Impact of the interaction of R207910 with rifampin on the treatment of tuberculosis studied in the mouse model

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The animal experimentation guidelines of Johnson & Johnson were followed. This study was approved by the Johnson & Johnson ethical committee for the use of animals.
ABSTRACT

New drugs are needed to shorten the duration of tuberculosis treatment. R207910, a diarylquinoline, is very active against *Mycobacterium tuberculosis* both *in vitro* and in mice. In healthy volunteers, co-administration of rifampin induced increased metabolism of R207910 resulting in a 50% reduction of exposure. We assessed the impact of reducing the dose of R207910 on efficacy when R207910 was combined with a background regimen of isoniazid, rifampin and pyrazinamide. Addition of 25 mg/kg or 12.5 mg/kg R207910 to the background regimen resulted in faster bacterial clearance and culture negativity. The difference in efficacy between the two doses was not statistically significant. The minimal bactericidal dose of R207910 when tested as combination was identical to that when tested in monotherapy.

Because of the drug-drug interaction in humans, the activity of R207910 in humans could be less than what was expected from mouse studies. Our data in the mouse model demonstrate that R207910 has significant activity even when exposure is reduced by 50% and when added to a strong background regimen of isoniazid, rifampin and pyrazinamide. In killing kinetic studies, the bactericidal effect of R207910 in mice was modest during the first week of treatment, but it increased in the following three weeks, while the bactericidal activity of isoniazid was limited to the first week of treatment.
INTRODUCTION

New antituberculosis drugs that have the potential to shorten the current duration of therapy for both active and latent tuberculosis, and to improve the efficacy of treatment regimens for multidrug-resistant TB (MDR-TB) are urgently needed.

R207910 (TMC207 or J), a diarylquinoline, is a new compound exhibiting a new mode of action (inhibition of ATP synthase) against mycobacteria (1, 6). The compound is being assessed in a phase IIb trial for the treatment of active tuberculosis in MDR-TB patients. In vitro, R207910 is active against sensitive and resistant strains of *M. tuberculosis* (1, 2). In mice, it accelerates bacterial clearance when combined with regimens against both sensitive and MDR-Mycobacterium tuberculosis (1, 10), *Mycobacterium leprae* (4) and *Mycobacterium ulcerans* (5). In the established murine model of tuberculosis, monotherapy with R207910 is as active as the standard WHO regimen combining rifampin (RIF or R), isoniazid (INH or H) and pyrazinamide (PZA or Z), and when combined with RHZ or HZ or RZ, the lungs of mice became culture negative after just 2 months of treatment (1).

When combined to pyrazinamide, R207910 acts synergically in the murine model (3). In fact, the combination of R207910 and PZA lead to a complete eradication of the 7.2 log10 CFU, present in the lungs of mice at the onset of treatment, after only 2 months of treatment. Such level of efficacy needed 4 months of treatment with the triple-drug combination RIF-moxifloxacin-PZA (11).

In humans, an extended Early Bactericidal Assay (eEBA) was conducted in patients who were treated for seven days with R207910 given at
25 mg, 100 mg and 400 mg per day. Patients treated either with 600 mg of RIF or 300 mg per day of INH were used as controls. This study showed that R207910 given at 400 mg per day showed a significant decrease in CFU counts in the sputum of treated patients in comparison with the pre-treatment levels (13).

A drug-drug interaction study was conducted in 16 healthy volunteers who received a single dose of 300 mg of R207910 alone and 7 daily doses of 10 mg/kg of RIF. The AUC_{0-336h} of R207910 after co-administration of RIF was about half when dosed alone, indicating that the metabolism of R207910 is induced by co-administration of RIF (unpublished data).

In the current study we aimed to determine the efficacy of different doses of R207910 in combination with RHZ in order to mimic the decrease of exposure to R207910 when combined to RIF. In addition, we wanted to measure the efficacy of R207910 and INH after one week of treatment, in order to compare it with the early bactericidal effect observed in patients.

