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Bioequivalence of azithromycin for oral suspension after a single dose 600 mg oral administration

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Background : *Azithromycin is a broad spectrum azalide antibiotic which was suggested to be first-line treatment in children for some respiratory tract infections, urethritis and mycobacterium avium complex infection with AIDs complications. The drug is well absorbed and has long half-life which supports a short time administration at higher dose regimens. Pharmacokinetic and bioequivalence of azithromycin have been extensively studied in many oral dosage forms at the standard dose of 500 mg; however, there is no bioequivalence study at higher dose carried out in an oral suspension dosage form.*

Objective : *To investigate bioequivalence of azithromycin powder for oral suspension with a single dose of 600 mg intended to be used as a high dose regimen for treatment of infectious diseases in children.*

Design : *A two-way crossover design.*

Setting : *Clinical study and drug assay were performed at Burachat Chaiyakorn Hospital, Bangkok and SGS Laboratory, Bangkok, respectively.*

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Materials and Methods : *Twenty-four healthy Thai volunteers participated in the study. The study design followed a two-way crossover design with two-treatment, two sequences and two periods with a 4-week washout period. The test product, Azith® (Siam Bheasach, Thailand), was compared with a reference product, Zithromax® (Pfizer, USA). Blood samples were collected for 168 h post-dose and plasma drug concentrations were determined by LC-MS. An individual plasma concentration-time profile from each treatment was analyzed for relevant pharmacokinetic parameters and its bioequivalence was evaluated between both formulations.*

Results : *The dose regimen was well tolerated in all volunteers. The drug was rapidly absorbed given* t_{max} *of 2-2.5 h for both products. The values of* C_{max}, AUC_{0-t} *and* $t_{1/2}$ *were 560.81 ± 231.94 ng/mL, 4655.15 ± 2015.31 ng.h/mL and 35.82 ± 13.65 h for the test product and 556.80 ± 308.13 ng/mL, 4583.67 ± 2066.49 ng.h/mL and 34.14 ± 14.31 h for the reference product, respectively. The 90% confidence intervals for the ratios of the test product to the reference product were within the bioequivalence acceptation range of 80-125%.*

Conclusion : *The result showed the bioequivalence between the test product and reference product in terms of both rate and extent of drug absorption after administration of a single oral dose of 600 mg azithromycin.*

Keywords : *Bioequivalence, azithromycin, suspension.*

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พรเลขา บรรหารศุภวาท, วิเชียร ธานินทร์ธราธาร, อุทัย สุวรรณกูฏ. การศึกษาชีวสมมูลของยาอะซิโทรมัยซินรูปยาน้ำแขวนตะกอนขนาด 600 mg รับประทานครั้งเดียว. จุฬาลงกรณ์–เวชสาร 2556 มี.ค. – เม.ย.; 57(2): 161 – 74

เหตุผลของการทำวิจัย : *อะซิโทรมัยซินเป็นยาปฏิชีวนะในกลุ่มอะซาไลด์ที่ออกฤทธิ์กว้าง ซึ่งได้รับการแนะนำให้ใช้เป็นลำดับแรกสำหรับรักษาอาการติดเชื้อในทางเดินหายใจ และทางเดินปัสสาวะในเด็ก รวมถึงการติดเชื้อไมโครแบคทีเรียม เอเวียม คอมเพล็กซ์ (mycobacterium avium complex) ในเด็กที่มีภาวะเอดส์แทรกซ้อน ตัวยาดูดซึมได้ดีและมีค่าครึ่งชีวิตยาวนาน ซึ่งคุณสมบัตินี้สนับสนุนรูปแบบการรักษาโดยให้ตัวยาในขนาดที่สูงขึ้นภายในระยะเวลาช่วงสั้น มีการศึกษาทางเภสัชจลนศาสตร์และชีวสมมูลของตัวยาอย่างกว้างขวางในหลากหลายเภสัชภัณฑ์ที่ขนาดการรักษามาตรฐาน 500 mg อย่างไรก็ตามยังไม่มีการ ศึกษาทางชีวสมมูลของตัวยาในรูปแบบยาน้ำแขวนตะกอนที่ขนาดการรักษาสูงกว่าขนาดการรักษามาตรฐาน*

