Microbiological Equivalence of Bacteriostatic and Bactericidal Activities of the Sera from Healthy Volunteers Receiving Generic Piperacillin/Tazobactam (Pipertaz^{R}) and Original Piperacillin/Tazobactam (Tazocin^{R})

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Background: *Several generic piperacillin/ tazobactam formulations have been approved by Thai Food and Drug Administration, Ministry of Public Health. Piperacillin/ tazobactam is a parenteral drug. Therefore, a study demonstrating a biological equivalence of generic piperacillin/ tazobactam is not required for drug registration in Thailand.*
Objective: *The study was to determine microbiological equivalence of serum bacteriostatic and bactericidal activities of the sera from healthy volunteers receiving generic piperacillin/ tazobactam (Pipertaz^{R}) and original piperacillin/tazobactam (Tazocin^{R}).*
Material and Method: *This was a randomized crossover study in 16 adult healthy volunteers. Each subject received 4.5 grams of Pipertaz^{R} and Tazocin^{R} in 50 ml of normal saline via intravenous infusion for 30 minutes. The blood samples were drawn at baseline prior to receiving the study drug, at 30 minutes after initiating infusion, and at 8 hours after initiating infusion. The serum bacteriostatic activity against E. coli ATCC 25922, K. pneumoniae, P. aeruginosa ATCC 27853 and A. baumannii was performed by disk diffusion The serum bactericidal activity against E. coli ATCC 25922 was performed by Serum Bactericidal Titre.*
Results: *The average inhibition zone diameter of the serum samples from the subjects while receiving Pipertaz^{R} against each tested organisms was < 1 mm smaller than that while receiving Tazocin^{R} and such difference was not significantly different. All serum samples collected at 30 minutes after initiating Tazocin^{R} and Pipertaz ^{R} had bactericidal titres 1:64 to 1:256 against E. coli ATCC 25922. All serum samples collected at 8 hours after initiating Tazocin^{R} and Pipertaz^{R} had bactericidal titres $\leq$ 1:2 against E. coli ATCC 25922. The differences of serum bactericidal titres of the serum samples collected at 30 minutes ($p = 0.7$) and 8 hours ($p = 1.0$) after initiating Tazocin^{R} and Pipertaz^{R} were not statistically significant.*
Conclusion: *The sera from healthy volunteers receiving Pipertaz^{R} contain bacteriostatic and bactericidal activities not significantly different from those receiving Tazocin^{R}.*

Keywords: *Microbiological equivalence, Generic drug, Piperacillin/ tazobactam*

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Piperacillin/tazobactam is an antibacterial agent. It is a combination of beta-lactam (piperacillin) and beta-lactamase inhibitor (tazobactam)[1]. Piperacillin/ tazobactam has a broad spectrum of *in vitro* activity against Gram-positive and Gram-negative pathogens, including *Pseudomonas aeruginosa* and anaerobes. Piperacillin/ tazobactam is a member drug in category D of the national drug list of Thailand. At Siriraj Hospital, piperacillin/ tazobactam is approved to be used for 1) confirmed or suspected infection due to *Pseudomonas aeruginosa*, 2) infection due to pathogen resistant to cephalosporins, aminoglycosides and fluoroquinolones, 3) empiric therapy for febrile neutropenia, 4) infection due to the pathogen being

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susceptible to other antibiotics but the patient is unable to receive such antibiotics, and 5) empiric therapy of nosocomial infections.

The original product of piperacillin/tazobactam is Tazocin^{R} from an innovator pharmaceutical company that has been used in Siriraj Hospital for many years. The cost of the original piperacillin/ tazobactam in Thailand is still high. In 2008, several generic products of piperacillin/tazobactam were approved by Thai Food and Drug Administration (FDA), Ministry of Public Health and became available in many hospitals. Piperacillin/tazobactam is a parenteral drug. Therefore, a study demonstrating a biological equivalence of generic piperacillin/tazobactam is not required for drug registration in Thailand.

