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Comparison between doxycycline—rifampin—amikacin and doxycycline—rifampin regimens in the treatment of brucellosis

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Summary
Background: Combination drug therapy of brucellosis leads to recovery of symptoms, shortening of symptomatic interval, and decrease in morbidity rate, but single drug therapy is associated with more relapse episodes and a higher rate of drug resistance. Different drug combinations have been evaluated in the treatment of brucellosis. Considering the failure of treatment and relatively high rate of relapse of the disease with the World Health Organization's (WHO) recommended therapeutic regimen, we evaluated a new regimen that we assumed would increase the success of treatment and decrease the rate of relapse. In this study we compare the standard regimen of the WHO, doxycycline—rifampin (DR), to triple therapy with doxycycline—rifampin—amikacin (ADR).
Methods: Two hundred and twenty-eight consecutive patients with brucellosis, who attended Hamedan Sina Hospital between 1999 and 2001, whether seen as outpatients or as inpatients, were enrolled in the study. The participants were randomly allocated to the DR group (receiving doxycycline 100 mg twice a day and rifampin 10 mg/kg body weight/day every morning, both taken orally for eight weeks) or the ADR group (receiving doxycycline 100 mg twice a day and rifampin 10mg/kg body weight/day every morning, both taken orally for eight weeks, plus 7.5 mg/kg amikacin intramuscularly twice a day for seven days). The patients were checked for the relief of symptoms, drug side-effects, and relapse of disease during the treatment and follow-up.
Results: Of the 228 patients enrolled, eight were withdrawn — four patients from the DR group and four from the ADR group. Of the remaining 220 participants (110 in the ADR group and 110 in the DR group), 107 were male (48.6%) and 113 were female (51.4%). Mean age was 35.7 ± 17 years

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in the ADR group and 37 ± 18.4 years in the DR group ($p = 0.5$). In the DR group, 97 (88.2%) and in the ADR group, 106 (96.4%) of the patients had relief of symptoms (a significant difference by Chi-square test ($p = 0.04$)). After completion of treatment, and at the sixth month follow-up, nine (9.3%) patients in the DR group and six (5.7%) in the ADR group experienced a relapse of the disease, with no significant difference ($p = 0.4$). Mild side-effects were found in only 10 patients, and none required discontinuation of the therapeutic regimen. Of these patients, four were from DR group and six from ADR group; no significant difference was observed ($p = 0.7$).

Conclusions: Given the fact that the ADR regimen had a higher efficacy and more rapid action in terms of relief of symptoms compared to the DR regimen, and that no significant difference in drug side-effects and disease relapse existed in the patients of either group, adding amikacin to the DR standard treatment regimen seems beneficial.

Introduction

Brucellosis is an important zoonotic disease. Brucella bacteria can infect patients via a number of different routes including skin wounds or scratches (in occupational contact), conjunctiva, air-borne particles, and consumption of contaminated livestock products.[1,2] Brucellosis has a wide range of manifestations from an acute febrile illness to mild non-specific complaints, and its duration varies from several days to several years.

More than half a million new cases from 100 countries are reported annually to the World Health Organization (WHO). The majority of cases are living in developing countries.[3] In Iran, brucellosis is a prevalent disease; it is endemic in some provinces including Khorasan, Gilan, Mazandaran, Chaharmahal and Bakhtiari, and Hamedan.[4] In 2001 the incidence of brucellosis in Iran was 25/100 000 population, and in 2004 the incidence had increased (38/100 000). In Hamedan (west of Iran) the incidence of brucellosis in 2004 was 121/100 000.

Combination drug therapy of brucellosis leads to recovery of symptoms, shortening of symptomatic interval, and a decrease in morbidity rate, but single drug therapy is associated with more relapse episodes and a higher rate of drug resistance.[5] In 1986 a doxycycline—rifampin (DR) regimen for six weeks was recommended by the WHO as the standard drug therapy for brucellosis.[2] In experimental studies, different drug therapy regimens have been compared, such as DR and doxycycline—streptomycin (DS). In one study, the DR regimen failed in 8% of cases compared with a 2% failure rate in DS therapy. The relapse rates were 16% and 5.3% in DR and DS groups, respectively.[6]

Given the fact that therapeutic drug regimens with aminoglycosides have higher therapeutic success rates, and the fact that long-term use of these agents is associated with significant nephrotoxicity, we decided to compare the drug regimen recommended by the WHO (DR) with a therapeutic regimen including doxycycline—rifampin and a short course of an aminoglycoside (amikacin for one week) (ADR).