วัตถุประสงค์ : *เพื่อศึกษาชีวสมมูลของยาอะซิโทรมัยซินในรูปแบบยาน้ำแขวนตะกอนขนาด 600 mg รับประทานเพียงครั้งเดียว โดยมีความมุ่งหมายเพื่อนำไปใช้ในการรักษาโรคติดเชื้อในเด็กด้วยขนาดยาที่สูงขึ้น*

รูปแบบการวิจัย : *การศึกษาแบบไขว้กันสองทาง*

สถานที่ทำการศึกษา : *การศึกษาทางคลินิก และการวิเคราะห์ปริมาณตัวยาสำคัญ ดำเนินการที่โรงพยาบาลบุรฉัตร ไชยากร, กรุงเทพมหานคร และ บริษัท SGS Laboratory กรุงเทพ ตามลำดับ*

ตัวอย่างและวิธีการศึกษา : *อาสาสมัครชายไทยสุขภาพดีจำนวน 24 คนเข้าร่วมการศึกษา รูปแบบการศึกษาเป็นแบบไขว้กันสองทางของสองกลุ่มตัวอย่าง (two-way crossover design with two-treatment, two-sequence and two-period) โดยมีระยะห่างระหว่างการทดสอบของสองกลุ่มตัวอย่าง (washout period) 4 อาทิตย์ ยาสามัญที่ใช้ในการศึกษาคือ Azith® (สยามเภสัช, ประเทศไทย) เทียบกับยาต้นแบบ Zithromax® (ไฟเซอร์, สหรัฐอเมริกา) อาสาสมัครจะถูกเก็บตัวอย่างเลือดภายในเวลา 168 ชั่วโมงหลังจากได้รับยา การวิเคราะห์ระดับยาในเลือดทำด้วยวิธี LC-MS ผลของระดับยาในเลือดของอาสาสมัครแต่ละคนเทียบกับเวลาจะถูกนำมาคำนวณตัวแปรทางเภสัชจลนศาสตร์และประเมินผลชีวสมมูลของยาทั้งสองตำรับ*

ผลการศึกษา : *อาสาสมัครทนต่อขนาดยาได้ดี ยาดูดซึมได้ดีโดยสามารถวัดปริมาณยาสูงสุดของทั้งสองตำรับได้ที่เวลา 2-2.5 ชั่วโมงหลังจากได้รับยา ค่า C_{max}, AUC_{0-t} และ $t_{1/2}$ ของยาสามัญเท่ากับ 560.81 ± 231.94 ng/mL, 4655.15 ± 2015.31 ng.h/mL และ 35.82 ± 13.65 ชั่วโมงตามลำดับ และของยาต้นแบบเท่ากับ 556.80 ± 308.13 ng/mL, 4583.67 ± 2066.49 ng.h/mL และ 34.14 ± 14.31 ชั่วโมงตามลำดับ ค่าความเชื่อมั่น (confidence interval) ที่ระดับ 90% ของอัตราส่วนระหว่างยาสามัญ และยาต้นแบบมีค่าอยู่ในเกณฑ์ที่ยอมรับได้คือ 80-125%*

สรุป : *จากการศึกษาสรุปได้ว่ายาทั้งสองตำรับมีชีวสมมูลกัน ทั้งทางด้านอัตราเร็วในการดูดซึมและขนาดการดูดซึมตัวยาสำคัญ หลังจากรับประทานยาในขนาด 600 mg เพียงครั้งเดียว*

คำสำคัญ : *ชีวสมมูล, อะซิโทรมัยซิน, ยาน้ำแขวนตะกอน.*

Azithromycin is a broad-spectrum azalide antibiotic, structurally related to erythromycin. It contains a methyl-substituted nitrogen in the lactone ring which improves acid stability and oral bioavailability compared to erythromycin.[1] The drug has unique pharmacokinetic characteristics including, long half-life, high tissue selectivity and ability to penetrate into phagocyte cells.[2 - 4] It is rapidly absorbed and distributed into many organs, allowing short onset of action and longer drug exposure time to pathogens at the infection site. Standard dosage regimens start with 500 mg on day 1, followed by 250 mg for four days or three daily dosages of 500 mg.[2] Due to its rapid absorption into the tissue and long half-life, higher dose regimens with a short-time administration are recommended in some treatments. A 1-g single dose is reported to be more effective than doxycycline given twice daily for 7 days in positive urethritis.[5] In community-acquired atypical pneumonia patients, a single dose as high as 1.5 g gives comparable efficacy to the standard dose of 500 mg once daily for 3 days.[6]