The objective of the study was to determine microbiological equivalence of bacteriostatic and bactericidal activities of the sera from healthy volunteers receiving generic piperacillin/tazobactam (Pipertaz^{R}) and original piperacillin/tazobactam (Tazocin^{R}).

Material and Method

The study protocol was approved by Siriraj Institutional Review Board and it was conducted at Siriraj Clinical Research Center and Microbiology Laboratory of Division of Infectious Diseases and Tropical Medicine, Department of Medicine, Faculty of Medicine Siriraj Hospital.

Study Population

The study was conducted in volunteers aged 18 to 45 years who had a body mass index of 18 to 25 kg/m^2. All subjects were healthy, had no history of beta-lactam allergy, and had not received any medications over the past 14 days prior to receiving the study drugs. It was estimated that 16 subjects were needed, based on the assumptions that the difference in the average inhibition zone diameters of the sera from healthy volunteers receiving original piperacillin/tazobactam and generic piperacillin/ tazobactam was within 2 mm; a standard error of inhibition zone diameter was 2 mm with 5% type I and 20% type II error.

Study Medications

The original piperacillin/tazobactam was Tazocin^{R} (Wyeth Co., Ltd.) and the generic piperacillin/ tazobactam was Pipertaz^{R} (manufactured by Reyoung Pharmaceutical Co., Ltd.). Each subject received 4.5 grams of Tazocin^{R} and Pipertaz^{R} in 50 ml of normal saline via intravenous infusion for 30 minutes.

Study Procedures

Clinical Study

This was a randomized crossover study with a wash-out period of 7 days. Each subject had 20 ml of blood samples drawn at baseline prior to receiving the study drug, at 30 minutes after initiating piperacillin/ tazobactam infusion and at 8 hours after initiating piperacillin/tazobactam infusion. The sera were separated and kept at -70°C before microbiological testing. Each subject was asked about any new symptoms experienced while receiving the study drugs.

Microbiological Study: Serum Bacteriostatic Activity by Disk Diffusion

The test was undertaken on the plate containing 10 ml of Mueller Hinton agar (Oxoid, UK) with 10^5 CFU/ml of *E. coli* ATCC 25922, *Klebsiella pneumoniae* (clinical isolate), *Pseudomonas aeruginosa* ATCC 27853 and *Acinetobacter baumannii* (clinical isolate). Four blank paper disks with diameter of 6 mm were placed on Mueller Hinton agar plate. Twenty-five microlitres of the sera taken from the same subject at the same blood drawing point were inoculated on the blank paper disks on the same plate. The plate was incubated at 35°C for 16 to 18 hours. The inhibition zone diameter was measured by vernier caliper and recorded.

Microbiological Study: Serum Bactericidal Activity by Serum Bactericidal Titre

Each serum sample was diluted with the pool serum samples taken from all subjects prior to receiving the study drug in order to achieve the final dilution titres from 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256 and 1:512. Each test tube contained 0.5 ml of the diluted serum sample and it was inoculated with 0.5 ml of *E. coli* ATCC 25922 at 10^5 CFUs/ml. The tube containing serum sample and bacteria was incubated at 35°C for 16 to 18 hours. Then, 10 microlitre of the inoculated serum sample was inoculated onto blood agar plate and the plate was incubated at 35°C for 16 to 18 hours. A serum bactericidal activity was present if $\leq$ 5 bacterial colonies were found on the blood agar plate ($\geq$ 99.5% bacterial killing).

Data Analysis

The data were compared by student t-test.

Results

Serum Bacteriostatic Activity

The average inhibition zone diameters of the

serum samples from the subjects while receiving Tazocin[R] and Pipertaz[R] against *E. coli* ATCC 25922, *K. pneumoniae*, *P. aeruginosa* ATCC 27853 and *A. baumannii* are shown in Table 1 to 4. The average inhibition zone diameters of the serum samples from the subjects while receiving Pipertaz[R] against each tested bacteria was ≤ 1 mm smaller than that from the subjects while receiving Tazocin[R]. The difference of the average inhibition zone diameters of the serum samples from the subjects while receiving Tazocin[R] and Pipertaz[R] was not significantly different.

