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# Treatment of Human Brucellosis with Doxycycline and Gentamicin

JAVIER SOLERA,[1]†* ALFREDO ESPINOSA,[1]† ELISA MARTÍNEZ-ALFARO,[1]† LORENZO SÁNCHEZ,[2]† PALOMA GEIJO,[3]† ELENA NAVARRO,[1]† JULIO ESCRIBANO,[4]† AND JOSÉ ANTONIO FERNÁNDEZ[4]†

*Department of Medicine, Unit of Infectious Diseases, Albacete General Hospital, Albacete,[1] Department of Medicine, Guadalajara Hospital, Guadalajara,[2] Department of Medicine, Unit of Infectious Diseases, Cuenca Hospital, Cuenca,[3] and Instituto de Desarrollo Regional, Division of Biotechnology, University of Castilla La Mancha,[4] Spain*

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**The objective of the present prospective, noncomparative, multicenter study was to assess the safety and efficacy of gentamicin and doxycycline therapy for human brucellosis. In the first part of the study, a cohort of 17 patients received 100 mg of doxycycline (or 5 mg/kg of body weight per day if the body weight was <40 kg) orally every 12 h for 45 days (cohort 1). In the second part of the study a subsequent cohort of 35 patients was treated with doxycycline at the same dosage for 30 days (cohort 2). All patients were treated intramuscularly with gentamicin at 240 mg (or 5 mg/kg per day if the body weight was <50 kg) once daily for the first 7 days. Both cohorts showed a favorable response during therapy, and there were no therapeutic failures. Relapse was noted in 1 (5.9%; 95% confidence interval [95% CI], 0.15 to 28.7%) of the 17 patients in cohort 1 and in 8 (22.9%; 95% CI, 10.4 to 40.1%) of the 35 patients in cohort 2. Nineteen patients (36.5%; 95% CI, 23.6 to 51.0%) had adverse effects, with no differences between cohorts, and no patients had a treatment-limiting adverse effect. The study indicates that the combination of doxycycline for 45 days and gentamicin for 7 days is an effective and well-tolerated therapy for human brucellosis. The relapse rates obtained with doxycycline treatment for 30 days appear to be higher than those obtained with doxycycline treatment for 45 days.**

Brucellosis due to infection with *Brucella melitensis* remains a major cause of zoonosis in many parts of the world, and it is associated with a considerable morbidity (14, 32). The combination of tetracycline and streptomycin is effective in the treatment of human brucellosis; however, relapse remains a significant problem (1, 5–7, 10, 11, 13, 20, 21, 25, 26). Investigation of alternative treatments for human brucellosis has been justified primarily by the frequency of relapse, the potential toxic effects associated with the use of streptomycin and tetracycline, and the inconvenience of parenteral administration of streptomycin.

Several reports have indicated that gentamicin is more active in vitro than streptomycin against clinical isolates of *Brucella* (15, 16). Waitz and Weinstein (31) found 14 strains of *Brucella* that were inhibited by 5 μg of gentamicin per ml, and Qadri et al. (24) found 114 strains of *B. melitensis* sensitive to ≤0.5 μg/ml. Recent data from Peru suggest that 21% of clinical isolates of *B. melitensis* are resistant to streptomycin (MIC range, 0.125 to 16.0 μg/ml), whereas 0% are resistant to gentamicin (MIC range, 0.25 to 2.0 μg/ml) (8). These properties make gentamicin an attractive possible alternative to streptomycin in tetracycline-aminoglycoside regimens for the treatment of brucellosis. However, clinical experience with gentamicin in the treatment of brucellosis is limited, and only a few cases in which adults were treated with gentamicin have been reported (2, 9, 22, 29, 33). Lubani et al. (19) treated 1,100 children with brucellosis, and no relapses were seen in 89 children receiving combinations of gentamicin with doxycycline or oxytetracycline. Gentamicin was given twice daily (b.i.d.) intramuscularly for only 5 days (5 mg/kg of body weight per day) with doxycycline or oxytetracycline for 3, 5, and 8 weeks. A short course of a 3-week doxycycline and a 5-day gentamicin combination could therefore be sufficient to achieve an optimal clinical response in patients with brucellosis.

