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Treatment of Human Brucellosis with Rifampin plus Minocycline

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Summary

In order to evaluate the efficacy and tolerability of a high intravenous dose of rifampin plus oral minocycline (administered daily for 3 weeks) for the treatment of acute brucellosis, we retrospectively reviewed the outcome of 239 consecutive patients (135 adults and 104 children) diagnosed and treated over a 17-year period in Italy. The combination used resulted in 100% response and a relapse rate lower than 2%. Fifty-two (30 adults and 22 children) (29.8%) complained of mild adverse effects including an increase in aspartate aminotransferase (>250 IU) observed in 12 cases and considered related to rifampin and in 11 cases a reversible hyperpigmentation of the tongue attributed to minocycline. A randomized prospective comparative study should be performed to confirm our encouraging results.

Key words: **Brucella, brucellosis, Italy, minocycline, rifampin, Sicily.**

INTRODUCTION

Brucellosis is one of the world's most widespread zoonoses; the disease is common in areas where domestic animals harboring *Brucella* spp. are raised, where adequate control measures are lacking and where the population has the custom of ingesting unpastorized milk or its products. In Sicily, the largest Italian island, brucellosis is highly endemic and has shown a marked resurgence in the last few years (14 cases per 100,000) [1]. However, fewer than 200 total cases per year (0.04 cases per 100,000 population) are reported in the United States [2].

Brucella spp. are intracellular pathogens that can survive and multiply within mononuclear phagocytes (monocytes and macrophages) of the reticuloendothelial system (RES). Localization within organs of RES may explain some of the clinical manifestations of systemic brucellosis, such as hepatosplenomegaly and the propensity for involvement of the skeletal system [3]. Lack of diagnosis or effective treatment may result in serious and sometimes life-threatening complications such as spondylitis, endocarditis and encephalitis.

Brucellae are sensitive *in vitro* to a number of antibiotics; however, clinical efficacy does not always correlate with *in vitro* susceptibility. Tetracycline compounds remain the most effective antibiotics for treating brucellosis. The treatment of choice for acute brucellosis is considered a 6-week regimen of tetracycline administered orally in combination with streptomycin (1 g/day intramuscularly for 2-3 weeks) [3]. Although other antibiotics have been used, no substantial improvement in relapse rates has been reported in association with any new treatment regimen in the past 45 years [4]. In 1986 the World Health Organization (WHO) recommended therapy with the combination of doxycycline (200 mg/day) plus rifampin (600-900 mg/day) both administered once daily by mouth for 6 weeks [5]. However, a recent randomized controlled trial involving 194 adult patients indicated that treatment with a rifampin/doxycycline combination was associated

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with more relapses than that of a streptomycin/tetracycline combination (16% and 5.3%, respectively) [6]. Experience with different treatments in childhood brucellosis is sparse [7-9] and generally tetracyclines are not used in children ≤8 years of age.

Since 1984 we have principally used the combination of intravenous rifampin (10 mg/Kg, max 600 mg) and oral minocycline (children 2.5 mg/Kg, max 100) twice a day for 3 weeks for the treatment of brucellosis both for adults and children (also <8 years). We preferred minocycline to doxycycline because of its higher liposolubility and better tissue penetration, as well as its superior oral bioavailability; furthermore the drug shows higher splenic and liver levels and is also active (not chelated) in the bone tissue. Minocycline also chelates calcium less than other tetracyclines and so it is theoretically less likely to cause tooth staining (due to the deposition of tetracycline/orthophosphate complexes) [10]. Half of each dose of rifampin was administered by means of parenteral route in 15 minutes, the other half in 2 hours, in order to achieve higher blood levels for a longer period afterward, approaching the kinetic profiles and improving the theoretical synergistic effect of rifampin and minocycline - each antibiotic acts on protein synthesis with a different mechanism (rifampin inhibits the mRNA synthetase, tetracyclines block the 30S subunit of the ribosome) [11].

In this study we have retrospectively reviewed the characteristic of 239 consecutive patients with brucellosis identified over a 17-year period to evaluate the efficacy and safety of this new scheme of therapy.