In research on brucellosis in Iran, *Brucella melitensis* has been reported as the most common type of Brucella. Zowghi and colleagues confirmed the high frequency of *Brucella melitensis* by extraction from infected sheep and goats. In this study 1014 samples from products of conception, including aborted fetuses of infected sheep and goats, were cultured at the Razi Institute, Tehran, Iran, between 1994 and 1996. Brucella was identified in 488 samples including 377 *Brucella melitensis* (biotype 1), 26 *Brucella melitensis* (biotype 2), 83 *Brucella melitensis* (biotype 3), and two *Brucella abortus*.[7] Due to this high frequency of *Brucella melitensis*, and to the high relapse rate of brucellosis that has been evident in Iran since 1997, the National Committee of Brucellosis of Iran proposed an eight-week therapy for brucellosis; on this basis we designed our eight-week study treatment period. Our main focus was to investigate the effect of agents that already have a proven profile of effectiveness, in a new combination.

Methods

We calculated the sample size based on a confidence interval of 95% ($\alpha = 0.05$) and a study power of 80% ($\beta = 0.20$). Our literature review showed that the probability of complete relief in patients receiving the DR regimen it is about 80% (P1) while for patients receiving the ADR regimen is about 94%. The probability of losing cases during the course of study was estimated to be 10% ($f = 10\%$) based on the results of other studies in the same setting. Based on these parameters the sample size required for each group was calculated to be 114 cases. So the total number of cases required for the study was 228.

In this study 228 consecutive patients with brucellosis who attended the Hamedan Sina Hospital between 1999 and 2001, whether seen as outpatients or as inpatients, were enrolled. A diagnosis of brucellosis was defined as: (1) brucellosis clinical features including fever, sweats, arthralgia, hepatomegaly, splenomegaly, and/or signs of focal disease with a $\geq$1/160 standard tube agglutination titer of antibodies to Brucella; or (2) a tissue sample or blood culture positive for Brucella bacteria; or (3) a four-fold increase in Wright titer in a two-week interval with compatible clinical findings.[2]

We did not use culture media (Castaneda) or rapid isolation techniques (e.g., BACTEC) for every case. Of the 228 patients, blood from 85 cases was cultured. For all patients who experienced a relapse or therapeutic failure, blood samples were cultured. In the absence of bacteriologic confirmation, a presumptive diagnosis can be made on the basis of high or rising titers of specific antibodies and characteristic clinical findings (fever, sweats, arthralgias, hepatomegaly, splenomegaly, and lymphadenopathy).[2]

In our study therapeutic failure was considered to have occurred if symptoms or signs of the disease persisted at the end of treatment, and relapse was defined by the reappearance of symptoms or signs of the disease or new positive blood cultures after therapy. A 2-mercaptoethanol (2-ME) Wright's test was carried out for all of the patients at the beginning of the study and at the end of the second and sixth months. Pregnant women, children under eight years of age, and patients with endocarditis and neurobrucellosis were excluded.

All the cases signed an informed consent document before the commencement of therapy. The ethics committee of the Hamedan University of Medical Sciences approved the study.

Using a table of random numbers, we assigned patients to receive either DR or ADR combination therapy. Patients in the DR group received doxycycline 100 mg twice a day plus rifampin 10 mg/kg body weight/day every morning, both taken orally for eight weeks. Patients in the ADR group received doxycycline 100 mg twice a day and rifampin 10 mg/kg body weight/day every morning, both taken orally for eight weeks, plus 7.5 mg/kg amikacin intramuscularly twice a day for seven days. Every patient was checked on days 7, 14, and 28, and at the end of therapy for drug side-effects. Compliance with treatment was emphasized in interviews conducted during the treatment period, and during the treatment phase this was checked at each visit (days 7, 14, 28, and at the end of therapy) by counting the pills remaining in drug containers. At these visits the subjects were asked whether they had missed any doses. For determination of aminoglycoside nephrotoxicity the patients were assessed at the outset and on days 7, 14, 28, and the end of therapy. If initial creatinine was normal, nephrotoxicity was either the elevation of creatinine to 1.5 or higher or an absolute increase of 0.4 mg/dL. Ototoxicity was assessed by audiometric study at the beginning of treatment and on day 14. Vestibular dysfunction was emphasized in interviews conducted during the treatment period.

At two months all the patients were checked for the presence of signs and symptoms, and laboratory results were studied. If recovery was achieved at this stage the treatment regimen was discontinued, but if signs and symptoms persisted the same therapeutic regimen was continued for a further four weeks. At three months, if the patient had made a full recovery, no further treatment was administered and a six-month follow-up was pursued. If the signs and symptoms persisted at the 12th week the patient was considered a case of therapeutic failure.

All patient information was recorded and analyzed with SPSS 10 software. A per protocol analysis was performed.

