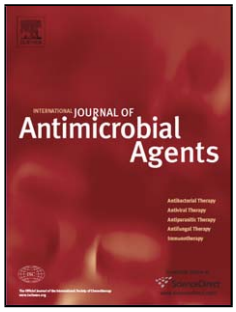
Efficacy of moxifloxacin or gatifloxacin as prophylaxis against experimental murine infection

Helen S. Atkins, Stephen Spencer, Simon D. Brew, Thomas R. Laws, Rachel E. Thirlwall, Alastair P. Macmillan, Timothy J.G. Brooks, Andrew J.H. Simpson



Title: Efficacy of moxifloxacin or gatifloxacin as prophylaxis against experimental murine *Brucella melitensis* infection

Authors: Helen S. Atkins, Stephen Spencer, Simon D. Brew, Thomas R. Laws, Rachel E. Thirlwall, Alastair P. MacMillan, Timothy J.G. Brooks, Andrew J.H. Simpson

PII: S0924-8579(09)00322-7
DOI: doi:10.1016/j.ijantimicag.2009.06.018
Reference: ANTAGE 3075

To appear in: *International Journal of Antimicrobial Agents*

Received date: 17-4-2009
Revised date: 12-6-2009
Accepted date: 15-6-2009

Please cite this article as: Atkins HS, Spencer S, Brew SD, Laws TR, Thirlwall RE, MacMillan AP, Brooks TJG, Simpson AJH, Efficacy of moxifloxacin or gatifloxacin as prophylaxis against experimental murine *Brucella melitensis* infection, *International Journal of Antimicrobial Agents* (2008), doi:10.1016/j.ijantimicag.2009.06.018

Efficacy of moxifloxacin or gatifloxacin as prophylaxis against experimental murine *Brucella melitensis* infection

Helen S. Atkins [a,*], Stephen Spencer [b], Simon D. Brew [b], Thomas R. Laws [a], Rachel E. Thirlwall [b], Alastair P. MacMillan [b], Timothy J.G. Brooks [a,1], Andrew J.H. Simpson [a]

[a] *Department of Biomedical Sciences, Defence Science and Technology Laboratory, Porton Down, Salisbury SP4 0JQ, UK*

[b] *Department of Statutory and Exotic Bacterial Diseases, Veterinary Laboratories Agency, Woodham Lane, New Haw, Addlestone, Surrey KT15 3NB, UK*

ARTICLE INFO

Article history:

Received 17 April 2009

Accepted 15 June 2009

Keywords:

Brucella

Antibiotic

Fluoroquinolone

Moxifloxacin

Gatifloxacin

Mouse

* Corresponding author. Tel.: +44 1980 614 755; fax: +44 1980 614 307.

E-mail address: hsatkins@dstl.gov.uk (H.S. Atkins).

[1] Present address: Health Protection Agency Centre for Emergency Preparedness & Response, Porton Down, Salisbury SP4 0JG, UK.

ABSTRACT

The prophylactic potential of moxifloxacin and gatifloxacin was assessed in comparison with doxycycline, an established therapeutic antibiotic, to limit or control infection by *Brucella melitensis* in an experimental mouse model, determined by reduced bacterial burden in the spleen. Although moxifloxacin was found to have a small protective effect when administered 6 h following infection, neither moxifloxacin nor gatifloxacin showed significant efficacy in vivo. In comparison, doxycycline provided significant protection when prophylaxis was started at 6 h, 7 days or 14 days following infection. Overall, these results confirm the utility of doxycycline in the prophylaxis of brucellosis and suggest that neither moxifloxacin nor gatifloxacin are likely to be valuable for post-exposure prophylaxis of *Brucella* infection.

1. Introduction

Brucellosis is a significant public health problem and, due to the lack of a licensed human vaccine, antibiotics remain the treatment of choice. However, relatively high relapse rates are associated with the World Health Organization's recommended treatment regimens of rifampicin plus doxycycline given for 6 weeks or doxycycline for 6 weeks plus streptomycin for 2–3 weeks [1]. Data are also lacking on prophylactic regimens following possible exposure to *Brucella* spp. Thus, there is a need to evaluate other antibiotic treatments. Clinical experience with fluoroquinolones such as ciprofloxacin for the treatment of brucellosis has been disappointing, however newer fluoroquinolones have been developed in recent years. The efficacy of two recent-generation fluoroquinolones, moxifloxacin and gatifloxacin, against *Brucella* spp. has been determined in vitro in a number of studies that include reported MIC_{90} values (minimum inhibitory concentration for 90% of the organisms) of 0.125 μg/mL [2], 1 μg/mL [3] or 8 μg/mL [4] for moxifloxacin and 2 μg/mL [3] or 4 μg/mL [5] for gatifloxacin. In one study, the MIC_{90} values for moxifloxacin and gatifloxacin against 160 strains of *Brucella melitensis* were found to be 1 μg/mL and 2 μg/mL, respectively [3], highlighting the efficacy of the antibiotics against a wide range of strains. However, the likely efficacy of these antibiotics in vivo is unclear. The only known report of moxifloxacin evaluation in a rat model of brucellosis indicated that it may be an alternative choice for antibiotic treatment of brucellosis [6]. There is no known evaluation of gatifloxacin treatment of brucellosis in vivo. In this study, we evaluated the potential efficacy of moxifloxacin and gatifloxacin for post-exposure

