In Vitro Susceptibility Test of Sitafloxacin against Resistant Gram-Negative Bacilli Isolated from Thai Patients by Disk Diffusion Method

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Objective: *To determine a correlation of minimum inhibitory concentration (MIC) of sitafloxacin determined by agar dilution method with inhibition zone diameter of sitafloxacin determined by disk diffusion method, and to determine inhibition zone, diameter breakpoints of sitafloxacin against resistant gram-negative bacilli isolated from Thai patients.*

Material and Method: *The study bacteria were 332 clinical isolates of gram-negative bacilli including ESBL-producing E. coli, ESBL-producing K. pneumoniae, P. aeruginosa and A. baumannii. Each isolate of the present study bacteria was tested for minimum inhibitory concentration (MIC) of sitafloxacin by agar dilution method and inhibition zone diameter of sitafloxacin by disk diffusion method.*

Results: *The MICs and inhibition zone diameters of sitafloxacin against gram-negative bacilli were well correlated (correlation coefficient -0.926, p-value <0.001). The inhibition zone diameter ≥15 mm had the least total error for determining susceptibility to sitafloxacin based on MIC value of sitafloxacin but the inhibition zone diameter ≥16 mm had less false susceptibility than that of ≥15 mm when compared with sitafloxacin MIC ≤2 mg/l that was considered susceptible. The inhibition zone diameter ≥19 mm had the least total error for determining susceptibility to sitafloxacin based on MIC value of sitafloxacin but the inhibition zone diameter ≥18 mm had less false susceptibility than that of ≥19 mm when compared with sitafloxacin MIC ≤1 mg/l that was considered susceptible.*

Conclusion: *For the susceptibility test of sitafloxacin against resistant gram-negative bacilli by disk diffusion method, the inhibition zone diameter ≥16 mm and ≥18 mm seem to be the appropriate breakpoints for susceptibility for resistant gram-negative bacilli isolated from urine and blood, respectively, since the serum concentration of sitafloxacin is rather low whereas the urinary concentration of sitafloxacin is much higher.*

Keywords: *Sitafloxacin, Inhibition zone, Minimum inhibitory concentration, Gram-negative bacilli, Susceptibility*

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Sitafloxacin (DU 6859a) is an oral fluoroquinolone. The mechanism of action of sitafloxacin involves inhibition of bacterial DNA gyrase and topoisomerase IV[1]. Bactericidal activity of sitafloxacin results from inhibition of these enzymes. The inhibitory activity of sitafloxacin against these enzymes was higher than that of the other fluoroquinolone antimicrobials used as comparators. Sitafloxacin also has a potent inhibitory activity against enzymes from fluoroquinolone-resistant bacteria. Sitafloxacin has a broad spectrum of antibacterial activity against aerobic and anaerobic gram-positive and gram-negative bacteria, as well as atypical bacteria including the strains that are resistant to other fluoroquinolones. Pharmacokinetics of sitafloxacin was generally favorable[2]. Oral administration of 100 mg of sitafloxacin was rapidly absorbed with an absolute bioavailability up to 90%. Food intake did not affect the rate and extent of absorption. The mean maximum concentration in serum of sitafloxacin was 1 mg/L after receiving sitafloxacin 100 mg with elimination half-life of 5 h. Sitafloxacin was primarily eliminated by the kidney. The area under the drug concentration-time curve was 5.5 mg.h/L. The serum protein binding of the drug was approximately 50%. The apparent volume of distribution exceeded 1.8 L/kg suggesting good tissue penetration. Sitafloxacin was recently approved

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in Japan and Thailand for the treatment of respiratory tract and genitourinary tract infections. Comparative in vitro activity study of sitafloxacin against bacteria isolated from Thai patients with urinary tract infections and lower respiratory tract infections revealed that sitafloxacin was much more active than other existing fluoroquinolones (ciprofloxacin, levofloxacin and moxifloxacin) in Thailand[3]. Sitafloxacin was active against some isolates of gram-negative bacilli that were resistant to the aforementioned fluoroquinolones, and it was also active against many strains of multidrug-resistant gram-negative bacilli including extended spectrum beta-lactamase (ESBL)-producing *E. coli*, ESBL-producing *K. pneumomiae*, and *carbapenem-resistant Acinetobacter baumannii*. Therefore, sitafloxacin might have a role in therapy of infections caused by the aforementioned multidrug-resistant gram-negative bacilli.

