Journal of Applied Pharmaceutical Science Vol. 4 (10), pp. 062-068, October, 2014
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2014.401012
ISSN 2231-3354

Model-Based Bioequivalence assessment of a commercial Azithromycin Capsule against Pfizer Zithromax® Tablet marketed in Jamaica

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ARTICLE INFO

Article history:
Received on: 11/05/2014
Revised on: 02/06/2014
Accepted on: 11/07/2014
Available online: 30/10/2014

Key words:
Azithromycin, capsule, tablet, bioequivalence, mechanistic pharmacokinetics

ABSTRACT

Clinical evidence indicated that effective substitution of azithromycin capsule with a tablet dosage form should be based on evidence of providing equivalent *in vivo* AUC/MIC ratio at the site of infection. This study was designed to compare the bioavailability of a generic azithromycin capsule and assess its bioequivalence with Zithromax tablets marketed in Jamaica. Healthy adult volunteers were recruited following official institutional protocols and randomly assigned to pre- and post wash-over periods of the tests. Sampled plasma levels were analyzed using validated HPLC method. Drug's bio-disposition mechanism in the subjects was determined using the Gastroplus® pharmacokinetics software. Model evaluation with Akaike Information Criterion (AIC) and Schwarz Criterion (SC) indicated one-compartment and two-compartment open models as the best for modeling azithromycin bioavailability from tablets and capsules respectively. However, statistical analysis showed no statistical significance between the respective bioavailability parameters of capsules and tablets, and they fell generally within the US FDA acceptance range of -20% to +25 % of reference product. Azithromycin release from tablets fits one-compartment while from capsules fits the two-compartment open models respectively. Azithromycin capsules were bioequivalent to its proprietary tablets and can be substituted in black male subjects if administered at least two hours before meals.

INTRODUCTION

Azithromycin is a15-membered ring, semi-synthetic macrolide antibiotic with two deoxysugars, derived from erythromycin through a methyl-substituted nitrogen atom in the lactone ring. Its chemical name is 9-deoxy-9a-azo-9a-methyl-9a-homoerythromycin A, with molecular weight 748.88 and chemical formula $C_{38}H_{72}N_2O_{12}$. Its chemical structure is shown below (USP 2012): Azithromycinis a bacteriostatic agent, which binds to the 50S ribosomal subunit of susceptible microorganisms and interferes with protein synthesis. Most of the sensitive organisms require an MIC of ≤ 2 µg/mL but *H. influenzae* requires an MIC of ≤4 µg/mL (MacDougal and Chambers, 2011). Rai *et al* (2012) reported an MIC of 24µg/mL for effective treatment of enteric fever in Indian population (Rai *et al.,* 2012) Azithromycin is more active against *H. influenzae* than clarithromycin and erythromycin. Its spectrum of activity covers a wide range of organisms including *M. catarrhalis*, *Chlamydia spp.*, *N. pneumophila*, *B. bugdorferi*, *Mycoplasma pneumoniae* and *H. pylori*. It also has considerable activity against some *M. avium-intracellularie* and some protozoa including *Toxoplasma gondii*, *Cryptosporidium* and *Plasmodium spp* (MacDougal and Chambers, 2011).

Azithromycin is effective against certain sexually transmitted infections such as the non-gonococci urethritis and cervicitis. Azithromycin showed comparative effectiveness as ciprofloxacin clinically and bacteriologically against sensitive and multidrug resistant (MDR) *S. typhi* in Egypt (Girgis *et al.,* 1999). It was more effective than doxycycline and ciprofloxacin on *Chlamydia trachomatis* and gonorrhea. It is currently used in the management of patients with cystic fibrosis (Wilms *et al.,* 2012)

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Azithromycin is widely distributed throughout the body and its steady-state volume of distribution is usually above 30 L/kg (DrugBank, 2013). Following absorption, azithromycin serum concentrations follow a two- or three-phase decline with a terminal elimination half-life of approximately 60 h (Foulds and Shepard, 1990; Foulds and Johnson, 1993).