In children, azithromycin is suggested to be the first-line treatment for respiratory tract infection caused by the Legionella species, *Mycoplasma pneumoniae*, and *Chlamydia pneumonia* and particularly the single-dose regimens are effective in the treatments of chlamydia urethritis, trachoma and mycobacterium avium-intracellulare complex infections in children with AIDs complications.[7] Children with febrile neutropenia administered with a single-dose regimen of 12 mg/kg suspension obtain proportionally higher serum drug concentrations than those treated with a standard dose of 5 mg/kg daily for 5 days.[8] Higher dose regimens of 20 mg/kg daily for 3 days or 30 mg/kg single dose are well tolerated and improve patient compliance of children with acute otitis media compared to amoxicillin/clavulanate treatment.[9-11]

In the view of bioequivalence evaluation, the two orally administered drug products proved to exhibit the same rate and extent of the absorption are considered to be bioequivalent.[12] There are extensive reports of pharmacokinetic and bioequivalence of azithromycin in tablet, capsule and oral suspension formulations with the standard dose of 500 mg;[13 - 17] however, the bioequivalence study at higher doses is limited. The present study is aimed to evaluate the bioequivalence of the two products of azithromycin powder for oral suspension, Azith® and Zithromax®, at the high dose of 600 mg (200 mg/ 5 mL). The administration was assigned with a whole bottle of 15 mL reconstituted suspension representing a practical regimen for children with a single oral dose.

Materials

Azithromycin powder for oral suspension (200 mg azithromycin/ 5 mL) used as a test product was Azith® (15-mL bottle, Lot no. B8AZ00109/1, Siam Bheasach, Thailand) compared to a reference product, Zithromax® (15-mL bottle, Lot no. 76413501, Pfizer, US).

Methods

Volunteers

Twenty-four healthy Thai male volunteers participated in the study. Their mean age was 25.08 ± 5.55 years with a range of 19 - 42 years. The mean body weight was 62.50 ± 5.50 kg with a range of 53-70 kg and the average height was 1.71 ± 0.05 m

with a range of 1.60 -1.80 m, corresponding to the body mass index of 21.33 ± 1.75 kg/m^2 with a range of 18.34 - 23.94 kg/m^2. All volunteers were healthy, based on their medical history, physical examination, vital signs and hematological and biochemical tests. Demographic characteristics and vital signs of the volunteers are summarized in Table 1 and their hematological and biochemical results are listed in Table 2. None was allergic to azithromycin and/or macrolides. All volunteers were instructed to abstain from any medicine intake and alcoholic preparations as well as smoking for two weeks prior to and throughout the study. The methods and conditions of the study were clearly explained to all volunteers. Informed consent was obtained from all volunteers before entering the study.

Study design

The study protocol has been approved by the Ethics Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University and the study was conducted in accordance with the Declaration of Helsinki. A two-way crossover design with two-treatment, two-sequence, and two-period was applied. The volunteers were randomly allocated into two groups of equal number. Each volunteer received both treatments based on randomized schedule with a washout period of 4 weeks. Drug assays were not advised of the product names being tested until the analytical work was completed.

After a 10-hour overnight fast, each volunteer was fitted with indwelling venous cannulas, and a blood sample of 5 mL was drawn for baseline plasma measurement. Then, the volunteer received an individually labeled dosage bottle filled with 15 mL of reconstituted suspension containing azithromycin 600 mg and instructed to swallow the whole suspension followed by a full glass of water (250 mL). Confirmed compliance was examined by a visual inspection of the oral cavity. The blood samples were collected from the antecubital vein at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 9, 12, 24, 48, 72, 96, 120, 144, and 168 h post-dose. No food and drink was allowed until 4 h after dosing. Thereafter, standard lunch and dinner meals were served after 4 and 9 h of dosing. The volunteers were required to remain at the study site for another 12 h after dosing.

