Correlation between Serum Doxycycline Concentrations and Serologic Evolution in Patients with Coxiella burnetii Endocarditis

J. M. Rolain, M. N. Mallet, and D. Raoult
Unité des Rickettsies, Centre National de Recherche Scientifique, Unité Mixte de Recherche 6020, Institut Fédératif de Recherche 48, Faculté de Médecine, Université de la Méditerranée, Marseille, France

The recommended treatment for Q fever endocarditis is a combination of doxycycline and hydroxychloroquine. We found a correlation between serum doxycycline concentrations and decreases in levels of phase 1 antibodies, in 24 patients with Q fever endocarditis. Patients who had a >2-fold decrease in levels of phase 1 antibodies had serum doxycycline concentrations higher than those of the other patients (mean ± SD, 5.29 ± 1.75 vs. 3.14 ± 1.40 μg/mL; P = .003). We recommend adjusting the posology of doxycycline to achieve a serum concentration of at least 5 μg/mL.

Coxiella burnetii is the etiological agent of Q fever, a worldwide zoonosis, and is a strictly intracellular bacterium that may cause either acute or chronic infection. C. burnetii is a potential agent of bioterrorism because it is resistant to heat and drying and can survive in the environment for months [1]. Acute Q fever is the primary infection and, in specific hosts, it may become chronic. The main clinical form of chronic Q fever is endocarditis, especially in patients with previous cardiac valve defects and/or with a cardiac valve prosthesis [2]. In fact, most patients with Q fever endocarditis will die of the disease if they do not receive antibiotic therapy.

Tetracyclines, especially doxycycline, remain the reference standard for treatment of acute Q fever, with fluoroquinolones as possible alternatives [2]. In vitro MICs for doxycycline against C. burnetii vary from 1 to 4 μg/mL [3, 4], and, in an embryonated egg model [5], 1 strain was reported to be resistant to tetracycline. Q fever endocarditis is one of the most problematic diseases to cure. It has been recommended that antibiotic combinations, such as doxycycline plus ofloxacin or doxycycline plus rifampin, be given for at least 3 years, but ~15% of patients experience relapse despite this prolonged antibiotic therapy [2, 6]. These results show that antibiotics, even when used in combination and for prolonged periods, do not cure most patients, because of the lack of bactericidal activity against intracellular C. burnetii [7]. C. burnetii multiplies in monocytes and macrophages, within a lysosome-fused acidic vacuole (pH ∼4.7), and bactericidal activity could be restored by alkalinization of the C. burnetii-containing vacuoles, with a lysosomotropic agent, such as chloroquine [7]. In vivo, this regimen was effective in patients with Q fever endocarditis [3], with <5% of patients experiencing relapse after 18 months of therapy [2, 8]. The dose of hydroxychloroquine is adjusted during treatment, whereas doxycycline is given to the patients in a standard regimen of 200 mg/day.

The current criteria for Q fever endocarditis recovery include the absence of clinical and echocardiographic abnormalities, an anti–phase 1 IgG titer <1:800, and an IgA titer <1:50 [3]. Nevertheless, there is no currently available clinical or biological criterion that allows the identification of patients who are at high risk of relapse or who received ineffective therapy during the course of the treatment. Because the MIC of doxycycline against C. burnetii and the achievable serum doxycycline concentration are predicted to be very close, we suspected that serum doxycycline concentration was critical in these patients and that posology had to be adjusted during treatment for some patients, to achieve an effective serum concentration.

Patients and methods. The diagnosis of chronic Q fever was established by serologic testing, using C. burnetii antigen phase 1 and phase 2, as described elsewhere [9], or by culture or polymerase chain reaction of biopsy samples. According to the Duke criteria [8], modified for C. burnetii, all patients had a definite diagnosis of Q fever endocarditis. Patients were treated with the combination of hydroxychloroquine (600 mg/day) and doxycycline (200 mg/day), as reported elsewhere [3]. Doses of hydroxychloroquine were adapted after evaluation of plasma concentrations, to maintain a mean ± SD concentration of ~1 ± 0.2 μg/mL [7]. All patients were monitored throughout 1 year of treatment, and, at each consultation, a physical examination, serologic testing, and measurement of serum doxycycline and hydroxychloroquine concentrations were performed. Two groups of patients were analyzed—those for whom the IgG and/or IgA phase 1 antibody titers had decreased for >2 dilutions (effective treatment) and those for
whom phase 1 antibody titers had decreased for ≤2 dilutions (ineffective treatment).