Azithromycin is available in Jamaica as intravenous and immediate-release (IR) oral formulations (Zithromax®). Currently, a 2-g single-dose regimen of azithromycin extended-release (ER) microsphere formulation (Zmax™) first approved by the US FDA in 2005 is now available in many countries including Jamaica. The 2-g single dose of azithromycin-ER produced a 2-fold higher serum C_{max} and 3-fold higher AUC_{0-24} of azithromycin on the day of dosing compared with a total dose of 1.5 g of azithromycin-IR tablets given as 500 mg daily for 3 days (Liu *et al.,* 2007).

In addition, the single-dose regimen provided higher exposure in white blood cells and at the infection site, including the lungs and sinus fluid on the day of dosing, with good gastrointestinal (GI) tolerability. A single dose of azithromycin has been suggested as a more effective and convenient treatment for STDs in women in resource-poor environments (Rustomjee *et al.,* 2002). Mouse survival experiments showed that azithromycin administration as single dose significantly increased survival rate with survival being inversely related to number of divided doses, thus indicating that Cmax is an important pharmacodynamics parameter for predicting clinical efficacy. Preclinical and early clinical studies in Japanese subjects suggest AUC/MIC ratio as the most predictive PK-PD parameter for azithromycin efficacy (Muto *et al.,* 2011). Although sub-inhibitory levels of azithromycin reduce exotoxin A, total protease, elastase and phospholipase C production by *Pseudomonas aeruginosa* as well as pneumolysin of high-level macrolide-resistant *Streptococcus pneumoniae* both *in vitro* and *in vivo* (Fukuda *et al.,* 2006), for therapeutic efficacy against many virulent organism, high AUC/MIC ratio appears to be the desirable goal of therapy.

Erratic absorption of azithromycin from capsules compared with tablets suggests the need for ensuring bioequivalence of marketed azithromycin capsules with standard tablets. High level of azithromycin resistance has been reported in England and Wales probably due, among other factors, to sub-therapeutic exposure following erratic absorption (Chilsolm *et al.,* 2009).

The Jamaica health policy and pharmacy law mandates the dispensing pharmacists to inform the patient and prescribers of the availability of generic alternatives to brand products with a view to saving cost and supporting wider coverage of the health budget. However, it is evident from the foregoing, that effective substitution of any tablet dosage with a capsule dosage form should be based on potential for providing equivalent *in vivo* AUC/MIC ratio at the site of infection following administration. The aim of this study was therefore to compare the bioavailability and assess the bioequivalence of generic azithromycin capsules with Zithromax tablets marketed in Jamaica.

MATERIALS AND METHODS

Drug products

Generic Azithromycin, 500 mg capsules Batch #500113 manufactured by MAC's Pharmaceutical and Cosmetics, Old Habour, Jamaica (code: MAC) and Pfizer Zithromax® 500 mg tablets Lot # 5186400707, manufactured by Pfizer, Mexico, S.A. de C.V. (code: ZMT) were obtained from independent Community Pharmacies in Jamaica. Details on product packages were documented including lot number, dose size, dates of manufacture and expiry.

Protocol and Approvals

The protocol involved a single blind, crossover design with equal number of subjects randomly assigned to MAC and ZMT respectively in the first and second phases of the study. The phase 1 and 2 of the study were separated by a washout period of two weeks. Random numbers obtained from a statistical table (Jones, 2005) were used to assign subjects to treatments. Approval by the Institutional Review Board of the Ministry of Health, Jamaica was obtained before the commencement of the study. Subjects were administered informed consent and signed copies of the form were documented. The study conduct was in compliance with the ethical principles from the Declaration of Helsinki and followed all the Good Clinical Practice (GCP) principles of the International Conference on Harmonization (ICH, 2005).

