Possible Implications of Doxycycline-Rifampin Interaction for Treatment of Brucellosis

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We studied the possible interaction between rifampin and doxycycline in 20 patients with brucellosis treated randomly with either doxycycline and streptomycin or doxycycline and rifampin. The doxycycline levels in the plasma of patients in the group treated with rifampin were significantly lower than those in the plasma of patients treated with doxycycline and streptomycin. Furthermore, clearance in patients treated with rifampin was significantly higher than that in patients treated with doxycycline and streptomycin, and consequently, the elimination half-life and the area under the concentration-time curve were significantly lower. There was no therapeutic failure or relapse in the group treated with doxycycline and streptomycin, whereas 2 of 10 patients in the group treated with doxycycline and rifampin had a therapeutic failure or relapse. The plasma doxycycline levels had an inverse correlation with plasma rifampin levels. In the group treated with rifampin, those who were rapid acetylaters had lower levels of doxycycline. In conclusion, combined treatment with rifampin reduces the levels of doxycycline in plasma. These data suggest that therapeutic failures or relapses may result from this interaction.

Brucellosis is still a serious public health problem in many parts of the world (43). Although the mortality rate as a result of this infection is low (12), it causes frequent complications, particularly those affecting the locomotor system, and these complications sometimes cause serious functional sequelae (3, 15).

The organisms of the genus Brucella are known to have a high degree of "in vitro" susceptibility to a number of antimicrobial agents (11, 36). However, their location, which is predominantly intracellular, protects them from the actions of many antibiotics. This explains to a great extent the tendency of this organism to cause illness with a long evolution and frequent relapses (41).

For several decades the treatment of choice for brucellosis was based on the association of tetracyclines and streptomycin (13, 29). Several studies carried out in the beginning of the 1980s showed that rifampin had excellent antibrugellar activity. This fact, together with its good intracellular penetration and ease of administration, made it a drug of great interest for the treatment of brucellosis (7, 16, 33).

Studies with animal models and humans showed that rifampin monotherapy for brucellosis resulted in a high number of therapeutic failures and relapses (20, 28). However, when it was used in combination with doxycycline there was a clear synergism and a good therapeutic efficacy (6).

In 1986, the Expert Committee on Brucellosis of the Food and Agriculture Organization-World Health Organization recommended the combination of doxycycline-rifampin as the treatment of choice for human brucellosis (24). Later studies showed that the combination doxycycline-rifampin was less effective in practice than the classical treatment of tetracycline and streptomycin (1, 14).

Although the doxycycline elimination process is not totally known, some data suggest that the liver may play an important part (10). Given that rifampin is metabolized through deacylation, that those data that do exist suggest that drug levels are lower in the plasma of patients who are slow acetylators, and that it has a potent enzymatic inductor effect (4), we designed the study described here to determine whether combination therapy with rifampin lowers plasma doxycycline levels and whether this circumstance is related to the acetylator genotype of the patient.

MATERIALS AND METHODS

Patient population. The present study was carried out with 19 patients with brucellosis in the Unit of Infectious Diseases of Málaga Regional Hospital. A diagnosis of brucellosis was made by the isolation of the organism or the presence of compatible clinical features together with the demonstration of specific antibodies at significant titers. Significant titers were considered to be ≥1/160 for seroagglutination and ≥1/320 by the anti-Brucella Coombs test. Blood cultures and serological tests were done by previously described methods (15).

Once the diagnosis was made, the patients were randomized into one of two therapeutic groups. Patients in group A were treated with doxycycline at 100 mg orally every 12 h for 6 weeks and streptomycin (sulfate) at 1 g intramuscularly daily for the first 3 weeks. Patients assigned to group B received the same dose of doxycycline plus rifampin: 600 mg/day orally for those who weighed between 50 and 60 kg, 900 mg/day for those who weighed between 61 and 80 kg, and 1,200 mg/day for those who weighed more than 80 kg. The single doses were given in the morning for 6 weeks. In patients with spondylitis, both schedules were maintained for 3 months.

Initially, the sample consisted of 19 patients: 9 were in group A and 10 were in group B. One patient assigned to group B was later treated with the schedule for patients in group A owing to a relapse a month after the end of the first treatment. The study sample was therefore composed of 20 cases, 10 in each therapeutic group.