Sample preparation and LC-MS analysis

All blood samples were collected in heparinized tubes. After centrifugation at 4000 rpm for 10 minutes, plasma was collected and stored at -20°C. The drug concentration was assayed using LC-MS (Agilent 1100/1946 D, USA) with the method modified from Zhong *et al*.[18] Briefly, a plasma sample of 1 mL was added with 50 μL of internal standard (clarithromycin 0.15 mg/mL in acetronitrile) and vortex mixed for 1 min. Then 6 mL of t-butyl methyl ether was added, vortex mixed for 1 min and centrifuged for 15 min at 5000 rpm. The organic supernatant was collected and evaporated. The residue was mixed with 5 mL acetronitrile and filtered through a 0.2 μm membrane. The solution of 10 μL was then injected into the LC-MS column and analyzed for drug concentration.

LC-MS method validation

Analytical method for azithromycin determination was validated for selectivity, accuracy, precision, linearity, recovery of extraction, lower limit

Table 1. Demographic characteristics and vital signs of 24 volunteers before study.

Subject no.	Demographic characteristics				Vital sign		
	Age (year)	Height (m)	Weight (kg)	BMI (kg/m^2)	Blood pressure (mmHg)	Pulse (/min)	Temperature (^{o}C)
1	19	1.70	53	18.34	105/62	61	36.9
2	23	1.70	64	22.15	115/64	82	37.0
3	21	1.79	63	19.66	124/74	65	37.1
4	24	1.75	67	21.88	115/70	80	37.0
5	23	1.74	69	22.79	120/60	70	36.9
6	23	1.72	57	19.27	115/68	70	37.0
7	28	1.79	65	20.29	125/75	68	37.0
8	42	1.60	55	21.48	128/76	80	37.1
9	22	1.65	65	23.88	120/80	70	37.0
10	22	1.80	67	20.68	125/70	75	37.1
11	21	1.71	60	20.52	120/80	85	37.0
12	23	1.63	57	21.45	125/83	75	37.1
13	25	1.70	60	20.76	92/64	65	36.9
14	22	1.75	60	19.59	110/75	70	37.0
15	24	1.67	56	20.08	125/76	65	37.0
16	39	1.70	69	23.88	102/72	68	37.0
17	26	1.72	70	23.66	125/83	85	37.1
18	26	1.71	70	23.94	120/80	82	37.0
19	21	1.72	70	23.66	125/83	80	37.1
20	33	1.70	63	21.80	122/73	65	36.9
21	27	1.80	64	19.75	112/70	62	37.0
22	22	1.70	58	20.07	118/70	80	37.0
23	23	1.67	65	23.31	110/70	72	36.9
24	23	1.67	53	19.00	115/82	75	37.0
Mean (SD)	25.08 (5.55)	1.71 (0.05)	62.50 (5.50)	21.33 (1.75)	117.21 (8.72)/ 73.38 (6.84)	72.92 (7.42)	37.00 (0.07)

Table 2. Results of hematological and biochemical tests of 24 volunteers before study.

