Doxycycline versus Azithromycin for the Treatment of Leptospirosis and Scrub typhus.

Running Title: Oral treatment of leptospirosis and scrub typhus

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ABSTRACT
Leptospirosis and scrub typhus are important causes of acute fever in Southeast Asia. Options for empirical therapy include doxycycline and azithromycin, but it is unclear whether their efficacy is equivalent. We conducted a multicenter, open, randomized controlled trial in adult patients presenting with acute fever (<15 days), without an obvious focus of infection, at 4 hospitals in Thailand between July 2003 and January 2005. Patients were randomly allocated to receive either a 7-day course of doxycycline or a 3-day course of azithromycin. Cure rate, fever clearance time and adverse drug events were compared between the two study groups. A total of 296 patients were enrolled in the study. The cause of acute fever was determined in 151 patients (51%): 69 patients (23.3%) had leptospirosis; 57 patients (19.3%) had scrub typhus; 14 patients (4.7%) had murine typhus; 11 patients (3.7%) had evidence of both leptospirosis and a rickettsial infection. The efficacy of azithromycin was non-inferior to doxycycline for the treatment of both leptospirosis and scrub typhus, with comparable fever clearance times in the two treatment arms. Adverse events occurred more frequent in the doxycycline group than in the azithromycin group (12.7% and 5.9% respectively, P= 0.02). In conclusion, doxycycline is an affordable and effective choice for the treatment of both leptospirosis and scrub typhus. Azithromycin was better tolerated than doxycycline, but is more expensive and less readily available.
INTRODUCTION

Leptospirosis is a zoonosis with a worldwide distribution (6). It is caused by pathogenic spirochetes of the genus *Leptospira* which are excreted in the urine of a variety of wild and domestic animals. Human infection occurs through direct contact with infected animals or via exposure to fresh water or soil contaminated by infected animal urine. Leptospirosis is an emerging infection in many countries including Thailand, where the annual number of reported cases has been increasing since 1997 (12). Scrub typhus is also a zoonotic diseases caused by *Orientia tsutsugamushi*, an obligate intracellular bacteria transmitted to humans by the bite of a larval leptotrombidium mite (14). Scrub typhus is an important cause of acute fever in the Western Pacific region (9).

Although classical presentations of leptospirosis and scrub typhus are well described, most patients present to hospital with non-specific signs and symptoms. Acute undifferentiated fever, i.e. acute fever without an obvious focus of infection, is the most common clinical presentation of both leptospirosis and scrub typhus (11). Early diagnosis of leptospirosis and scrub typhus are essential since antibiotic therapy provides greatest benefit when initiated early in the course of illness (2, 9). Diagnosis of early phase of either leptospirosis or scrub typhus is hampered by its non-specific presentation. The lack of widely available, sensitive and rapid methods for laboratory diagnosis of both diseases is an important clinical problem when managing patients presenting with acute undifferentiated fever, making it difficult to select appropriate empirical antimicrobial therapy. Doxycycline is potentially an excellent choice of initial antimicrobial treatment for such individuals, though there has been a report of doxycycline- resistant scrub typhus in Northern Thailand (13). Although expensive at present azithromycin may be an excellent alternative, particularly when resistance is suspected. We report here the results of a multi-centre open randomized
controlled trial comparing the efficacy and tolerability of doxycycline and azithromycin for
the treatment of acute undifferentiated fever suspected of either leptospirosis or scrub typhus
in areas of high leptospirosis and scrub typhus endemicity.