Since no proof of these good results has subsequently been reported, we conducted a prospective, noncomparative, multicenter investigation to assess the safety and efficacy of gentamicin and doxycycline in the treatment of adults with brucellosis and to determine the desirability of comparing this combination with standard therapy in larger studies in the future.

## MATERIALS AND METHODS

**Patients.** The inclusion and exclusion criteria, the methods used to make a microbiological diagnosis and the determine clinical toxicity, follow-up period, and definitions of response were almost identical to those in our previous studies (25–27). In brief, eligible patients had brucellosis as defined below and were older than 8 years of age. The diagnostic criteria were (i) isolation of a *Brucella* species from blood or other fluids or tissues or (ii) the finding of ≥1:160 titer of antibodies to *Brucella* by a standard tube agglutination method in association with compatible clinical findings (fever, sweats, arthralgias, hepatomegaly, splenomegaly, or signs of focal disease). Patient exclusion criteria were pregnancy or nursing, known or suspected hypersensitivity or other contraindication to tetracyclines or aminoglycosides, severe concomitant disease, and effective antimicrobial therapy within 7 days before entering the study. We also excluded one patient with central nervous system involvement, four patients with spondylitis, and one patient with femur osteomyelitis, since these patients received a much longer course of antibiotic therapy and a different follow-up. Patients could enter the study once only. The study was approved by the institutional review board at each center, and informed consent was obtained from all patients.

**Study design.** The study was a prospective, multicenter, open-label phase II clinical trial of two treatment regimens. Regimen 1 consisted of the administration of doxycycline (Retens; Wassermann, Barcelona, Spain) at a dosage of 100 mg (5 mg/kg per day if the body weight was <40 kg) twice a day orally for 45 days plus gentamicin (Gevramicin; Schering Plough, Madrid, Spain) at a dosage of 240 mg (5 mg/kg per day if the body weight was <50 kg) intramuscularly once daily throughout the first 7 days of treatment. Regimen 2 was the same except that the doxycycline was given for only 30 days. Patients in cohort 2, who received regimen 2, were enrolled immediately after those in cohort 1, who received regimen 1. Patients could not receive any other antibiotics, but they could be given analgesics, anti-inflammatory agents, antacids, or histamine $H_2$-receptor antagonists as required. Patients received the study drugs unless treatment-limiting toxicity was encountered, and they were categorized according to the end points, defined as described below.

* Corresponding author. Mailing address: Unidad de Enfermedades Infecciosas, Hospital General, C/ Hermanos Falcó S/N, 02006 Albacete, Spain. Phone: 34-67-597100. Fax: 34-67-597121.

† Investigator representing the Grupo de Estudio de Castilla-La Mancha de Enfermedades (GECMEI). Other members of the GECMEI who participated in this study are listed in the appendix.

**Definition of end points.** The main end points of interest were therapeutic failure due to lack of efficacy and relapse of brucellosis. Therapeutic failure due to lack of efficacy was defined by symptoms or signs of the disease that persisted at the end of treatment. A relapse of brucellosis was defined by the reappearance of symptoms or signs of the disease or new positive blood cultures during the 12 months after therapy. Safety was assessed on the basis of all reported adverse events and the results of laboratory tests and other investigations. Clinical adverse events were recorded and evaluated for severity, outcome, and relation to the study drugs.