MATERIALS AND METHODS

Antimicrobial agents. The following compounds were provided by Johnson & Johnson (Beerse, Belgium): RIF, INH, PZA and R207910.

*Mycobacterium tuberculosis* strain. The drug sensitive H37Rv strain of *M. tuberculosis* was obtained from Pr. Jarlier (Paris, France) and grown on Löwenstein-Jensen medium. Colonies were subcultured in 7H9 broth + 10% OADC (Difco, le Pont de Claix, France) for 7 days at 37°C. The turbidity of the resulting suspension was adjusted with PBS to match that of a Mac Farland 2
suspension that is estimated to have $10^8$ CFU/ml of microorganisms and was used for mouse inoculation. The minimal inhibitory concentrations (MIC, in µg/ml) determined on 7H11 agar medium for the H37Rv strain were 0.25, 0.06 and 0.03 for RIF, INH and R207910, respectively. The MIC of PZA when determined on Löwenstein-Jensen medium at pH 5.5 was 10.

**Infection of mice.** In experiments I and II (drug-drug interaction and JZ in the established infection model studies), one hundred fifty and sixty two female 4-week-old outbred Swiss mice, were purchased from the Janvier Breeding Center (Le Genest Saint-Isle, France), and were inoculated in the tail vein with 0.2 ml of a bacterial suspension containing $3.5 \times 10^7$ and $3 \times 10^6$ colony forming units (CFU) of *M. tuberculosis* H37Rv, respectively.

**Chemotherapy.** In experiment I (drug-drug interaction study), following infection, mice were randomly allocated to two control groups and four test groups (Table 1), each consisting of 24 to 30 mice. The first group was a negative control group, in which mice were infected but left untreated. The second group was a positive control group in which mice were treated with the standard regimen for drug-susceptible tuberculosis, *i.e.*, 2 months of the combination RHZ (15). Mice of the third, fourth, fifth and sixth groups were treated with different dosages of R207910 (3, 6.25, 12.5 and 25 mg/kg) in combination with RHZ.

In experiment II (JZ in the established infection model), mice were randomly allocated following infection to two control groups and three test groups (Table 2), each consisting of 5 to 18 mice. The first group was a
negative control group, in which mice were infected but not treated. The second was a positive control group in which mice were treated with the first-line drug INH. Mice of the third, fourth and fifth groups were treated with either R207910 at 25 mg/kg alone or with the combination of R207910 compound used at 2 different dosages (2.5 and 25 mg/kg) with PZA.

In experiments I and II, treatment was initiated 2 weeks after infection in order to mimic a large bacterial population as observed in human cavities, and was administered five days a week.

In experiment I, to provide baseline values, 6 and 12 infected and untreated mice were sacrificed on days 1 and 14 after infection (D-13 and D0 in relation to the initiation of treatment). For all the RHZ and the RHZJ groups, sacrifices were carried out after 2, 4, 6 and 8 weeks of treatment. In experiment II, to provide baseline values, 8 infected and untreated mice were sacrificed on day 14 (D0) after infection and the treated mice were sacrificed on week 1 (W1), week 2 (W2) and week 4 (W4) post-infection.

In both experiments, solutions of R207910 and RIF were prepared in a 20% hydroxypropyl-β-cyclodextrin, those of PZA and INH were prepared in 10% hydroxypropyl-β-cyclodextrin and water, respectively, and were stored at 4 °C. All the drugs were given orally by gavage.

The drugs were administered in the following dosages: 10, 25 and 150 mg/kg/day for RIF, INH and PZA, respectively. Based on AUC, these dosages, which are similar to those used in previous experiments (1, 8, 9, 10, 14), were chosen as equipotent with the usual dosages administered to humans.
Assessment of infection and treatment. The severity of infection and the effectiveness of treatments were assessed by survival rate, spleen weight, gross lung lesion (scored from 0 to ++, the latter referring to a lung that was extensively occupied by tubercles), and numbers of colony forming-units (CFU) in the spleens.