The Clinical and Laboratory Standards Institute (CLSI) has not recommended minimum inhibitory concentration (MIC) and inhibition zone, diameter breakpoints for sitafloxacin against multidrug-resistant gram-negative bacilli. A study on interpretive criteria for susceptibility tests with sitafloxacin tested against 100 isolates of *Haemophilus influenzae* and 102 isolates of *Neisseria gonorrhoeae* was reported[4]. The proposed *H. influenzae* susceptibility testing criteria were for sitafloxacin susceptible at ≤1 mg/l (5-microgram disk zone diameter correlate, ≥19 mm) or ≤2 mg/l (disk zone diameter correlate, ≥16 mm). The gonococcal susceptibility testing criteria were for sitafloxacin susceptible at ≤0.12 mg/l (disk zone diameter correlate, >34 mm). Another study on interpretive criteria for susceptibility tests with sitafloxacin tested against 494 bacterial isolates including 255 *Enterobacteriaceae*, 45 *enterococci*, 90 *staphylococci*, 37 *Pseudomonas* spp., 14 *Xanthomonas maltophilia*, 16 *Acinetobacter* spp., 30 *Moraxella catarrhalis*, and seven other organisms[5]. The interpretive criteria were proposed for two susceptible breakpoints: ≥16 mm for MIC ≤2 mg/l, and ≥19 mm for MIC ≤1 mg/l. These criteria demonstrated at least 97% absolute interpretive agreement between test methods.

The objectives of the present study were to determine a correlation of minimum inhibitory concentration (MIC) of sitafloxacin determined by broth dilution method, with inhibition zone diameter of sitafloxacin determined by disk diffusion method, and to determine inhibition zone, diameter breakpoints of sitafloxacin against resistant gram-negative bacilli isolated from Thai patients.

Material and Method

Study bacteria

They were 332 clinical isolates of gram-negative bacilli from different hospitalized patients with lower respiratory tract infections, urinary tract infections or bacteremia including ESBL-producing *E. coli* (n = 88), ESBL-producing *K. pneumoniae* (n = 84), *P. aeruginosa* (n = 80) and *A. baumannii* (n = 80).

Minimum inhibitory concentrations (MIC) determination

The MICs of sitafloxacin against gram-negative bacilli were determined by standard agar dilution method according to the clinical and laboratory standards institute (CLSI) 2010. Inoculum preparation of gram-negative bacteria was made by broth method, adjusted to 0.5 McFarland turbidity and then the bacterial suspension diluted with cation adjusted Mueller Hinton broth (BBL, Becton Dickinson, USA) to 10^6 CFU/mL. Final inoculum of approximately 10^6 CFU/mL was used and applied to the medium by multipoints spot inoculators. The inoculated agars were incubated at 35°C for 18-24 hours in ambient air. The MIC was defined as the lowest concentration of antimicrobial agent that inhibited visible growth on agar. The control strain was *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853.

Inhibition zone diameter determination

The inhibition zone diameters of sitafloxacin against gram-negative bacilli were determined by standard disk diffusion method using 5-microgram disk of sitafloxacin.

Data analyses

A correlation of MICs of sitafloxacin with inhibition zone diameters of sitafloxacin was determined by Spearman correlation. A p-value <0.05 was considered statistically significant. The inhibition zone, diameter breakpoints of sitafloxacin against ESBL-producing *E. coli*, ESBL-producing *K. pneumomiae, P. aeruginosa and Acinetobacter baumannii* were analyzed by descriptive statistics. MIC of sitafloxacin is considered the gold standard for sitafloxacin susceptibility. False resistance is defined as when bacterial strain is susceptible to sitafloxacin by MIC criteria but is resistant to sitafloxacin by specified inhibition zone diameter, whereas false susceptibility is defined as when bacterial strain is resistant to sitafloxacin by MIC criteria but is susceptible to sitafloxacin by specified inhibition zone diameter.

