ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1990, p. 881–883
0066-4804/90/050881-03$02.00/0

Vol. 34, No. 5

Multicenter Prospective Study of Treatment of *Brucella melitensis* Brucellosis with Doxycycline for 6 Weeks plus Streptomycin for 2 Weeks

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Received 31 July 1989/Accepted 17 February 1990

The effectiveness of treating human brucellosis caused by *Brucella melitensis* with a 6-week course of doxycycline plus streptomycin for 2 of those weeks was analyzed by a multicenter prospective study of 139 patients. Subjects with central nervous system involvement, endocarditis, or spondylitis were excluded from the study. All but 5 of the 139 patients completed the full treatment schedule and became afebrile in the first week of therapy. Four patients suffered relapses during the follow-up period. Of the five patients who did not complete the treatment, two left because of adverse secondary effects (1.4%), another two left for noncompliance with the treatment (1.4%), and the remaining patient was considered a therapeutic failure because his symptoms persisted after the first week of therapy (0.7%). We conclude that the combination of doxycycline and streptomycin is an effective treatment for the types of brucellosis included in our study.

Human brucellosis is an important ongoing public health problem. Although the incidence of this infection varies from one geographic area to another, the Mediterranean region is one of the most heavily affected (J. Fuente, Proc. Int. Meet. Brucellosis, Madrid, p. 161–172, 1985). In spite of the harm that this disease may cause, there still is not a completely effective treatment or method of preventing the frequent relapses that are encountered.

Although a 21-day treatment schedule of tetracycline plus streptomycin is effective in relieving the initial symptom (8, 9, 23, 25), relapses frequently occur. The number of recurrences can be reduced by extending the tetracycline administration period to 30 to 45 days (5, 7, 13, 17, 20). The use of co-trimoxazole, even during prolonged treatment, has been discontinued because of the large number of relapses encountered with its use (25). In later trials, because of its good in vitro antibrucellar activity and the satisfactory results obtained with experimental animals, rifampin was assessed (18, 22). However, because of relapses of the disease, its use as a single agent for the treatment of human brucellosis was not acceptable (6, 14, 19). Most comparative studies have shown that the combination of rifampin and tetracycline, recommended by the World Health Organization (11), is less effective than tetracycline plus streptomycin (4, 7, 13, 20).

In the present work, we treated human brucellosis with a 6-week course of doxycycline combined with an initial 2-week course of streptomycin. Drug tolerance of patients and compliance with this schedule were also assessed in a subset of patients.

MATERIALS AND METHODS

A prospective study of 139 patients diagnosed as having brucellosis between 1985 and 1987 at Virgen del Rocio Hospital in Seville (84 cases), the Carlos Haya Hospital in Malaga (29 cases), and the Comarcal de la Axarquía in Velez-Malaga (26 cases) was carried out to evaluate the safety of and the tolerance of patients to doxycycline plus streptomycin and the efficacy of this combination in eliminating symptoms and preventing relapses of the disease. The hospitals are located in southern Spain, where brucellosis is endemic.

Patients with brucellosis with central nervous system involvement, endocarditis, or spondylitis, which are considered severe complications of this disease, were excluded from the study. Individuals who received antimicrobial therapy prior to the study, children under 7 years of age, pregnant women, and patients allergic to the drugs employed where also eliminated.

The diagnostic criteria were isolation of a *Brucella* sp. from blood or other tissues or fluids and positive serology in the presence of compatible clinical findings. The serological criteria were two or more of the following: positive rose bengal test, Wright seroagglutination assay titers equal to or higher than 1/160, and indirect immunofluorescence assay titers higher than 1/100.

Standard procedures were used in the serological testing (1, 12, 16). *Brucella abortus* 99 antigen (Webridge strain) (Bio-Mérieux, Lyon, France) was employed, as well as fluorescein-labeled anti-human immunoglobulins (multivalent anti-immunoglobulin and monospecific anti-immunoglobulin G, anti-immunoglobulin A, and anti-immunoglobulin M) (Behringwerke, Marburg, Federal Republic of Germany) for the immunofluorescence technique.