Demographic characteristics and medical assessment of the Subjects

Twenty (20) adult male subjects were recruited but only 12 of them completed the study for non-medical and non-adverse event-related reasons. The average age of subjects that completed the study was 32 years (range: 22 – 42 years), while their average body weight was 73 Kg (57 – 98.5 Kg). The vital signs of subjects were used to enlist them in the study and were monitored at regular intervals during the study and at the completion of sampling. Average body temperature was 35.75 (± 0.62) ˚C, average pulse was 19.33 (±2.74) min^{-1} and average heart rate was 74.50 (±10.06) min^{-1}. These generally fell within the literature "normal" ranges and assured the physical fitness of the subjects to participate in the study (Troutman, 2002). The subjects reported no adverse effect of drug products during or after the study.

Drug administration and sample collection

The subjects were fasted overnight and a single dose of either the brand Zithromax (ZMT) 500 mg tablet or generic capsule (MAZ) 500 mg capsule was given orally with about 250 ml of water. Three hours after administration of drug, subjects were fed on a local diet comprising of steamed banana, yam and dumpling with some ackee and salt fish two hours after the start of sample collection. After a two-weeks wash out period, the subjects were crossed-over: 6 that received 500 mg ZMT tablets were given 500 mg MAZ capsules each, while the other 6 were given one ZMT 500 mg tablet each. Blood samples were collected from the

subclavian vein at the pre-determined time intervals (0 h, 1 hr, 1.5 hr, 2.0 hr, 2.5 hr, 3.0 hr, 4 hr, 6 hr 8 hr, 12 hr, & 24 hr.) into non-heparinized tubes. Each sample was allowed to clot and then centrifuged at 12,000 rpm for 10 minutes. The serum fraction was then carefully separated and stored at -20 °C in coded 96-plate wells until assayed.

Sample analysis

Chromatographic conditions

The HPLC system consisted of a Perkin Elmer 200 Series Autosampler, Series 200 pump, Colouchem Electrochemical III detector by ESA, Zirchrome PS 150mm x 4.6mm column, Hot Sleeve Column Heater (Analytical Sales and Services, NY) and Biotrap MS and C8 (Chromtech, UK). The detector was set at channel 1 (650mV Sensitivity of 2 µA) while channel 2 was at 870mV and sensitivity of 2 µA. The analytical mobile phase consisted of acetonitrile 35 %, methanol 8.75 % and 26.25 mMol /L phosphate buffer 56.25 % at pH 7.04 at the flow rate of 1ml/minute. The extraction mobile phase was acetonitrile 7 % and deionized water 93 %. Twenty (20) µL of serum sample prepared as described under assay was injected onto the column and the column temperature was maintained at 45°C.

Method development

Serum samples of concentrations 1000 ng/mL, 500 ng/mL, 250 ng/mL, 125 ng/mL, 62.5 ng/mL were prepared. The blank and 100 µL of each were injected into the system and the calibration curve was constructed. In addition, a 100 µL of the Azithromycin 1000 ng/ml in plasma was injected repeatedly (5 times) to check reproducibility.

During the run, another sample containing 1000 ng/mL was prepared and 50 µL was injected at various times during the run to check inter-day and intra-day variability. System suitability was checked according to USP specifications while method specificity, linearity, accuracy, precision, detection limit, quantification limit, range and robustness were evaluated based on ICH Harmonized Tripartite Guideline (ICH, 2001).

Data treatment and analysis

Plasma concentration-versus time data were treated with Gastroplus® modeling and simulation software (Simulations Plus Inc., CA) and the various pharmacokinetic parameters were obtained and used to calculate the bioequivalence of MAZ to ZMT. The model that best describes the profiles was generated with the software. Various statistics for testing significance of any observed differences in parameters between capsules and tablets were also documented.

RESULTS AND DISCUSSION

Subject demographic and vitas

Twelve of 17 subjects recruited completed the study. Subjects' demographic data and vitas are shown in Table 1. All subjects were black, male Jamaicans with body vitas falling within the 'normal' general population ranges (Troutman, 2002). No adverse drug event was reported during the 24-hr test period, two weeks washout period and one month of post study follow-up period.