MATERIAL AND METHODS

Patients and study sites: This study was conducted between July 2003 and January 2005 at 4 hospitals in Thailand. Three hospitals are in the northeast part of the country (Udonthani Hospital, Udonthani Province, Maharat Nakhon Rachasima Hospital, Nakhon Rachasima Province, and Chaiyapoom Hospital, Chaiyapoom Province), and one hospital is in the south (Chumphon Hospital, Chumphon Province). Included in the trial were adult patients (> 14 years) with suspected leptospirosis or scrub typhus— that is, patients who presented with acute fever (oral temperature, $\geq 38.0^\circ$ C for <15 days) in the absence of an obvious focus of infection, and who in the opinion of the attending physician could receive oral antimicrobial treatment. Patients who were unable to take oral medications, those who were pregnant or breastfeeding, those with a history of allergy to macrolides or tetracyclines, those who had positive malarial blood smear, clinical dengue virus infection consistent with WHO criteria (8), severe leptospirosis or scrub typhus related complication, and those who had a definite history of receiving treatment active against leptospirosis or scrub typhus for more than 48 hours before enrollment were excluded. The study protocol was approved by the Ethical Review Subcommittee of the Public Health Ministry of Thailand and written inform consent was obtained from all study volunteers.
Sample size requirements: The study was designed to test that azithromycin had non-inferior
efficacy compared with doxycycline for the treatment of both leptospirosis and scrub typhus.
Assuming a 90% cure rate for doxycycline in both diseases, a relative different of ≥ 20%
between the cure rates of the 2 groups was defined as nonequivalent. On the basis of a one-
sided 0.05 significant level and 90% power respectively, to reject the null hypothesis that the
two treatments was not equivalent, testing of non-inferior efficacy required that at least 28
patients with confirmed leptospirosis and scrub typhus in each treatment group complete the
trial protocol.

Randomization and Study protocol: Independent, computer generated, simple random
allocation sequences were prepared for each study hospital by the investigator team in
Bangkok. These were sealed in an opaque envelope and numbered. The investigator in each
study hospital assigned study participants to their treatment groups after opening the sealed
envelope. Patients were randomly allocated to receive either oral doxycycline (Siam
Pharmaceutical, Thailand) 200 mg on the first dose followed by 100 mg every 12 hrs for 7
days, or a 3- day course of azithromycin (Pfizer International) 1 g initially followed by 500
mg once daily for 2 days. History, physical examination findings and results of laboratory
investigations were recorded on standardized case record forms. During hospitalization,
temperature was recorded orally every 4 hours. Baseline investigations included a full blood
count, plasma glucose and electrolytes, serum urea and creatinine, liver function tests, two
aerobic blood cultures, urine analysis and chest radiography. Five milliliters of blood were
placed in a sterile heparinized bottle for leptospire culture using EMJH medium. Leptospira
were cultured and identified using standard methodology (17).
Patients were discharged when defervescence had been achieved and maintained for at least 48 hours. Follow-up visit was scheduled for 1-2 weeks after initial sampling to obtain convalescent serum samples for serological analysis. Sera were stored at –20°C until tested.

Data collection was done by the study team who was unaware of the study hypothesis.

**Confirmation of leptospirosis and scrub typhus:** Leptospirosis was confirmed on the basis of World Health Organization’s criteria for leptospirosis (2). Acute and follow up sera were tested by the microscopic agglutination test (MAT) as previously described (2). Reference leptospira from 24 serogroups including serovars known to be prevalent in Thailand were used as the antigen in the MAT. The diagnosis of leptospirosis was made by either the isolation of leptospires from blood, or positive serologic tests which were defined as either a fourfold or greater rise in antibody titer, or a titer of at least 1:400 on a single specimen.

Scrub typhus was diagnosed serologically by a microimmunofluorescence assays that employed either a combination of 3 *O. tsutsugamushi* strains (Karp, Gilliam and Kato) as antigen. Total anti-rickettsial immunoglobulins, IgG- and IgM-specific antibody were assayed as described previously (4). Criteria for the diagnosis of scrub typhus were either a fourfold or greater rise in IFA titers between paired serum samples, or a titer of at least 1:400 or greater on a single specimen.

**Analysis of results:** The efficacy of treatment was analyzed on the intention to treat and a subgroup analysis basis. Intention to treat analysis was based on the number of patients who entered the study- 145 doxycycline treated patients and 151 azithromycin treated patients. Subgroup analysis was based on the number of patients who complete the treatment and had laboratory confirmed leptospirosis and scrub typhus. The primary efficacy outcome was evaluated according to the following definitions. “Cure” was defined as the resolution of
fever within 5 days after initiating the antimicrobial treatment. “Failure” was defined either persistent fever or the development of any complication, after at least 48 hours of treatment.

