Maejo Int. J. Sci. Technol. **2023**, *17*(03), 279-287

Maejo International Journal of Science and Technology

ISSN 1905-7873
Available online at www.mijst.mju.ac.th

Full Paper

Therapeutic effects of doxycycline combined with azithromycin and levofloxacin on tsutsugamushi disease and its influence on neutrophil count and lymphocyte count

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Received: 20 July 2023 / Accepted: 27 December 2023 / Published: 31 December 2023

Abstract: To assess the therapeutic effects of doxycycline combined with azithromycin and levofloxacin on tsutsugamushi disease (TD) and its influence on neutrophil count (NE) and lymphocyte count (LY), a retrospective analysis was performed on the clinical data of 108 patients with TD hospitalised from June 2019 to June 2021. The patients were divided into control group (n=51) receiving doxycycline and observation group (n=57) receiving doxycycline, azithromycin and levofloxacin. Their recovery indices, serum indicators [neutrophil count (NE), lymphocyte count (LY), neutrophil-to-lymphocyte ratio (NLR), C-reactive protein (CRP), D-dimer (D-D) and procalcitonin (PCT)], disease severity [acute physiology and chronic health evaluation (APACHE II) scores], adverse reactions and prognoses (recurrence rate and mortality rate) were compared. Results show that the observation group took a shorter time than the control group in fever abatement, disappearance of other clinical symptoms and signs, and recovery of laboratory indicators to a normal level ($P<0.05$). Its neutrophil count, NLR, CRP, D-D, PCT and APACHE II score was also lower while LY was higher than the control group ($P<0.05$). No mortality occurred in the two groups. In terms of adverse reactions, the two groups had similar incidence and recurrence rates ($P>0.05$).

Keywords: tsutsugamushi disease, scrub typhus, doxycycline, levofloxacin, azithromycin, neutrophil, lymphocyte

INTRODUCTION

Tsutsugamushi disease (TD), also known as scrub typhus, is an acute infectious disease of natural focus induced by *Orientia tsutsugamushi* (Ot), with rats as the major source of infection [1].

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As a systemic disease, it is manifested in a variety of clinical symptoms such as fever, lymphadenectasis, skin ulcer, rashes and eschar. Favourable prognoses are common in most patients after treatment, in contrast to induced dysfunctions of single or multiple organs including the respiratory tract, the nervous system, liver and heart in some patients several weeks after onset [2]. Ot releases toxins to cause proliferation of mononuclear macrophages and systemic diffuse small-vessel vasculitis, resulting in swelling and degeneration of organs and tissues and cell necrosis [3]. Meanwhile, inflammatory responses cannot be controlled effectively due to the release of a large number of cytokines under the stimulation by pathogens, which induces the systemic inflammatory response syndrome and thus aggravates organ dysfunction [4]. The use of antibiotics such as tetracycline, chloramphenicol, quinolinones and macrolides is common for the treatment of TD. As a drug of tetracycline antibiotics, doxycycline is considered the preferred drug for the treatment of TD. However, considering that drug-resistant Ot strains may emerge due to poor clinical effect of single-drug administration, combined drug therapy is commonly used to treat this disease. Both azithromycin (a macrolide antibiotic) and levofloxacin (a quinolinone antibiotic) have antibacterial effects on multiple common pathogenic bacteria in the clinic [5]. However, there are no clear guidelines or authoritative studies regarding the efficacy and safety of the use of doxycycline combined with azithromycin and levofloxacin. Therefore, a retrospective analysis was conducted in this study to compare the therapeutic effects of doxycycline in combination with these two drugs on TD and to observe their influences on serum neutrophil count (NE) and lymphocyte count (LY).

MATERIALS AND METHODS

General Data

The clinical data of 108 patients with TD hospitalised from June 2019 to June 2021 were retrospectively analysed. The patients were divided into two groups according to different medication regimens. The control group consisted of 51 patients consisting of 21 males and 30 females aged 48-67 years (average 57.50±8.92 years). The time from onset to admission was 1-5 d, annually averaged 3.12±1.74 d. The highest body temperature reached 38.6-40.2°C, with an average of 39.36±0.82°C. The complications included skin eschar (n=51), cough (n=24), dyspnea (n=17), chest tightness (n=16), headache (n=16), oliguria (n=9) and skin rashes (n=10). Five patients manifested single-organ dysfunction while 9 patients showed dysfunction of multiple organs. Decreased platelets (n=19), increased alanine aminotransferase (ALT) (n=39), increased leukocytes (n=20), decreased leukocytes (n=14) and increased total bilirubin (TBiL) (n=18) were observed in the laboratory tests.