**Clinical and laboratory assessment.** Patients were monitored for therapeutic efficacy and signs of drug toxicity by analyzing clinical data; complete blood counts (with differential and platelet counts) and erythrocyte sedimentation rates; urinalysis; measurements of the creatinine, aspartate aminotransferase, alkaline phosphatase, albumin, total protein, bilirubin, and electrolyte levels; *Brucella* serology; and blood culture. The patients were evaluated initially, on day 7, and at the end of therapy. During these visits, the subjects were asked whether they had missed any dosings. After ending the therapy, the patients were reassessed at months 1, 2, 3, 6, 9, and 12, as well as whenever clinical symptoms reappeared. Diagnosis of spondylitis, sacroiliitis, and hip arthritis was made by appropriate findings on physical examination and radiological, bone scintigraphy, or magnetic resonance studies. Cochlear and vestibular toxicities were assessed clinically. The concentrations of gentamicin in serum were monitored by assaying serum between day 5 and day 7 of treatment. Samples used to determine peak concentrations were taken 1 h after intramuscular injection, and those used to determine trough concentrations were taken immediately before the drug was administered. Assays of serum gentamicin concentrations were performed with the Abbott Therapeutic Drug Monitoring System (TDX; Abbott Cientifica, Madrid, Spain). Doxycycline concentrations were measured in serum obtained from whole-blood samples withdrawn just before and 1 h after administration. The extraction was performed by the method described by Leenheer and Nelis (18).

**Microbiological studies.** The standard tube agglutination test, the rose bengal test, and the anti-*Brucella* Coombs' test were done by standard methods (4) with commercial reagents (Knickerbocker, Barcelona, Spain). Blood cultures were performed as reported previously (25), and the cultures were incubated for 6 weeks by using the BACTEC NR-730 system (Becton Dickinson-Spain, Madrid, Spain). All isolates were identified as recommended by Hausler et al. (17). Twenty-two of the isolated strains were sent to a reference center (Laboratorio Regional de Brucelosis, Valladolid, Spain) for confirmation and biotyping. All isolated *Brucella* strains were identified as *Brucella melitensis.*

**Statistical analysis.** Values of the continuous variables are expressed as means ± 1 standard deviation (SD) or as medians and ranges. Confidence intervals for response and relapse were calculated by using the normal approximation to the binomial by Epi Info, version 6 (12). The chi-square test, Fisher's exact test, the *t* test, and the Wilcoxon-Mann-Whitney rank-sum test were used as appropriate (3). *P* values of less than 0.05 by two-sided tests were considered to indicate statistical significance.

## RESULTS

**Study patients.** Between November 1994 and December 1995 we enrolled 52 patients at the three hospitals participating in the study. Cohort 1 consisted of 17 patients who received doxycycline for 45 days, and cohort 2 consisted of 35 patients who received doxycycline for 30 days, both combined with gentamicin during the first 7 days. There were no significant differences between the two cohorts in any of the characteristics listed in Table 1. The median follow-up was 12 months (range, 3 to 18 months). Forty-eight patients (92%) completed at least a 6-month follow-up period. Six patients were lost to follow-up; therefore, data for these patients were censored at the last visit. Two patients discontinued the treatment after 36 and 27 days, respectively, but they were monitored nevertheless.

**Response to therapy.** Both cohorts showed a favorable response during therapy, and there were no therapeutic failures. Despite clinical improvement, at the end of the first week of treatment the proportion of patients whose blood culture remained positive was 17% (7 of 48), with no differences between either cohort. At the end of treatment, all patients had negative blood cultures. Among the 52 patients included in this study, 9 (17.3%; 95% confidence interval [95% CI], 8.2 to 30.3%) had relapses within the first 6 months after therapy, with no differences between the patients in the two cohorts.

After a median follow-up of 12 months, there was one relapse among the 17 patients (5.8%; 95% CI, 0.1 to 26.0%) in cohort 1. Knee arthritis was diagnosed at the baseline in this previously asymptomatic human immunodeficiency virus (HIV)-seropositive patient, and *B. melitensis* was isolated from the synovial fluid (Table 2). The patient responded promptly to the antibiotic therapy, but he developed fever and orchitis 2 months later. After a new course of antibiotic therapy with doxycycline for 45 days and gentamicin for 14 days, the orchitis was resolved.

