Brief Communication

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In Vitro Activity of Tigecycline Against *Orientia tsutsugamushi*

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Scrub typhus is a zoonosis caused by *Orientia tsutsugamushi* (*O. tsutsugamushi*) occurring mainly in autumn in Korea. The need of new antibiotics has arisen with a report on strains resistant to antibiotics and chronic infection. This study aims to identify susceptibility of tigecycline *in-vitro* as a new therapeutic option for *O. tsutsugamushi*. Antibacterial activity of tigecycline against the *O. tsutsugamushi* was compared with doxycycline using flow cytometry assay. The inhibitory concentration 50 (IC_{50}) was 3.59×10^{-3} μg/mL in doxycycline-treated group. Whereas in 0.71×10^{-3} μg/mL tigecycline-treated group. These findings indicate that tigecycline may be a therapeutic option for the treatment of scrub typhus.

Key Words: *Orientia tsutsugamushi*, scrub typhus, tigecycline, IC_{50}

Scrub typhus is a febrile disease caused by *Orientia tsutsugamushi* (*O. tsutsugamushi*), which is a gram negative and obligate intracellular bacterium. Symptoms vary from a simple febrile illness to a life threatening fatal infection, complicated with multi-organ dysfunction. A variety of antibiotics such as chloramphenicol, doxycycline and azithromycin have shown an effective bactericidal activity against scrub typhus. However, chloramphenicol poses the risk of relapse and aplastic anemia as a serious adverse reaction and it is not recommended during pregnancy.[1,2] Tetracycline was reported to be more effective than chloramphenicol but its use is not recommended for pregnant women and children because of its teratogenic potential.[3] Doxycycline is effective against *O. tsutsugamushi*, but a potential treatment failure looms in the emergence of a drug-resistant strain and possibility of chronic infection.[4-6] Azithromycin shows efficacy *in vitro* to the doxycycline-resistant *O. tsutsugamushi* demonstrating therapeutic potential to drug-resistant strains.[3] Nevertheless, one needs to be cautious in applying azithromycin since therapeutic failure and relapse have been reported in studies analyzing drug efficacy in treating scrub typhus.[7] As described, various antibiotics have demonstrated diverse aspects of therapeutic effects against scrub typhus, however, along with reports of resistant strains to *O. tsutsugamushi*, there is a need to search for new antibiotics that are more effective yet safe against *O. tsutsugamushi*.

Tigecycline is a novel glycylcycline and an antimicrobial agent that has shown excellent activity against many multidrug-resistant organisms and intracellular bacteria in *in vivo* and *in vitro* studies.[8] The intracellular penetration of tigecycline may contribute to the growth inhibition of intracellular bacterial pathogens.[9] Spyridaki, et al.[10] showed that tigecycline was effective in inhibiting stringent intracellular bacteria, *Coxiella burnetii* (*C. burnetii*). However, activity of tigecycline against *O. tsutsugamushi* has not yet been assessed. In this study, we intended to compare the effects of tigecycline and doxycycline against *O. tsutsugamushi in vitro*.

The flow cytometric assay has been used to examine antibiotic susceptibility in many intracellular pathogens. Dessus-Babus, et al.[11] documented antibiotic susceptibility for *Chlamydia*