Results

Of the 228 patients enrolled in the study, eight were withdrawn (four in the DR group and four in the ADR group): five patients did not take the prescribed drugs correctly (three in the DR group and two in the ADR group), and three patients had a follow-up period of less than six months (one in the DR group and two in the ADR group). Of the remaining 220 participants (110 in each group), 107 were male (48.6%) and 113 were female (51.4%). In the ADR group 49% were men and in the DR group 48.2% were men, which made the two groups comparable in terms of sex distribution ($p = 0.9$ for group difference). Of all participants, 110 (50%) lived in urban areas and the rest in rural regions. Mean age was 35.7 ± 17 years in the ADR group and 37 ± 18.4 years in the DR group ($p = 0.5$).

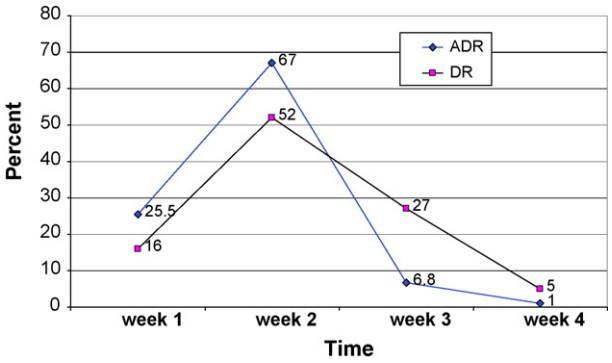

Figure 1 Percentage of patients with fever over the course of the first four weeks of treatment with either doxycycline—rifampin—amikacin (ADR) or doxycycline—rifampin (DR).

From 85 positive blood cultures (40 in the DR group and 45 in the ADR group) only 16 patients had a blood culture positive for *Brucella melitensis* (seven in the DR group and nine in the ADR group). Sixty-nine cases had a negative blood culture. Blood culture results were positive in one patient in the relapse group and one patient in the failure group (both in the DR group). Relief of symptoms was found in 97 (88.2%) patients in the DR group and in 106 (96.4%) patients in the ADR group. This was found to be a significant difference by Chi-square test ($p = 0.04$, 95% CI 0.008—0.15). In the ADR group 85 (80.2%) were treated for eight weeks and the rest were treated for 12 weeks while in the DR group 60 (61.9%) were treated for eight weeks and 37 (38.1%) received treatment for 12 weeks ($p = 0.006$).

Particular care was given to record fever relief as accurately as possible because fever had an important place in the monitoring of patient treatment. At baseline 203 (92.3%) of the patients had fever (103 in the ADR group and 100 in the DR group). In the DR group, at the end of the second week 68 cases (68%) were afebrile and at the end of the third week 95 cases (95%) were afebrile. In five patients (5%) fever persisted until the fourth week. In the ADR group, at the end of the second week 95 cases (92.2%) were afebrile and at the end of the third week 102 cases (99%) were afebrile; only one case was febrile until the fourth week (Figure 1). This difference between the two groups was significant ($p = 0.0001$, CI 0.15—0.35).

As shown in Table 1, after the completion of treatment, at the six-month follow-up, nine (9.3%) patients in the DR group and six (5.7%) in the ADR group experienced a relapse of the disease, with no significant difference ($p = 0.4$). At two and six months the 2-ME titer was checked. Of all patients, 188 were 2-ME negative at six months. In the DR group, 43 (39.1%) and 45 (40.9%) patients were 2-ME negative at the second and sixth months, respectively. In the ADR group at two- and six-months, 57 (51.8%) and 43 (39.1%) were 2-ME negative, respectively, and the difference was significant ($p = 0.03$, CI 0.01—0.27).

Mild side-effects were found in only 10 patients. Of these, four were from the DR group and six from the ADR group; no

Table 1 Demographic and treatment outcome comparisons between the two treatment groups

	DR group $n = 110$	ADR group $n = 110$	p Value
Male sex	48.2%	49%	0.9
Percentage living in urban areas	50%	50%	1
Mean age ± SD (years)	37 ± 18.4	35.7 ± 17	0.5
Symptom relief	88.2%	96.4%	0.04
Fever relief at 2 weeks	68%	92.2%	0.0001
Drug side-effects	3.6%	5.5%	0.7
Relapse of disease	9.3%	5.7%	0.4

DR, doxycycline-rifampin; ADR, doxycycline-rifampin-amikacin.

significant difference was observed ($p = 0.7$). In both groups, the treatment was usually well tolerated, although some patients had mild and reversible adverse effects. In no case were adverse effects severe enough to warrant discontinuation of therapy. In the DR group two patients had mild gastric complaints, one patient had vomiting, and one patient had genital candidiasis. In the ADR group four patients had mild gastric complaints and two patients had phototoxicity. At the end of therapy there was no impairment of renal function or ototoxicity in any patients.