Among the 35 patients included in cohort 2 (median follow-up, 12 months), 8 had relapses (22.9%; 95% CI, 10.4 to 40.1%). Relapse was confirmed by the isolation of *Brucella* from the blood of all of these patients. Five of the 8 patients had clinical relapses, with characteristic clinical findings; in two of them the clinical findings appeared 2 and 3 weeks after the blood cultures became positive again. A patient who had a relapse developed sacroiliitis. Two patients had bacterial relapses without clinical signs or symptoms, and one other patient had only mild clinical symptoms, despite new positive blood cultures. The clinical characteristics of these patients are summarized in Table 2.

All patients with relapses were retreated with the same antibiotic regimen. The clinical responses were excellent in all

TABLE 1. Clinical characteristics of patients with brucellosis at study entry[a]

| Characteristic | Cohort 1 | Cohort 2 |
|---|---|---|
| No. of patients | 17 | 35 |
| Mean ± SD age (yr) | 26.3 ± 14.8 | 30.6 ± 14.0 |
| Male gender (no. [%]) | 12 (71) | 24 (69) |
| No. (%) with risk factor for brucellosis[b] | | |
| Occupational exposure | 9 (52) | 18 (51) |
| Ingestion of unpasteurized dairy products | 10 (59) | 14 (40) |
| No. (%) who previously had brucellosis | 3 (18) | 2 (6) |
| Mean ± SD duration of symptoms before therapy (days) | 26.5 ± 17.9 | 30.3 ± 31.3 |
| Mean ± SD (kg) | 61.3 ± 16.7 | 67.4 ± 12.7 |
| Mean ± SD serum creatinine concn (mg/dl) | 0.97 ± 0.17 | 0.96 ± 0.18 |
| No. (%) with positive blood cultures[c] | 11 (68) | 21 (60) |
| Median agglutination titer (range)[d] | 640 (80–10,240) | 320 (40–20,480) |
| No. (%) with focal disease | 6 (35) | 6 (17) |
| Sacroiliitis | 4 | 3 |
| Knee arthritis | 2 | 0 |
| Ankle arthritis | 0 | 1 |
| Orchitis | 0 | 2 |
| Follow-up (median no. of mo [range]) | 12 (4–18) | 12 (3–18) |
| No. (%) of patients followed for: | | |
| <6 mo | 1 (6) | 3 (9) |
| <12 mo | 3 (18) | 6 (17) |

[a] Cohort 1 received doxycycline for 45 days and gentamicin for 7 days. Cohort 2 received doxycycline for 30 days and gentamicin for 7 days.
[b] Some patients had more than one risk factor.
[c] In another patient *Brucella* was isolated from the joint fluid of knee.
[d] Reciprocal of the standard tube agglutination titer.

TABLE 2. Characteristics of nine patients with brucellosis relapse[a]

| Regimen and patient no. | Age (yr), gender | Before therapy | | | | Time to relapse (mo) | Blood culture result at relapse | Comment (diagnostic procedure) |
|---|---|---|---|---|---|---|---|---|
| | | Duration of symptoms (days) | Blood culture result | STA titer[b] | Focal disease | | | |
| 45-day doxycycline, 7-day gentamicin regimen | | | | | | | | |
| 1 | 35, M | 13 | ND | 10,240 | Knee arthritis[c] | 2 | ND | Ex-IDU HIV-seropositive patient with no HIV-related complications and CD4 count of 658/mm$^3$; clinical relapse with orchitis |
| 30-day doxycycline, 7-day gentamicin regimen | | | | | | | | |
| 2 | 36, F | 28 | + | 320 | None | 2 | + | Malaise, asthenia, sweats, arthralgias, myalgias, headache |
| 3 | 17, F | 25 | + | 160 | None | 0.5 | + | Treatment compliance, 90%; fever, anorexia, and lumbar pain 2 weeks after positive blood culture |
| 4 | 46, M | 20 | + | 320 | None | 2 | + | Fever, sweats, arthralgias, malaise |
| 5 | 13, M | 6 | + | 320 | None | 2 | + | Bacterial relapse without symptoms; therapy failed two times after relapse[d] |
| 6 | 23, M | 15 | + | 2,560 | None | 6 | + | Fever, malaise, arthralgias |
| 7 | 67, M | 20 | + | 20,480 | None | 6 | + | Bacterial relapse with only mild symptoms (arthralgias and myalgias). |
| 8 | 32, M | 35 | + | 320 | None | 1 | + | Two relapses; fatigue, malaise, and intermittent elbow pain for several weeks after the second relapse[d] |
| 9 | 15, M | 9 | + | 5,120 | None | 1.5 | + | Fever and low back pain; sacroiliitis (MRI) |