Table 1: Demographic and vital characteristics of subjects.

Subject characteristics	Average Values	SD	Range
Age (yr.)	31.2	5.7	22-42
Height (m)	1.707	0.083	1.587-1.902
Weight (kg)	72.8	10.9	57-98.5
BMI*(kg/m^2)	24.8	1.2	22.6-27.2
Body temperature (°C)	35.8	0.6	35 – 37
Respiratory rate (min^{-1})	19.3	2.7	12 – 24
Heart rate (min^{-1})	74.5	10.1	60 – 90
Systolic BP (Hg)	122.5	11.9	102 – 140
Diastolic BP (Hg)	80.7	8.0	60 – 90

HPLC system suitability for assay of azithromycin in plasma samples

Figs. 1 shows the HPLC calibration curve of azithromycin in plasma fluid. The system suitability was assessed (and compared with the USP specifications) as follow: Resolution – 7.54 (NLT 2.5 between azaerythromycin A and azithromycin), column efficiency – 2884.86 (NLT 1000 theoretical plates), tailing factor – 1.356 (0.9–1.5), relative standard deviation (RSD) – 2.0% (NMT 2.0%).

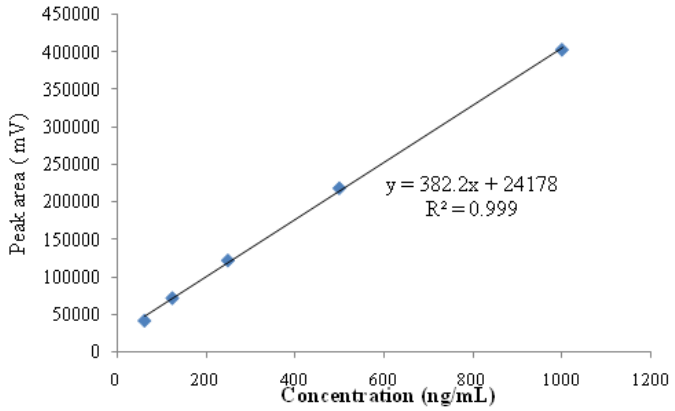

Fig. 1: Calibration curve for azithromycin in plasma fluid.

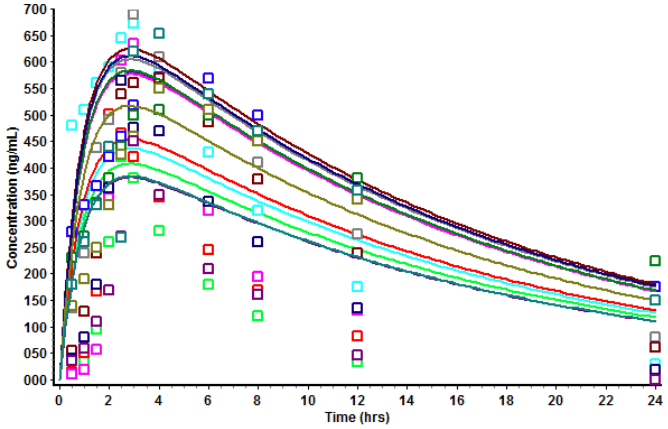

Fig. 2: Superimposed individual Cp vs. time profiles of subjects after administration of 500 mg azithromycin tablets.

Bioavailability of azithromycin from tablets and capsules

The individual plasma concentration-time profiles for the twelve subjects who took either 500 mg of azithromycin capsule (MAZ) or Pfizer's Zithromax® (ZMT) are shown in Figs. 2 and 3 respectively. Average Cp versus time plots are presented in Fig. 4. Significant overlaps at equivalent time points were observed indicating similarity in the blood level-time profiles. However, variation in individual subject's absorption at earlier time points appears to be higher with ZMT than with MAZ (higher values of standard deviation, longer error bars).