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trachomatis by a flow cytometry, and Kim, et al.[12] also demonstrated that bacterial growth in a doxycycline resistant strain could be measured quantitatively using this assay. In the present study, ECV304 infected with *O. tsutsugamushi* Boryong strain was cultured with media containing two-fold dilutions of antibiotics from 0.2 μg/mL to 3.9×10^{-4} μg/mL, except the positive controls which was infected cells with *O. tsutsugamushi* without antibiotics and the uninfected cells (negative control). Six days later, infected cells in each well were collected. Fixation-permeabilization and staining using FITC-conjugated FS15 monoclonal antibody were performed to detect intracellular *O. tsutsugamushi*. Each set of test was repeated three times. We then assigned resistance (R), intermediate susceptibility (I), and susceptibility (S) to evaluate the antibiotic efficacy. "R" indicates the percentage of infected cells more than 50% of the positive control and "I" the percentage value between 50 and 10% of the positive control, and "S" below 10% of the positive control. The minimal inhibitory concentration (MIC) was defined as the highest concentration of antibiotics at flow cytometry value was reduced by 10% less than the value of positive control. MIC of tigecycline and doxycycline were 0.0016 μg/mL and 0.0125 μg/mL, respectively, demonstrating that treatment with doxycycline may result in a compromised treatment outcome if the dosage was not sufficient (Table 1). We also calculated the value of the half maximum inhibitory concentration (IC_{50}) from mean fluorescence intensity measured by flow cytometry: IC_{50} represents the antibiotic concentration required to reduce the percentage of infected cells by 50% compared with antibiotic-free infected cells (positive control). IC_{50} values can be more objective and precise compared to MIC value obtained by microscopic readings. As shown in Fig. 1, the IC_{50} curve shape exhibited different patterns of inhibition for each antibiotic. The IC_{50} curve shape of the doxycycline-treated group showed that the effect of bacterial growth inhibition was gradually decreased from 0.00625 μg/mL to the lowest concentration, whereas the activity of tigecycline had steeply dropped at 0.00078 μg/mL, indicating that tigecycline suppresses the growth of *O. tsutsugamushi* more efficiently than doxycycline at the same concentration. The IC_{50} values of group treated with doxycycline and tigecycline were 3.59×10^{-3} μg/mL and 0.71×10^{-3} μg/mL, respectively, thus IC_{50} values for the tigecycline treated group being remarkably lower than for the doxycycline treated group.

For the treatment of infectious diseases, the antibiotic concentration within an infected tissue is important. Since *O. tsutsugamushi* replicates in endothelial cells and macrophages, it is necessary to achieve sufficient antibiotic concentration within blood and tissue.[13,14] *C. burnetii* has been reported to cause persistent and recurrent infection by its ability to live inside phagocytic cells.[15] *O. tsutsugamushi* can also cause chronic infections, a phenomenon regarded to be related to its characteristic of residing inside cells. Thus, an effective treatment against *O. tsutsugamushi* would require antibiotics that respond well in the intracellular environment.[10]

Tigecycline, a new antibiotic belonging to a family of glycylcyclines, was approved by the U.S. Food and Drug Administration

Table 1. Percentage of Infected Cells in Doxycycline and Tigecycline of Various Concentration

	Percentage of infected cells (%)											
Concentration of antibiotics (μg/mL)	N.C	0.2	0.1	0.05	0.025	0.0125	0.0063	0.0031	0.0016	0.0008	0.0004	P.C
Doxycycline	1	1 (S)	2 (S)	3 (S)	3 (S)	3 (S)	17 (I)	24 (R)	31 (R)	37 (R)	38 (R)	41
Tigecycline	1	0 (S)	0 (S)	0 (S)	1 (S)	1 (S)	1 (S)	2 (S)	4 (S)	27 (I)	50 (R)	60

N.C, negative control; P.C, positive control; S, susceptible; I, intermediate susceptibility; R, resistant.