prophylaxis and treatment of experimental brucellosis in an established BALB/c mouse model.

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% CO_2. Bacteria were harvested into 20 mL of PBS and the bacterial suspension was standardised to ca. 10^{10} 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

Moxifloxacin (Bayer) and gatifloxacin tablets (Bristol-Myers Squibb) were dissolved in distilled water to 100 mg/mL, and doxycycline powder (Sigma) was dissolved in distilled water to 40 mg/mL. Antibiotic solutions were prepared freshly each day and sterilised through a 0.2 μm filter.

2.3. Animals

The efficacy of moxifloxacin and gatifloxacin compared with doxycycline as prophylaxis for *B. melitensis* infection was evaluated in groups of five female, 6–7-week-old, BALB/c mice (obtained from Harlan, UK). All experimental work was conducted in flexible film isolators according to a UK Home Office licence.

In this study, mice were orally administered 100 μL of distilled water (as control) or antibiotic solution (equivalent to 40 mg/kg in a 20 g mouse) twice daily for 14 days. The antibiotic treatment was started at 6 h, 7 days or 14 days following challenge with ca. 10^3 CFU of *B. melitensis* H38 by intraperitoneal injection. Animals were culled at 7, 14, 21, 28, 35, 42, 49 or 56 days, as appropriate, after challenge to evaluate the effect of the antibiotics over time. At post-mortem, spleens were removed, homogenised in 3 mL of distilled water using a Colworth stomacher (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% CO_2).

2.4. Statistical analyses

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

3. Results and discussion

Although MIC data indicate that the fluoroquinolones moxifloxacin and gatifloxacin are effective at killing *Brucella* spp. in vitro, data from this study suggest that these antibiotics are relatively ineffective at preventing a *B. melitensis* infection in vivo. Moxifloxacin applied at 6 h post infection had a small effect (ca. five-fold drop on average) on the bacterial load within the spleen compared with the sham-treated control ($P < 0.01$) (Fig. 1a). Moxifloxacin also had a transient effect when applied at 14 days post infection, significantly reducing the number of bacteria within the spleen at 7 days after the onset of treatment (i.e. 21 days post infection) ($P < 0.05$) (Fig. 1c). In all other circumstances moxifloxacin had no effect ($P > 0.05$). Gatifloxacin had no effect on bacterial numbers within the spleen at any time points or for any treatment regimes ($P > 0.05$) (Fig. 1).

These data also confirm the utility of doxycycline in the prophylaxis of *Brucella* infection. Significant protection was observed in animals treated with this antibiotic compared with those given distilled water, evidenced by a reduced bacterial load in the spleen ($P < 0.01$ in all cases). Doxycycline administered at all treatment times evaluated reduced the bacterial load ca. 3000-fold by 7 days after onset of treatment. However, at the end of antibiotic treatment the numbers of bacteria in the spleens of mice treated from Day 14 post infection had ca. 400 000-fold fewer bacteria than those given the control. Conversely, mice treated at 6 h or 7 days post infection had only ca. 500-fold fewer bacteria than the control. This difference was found to be statistically significant using Bonferroni's post-test on the treatment-associated bacterial

load reductions ($P < 0.01$). This indicates the role of factors other than the effects of treatment. Conceivably, the maturity of the immune response and/or the increase in innate immune agonists (in the form of *Brucella* bacterial burden) in mice treated with doxycycline from Day 14 post infection may contribute to the bacterial clearance observed.

Our data are consistent with the reported poor efficacy of fluoroquinolones for treating brucellosis compared with doxycycline and suggest that neither moxifloxacin nor gatifloxacin are likely to be valuable as single agents for post-exposure prophylaxis of *Brucella* infections. The data provide evidence that doxycycline alone may have some value, but further studies on antibiotic combinations for post-exposure prophylaxis of *Brucella* infection are also indicated.