Plasma doxycycline concentrations were analyzed by use of high-performance liquid chromatography (HPLC). Serum samples were frozen for 1–12 months, and doxycycline concentrations were measured retrospectively, to investigate both the stability of the drug in frozen serum samples and the correlation between plasma and serum concentrations. All subsequent measurements of doxycycline were performed retrospectively, using frozen (−20°C) serum samples.

Measurement of doxycycline was performed after extraction from plasma or serum samples, as described elsewhere [10]. In brief, 1 mL of plasma or serum was mixed with 500 μL of an aqueous solution of ascorbic acid (6%), 100 μL of a solution of internal standard (5 μg/mL oxytetracycline), and 500 μL of 1 mol/L monosodium phosphate–sulfite buffer (pH 6.1). After gently mixing for 30 s, 3 mL of ethyl acetate was added for antibiotic extraction. The mixture was vortexed for 5 min and centrifuged at 2000 g for 10 min. Two milliliters of the upper layer (organic phase) was transferred to a conical tube containing 100 μL of ascorbic acid in methanol (0.2%). The solvent was evaporated, the residue was dissolved in 50 μL of mobile phase (i.e., bidistilled water-acetonitrile [73/27, vol/vol] adjusted to a pH of 2.5 with 1 N H3PO4), and the whole sample was passed onto the chromatograph column (flow rate, 1.0 mL/min). The HPLC system was a Merck L6200A (Merck SA) equipped with an L4250 UV detector set at 350 nm. The column was a Merck Lichrospher 100 RP18 (5 μm × 15 cm) preceded by a guard column filled with Merck Lichrospher 100 RP18 and end-capped (5-μm particle size). For each patient, each sample was analyzed in duplicate.

Hydroxychloroquine serum levels were measured as described elsewhere [3]. An indirect fluorescent antibody assay was used in this study, as described elsewhere [3].

Antibiotic concentrations are given as mean ± SD. Student’s t test was used for comparison of treatment efficacy and serum antibiotic concentrations.

Results. Sixteen men and 8 women were included in the study, from 2001 to 2002. Their mean age was 61.1 years (range, 35–82 years) for the men (n = 17) and 52.6 years (range, 26–65 years) for the women (n = 7). Thirteen patients had infected aortic valves, and 11 patients had infected mitral valves. For 12 patients, the infected valve was a prosthetic. All patients experienced photosensitivity of the hands during the treatment. During the 1-year study, all 24 patients were examined and monitored at least 4 times by one of us (D.R.). For the entire population of patients, the mean serum hydroxychloroquine concentration after 1 year of treatment was 1.5 ± 0.7 μg/mL (range, 0.8–2.6 μg/mL; n = 24 patients). Comparisons of doxycycline concentrations in 25 fresh plasma samples and 25 frozen serum samples (1–12 months after being frozen) showed no difference in mean concentrations and revealed a positive correlation with serum concentrations (ρ = 0.92). Thus, all experiments were performed with frozen serum samples. Overall, for the 24 treated patients, the mean serum doxycycline concentration after 3 months of treatment was 3.02 ± 1.89 μg/mL (range, 0.06–8.5 μg/mL; n = 24 patients), whereas, after 1 year of treatment at the same dosage, it was 4.75 ± 2.8 μg/mL (range, 0.06–12.92 μg/mL; n = 24 patients). Thus, serum doxycycline concentration was significantly increased after 1 year (P = .015, Student’s t test). The serum doxycycline concentrations after 3 months and 1 year of treatment were correlated with decreases in IgG and IgA phase 1 antibody titers. A statistical correlation (ρ = 0.61; P < .05) between decreases of phase 1 antibody titers and serum doxycycline concentrations was also found (figure 1). Patients who had a >2-fold decrease in IgG and IgA phase 1 antibody titers after 1 year of treatment had serum doxycycline concentrations of 5.29 ± 1.75 μg/mL (95% confidence interval [CI], 4.07–6.51 μg/mL; n = 8 patients), whereas those with a ≤2-fold decrease had serum doxycycline concentrations of 3.14 ± 1.40 μg/mL (95% CI, 2.46–3.82 μg/mL; n = 16 patients) (figure 2). This difference in serum concentrations was statistically significant (P = .003, Student’s t test). This difference was also significant after 3 months of treatment, with a serum concentration of 6.65 ± 3.46 μg/mL (95% CI, 4.25–9.05 μg/mL; n = 8 patients) for patients with a >2-fold decrease in C. burnetii phase 1 antibody titers, versus 3.80 ± 2.02 μg/mL (95% CI, 2.81–4.79 μg/mL; n = 16 patients) for patients with a ≤2-fold decrease (P = .017, Student’s t test).

