar medium. The resulting bacteria were counted after 21 days of incubation at 37°C. This procedure avoided the possibility of accidental reduction in bacterial viability as a result of carryover of drugs to the solid medium.\textsuperscript{10,11}

rifabutin, to all the members of the \textit{M. tuberculosis} complex by (i) the 1% proportional method using Middlebrook 7H11 agar medium and (ii) the Bactec radiometric method using 7H12 broth.

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Rifapentine and its metabolite against mycobacteria

Isolates were in the range 0.03–0.06 mg/L and 0.125–0.25 mg/L, respectively, while the MICs obtained by the 7H11 agar method were about one or two dilutions higher (range 0.125–0.25 mg/L for rifapentine and 0.25–0.50 mg/L for its metabolite). The MICs of rifabutin were comparable to those of rifapentine and the MICs of rifampicin were comparable to those of 25-O-desacetylrifapentine (Table). Similar Bactec MICs for rifapentine and its metabolite were obtained for *M. africanum* (range 0.03–0.125 and 0.125–0.50 mg/L, respectively) and *M. bovis* (0.063–0.25 and 0.125–1.0 mg/L, respectively). As for *M. tuberculosis*, MICs determined using the 7H11 agar medium were generally one or two dilutions higher than those obtained using the Bactec broth, although for some isolates the MICs were identical irrespective of the method used (Table). The inhibitory activity of rifapentine was roughly comparable to that of rifabutin, while the activity of its 25-desacetyl metabolite was comparable to that of rifampicin.

It is interesting to note that the activity of all the rifamycins tested was highest against *M. bovis* BCG (MIC range 0.008–0.063 mg/L for rifapentine and 0.016–0.125 mg/L for its metabolite). For this species, MICs of rifapentine were either comparable to that of rifabutin or one dilution lower, and the MICs of 25-O-desacetylrifapentine were either comparable to that of rifampicin or one dilution lower (Table). MICs determined using the 1% proportional method on 7H11 agar medium were not always one or two dilutions higher for *M. bovis* BCG, unlike most other species (Table).

The three isolates of *M. tuberculosis* with a high degree of resistance to rifampicin (MICs >32.0–>64.0 mg/L) were also resistant to all the drugs tested, including rifapentine; however, the MICs of rifabutin in this case were lower than that of rifapentine (Figure 1, Table).

The bactericidal effect of the four rifamycins against the isolates tested is compared in Figure 2. The results obtained for fixed concentrations (0.125, 0.25 and 0.5 mg/L) of each drug against representative drug-sensitive isolates of *M. tuberculosis* (Figure 2a), *M. africanum* (Figure 2b) and *M. bovis* and *M. bovis* BCG (Figure 2c) show that the bactericidal activity of the 25-desacetyl metabolite was comparable to that of rifampicin, and that rifapentine used at the same concentration was significantly more bactericidal than rifampicin (it resulted in a 2-log higher killing at 0.5 mg/L). For a significant proportion of drug-sensitive isolates, rifapentine was also more bactericidal than rif-

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**Figure 1.** Typical Bactec results illustrating the radiometric inhibition of bacterial growth by varying concentrations of rifapentine (a and c), rifabutin (b and f), 25-O-desacetylrifapentine (c and g) and rifampicin (d and h). The results compare the inhibitory activity of the four compounds for a drug-susceptible strain of *M. tuberculosis* (type strain H37Rv; panels a–d), and a rifampicin-resistant clinical isolate (strain 920492; panels e–h). The radiometric curves in a–d are shown for drug concentrations of 0.031 (▼), 0.063 (○), 0.125 (□) and 0.25 mg/L (□), and in e–h for drug concentrations of 4 (●), 8 (○), 16 (▲) and 32 mg/L (△). Each plot represents the result of a single incubation. ×, Control; ●, 1/100 dilution of control.
butin, with a 1-log higher killing effect (Figure 2). In contrast, none of the rifamycins used showed remarkable bactericidal activity against rifampicin-resistant *M. tuberculosis* clinical isolates (Figure 2d). In conclusion, irrespective of the method used, the rifapentine MICs for all rifampicin-susceptible isolates of *M. tuberculosis* complex (*M. tuberculosis, M. africanum, M. bovis and M. bovis BCG*) were ≤0.5 mg/L, whereas resistance to rifamycins was always associated with much higher MICs (Table). We suggest, therefore, that a rifapentine breakpoint concentration of 1.0 mg/L is suitable for distinguishing drug-susceptible and drug-resistant clinical isolates by the Bactec radiometric method or the 1% proportional method using 7H11 agar. However, a better correlation of rifapentine and rifampicin MICs would require studies on a number of *M. tuberculosis* isolates with varying levels of rifampicin susceptibility.

