Effect of timing and duration of azithromycin therapy of leptospirosis in a hamster model

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Objectives: Azithromycin is not associated with significant adverse effects or restricted usage in certain populations unlike standard antileptospirosis agents. In this study, the utility of short courses of azithromycin in treating or preventing leptospirosis was investigated in a lethal hamster model.

Methods: All hamsters were infected intraperitoneally with $10^5$ leptospires. In experiment one, animals received 5 mg/kg of doxycycline or 10 mg/kg of azithromycin via intraperitoneal injection beginning on the second day after infection and continuing once daily for 1, 2, 3 or 5 days. In experiment two, animals received 1 or 2 day courses of azithromycin initiated 2 or 4 days following infection, or 4 days prior to infection.

Results: All untreated control animals died between the sixth and ninth day following infection. In experiment one, survival rates in the doxycycline groups were 0, 50, 80 and 100% for those animals treated for 1, 2, 3 and 5 days, respectively. Except for the 1 day treatment group (which had an 80% survival), there was 100% survival in all azithromycin-treated groups. In experiment two, all animals treated after infection survived until study completion. No animals survived with 1 day of therapy started 4 days prior to infection while only 20% survived if they received 2 days.

Conclusions: These results suggest short-course therapy with azithromycin, even started well after infection, is efficacious in preventing mortality from acute leptospirosis.

Keywords: treatment, prophylaxis, animal model

Introduction

Leptospirosis is a worldwide zoonotic infection of protean manifestations. Current therapeutic options are dictated by severity of presentation and can include symptomatic support and directed antimicrobial therapy. While research into antimicrobial therapy for leptospirosis has been ongoing for more than half a century, proven therapeutic options based on randomized, controlled human studies are limited to doxycycline, penicillin, cefotaxime and ceftriaxone. The use of doxycycline and these other agents can be limited by toxicity, hypersensitivity and contraindications among selected patient populations. Azithromycin is an antimicrobial agent notable for its ease of administration, minimal adverse effects and lack of contraindications during pregnancy or childhood. Prior in vitro work has revealed azithromycin to have lower MICs than any of the proven agents. In addition, recent work from our institution has shown that azithromycin has significant efficacy in treating acute leptospirosis in our lethal hamster model. Specifically, azithromycin produced 100% survival in treated hamsters over a wide range of doses (6.25–200 mg/kg) and with as little as 3 days of treatment at high (100 and 200 mg/kg) doses. These results led to speculation that azithromycin might prove useful as a short-course treatment for acute leptospirosis or as a prophylactic agent using near human equivalent doses. This report describes our investigations into timing and duration of azithromycin treatment in managing acute leptospirosis in a lethal hamster model.

Materials and methods

Animal model

In this model, female golden Syrian hamsters (Mesocricetus auratus) with a weight range of 50–100 g (Harlan, Indianapolis, IN, USA)

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were inoculated intraperitoneally with 0.5 mL of Ellinghausen McCullough Johnson Harris (EMJH) medium (Becton Dickinson, Sparks, MD, USA) containing 107 leptospires, as determined by Petroff–Hauser counting chamber and dark-field microscopy. All trials utilized Leptospira interrogans serovar Portlandve (CA-12-029/CDC Nic 1808) obtained from Dr David Haake (University of California Los Angeles). This model produces 100% mortality among untreated hamsters within 6–9 days following infection. Once infected, animals were observed several times a day for 21 days. Any hamster showing evidence of significant pain or distress, or characteristics of a moribund state, was euthanized humanely. At the conclusion of all experiments (day 21) all surviving animals were euthanized humanely. All animal experimentation was conducted under protocols approved by our Institutional Animal Care and Use Committee, and monitored by the facility veterinarian to ensure compliance with ethical standards of animal care.