The observation group comprised 57 patients consisting of 22 males and 35 females aged 48-68 years (average 58.07±10.83 years). The time from onset to admission was 1-6 d, annually averaging 3.47±1.96 d. The highest body temperature went up to 38.5-40.3°C, with an average of 39.25±0.74°C. The complications included skin eschar (n=57), cough (n=25), dyspnea (n=19), chest tightness (n=18), headache (n=19), oliguria (n=11) and skin rashes (n=8). Single-organ dysfunction was observed in 7 patients while dysfunction of multiple organs was identified in 11 cases. Decreased platelets (n=23), increased ALT (n=42), increased leukocytes (n=20), decreased leukocytes (n=16) and increased TBiL (n=21) were observed in the laboratory tests. There were no significant differences in general data between the two groups ($P>0.05$), which were comparable.

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This study was approved by the Medical Ethics Committee of Affiliated Nantong Hospital 3 of Nantong University, China (Approval No. EC202209101).

Inclusion and Exclusion Criteria

Inclusion criteria were set as follows: 1) patients who met the diagnostic criteria for TD [6]; 2) those who had a history of field activities in places like grassland and infectious areas; 3) those who were clinically presented with sudden fever and specific skin eschar or ulcer; 4) those who had an agglutination titer of *Proteus* oxidase-Kitasato antigen of 1:60 or increased titer of double sera in early and late stages by more than 4 times; 5) those with a good clinical response to doxycycline 48 h after administration; and 6) those with complete clinical data.

Exclusion criteria involved: 1) patients with acute myocardial infarction; 2) those with immune or hematological disorders; 3) those with malignant tumours; 4) those with acute infection-induced fever due to other reasons; 5) those with immunosuppression; and 6) those who did not adhere to the standardised treatment during the study period.

Methods

General treatment strategies are as follows. The patients were suggested for better bed rest, a liquid or semi-liquid diet, and maintenance of favourable oral health. In addition, they were given maintenance of acid-base balance, water supply and correction of electrolytes. Corresponding therapies were administered based on patients' symptoms (e.g. antipyretic analgesics for those with fever and diuretics, cardiotonic agents for those with heart failure, and use of enzyme reduction and liver protection therapies for those with liver function damage).

Medications are detailed as below. Patients of both groups received doxycycline hydrochloride tablets (lot no. NMPA H32021266, Jiangsu Lianhuan Pharmaceutical Co., specification: 0.1 g) orally (100 mg/time, twice/d). Doxycycline hydrochloride for injection (lot no. NMPA H20060405, China Meheco Kangli Pharma Co., specification: 0.1 g) was administered by intravenous drip infusion, at a dose of 200 mg for 1 d followed by 100-200 mg/d according to the disease conditions. Adverse reactions mainly involved gastrointestinal reactions and allergic reaction, rarely angioneurotic edema and urticaria, and occasionally thrombocytopenia, hemolytic anemia, headache and vomiting.

In addition, azithromycin tablets (lot no. NMPA H10960167, Pfizer Inc., specification: 0.25 g) were also given orally in the observation group at an initial dose of 500 mg/d followed by 250 mg/d after fever abatement. Azithromycin for injection (lot no. NMPA H20051466, Yabao Pharmaceutical Group Co., specification: 2 mL:0.1 g) was administered by intravenous drip infusion, at a dose of 200 mg/time and twice/d for 1 d followed by 100 mg/time and twice/d. Common adverse reactions included gastrointestinal reactions, dizziness, dyspnea, anorexia and skin rashes. Also, in the observation group levofloxacin hydrochloride for injection (lot no. NMPA H20040313, Shanxi Weiqida Guangming Pharmaceutical Co., specification: 0.2 g) at a dose of 300-400 mg/d was given by intravenous drip infusion, and adverse reactions mainly included gastrointestinal reactions, abdominal discomfort, headache, insomnia and allergic reaction, and occasionally phlebitis and epilepsy. The treatment period lasted for 7-10 d.

Observation Indices

Clinical indices, namely the time taken for fever abatement, disappearance of other clinical

symptoms and signs, and recovery of laboratory indicators to a normal level were recorded.

Serum indicators were also detected. Four mL of peripheral venous blood was drawn from each patient before treatment and at 7 d after treatment. After standing at room temperature for 20 min., the blood samples were centrifuged at 3,000 r/min. for 15 min. and then the supernatant was dispensed into separate 1.5-mL EP tubes and stored at -80°C. Blood routine tests were performed using a Sysmex XE-2100 automated hematology analyser (Japan) to record NE and LY and the neutrophil-to-lymphocyte ratio (NLR) was calculated. The levels of C-reactive protein (CRP) and D-dimer (D-D) were detected *via* enzyme-linked immunosorbent assay (ELISA) using a Siemens ADVIA1800 automatic biochemistry analyser (Siemens, Germany), and the procalcitonin (PCT) level was tested by electrochemiluminescence using a Roche E170E601 automatic electrochemiluminescence immunoassay analyser (Switzerland).