[a] −, negative; +, positive; ND, not done; MRI, magnetic resonance imaging; Ex-IDU, previous intravenous drug user; M, male; F, female
[b] Reciprocal of the standard tube agglutination (STA) titer.
[c] *B. melitensis* was isolated from the joint fluid.
[d] See text with regard to clinical evolution.

patients, but two patients (25%) required three or more courses of therapy. Case reports for these patients are presented here. The first patient was a 13-year-old male who had positive blood cultures 2 months after the completion of therapy. The patient was asymptomatic, and a new course of doxycycline for 30 days and gentamicin for 7 days was administered. However, *Brucella* was once again isolated from the blood of this asymptomatic child after the patient completed the therapy. Then, monotherapy with doxycycline was administered for 30 days. Although at the end of therapy *Brucella* was isolated from blood cultures, the patient remained without signs and symptoms. An extensive evaluation was done to rule out endocarditis and splenic or hepatic abscesses, including echocardiography and abdominal ultrasonography. Treatment with doxycycline at 200 mg/day for 90 days and streptomycin at 1.0 g/day for 2 weeks was administered. He was observed for more than 6 months after retreatment and showed no evidence of another relapse.

The second patient was a 32-year-old male, and he had positive blood cultures 1 month after therapy without clinical findings. A new course of doxycycline for 30 days and gentamicin for 7 days was administered. Ten weeks later, he began experiencing fever, sweats, easy fatigue, malaise, and neck and elbow pain. Clinical suspicion of relapse was confirmed by isolation of *Brucella* from the blood culture. Treatment with doxycycline for 45 days and gentamicin for 7 days was administered. During the third day of treatment, the patient became afebrile, and he felt well enough to return to work. Fatigue, malaise, and intermittent elbow pain persisted for several more weeks, but no relapse occurred during the following 6 months.

**Adverse effects.** Adverse effects were reported for 19 (36.5%; 95% CI, 23.6% to 51.0%) of the 52 patients: 7 patients had photosensitivity rash, 5 patients had epigastric discomfort, 6 patients had nausea, 3 patients had vomiting, 2 patients had heartburn, 1 patient had anorexia, and 1 patient had an erythematous rash on the palms. Most of these reactions were classified as mild, and no toxic effects led to the discontinuation of the study therapy. Adverse effects occurred with similar frequencies in both cohorts: 5 of 17 patients (29%) in cohort 1 and 14 of 35 patients (40%) in cohort 2 ($P > 0.2$). The most frequently observed adverse effects were phototoxicity and gastrointestinal complaints, both of which were considered to be related to doxycycline. The intramuscular administration of gentamicin was well tolerated. Mild pain at the site of injection was rare, and no change in therapy was required. Laboratory studies did not reveal any drug-related hematopoietic, renal, or hepatic abnormalities. The mean serum creatinine concentrations at the baseline and after 7 days of gentamicin treatment were 0.92 ± 0.19 and 0.95 ± 0.13 mg/dl, respectively ($P > 0.2$). The concentrations of gentamicin and doxycycline in plasma were measured in five patients. The mean peak and trough serum gentamicin levels were 15.2 ± 5.1 mg/liter (range, 11.0 to 23.6 mg/liter) and 0.22 ± 0.24 mg/liter (range, 0.10 to 0.60 mg/liter), respectively. The mean peak and trough serum doxycycline levels were 5.96 ± 2.08 μg/ml (range, 2.91 to 7.58 μg/ml) and 3.56 ± 0.96 μg/ml (range, 2.18 to 4.39 μg/ml), respectively.