Subject no.	AP (U/L)	AST (U/L)	ALT (U/L)	BUN (mg/dL)	Creatinine (mmHg)	FBS (mg/dL)	Hemoglobin (g/dL)	Hematocrit (%)	WBC ($x10^3/\mu L$)	Platelets ($x10^3/\mu L$)	RBC ($x10^6$/cu.mm)	Total bilirubin (mg/dL)	Anti-HIV
1	88	37	16	12	0.9	94	14.0	42	5.3	209	4.37	0.42	Negative
2	41	25	24	12	1.1	88	14.2	42	9.3	180	5.30	0.38	Negative
3	48	31	20	10	0.8	88	14.8	45	8.4	150	5.03	0.30	Negative
4	48	36	31	10	1.1	95	14.0	42	6.4	188	4.08	0.30	Negative
5	49	38	21	10	0.8	81	14.0	42	10.6	210	4.64	0.65	Negative
6	48	27	15	12	0.8	95	14.2	42	5.7	205	4.68	0.32	Negative
7	39	27	20	11	0.9	76	14.2	43	6.7	150	5.03	0.30	Negative
8	52	33	31	15	1.2	81	15.6	45	8.2	240	5.06	0.47	Negative
9	45	25	21	10	1.0	87	15.2	45	5.6	246	5.13	0.30	Negative
10	63	38	25	10	0.9	99	14.5	43	7.8	167	4.35	0.45	Negative
11	42	31	29	10	0.8	70	14.9	43	9.5	213	5.13	0.30	Negative
12	54	37	28	14	1.2	87	14.0	42	9.3	300	5.59	0.30	Negative
13	48	31	25	12	1.1	82	15.7	45	8.1	207	5.55	0.32	Negative
14	112	33	38	11	0.8	84	15.9	45	9.8	247	5.38	0.35	Negative
15	78	38	29	10	0.9	107	14.7	42	5.6	219	5.90	0.58	Negative
16	51	38	36	13	1.0	80	14.0	42	4.8	150	4.37	0.56	Negative
17	49	24	38	12	1.1	74	17.4	45	9.0	224	5.17	0.52	Negative
18	54	38	29	14	1.2	88	14.2	43	9.5	202	5.78	0.34	Negative
19	44	32	12	11	0.9	82	15.8	45	9.3	292	5.91	0.30	Negative
20	72	35	12	11	0.8	87	15.0	45	7.4	151	4.98	0.52	Negative
21	69	19	8	12	1.1	75	14.7	43	6.0	150	4.56	0.35	Negative
22	107	38	35	12	0.8	86	15.1	45	7.1	150	4.83	0.45	Negative
23	57	38	25	12	1.0	85	14.0	42	9.2	273	4.75	0.32	Negative
24	45	34	28	14	1.3	90	15.5	45	11.0	205	4.61	0.47	Negative
Mean	58.46	32.63	24.83	11.67	0.98	85.88	14.82	43.46	7.90	205.33	5.01	0.40	-
(SD)	(19.86)	(5.55)	(8.31)	(1.49)	(0.16)	(8.37)	(0.85)	(1.38)	(1.80)	(45.19)	(0.51)	(0.11)	

Abbreviations: AP-Alkaline phosphatase, AST-Aspartate aminotransferase, ALT-Alanine aminotransferase, BUN-Blood urea nitrogen, FBS-Fasting blood sugar, WBC-White blood cell count and RBC-Red blood cell count

of quantification (LLOQ) and stability prior to the study[19]. Selectivity was indicated as no interference peaks of plasma proteins and/or endogenous substances. Accuracy, precision and recovery of extraction were tested with 5 replicate sets of 3 plasma drug concentrations, i.e., low, medium and high concentrations of quality control (QC) samples, representing the entire range of the measurable concentrations. Accuracy was in the acceptance criteria of 85 - 115% and precision was not exceeded the limit of 15%. Recovery of extraction was 85 - 90%. Linearity was achieved with standard samples of 9 different concentrations in the range of 10-1500 ng/mL with the coefficient of determination (r^2) more than 0.99. LLOQ was 10 ng/mL determined from 5 replicate sets of the lowest plasma drug concentrations. Stability study was performed with QC samples stored under 4 conditions, i.e., long-term stability (-20 °C, 8 weeks), freeze and thaw stability (3 cycles of -20 °C freezing for 24 h, following by completely thawing at room temperature), short-term stability (-20 °C freezing for 24 h, following by completely thawing at room temperature) and post-preparative stability (sample after extraction placed in an autosampler for 12 h). Acceptance criteria for stability study included 80 -120% drug recovery with coefficient of variation of estimated concentration for precision of not exceed 20%. Plasma azithromycin could be stored at -20 °C up to 10 weeks and stable under room condition for 6 hours as well as stressed conditions following freeze-thaw 3 cycles.

For routine drug analysis, the method was validated where QC samples and a series of standard concentrations with the internal standard were randomly and alternately injected into the column and analyzed as one batch. The analytical run is acceptable when (i) 75% or a minimum of 6 from 8 standards when back-calculated fall within ± 15%, except for LLOQ, when it should be ± 20% of the nominal value, and (ii) at least 67% or 4 out of 6 of the QC samples fall within ± 15% of their respective nominal values while the rest 33% but not all replicates at the same concentration may be outside ± 15% of the nominal value.[19] The drug concentration was calculated from the corresponding routine standard curve.