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TABLE 1. Clinical characteristics of patientsa

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (no. male/no. female)</td>
<td>7/3</td>
<td>6/4</td>
</tr>
<tr>
<td>Age (yr)b</td>
<td>35.3 ± 18.8</td>
<td>32.1 ± 12.1</td>
</tr>
<tr>
<td>Wt (kg)b</td>
<td>70.7 ± 16.7</td>
<td>74 ± 11.4</td>
</tr>
<tr>
<td>Duration of symptoms (days)b</td>
<td>67.5 ± 73.2</td>
<td>53.7 ± 35.8</td>
</tr>
<tr>
<td>Blood culture positive (%)</td>
<td>67</td>
<td>67</td>
</tr>
<tr>
<td>Focal forms (%)</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>

a None of the differences between patients in groups A and B was significant.
b Values are means ± standard deviations.

All of the patients included in the study had normal hepatic and renal functions, and none of them received any drugs or took any alcohol during the treatment period.

From days 7 to 10 after the start of treatment, once the equilibrium phase of doxycycline had been reached, the patients were admitted to the hospital 12 h prior to the commencement of the study and remained in the hospital until the conclusion of the pharmacological part of the study.

Doxycycline and rifampin levels were measured in plasma obtained from whole-blood samples withdrawn just before (point 0) and 3, 6, 9, and 12 h after the administration of doxycycline and rifampin or doxycycline and streptomycin. They were analyzed by high-pressure liquid chromatography with Perkin-Elmer equipment consisting of a binary pump (LC-250), a manual sample injector with bypass and buffer volume of 20 µl, a diode array detector (LC-235, operated at 0.005 AV and a wavelength of 340 nm), an analytic column (Lichrospher 100RP-18; 5 µm, 125 by 4 mm [inner diameter]). A solution of 0.1 M citric acid-acetonitrile (20:80; vol/vol) at a flow rate of 1 ml/min was used as a mobile phase for doxycycline, and for rifampin a solution of acetonitrile-acetate buffer (0.1 M sodium acetate with 1% triethanolamine adjusted to pH 4 with acetic acid [55:45; vol/vol]) was used at a flow rate of 1 ml/min.

The doxycycline and rifampin standards were kindly provided by Pfizer Corporation (Brussels, Belgium) and Marion Merrell Dow (Madrid, Spain). As an internal standard, papaverine chloride was used. The other solvents and reactive agents used were of analytical grade.

The stock standard solutions were prepared by dissolving doxycycline HCl (1 mg/ml) and rifampin (2 mg/ml) in methanol. Papaverine chloride was dissolved in acetonitrile-water (90:10; vol/vol) at a concentration of 2 mg/ml. These solutions were stable for a period of at least 30 days at 20°C. By using the stock solutions and through dissolution in icteric human plasma, the working standards of doxycycline and rifampin were prepared at concentrations of 2.5 and 10 µg/ml, respectively. The internal standard of papaverine was prepared by dissolving the stock solution in acetonitrile at a concentration of 20 µg/ml.

Both methods were found to be precise. The calibration graphs for rifampin and doxycycline were linear over concentration ranges of between 0.5 and 25 µg/ml and 0.2 and 5 µg/ml, respectively.

The intra- and interassay coefficients of variation for doxycycline concentrations of 0.2 and 5 µg/ml were 4.1 and 2.1% and 6.2 and 3.5%, respectively. For rifampin the intra- and interassay coefficients of variation for concentrations of 2.0 and 25 µg/ml were 4.2 and 1.4% and 5.0 and 1.8%, respectively.

The processing of the samples involved several steps. The combination of 0.5 ml of problem serum with 0.5 ml of the papaverine solution was mechanically agitated for 7 min and was centrifuged at 9,500 rpm for 10 min. After separation of the supernatant fraction, 20 µl was injected directly into the chromatograph. Each sample was analyzed three times.