In experiment I, at D-13, D0, 2 and 4 weeks of treatment, the number of CFU in the spleens were determined by plating six serial 10-fold dilutions of homogenized suspensions onto Löwenstein-Jensen plates. At 6 and 8 weeks of treatment, the entire suspension prepared from each individual spleen, expected to contain only a few bacilli, was plated without dilution on 12 Löwenstein-Jensen plates. In experiment II, at D0, W1 and W2 of treatment, the number of CFU in the spleens were determined by plating 6 serial 10-fold dilutions of homogenized suspensions onto Löwenstein-Jensen plates. At W4 post-infection, the entire suspension prepared from each individual spleen, expected to contain only a few bacilli, was plated without dilution on 12 Löwenstein-Jensen plates.

In both experiments, results of the cultures were recorded after incubation at 37°C for 4 weeks. The bactericidal effect of the treatment is defined as a significant decrease of the mean number of CFU in the treated group compared to pre-treatment value.

Statistical analysis. In experiments I and II, the Student t test was used to analyze the spleen weights and CFU counts data. Since multiple comparisons were made, the Bonferroni correction of the p value was used. Since 6 and 5
groups were compared the p values were adjusted to 0.0033 and 0.005, respectively.

RESULTS

Survival rate. In experiment I, all 12 infected and untreated mice died between day 14 and day 28 post-infection. The mortality rates in the treated groups were 4, 10, 3, 1 and 1 mouse in the RHZ, RHZJ3, RHZJ6.25, RHZJ12.5 and RHZJ25 groups, respectively. The high mortality rates observed were due to the high CFU counts achieved in mice at the time treatment was started (7.92 log10 CFU in the spleens). In experiment II, all untreated mice were dead by W2. Mortality of treated mice was limited and related to gavage accidents: two mice died in the R207910 at 25 mg/kg group and one in the ZJ2.5 treated group.

Spleen weight. In experiment I, the mean spleen weight was 172±20 mg one day post-infection. It increased significantly 2 weeks later to reach 437±94 mg (p=0.001). Two weeks of RHZ or RHZJ (J given at 3, 6.25, 12.5 and 25 mg/kg) were not able to reduce the increase of the mean spleen weight. Four, 6 and 8 weeks of treatment with RHZ or RHZJ (at all concentrations) were able to prevent further increases of spleen weight (p>0.0033). In experiment II, the mean spleen weight of infected and untreated mice increased significantly from 125±16 mg on day 1 post-infection to 441±145 mg on D14 (p=0.0001) at the time treatment was started. One, 2 and 4 weeks of treatment with either INH,
R207910 or the ZJ combinations were able to prevent further spleen weight increases but did not reduce them (p>0.005).

**Gross Lung lesions.** In experiment I, at two weeks post-infection, all 12 infected and untreated mice harbored severe (++) gross lung lesions. After two weeks of treatment, there was no change in gross lung lesions. The number of mice displaying severe lung lesions decreased after 4 weeks of treatment. After 6 and 8 weeks of treatment, almost all mice treated with different doses of R207910 in combination with RHZ displayed (+) moderate gross lung lesions and a few mice receiving at least 6.25 mg/kg of R207910 were free of lesions. In experiment II, gross lung lesions developed in the infected and untreated mice at 2 weeks post-infection (++ score). Treatment with INH, R207910 at 25 mg/kg, ZJ2.5 and ZJ25 for 1, 2 or 4 weeks did not improve the lung lesion scores.