Disease severity was evaluated: The Acute Physiology and Chronic Health Evaluation (APACHE II) scoring system (involving 3 items: acute physiology score, age and chronic health score, totally scoring 0-71 points) was employed to assess the recovery of patients on 1, 3 and 7 d after treatment. A lower score indicated a better outcome, and a score >15 points represented a severe case.

Adverse drug reactions occurring in patients were recorded. With regard to prognosis, the recurrence rate and mortality rate of TD patients within 1 month after the treatment were calculated.

Statistical Analysis

SPSS 23.0 software was employed for statistical analysis. Measurement data were expressed as $\bar{x} \pm s$. The *t*-test was used for inter-group comparisons and the *F*-test was utilised for intra-group comparisons at different time points. Count data were expressed as percentage (%) and analysed by the χ^2 test. $P<0.05$ was considered statistically significant.

RESULTS

Clinical Indices

The observation group took a shorter time than the control group in fever abatement, disappearance of other clinical symptoms and signs, and recovery of laboratory indicators to a normal level ($P<0.05$) (Table 1).

Table 1. Clinical indices (x ± s, d)

Group	Days taken for fever abatement	Days taken for disappearance of other clinical symptoms and signs	Days taken for recovery of laboratory indicators to a normal level
Control (n=51)	5.40±1.19	6.12±1.47	7.84±1.96
Observation (n=57)	4.08±0.72	5.39±1.18	5.73±1.49
t	7.057	2.859	6.336
P	<0.001	0.005	<0.001

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Serum Indicators and APACHE II Scores

The levels of serum indicators were compared between observation group and control group before treatment, which showed no significant differences (P>0.05). After treatment, the observation group displayed lower levels of NE, NLR, CRP, D-D and PCT and a higher level of LY than the control group (P<0.05) (Table 2). The APACHE II scores were lower in the observation group than those in control group at 3 and 7 d after treatment (P<0.05) (Table 3).

Table 2. Serum indicators (x ± s)

Group	NE ($\times10^9$/L)		LY ($\times10^9$/L)		NLR		CRP (mg/L)		D-D (mg/L)		PCT (ng/mL)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control (n=51)	10.53±4.68	4.72±1.17#	0.86±0.35	2.04±0.63#	12.24±5.82	2.31±0.68#	38.93±7.42	3.55±0.79#	0.64±0.18	0.32±0.11#	0.96±0.37	0.43±0.16#
Observation (n=57)	10.19±4.37	3.39±0.94#	0.83±0.30	2.80±0.71#	12.27±5.95	1.21±0.40#	39.10±7.78	2.94±0.56#	0.68±0.20	0.21±0.07#	0.94±0.35	0.29±0.10#
t	0.390	6.542	0.480	5.855	0.026	10.374	0.116	4.666	1.088	6.266	0.289	5.513
P	0.697	<0.001	0.632	<0.001	0.979	<0.001	0.908	<0.001	0.279	<0.001	0.773	<0.001

# P<0.05 cf. before treatment in the same group

Table 3. APACHE II scores (point, x ± s) on 1, 3 and 7 d after treatment

Group	1 d	3 d	7 d	*F*
Control (n=51)	9.92±1.49	6.68±0.91	3.00±0.56	545.578
Observation (n=57)	9.76±1.28	5.20±0.78	2.00±0.39	1084.105
t	0.600	9.100	10.859	
P	0.550	<0.001	<0.001	

Adverse Reactions and Prognosis

No mortality occurred in the two groups. In terms of adverse reactions, an incidence of 3.51% and 5.88% and a recurrence rate of 1.75% and 3.92% in the observation group and control group respectively indicated no significant differences (P>0.05) (Table 4).

Table 4. Adverse reactions and prognosis [n (%)]

Group	Gastrointestinal reaction	Allergic reaction	Headache	Total incidence	Recurrence rate
Control (n=51)	2 (3.92)	1 (1.96)	0 (0.00)	3 (5.88)	2 (3.92)
Observation (n=57)	1 (1.75)	0 (0.00)	1 (1.75)	2 (3.51)	1 (1.75)
χ^2				0.343	0.468
P				0.558	0.494