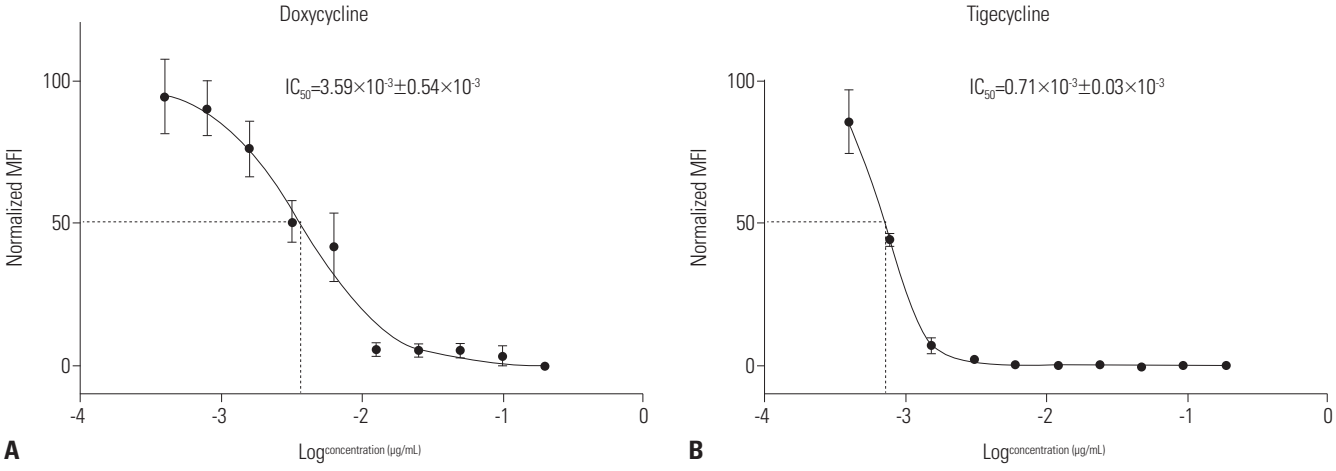

Fig. 1. The inhibitory concentration 50 (IC_{50}) of doxycycline (A) and tigecycline (B) against *Orientia tsutsugamushi* (*O. tsutsugamushi*). IC_{50} of doxycycline and tigecycline, in ECV304 cells infected with *O. tsutsugamushi*. IC_{50} calculated from mean fluorscence intensity (MFI) at different concentrations of antibiotics using flow cytometry assay. This result shows that tigecycline inhibits the growth of bacteria better than doxycycline.

for the treatment of complicated skin and soft tissue infections and intra-abdominal infection in 2005.[16] It has a broad antibacterial activity. In particular, it has been suggested that tigecycline can be used to treat intracellular bacteria, because of its high capability to penetrate into tissue cells and remain accumulated inside.[17] Tigecycline's various effects have been reported against intracellular bacteria such as *Legionella pneumophila*, *Chlamydia pneumoinae*, and *Salmonella* species.[18-20] Tigecycline typically displays a large volume of distribution of 7–10 L/kg, extensively spreading to the bone or lung tissues and bile.[21] The tigecycline concentration, when administered to human polymorphonuclear neutrophils, rose rapidly, increasing the extracellular concentration by 20–30 fold.[22] With an excellent cell penetration property and an ability to maintain high cellular concentration, tigecycline possesses an advantageous characteristic in treating intracellular bacteria. In the present study, we found that MIC and IC_{50} values of tigecycline were 0.0016 μg/mL and 0.0007 μg/mL, respectively. Pharmacokinetic study of tigecycline showed that the maximum and minimum plasma concentrations were 0.72±0.24 μg/mL and 0.10±0.05 μg/mL, respectively, after administration of 100 mg followed by subsequent dosage of 50 mg (or twice every 12 hours). These values were remarkably higher than the MIC and IC_{50} values demonstrated in the present study. Tigecycline has excellent tissue penetration, having a high ratio of intracellular to extracellular drug concentration, and maintaining an adequate level of blood concentration. All of these characteristics make it well suited to treating intracellular bacteria like *O. tsutsugamushi*. Therefore, we may expect sufficient therapeutic effects against *O. tsutsugamushi in vivo*.[21]

Tigecycline, a semi-synthetic tetracycline derivatives, is stable for tetracycline-specific efflux pumps and is thus expected to work against resistant strains.[23] In Thailand, strains resistant to doxycycline, a representative antibiotic drug against scrub typhus, have been reported[4] and no drugs that would work effectively against these antibiotic-resistant strains have been generated to date. In the present study, we did not investigate tigecycline activity against drug-resistant strains. However, since tigecycline displayed sufficient efficacy to other strains resistant to a diverse group of drugs, we can envision therapeutic effects of tigecycline against strains that are resistant to doxycycline as well. Follow-up studies investigating this point would be necessary.

Tigecycline showed better antibiotic efficacy than doxycycline. In addition to strains reasistant to doxycycline, tigecycline might be applied to severe scrub typhus cases.

In summary, our present study has shown that tigecycline sufficiently suppressed the activity of *O. tsutsugamushi in vitro*, and tigecycline can be expected to be an alternative drug for treating scrub typhus patients.

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