Discussion. During treatment of Q fever endocarditis, monitoring of patients can be done easily by serologic testing, and, with effective therapy, follow-up should show a decrease in phase 1 antibody titers. In the present study, we measured the serum doxycycline concentration in 24 patients treated with a standard dose of doxycycline (200 mg/day orally) and hydroxychloroquine.

Figure 1. Correlation between serum doxycycline concentration and dilution decrease of phase 1 antibody (IgG and IgA), after 1 year of treatment with doxycycline (200 mg/day orally) and hydroxychloroquine.
Serum doxycycline concentration and dilution decrease of phase 1 antibody (IgG and IgA), after 1 year of treatment with doxycycline (200 mg/day orally) and hydroxychloroquine. The first group (≤2 dilutions) corresponds to patients for whom the no. of reciprocal dilutions decrease of phase 1 antibody titers was ≤2 dilutions, whereas the second group corresponds to patients for whom the no. of reciprocal dilutions was >2 dilutions.

Droxychloroquine. Our results were consistent and reproducible with either fresh plasma or frozen serum samples. Moreover, because the half-life of doxycycline is very long (14–24 h), each measurement was performed during the plateau, consistently reflecting the mean available serum concentration. Twelve months after therapy began, we noted that the serum concentration reflecting the mean available serum concentration. Twelve months after therapy began, we noted that the serum concentration of doxycycline varied from 0.06 to 12.92 µg/mL. This wide heterogeneity in doxycycline levels was also found in patients with small, asymptomatic, abdominal aortic aneurysms [11]. This marked interpatient variation is difficult to explain, although low serum doxycycline concentrations might occur with the use of some psychotropic drugs and/or alcohol. Moreover, because doxycycline was administered orally, it is also possible that, for some patients, certain foods or drugs, such as antacids, inhibited the bioavailability of doxycycline [12, 13]. Because doxycycline can cause various adverse effects, such as epigastric burning, nausea, vomiting, or hyperpigmentation of the skin after exposure to the sun, lack of compliance of patients may also explain the wide variation in serum doxycycline concentrations. Moreover, one may hypothesize that bioavailability of the drug differs from patient to patient because of weight or clearance of the drug and, thus, that dosage of doxycycline needs to be adjusted for each patient. In our experience, the same standard dose of doxycycline was given to all patients whether they weighed 50 or 110 kg. Interestingly, we found that the mean serum doxycycline concentration in our patients increased during long-term administration of drugs [14].

It is possible to speculate that patients with higher serum doxycycline concentrations were treated more efficiently. The positive correlation between serum doxycycline concentrations and decreases in C. burnetii phase 1 antibody titers found in this study is in accordance with this hypothesis. A decrease in phase 1 IgG antibody titers <1:800 remains the main predictive criterion of clinical cure [3]. In general, the antibody titers decrease slowly with treatment [3]. However, the kinetics of antibody titer decrease in patients treated with doxycycline may vary, suggesting that some patients should be treated for >18 months to be cured. Moreover, the differences found in the present study show that patients with serum doxycycline concentrations ≤5 µg/mL had a lessened decreases in antibody titers, suggesting that treatment was not sufficiently effective. One may hypothesize that higher doses of doxycycline would be more effective in the treatment of Q fever endocarditis. Forslin et al. [15] have demonstrated that, when the oral dose of doxycycline increases, the serum concentration increases accordingly.

It is interesting to note that MICs found for doxycycline against C. burnetii may vary from 1 to 4 µg/mL [3, 4], and, thus, failures or relapses in these patients may be explained by an inappropriate serum doxycycline concentration. Thus, treatment and monitoring of patients with Q fever endocarditis should include evaluation of the antibiotic susceptibility of the isolate, when available, and measurement of serum doxycycline concentrations. Because our data show that a difference in serum doxycycline concentration can be observed after 3 months, we recommend measuring the serum doxycycline concentration after 3 months of therapy and adjusting the dosage to achieve a serum concentration of 5 µg/mL. Considering the low and erratic levels produced by the usual 200-mg dose of doxycycline, it is possible that higher doses (400 mg) may be justified in the treatment of some cases of Q fever endocarditis, especially for patients who are infected with strains with higher MICs against doxycycline.

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References

5. Spicer AJ, Peacock MG, Williams JC. Effectiveness of several antibiotics in suppressing chick embryo lethality during experimental infections by Coxiella burnetii, Rickettsia typhi, and R. rickettsii. In: Burgdorfer