Assay performance. The pharmacokinetic parameters elimination half-life (t1/2b), total body clearance (CL), and area under the concentration-time curve (AUC) were analyzed by an open two-compartment model. Since we had access to only limited information about the absorption phase, in the present study we used the absorption constant values referred to in the studies of Salvin and Honin (37) and considered the bioavailability of doxycycline by the oral route to be 90%.

Acetylator genotype evaluation. For evaluation of the acetylator genotype, 20 ml of venous blood anticoagulated with EDTA was obtained and immediately stored at −80°C until it was processed.

The acetylator genotype was evaluated by PCR-based allelespecific amplification as described previously (9). Three common mutations at the NAT-2 gene locus were studied, namely, a C residue at position 481 (481C) to 481T, 590G to 590A, and 857G to 857A. All of these mutations lead to the slow acetylator phenotype. The subjects homozygous or heterozygous for the wild-type allele were classified as rapid acetylators, whereas the subjects homozygous for mutant alleles were classified as slow acetylators.

Once the treatment was concluded the patients were followed clinically and bacteriologically monthly for at least 6 months. Therapeutic failure was considered to be the persistence of symptoms after the third week of treatment, and relapse was considered to be the reappearance of symptoms once the treatment had finished.

Statistical analysis. Statistical analysis of the results was performed by using the Statistical Package for Social Sciences. To assess the difference between mean values the Student t test and the Wilcoxon test were used. The Pearson correlation coefficient was used to study the relation between variables. A P value of less than 0.05 was considered significant.

RESULTS

Of the total number of patients included in the study, 13 (65%) were men and 7 (35%) were women. The mean age was 33.3 ± 15.6 years (r = 17 to 69).

The duration of the symptoms was 60.2 ± 55.7 days. Three or more blood samples from 18 patients were cultured, with a positive result for 12 (66.6%) patients. The isolated organism was Brucella melitensis biovar 1 for all 12 patients.

TABLE 2. Levels of doxycycline in plasma

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Group A</th>
<th>Group B</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD doxycycline level (µg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.72 ± 1.57</td>
<td>1.05 ± 0.67</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>6</td>
<td>3.97 ± 1.91</td>
<td>2.73 ± 1.29</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>9</td>
<td>3.58 ± 1.47</td>
<td>2.73 ± 1.29</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>12</td>
<td>2.78 ± 1.03</td>
<td>1.35 ± 0.43</td>
<td>P &lt; 0.0005</td>
</tr>
</tbody>
</table>

a NS, not significant.

TABLE 3. Pharmacokinetic parameters for doxycycline

<table>
<thead>
<tr>
<th>Group</th>
<th>t1/2b (h)</th>
<th>CL (liters/h)</th>
<th>AUC (µg · h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10.59 ± 4.71</td>
<td>1.55 ± 0.73</td>
<td>72.6 ± 35.3</td>
</tr>
<tr>
<td>B</td>
<td>4.32 ± 2.26</td>
<td>3.59 ± 1.79</td>
<td>30.4 ± 12.3</td>
</tr>
</tbody>
</table>

a P was < 0.005 for all comparisons. Values are means ± standard deviations.
Eight patients (40%) presented with a focal form of disease. Four cases of epididymitis, two cases of spondylitis, one case of sacroiliitis, and one breast abscess were detected.

Both groups of patients were homogeneous for the more relevant clinical parameters (Table 1).

The levels of doxycycline at the baseline (time zero) and 6, 9, and 12 h after ingestion of the drug were significantly lower in group B patients (Table 2). Furthermore, both $t_{1/2}$ and AUC were significantly lower in group B patients. Conversely, CL was increased significantly in patients treated with doxycycline plus rifampin (Table 5).

The levels of rifampin in the plasma of group B patients were 0.68 ± 0.41, 14.8 ± 6.00, 8.3 ± 2.9, 4.8 ± 2.2, and 2.2 ± 1.5 mg/liter at 0, 3, 6, 9 and 12 h, respectively, after ingestion of the drug.

We detected an inverse relation between the levels of doxycycline and rifampin which was statistically significant at 6 and 9 h ($r = -0.851$ and $-0.719; P = 0.005$ and $P = 0.02$, respectively) (Table 4).

