ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1993, p. 1831–1834
0066-4804/93/091831-04$02.00/0

Vol. 37, No. 9

Quinolones in Treatment of Human Brucellosis: Comparative Trial of Ofloxacin-Rifampin versus Doxycycline-Rifampin

MURAT AKOVA, ÖMRÜM UZUN, H. ERDAL AKALIN,* MURAT HAYRAN, SERHAT ÜNAL, AND DENIZ GÜR

Department of Medicine, Section of Infectious Diseases, Hacettepe University School of Medicine, Hacettepe, 06100 Ankara, Turkey

Received 12 March 1993/Returned for modification 29 April 1993/Accepted 29 June 1993

Quinolones have been reported to be active against *Brucella* species in vitro. In this prospective randomized study, the efficacy and safety of the combination of ofloxacin plus rifampin were compared with the efficacy and safety of doxycycline plus rifampin, both combinations administered for a 6-week period in treatment of brucellosis. Sixty-one patients were enrolled in the study, and 49 had blood or bone marrow cultures positive for *Brucella melitensis*. Thirty patients received 200 mg of doxycycline plus 600 mg of rifampin once daily, and 31 patients were treated with 400 mg of ofloxacin plus 600 mg of rifampin once daily for 6 weeks. Nine patients in each group had complications of the disease. There was one therapeutic failure in the ofloxacin-rifampin treatment group, and one patient from each group relapsed (3.3% of those in the doxycycline-rifampin treatment group versus 3.2% of those in the ofloxacin-rifampin treatment group). Gastric discomfort was the major side effect observed in 13 patients (43.3%) who received doxycycline plus rifampin, whereas only 2 patients (6.5%) treated with ofloxacin plus rifampin complained of gastric irritation. These results suggest that the combination of ofloxacin plus rifampin administered for 6 weeks is as effective as doxycycline plus rifampin given for the same period, regardless of the presence of complications of the disease.

Brucellosis is a major health problem worldwide, especially in developing countries. The best regimen for the treatment of acute brucellosis is not clearly determined (2). *Brucella* species are facultative intracellular parasites; therefore, complete eradication of the microorganism is difficult to achieve, and relapses are common.

The fluoroquinolones have excellent bactericidal activity against a variety of bacteria. In addition, they penetrate well into leukocytes and macrophages, which makes them suitable agents in treatment of intracellular infections. Several studies have reported the in vitro activity of quinolones against *Brucella* species (6, 12, 13, 15, 22). However, clinical trials of monotherapy with ciprofloxacin (4, 17) or ofloxacin (3, 6) have yielded relapse rates ranging from 16 to 66%. The numbers of enrolled patients have been relatively small in these trials, making it difficult to interpret the discrepancy among these results. Nevertheless, relapse rates with the quinolones have been considerably higher than those with conventional antibiotic regimens. It may be expected that the addition of another antimicrobial agent active against *Brucella* species may lower the relapse rates. In this prospective randomized study, the efficacy and safety of the combination of ofloxacin plus rifampin were compared with the efficacy and safety of doxycycline plus rifampin, both combinations administered for a 6-week period in treatment of brucellosis.

MATERIALS AND METHODS

Between March 1989 and March 1992, 61 adult patients with brucellosis admitted consecutively to Hacettepe University hospital were enrolled in the study. Patients with endocarditis or neurobrucellosis were excluded. Individuals who received antimicrobial therapy prior to the study, pregnant women, and patients allergic to any of the drugs employed in the regimens were also not included in the study.

The diagnostic criteria were a standard tube agglutination titer of 1/160 or more for anti-*Brucella* antibodies in the presence of compatible clinical findings (fever, night sweats, arthralgia, hepatomegaly, splenomegaly, and lymphadenopathy) and isolation of a *Brucella* sp. from blood or bone marrow cultures.

Sacroiliitis, spondylitis, peripheral arthritis, and orchitis were defined by appropriate findings on physical examination and relevant radiographic, radionuclide, and tomographic studies. Briefly, pain over the involved vertebral bodies or sacroiliac joints, accompanied by isotope accumulation in a radionuclide scan, was defined as sacroiliitis or spondylitis. Narrowing and/or irregularity of the sacroiliac articular spaces in plain X-ray films were considered to support the diagnosis of sacroiliitis. The time to defervescence was established as the number of days elapsed from the start of therapy until the patient became afebrile. Therapeutic failure was defined as the persistence of symptoms or signs of the disease, or both, at the end of therapy. Reappearance of symptoms or signs or new positive blood or bone marrow cultures during the 12 months after therapy was considered relapse.

