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Trimethoprim/sulfamethoxazole (co-trimoxazole) prophylaxis is effective against acute murine inhalational melioidosis and glanders

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ABSTRACT

Burkholderia pseudomallei is the causative agent of the disease melioidosis, which is prevalent in tropical countries and is intractable to a number of antibiotics. In this study, the antibiotic co-trimoxazole (trimethoprim/sulfamethoxazole) was assessed for the post-exposure prophylaxis of experimental infection in mice with *B. pseudomallei* and its close phylogenetic relative *Burkholderia mallei*, the causative agent of glanders. Co-trimoxazole was effective against an inhalational infection with *B. pseudomallei* or *B. mallei*. However, oral co-trimoxazole delivered twice daily did not eradicate infection when administered from 6 h post exposure for 14 days or 21 days, since infected and antibiotic-treated mice succumbed to infection following relapse or immunosuppression. These data highlight the utility of co-trimoxazole for prophylaxis both of *B. pseudomallei* and *B. mallei* and the need for new approaches for the treatment of persistent bacterial infection.

1. Introduction

Burkholderia pseudomallei and *Burkholderia mallei* are close phylogenetically related species and are the causative agents of melioidosis and glanders, respectively, which are diseases both of animals and humans. Human melioidosis is a severe disease that is endemic in tropical countries. Melioidosis may present as an acute infection with pneumonia and septicaemia and can be rapidly progressive with high mortality rates. However, subacute and chronic forms of melioidosis also exist, and re-activation following sometimes lengthy latent periods is common [1], possibly due to the ability of the organism to survive intracellularly for long periods. The natural mode of transmission is thought to be predominantly through inoculation into skin abrasions or by inhalation, with incubation periods ranging from 1 day to 21 days. However, there is increasing evidence that inhalation following aerosolisation of *B. pseudomallei* may account for the high mortality of melioidosis cases that occur after severe weather events [2]. In comparison, glanders is primarily an equine disease and, although infections in humans are uncommon, *B. mallei* has been used as a biological weapon, both in World War I and World War II [3]. Like *B. pseudomallei*, *B. mallei* is highly infectious by the respiratory route and has a high mortality rate if left untreated [4].

No vaccines are currently available for melioidosis or glanders. Furthermore, there are relatively few data available on the prophylaxis of melioidosis or glanders to inform current guidance on the management of human exposures [5]. The current recommended treatment for acute melioidosis infection is high-dose intravenous ceftazidime or a carbapenem for at least 10–14 days, followed by lengthy oral eradication therapy [6]. Both organisms are susceptible to the tetracyclines, co-trimoxazole [a fixed-ratio (1:5) combination of trimethoprim and sulfamethoxazole], amoxicillin/clavulanic acid, third-generation cephalosporins and chloramphenicol [7]. Oral regimens comprising co-trimoxazole and doxycycline have been shown to be as effective as the conventional four-dose regimen of chloramphenicol, co-trimoxazole and doxycycline for eradication therapy in melioidosis [8]. Co-trimoxazole alone has been used successfully in Australia [6], but doxycycline alone was disappointing as eradication therapy in Thailand and is not recommended [9]. Since there is little evidence available related to antibiotic treatment of glanders in humans, it is recommended that cases should be treated with the same regimens as used for melioidosis [5].

The BALB/c mouse is considered to be an appropriate model for the study of melioidosis and glanders [10,11]. In this study, the efficacy of co-trimoxazole was assessed for use as post-exposure prophylaxis (PEP) in experimental BALB/c models of inhalational infection with *B. pseudomallei* and *B. mallei*.

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2. Materials and methods

2.1. Bacteria

All bacteriological procedures were carried out in a Class III microbiological safety cabinet within an Advisory Committee on Dangerous Pathogens (ACDP) Containment Level 3 laboratory. *B. pseudomallei* strain K96243 was prepared as previously described [12]. *B. mallei* strain ATCC 23344 was cultured in nutrient broth (bioMérieux, Basingstoke, UK) with incubated shaking at 180 rpm at 37 °C for 48 h. Aliquots were stored in 40% glycerol at −80 °C. All bacteria were routinely enumerated on nutrient agar (bioMérieux) cultured at 37 °C for 48–72 h.

