



HAL
open science

Evaluation of azithromycin, trovafloxacin and grepafloxacin as prophylaxis against experimental murine infection

Helen S. Atkins, Stephen Spencer, Simon D. Brew, Dominic C. Jenner, Armine M. Sefton, Alastair P. Macmillan, Timothy J.G. Brooks, Andrew J.H. Simpson

► To cite this version:

Helen S. Atkins, Stephen Spencer, Simon D. Brew, Dominic C. Jenner, Armine M. Sefton, et al.. Evaluation of azithromycin, trovafloxacin and grepafloxacin as prophylaxis against experimental murine infection. *International Journal of Antimicrobial Agents*, 2010, 36 (1), pp.66. 10.1016/j.ijantimicag.2009.10.003 . hal-00594501

HAL Id: hal-00594501

<https://hal.science/hal-00594501>

Submitted on 20 May 2011

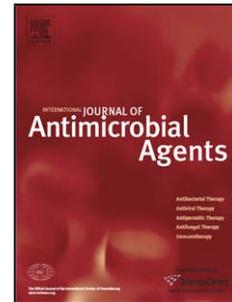
HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Evaluation of azithromycin, trovafloxacin and grepafloxacin as prophylaxis against experimental murine *Brucella melitensis* infection

Authors: Helen S. Atkins, Stephen Spencer, Simon D. Brew, Dominic C. Jenner, Armine M. Sefton, Alastair P. MacMillan, Timothy J.G. Brooks, Andrew J.H. Simpson



PII: S0924-8579(09)00466-X
DOI: doi:10.1016/j.ijantimicag.2009.10.003
Reference: ANTAGE 3155

To appear in: *International Journal of Antimicrobial Agents*

Received date: 2-10-2009
Accepted date: 2-10-2009

Please cite this article as: Atkins HS, Spencer S, Brew SD, Jenner DC, Sefton AM, MacMillan AP, Brooks TJG, Simpson AJH, Evaluation of azithromycin, trovafloxacin and grepafloxacin as prophylaxis against experimental murine *Brucella melitensis* infection, *International Journal of Antimicrobial Agents* (2008), doi:10.1016/j.ijantimicag.2009.10.003

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

**Evaluation of azithromycin, trovafloxacin and grepafloxacin
as prophylaxis against experimental murine *Brucella
melitensis* infection**

Helen S. Atkins ^{a,*}, Stephen Spencer ^b, Simon D. Brew ^b, Dominic C. Jenner ^a,
Armine M. Sefton ^c, 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*

^c *Centre for Infectious Disease, Barts and The London, Queen Mary's School
of Medicine and Dentistry, Whitechapel, London E1 2AD, UK*

ARTICLE INFO

Article history:

Received 2 October 2009

Accepted 2 October 2009

Keywords:

Brucella

Macrolide

Fluoroquinolone

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.

© Crown Copyright 2009. Published with permission of the Defence Science and Technology Laboratory on behalf of the Controller of HMSO.

ABSTRACT

The prophylactic potential of the azalide azithromycin as well as the fluoroquinolones trovafloxacin and grepafloxacin was assessed for the control of infection with *Brucella melitensis* in an experimental mouse model, determined by reduction in splenic bacterial burden. Trovafloxacin showed limited protective efficacy when administered 2 h following a low-dose *B. melitensis* challenge, whereas grepafloxacin was ineffective. In comparison, azithromycin provided significant control of infection both following low- and high-dose challenges. Overall, the data confirm the potential utility of azithromycin in the prophylaxis of brucellosis and suggest that neither trovafloxacin nor grepafloxacin would likely be valuable for post-exposure prophylaxis of *Brucella* infection.

1. Introduction

Brucellosis, caused by *Brucella* spp., remains a public health problem in many areas of the world. However, relatively high relapse rates are associated with the World Health Organization's recommended treatment regimens (rifampicin plus doxycycline for 6 weeks, or doxycycline for 6 weeks plus streptomycin for 2–3 weeks) [1]. Thus, there is a need to evaluate other antibiotic treatments. In particular, data are lacking regarding suitable post-exposure antibiotic prophylaxis, which would ideally be a single-agent, short-course, oral regimen.