Acknowledgments: The authors gratefully acknowledge the technical assistance provided by Dr Jackie Steward and the Brucella Research Team and the Animal Services Unit at Veterinary Laboratories Agency in this study.

Funding: This work was funded by the UK Ministry of Defence.

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.

References

[1] Roushan MRH, Mohraz M, Hajlahmadi M, Ramzani A, Valayati AA. Efficacy of gentamicin plus doxycycline versus streptomycin plus doxycycline in the treatment of brucellosis in humans. Clin Infect Dis 2006;42:1075–80.

[2] Kilic S, Dizbay M, Cabadak H. In vitro activity of tigecycline, tetracycline and fluoroquinolones against *Brucella melitensis.* J Chemother 2008;20:33–7.

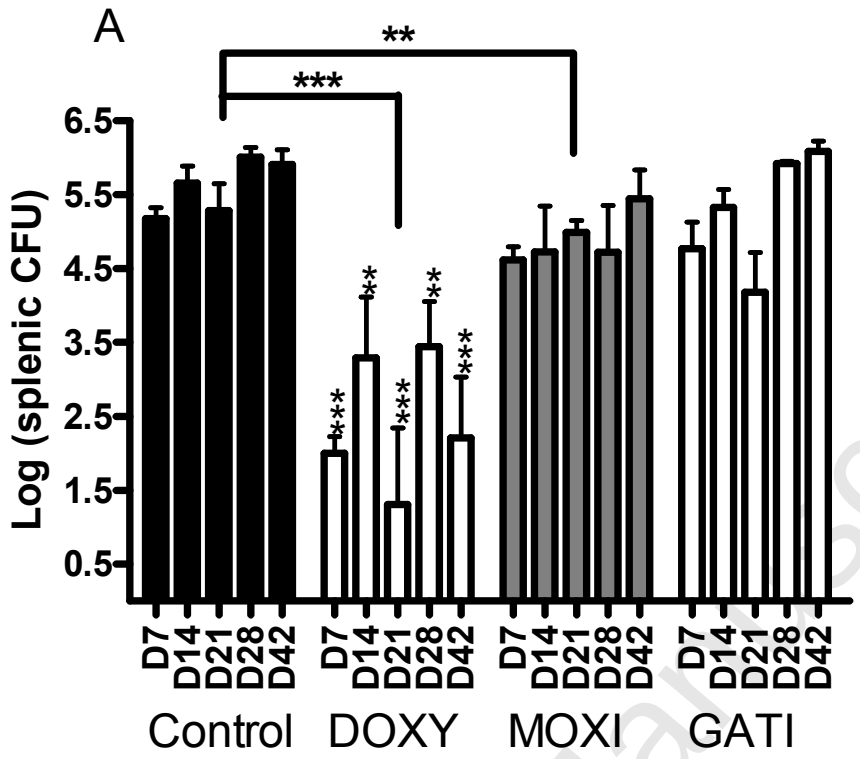
[3] Trujillano-Martin I, Garcia-Sanchez E, Martinez IM, Fresnadillo MJ, Garcia-Sanchez JE, Garcia-Rodriguez JA. In vitro activities of six new fluoroquinolones against *Brucella melitensis.* Antimicrob Agents Chemother 1999;43:194–5.

[4] Yamazhan T, Aydemir S, Tunger A, Serter D, Gokengin D. In vitro activities of various antimicrobials against *Brucella melitensis* strains in the Aegean region in Turkey. Med Princ Pract 2005;14:413–6.