2.2. Minimum inhibitory concentration (MIC) assays

MICs for co-trimoxazole were determined using the broth microdilution method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [13].

2.3. Animals

All animal studies were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986 and the Codes of Practice for the Housing and Care of Animals used in Scientific Procedures 1989. Female BALB/c mice (Charles River Laboratories, Maidstone, UK) aged 6–12 weeks were randomised into cages. Bacterial challenge studies were performed within an ACDP animal Containment Level 3 facility. Mice were allowed free access to water and rodent diet (Harlan Teklad, Bicester, UK) and underwent a 7-day acclimatisation period before any procedures were undertaken.

2.4. Determination of co-trimoxazole concentration following oral dosing

Groups of five mice were administered one oral dose of 4.8 mg of co-trimoxazole (0.8 mg trimethoprim, 4 mg sulfamethoxazole). Blood was collected under terminal anaesthesia in lithium heparin tubes at 1, 3, 5, 7 and 12 h after dosing and the plasma was separated by centrifugation at 5000 rpm. Plasma samples were analysed by quantitative high-performance liquid chromatography (HPLC) with a diode array detector (DAD) detection system to determine sulfamethoxazole and trimethoprim concentrations. An Agilent 1100 HPLC-DAD system (Agilent, Wokingham, UK) was used, with separation performed on a Phenomenex Luna® C18(2) analytical column (Phenomenex, Macclesfield, UK) (150 mm × 4.6 mm, 5 μm particle size). The aqueous mobile phase (phase A) consisted of 0.1% trifluoroacetic acid in water, and the organic phase (phase B) consisted of methanol. The gradient programme was as follows: 5% B (0 min) to 80% B (6–11 min). The column was run at ambient temperature with the flow rate set at 0.8 mL/min and the DAD wavelength set at 270 nm. Sulfamethoxazole (Sigma, Gillingham, UK; purity 98% w/w) and trimethoprim (Sigma; purity 98% w/w) standards (2–1000 μg/mL) were generated in mouse plasma and were analysed with the experimental samples. Briefly, 70 μL of each standard and sample was extracted utilising a protein precipitation step by addition of 140 μL of acetonitrile, vortexed and centrifuged for 15 min at 12,000 rpm. Then, 150 μL of the supernatant was transferred to a fresh vial and taken to dryness in a centrifugal rotary evaporator at 40 °C. The residue was reconstituted in 100 μL of 5% methanol in water and 20 μL was injected onto the LC-DAD system for analysis. All data were collected and quantitated using ChemStation software v.04 (Agilent). Calibration curves were linear, fit with no weighting to a $y = mx + C$ equation. The mean antibiotic concentration–time profile was generated from the individual plasma concentrations, and non-compartmental pharmacokinetic analysis of these profiles was completed using WinNonlin Phoenix v.6.1 (Pharsight Corp., St Louis, MO). The maximum drug concentration (C_{max}), area under the concentration–time curve (AUC) and terminal half-life ($t_{1/2}$) were calculated.

2.5. Antibiotic dosing

Co-trimoxazole oral suspension (Septrin®; GlaxoSmithKline Laboratories, Uxbridge, UK) was administered to mice twice daily at 4.8 mg/mouse (240 mg/kg). The dosing schedule was calculated using quantitative HPLC with ultraviolet detection (HPLC-UV)-determined antibiotic levels in mouse plasma that maintained antibiotic levels in plasma above the MIC (32/16 μg/mL trimethoprim/sulfamethoxazole). Infected untreated control mice were administered sterile deionised water for 14 days. Each animal was dosed orally as previously described [14].