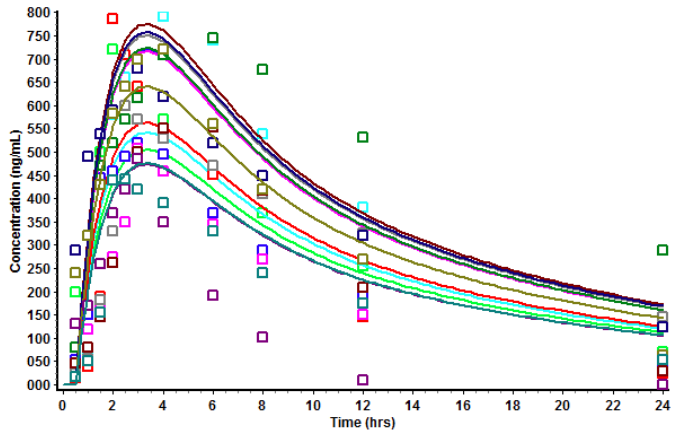

Fig. 3: Superimposed individual Cp vs. time profiles of subjects after administration of 500 mg azithromycin capsules.

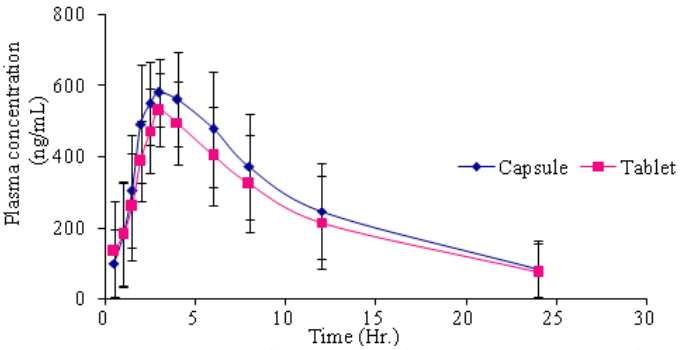

Fig. 4: Mean plasma levels of azithromycin following administration of 500 mg each of MAC'S capsule and Zithromax tablet.

Pharmacokinetic parameters

The subjects Cp.vs. time profiles were input into the GatsroPlus™ as oral plasma data (.opd) files and the non-compartmental analysis (NCA) results were generated (Table 2). The individual subject's weights were entered and the pharmacokinetic profiles were solved for one to three compartmental models. The system utilized the Hooke and Jeeves Pattern search with the error weighting set at 1/Yhat^2. The system returned solutions for NCA, one, two and three compartment models (Tables 3, 4 and 5) with Akaike Information Criterion (AIC) and Schwarz Criterion (SC) according to the equations:

AIC = (#Pts)*Log(Obj)+2(#Parameters) .. (1)

SC = (#Pts)*(Log(Obj) + (#Parameters)*(Log(#Pts) .. (2)

The AIC and SC utilize results of model evaluation to specify the model that best fit the *Cp*. vs. *t* data obtained from capsule and tablet dosage forms respectively. Critical two tail values are greater than calculated *t*-statistics and *p* values are much greater than 0.05 (cut off point for significance at 95 % confidence level), hence there is no statistically significant difference between NCA parameters of test capsules and reference tablets.