Of the 19 patients studied, 6 (31.5%) were rapid acetylators: 2 (20%) were in group A (1 was homozygous and 1 was heterozygous) and 4 (40%) were in group B (2 were homozygous and 2 were heterozygous).

The levels of rifampin in plasma were greater in the rapid acetylators. These differences were statistically significant at 3 and 6 h (Table 5). Inversely, the levels of doxycycline were lower in the rapid acetylators treated with rifampin, although these differences had no statistical significance (Table 6).

Although only two patients in the group treated with the doxycycline-streptomycin schedule were rapid acetylators, our results appear to indicate that in this group there were no differences in doxycycline levels by acetylator genotype (data not shown).

Eighteen patients, 90% of the total, were cured with just one course of therapy. The other two patients, both in group B, presented with a relapse and a therapeutic failure, respectively. The first patient relapsed 1 month after the end of the treatment, with fever and low back pain caused by spondylitis which was cured after a new course of doxycycline plus rifampin for 3 months. The second patient had right orchiopididymitis that failed to respond to therapy. He was therefore changed to the therapeutic schedule for group A patients, and the symptomatology disappeared after 15 days, with a definitive cure. The levels of doxycycline in the plasma of this patient while receiving both therapeutic schedules are shown in Fig. 1.

Overall, all 10 group A patients were cured, whereas there were two failures among group B patients. There were no differences in the AUCs for the two therapeutic failure and relapse patients and the remainder of the group treated with rifampin.

**DISCUSSION**

For more than 40 years tetracyclines have been the basis of treatment for brucellosis (32, 35). Furthermore, the use of tetracyclines in monotherapeutic schedules is known to be less effective than the use of tetracyclines in association with a second antibacterial drug (17). Once it was established that the combination of tetracycline and streptomycin presented in vitro synergistic antibacterial activity and that it was highly effective, this schedule became the treatment of choice for brucellosis (23). However, the vestibular toxicity of streptomycin-

**TABLE 4. Relation between levels of rifampin and doxycycline**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Correlation coefficient (r)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>-0.143</td>
<td>NS*</td>
</tr>
<tr>
<td>3</td>
<td>-0.319</td>
<td>NS</td>
</tr>
<tr>
<td>6</td>
<td>-0.851</td>
<td>$P &lt; 0.005$</td>
</tr>
<tr>
<td>9</td>
<td>-0.719</td>
<td>$P &lt; 0.05$</td>
</tr>
<tr>
<td>12</td>
<td>-0.513</td>
<td>NS</td>
</tr>
</tbody>
</table>

* NS, not significant.

**TABLE 5. Plasma rifampin levels**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Plasma rifampin level (µg/ml) in*</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rapid acetylators</td>
<td>Slow acetylators</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.58 ± 010</td>
<td>0.74 ± 051</td>
</tr>
<tr>
<td>3</td>
<td>20.6 ± 2.37</td>
<td>11.9 ± 4.99</td>
</tr>
<tr>
<td>6</td>
<td>10.9 ± 1.88</td>
<td>7.04 ± 2.55</td>
</tr>
<tr>
<td>9</td>
<td>6.65 ± 2.62</td>
<td>3.85 ± 1.50</td>
</tr>
<tr>
<td>12</td>
<td>3.03 ± 2.43</td>
<td>1.77 ± 0.73</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviations.

* NS, not significant.

**TABLE 6. Levels of doxycycline in patients treated with rifampin**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Doxycycline level (µg/ml) in*</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rapid acetylators</td>
<td>Slow acetylators</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.95 ± 0.43</td>
<td>1.03 ± 0.75</td>
</tr>
<tr>
<td>3</td>
<td>2.35 ± 0.52</td>
<td>3.06 ± 1.55</td>
</tr>
<tr>
<td>6</td>
<td>1.42 ± 0.32</td>
<td>2.72 ± 1.20</td>
</tr>
<tr>
<td>9</td>
<td>1.24 ± 0.19</td>
<td>1.42 ± 0.35</td>
</tr>
<tr>
<td>12</td>
<td>0.65 ± 0.35</td>
<td>0.74 ± 0.41</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviations.

* NS, not significant.