2.6. Bacterial challenge studies

Groups of 12 mice were exposed to a calculated retained dose of ca. 2×10^2 CFU or 2×10^3 CFU of aerosolised *B. pseudomallei* K96243 or with a calculated retained dose of ca. 3×10^4 CFU of *B. mallei* ATCC 23344. To generate the aerosols, a three-jet Collison nebuliser (manufactured at the Defence Science and Technology Laboratory, Salisbury, UK) containing 10 mL of ca. 2×10^7 CFU/mL or 2×10^8 CFU/mL of *B. pseudomallei* K96243 or 1×10^9 CFU/mL of *B. mallei* ATCC 23344 was used to generate and deliver aerosol particles. The particle size of the aerosol produced in this manner is in the range of 1–3 μm. The aerosol was conditioned in a modified Henderson apparatus [15]. Mice were placed in a nose-only exposure chamber and were exposed for 10 min to a dynamic aerosol. The aerosol stream was maintained at 50–55% relative humidity and 22 ± 3 °C. The concentration of *B. pseudomallei* and *B. mallei* in the airstream was determined by taking samples from the exposure chamber using an All Glass Impinger (AGI-30; Ace Glass, Vineland, NJ) [16] operating at 11.5 L/min, containing 10 mL of sterile phosphate-buffered saline (Life Technologies Ltd., Paisley, UK). Impinger samples were plated onto nutrient agar for enumeration. Each test group was challenged with aliquots of the same bacterial suspension, and impinger counts from each run were enumerated to confirm the calculated dose that mice from each run received. This was calculated by applying the Guyton formula [17]. It was assumed that each mouse retained 40% of the organisms inhaled [18].

Groups of mice exposed to *B. pseudomallei* or *B. mallei* were administered co-trimoxazole orally for 14 days or 21 days, initiated at 6 h or 24 h post exposure. Control groups of infected, untreated mice were administered sterile deionised water orally for 14 days. Where appropriate, at 39 days post exposure, surviving mice were administered dexamethasone (5 mg once daily for 5 days) by intraperitoneal injection. All mice were observed twice daily for clinical signs of disease for 56 days (*B. pseudomallei*) or 74 days (*B. mallei*) post exposure, and mortality was recorded.

2.7. Bacteriology

Lungs and spleen were removed from individual animals prior to dexamethasone treatment of the remaining animals, where appropriate, or on termination of the experiments for culture of viable *B. pseudomallei* or *B. mallei*. Tissues were homogenised in nutrient broth, serially diluted and enumerated on nutrient agar.

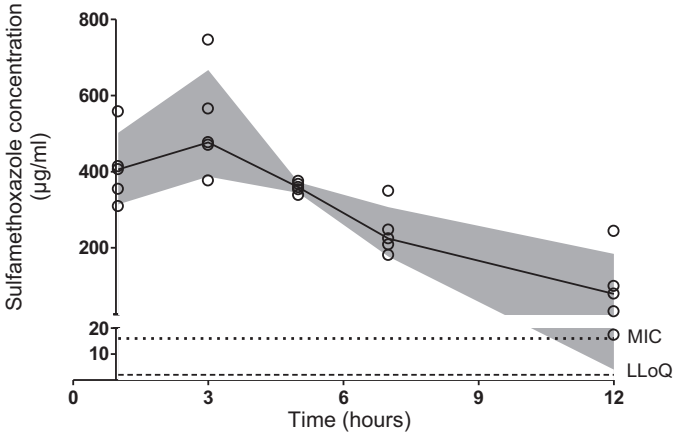

Fig. 1. Individual sulfamethoxazole concentrations (circles), median (solid line) and standard deviation (grey area) over time in plasma of mice following a single oral dose of 4.8 mg of co-trimoxazole (0.8 mg trimethoprim, 4 mg sulfamethoxazole). The minimum inhibitory concentration (MIC) (16 μg/mL for *B. pseudomallei* K96243) and the lowest limit of quantitation (LLoQ) (2 μg/mL) are shown for information.

2.8. Statistical analysis

Analysis of survival rates between mice in individual antibiotic groups was performed using GraphPad Prism v.4.0 (GraphPad Software Inc., La Jolla, CA). The Mantel–Haenszel log-rank test was used to compare survival rates between groups. Analysis of bacterial burden within organs was performed using the Mann–Whitney test.

3. Results

3.1. Minimum inhibitory concentrations of co-trimoxazole

The MIC of co-trimoxazole was determined as 32/16 μg/mL (trimethoprim/sulfamethoxazole) and 8/32 μg/mL for *B. pseudomallei* K96243 and *B. mallei* ATCC 23344, respectively.