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DISCUSSION

Ot, an obligate intracellular parasitic bacterium, cannot survive without a host and mainly reproduces in small-vessel endothelial cells and reticuloendothelial cells [7]. When the cells are invaded, a large amount of toxins may be released into the blood and thus induce acute vasculitis, peripheral vasculitis, degeneration, hyperemia and edema of parenchymal organs, eventually leading to dysfunction of multiple organs [8]. Antibiotics are considered a major means for the treatment of TD, but only those with high liposolubility and ability to pass through the cell membrane of the host are therapeutically effective for Ot [9]. Aminoglycoside and β-lactam antibiotics, for instance, are ineffective for Ot because they cannot enter the cytoplasm through the cell membrane of the host [10]. In addition, since the absolute immunity to Ot cannot be achieved until two weeks after infection in most circumstances and early administration of antibiotic therapy is unable to guarantee enough time for the body to generate effective immune responses, relapse may occur after a short-course treatment at the early stage [11]. Therefore, the treatment duration in this study was specified as 7-10 d according to the conditions of the patients, and all included subjects completed the course of treatment.

Chloramphenicol was used as the most common antibiotic for treatment of TD in clinical practice. However, chloramphenicol, which may induce serious adverse reactions such as bone marrow depression and irreversible aplastic anemia, has been currently replaced by doxycycline [12]. This drug has certain inhibitory effect on Ot based on the mechanism of action in which it interferes with the binding of the action site on 30S subunit (a bacterial ribosome) to the amino-acyl transfer RNA by binding to the 30S subunit, and also blocks the amino-acyl transfer RNA from reaching the messenger RNA. In this manner it inhibits the extension of peptide chains during protein synthesis, thus obstructing protein synthesis and eventually achieving the antibacterial effect [13, 14]. A previous study reported that doxycycline is also effective for vascular inflammation in a selective manner and able to efficiently reduce the numbers of NE and cytotoxic T lymphocytes in vascular walls, thus playing a strong anti-inflammatory role [15]. Nevertheless, some patients with TD do not respond well to doxycycline, which may be attributed to the disease's resistance to doxycycline [16].

Guan et al. [17] revealed that both doxycycline and azithromycin were substantially effective in treating severe TD. In a study conducted by Wangrangsimakul et al. [18] both doxycycline and azithromycin were able to interfere with protein synthesis of the bacteria and affect the growth cycle of the germs. As shown by the results of this study, the observation group needed less time than the control group for fever abatement, disappearance of other clinical symptoms and signs, and recovery of laboratory indicators to a normal level, indicating that the clinical efficacy of doxyclycline combined with azithromycin and levofloxacin is superior to that of doxycycline alone in treating TD.

The 70S ribosome serves as the major action site of azithromycin, which enters the bodies of bacteria through their cell membrane and subsequently binds to the bacterial ribosome 50S subunit. In this manner, it inhibits the translocation of messenger RNA ribosomes, thus suppressing the protein synthesis of bacteria to achieve the antibacterial effect [19]. Moreover, azithromycin has a curative effect in blocking Ot reproduction due to its high concentration at infection sites and within the bacteria [20].

The antibacterial mechanism of levofloxacin is based on its inhibitory role on the activity of DNA gyrases of bacteria and RNA and protein syntheses. However, levofloxacin is reported to be

active against Gram-negative bacteria, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*, but possibly has a weaker antibacterial effect on Ot [21]. According to a previous study on *in vitro* activity of levofloxacin on obligate intracellular bacterium Ot, the antibiotic effect of levofloxacin is inferior to that of macrolides [22].

Neutrophil, the first line of defense of the body in the face of inflammatory and immune responses, has the functions of chemotaxis, phagocytosis, adhesion and sterilisation. When Ot releases toxins to damage organs, it also releases a large number of inflammatory cytokines and destructive enzyme factors that will cause a systemic inflammatory response. Lymphocyte works as a key cell component that participates in the immune response of the body. With the ability to promote lymphocyte apoptosis, inflammatory response contributes to increase in the NLR value. In comparison to inflammatory cytokines such as CRP and PCT that are commonly used in clinical practice, NE and LY are more stable indicators in evaluating the severity of the body infection. As shown by the results of this study, the observation group has lower levels of NE, NLR, CRP, D-D and PCT, but a higher level of LY than the control group, suggesting that a combined therapy of doxycycline and azithromycin has strong anti-inflammatory and anti-infection effects against TD. This further confirms the effectiveness of doxycycline combined with azithromycin in inhibiting Ot reproduction. Both groups showed relatively mild adverse reactions and a low recurrence rate with no mortality reported, indicating that both uses of doxycycline combined with azithromycin or levofloxacin are highly safe.

CONCLUSIONS

The use of doxycycline in combination with azithromycin and levofloxacin is effective for the treatment of TD.

AUTHORS' CONTRIBUTION

X. C. and L. J. contributed equally to this work.

ACKNOWLEDGEMENTS

This work was supported by Nan Tong Health and Health Committee, Nan Tong City (Funding Nos. QN2022042, MS2022060 and MS2022061).

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