Pharmacokinetic and bioequivalence analysis

An individual plasma azithromycin concentration-time profile from each treatment was analyzed for relevant pharmacokinetic parameters: peak plasma drug concentration (C_{max}), and time to peak plasma drug concentration (t_{max}). The area under the curve from time zero to the last point of collection (AUC_{0-t}) was calculated using linear trapezoidal rule and that from time zero to infinite time ($AUC_{0-\infty}$) was calculated as $AUC_{0-t} + C^*/K_e$ where C^* was the last measurable drug concentration and K_e was the terminal elimination rate constant. At least three points during the terminal Ln-linear phase were used to obtain an accurate estimate of K from linear regression. For bioequivalence evaluation, analysis of variance (ANOVA) at significance level of $\alpha = 0.05$ of the C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values based on Ln-transformed data were interpreted. The two products were considered bioequivalent when 90% confidence interval (CI) of Ln-transformed data of the corresponding parameter when transferred back to normal data was within 80 - 125%.[12, 20] Comparison of t_{max} was performed with nonparametric statistics using a Wilcoxon signed rank test based on their median values.

Results and discussion

All volunteers participated and completed the study on the assigned schedule. The dose regimen was well tolerated in all volunteers without any side effects and/or intoxication. Mean plasma drug concentrations-time profiles are illustrated in Figure 1 and all pharmacokinetic parameters are summarized in Table 3. The values of C_{max}, t_{max}, AUC_{0-t}, $AUC_{0-\infty}$ and $t_{1/2}$ were 560.81 ng/mL, 2.08 h, 4655.15 ng.h/mL, 5468.09 ng.h/mL and 35.82 h for the test product and 556.80 ng/mL, 2.46 h, 4583.67 ng.h/mL, 5391.76 ng.h/mL and 34.14 h for the reference product, respectively.

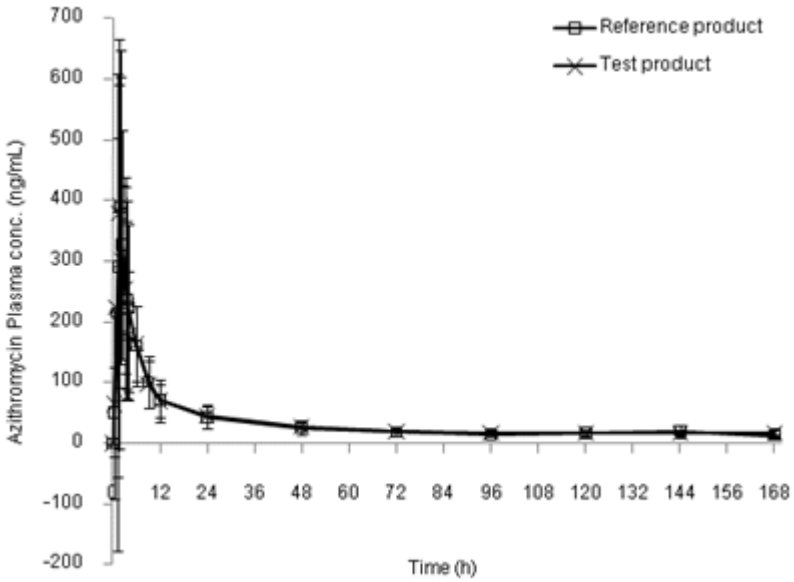

Figure 1. Mean plasma concentration of azithromycin after administration of 15-mL (600 mg) reconstituted oral suspension of the test product, Azith®, and reference product, Zithromax® (*n* = 24).

Table 3. Mean pharmacokinetic parameters of azithromycin from 24 volunteers following oral administration of 15-mL reconstituted oral suspension containing azithromycin 600 mg (*n* = 24).

Parameters		Products	
		Test product (Azith®)	**Reference product (Zithromax®)**
C_{max}	(ng/mL)	560.81 ± 231.94	556.80 ± 308.13
t_{max}	(h)	2.08 ± 1.02	2.46 ± 0.99
AUC_{0-t}	(ng.h/mL)	4655.15 ± 2015.31	4583.67 ± 2066.49
$AUC_{0-\infty}$	(ng.h/mL)	5468.09 ± 2253.53	5391.76 ± 2333.36
$t_{1/2}$	(h)	35.82 ± 13.65	34.14 ± 14.31

Table 4 summarizes ANOVA results of pharmacokinetic parameters based on the Ln-transformed data. The sequence and formulation effects of the C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values were not statistically significant ($p > 0.05$). For the comparison of t_{max}, both products showed the same median values of t_{max} at 2.0 h with no significant difference between the products ($p > 0.05$). The results of parametric 90% CI for the ratio of the C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ are listed in Table 5. All parameters gave the values of 90% CI within the acceptable range of 80 - 125%.