3.2. Determination of co-trimoxazole concentration following oral administration

Since co-trimoxazole may be delivered orally, an attribute that is useful for the potential widespread use of the antibiotic following a deliberate release, a study was performed to determine the concentration of co-trimoxazole in murine plasma following oral administration (Fig. 1). Using quantitative HPLC-UV, the lower limit of quantitation for trimethoprim was 2 μg/mL. None was detectable in the plasma samples. Pharmacokinetic parameters were determined for sulfamethoxazole (C_{max} = 527 μg/mL; AUC = 3465 h μg/mL; $t_{1/2}$ = 3.7 h). At 12 h, the average concentration of sulfamethoxazole remaining in the plasma was 94 μg/mL, which is above the MICs of 16 μg/mL for *B. pseudomallei* K96243 and 32 μg/mL for *B. mallei* 23344. Therefore, a 12-hourly dosing regimen was selected for antibiotic efficacy studies.

3.3. Efficacy of co-trimoxazole against exposure to aerosolised B. pseudomallei

Since *B. pseudomallei* can cause inhalational melioidosis, an experiment was performed to demonstrate the efficacy of co-trimoxazole against *B. pseudomallei* K96243 delivered by the inhalational route (Fig. 2). Groups of 12 mice were exposed to two challenge doses of aerosolised *B. pseudomallei* over four runs of the apparatus. The calculated retained dose was either 2×10^3 CFU (Fig. 2B) or 2×10^2 CFU (Fig. 2A). All infected, untreated mice

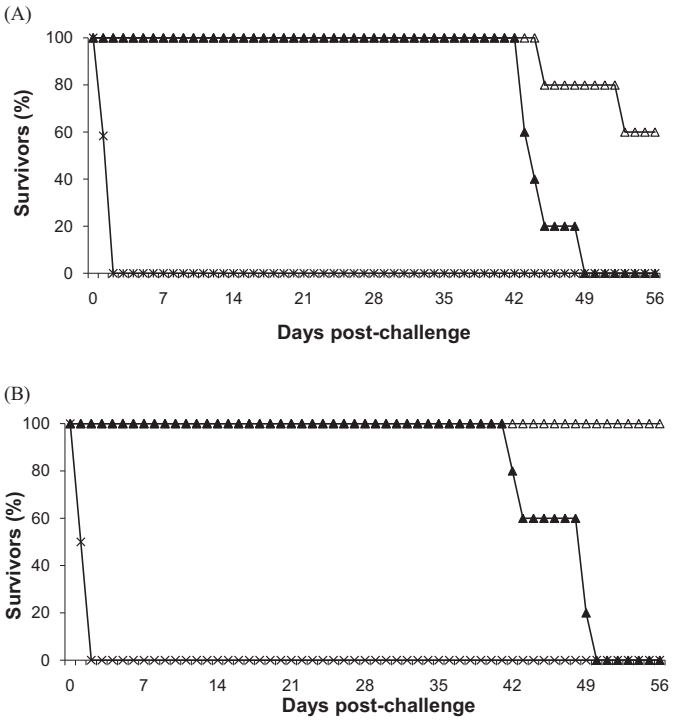

Fig. 2. Survival of mice challenged with *B. pseudomallei* K96243 by the inhalational route and treated with trimethoprim/sulfamethoxazole (co-trimoxazole). Groups of 12 female BALB/c mice were challenged with (A) 2×10^2 CFU or (B) 2×10^3 CFU of *B. pseudomallei* K96243 by the inhalational route and were treated with co-trimoxazole (triangles) for 14 days initiated at 6 h post challenge. Controls (crosses) received sterile water. In each group, at Day 36 two mice were culled for bacterial load enumeration; five mice were administered dexamethasone via the intraperitoneal route for 5 days from Day 39 post infection (solid symbols) and five mice did not receive dexamethasone (open symbols).