PATIENTS AND METHODS

The "G. Di Cristina" Hospital in Palermo is the largest children's hospital in Sicily. It is a tertiary-care university hospital with 350 beds serving a population of about 1,000,000 in the Palermo district and, in addition, the hospital serves as referral pediatric hospital for all of western Sicily. The infectious diseases department has 40 beds for inpatient children and 5 beds both for adult and children outpatients.

All patients with brucellosis consecutively diagnosed from March 1984 through December 2000 were included in this review. A patient was considered to have brucellosis if he met at least 2 of the following criteria: 1) isolation of *Brucella* spp. from blood or other fluids or tissue; 2) finding of a ≥1:160 standard tube agglutination titer of antibodies to *Brucella* spp.; 3) presence of clinical signs compatible with brucellosis (fever, sweats, arthralgias, hepatomegaly, splenomegaly, and/or signs of focal disease). Fever was defined as a axillary temperature ≥38.0°C. Standard tube agglutination testing, the Rose-Bengal test, and the Coombs test for antibodies to *Brucella* species were done according to standard methods with commercial reagents.

Diagnosis of spondylitis or arthritis was made on the basis of physical examination and with use of radiological or magnetic resonance imaging studies.

The treatment schedule consisted of the administration of intravenous rifampin (Rifadin® Lepetit) (children 10 mg/Kg, max 600 mg) and oral minocycline (Minocin® Lederle) (children 2.5 mg/Kg, max 100 mg) twice daily for 3 weeks. Each dose of rifampin was diluted in 250 ml of 5% glucose solution, half of which was administered intravenously in 15 minutes and the other half in 2 hours. For some patients with focal disease the same treatment was prolonged for 6 weeks with rifampin given orally in the last 3 weeks.

Other schedules were based on the physician's preference or if the patient was not able to come to hospital every day for the intravenous treatment.

Clinical response was assessed at the end of treatment and was defined as cure (defervescence, restoration of laboratory parameters, or reduction of spleen size and disappearance of symptoms) or failure (persistent or worsening clinical and laboratory findings). Relapse was defined by the reappearance of symptoms or signs of the disease or by new positive blood culture after therapy.

Most of the patients were monitored for therapeutic efficacy and signs of drug toxicity through obtainment of the following clinical data: determination of blood cell counts and erythrocyte sedimentation rates, urinalysis, measurements of the creatinine, aspartate aminotransferase, alkaline phosphatase, bilirubin and electrolyte levels, *Brucella* serology and blood culture.

Following therapy, most patients were re-examined on an outpatient basis at regular 3-month intervals for 18 months as well as whenever clinical symptoms reappeared. While preparing this article, whenever feasible, we contacted patients or their parents by telephone to gather further follow-up data. Informed consent was obtained from the patients or from their parents and the guidelines of the G. Di Cristina Hospital were followed in the conduct of the clinical research.

RESULTS

From March 1984 to December 2000, 239 consecutive patients (135 adults and 94 children) were diagnosed with brucellosis and 174 treated with the above protocol. Admissions were evenly distributed throughout the years studied. All patients were HIV-negative. In all cases the disease was contracted in western Sicily, in 39 cases (9 clusters) patients belonged to the same family and contagion

was due to the ingestion of the same dairy products. The characteristics of the patients are summarized in *Table 1*.

All the children were admitted as inpatients and stayed in hospital during the treatment period; all the adults were treated as outpatients and 80 of them came to hospital twice daily for the treatment.

Therapeutic failure was not observed in any of the patients. Among the 174 patients treated with our scheme, 3 cases (2 children +1 adult) (1.7%) relapsed while 10 of the 65 (15.3 %) patients treated with other protocols did. Twelve of the 13 patients experiencing relapses were treated with a 3-week course of rifampin-minocycline, the remaining patient with a streptomycin/doxycycline combination; all these patients recovered without any sequel (*Table 2*).

The mean time to defervescence was 3.5 days (range, 1 - 6 days) in the 174 patients treated with our scheme. Fifty-two (30 adults and 22 children) (29.8%) experienced one or more adverse effects during therapy: epigastric discomfort in 36 cases; nausea in 28; diarrhea in 8, vomiting in 3, dizziness in 22; phlebitis at the infusion site in 6. In 12 patients a mild erythematous rash and asthenia occurred after the first administrations of rifampin. These reactions were classified as grade (I-II) according to WHO and did not require any discontinuation of the treatment. In 12 patients a marked increase of aspartate aminotransferase (>250 IU) was noted after the first week, and in 6 after the second week; these changes were considered to be related to rifampin; in 11 cases its administration was interrupted for a week, in four cases rifampin was replaced with streptomycin. No patients complained of teeth discoloration. Hyperpigmentation of the tongue was observed in 6 children and 5 adults and resolved spontaneously 2 weeks after discontinuation of therapy.