Table 4. Analysis of variance of pharmacokinetic parameters (Ln-transformed data) after dosing of azithromycin 600 mg reconstituted oral suspension.

Source of variation	df	Sum of squares	Mean square	Computed F	Tabulated F	Sig.level
C_{max}						
Total	47	9.7398	-	-	-	-
Sequence	1	0.0013	0.0013	0.004	4.30	NS
Subjects (sequence)	22	6.7366	0.3062	3.005	2.05	S
Period	1	0.7326	0.7326	7.189	4.30	S
Formulation	1	0.0275	0.0275	0.270	4.30	NS
Error	22	2.2418	0.1019	-	-	-
AUC_{0-t}						
Total	47	13.6811	-	-	-	-
Sequence	1	0.0038	0.0038	0.009	4.30	NS
Subjects (sequence)	22	9.5074	0.4321	2.352	2.05	S
Period	1	0.1271	0.1271	0.692	4.30	NS
Formulation	1	0.0009	0.0009	0.005	4.30	NS
Error	22	4.0419	0.1837	-	-	-
$AUC_{0-\infty}$						
Total	47	13.1611	-	-	-	-
Sequence	1	0.0102	0.0102	0.025	4.30	NS
Subjects (sequence)	22	8.8621	0.4028	2.161	2.05	S
Period	1	0.1875	0.1875	1.006	4.30	NS
Formulation	1	0.0007	0.0007	0.004	4.30	NS
Error	22	4.1006	0.1864	-	-	-

NS: Not significant difference at $p > 0.05$

S: Significant difference at $p < 0.05$

Table 5. 90% confidential intervals for the ratio of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values.

Pharmacokinetic parameter	Confidential interval (%)
C_{max}	89.56 – 122.89
AUC_{0-t}	80.18 – 122.58
$AUC_{0-\infty}$	80.13 – 122.94

Discussion

Azithromycin was rapidly absorbed giving t_{max} of approximately 2 h for the test product and 2.5 h for the reference product. The result is consistent with a previous report which t_{max} of 2.5 h was observed in volunteers after dosing of 500 mg azithromycin oral suspension.[13] The C_{max} values of both products were approximately 560 ng/mL, showing a dose proportional relation of azithromycin reported previously where the C_{max} values of 323 - 524 ng/mL were obtained after single dosing of 500 mg oral administration[13-17] and the C_{max} value of 1460 ng/mL was obtained after single dosing of 1500 mg.[21]

The AUCs of both products were in the range of 5400-5500 ng.h/mL and the drug half-life lasted 34 - 36 h. Naji *et al.*[13] reported higher AUCs and longer half-life in Jordanian volunteers administered with the lower dose of 500 mg oral suspension. However, the AUCs and half-life were found to be higher and longer than those reported by Boonleang *et al.*[15] when Thai volunteers were administered with a single dose of 2 x 250 mg capsules. This indicates the difference in drug disposition rate in different populations.

Statistical analysis showed no significant difference in C_{max}, t_{max} and AUC between both products. There was no significant influence of sequence or formulation on C_{max}, AUC_{0-t} and $AUC_{0-\infty}$. However, the subject effects of these three parameters were significant due to wide variation of the data sets. Significance of the period effect of the C_{max} was also observed but not for the AUCs. This could be relevant to the high variation in the C_{max} values more than in the AUCs values. Intravariability of volunteers which were most difficult to control might probably contribute to this variation. To determine bioequivalence after a single dose, C_{max} and AUC parameters should be taken into consideration. The values of 90% CI of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were within the acceptable range of 80 - 125%. Therefore, it could be concluded that the test product was bioequivalent to the reference product in terms of both rate and extent of drug absorption.

Conclusively, the bioequivalence of the two products of azithromycin powder for oral suspension was studied after a single oral dose of 600 mg administration. Both products were well tolerated and the dose proportional effect was observed compared to previous research works. Pharmacokinetic results and statistic analysis revealed the 90% CIs in the acceptable range, indicating the bioequivalence of the test product, Azith®, and the reference product, Zithromax®.

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