Results

The correlation between MICs and inhibition zone diameters of sitafloxacin against resistant gram-negative bacilli is shown in Fig. 1. The correlation coefficient was -0.926 (p-value <0.001). The correlation between MICs and inhibition zone diameters of sitafloxacin against each type of gram-negative bacilli (ESBL-producing *E. coli*, ESBL-producing *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*) also showed statistically significant negative correlations with the correlation coefficient varying from -0.82 to -0.92. The agreements between sitafloxacin MIC ≤2 mg/l and inhibition zone diameter ≥15, ≥16 and ≥17 mm are shown in Fig. 2 and Table 1. The inhibition zone diameter ≥15 mm had the least total error but the inhibition zone diameter ≥16 mm had less false susceptibility than that of ≥15 mm. The agreements between sitafloxacin MIC ≤1 mg/l and inhibition zone diameter ≥18, ≥19 and ≥20 mm are shown in Fig. 3 and Table 2. The inhibition zone diameter ≥19 mm had the least total error but the inhibition zone diameter ≥18 mm had less false susceptibility than that of ≥19 mm.

Discussion

Sitafloxacin showed good in vitro activity against resistant gram-negative bacteria isolated from Thai patients with urinary tract infections and lower respiratory tract infections[3]. Sitafloxacin has been approved by Thai FDA and has been available for clinical use in Thailand since 2011. Since some strains of resistant gram-negative bacteria are still resistant to sitafloxacin, it is necessary to perform in vitro susceptibility tests of sitafloxacin against the bacteria isolated from the patients in clinical setting. Determination of sitafloxacin MIC is not available in all microbiology laboratories in Thailand. Therefore, the disk diffusion method needs to be used for determination of sitafloxacin susceptibility. The clinical and laboratory standards institute (CLSI) does not have inhibition zone, diameter breakpoints for sitafloxacin against resistant gram-negative bacilli because sitafloxacin is not widely available in other countries except Japan and Thailand. The existing interpretive criteria for susceptibility test of sitafloxacin by disk diffusion were derived from several studies in bacteria isolated from Western countries many years ago that did not include many strains of resistant gram-negative bacilli[4,5]. Therefore, such interpretive criteria for susceptibility test of sitafloxacin by disk diffusion might not be applicable to resistant gram-negative bacilli isolated from Thai patients. The present study revealed that the inhibition zone diameters of sitafloxacin were well correlated with MIC of sitafloxacin against resistant gram-negative bacteria. Therefore, the disk diffusion method should be useful for determination of sitafloxacin susceptibility against resistant gram-negative bacilli. The present study showed that the most appropriate inhibition zone, diameter breakpoint of sitafloxacin of ≥16 mm for bacterial isolate with MIC ≤2 mg/l was the same as that suggested in a previous

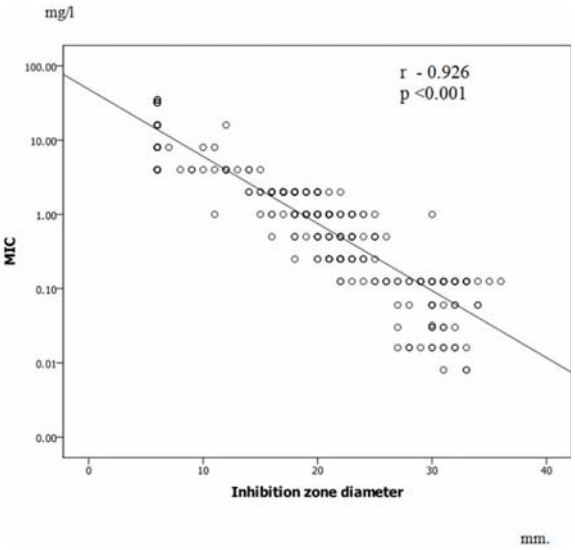
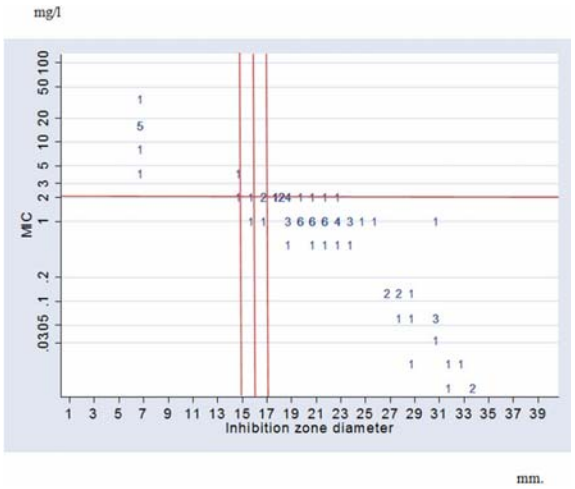


