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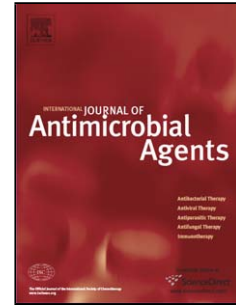


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**Efficacy of ciprofloxacin versus doxycycline as prophylaxis
against experimental murine *Brucella melitensis* infection**

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Abstract

The prophylactic potential of ciprofloxacin was assessed in comparison with doxycycline, an established therapeutic antibiotic, to limit or control infection by *Brucella melitensis* in an experimental mouse model. Ciprofloxacin treatment reduced bacterial loads in the spleens of challenged mice when administered prior to or at the same time as the bacterial challenge. In comparison, doxycycline provided much greater reductions in bacterial counts, even when treatment was initiated after infection. Doxycycline was able to protect against *B. melitensis* when administered for 5 days from 24 h after infection and for at least 28 days after cessation of the antibiotic. Overall, these results confirm that ciprofloxacin is less effective than doxycycline but suggest that it may have some utility in providing protection against low-level infections. Combination studies are indicated.

1. Introduction

Brucellosis is a significant public health problem, particularly in the Mediterranean and some developing countries. There is no vaccine available that is suitable for use in humans and antibiotic therapy remains the treatment of choice. The World Health Organization (WHO) recommends a treatment regimen for brucellosis of doxycycline plus rifampicin given for 6 weeks or doxycycline for 6 weeks plus streptomycin for 2–3 weeks [1]. However, the relapse rates associated with doxycycline/rifampicin and doxycycline/streptomycin regimens are 4.6–24% and 5–8%, respectively [2]. A recent meta-analysis suggested the use of combination regimens containing an aminoglycoside, although it has been claimed that the oral regimen is preferred. There remains a need to evaluate other antibiotic treatments. There is also a paucity of data regarding suitable antibiotic post-exposure prophylaxis, which would ideally be a single-agent, short-course, oral regimen.

Since *Brucella* spp. are considered potential bioterrorism agents [3], it may be useful to consider antibiotics currently recommended for the prophylaxis of other bioterrorism agents in order to expedite the use of a single antibiotic regimen for use in a bioterrorism outbreak scenario. The fluoroquinolone ciprofloxacin shows efficacy in treating the potential bioterrorism agents *Yersinia pestis* [4], *Francisella tularensis* [5] and *Bacillus anthracis* [6] and is recommended for post-exposure prophylaxis of infection with these organisms. Ciprofloxacin could also be potentially useful for prophylaxis of *Brucella* infection, despite a lack of efficacy in established infections. The efficacy of ciprofloxacin against *Brucella* spp. has been determined in vitro in

a number of studies, which include reported MIC₉₀ values (minimum inhibitory concentration for 90% of the organisms) of 0.19 µg/mL [7,8] and 0.25 µg/mL [9]. In comparison, the only known report of ciprofloxacin evaluation in a murine model of brucellosis indicates that the antibiotic does not afford significant protection against the disease [10]. However, ciprofloxacin administered with doxycycline or rifampicin appears to show some efficacy against brucellosis in humans [11,12]. Thus, the in vivo efficacy of ciprofloxacin against brucellosis is unclear and merits further investigation. This study aimed to determine the likely utility of ciprofloxacin compared with doxycycline for the prophylaxis of *Brucella* infection using a murine model of infection.

2. Materials and methods

2.1. Bacteria

Brucella melitensis strain H38 was used as the challenge strain in this study. Lyophilised ampoules of the strain were reconstituted with 100 µL of phosphate-buffered saline (PBS), streaked onto serum dextrose agar (SDA) plates and incubated for 3 days at 37 °C in an atmosphere containing 10% CO₂. Bacteria were harvested into 20 mL of PBS and the bacterial suspension was standardised to ca. 10¹⁰ colony-forming units (CFU)/mL prior to dilution to appropriate concentrations of inocula. The concentrations were determined retrospectively by enumeration of ten-fold dilutions of the inocula on SDA plates.

2.2. Antibiotics

Doxycycline powder (Sigma, Poole, UK) was dissolved in PBS and ciprofloxacin tablets (Bayer, Newbury, UK) was dissolved in distilled water to a working concentration of 8 mg/mL. Antibiotics were prepared freshly each day and sterilised through a 0.2 µm filter.