**Killing kinetics for RHZ and RHZJ regimens.** Between day one and day fourteen post-infection, when the treatment was started, mean CFU counts in the spleens increased from $6.56\pm0.22$ to $7.92\pm0.23$ log10 CFU (p=0.0001) (Figure 1). Treatment with RHZ resulted in a potent bactericidal activity in the first two weeks (-3.0 log10 CFU), followed by a weaker bactericidal effect in the following 2-week intervals (-1.4, -1.5, and –0.8 log10 CFU, respectively). The addition of 3 or 6 mg/kg of R207910 to the RHZ regimen slightly improved its efficacy after 2 weeks of treatment (0.2 log10 CFU, p=0.36 and 0.8 log10 CFU, p=0.003, respectively), but no additional bactericidal activity was observed between week 2 and week 8 (p>0.0033). However, only one out of 5
mice became culture negative after 8 weeks of treatment with RHZ against 4 out of 6 mice treated with RHJZ6.25. Use of R207910 at 12.5 and 25 mg/kg resulted in very significant additional bactericidal activity, removing an additional 1.5 to 2.0 log₁₀ CFU (compared to RHZ) after just two weeks, and an additional 3.0 to 3.4 after 4 weeks of treatment (p<0.0033). The killing kinetics in subsequent weeks could not be assessed, as less than 0.5 log₁₀ CFU survived after the first 4 weeks of treatment with these combinations (p>0.0033). In the group combining 25 mg/kg of R207910 with RHZ, all mice became culture negative after just 6 weeks of treatment.

**Killing kinetics for INH, PZA, R207910 and PZA-R207910 combinations.**

INH killed 0.6 log₁₀ CFU during the first week (p=0.27 vs. untreated controls) and displayed no further killing during the second week of treatment (Figure 2). R207910 at 25 mg/kg had similar activity during the first week (0.6 log₁₀ CFU, p=0.028 vs. untreated controls) but, in contrast to INH, displayed accelerated killing in the second week (1.3 log₁₀ CFU), and killing continued during the weeks 3 and 4 (average 0.95 log₁₀ CFU per week, p=0.001 vs. untreated controls). Compared to R207910 25 mg/kg monotherapy, the combination with PZA (ZJ25) killed an extra 0.8 log₁₀ CFU after one week and 1.2 log₁₀ CFU after 4 weeks (p=0.0001). The combination of PZA with a 10-fold lower dose of R207190 (ZJ2.5) matched the activity of R207910 25 mg/kg monotherapy during the first two weeks, but did not kill any additional bacilli in the weeks 3 and 4.
DISCUSSION

A pharmacokinetic interaction with rifampin reduces the AUC of R207910 by 50% in humans (unpublished data). Because of this drug-drug interaction, the expected efficacy in humans may be less than that observed in mice. To estimate the effect of this interaction on efficacy in humans, we studied lower doses of R207910 in combination with RHZ in the mouse model. When dosed at 6.25, 12.5 and 25 mg/kg, R207910 was able to improve the activity of the RHZ regimen by decreasing the CFU counts in the spleens after 2, 4, 6 and 8 weeks of treatment and by increasing the proportion of mice having negative cultures after 8 weeks of treatment. The Minimal Bactericidal Dose (MBD, leading to at least 2 log10 CFU killing) when used in combination with RHZ was 12.5 mg/kg. This is in full agreement with the MBD of R207910 when used in monotherapy (1). That an identical MBD is obtained when R207910 is used in monotherapy and in combination with RHZ, suggests that a strong background regimen (RHZ) is not able to conceal the activity of even the lowest bactericidal dose of R207910. This is a surprising finding, as this was so far not observed with other new anti-TB drugs. When combined with RHZ during 8 weeks, PA-824 when used at its MBD of 100 mg/kg was not able to increase the bactericidal activity of the RHZ regimen (12). When moxifloxacin was given at 100 mg/kg 5 days a week for 8 weeks in combination with RHZ, the increase in bactericidal activity was modest (11). The addition of sparfloxacin to RHZ for 8 weeks did not improve the activity of RHZ (7).
We studied the speed and extent of killing of some individual drugs and combinations in a subsequent experiment (Figure 2). The combination of PZA and R207910 at 25 mg/kg killed about 1 log10 CFU more compared to R207910 alone. The combination of PZA with R207910 at 2.5 mg/kg, a dose well below the MBD, was as active as R207910 at 25 mg/kg during the first two weeks of treatment. The synergy between R207910 and PZA was able to compensate for a 10-fold decrease in the R207910 dose in that time frame (Figure 2). It is not clear why this ZJ2.5 combination did not succeed in further killing in the third and fourth treatment week. The addition of RH to the ZJ25 group (RHZJ25 in Figure 1 compared to ZJ25 in Figure 2) resulted in accelerated killing, achieving culture negativity in all animals after just 6 weeks of treatment. The contribution of ZJ to the efficacy of this RHZJ combination appeared at least as important as the contribution of RH.