The NCA indicates comparability of a number of parameters between capsule and tablet as shown in Table 2. For bioequivalence, US FDA (US DHHS/FDA/CDER, 2003) requires value of parameters not falls within -20% and plus +25 % bounds. Hence, from the product perspective, the generic capsule appears to be bioequivalent to the brand tablet in fasted adult healthy Jamaican black subjects. Literature information generally supports the bioequivalence of azithromycin capsule with tablet dosage form in fasted but not in fed state (Curatolo *et al.*, 2010; 2011). Food has been reported to cause significant degradation of azithromycin to desethyl azithromycin when administered in capsule dosage form (Curatolo *et al.*, 2011). Analysis of bioavailability data with one-compartment model also indicates similarity between capsules and tablets in a number of parameters (Table 3). The main differences between capsule and tablet pharmacokinetic parameters were found in the absorption rate constant (K_a), the elimination rate constant (K_{10}), lag time (T_{lag}), AUC, AUMC and mean residence time (*MRT*) and weighted volume of distribution (*Vd/F*). Faster absorption from MAZ's capsules could be due to the presence of a surfactant in the capsule formulation (Drug literature), which is also reflected in the much higher apparent physiologic volume of distribution than the tablets. According to Jones (1987), the rate-limiting step in disintegration and drug release from capsule is the formulation. Hence, capsule formulations, which are made hydrophilic and readily dispersible, would produce faster drug dissolution than from tablets. The slow post-disintegration dispersion of encapsulated particles may explain the intensity of azithromycin-food interaction that have been reported with capsule dosage form (Curatolo *et al.*, 2010; 2011). The use of surfactants in azithromycin capsule formulation evidently enhanced the particle dispersion after shell disintegration. Absorption rate constants obtained from NCA analysis indicate that capsule and tablet proceeded at about the same rate (Table 2).

Table 2: Non-compartmental analysis (NCA) of comparative bioavailability of azithromycin capsule and tablets.

Parameters	Capsule (Test)	Tablet (Reference)	Ratio (%) Test/Ref
AUC (µg.h/mL)	7602	6676	113.87
AUMC (µg.h^2/mL)	90370	80210	112.67
MRT (h)	11.89	12.01	99.00
CL/F (L/h)	65.77	74.98	87.72
K (h^{-1})	0.089	0.087	102.30
V_{ss}/F (L)	781.8	899.8	86.89

Definitions of parameters in Table 2: AUC - area under the plasma concentration-time plot; AUMC - area under the first moment curve; MRT - mean residence time; K – elimination rate constant; V_{ss}/F - post-absorption volume of distribution at steady state; CL/F – post absorption total clearance.

Table 3: One-compartment model analysis of the bioavailability of azithromycin from capsule and tablet.

Parameters	Capsule (Test)	Tablet (Reference)	Ratio (%) Test/Ref	2-tailed t-test {Ref Tvalue for df 22@0.05=2.074; @0.01 = 2.89)}
AUC (µg.h/mL)	7602(33.93%)	6676(25.17%)	113.87	1.04**
AUMC (µg.h^2/mL)	67050 (33.93%)	64330 (25.17%)	104.23	0.58**
MRT (h)	8.783 (23.99%)	9.778 (17.80%)	89.82	-1.26**
CL/F (L/h)	65.49 (23.99%)	75.99 (17.80%)	86.18	-1.75**
C_{max} (ng/mL)	520	457	113.78	
T_{lag} (h)	-	0.114 (266.81)		-
K_a	0.348 (34.64%)	0.102(17.8%)	3.41	6.99
K_{10}(h^{-1})	0.114 (23.99%)	*0.545 (46.29%)	20.92	-1.11**
$T_{1/2}$ (h)	6.088 (23.99)	6.777 (17.8%)	89.83	-1.26**
$V_{d/F}$ (L)	575.2 (0.04%)	743.1(0.02%)	77.40	-2123.37
R^2	0.8289	0.8824	93.94	-

Definitions of parameters in Table 3: AUC - area under the plasma concentration-time plot; AUMC - area under the first moment curve; K_{10} - elimination rate constant from central compartment; K_a - absorption rate constant; C_{max} - maximum plasma concentration; MRT - mean residence time; $T_{1/2}$ - elimination half-life; Vd/F - post-absorption volume of distribution.

Table 4: Two-compartmental model analysis of bioavailability data obtained from subjects who were administered 500 mg of either azithromycin capsule or tablet.