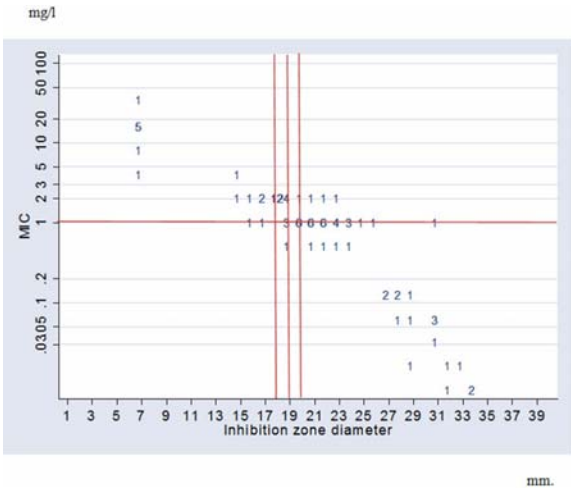
Fig. 1 The correlation between MICs and inhibition zone diameters of sitafloxacin against 332 isolates of Gram negative bacilli.



NB = the number in figure is the number of the isolates.

Fig. 2 Agreements between sitafloxacin MIC ≤2 mg/l and inhibition zone diameter 15 mm, 16 mm and 17 mm.

study[5]. However, the most appropriate inhibition zone diameter breakpoint of sitafloxacin of ≥18 mm for bacterial isolate with MIC ≤1 mg/l observed in the present study was different from ≥19 mm that was suggested in a previous study[5]. The single-dose pharmacokinetics of sitafloxacin after oral administration of sitafloxacin to fasted Japanese male volunteers showed that the mean maximum concentrations of sitafloxacin in serum were 0.29, 0.51, 1.00, and 1.86 mg/l after receiving 25, 50, 100, and 200 mg, respectively. These serum concentrations were rather low when MIC breakpoints of 1 mg/l and 2 mg/l were taken into account. However, the mean concentrations of sitafloxacin in the urine samples obtained 12 to 24 h after oral doses of 25, 50, 100, and 200 mg were 5.3, 9.0, 14.1 and 41.8 mg/l, respectively. The aforementioned urinary concentrations at 12 to 24 h after oral administration were still much higher than 1 mg/l and 2/mg/l that are considered MIC breakpoints of sitafloxacin. Therefore, MIC breakpoint 1 mg/l might be considered as the breakpoint for blood isolate of bacteria, and MIC breakpoint 2 mg/l should be considered as the breakpoint for urinary isolate of bacteria. It should be kept in mind that the suggested interpretive inhibition zone diameter breakpoint of sitafloxacin of ≥16 mm for bacterial isolate with MIC ≤2 mg/l and that of ≥18 mm for bacterial isolate with MIC ≤1 mg/l observed in the present study are not in perfect agreement with MIC. Therefore, caution should be exercised when prescribing sitafloxacin for empiric therapy of patient with severe infection caused by suspected resistant gram-negative bacteria. Sitafloxacin should be considered for therapy of mild to moderate infection due to documented resistant gram-negative bacteria that are resistant to other oral agents, yet susceptible to sitafloxacin.



NB = the number in figure is the number of the isolates.

Fig. 3 Agreements between sitafloxacin MIC ≤1 mg/l and inhibition zone diameter 18 mm, 19 mm and 20 mm.