The secondary outcome measure was the time to defervescence, which was defined as the interval between the time at which the first dose of the study drug was administered and the time at which the oral temperature first returned to ≤ 37.5°C and was maintained for two consecutive measurements without antipyretics.

Patients were assessed for adverse events. “Adverse events” were defined as symptoms or signs that developed after the study drug administration and had not been reported prior to the administered of the first dose of the antibiotic. Analyses of baseline characteristics and adverse events were done on the intention to treat basis.

Statistical analysis: All statistical analyses were performed using SPSS, version 13.5 (SPSS). Pearson’s or Fisher’s exact tests were used to compare rates and proportions, as appropriate. Mann-Whitney U tests were used to analyze continuous variables that were not normally distributed. Independent -sample t tests were used to compare normally distributed variables. Times to fever clearance were compared using the log-rank test. All P values were 2-tailed; P ≤ 0.05 was considered to be statically significant.

RESULTS

A total of 348 patients were initially evaluated and 52 patients were excluded prior to randomization (25 patients did not have fever during the baseline examination, 19 patients had severe complications such as hypotension, acute renal failure on admission, and 8 patients did not agree to admit to the hospital). Therefore 296 patients (145 patients in doxycycline group and 151 patients in azithromycin group respectively) were randomized. Recruitment by site was as follows: 137 patients at Udonthani (46.3%); 86 patients at Chumphon (29.1%); 39 patients at Nakhon Rachasima (13.2%); and 34 patients at Chaiyapoom (11.5%). Most
patients (69.3%) were male and the median age was 36 (range 15-88) years. Most patients (69.6%) were agricultural workers, mainly rice farmers. The median duration of fever prior to presentation to hospital was 4.5 days (range 1–15). Forty-three patients received antibiotic treatment within 48 hours prior to enrollment in the study (16, 11%) in doxycycline group and 27, 17.9% in the azithromycin group respectively, P = 0.095). Most of them received either a single dose of ceftriaxone injection or oral doxycycline at the district hospital or primary health care center. The distribution of prior antimicrobial treatment was similar in both treatment groups (data not shown here).

Eighty-nine patients (30.1%, 42 patients in the doxycycline group and 47 patients in the azithromycin group respectively) were lost to follow up after discharge from the hospital. The median duration of follow up was 15 days in both groups, with ranges of 6–120 days in doxycycline group, and 6–150 days in azithromycin group respectively. All patients provided blood sample for culture isolation. Among patients who lost to follow up, second serum sample was obtained on day 3 to day 5 of admission in 23 patients. Therefore 66 patients provided only acute serum.

The causes of acute fever were obtained in 151 out of 296 patients (51%). Of these, the diagnosis was leptospirosis in 69 patients (23.3%), scrub typhus in 57 patients (19.3%), murine typhus in 14 patients (4.7%), evidence of leptospirosis and scrub or murine typhus co-infection in 11 patients (3.7%). The diagnosis of leptospirosis was made by the isolation of leptosprires from blood in 10 patients, a fourfold or greater rise in the MAT titer in 45 patients, and a single titer of 1:400 or greater in 14 patients. The diagnosis of scrub typhus was confirm by a fourfold or greater rise in IFA titers in 34 patients, and a single titer of 1:400 or greater in 23 patients. The distribution of the causes of fever was not significantly different between the two study groups.
In the intention to treat analysis, treatment failure was observed in 3 patients in the doxycycline group, and 4 patients in the azithromycin group ($P=0.12$, Table 1). In addition, severe adverse event occurred in 2 patients in the doxycycline group. Definite diagnosis was not obtained in these patients. Overall the cure rate of azithromycin was non-inferior to doxycycline; 96.5% in the doxycycline group and 97.4% in azithromycin group, with the difference of 0.9% (90% CI -4.6% and 2.8%).

In patients with laboratory confirmed leptospirosis, only 1 patient in the azithromycin group did not show defervescence within 5 days after treatment initiation. Within 48 hours after initiation of treatment, 19 patients (55.9%) in the doxycycline group and 23 patients (65.7%) in the azithromycin group became afebrile ($P=0.33$). Median time to fever clearance was 45 hours (range 8-118 hours) in doxycycline group and 40 hours (range 8-136 hours) in azithromycin group respectively, $P=0.45$.