Serum Bactericidal Activity

Bactericidal activity of the serum samples from the subjects while receiving Tazocin[R] and Pipertaz[R] against *E. coli* ATCC 25922 are shown in Table 5 and 6. All serum samples collected at 30 minutes after initiating Tazocin[R] and Pipertaz[R] had bactericidal titres 1:64 to1:256. All serum samples collected at 8 hours after initiating Tazocin[R] and Pipertaz [R] had bactericidal titres ≤ 1:2. The differences of serum bactericidal titres of the serum samples collected at 30 minutes (p = 0.7) and 8 hours (p = 1.0) after initiating Tazocin[R] and Pipertaz[R] were not statistically significant.

Adverse Events

Six subjects had adverse events (dizziness, itching, metallic taste, headache, abdominal pain) while receiving Tazocin[R]. Four subjects had adverse events (drowsiness, skin rash, metallic taste, headache) while receiving Pipertaz[R]. All adverse events were mild and transient without any specific treatments.

Discussion

An ideal generic drug product is one that is chemically equivalent, bioequivalent and therapeutically equivalent to an innovator or first version of the drug product approved by the FDA. If

Table 1. Serum bacteriostatic activity against *E. coli* ATCC 25922

Drug	Mean (standard deviation) of inhibition zone diameter (mm)		
	Baseline	30 min after initiating infusion	8 h after initiating infusion
Tazocin[R]	no zone	24.9 (2.2)	no zone
Pipertaz[R]	no zone	24.4 (1.8)	no zone
p		0.56	

Table 2. Serum bacteriostatic activity against *Klebsiella pneumoniae*

Drug	Mean (standard deviation) of inhibition zone diameter (mm)		
	Baseline	30 min after initiating infusion	8 h after initiating infusion
Tazocin[R]	no zone	27.2 (2.3)	no zone
Pipertaz[R]	no zone	26.7 (2.1)	no zone
p		0.54	

Table 3. Serum bacteriostatic activity against *Pseudomonas aeruginosa* ATCC 27853

Drug	Mean (standard deviation) of inhibition zone diameter (mm)		
	Baseline	30 min after initiating infusion	8 h after initiating infusion
Tazocin[R]	no zone	23.0 (1.5)	no zone
Pipertaz[R]	no zone	22.8 (1.4)	no zone
p		0.74	

Table 4. Serum bacteriostatic activity against *Acinetobacter baumannii*

Drug	Mean (standard deviation) of inhibition zone diameter (mm)		
	Baseline	30 min after initiating infusion	8 h after initiating infusion
Tazocin®	no zone	15.8 (1.9)	no zone
Pipertaz®	no zone	15.8 (1.6)	no zone
p		0.95	

Table 5. Serum bactericidal titre against *E. coli* ATCC 25922 from the subjects receiving Tazocin®

Subject	Baseline	30 min after initiating infusion	8 h after initiating infusion
1	< 1 : 2	1 : 256	1 : 2
2	< 1 : 2	1 : 64	< 1 : 2
3	< 1 : 2	1 : 128	< 1 : 2
4	< 1 : 2	1 : 128	1 : 2
5	< 1 : 2	1 : 128	< 1 : 2
6	< 1 : 2	1 : 256	1 : 2
7	< 1 : 2	1 : 128	< 1 : 2
8	< 1 : 2	1 : 128	< 1 : 2
9	< 1 : 2	1 : 128	< 1 : 2
10	< 1 : 2	1 : 256	1 : 2
11	< 1 : 2	1 : 256	1 : 2
12	< 1 : 2	1 : 256	< 1 : 2
13	< 1 : 2	1 : 64	< 1 : 2
14	< 1 : 2	1 : 64	1 : 2
15	< 1 : 2	1 : 128	1 : 2
16	< 1 : 2	1 : 128	< 1 : 2