Clinical experience with fluoroquinolones such as ciprofloxacin for treatment of brucellosis has been disappointing. However, newer fluoroquinolones have been developed in recent years, with reported MIC₉₀ values (minimum inhibitory concentration for 90% of organisms) for *Brucella melitensis* ranging from 0.5 µg/mL [2] to 1 µg/mL [3] for trovafloxacin and from 1 µg/mL [2] to 2 µg/mL [3] for grepafloxacin. Similarly, low MIC₉₀ values for *B. melitensis* have been reported using the azalide azithromycin [4,5], and a limited number of in vivo efficacy studies have shown that this antibiotic significantly reduces *Brucella* infection [6–8]. Unfortunately, grepafloxacin has been withdrawn from the market, whilst use of trovafloxacin has been severely limited due to safety concerns [2]. However, prior to their withdrawal we initiated studies to evaluate the potential utility of trovafloxacin and grepafloxacin as well as azithromycin for the treatment of brucellosis. Here we describe an assessment of the potential efficacy of these antibiotics for post-exposure

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

2. Materials and methods

2.1. Antibiotics

Antibiotic solutions were prepared freshly each day. Azithromycin (Pfizer, Sandwich, UK) was prepared by dissolving in a minimal volume of 98% ethanol (Sigma, Poole, UK) and subsequently dissolving in 0.5% methylcellulose (Sigma) in distilled water to achieve a dose of 40 mg/kg in a volume of 50 μ L/mouse. Trovafloxacin (Pfizer) and grepafloxacin (GlaxoSmithKline, Stevenage, UK) were dissolved in 0.5% methylcellulose in distilled water to achieve a dose of 40 mg/kg in a volume of 20 μ L/mouse.

2.2. Animals

The efficacy of azithromycin, trovafloxacin and grepafloxacin as prophylaxis for *B. melitensis* infection was evaluated in groups of five 6–7-week-old female BALB/c mice (Harlan, UK). In this study, four separate experiments were performed. Mice were dosed orally once daily for 14 days with 50 μ L of azithromycin solution, 20 μ L of trovafloxacin or grepafloxacin solution (each equivalent to 40 mg/kg in a 20 g mouse) or distilled water (as antibiotic diluent control). Antibiotic treatment was started at 2 h following challenge with a low [ca. 10^2 – 10^3 colony-forming units (CFU)] or high (ca. 10^6 – 10^7 CFU) dose of *B. melitensis* strain H38 by intraperitoneal injection, prepared as described

previously [9,10]. Animals were culled at 24 h, 14 days or 28 days after the last antibiotic dose to evaluate the effect of antibiotics over time. At post-mortem, spleens were removed and homogenised in 3 mL of distilled water using a Colworth stomacher® (Seward Ltd., London, UK) and bacterial loads were determined following enumeration of ten-fold serial dilutions on serum dextrose agar plates incubated for 3 days at 37 °C in air + 10% CO₂.

2.3. Statistical analyses

Data were transformed into log₁₀ CFU and 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

Splenic bacterial load data are shown in Fig. 1. Although MIC data indicate that the fluoroquinolones trovafloxacin and grepafloxacin are effective at killing *Brucella* spp. in vitro, the data from this study suggest that these antibiotics are relatively ineffective at preventing a *B. melitensis* infection in vivo.

Trovafloxacin administered 2 h post infection to mice given a low challenge of *B. melitensis* reduced the bacterial load within the spleen compared with the sham-treated control ($P < 0.05$) on Days 1 and 14 post treatment. However, the protective effect of trovafloxacin was not observed on Day 28 post treatment, indicating relapse. Overall, trovafloxacin was not effective against a

high challenge of *B. melitensis*, and grepafloxacin was ineffective against both challenge doses.