Parameters (Units)	Capsule	Tablet	Test/Ref Ratio (%)	Calculated *t*-difference	*Critical two-tail *t* value	P values
CL/F (L/h)	65.08	67.21	96.8	0.077	0.94	0.53
Vc/F (L)	356.9	241.3	147.9**	0.49	0.63	0.31
CL2/F (L/h)	106.6	147.7	72.2**	0.117	0.91	0.55
V_2/F (L)	549.6	632	87.0	0.793	0.44	0.78
(CL/F)/kg (L.h/kg)	0.894	0.923	96.9	0.077	0.94	0.53
(Vc/F)/kg (L/kg)	4.903	3.316	147.9**	0.491	0.63	0.31
(CL_2/F)/kg ((L.h/kg)	1.465	2.03	72.2**	0.117	0.91	0.55
(V_2/F)/kg(L/kg)	7.55	8.682	87.0	0.793	0.44	0.78
K_{10} (h^{-1})	0.182	0.278	65.5**	0.368	0.72	0.64
K_{12} (h^{-1})	0.299	0.612	48.9**	0.208	0.84	0.58
K_{21}(h^{-1})	0.194	0.234	82.9	0.07	0.94	0.35
T_{lag}(h)	0.5052	0	-	-	-	-
K_a (h^{-1})	0.361	0.235	153.6**	0.455	0.65	0.33
C_{max}(µg/mL)/mg Dose)	1.55^-03	1.25^-03	124.0	-	-	-
$t_{½}$ (h)	12.11	11.32	107.0	-	-	-
R^2	0.6504	0.4667	-			

Definitions of parameters in Table 4: AUC - area under the plasma concentration-time plot; Ka - absorption rate constant; Cmax - maximum plasma concentration; AUMC - area under the first moment curve; MRT - mean residence time; K – elimination rate constant; T1/2 - elimination half-life; Tlag - lag time of absorption phase ;Vss/F - post-absorption volume of distribution at steady state; CL/F – post absorption total clearance. (p> 0.05 indicates insignificant difference between means of capsule and tablets parameters). *For 2-tailed t-test, reference t-value for df 22 at 0.05 = 2.074; at 0.01 = 2.819). **Outside the -20 to +25 % range.

Table 5: Three-compartment model analysis of the bioavailability of azithromycin from capsule and tablet.

Parameters	Test Capsule (CV)	Reference Tablet (CV)	Ratio (%) Test/Ref	2-tailed t-test {Ref Tvalue for df 22@ 0.05=2.074; @0.01 = 2.89)}
C_{max} (µg/L)	525 (16.08)	494 (19.31)	106.28	1.229**
CL/F (L/h)	12.41(297.03)	64.79(16.17)	19.23	-4.735
Vc/F (L)	364.2(0.03)	425.9(1.87)	85.51	-26.833
CL_2/F (L/h)	37.72(77.05)	58.49(82.32)	64.49	-1.279**
V_2/F (L)	3026.8(0.01)	356.5(1.83)	849.03	1416.317
CL_3/F (L/h)	34.37(97.37)	11.72(126.21)	293.26	2.144*
V_3/F (L)	2516.3(0.02)	3169.7(0.00)	79.39	-4497.430
K_a (h^{-1})	0.244(30.72)	0.386(33.25)	63.21	-3.309
K 10(h^{-1})	0.034(297.03)	0.152(16.28)	22.36	-3.931
K $_{12}$(h^{-1})	0.104(77.05)	0.137(82.35)	75.91	-0.826**
K $_{21}$(h^{-1})	0.012(77.05)	0.164(82.34)	7.32	-3.890
K $_{13}$(h^{-1})	0.094(97.37)	0.028(126.23)	335.71	2.330*
K $_{31}$(h^{-1})	0.014(97.37)	0.004(126.21)	350	2.383
$T_{1/2}$ (h)	380.4 (0.00)	222.8(0.00)	170.74	-
R^2	0.8567	0.9223	92.89	