Blood and bone marrow cultures were performed by inoculating samples from all patients before treatment into Castaneda medium and incubating them for 30 days in case of no growth. Standard processing techniques were used (23). Susceptibilities of isolated organisms to ofloxacin, rifampin, and doxycycline were determined by the standard tube dilution method. The inoculum consisted of approximately 10^8 CFU/ml and was suspended in tryptic soy broth (Difco Laboratories, Detroit, Mich.). Bactericidal agent titers in serum were determined by the method described by Reller and Stratton (20). Sera were taken for measurement at the fifth day of treatment, immediately before (for trough-

* Corresponding author.

TABLE 1. Characteristics of patients treated with doxycycline plus rifampin or ofloxacin plus rifampin[a]

Characteristic	Value for group	
	DR	OR
Total no. of patients	30	31
Female/male	14/16	17/14
Age range (yr)	18–72	18–70
Mean age ± SD (yr)	34.4 ± 14.3	37.8 ± 15.1
Epidemiologic contact (*n*)		
Occupational exposure	2	
Ingestion of dairy products	27	29
None	1	2
No. (%) of patients with a positive blood and/or bone marrow culture	23 (77)	26 (84)
Median titers of bactericidal agent in serum (trough, peak)	1/2, 1/4	1/2, 1/4
Agglutination titers		
Range	1/160–1/2,560	1/160–2,560
Median	1/640	1/640
Mean duration of symptoms ± SD (days)	31.4 ± 21.7	37.1 ± 24.5
Complications (no.)		
Spondylitis	3	3
Hip arthritis	3	2
Knee arthritis	1	2
Sacroiliitis	1	2
Orchitis	1	
Total	9	9

[a] No statistically significant differences were present between the two treatment groups.

level measurement) and 2 h after (for peak-level measurement) the antibiotics were given.

Informed consent was obtained from the patients, and the trial protocol was approved by the hospital ethical committee. All patients were hospitalized for at least 2 weeks at the beginning of treatment in order to monitor clinical response and potential side effects. Complete physical examinations were performed, and complete blood counts; erythrocyte sedimentation rates; and serum aspartate aminotransferase, serum alanine aminotransferase, bilirubin, alkaline phosphatase, and creatinine levels were determined at baseline, at the end of the first and second weeks of treatment, and on all scheduled follow-up visits. Additional studies were performed as needed.

The patients were then randomized in a nonblinded fashion to receive 200 mg of doxycycline plus 600 mg of rifampin per day (DR group) or 400 mg of ofloxacin plus 600 mg of rifampin per day (OR group) for a total of 6 weeks. All medications were administered once daily. The patients were reassessed on an outpatient basis after being discharged from hospital. Blood cultures and clinical evaluations were done monthly during the first 3 months posttherapy and every 3 months thereafter for at least one year.

The chi-square test with Yates' correction and the Fisher exact test were used for statistical analysis of data.

RESULTS

Thirty patients (14 females and 16 males) received doxycycline plus rifampin, and 31 (17 females and 14 males) received ofloxacin plus rifampin (Table 1). No statistically significant differences were found between the two groups of patients. Blood or bone marrow cultures were positive for 23 (77%) of the patients in the DR group and 26 (84%) of those in the OR group. All microorganisms isolated were *Brucella melitensis* and were found to be susceptible to doxycycline (MIC for 90% of the strains [MIC_{90}], 0.50 μg/ml; range, 0.03 to 0.50 μg/ml), rifampin (MIC_{90}, 0.50 μg/ml; range, 0.03 to 1.0 μg/ml), and ofloxacin (MIC_{90}, 0.50 μg/ml; range, 0.008 to 0.50 μg/ml). Bactericidal agent titers in serum were similar in both groups, with a median of 1/2 for trough levels and 1/4 for peak levels.

TABLE 2. Outcome of treatment in patients receiving doxycycline plus rifampin or ofloxacin plus rifampin

Parameter	Value for group	
	DR	OR
Mean time (days) to defervescence (range)	5.1 (2–10)	6.3 (2–18)
No. cured (%)	30 (100.0)	30 (96.8)
No. of therapeutic failures (%)		1 (3.2)
No. relapsed (%)	1 (3.3)	1 (3.2)
No. with side effects (%)		
Gastric discomfort	13 (43.3)[a]	2 (6.5)
Skin rash	1 (3.3)	1 (3.2)
Mean follow-up time ± SD (mo)	14.2 ± 5.6	15.1 ± 5.9

[a] $P < 0.005$. (Values for all other parameters did not differ significantly between the two treatment groups.)