2.3. Animals

The efficacy of ciprofloxacin or doxycycline for treating *B. melitensis* infection was evaluated in groups of five 6–7-week-old, female, BALB/c mice (Harlan UK Ltd., Bicester, UK). All experimental work was conducted in flexible film isolators and according to a UK Home Office licence. Briefly, mice were administered 100 µL of PBS or antibiotic solution (equivalent to 40 mg/kg in a 20 g mouse) twice daily by subcutaneous injection. The antibiotic treatment was started either at 48 h prior to challenge (and continued for 7 days), at the same time as challenge (continued for 5 days) or 24 h after challenge (continued for 5 days). Mice were challenged with either a low dose (10^2 – 10^3 CFU) or a high dose (10^6 – 10^7 CFU) of *B. melitensis* H38 by intraperitoneal injection (100 µL). Animals were culled at either 48 h or 28 days after the final antibiotic administration. At post-mortem, spleens were removed and homogenised in 5 mL of distilled water using a stomacher (Colworth, London, UK) and bacterial loads were determined following enumeration of ten-fold serial dilutions on SDA plates (incubated for 3 days at 37 °C in air plus 10% CO₂).

2.4. Statistical analyses

Data were transformed into \log_{10} CFU and analysed using GraphPad Prism V4.0. *P*-values were calculated using one-way analysis of variance (ANOVA) to calculate the effect of treatment compared with the control, using Bonferroni's post-test used to compare individual time points with the control.

3. Results and discussion

Although MIC data indicate that the fluoroquinolone ciprofloxacin is effective at killing *Brucella* spp., this study suggests that it is less effective than doxycycline at preventing *B. melitensis* infection in vivo (Fig. 1). However, ciprofloxacin does offer some protection in the murine model against *B. melitensis*, measured by a reduction in bacterial load in the spleen compared with control. Specifically, when administered 48 h prior to infection with low or high challenge doses, a 7-day course of ciprofloxacin reduced splenic bacterial counts at 48 h after cessation of treatment (Fig. 1A; $P < 0.05$; Fig. 1B, $P < 0.01$). Similarly, when administered at the same time as infection, the antibiotic had a limited effect (Fig. 1B; $P < 0.05$). However, ciprofloxacin was ineffective when started 24 h post challenge both at 48 h and 28 days after antibiotic treatment (Fig. 1C,D). In comparison, our data confirm the utility of doxycycline for preventing *Brucella* infection. Significant protection was observed in mice treated with this antibiotic in all groups, i.e. either prior to exposure, at the same time as exposure or 24 h after exposure to either a low (Fig. 1A,C) or high challenge dose (Fig. 1B,D). Furthermore, this significant

protection was observed 28 days after the cessation of antibiotic treatment (Fig. 1C,D; $P < 0.001$ in all cases).

Although MIC data indicate that doxycycline is more effective against *Brucella* spp. than ciprofloxacin [7,9,10], it is surprising that ciprofloxacin is not more effective in treating brucellosis in vivo. Data reported by Shasha et al. [10] indicate that mice treated with ciprofloxacin for 14 days or 21 days do not clear a *B. melitensis* infection. Our data are consistent with the relatively poor efficacy of ciprofloxacin for treating brucellosis compared with doxycycline but highlight the ability of ciprofloxacin potentially to provide a low level of protection. This might be exploited in combination prophylaxis regimens. Further studies on antibiotic combinations that include a fluoroquinolone for post-exposure prophylaxis of *Brucella* infection are indicated.

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Competing interests: None declared.

Ethical approval: All animal studies were carried out in accordance with the Animals Scientific Procedures Act 1986 and the Codes of Practice for the Housing and Care of Animals used in Scientific Procedures 1989.

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Fig. 1. Protective efficacy of ciprofloxacin and doxycycline against *Brucella melitensis* infection in the mouse model. Groups of five BALB/c mice were challenged with ca. 10^2 – 10^3 colony-forming units (CFU) (A,C) or 10^6 – 10^7 CFU (B,D) of *B. melitensis* H38 and treated with ciprofloxacin (Cipro) or doxycycline (Doxy) starting at 48 h prior to challenge (PRE, for 7 days), the same time as challenge (CO, for 5 days) or 24 h after challenge (POST, for 5 days). Control animals were treated with phosphate-buffered saline (PBS) for 7 days (Control 1 for PRE and CO groups) or 8 days (Control 2 for POST groups). At 48 h (A,B) or 28 days (C,D) after the final antibiotic administration, bacterial loads in the spleens were enumerated. Significance markers (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) are indicative of one-way analysis of variance (ANOVA) with Bonferroni's post test; ns, not significant.