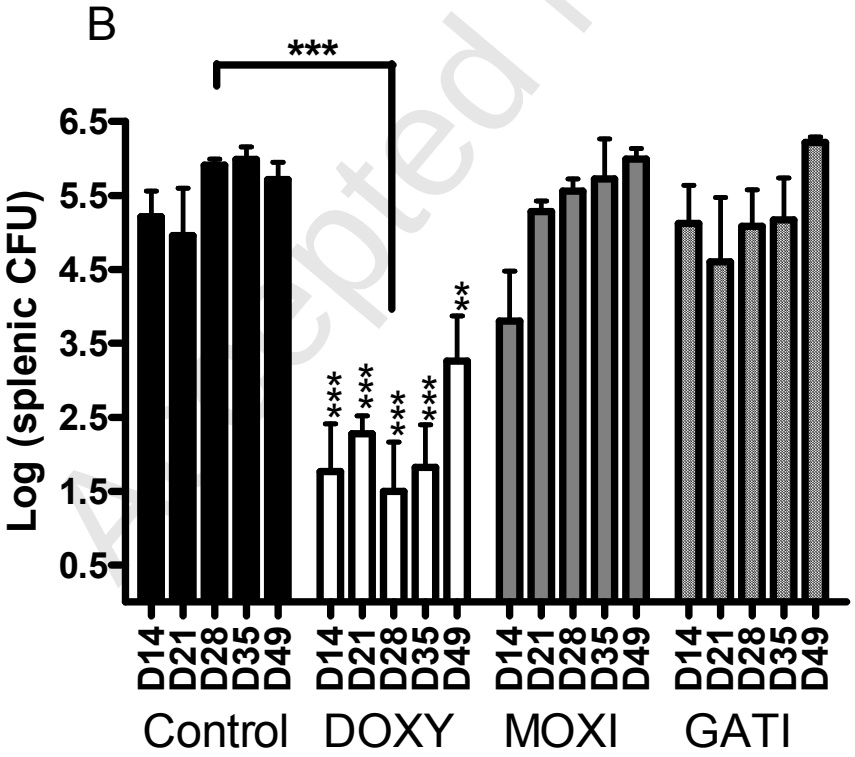
[5] Kocagöz S, Akova M, Altun B, Güur D, Hasçelik G. In vitro activities of new quinolones against *Brucella melitensis* isolated in a tertiary-care hospital in Turkey. Clin Microbiol Infect 2002;8:240–2.

[6] Sezak N, Kuruuzum Z, Cakir N, Yuce A. Comparison of rifampicin and moxifloxacin efficacy in an experimental model of animal brucellosis. J Chemother 2008;20:58–62.

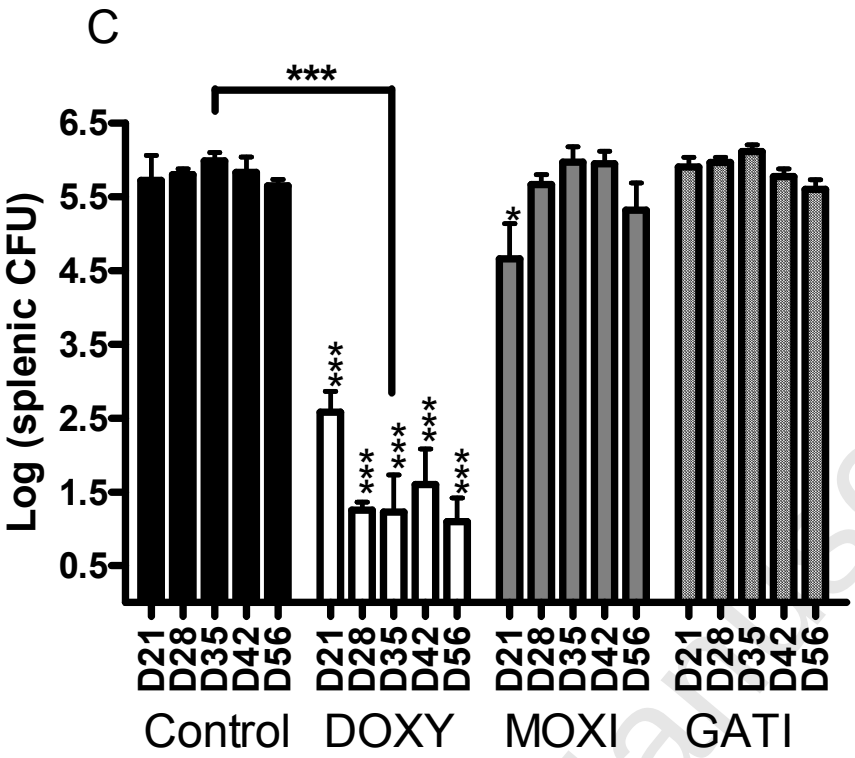
Fig. 1. Protective efficacy of moxifloxacin, gatifloxacin and doxycycline against *Brucella melitensis* infection. Groups of five BALB/c mice were challenged with ca. 10^3 colony-forming units (CFU) of *B. melitensis* H38 by intraperitoneal injection and treated with doxycycline (DOXY), moxifloxacin (MOXI) or gatifloxacin (GATI) or a distilled water sham orally, twice a day for 14 days. Antibiotic treatment started at (A) 6 h, (B) 7 days or (C) 14 days after infection. Bacterial loads in the spleens were enumerated at 7, 14, 21, 28, 35, 42, 49 or 56 days, as appropriate. Each bar is representative of the mean bacterial count of five mice ± the standard error of the mean. Significance markers (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) are indicative of a two-way analysis of variance (ANOVA) (brackets between conditions) or Bonferroni's post tests (markers above bars).



1



2



3