Nine patients in each group suffered from complications of the disease, including spondylitis (three each in the DR and OR groups), hip arthritis (three in the DR and two in the OR group), knee arthritis (one in the DR and two in the OR group), sacroiliitis (one in the DR and two in the OR group), and orchitis (one in the DR group). The duration of symptoms prior to commencement of therapy ranged from 7 to 60 days (31.4 ± 21.7) in patients who received doxycycline plus rifampin and 10 to 70 days (37.1 ± 24.5) in those treated with ofloxacin and rifampin ($P > 0.5$).

When only patients with positive cultures were analyzed, the two regimens produced similar therapeutic outcomes ($P = 0.9$). The mean time to defervescence was 5.1 days (range, 2 to 10 days) for patients in the DR group and 6.3 days (range, 2 to 18 days) for patients in the OR group (Table 2). Blood cultures during treatment were negative for all patients. One patient in the OR group who had sacroiliitis continued to have pain at the end of treatment, although fever disappeared and cultures became negative, and was considered a therapeutic failure. Relapse occurred in one patient from each group. Symptoms recurred 4 months after the completion of therapy in a patient treated with ofloxacin and rifampin. Another patient treated with doxycycline and rifampin had fever and back pain recurring 6 months after therapy, showing a rising titer in an agglutination test. Blood cultures were negative in both cases. These patients received ofloxacin plus rifampin and showed good responses to therapy.

Gastric discomfort was the most frequent side effect in the DR group (occurring in 43.3% of patients), whereas only two patients in the OR group (6.5%) complained of gastric irritation ($P < 0.005$). One patient in each group experienced skin rash. These side effects were not severe enough in any of the patients to warrant discontinuation of therapy. No other side effects attributable to the study drugs were noted, and compliance, monitored throughout the therapy, was complete.

DISCUSSION

Although a number of clinical trials have been performed during the past 40 years since Magill and Killough (19) suggested tetracycline plus streptomycin for the treatment of human brucellosis, the optimal treatment modality is an unsettled issue. Most antibiotics active against *Brucella* species resulted in control of initial symptoms; however, relapse rates were found to be high during follow-up evaluations. *Brucella* species are intracellular organisms; therefore, any treatment regimen should consist of an antibiotic(s) capable of penetrating mononuclear phagocytes in order to prevent relapses.

The combination of 3 weeks of tetracycline administration plus 2 weeks of streptomycin administration was recommended as the treatment of choice for human brucellosis by the World Health Organization in 1971 (9). It was possible to lower the relapse rates of 15 and 26% observed previously (8, 16) to 3 and 8% by prolonging the administration of tetracycline or doxycycline to 4 or 6 weeks (1, 5, 7). Rifampin, which was very active against *Brucella* species, showed excellent penetration of cells. Trials employing rifampin alone were discouraged because of the high relapse rates and the possibility of development of resistance (18, 21). The combination of rifampin plus doxycycline, administered for 6 weeks, resulted in failure rates of 0 to 13% (1, 5, 7, 16) and was recommended by the World Health Organization in 1986 in treatment of brucellosis (10).

In vitro studies of ciprofloxacin and ofloxacin against *B. melitensis* have shown excellent results. However, clinical trials employing fluoroquinolones alone have yielded contradictory results. In our own experience with ofloxacin, 21 patients, including those with complications of the disease or endocarditis, showed a relapse rate of 16% (3), whereas Lang et al. (17) reported a relapse rate of 66% in 6 patients treated with ciprofloxacin alone.

The results of the present study indicate that 6 weeks of treatment with ofloxacin plus rifampin is as effective as 6 weeks of treatment with doxycycline plus rifampin. Initial response rates were 100% for patients receiving doxycycline plus rifampin and 97% for those treated with ofloxacin plus rifampin. Relapse rates were also comparable (1 of 30 patients [3.3%] in the DR group and 1 of 31 patients [3.2%] in the OR group).