In patients with laboratory confirmed scrub typhus, treatment failure occurred in 1 patient in azithromycin group. Median time to fever clearance was 48 hours (range 16-120 hours) in doxycycline group and 60 hours (range 12-128 hours) in azithromycin group respectively, $P=0.13$. However within 48 hours after initiation of treatment, a significant higher proportion of doxycycline- treated group (16 patients, 59.3%) became afebrile, compared with azithromycin- treated group (9 patients, 30%), $P=0.03$. The analysis on patients, who did not receive prior antimicrobial treatment, revealed similar results to the analysis of all laboratory-confirmed cases.

No relapse was observed in either group over the follow-up period. Kaplan-Meier curves on the time to defervescence compared between doxycycline group and azithromycin group in patients with laboratory confirmed leptospirosis and in patients with laboratory confirmed scrub typhus are shown in figure 1 and figure 2 respectively.
There were no deaths or serious adverse events in either group. Adverse drug events occurred significantly more frequent in the doxycycline-treated group than in the azithromycin-treated group (Table 2). Doxycycline was discontinued in 2 patients with unknown diagnosis due to adverse reaction, rash and severe vomiting in one each. They were switched to ceftriaxone injection in one patient, and to oral azithromycin in another. Most patients, who developed nausea and/or vomiting after treatment, had a history of nausea or vomiting prior to admission. In patients without this history, only 6 patients in doxycycline group and 2 patients in azithromycin group developed nausea and/or vomiting after treatment.

DISCUSSION

Leptospirosis and scrub typhus are recently recognized as common causes of acute undifferentiated febrile illness in rural southeast Asia (1,5,11). Clinical presentations of these infections vary widely from acute flu-like syndrome, with or without signs of organ dysfunction such as jaundice or renal insufficiency, to multi-organ dysfunction mimicking severe sepsis syndrome (10). Leptospirosis and scrub typhus were ascertained in approximately half of the patients in this study, and were consistent with those found in previous studies in this region (1,5,11). Most cases occurred in men, in the working age group, and in an occupational setting. Approximately 3% of people traveling internationally for short period report fever that requires prompt attention (14). Leptospirosis and scrub typhus in travelers are increasingly recognized as important causes of fever and illness in returning travelers (7).

Diagnosing the causative infections in these fever cases is difficult during the acute phase, and yet appropriate treatment is essential for rapid recovery and the prevention of
complications. Presumptive antimicrobial therapy is recommended whenever a case of either
leptospirosis or scrub typhus is suspected. Oral doxycycline is the standard treatment for mild
cases of leptospirosis and scrub typhus (14, 17). Clinical studies comparing the efficacy of
different antimicrobial treatments for mild leptospirosis and scrub typhus are limited.
Leptospires are sensitive to most antimicrobials in vitro, including macrolides (6). Results of
this study confirmed that oral azithromycin was non-inferior to doxycycline in patients with
confirmed leptospirosis.

A single, oral, 500mg. dose of azithromycin was shown to be an effective alternative
antimicrobial treatment for mild cases of scrub typhus in a recent study in Korea (3). A 3-day
course of azithromycin and a 7-day course of doxycycline were selected for study in Thailand
because the awareness of the emergence of doxycycline resistant O. tsutsugamushi in the
north of the country (13, 15). However all doxycycline treated patients became afebrile within
5 days after an initiation of treatment. Azithromycin was found to be as effective as
doxycycline in patients with confirmed scrub typhus, although the proportion of patients who
became afebrile within 48 hours after azithromycin treatment was significantly lower than in
doxycycline treated patients. Clinical responses of scrub typhus depend on both the
antimicrobial susceptibility of various O. tsusugamshi strains and severity of the disease.
Studies with larger number of patients are needed to confirm this finding.