Table 6. Serum bactericidal titre against *E. coli* ATCC 25922 from the subjects receiving Pipertaz®

Subject	Baseline	30 min after initiating infusion	8 h after initiating infusion
1	< 1 : 2	1 : 128	< 1 : 2
2	< 1 : 2	1 : 64	< 1 : 2
3	< 1 : 2	1 : 256	1 : 2
4	< 1 : 2	1 : 128	< 1 : 2
5	< 1 : 2	1 : 128	< 1 : 2
6	< 1 : 2	1 : 128	1 : 2
7	< 1 : 2	1 : 128	< 1 : 2
8	< 1 : 2	1 : 128	< 1 : 2
9	< 1 : 2	1 : 64	< 1 : 2
10	< 1 : 2	1 : 128	1 : 2
11	< 1 : 2	1 : 128	< 1 : 2
12	< 1 : 2	1 : 128	< 1 : 2
13	< 1 : 2	1 : 128	1 : 2
14	< 1 : 2	1 : 128	1 : 2
15	< 1 : 2	1 : 128	< 1 : 2
16	< 1 : 2	1 : 256	1 : 2

a generic drug is clearly shown to be chemically equivalent, bioequivalent and therapeutically equivalent to an original drug, it can be substituted for the original product with much lower cost. Although the randomized controlled trial of generic piperacillin/ tazobactam is ideal for assessing a therapeutic equivalence, it is not feasible because the study needs a large number of patients, has a very high cost and requires a long duration of study. Moreover, the data on therapeutic equivalence of generic drug from randomized controlled trials are not required for drug registration with Thai FDA. Neither is bioequivalence study of a generic intravenous drug required for drug registration. Bioequivalence study of an oral generic drug is mandatory for drug registration in Thailand. Measurement of plasma levels or a bioequivalence study of non-antibiotic generic drugs is logical since their activity is quite difficult to determine. However, antimicrobial activity of generic antibiotics can be easily determined and their antimicrobial activity can be compared with that of the original antibiotic. This type of study could be called microbiological equivalence. Zuluaga AF et al reported an application of microbiological assay to determine pharmaceutical equivalence of generic intravenous antibiotics[3]. The proposed method was based on the concentration-dependent variation of the inhibitory effect of antibiotics on reference bacteria (*B. subtilis* ATCC 6633, *S. aureus* ATCC 6538p and *S. epidermidis* ATCC 12228) in a seeded agar, producing a concentration-response linear relationship with two parameters: y-intercept (concentration) and slope (potency). The proposed method allowed rapid, cost-saving, precise, and accurate determination of pharmaceutical equivalence of drugs in pharmaceutical dosage-form, and may be used as a technique for testing generic antibiotics prior to their approval for human use. However, such a proposed method is still an *in vitro* test and its results may not reflect microbiological activity *in vivo*. The method used in our study should be more valid because 1) we used the serum samples collected from the subjects who received original and generic antibiotics instead of using the original and generic drugs for direct testing, 2) we used the organisms that were the target of the study drug and 3) we determined bactericidal activity in addition to bacteriostatic activity. We proposed serum bactericidal activity assay as a method to determine microbiological equivalence because the serum bactericidal titres were found to be associated with microbiological and clinical outcomes of treatment of several bacterial infections in some other studies[4-9].

Several studies on antimicrobial activity of generic piperacillin/ tazobactam revealed that some formulations contained equivalent antimicrobial activity and many formulations had significantly less antimicrobial activity when compared with original piperacillin/ tazobactam[10-12]. Therefore, it is crucial that each generic piperacillin/tazobactam formulation should be tested for antimicrobial activity in order to be certain that it could replace original piperacillin/tazobactam.