The outcome of treatment for patients with spondylitis deserves special attention, since discouraging results have been reported (5). In our series, all patients with spondylitis were successfully treated with either doxycycline plus rifampin or ofloxacin plus rifampin. A single patient with sacroiliitis was considered to have failed to respond to therapy with ofloxacin plus rifampin on the basis of persisting severe pain. Although this clinical condition has been regarded as reactive arthritis rather than as an infectious activity of brucellosis by some authors (14), the fact that this particular patient's pain was not relieved by anti-inflammatory drugs but responded to a further course of therapy with doxycycline plus rifampin indicated the failure of the ofloxacin-rifampin regimen.

An important difference between the treatment groups was the low incidence of side effects with ofloxacin plus rifampin (9.7% of patients) compared with that seen with doxycycline plus rifampin (46.7% of patients). Gastric discomfort was observed in 43.3% of the patients receiving doxycycline plus rifampin, despite the fact that doxycycline was given after meals in all cases. The incidence of gastric discomfort was very low (6.5%) in patients treated with ofloxacin plus rifampin. However, this side effect was usually very mild in both treatment groups and never led to premature discontinuation of therapy.

The unacceptably high relapse rates observed with quinolone monotherapy, despite the excellent MICs of these drugs, have led the investigators to evaluate the activity of quinolones, as well as those of other antibiotics, at low pH levels which compare to that achieved in phagolysosomes. Garcia-Rodriguez et al. (11) reported a two- to fourfold increase in MICs of fluoroquinolones when the pH of the medium was lowered to 5. In vitro data from our laboratory yielded similar results, with a four- to eightfold increase in the MICs of ciprofloxacin and ofloxacin at pH 5.0 (unpublished data). The fact that quinolones are no more bactericidal against *Brucella* species when the pH of the test medium is lowered to 5.0 seems to explain the failure of quinolone monotherapy. On the other hand, data from our laboratory showed a two- to fourfold decrease in the MICs of rifampin against *B. melitensis* at pH 5.0 (unpublished data). On the basis of these observations, it is tempting to speculate that rifampin compensates for the decreased bactericidal effect of quinolones in the phagolysosome, and this might explain the lower relapse rate obtained with a combination of ofloxacin plus rifampin than with ofloxacin monotherapy. Nevertheless, more data are needed for further clarification.

In conclusion, the combination of ofloxacin plus rifampin administered for 6 weeks is as effective as doxycycline plus rifampin given for the same period. However, some caution is appropriate considering the relatively small sample size in the trial. In order to detect a minimum difference of 10% between two groups of patients, the beta error would be 0.44. On the other hand, a true difference between regimens (i.e., alpha = 0.05 and beta = 0.20) would require 58 patients to be allocated to each of the treatment groups. The reason which deterred us from increasing the size of the study group to that level was a time constraint (approximately five additional study years would be required). Complications such as spondylitis responded well to either regimen. The low rate of side effects with the ofloxacin-rifampin regimen gives this regimen an advantage over the doxycycline-rifampin combination and may have an impact on compliance to therapy.

REFERENCES

1. **Acocella, G., A. Bertrand, J. Beytout, J. B. Durrande, J. A. Garcia-Rodriguez, J. Kosmidis, M. Micoud, M. Rey, M. R. Zapata, J. Roux, and J. P. Stahl.** 1989. Comparison of three different regimens in the treatment of acute brucellosis: a multicenter multinational study. J. Antimicrob. Chemother. **23:**433–439.
2. **Akalin, H. E.** 1989. Therapy of human brucellosis, p. 219–243. *In* E. Tümbay, S. Hilmi, and Ö. Ang (ed.), Brucella and brucellosis in man and animals. Proceedings of a symposium held under the auspices of the Federation of European Microbiological Societies. Turkish Microbiological Society, Izmir.
3. **Akalin, H. E., S. Ünal, D. Gür, and M. Baykal.** 1990. Ofloxacin in the treatment of brucellosis. Eur. J. Clin. Microbiol. Infect. Dis. **1990:**326–328. (Special issue: Proceedings of the Third International Symposium on Quinolones, Vancouver, Canada, 1990.)
4. **Al-Sibai, M. B., M. A. Halim, M. M. El-Shaker, B. A. Khan, and M. H. Qadri.** 1992. Efficacy of ciprofloxacin for the treatment of *Brucella melitensis* infections. Antimicrob. Agents Chemother. **36:**150–152.
5. **Ariza, J., F. Gudiol, R. Pallares, P. F. Viladrich, G. Rufi, J. Corredioira, and M. Miravitlles.** 1992. Treatment of human brucellosis with doxycycline plus rifampin or doxycycline plus