This study was not a randomized double-blinded study. To reduce information bias,
baseline data and outcome measurements used in this study were based on well defined
criteria, evaluated by an independent investigator, and the diagnosis was blinded to the
statistician until all other data were cleaned and the database was locked. The cure rate in
patients with unknown diagnosis in this study was similar to those with confirmed
leptospirosis or scrub typhus. It was not possible to state that a proportion of these patients
could truly have leptospirosis, or scrub typhus or their illnesses were due to other diseases, because we did not obtain paired sera from some of them, and early treatment has been reported to abrogate the rise in antibody titers between paired samples which are required to identify serologically-confirmed cases. This is the limitation for the generalizability of results of this study.

In summary doxycycline and azithromycin were found to be highly effective against both leptospirosis and scrub typhus. Both drugs were also effective as an initial empirical treatment in patients presented with acute fever without an obvious focus of infection, and no bacterial infection was evident after admission. Azithromycin is an appropriate alternative antimicrobial treatment, in areas where doxycycline-resistant scrub typhus is prevalent and also in children under 8 years old and in pregnancy whose doxycycline is contraindicated. Azithromycin was better tolerated than doxycycline but it is more expensive (approximately 10 $ versus 2 $ per treatment course) and less readily available.

ACKNOWLEDGEMENTS

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REFERENCES


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Legend to Figures

Figure 1. Time to defervescence in hours after treatment in patients with confirmed leptospirosis.

Figure 2. Time to defervescence in hours after treatment in patients with confirmed scrub typhus.
Table 1. Demographic data, final diagnosis and outcome of all 296 patients included in the study (Intention to treat analysis).

<table>
<thead>
<tr>
<th></th>
<th>Doxycycline group (n=145)</th>
<th>Azithromycin group (n=151)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>101/44</td>
<td>104/47</td>
<td>0.88</td>
</tr>
<tr>
<td>Median age in year (range)</td>
<td>38 (15-79)</td>
<td>38 (15-88)</td>
<td>0.78</td>
</tr>
<tr>
<td>Median days of illness (range)</td>
<td>4 (1-14)</td>
<td>5 (1-15)</td>
<td>0.09</td>
</tr>
<tr>
<td>Final diagnosis, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Leptospirosis</td>
<td>34 (23.4)</td>
<td>35 (23.2)</td>
<td>0.96</td>
</tr>
<tr>
<td>- Scrub typhus</td>
<td>27 (18.6)</td>
<td>30 (19.9)</td>
<td>0.79</td>
</tr>
<tr>
<td>- Murine typhus</td>
<td>6 (4.1)</td>
<td>8 (5.3)</td>
<td>0.64</td>
</tr>
<tr>
<td>- Leptospirosis and rickettsioses</td>
<td>6 (4.1)</td>
<td>5 (3.3)</td>
<td>0.71</td>
</tr>
<tr>
<td>- Unknown</td>
<td>72 (49.7)</td>
<td>73 (48.3)</td>
<td>0.82</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Median (range) of fever clearance, hr</td>
<td>48 (8-336)</td>
<td>48 (8-188)</td>
<td>0.57</td>
</tr>
<tr>
<td>- Successful</td>
<td>140 (96.5)</td>
<td>147 (97.4)</td>
<td>0.15</td>
</tr>
<tr>
<td>- Treatment failure</td>
<td>3 (2)</td>
<td>4 (2.6)</td>
<td></td>
</tr>
<tr>
<td>- Stop treatment due to adverse events</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. List of adverse events compared between the two treatment groups (intention to treat analysis).

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>Doxycycline Group (n=145)</th>
<th>Azithromycin Group (n=151)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>40 (27.6)</td>
<td>16 (10.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nausea</td>
<td>3 (2.1)</td>
<td>1 (6.3)</td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>22 (15.2)</td>
<td>10 (6.9)</td>
<td></td>
</tr>
<tr>
<td>Nausea and vomiting</td>
<td>10 (6.9)</td>
<td>1 (1.1)</td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1 (1.1)</td>
<td>1 (1.6)</td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>1 (0.6)</td>
<td>0 (0.5)</td>
<td></td>
</tr>
<tr>
<td>Rash</td>
<td>1 (0.6)</td>
<td>3 (2.1)</td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td>2 (1.2)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
Leptospirosis

Figure 1. Proportion of patients remaining febrile over time to defervescence.}

- Doxycycline group
- Azithromycin group
Figure 2.