At least three blood cultures each were performed for 86 patients under optimal conditions (defined as absence of antimicrobial treatment in the patient during the preceding 48 h, inoculation of samples in Ruiz-Castañeda medium, and maintenance of the blood culture for 30 days). Standard processing procedures were used (21). Upon patient admission to the study, a clinical examination, a laboratory analysis (complete blood count and measurement of the following: erythrocyte sedimentation rate, serum glutamic oxalacetic transaminase, serum glutamic pyruvic transaminase, bilirubin, alkaline phosphatase, uremia, and urine), and a chest X ray were carried out. Additional studies were performed according to the symptom of the patients.

The patients were then administered a 6-week course of 100 mg of oral doxycycline every 12 h, plus 1 g of intramus-

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cular streptomycin sulfate during the first 2 weeks of treatment.

The patients were reassessed during, at the end of, and at 3 and 6 months following the end of the treatment, as well as whenever clinical symptoms reappeared. During each check-up, the clinical, analytical, and serological assessments that were performed upon admission to the study were carried out. New blood samples for cultures were collected from patients suspected of having a relapse of the disease.

Treatment was considered a failure if fever or focal symptoms persisted after 7 days of treatment. Relapse was diagnosed if symptoms reappeared after the end of the therapy. Only patients who were monitored for 6 months or longer were included in the evaluations of the relapses. The result was considered unassessable if a patient voluntarily decided to discontinue therapy without a justifiable medical reason.

A statistics package furnished by Epi-Info (Atlanta, Ga.) was used to carry out the statistical analysis.

RESULTS

Thirty-five women (25.2%) and 104 men (74.8%), ranging in age from 8 to 72 years (mean ± standard deviation, 32.5 ± 15.5 years), were studied. The majority of these individuals lived in rural districts (80.6%), but some resided in urban areas (19.4%). More than half of the patients had had contact with goats (58.2%) and had ingested unpasteurized milk (66.9%); both risk factors were present in 38.1% of the patients. Twelve individuals had no known risk factors.

On the average, the diagnosis was confirmed 34.46 ± 45.14 days (mean ± standard deviation) after the onset of symptoms. Brucellosis without complications was diagnosed in 121 cases (87.1%), and 18 patients (12.9%) suffered from complicated forms of the disease: 15 cases of osteoarticular complications (12 cases of sacroiliitis, 2 cases of arthritis of the knee, and 1 case of arthritis of the shoulder), 3 cases of orchitis, 2 cases of granulomatous hepatitis, 1 case of hepatic brucelloma, 1 case of perisplenitis, and 1 case of pneumonitis. Five patients had two complications each. The blood cultures from 61 of 86 patients were positive (70.9%), and *Brucella melitensis* was isolated in all of them. The rose bengal assay was positive for 131 of 139 patients (94.2%), the tube agglutination assay was positive for 103 of 130 patients (79.2%), and indirect immunofluorescence was positive in 74 of 97 patients (76.2%).

All but 5 of the 139 patients who began the study adhered to the full treatment schedule and were afebrile and asymptomatic within a week after the onset of therapy. Of the five patients who did not complete the study, one (0.7%) was considered a therapeutic failure and was put on a modified treatment regimen because of persistent joint symptoms, two (1.4%) were taken off treatment because of adverse secondary effects (itchy rash and ototoxicity), and the remaining two (1.4%) left the study voluntarily. Six patients suffered from slight secondary effects (three cases of photosensitivity, two cases of epigastric pain, and one case of a Herxheimer-like reaction) that did not necessitate suspension of treatment.

Relapses were suffered by 4 (3.9%) of the 102 patients who were monitored for at least 6 months after discontinuing treatment. These four individuals had brucellosis without complications. The remaining 32 patients did not complete the 6-month follow-up period; contact with 15 was lost after the first month, and contact with 7 following the third month, but none of these individuals suffered from a recurrence of the disease during that time.

DISCUSSION

In recent years, a number of therapeutic trials dealing with the treatment of human brucellosis have been carried out. Most of these studies resulted in control of the initial symptoms, but the therapies used in these studies did not prevent relapses in every case. Relapse does not necessarily indicate a decrease in microbial susceptibility in vitro (3) but does reflect the difficulties in eradicating *Brucella* spp. from mononuclear phagocytes (24).