succumbed to infection by 48 h post challenge at both challenge doses. All mice administered co-trimoxazole twice daily for 14 days, starting 6 h post exposure, survived to Day 39. At this time, two mice per group were culled to determine the level of colonisation in the spleen and lungs. *B. pseudomallei* was isolated from the organs. One-half of the remaining mice received dexamethasone treatment from Day 39 and all subsequently succumbed to infection. In groups of co-trimoxazole-treated mice that did not receive dexamethasone, survival rates at the end of the experiment (Day 56) were 60% and 100% following exposure with 2×10^2 CFU or 2×10^3 CFU, respectively. Therefore, co-trimoxazole significantly increased survival in comparison with the untreated controls following exposure to 2×10^2 CFU ($P = 0.0016$) or 2×10^3 CFU ($P = 0.0020$); however, administration of dexamethasone significantly increased mortality when administered to groups receiving 2×10^2 CFU ($P = 0.0086$) or 2×10^3 CFU ($P = 0.0021$). Post-mortem examination of mice that succumbed to infection throughout the experiment revealed hepatomegaly and splenomegaly. All surviving mice exhibited clinical signs of infection, and at post-mortem examination all mice had splenomegaly with multiple abscesses. *B. pseudomallei* was recovered from the lungs and spleen of all surviving mice (Fig. 3). There was no significant difference ($P > 0.05$) in bacterial load in the organs at Day 56 between mice challenged with 2×10^2 CFU or 2×10^3 CFU.

3.4. Efficacy of co-trimoxazole against exposure to aerosolised B. mallei

Co-trimoxazole demonstrated significant efficacy against *B. pseudomallei*-induced mortality, despite the MIC for *B. pseudomallei*

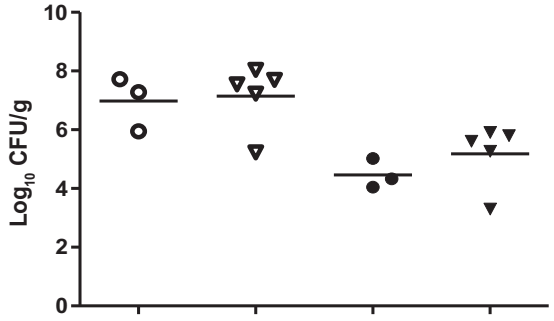

Fig. 3. Bacterial counts (CFU/g) in the spleen (open symbols) and lungs (solid symbols) of mice that survived to Day 56 following challenge with *B. pseudomallei* K96243 at 2×10^2 CFU (circles) or 2×10^3 CFU (triangles) by the inhalational route and treated with trimethoprim/sulfamethoxazole (co-trimoxazole).

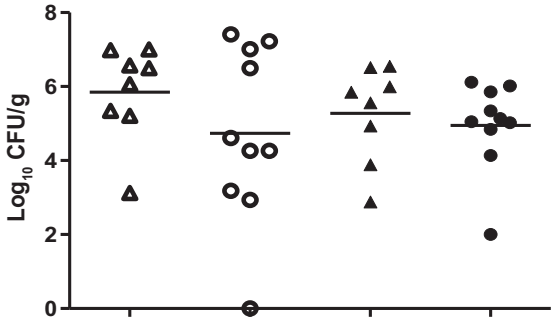

Fig. 5. Bacterial counts (CFU/g) in the spleen (open symbols) and lungs (solid symbols) of mice that survived to Day 74 following challenge with 3×10^4 CFU of *B. mallei* ATCC 23344 by the inhalational route and treated with trimethoprim/sulfamethoxazole (co-trimoxazole) for 14 days from 6 h (triangles) or 24 h (circles) post infection.