streptomycin. A randomized double-blind study. Ann. Intern. Med. **117**:25–30.
6. **Baykal, M., H. E. Akalin, M. Firat, and A. Serin.** 1989. In vitro activity and clinical efficacy of ofloxacin in infections due to *Brucella melitensis*. Rev. Infect. Dis. **11**(Suppl. 5):S993–S994.
7. **Colmenero, J. D., S. Hernandez, J. M. Reguera, F. Cabrera, F. Rius, and A. Alonso.** 1989. Comparative trial of doxycycline plus streptomycin versus doxycycline plus rifampin for the therapy of human brucellosis. Chemotherapy (Basel) **35**:146–152.
8. **Feiz, J. M., H. Sabbaghian, and F. Sohrabi.** 1973. A comparative study of therapeutic agents used for the treatment of acute brucellosis. Br. J. Clin. Pract. **27**:410–413.
9. **Food and Agricultural Organization-World Health Organization.** 1971. Food and Agricultural Organization-World Health Organization expert committee on brucellosis, 5th report. WHO Tech. Rep. Ser. **464**:82.
10. **Food and Agricultural Organization-World Health Organization.** 1986. Food and Agricultural Organization-World Health Organization expert committee on brucellosis, 6th report. WHO Tech. Rep. Ser. **740**:56–57.
11. **Garcia-Rodriguez, J. A., J. E. Garcia Sanchez, and I. Trujillano.** 1991. Lack of bactericidal activity of new quinolones against *Brucella* spp. Antimicrob. Agents Chemother. **35**:756–759.
12. **Garcia-Rodriguez, J. A., J. E. Garcia-Sanchez, I. Trujillano, and J. L. Munoz Bellido.** 1989. In vitro activity of new quinolones against *Brucella* species. Rev. Infect. Dis. **11**(Suppl. 5):S992–S993.
13. **Gobernado, M., E. Canton, and M. Santos.** 1984. In vitro activity of ciprofloxacin against *B. melitensis*. Eur. J. Clin. Microbiol. **3**:371.
14. **Gotuzzo, E., and C. Carillo.** 1988. Brucellar arthritis, p. 31–41. *In* L. Espinoza (ed.), Infections in the rheumatic diseases. Grune and Stratton, Orlando, Fla.
15. **Khan, M. Y., M. Dizon, and F. W. Kiel.** 1989. Comparative in vitro activities of ofloxacin, difloxacin, ciprofloxacin, and other selected antibiotics against *Brucella melitensis*. Antimicrob. Agents Chemother. **33**:1409–1410.
16. **Kosmidis, J., A. Karagounis, J. Tselentis, and G. K. Daikos.** 1982. The combination of rifampin-doxycycline in brucellosis is better than the WHO regimen. Chemotherapia **1**(Suppl. 4):107.
17. **Lang, R., R. Raz, T. Sacks, and M. Shapiro.** 1990. Failure of prolonged treatment with ciprofloxacin in acute brucellosis. J. Antimicrob. Chemother. **26**:841–846.
18. **Llorens, J., and R. M. Busquets.** 1990. Brucellosis treated with rifampin. Arch. Dis. Child. **55**:486–488.
19. **Magill, G. B., and J. H. Killough.** 1953. Oxytetracycline-streptomycin therapy in brucellosis due to *Brucella melitensis*. Arch. Intern. Med. **23**:204–211.
20. **Reller, L. B., and C. W. Stratton.** 1977. Serum dilution test for bactericidal activity. II. Standardization and correlation with antimicrobial assays and susceptibility tests. J. Infect. Dis. **136**:196–204.
21. **Rodriguez-Creixems, M., L. Buzon, A. Meseguer, J. Martinez-Beltran, and E. Bouza.** 1980. Rifampin as a single agent in the treatment of acute brucellosis in humans, p. 1064–1066. *In* J. D. Nelson and C. Grassi (ed.), Current chemotherapy and infectious disease. Proceedings of the 11th International Congress of Chemotherapy and the 19th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
22. **Rubinstein, E., R. Lang, B. Shasha, B. Hagar, L. Diamanstein, G. Joseph, M. Anderson, and K. Harrison.** 1991. In vitro susceptibility of *Brucella melitensis* to antibiotics. Antimicrob. Agents Chemother. **35**:1925–1927.
23. **Ruiz-Castaneda, M.** 1961. Laboratory diagnosis of brucellosis in man. Bull. W.H.O. **24**:73–84.