Antimicrobial agents

Doxycycline, in powder formulation, was purchased directly from Sigma-Aldrich (St Louis, MO, USA) and Bedford Laboratories (Bedford, OH, USA). Azithromycin, in parenteral commercial formulation, was obtained from Pfizer (Groton, CT, USA). Manufacturer recommendations were followed in the preparation and storage (if necessary) of all drug solutions. Previous in vitro work at our institution demonstrated doxycycline and azithromycin to have median MICs of 0.063 and ≤0.016 mg/L, respectively, against the experimental strain of Leptospira used in this study (M. E. Griffith, D. R. Hospenthal and C. K. Murray, unpublished data).

Therapeutic trials

In each experiment, hamsters were divided into groups of 10 and then infected, with one group not given antimicrobial therapy to serve as untreated controls. Group size was derived from the initial statistical development of the animal model in which it was found that data from a minimum of 30 subjects, divided among all experimental groups, were required to perform satisfactory analysis versus a control. Given a minimum of three experimental groups in each trial, a group size of 10 hamsters was chosen. All antimicrobials were administered once daily via intraperitoneal injection. Each experiment was performed only once.

In the first experiment, the therapeutic efficacy of different durations of doxycycline and azithromycin treatment was evaluated. Beginning 2 days after infection, groups selected for treatment received 1, 2, 3 or 5 day courses of either 10 mg/kg of azithromycin or 5 mg/kg of doxycycline. Bioequivalence for azithromycin activity between hamsters and humans is not available; therefore, antimicrobial dosages were selected to be the closest approximation to a weight-based human comparable dose previously shown to produce 100% survival over a 5 day treatment course in the same animal model.6 Based upon the efficacy of the first experiment a second experiment was performed evaluating the therapeutic efficacy of short courses of azithromycin therapy over a range of starting times after or before infection. Groups selected for treatment received 1 or 2 day courses of 10 mg/kg of azithromycin, beginning either 2 or 4 days after infection, or 4 days prior to infection. With its reported long elimination half-life of near 72 h, we wished to evaluate azithromycin’s ability to prevent the development of infection. As doxycycline is currently used as weekly prophylaxis, our use of azithromycin 4 days before infection and 2 or 4 days post-infection, mirrors the 7 day interval of weekly prophylaxis. This attempts to assess if azithromycin could play a role in preventing infection as well as treating incubating disease.

Statistical analysis

Experimental results from individual treatment groups were analysed using the Kruskal–Wallis test (non-parametric ANOVA), and then compared by the Mann–Whitney rank sum test. P values of ≤0.01 were considered significant.

Results and discussion

Current roles for antimicrobials in leptospirosis include therapy for acute infection and prevention of clinical disease with weekly prophylaxis.1,7 The small number of antimicrobial agents that have been proven to be effective by randomized, controlled studies have limitations in their applicability in certain patient populations due to toxicities, contraindications, cost or complex administration. Azithromycin is generally available, easy to administer, has minimal toxicity and is not restricted from use in pregnancy or childhood.4 In multiple in vitro studies azithromycin and the other macrolides have shown themselves capable of inhibiting many different strains of Leptospira, consistently demonstrating MICs and minimum bactericidal concentrations equal or superior to any of the traditional agents.5,8 Likewise, azithromycin and the other macrolides have performed well in various in vivo animal studies in preventing mortality.6,9

In our first experiment all hamsters treated with more than 1 day of azithromycin (2, 3 or 5 days) survived, with 1 day of therapy yielding an 80% rate of survival (Figure 1a). All treatment regimens produced a significant survival advantage when compared with no treatment (P < 0.001). In the subsequent experiment, there was 100% survival among all animals treated with azithromycin beginning 2 or 4 days after infection (Figure 2). Hamsters treated with azithromycin prior to infection had 100% mortality if treated with one dose. The mortality decreased to 80% if animals received 2 days of azithromycin.