Discussion

The two groups were similar in terms of age, sex, and where they lived (urban/rural locations). This showed that the two groups were similar enough to make a comparison between the results possible.

The male to female ratio was nearly one in our study. In industrial countries this ratio is 5/1 to 6/1, which is not comparable with that of our study.[8] Also in two other studies performed by Hashemi[9] and Haddadi[8] in Hamedan and Tehran, respectively, the prevalence of disease among women was higher than that reported in reference books, which might be the result of high non-pasteurized dairy consumption by women, and of women working in animal husbandry with men.

In this study we aimed to compare the efficacy of this new treatment regimen and the regimen recommended by the WHO. The rate of symptom relief in the ADR group was significantly higher than that in the DR group. During the first eight weeks of therapy, the ADR group had a significantly better response to treatment compared to the DR group. In other words, patients with the ADR treatment regimen experienced a more rapid and better recovery.

In a study by Haddadi[8] the recovery rate with the DR regimen was 88% and with a co-trimoxazole—rifampin—gentamicin regimen was 90.5%, which was higher than that of the standard regimen recommended by the WHO. In one study in Spain in 1995, the failure rate with the DR regimen was 8% and with a doxycycline—streptomycin regimen was 2%, which was comparable to our findings (11.8% failure rate with DR).[6] However a study in Saudi Arabia in 2001 reported a high resistance to rifampin and streptomycin during the previous 10 years, and as a result a 12-week treatment with this regimen was recommended for all patients with brucellosis.[10] In a cohort study in Spain in 1997, the efficacy and rate of complications of a 45-day treatment with doxycycline—gentamicin on human brucellosis was investigated. In none of the patients did therapeutic failure occur. Only in one case (5.9%) did the disease relapse after treatment.[11]

Relief of fever in patients under treatment is an appropriate marker for evaluating the rate of response to therapy. In our study 92.3% of the patients had fever at baseline, which is comparable to other reports (93—95%).[2—4] The fever relief in the ADR group was significantly higher than that in the DR group at two weeks, and the persistence of fever at the fourth week of treatment was significantly less in the ADR group than that observed in the DR group. This suggests that the new regimen is more efficient and acts faster on the relief of symptoms. In a study by Hashemi,[9] in the DR group fever was relieved in 67.7% of patients at two weeks and in 96.6% of patients at three weeks. The results are similar to our findings.

Different studies have been performed on the relapse rate of brucellosis after an appropriate course of treatment, by socio-economic status and treatment regimen, and differing results have been reported. For example in a Turkish study in 1999, the results of two different drug regimens, ciprofloxacin—rifampin and doxycycline—rifampin (DR), were compared in a group of 40 patients with brucellosis. In this study the relapse rate in the DR group was 15% while it was 10% in the other group.[12] Also in a similar study in 1999 in Spain the relapse rate was 12.5% with a netilmicin—doxycycline regimen.[13] In another study of the DR regimen this rate was 16%, but in a study in Turkey in 2002 the relapse rate with the DR regimen was 6.7%.[14] In our study the relapse rate in the DR group was less than that reported in other studies[15], and the ADR group had a lower relapse rate than that of this standard treatment, though this was not significant.

In another study in Iran two antibiotic regimens — co-trimoxazole—doxycycline (CD) vs. co-trimoxazole—rifampin (CR) — were compared over a period of two months. Failure of treatment plus relapse was 15.7% in the CD group and 26.4% in the group taking the CR regimen.[16]

In patients with brucellosis, even after appropriate antibiotic therapy, a relapse rate of 10% has been reported in the literature. This might be due to the intracellular position of the organism, which protects them from the effects of antibiotics and from the immune mechanisms of the host.[2,3]

More patients were negative for the 2-ME test at two- and six-months in the ADR group, which means that not only did this test become negative in more patients undergoing the ADR regimen, but this also happened in a shorter time.

Drug side-effects are another feature that may limit treatment. Since in the ADR regimen three drugs were used, and because aminoglycosides have multiple side-effects, we expected a higher rate of side-effects compared to the DR group, but the difference was not significant. In fact we did not have any severe complications in either of the groups that might have led to any discontinuation of the treatment. In the short-term use of amikacin (seven days) we did not have any aminoglycoside-specific side-effects such as nephrotoxicity or ototoxicity.

Given the fact that the ADR regimen had higher efficacy and more rapid action in terms of relief of symptoms compared to the DR regimen, and that no significant difference in drug side-effects and disease relapse existed in the patients of either group, adding amikacin to the DR standard treatment regimen seems beneficial.

Conflict of interest: No conflict of interest to declare.

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