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Efficacy of minocycline and tigecycline in a hamster model of leptospirosis☆,☆☆

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Abstract

Leptospirosis is a widespread zoonotic infection characterized by acute febrile illness. Severely ill patients may require empiric treatment with broad-spectrum antibiotics prior to definitive diagnosis. We evaluated the efficacy of minocycline and tigecycline against leptospirosis in a hamster model. Hamsters were treated with either minocycline (5, 10, or 25 mg/kg per day) or tigecycline (5, 10, or 25 mg/kg per day) for 5 days. Controls included untreated animals and doxycycline-treated animals (5 mg/kg per day). Nine days after infection, all untreated animals were dead. All treated hamsters survived to the end of study (day 21). Study groups showed significantly improved survival compared to the untreated group ($P < .01$). Minocycline and tigecycline showed survival benefit comparable to the standard treatment, doxycycline. In the absence of doxycycline, minocycline may be considered as an alternative, while tigecycline may be useful in the management of severely ill patients prior to a definitive diagnosis.
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1. Introduction

Leptospirosis is a zoonotic infection characterized by acute febrile illness and caused by the spirochetes of the genus *Leptospira*. In addition to nonpathogenic serovars, over 200 pathogenic serovars of *Leptospira* have been described (World Health Organization, 2003). Leptospirosis is a public health threat worldwide, particularly in the tropics and subtropics, and is spread through excretion of leptospires in the urine of infected animals. Disease presentation varies widely, from a mild, self-limited febrile illness to a life-threatening syndrome involving vasculitis of multiple organ systems and a mortality of up to 50% (McBride et al., 2005; Spichler et al., 2008; World Health Organization, 2003). Treatment of leptospirosis is typically based on severity and duration of symptoms and usually includes supportive care in addition to antimicrobials. Currently, antimicrobial therapy for leptospirosis includes oral or intravenous (IV) doxycycline, IV penicillin G, IV third-generation cephalosporins, or, in milder cases, oral amoxicillin or macrolides (Levett, 2001; World Health Organization, 2003). Diagnosis of leptospirosis is most often based on serologic testing, some of which is time and labor intensive and may take several days to produce results (Effler et al., 2002). Rapid tests often lack sensitivity within the first week of illness (McBride et al., 2005). Therefore, patients who are severely ill may require empiric treatment with broad-spectrum antibiotics prior to definitive diagnosis of leptospirosis.

Minocycline and tigecycline are members of the tetracyclines, a group of broad-spectrum, bacteriostatic antibiotics.

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Tetracyclines have activity against many Gram-positive and Gram-negative microorganisms, including anaerobes, as well as rickettsiae, chlamydiae, mycoplasmas, and some protozoa. Susceptibility patterns produced by most tetracycline drugs are similar. However, tigecycline, a glycylcycline derivative of minocycline, is not affected by some of the common mechanisms of tetracycline resistance and therefore remains effective against a wider range of bacteria that may be resistant to older tetracyclines, including doxycycline and minocycline (Chambers and Deck, 2009; Chopra and Roberts, 2001). Minocycline is an older tetracycline compound that is typically available and inexpensive throughout the world, making it a potential substitute for doxycycline when that drug is unavailable. If found to be effective against leptospires, tigecycline may be useful in empiric treatment of acute febrile illnesses of unknown etiology when leptospirosis is included in the differential diagnosis, allowing coverage of a wide range of other infectious diseases. In the study reported herein, we evaluate the efficacy of minocycline and tigecycline in a hamster model of acute leptospirosis.

2. Materials and methods

2.1. Animal model

All animal experimentation was conducted under a protocol approved by the local Institutional Animal Care and Use Committee. Female Golden Syrian hamsters (*Mesocretius auratus*), 4-6 weeks old and 75-100 g in weight (Harlan Sprague Dawley, Indianapolis, IN, USA) were employed. All animals were infected with 10^5 *Leptospira interrogans* serovar Portlandvere by intraperitoneal (IP) injection as previously described (Moon et al., 2006). All animals were monitored at least twice daily for 21 days, and those exhibiting signs of significant pain or distress or characteristics of a moribund state were humanely euthanized. All animals surviving to day 21 were euthanized at that time. Blood samples for culture were obtained via cardiac puncture at the time of death to determine the presence of spirochetemia.

2.2. Antimicrobial agents

Doxycycline hyclate (Bedford Laboratories, Bedford, OH, USA) and tigecycline (Wyeth Pharmaceuticals, Philadelphia, PA, USA) were purchased in their commercially available, parenteral formulations. These were reconstituted in normal saline under aseptic conditions prior to use. Minocycline (Sigma-Aldrich, St. Louis, MO, USA) was purchased in powder form and dissolved in normal saline solution. All were diluted to appropriate concentrations to allow for administration of each dose in a volume of 0.5 mL (5 mg/kg of doxycycline or 5, 10, or 25 mg/kg of minocycline or tigecycline, based on mean animal weight per group). All reconstituted drug solutions were stored at 4 °C in the dark between uses and allowed to come to room temperature prior to administration. Previous in vitro work in our institution showed median MICs of 0.125 μg/mL for both minocycline and tigecycline on repeated broth microdilution susceptibility testing against this strain (Hospenthal, D.R., unpublished results). Doxycycline was demonstrated to have a median MIC of 0.06 μg/mL (Moon et al., 2007).

2.3. Therapeutic trials

After infection, animals were divided into 8 groups: 1 group of 5 untreated controls and 7 groups of 10 treated animals, including a treated control group of 10 animals. Treated control animals received doxycycline 5 mg/kg IP once daily on days 2 to 6 after infection. The doxycycline dose for the treated control group was chosen based on previously determined effective dose in a hamster model of leptospirosis (Moon et al., 2006). Groups of 10 animals received 1 of 3 doses of minocycline (5, 10, or 25 mg/kg) IP once daily or 1 of 3 tigecycline doses (5, 10, or 25 mg/kg) IP once daily on days 2 to 6 after infection.

2.4. Blood cultures

Sterile conical tubes containing 10 mL of semi-solid 0.2% Ellinghausen-McCullough-Johnson-Harris media were inoculated with 2–3 drops of whole blood obtained at the time of death of each animal. Culture tubes then were incubated at 30 °C. At 1–2-week intervals, a 0.1-mL sample of each culture was obtained approximately 1 cm from the top of each culture surface (in positive cultures this location commonly has a cloudy area of *Leptospira* growth termed the Dinger zone) and examined for the presence of leptospires by dark field microscopy. Cultures were deemed negative if no leptospires were visualized by 6 weeks after inoculation of each culture.

2.5. Statistical analysis

SPSS version 16.0 (SPSS, Chicago, IL, USA) was used to create Kaplan–Meier plots for each study group. Survival differences between study groups were compared by the log rank test. P values of $< .05$ were considered significant.

3. Results

All of the untreated animals became moribund and were euthanized by day 9 (Fig. 1). All of the doxycycline-treated control animals survived to day 21. All animals in the minocycline and tigecycline treatment groups survived to day 21 without evidence of disease. Overall, survival for each of the experimental treatment groups was significantly improved compared to untreated controls ($P < .01$). On the contrary, there was no difference between the experimental minocycline- and tigecycline-treated groups and the doxycycline-treated control group. Blood cultures from 4 of 5 of the untreated animals became positive for spirochete growth