The effectiveness of 5 days of therapy is consistent with our prior work with azithromycin.6 Previous researchers have also found that 3 days of therapy or longer with other macrolides, including erythromycin, oleandomycin and carbomycin, can prevent mortality in various animal models of leptospirosis.9,10 However, our success with shorter courses (1 or 2 days) and with delaying the therapy of azithromycin has not been reported by other researchers in mortality studies with macrolides.

For doxycycline, the present study demonstrated a proportional response between duration of therapy and rate of survival, with 0, 50, 80 and 100% survival noted for all animals treated with 1, 2, 3 and 5 days of therapy, respectively (Figure 1b). While a single treatment with doxycycline failed to prevent mortality, a significant delay in time to death was noted compared with untreated controls (P < 0.001).

As with azithromycin, the survival of all hamsters treated with 5 days of doxycycline is in agreement with our previously reported results.6 With regards to 2 and 3 day therapy, previous researchers have demonstrated similar durations of doxycycline to be capable of preventing mortality and clearing all leptospires from tissues and fluids within hamster models.9,11 Observations of the effects of similar single-dose doxycycline therapy by others have been mixed, with one group reporting complete lack of
Untreated controls

Efficacy with oral dosages below 40 mg/kg, and another reporting universal survival with an oral dosage of 28.8 mg/kg.12,13

In this study we have expanded upon azithromycin’s potential by demonstrating its effectiveness against leptospirosis with even a single dose given at varying times after infection but prior to the development of clinical illness. These new data suggest a role for azithromycin as a potential prophylactic agent. At present, the only agent with any demonstrated chemoprophylactic efficacy against leptospirosis is doxycycline.7 In human studies, a weekly 200 mg dose of doxycycline has been shown to reduce the development of clinical leptospirosis by up to 95% compared with untreated controls within an endemic region. However, seroconversion among the treated populations of these studies indicates that doxycycline prophylaxis does not prevent the infection itself, merely the development of clinical disease. Further, pharmacokinetically, doxycycline has neither a long plasma half-life (15–22 h) nor a long elimination half-life, which may preclude its capability of suppressing infection during once-weekly dosing. Overall, this suggests that doxycycline functions as a post-exposure prophylactic (abortive) agent, rather than a pre-exposure (preventative) one. However, one animal study revealed doxycycline given 1 day prior to infection resulted in 100% survival (preventative) one. However, one animal study revealed doxycycline given 1 day prior to infection resulted in 100% survival implying possible preventive prophylactic activity.12

Azithromycin shares many pharmacodynamic properties with doxycycline, and thus might function in a similar fashion. Both agents are characterized by rapid absorption, excellent tissue penetration, similar mechanisms of action (inhibition of protein synthesis) and similar peak serum concentrations at standard dosing. Furthermore, azithromycin, while it has a long elimination half-life due to its rapid cellular uptake and retention, is similar to doxycycline in that it does not maintain high serum concentrations.14

Our study has two main limitations. First, we used intraperitoneal administration of medications because of ease of administration. It is not clear how this corresponds to oral dosing, which would obviously be used in human trials. On this topic, there is conflicting evidence in the medical literature. In one study involving erythromycin treatment of leptospirosis in guinea pigs, oral therapy was more effective than intraperitoneal injection.9 In contrast, in a study examining Legionella therapy with azithromycin in guinea pigs, oral therapy was less effective than intraperitoneal.15 Despite there being no clear data to compare the two routes of administration, we feel that our results are generalizable because of the good bioavailability of azithromycin when given orally. A second limitation of our study is that our model focuses on mortality and not all of the different presentations of human leptospirosis, which range from asymptomatic to fatal disease. This range of presentations must be considered in the development of future human clinical trials.

In conclusion, the results of our study indicate that even single-dose azithromycin therapy is effective in preventing mortality in an acute lethal hamster model of infection. In light of its efficacy, lack of contraindications in populations such as pregnant women and children, wide availability, ease of use and oral formulation, single-dose azithromycin treatment merits further evaluation as an agent for post-exposure prophylaxis for acute leptospiral infections in humans.

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Transparency declarations

None to declare.

References


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