The median follow-up was 9 months (range, 3-18 months). Two hundred nine patients (87.4%) were followed up for at least 6 months. Five patients were lost to follow up; 40 patients were contacted by telephone interview.

DISCUSSION

One of the major concerns regarding the treatment of brucellosis is the high rate of relapse, ranging from 5% to 17% when employing either the combination of streptomycin plus doxycycline or rifampin plus doxycycline. The latter is endorsed by the WHO [6].

In a recent study the rifampin minimal inhibitory concentrations (MIC_S) for 50% and 90% of 86 isolates of *B. melitensis* were 2.5 mg/L and 4.0 mg/L respectively and rifampin has been found to be bactericidal for *Brucella* at a concentration of 4x the MIC between 24 and 48 hours [12]. Rifampin concentrates in phagocytic cells [13-14], and its activity is increased 2- to 8-fold at pH 5.0 [15], the pH of the

TABLE 1 - *Baseline characteristics of the 239 patients with brucellosis.*

Variables	**Adults n=135**	**Children n=104**
Median age (years) (range)	37 (15-86)	7 (1-14)
N. of males (%)	73 (54 %)	54 (51.9 %)
N. (%) with risk factor for brucellosis		
Occupational exposure	28 (25.9 %)	
Ingestion of unpasteurized dairy products	70 (51.8 %)	73 (70.1 %)
Duration of symptoms before therapy: median n° of days (range)	30 (4-360)	15 (4-180)
Median AST UI/L (range)	34 (8 -388)	58 (18-498)
N. with AST >100 IU (%)	17 (12.5)	29 (27.8)
Median agglutination titer (range)	320 (20 - 5,120)	908.5 (40-10,240)
N. with focal disease		
Spondylitis	2	
Coxo-phemoral arthritis	1	2
Gonarthritis	3	
Orchitis	1	
Encephalitis		1
Annexitis*	1	

*A 23-year old women, in which *B. melitensis* was cultured from the fluid present in the pouch of Douglas. She underwent left ovarectomy and salpingectomy.

TABLE 2 - *Treatment * and outcome of the 239 patients with brucellosis.*

	N.	Focal disease	Cure	Relapse	Adverse effects
Adults					
Rifampin i.v. + minocycline x 21d	80	4	80/80	1/80	30/80
Rifampin i.v. x 7d followed by rifampin p.o. x 14 d + minocycline x 21d	19	0	19/19	3/19	4/19
Rifampin p.o. + minocycline x 21d	11	0	11/11	2/11	3/11
Rifampin i.v. + minocycline x 21d followed by rifampin p.o. + minocycline x 21d (total 42 d)	13	4	13/13	0/13	12/13
Other treatment**	12	0	12/12	5/12	4/12
Children					
Rifampin i.v. + minocycline x 21d	94	1	94/94	2/94	22/94
Other treatment§	10	2	10/10	0/10	3/10

*In all cases rifampin was dosed at 10 mg/Kg (max 600 mg) twice daily. **Nine cases received: rifampin alone or combination of rifampin with ofloxacin or with doxycycline, or streptomycin + doxycycline, or minocycline + pefloxacin, in all the cases x 21 d; 3 cases received rifampin i.v. + doxycycline x 21d followed by rifampin p.o. + doxycycline x 21d (total 42 days). §Six cases were administered rifampin i.v. + doxycycline x 21d; 1 case rifampin i.v. + doxycycline + chloramphenicol x 21d; 1 case streptomycin + doxycycline x 21 days, 1 case rifampin p.o. + minocycline x 21 d; 1 case rifampin i.v. + minocycline x 21d followed by rifampin p.o. + minocycline x 21d (total 42 d).

phagolysosome of macrophages in which *Brucella* spp. grows and replicates [16].