Some interesting observations could be made regarding killing kinetics. First, the efficacy of R207910 monotherapy during the first week was not impressive (Figure 2, 0.65 log10 CFU, p=0.0028 vs. untreated controls), although matching that of INH (Figure 2, 0.59 log10 CFU, p=0.9 vs. R207910). In the first early bactericidal activity trial performed in TB-patients, the 400 mg dose of R207910 killed 0.77 log10 CFU in the first week of treatment against 1.88 log10 CFU for INH (13). That INH had greater efficacy in the one week human study compared to the mouse study may be explained by the presence of more rapidly replicating bacilli in the human cavities. Interestingly, INH did not kill any additional bacilli during the second week in the mouse study, while killing by R207910 accelerated in the second week (1.2 log10 CFU), and continued in the last two weeks (0.8 log10 CFU).
average per week). The ZJ25 combination killed 1.4 log10 CFU in both the first and second week, slowing down to 0.95 log10 CFU per week in the subsequent two weeks. The RHZ combination killed 3.0 log10 CFU in the first 2 weeks, and 1.4, 1.5 and 0.8 log10 CFU in the subsequent two week intervals. The addition of 25 mg/kg R207910 to that regimen killed an extra 2.0 log10 CFU in the first two weeks, and an extra 3.4 log10 CFU in the next two weeks, or 7.8 log10 CFU in total, with four out of six animals reaching culture negativity after just 4 weeks of treatment. The combination of potent drugs apparently compensated for the decreased killing speed over time of individual drugs.

In conclusion, our study suggests that the addition of R207910 to the standard regimen RHZ remains an interesting option to study in clinical trials, despite the observed drug-drug interactions between rifampin and R207910. As a rapid rate of kill may or may not translate into a durable effect in patients, relapse rates obtained with reduced doses of R207910 should be studied in the mouse model.
REFERENCES


TABLE 1: Experimental design for drug-drug interactions study (Experiment I).

<table>
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<th>Treatment groups (mg/kg)</th>
<th>No. of mice</th>
<th>Dates of sacrifices</th>
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<td>D-13</td>
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<tr>
<td>Control</td>
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<td>6</td>
</tr>
<tr>
<td>RHZ</td>
<td>24</td>
<td>-</td>
</tr>
<tr>
<td>RHZJ3</td>
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<td>-</td>
</tr>
<tr>
<td>RHZJ6.25</td>
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<td>-</td>
</tr>
<tr>
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<td>-</td>
</tr>
<tr>
<td>RHZJ25</td>
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</tr>
<tr>
<td>Total number of mice</td>
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<td>6</td>
</tr>
</tbody>
</table>

Dosages:
- Isoniazid (H): 25 mg/kg, Rifampin (R): 10 mg/kg, Pyrazinamide (Z): 150 mg/kg
- R207910(J): 3, 6.25, 12.5 or 25 mg/kg
TABLE 2. Early bactericidal activity of R207910 (J) and other antituberculosis drugs against *Mycobacterium tuberculosis* infection in the established infection mouse model (Experiment II).

<table>
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<tr>
<th>Treatment groups (5 days/week)</th>
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<tr>
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<tr>
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<tr>
<td>J25</td>
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<td>ZJ2.5</td>
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<tr>
<td>Total number of mice</td>
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<td>8</td>
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</table>
Fig. 1: Bactericidal activity of daily (5/7) RHZJ combinations in comparison with standard daily therapy with RHZ in *M. tuberculosis* infected mice. Error bars represent the standard error of the mean.
Fig. 2: Bactericidal activity of daily (5/7) JZ combinations in comparison with isoniazid in *M. tuberculosis* infected mice. Error bars represent the standard error of the mean.