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Table 1. Agreements between sitafloxacin MIC ≤2 mg/l and inhibition zone diameter 15 mm, 16 mm and 17 mm

Zone diameter	False resistance	False susceptibility	Total error
≥15 mm	3 (0.9%)	1 (0.3%)	4 (1.2%)
≥16 mm	6 (1.8%)	0	6 (1.8%)
≥17 mm	21 (6.3%)	0	21 (6.3%)

Table 2. Agreements between sitafloxacin MIC ≤1 mg/l and inhibition zone diameter 18 mm, 19 mm and 20 mm

Zone diameter	False resistance	False susceptibility	Total error
≥18 mm	23 (6.9%)	6 (1.8%)	29 (8.7%)
≥19 mm	16 (4.8%)	11 (3.3%)	27 (8.1%)
≥20 mm	33 (9.9%)	7 (2.1%)	40 (12%)

Potential conflicts of interest

None.

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การทดสอบความไวของแบคทีเรียกรัมลบดื้อยาต่อ sitafloxacin ด้วยวิธี disk diffusion

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วัตถุประสงค์: เพื่อทราบความสัมพันธ์ระหว่างปริมาณ minimum inhibitory concentration (MIC) ที่ตรวจด้วยวิธี agar dilution กับขนาดของ inhibition zone diameter ของยา sitafloxacin ที่ตรวจด้วยวิธี disk diffusion และเพื่อทราบขนาดของ inhibition zone diameter ของยา sitafloxacin ที่แสดงว่าแบคทีเรียกรัมลบดื้อยาที่แยกจากผู้ป่วยไทยไวต่อยา sitafloxacin

วัสดุและวิธีการ: แบคทีเรียกรัมลบดื้อยาจำนวน 332 สายพันธุ์ที่แยกได้จากผู้ป่วย เชื้อดังกล่าวได้แก่ ESBL-producing E. coli, ESBL-producing K. pneumoniae, P. aeruginosa และ A. baumannii นำเชื้อแต่ละสายพันธุ์มาตรวจ MIC ของยา sitafloxacin ด้วยวิธี agar dilution และ inhibition zone diameter ต่อยา sitafloxacin ด้วยวิธี disk diffusion

ผลการศึกษา: MIC และ inhibition zone diameter ของยา sitafloxacin ต่อแบคทีเรียกรัมลบดื้อยา มีความสัมพันธ์กันดีโดยมี correlation coefficient -0.926 (p-value <0.001) ขนาดของ inhibition zone diameter ≥15 มม. มีความคลาดเคลื่อนรวมในการทำนายความไวต่อยา sitafloxacin ด้วย MIC น้อยที่สุด แต่ขนาด inhibition zone diameter ≥16 มม. มีความไวลวงน้อยกว่า inhibition zone diameter ≥15 มม. เมื่อเปรียบเทียบกับเชื้อที่ไวต่อยา sitafloxacin ด้วยเกณฑ์ MIC ≤2 มก./ล. ขนาดของ inhibition zone diameter ≥19 มม. มีความคลาดเคลื่อนรวมในการทำนายความไวต่อยา sitafloxacin ด้วย MIC น้อยที่สุด แต่ขนาด inhibition zone diameter ≥18 มม. มีความไวลวงน้อยกว่า inhibition zone diameter ≥19 มม. เมื่อเปรียบเทียบกับเชื้อที่ไวต่อยา sitafloxacin ด้วยเกณฑ์ MIC ≤1 มก./ล.

สรุป: การทดสอบความไวของยา sitafloxacin ต่อแบคทีเรียกรัมลบดื้อยาด้วยวิธี disk diffusion ขนาดของ inhibition zone diameter ≥16 มม. และ ≥18 มม. น่าจะเหมาะสมในการแปลว่าเชื้อที่แยกจากปัสสาวะและเลือดไวต่อยา sitafloxacin ด้วย MIC ≤2 มก./ล. และ ≤1 มก./ล. ตามลำดับ เนื่องจากระดับยา sitafloxacin ในซีรัมค่อนข้างต่ำ ส่วนระดับยา sitafloxacin ในปัสสาวะสูงกว่ามาก