**FIG. 1.** Doxycycline levels in the plasma of patient 12 during the doxycycline-rifampin schedule (■) and after a change to doxycycline-streptomycin (△)
cin and the need to administer it through the parenteral route have kept alive interest in finding an alternative treatment.

Rifampin has good antibrucellar activity and intracellular penetration. It acts synergistically with doxycycline, is easy to administer, and causes few side effects. These numerous reasons promoted its addition to doxycycline in the treatment of brucellosis (24).

Several studies showed, however, that despite its apparent advantages, the doxycycline-rifampin schedule seems to be somewhat less effective than the classical treatment (1, 2, 14, 40). A recently published meta-analysis of the efficacy of the combination of rifampin and doxycycline in the treatment of human brucellosis shows that with this combination there are significantly higher numbers of relapses than with the classical treatment of streptomycin and doxycycline (39).

Rifampin is a potent inducer of hepatic microsomes, which are able to accelerate the metabolism and to lower the levels of an array of drugs in plasma (5, 34, 42). On the other hand, even though the biotransformation of doxycycline is only partially known, there are data suggesting considerable hepatic metabolism (10). It is therefore reasonable to infer that its association with rifampin might accelerate the metabolism process and lower its levels in plasma.

In two previous studies, Bessard et al. (8) and Garraffo et al. (19) reported the decrease in the levels of doxycycline in plasma and the alteration of its pharmacokinetic parameters in two groups of five and seven patients treated with doxycycline and rifampin, respectively.

To date no other investigators have carried out a comparative study of the levels of doxycycline in the plasma of two groups of patients with brucellosis treated with doxycycline-streptomycin and doxycycline-rifampin to relate their efficacies to the levels of doxycycline in plasma.

The results of the present study coincide with those of Bessard et al. (8) and Garraffo et al. (19). The levels of doxycycline in the plasma of patients treated with rifampin decreased significantly compared with the levels in the plasma of those who received streptomycin. Likewise, $t_{1/2}$ and AUC were also reduced significantly, whereas the doxycycline CL was increased significantly.

Although the number of patients included in the study was small and it was not our intention to demonstrate differences in the therapeutic efficacies of the two schedules, we nevertheless once again observed that the number of therapeutic failures or relapses was greater in the group treated with the doxycycline-rifampin schedule.

This finding supports those of previous clinical trials, which showed a lower degree of efficacy of the doxycycline-rifampin schedule compared with that of the classical treatment (1, 2, 14, 39, 40).

Rifampin exerts its enzymatic inducer effect by causing a marked proliferation of the smooth endoplasmic reticulum and increasing the hepatic content of cytochrome P-450 (22). Some studies have suggested that this inducer effect is related to both the dose of the drug and the duration of treatment (31). Our results seem to support this hypothesis, since the levels of doxycycline in plasma had an inverse relationship to those of rifampin.

Although the elimination process is only partially known, a considerable proportion of doxycycline may be metabolized in the liver, since it does not accumulate in patients with renal failure (21).

In a previous study patients treated with equal doses of rifampin and doxycycline showed different doxycycline levels, which could apparently indicate interindividual differences in the sensitivity to the enzymatic inducer effect of rifampin (18).

These differences can also be related to the existence of slow metabolizers in the study population (30).

The decrease in the levels of doxycycline in the plasma of patients who are rapidly acetylated appears to be related to the higher levels of rifampin in this group. Currently, there are no data to explain the apparent relation between the rapid acetylator genotype and the increased levels of rifampin in plasma. This relation could be explained in part by an acetylation-deacetylation imbalance of this drug. This disequilibrium has been described in the metabolism of numerous chemical compounds, such as acrylamides (26, 27), 4-aminobiphenyl (25), and sulfadimethoxine (38).

In conclusion, the results of our study indicate that rifampin lowers the levels of doxycycline in the plasma in of patients receiving the combined treatment, and this is likely to result in lower therapeutic efficacy in patients with brucellosis. Finally, we suspect that the discrepancies in the therapeutic efficacies of the combination doxycycline-rifampin compared with that of the doxycycline-streptomycin schedule found in previous clinical trials could be explained by the different prevalences of patients with slow metabolizers and acetylator genotypes in the study populations.

ACKNOWLEDGMENTS

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