## DISCUSSION

It has been known for nearly five decades that patients who have brucellosis develop relapses, despite treatment with several antibiotic regimens (16, 32). Brucellae are facultative intracellular pathogens, and this fact seems to be responsible for

the high incidence of relapses (28). The relapse rate of 5.9% in patients treated with doxycycline for 45 days and gentamicin for the first 7 days (cohort 1) in this study is almost identical to the rates in our two previous trials with doxycycline for 45 days plus streptomycin for 14 days (25, 26). Other studies of patients with brucellosis due to *B. melitensis* who received treatment with doxycycline for 6 weeks and streptomycin for 2 to 3 weeks yielded relapse rates of 0 to 5% (1, 7, 10, 21). This 45-day doxycycline and 7-day gentamicin combination may therefore be sufficient to achieve an optimal clinical response, and it could be the regimen of choice instead of the classic treatment with doxycycline and streptomycin. The decrease in the duration of aminoglycoside injection therapy, from 14 to 21 days for streptomycin to 7 days for gentamicin, constitutes an advantage with respect to toxicity, cost, and ease of administration. Since the potential toxicity of aminoglycoside therapy is time dependent, a 7-day course is prefereable to a longer one. On the other hand, we recently demonstrated a 12.5% relapse rate with a 45-day doxycycline and 7-day netilmicin regimen (27), which in terms of efficacy is similar to the doxycycline and gentamicin combination used in the present study. Both regimens have certain disadvantages (e.g., seven injections are administered), but the doxycycline-netilmicin regimen has a higher cost.

However, the 23% relapse rate in patients treated with doxycycline for 30 days and gentamicin for 7 days (cohort 2) in this study was higher than that in previous studies in which doxycycline was given for 30 days and streptomycin was given for 2 to 3 weeks (with relapse rates ranging from 5 to 7.1%) (5, 11). These results could mean that with short courses of tetracyclines, a long duration of aminoglycoside treatment appears to be necessary to obtain lower relapse rates.

The outcome for our patient with HIV coinfection deserves comment. In southern Europe acute brucellosis has been reported in immunosuppressed patients with AIDS (23, 30). Although most patients with acute brucellosis as a complication of HIV infection initially responded to treatment with antibiotics, relapses were common and cures were difficult to achieve (23, 30), as happened with our patient. Infection with *Brucella* species as a complication of AIDS may require prolonged antibiotic therapy.

In summary, this study demonstrates that the combination of doxycycline for 45 days and gentamicin for 7 days is an effective and well-tolerated therapy for brucellosis. This combination is relatively inexpensive; gentamicin can be given intramuscularly once daily, and it is suited to outpatient therapy. The combination also appears promising for the treatment of brucellosis in patients with focal disease such as sacroiliitis and peripheral arthritis. However, the most convenient duration of doxycycline in the doxycycline-gentamicin regiment requires prospective randomized trials. Such a clinical trial is being performed by our group.

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## APPENDIX

In addition to the study authors, the following persons and institutions participated in this trial, entered research subjects, or both: at Albacete Hospital, Lourdes Sáez, Miguel Angel Barba, Francisco Medrano, and José Antonio Sáez-Barcelona; at Cuenca Hospital, María Dolores Ruiz-Ribó and Alberto Roldán; and at Guadalajara Hospital, Manuel Rodríguez-Zapata.

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