being 32/16 μg/mL. MICs were also obtained for co-trimoxazole against *B. mallei* strain ATCC 23344, indicating a similar level of activity. To determine whether co-trimoxazole was also efficacious against *B. mallei* infection, a further experiment was performed in which groups of 12 mice exposed to aerosolised *B. mallei* were administered co-trimoxazole orally for 14 days, initiated at 6 h or 24 h post exposure. The calculated retained dose for mice was ca. 3×10^4 CFU. All infected, untreated mice succumbed to infection by 96 h post exposure. In comparison, 67% of mice administered co-trimoxazole from 6 h (Fig. 4) survived to the end of the experiment at Day 74. When antibiotic administration was delayed to 24 h post exposure, co-trimoxazole offered 83% protection (Fig. 4). Co-trimoxazole offered significantly better protection in comparison with the untreated controls when administered at either 6 h or 24 h ($P < 0.0001$), but there was no significant advantage in delivering the antibiotic at 6 h ($P = 0.3460$). Dexamethasone was not used in this experiment since mice started to show clinical signs of infection by Day 30, indicating relapse. Following post-mortem, *B. mallei* was detected in the spleen of 100% and 92% of the survivors in the 6 h and 24 h treatment groups, respectively. The lungs of all mice were colonised with bacteria ranging from 7×10^2 to 4×10^7 CFU/g. Analysis of the bacterial load showed that there was no significant difference ($P > 0.05$) between the bacterial burden in mice treated with co-trimoxazole from 6 h or 24 h post challenge (Fig. 5). The presence of *B. mallei* in the organs indicates that a chronic infection was present in all surviving mice.

3.5. Efficacy of co-trimoxazole administered for 21 days

These studies demonstrated that a 14-day regimen of orally delivered co-trimoxazole provided PEP against *B. pseudomallei* infection but did not eradicate infection. Thus, we postulated that a longer course of the antibiotic may enable effective clearance of the organism. Groups of 12 mice were exposed to aerosolised *B. pseudomallei.* The calculated retained dose in mice was ca. 2×10^2 CFU. In this experiment, all infected, untreated mice succumbed to infection by 72 h post exposure, whilst, as in the previous experiment, co-trimoxazole was effective at providing protection against infection with *B. pseudomallei.* However, two mice given a 14-day regimen of co-trimoxazole initiated at 6 h post exposure succumbed to the infection at 28 days and 44 days post exposure, respectively, and 44% of the mice survived until the end of the experiment at Day 71 (Fig. 6). In comparison, mice given a 21-day regimen of co-trimoxazole were protected until Day 50 post exposure, when they started to succumb to infection. At the end of the experiment survival was 83% (Fig. 6). Co-trimoxazole offered significantly better protection in comparison with the untreated controls when administered for either 14 days or 21 days ($P < 0.0001$), and 21 days treatment offered significantly better survival ($P = 0.0199$) in comparison with 14 days treatment; however, the time to death following cessation of antibiotic was not significantly different in mice administered co-trimoxazole for 14 days or 21 days ($P = 0.0988$).

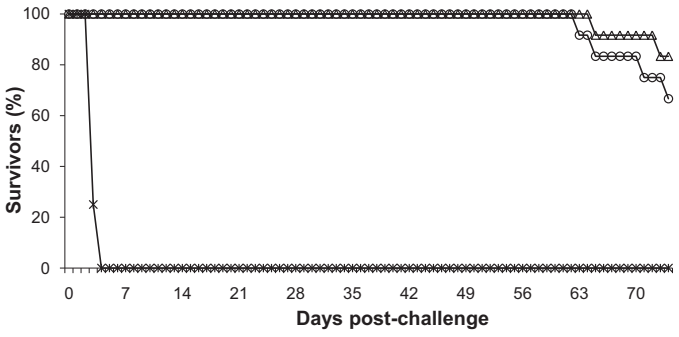

Fig. 4. Survival of mice challenged with *B. mallei* ATCC 23344 by the inhalational route and treated with trimethoprim/sulfamethoxazole (co-trimoxazole). Groups of 12 female BALB/c mice were challenged with ca. 3×10^4 CFU of *B. mallei* ATCC 23344 by the inhalational route and were treated with co-trimoxazole for 14 days initiated at 6 h (circle) or 24 h (triangle) post challenge. Controls (crosses) received sterile water.