**Discussion**

Rifampicin is equally active against actively multiplying and resting tubercle bacilli. One study has shown that,
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when used in a three-drug combination with isoniazid and streptomycin for 9 months, it is effective for almost all tuberculosis patients. These findings were the basis for the recommendation of the standard 6 month short-course chemotherapy, which now also includes pyrazinamide and ethambutol. When adequately prescribed and taken regularly, the standard short-course chemotherapy has been extremely effective in reducing the burden of tuberculosis. However, lack of compliance by those living in developing countries or in unfavourable socio-economic conditions in developed countries has resulted in the emergence of a significant proportion of multiple drug-resistant (MDR) tubercle bacilli that are simultaneously resistant to isoniazid and rifampicin. Consequently, the search for new antituberculosis drugs and for the new derivatives of existing drugs with improved pharmacokinetic and antibacterial properties has attracted considerable interest.

In this context, the recent approval by the United States Food and Drug Administration of rifapentine, a cyclopentyl-substituted rifamycin, has made this the first new antituberculosis drug to be licensed in 25 years. After the intensive first phase of the short-course chemotherapy, when rifapentine is administered twice weekly for 2 months, just one dose of rifapentine once a week is reportedly sufficient for the next 4 months instead of the present twice-weekly dose of rifampicin. This is expected to increase compliance, thereby decreasing the probability of the emergence of secondary resistance to rifamycins in general.

The MICs of rifapentine and its 25-desacetyl metabolite against drug-susceptible isolates of \textit{M. tuberculosis}, \textit{M. africanum}, \textit{M. bovis} and \textit{M. bovis} BCG (Table) are easily achievable in humans (the C\textsubscript{max} of rifapentine and 25-O-desacetylrifapentine are about 15 mg/L and 4–6 mg/L, respectively, after oral administration of a single 600 mg oral dose). At a concentration of 0.5 mg/L, rifapentine results in a 2–4 log reduction in initial bacterial inoculum, and its 25-desacetyl derivative can reduce the initial bacterial inoculum by 1–2 logs at the same concentration (Figure 2), so the achievable serum concentrations of these two compounds represent a C\textsubscript{max}/MBC ratio of about 10 for 25-O-desacetylrifapentine and of ≈30 for rifapentine.

We also compared the results obtained with the reference compound rifampicin and a more recent drug, rifabutin. Rifabutin, a spiro-piperidyl rifamycin S compound previously known as LM 427, shows considerably greater in vitro activity than rifampicin against both \textit{M. tuberculosis} and \textit{Mycobacterium avium}, and has recently been used successfully to treat patients with newly diagnosed pulmonary tuberculosis. Like rifapentine, rifabutin has a prolonged half-life. These two agents have comparable activity against \textit{M. tuberculosis} (reference 7 and results of this study). However, the achievable serum concentration of rifabutin, 0.34 mg/L, allows a considerably lower C\textsubscript{max}/MBC ratio (1–2) than for rifapentine (about 30) and 25-O-desacetylrifapentine (10) (see above). Consequently, the in vitro antituberculosis activity of rifapentine (reference 5 and 7 and this study), its pharmacokinetic properties and its recently described efficacy in treating tuberculosis in models of experimental infection and in human clinical trials alike argue in favour of its use as a first-line antituberculosis drug.

However, the emergence of acquired resistance to rifabutin during unsuccessful chemotherapy of a patient
with a rifampicin-containing regimen\textsuperscript{22} to rifamycins in patients treated with rifapentine\textsuperscript{23} and to rifampicin in patients receiving rifabutin prophylaxis\textsuperscript{24} calls for the utmost care and vigilance while using the newer rifamycins. Their higher activity can neither compensate for the emergence of acquired resistance in patients as a result of lack of compliance, nor avoid the emergence of cross-resistance to new generations of rifamycins in patients infected with resistant strains of tubercle bacilli that are unknowingly subjected to a standard short-course chemotherapy regimen for long periods because of a delayed diagnosis of drug resistance.

References


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