These data also confirm the utility of azithromycin for prophylaxis of *Brucella* infection. Overall, 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 ($P < 0.001$). Azithromycin administered 2 h after a low challenge dose of *B. melitensis* afforded significant protection against *B. melitensis* infection. On Days 1 or 14 post treatment, four mice and three mice, respectively, were found to have no detectable bacterial loads in their spleens following the low-dose *B. melitensis* challenge. However, bacteria were detectable on Day 28 post treatment, indicating partial relapse. Azithromycin also significantly reduced splenic bacterial loads at all three time points compared with control treatment following a high challenge dose of *B. melitensis*.

Overall, these data are consistent with our previous reports of the poor efficacy of fluoroquinolones for treating brucellosis [9,10] and suggest that neither trovafloxacin nor grepafloxacin would likely be valuable as single agents for post-exposure prophylaxis of *Brucella* infections. However, the data provide additional evidence that azithromycin alone may have some value. Further studies on antibiotic combinations involving azithromycin for post-exposure prophylaxis of *Brucella* infection are warranted.

Acknowledgments

The authors gratefully acknowledge the technical assistance provided by 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.

Ethical approval

All experimental work was conducted in flexible film isolators according to a UK Home Office licence. 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] Kocagoz S, Akova M, Altun B, Gur D, Hascelik 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.
- [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] Landinez R, Linares J, Loza E, Martinez-Beltran J, Martin R, Baquero F. In vitro activity of azithromycin and tetracycline against 358 clinical isolates of *Brucella melitensis*. *Eur J Clin Microbiol Infect Dis* 1992;11:265–7.
- [5] Akova M, Gur D, Livermore DM, Kocagoz T, Akalin HE. In vitro activities of antibiotics alone and in combination against *Brucella melitensis* at neutral and acidic pHs. *Antimicrob Agents Chemother* 1999;43:1298–300.
- [6] Lang R, Shasha B, Ifrach N, Tinman S, Rubinstein E. Therapeutic effects of roxithromycin and azithromycin in experimental murine brucellosis. *Chemotherapy* 1994;40:252–5.
- [7] Domingo S, Gastearena I, Vitas AI, Lopez-Goni I, Dios-Vieitez C, Diaz R, et al. Comparative activity of azithromycin and doxycycline against *Brucella* spp. infection in mice. *J Antimicrob Chemother* 1995;36:647–56.

- [8] Domingo S, Gamazo C. Inadequate azithromycin activity against *Brucella melitensis* in mice with acute or chronic infections. *J Chemother* 1996;8:55–8.
- [9] Atkins HS, Spencer S, Brew SD, Jenner DC, Russell P, MacMillan AP, et al. Efficacy of ciprofloxacin or doxycycline as prophylaxis against experimental murine *Brucella melitensis* infection. *Int J Antimicrob Agents* 2009;34:474–6.
- [10] Atkins HS, Spencer S, Brew SD, Laws TP, Thirlwall RE, MacMillan AP, et al. Efficacy of moxifloxacin or gatifloxacin as prophylaxis against experimental murine *Brucella melitensis* infection. *Int J Antimicrob Agents* 2009;34:471–3.

Fig. 1. Protective efficacy of azithromycin, trovafloxacin and grepafloxacin against *Brucella melitensis* infection. Groups of five BALB/c mice were challenged with (A) ca. 10^2 – 10^3 colony-forming units (CFU) or (B) ca. 10^6 – 10^7 CFU of *B. melitensis* H38 by intraperitoneal injection and were treated with azithromycin (Azith), grepafloxacin (Grep), trovafloxacin (Trova) or distilled water orally, starting at 2 h post infection, daily for 14 days. Bacterial loads in the spleens were enumerated at 1, 14 or 28 days after cessation of treatment. Each bar is representative of the \log_{10} mean bacterial counts per spleen of five mice \pm standard error of the mean. Significance markers (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) are indicative of two-way analysis of variance (ANOVA) (brackets above treatments) with a Bonferroni's post-test (markers above individual bars).

1