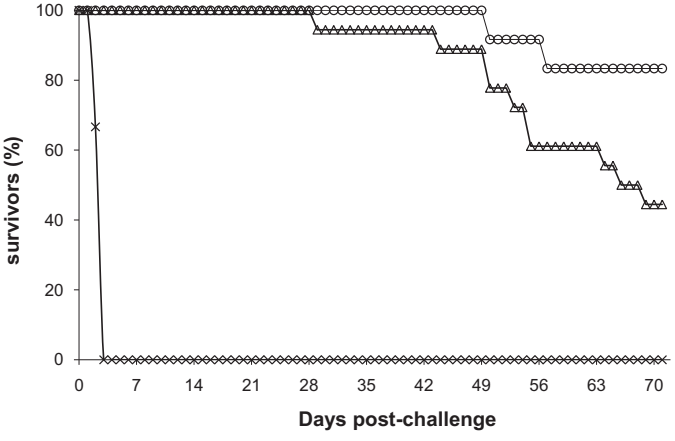

Fig. 6. Survival of mice challenged with *B. pseudomallei* K96243 by the inhalational route and treated with trimethoprim/sulfamethoxazole (co-trimoxazole) for 14 days or 21 days. Groups of 12 female BALB/c mice were challenged with 2×10^2 CFU of *B. pseudomallei* K96243 by the inhalation route and were treated with co-trimoxazole for 14 days (triangles) or 21 days (circles) initiated at 6 h post challenge. Controls (crosses) received sterile water.

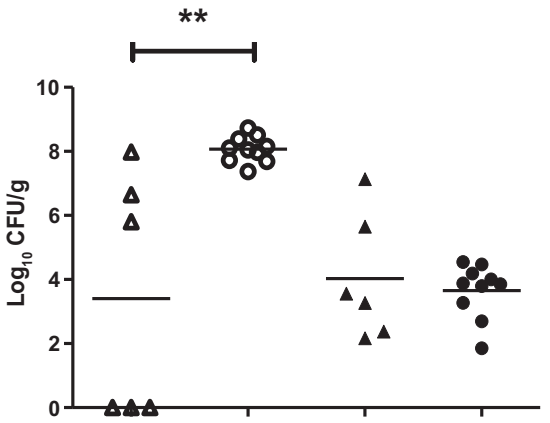

Fig. 7. Bacterial counts (CFU/g) in the spleen (open symbols) and lungs (solid symbols) of mice that survived to Day 71 post challenge with 2×10^2 CFU of *B. pseudomallei* K96243 by the inhalational route and treated with co-trimoxazole for 14 days (triangles) or 21 days (circles) initiated 6 h post challenge. ** $P < 0.01$.

Bacterial load in the organs of surviving mice at Day 71 showed that the mice treated for 14 days with co-trimoxazole had significantly less bacteria in the spleen than those treated for 21 days ($P < 0.01$). However, the lungs were colonised with up to 4×10^4 CFU/g (Fig. 7) and there was no significant difference between bacterial loads in lungs following the 14-day or 21-day treatment regimens ($P > 0.05$). These data indicate that the longer treatment regimen did not result in clearance of *B. pseudomallei*, only extending the time taken to succumb to infection.

4. Discussion

Treatment for acute melioidosis usually involves high-dose intravenous ceftazidime or a carbapenem, followed by prolonged oral eradication therapy to reduce the risk of relapse [6]. However, there is relatively little experimental data demonstrating antimicrobial prophylaxis for melioidosis and glanders upon which to build recommendations. The current UK guidance for PEP recommends co-trimoxazole or doxycycline, based on data from animal experiments, as there is no evidence of the protective efficacy of antibiotic PEP in preventing human melioidosis or glanders [5]. Particularly, there is little experimental evidence relating to the performance of antibiotics against infection with aerosolised *B. pseudomallei* or *B. mallei*, which is considered the likely route of infection resulting from a biological attack. In human cases of melioidosis, treatment with doxycycline alone proved to be disappointing during the eradication phase of treatment [9]. In comparison, co-trimoxazole alone was used successfully for the eradication phase in Australia [6], suggesting that co-trimoxazole may be effective when used as prophylaxis against melioidosis.

In this study, the efficacy of co-trimoxazole for PEP of infection with *B. pseudomallei* and *B. mallei* has been evaluated. The MICs of these antibiotics for *B. pseudomallei* and *B. mallei* are consistent with those previously reported [19,20], generally reflecting the relative resistance of *Burkholderia* spp. to antibiotic therapy.