Our study found that the sera from healthy volunteers receiving Pipertaz^{R} contain bacteriostatic and bactericidal activities not significantly different from those receiving Tazocin^{R}. However, it should be noted that bacteriostatic and bactericidal activities of the serum samples taken from the subjects at 8 hours after receiving a 4.5 grams dosage of both formulations of piperacillin/tazobactam had minimal or no antimicrobial activity. Therefore, a more frequent administration or prolong infusion or continuous administration of piperacillin/tazobactam for therapy of serious infections should be considered[13].

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Potential conflicts of interest

None.

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ความเท่าเทียมกันของฤทธิ์ยับยั้งเชื้อ และฤทธิ์ทำลายเชื้อแบคทีเรียโดยซีรัมของอาสาสมัครที่ได้รับยาสามัญ piperacillin/tazobactam (Pipertaz^R) และยาต้นแบบ piperacillin/tazobactam (Tazocin^R)

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ภูมิหลัง: *ยาสามัญ piperacillin/tazobactam หลายขนานได้รับการรับรองจากคณะกรรมการอาหารและยา กระทรวงสาธารณสุข ยา piperacillin/tazobactam เป็นยาฉีดเข้าหลอดเลือดดำ ดังนั้นการขึ้นทะเบียนยาดังกล่าวจึงไม่ต้องมีข้อมูลชีวสมมูลของยา*

วัตถุประสงค์: *เพื่อทราบความเท่าเทียมกันทางจุลชีววิทยาของยาสามัญ piperacillin/tazobactam (Pipertaz^R) และยาต้นแบบ piperacillin/tazobactam (Tazocin^R) ในการยับยั้ง และทำลายแบคทีเรีย*

วัสดุและวิธีการ: *การศึกษานี้เป็นการศึกษาชนิด randomized crossover ในอาสามัครสุขภาพแข็งแรงจำนวน 16 คน อาสาสมัครแต่ละคนได้รับยา Pipertaz^R และยา Tazocin^R ขนาด 4.5 กรัมผสมในน้ำเกลือ 50 มล. หยดเข้าหลอดเลือดดำนาน 30 นาที ซีรัมจากอาสามัครเก็บก่อนได้รับยาที่ 30 นาทีหลังเริ่มยา และที่ 8 ชั่วโมงหลังเริ่มยานำไปทดสอบฤทธิ์ยับยั้งเชื้อ E. coli ATCC 25922, K. pneumoniae, P. aeruginosa ATCC 27853 และ A. baumannii ด้วยวิธี disk diffusion และฤทธิ์ทำลายเชื้อแบคทีเรีย E. coli ATCC 25922 ด้วยวิธี serum bactericidal titre*

ผลการศึกษา: *ขนาดเฉลี่ยของ inhibition zone diameter ของซีรัมจากอาสาสมัครขณะได้รับ Pipertaz^R ต่อเชื้อแบคทีเรียทุกชนิดมีขนาดเล็กกว่าขณะที่ได้รับ Tazocin^R น้อยกว่า 1 มม. และความแตกต่างนี้ไม่มีนัยสำคัญทางสถิติ ซีรัมที่เก็บจากอาสาสมัครทุกรายที่ 30 นาทีหลังเริ่มยามีฤทธิ์ทำลายเชื้อแบคทีเรีย E. coli ATCC 25922 1:64 ถึง 1:256 ซีรัมที่เก็บจากอาสาสมัครทุกรายที่ 8 ชั่วโมง หลังเริ่มยามีฤทธิ์ทำลายเชื้อแบคทีเรีย E. coli ATCC 25922 ≤ 1:2 ความแตกต่างของฤทธิ์ในการทำลายเชื้อในซีรัมหลังได้รับ Tazocin^R และ Pipertaz^R ไม่แตกต่างกันอย่างมีนัยสำคัญ*

สรุป: *ซีรัมจากอาสาสมัครขณะได้รับ Pipertaz^R และ Tazocin^R มีความเท่าเทียมกันทางจุลชีววิทยาในการยับยั้งและทำลายแบคทีเรีย*