After an intravenous dose of 600 mg the C_{max} of rifampin is 15 mg/L, while after an oral dose of 900 mg the C_{max} of rifampin (after 3 hours) is 12 mg/L. The amount of free rifampin, unbound to plasma proteins (e.g. the active fraction), is on average 17.5% (range, 10-25%) so it will be theoretically 2.6 mg/L and 2 mg/L respectively for the i.v. 600 mg and for the orally administered 900 mg. The latter value is below the MIC_{50} value. The mean half-life is 4 hours (range, 2-6 hours) [17]. With a second i.v. 600 mg dose after 12 h we should guarantee concentrations higher than the MIC of 2.5 mg/L for at least 8 hours.

In the previously cited study [12] minocycline was the most active antibiotic *in vitro* against *B. melitensis* showing the lowest MIC among all the other conventional anti-brucella antibiotics (streptomycin, cotrimoxazole, rifampin). After a single oral dose of 200 mg the C_{max} of minocycline is 4 mg/L and that of doxycycline 3.5 mg/L, then the drug concentration decreases with a half-life of 16 hours for minocycline and 18 hours for doxycycline, but the amount unbound to plasma proteins (e.g. the active fraction) is on average 32% for minocycline and only 12.5% for doxycycline, so the free minocycline is theoretically more than double the free doxycycline [18].

Based on the above reported pharmacokinetic considerations, we adopted a different regimen; the dose of rifampin was higher than generally recommended and the intravenous route of administration allowed a more favorable kinetic profile against brucellae.

Favorable results employing minocycline alone or in combination with rifampin for the therapy of brucellosis have been previously reported by Italian researchers during the mid-eighties (relapse rate of 3.4 –4%) [4].

The results of our retrospective study show that the combination of intravenous rifampin plus oral minocycline administered for 3 weeks obtained the lowest relapse rate (1.7%) so far reported for treatment of human brucellosis. In fact, in a randomized controlled trial comparing the combination therapy endorsed by WHO i.e. (doxycycline plus rifampin) versus streptomycin plus doxycycline, the relapse rates were 16% and 5.3%, respectively [6]; other studies did not report better results [3-5,19].

The likely explanation of this impressive success is probably due to the higher dose of rifampin used and the parental route of administration coupled with a therapeutic profile of minocycline that is possibly better than that of doxycycline. However, these assertions are speculative and largely unproved since no pharmacokinetic study was performed in our patients.

The most frequently observed adverse effects were dizziness and gastrointestinal symptoms, both considered to be related to minocycline.

Reports of minocycline-induced hyperpigmenta-

tion affecting the oral mucous membranes are rare. Although minocycline is the only tetracycline associated with oral mucosal pigmentation, its cause remains unknown. It has been suggested that either a minocycline-metabolite complex or melanin, iron and calcium-containing granules are the source of the pigment. Oral pigmentation develops at sites predisposed to oral trauma such as the tongue. The pigmentation appears to be unrelated to the duration of minocycline therapy or the cumulative dose, and resolves completely when the drug is discontinued [20].

However, several issues should be considered in interpreting the results of this study: first, the retrospective nature of the study and the absence of a predefined clinical end-point are recognized as a source of potential bias; second, the higher dose of rifampin used might be responsible for an increase in adverse effects; however the overall percentage of adverse effects was similar to that reported with standard regimens [5]; furthermore the parental route of administration of rifampin requiring prolonged hospitalization may be considered unfeasible in most institutions due to the cost and inconvenience to the patient. These considerations must be weighed with the cost of retreatment in case of relapse and the possible severe and unresponsiveness to therapy of organ complications. The last issue regards the use of tetracycline in children under 8 years of age, but doxycycline is recommended by the American Academy of Pediatrics for the treatment of Rocky Mountain spotted fever at any age [21]. However, no patients complained of teeth discoloration at the end of follow-up, and it is not mentioned in the works of other authors who used minocycline in children [11,22], suggesting the possibility of using this antibiotic for a short period among pediatric patients.

In conclusion, we believe that, based on our results, even if the WHO recognizes doxycycline and oral rifampin as the standard therapy of brucellosis, it would be interesting to perform a randomized, prospective, comparative study to confirm the excellent results obtained with the schedule used in our study.

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