The clinical manifestations of melioidosis have been shown, in part, to be dependent on the route of infection and although any organ system may be involved in human *B. pseudomallei* infection, the lungs, liver and spleen are the primary targets of pathological involvement [21,22]. Furthermore, inhalation of aerosolised *B. pseudomallei* results in a more acute infection in mice than other infection routes [10]. Thus, our studies to evaluate co-trimoxazole for prophylaxis of aerosolised *B. pseudomallei* infection offer a robust and relevant test of the antibiotic. These studies demonstrate that co-trimoxazole is effective for prophylaxis of inhalational melioidosis. These findings are in agreement with a recent report by Sivalingam et al. in which co-trimoxazole was found to be highly effective in protecting mice infected with aerosolised *B. pseudomallei* K96243 [23]. In that study, co-trimoxazole was administered up to 24 h post exposure for 10 days and the mice were observed until 21 days post infection, reporting 100% protection. At this time, *B. pseudomallei* could not be cultured from the lungs, liver and spleen. However, the current study has demonstrated that, although organs may appear to be sterile following co-trimoxazole prophylaxis, *B. pseudomallei*-induced relapse can be initiated by immunosuppression using dexamethasone or, if left for longer, infection will manifest in the mice. In each of our experiments using co-trimoxazole, we did not see complete protection afforded by this antibiotic against *B. pseudomallei*.

Infection of humans with *B. pseudomallei* causes melioidosis that may manifest as a chronic form or as an asymptomatic latent infection [6]. Yet the site of *B. pseudomallei* persistence and subsequent relapse are unknown. It is perhaps this ability of the organism to cause a persistent infection that makes true eradication of *B. pseudomallei* a significant challenge. The current studies indicate that use of co-trimoxazole alone may not fully eradicate infection. Some studies have demonstrated that *B. pseudomallei* as a biofilm has a MIC 200-fold higher than that of planktonic cells [24]. The clinical significance of this is not yet known, although it is thought that the virulence of *B. pseudomallei* does not correlate with biofilm formation [24]. It is possible, however, that formation of biofilm in vivo or the generation of genetically different populations of *B. pseudomallei* may contribute to the resistance to antibiotic therapy.

Similarly to treatment of *B. pseudomallei* infection, co-trimoxazole showed efficacy against *B. mallei* in a mouse model of inhalational glanders, preventing the development of acute infection. Whilst both *B. pseudomallei* and *B. mallei* are intracellular organisms and require cellular immunity to control infection [25], *B. mallei* is not known to cause a latent infection. However, treatment with co-trimoxazole was not able to eradicate *B. mallei* and a subsequent relapse of infection occurred.

A pharmacokinetic parameter has not yet been identified as an appropriate marker of efficacy for co-trimoxazole. However, limited published data have suggested that clinical efficacy correlates with the proportion of time the antibiotic concentration exceeds the MIC of the infecting organism [26]. Relapse rates of melioidosis following treatment with co-trimoxazole are high in Thailand and one explanation for this is the use of a suboptimal dosing regimen of 160/800 mg co-trimoxazole (trimethoprim/sulfamethoxazole) every 12 h, leading to insufficient time for which the concentration of antibiotic was above the MIC [27].

In this study, the pharmacokinetic parameters showed that the dosing regimen of 4.8 mg per mouse resulted in plasma concentrations of sulfamethoxazole that were above the MIC for 12 h, and therefore for the full duration of treatment of 14 days for both *B. pseudomallei* K96243 and *B. mallei* ATCC 23344. However, a chronic infection was established even with extended therapy of 21 days for *B. pseudomallei* infection. This suggests that the currently recommended prophylaxis of co-trimoxazole for 7 days for *Burkholderia* pathogens [5] may not be adequate to prevent the development of a chronic infection, and for this reason further study is warranted.

In summary, PEP with co-trimoxazole prevented the onset of acute melioidosis and acute glanders and therefore offers effective PEP for these diseases. However, a chronic infection was established, suggesting that the antibiotics had failed to eliminate colonising bacteria and did not prevent the establishment of latent or persistent infection. This is consistent with clinical experience where a high recurrence rate occurs, even after apparently successful antimicrobial therapy. Future strategies to eradicate persistent infection are required for *Burkholderia* spp.

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Competing interests: None declared.

Ethical approval: All animal studies were carried out in accordance with the UK 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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