Treatment with tetracycline plus streptomycin was initiated in the 1950s by Magill and Killough (15), and the World Health Organization recommended this combination of drugs as the treatment of choice for human brucellosis in 1971 (10). This combination has a synergistic effect on *Brucella* organisms (22). Since trials with the recommended treatment of 3 weeks of tetracycline plus 2 weeks of streptomycin resulted in a relapse rate that ranged from 15 to 26% (9, 13, 25), a number of modifications to the schedule have been made since that time. Prolonging the administration of tetracycline from 21 to 30 or 45 days reduces the number of therapeutic failures and lowers the relapse rate to between 2.8 and 8.4% (5, 7, 13, 17, 20). In addition, the introduction of doxycycline has improved the therapeutic compliance level (7, 13, 20).

The introduction of rifampin into the arsenal of antibrucellar agents was promising because of its excellent in vitro activity and the satisfactory trials carried out on experimental animals (18, 22). However, its use as a single agent for treatment has been discontinued because of the large number of relapses that are associated with its use and the possibility of the appearance of drug resistance (6, 14, 19). In recent years, discouraging results of studies employing rifampin combined with doxycycline, showing a relapse rate ranging from 0 to 38%, have been reported (4, 7, 13, 20).

When treatment with doxycycline and rifampin was compared with treatment with tetracycline and streptomycin over a period of 30 days, a significant difference in the relapse rates, which reached 38.8 and 7.1%, respectively, became evident (4). Other authors have compared doxycycline plus rifampin for 6 weeks with doxycycline plus streptomycin for 4 weeks, and they too have found favorable results for the latter treatment, although with high relapse rates of 13.4 and 8.4%, respectively (7). Two randomized trials compared the efficacy of rifampin plus doxycycline with that of streptomycin plus doxycycline, which were administered over a period of 6 weeks to a reduced number of patients and which resulted in a relapse rate of 0 to 13% with doxycycline and rifampin and 0 to 7% with streptomycin and doxycycline (13, 20). These results imply that treatment of brucellosis with streptomycin and tetracycline is more efficacious than treatment with doxycycline and rifampin. Nevertheless, the doxycycline and rifampin treatment was recommended not long ago by the World Health Organization as the treatment of choice for human brucellosis (11).

The information obtained from the aforementioned studies implies that better results in brucellosis treatment are obtained when treatment is prolonged to 6 weeks and streptomycin and doxycycline are used. However, only a small number of patients were included in the studies in which streptomycin and doxycycline were used over a period of 6 weeks (13, 20). Our study constitutes the first to use large

series of patients with brucellosis who were treated with doxycycline and streptomycin for 6 and 2 weeks, respectively, and is the first known study in which cases complicated with sacroiliitis are included.

The initial response in our study was excellent. There was only one therapeutic failure, and all the remaining patients became afebrile and asymptomatic during the first week of treatment. The percentage of patients that completed the entire treatment schedule was very high (98.6%), despite the fact that the streptomycin was injected intramuscularly. Our study showed that doxycycline plus streptomycin was safe and well tolerated by most patients; additionally, in only two of our cases (1.4%) was the therapy discontinued because of undesirable side effects.

The risk of relapse is the most important parameter to consider when a therapy for human brucellosis is being assessed. Ninety percent of the recurrences appear within the first 4 months after treatment is discontinued (2). In our study, only 4 (3.9%) of the 102 patients who were monitored for 6 or more months after concluding treatment suffered relapses.

Sacroiliitis, along with spondylitis, has always been one of the most frequent complications of brucellosis (23). It was traditionally thought that a prolonged treatment of at least 3 months was necessary in order to achieve recovery (5). Thirteen of our patients had sacroiliitis, and all of them recovered, with no sequelae or relapses, after only 6 weeks of treatment.

On the basis of our results, we conclude that the combination of a 6-week course of doxycycline and a 2-week course of streptomycin is well tolerated and easy to comply with, and above all, it considerably reduces the number of relapses. In our opinion, this combination should be the treatment of choice for brucellosis without complications as well as for the focal forms outlined in our study.

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