Definitions of parameters in Table 5: AUC - area under the plasma concentration-time plot; AUMC - area under the first moment curve; K_{10} - elimination rate constant from central compartment; K_a - absorption rate constant; C_{max} - maximum plasma concentration; MRT - mean residence time; $T_{1/2}$ - elimination half-life; V_d/F - post-absorption volume of distribution; CL/F – post absorption total clearance; T_{lag} - lag time of absorption phase CV = coefficient of variation. *No significance difference at p=0.05, **No significance difference at p = 0.01

Although capsule absorption rate from one compartmental analysis appears much higher than for tablet, two-compartmental treatment *Ka* was completely opposite (Table 3 and 4). Similarly, the lag time was reversed between one-compartmental and two-compartmental treatments (Tables 3 and 4). These observations indicate the significance of selecting the right model for pharmacokinetics treatment of data from different dosage forms. Between one-compartment and two compartment models, there were flip-flops in the values of K_a, T_{lag} and C_{max}. This is not surprising since the absorption determines the plasma concentration over time, which in turn determines the rate and pattern of extravascular distribution.

However, the large inter-subject variability, characteristic of biological data, as observed in these parameters does not enable any statistical significance to be manifest. Therefore, as shown in Table 1, calculated *t*-statistical values were generally lower than the critical two-tail reference cut off points and the *p* values are generally greater than 0.05. Hence, although the nominal values of pharmacokinetic parameters suggest differences between capsule and tablets, these differences are not statistically significant owing to the high inter-subject variability.

As indicated by the Akaike and Schwarz information criteria, one-compartment model is the best fitting for tablet whereas a two-compartment model is the best model for describing the bioavailability of data from azithromycin capsule. The differences in the physico-mechanical properties of capsules and tablets could be responsible for the model-dependent pharmacokinetic behavior of azithromycin in fasted human subjects. It is well known that drug dissolution could occur from intact tablets, granules and fine particles, and that exponential release of drug occurs after granules have been finely dispersed (Wagner, 1969; Adebayo and Itiola, 2003). High compression force employed in tablet preparation provides the desirable mechanical strength to reduce friability and prevent loss of tablet material in transportation and use. However, deactivation of the bonding forces created during compression, in order to initiate tablet disintegration and dispersion of granular particles in fine state of subdivision, is time-dependent and may impart more gradual and phased release pattern to drugs in tablet form (Kitazawa *et al.*, 1977).

This is particularly important for azithromycin, a BCS Class 2 drug with solubility of 0.1-1mg/mL (Curatolo *et al.*, 1998; Wu and Benet, 2005; Takagi *et al.*, 2006). On the other hand, capsule preparation involves limited compression force and, once the shells disintegrate, normally disperses the drug in finely divided state of subdivision. In addition, modern formulations of hard gelatin capsule shells ensure rapid disintegration at body temperature while hypromellose capsules are soluble at temperatures as low as 10 °C (Chiwele *et al.*, 2000). The presence of a surfactant in the capsule formulation used in this study evidently contributed to the fast dispersion of the encapsulated drug after gelatin shell disintegration. The dosage form effect on bioavailability affirms the necessity to compared drug products in similar dosage form whenever possible. When the dosage forms are not exactly the same, potential effect of dosage form on the pharmacokinetic parameters should be carefully considered. The results of this study generally indicate that azithromycin follows dosage form-dependent post-absorption distribution patterns. Generally, NCA indicates no significant difference in pharmacokinetic parameters ($p > 0.05$). Although one- and two-compartmental analyses showed some differences in nominal value of parameters, these could be treated as effects of residuals and, hence, did not enable statistical significance to be manifest between capsule and tablet parameters. Dosage regimen-dependent differences in post-absorption distribution half-lives of azithromycin have been reported (Ritschel, 1985; Gandhi *et al.*, 2004). In addition, literature information indicates that azithromycin plasma concentration shows polyphasic decline with regimen-dependent mean half-lives of 27.9 h (three-day regimen) and 35.8 h (five-day regimen) (Wildfeuer *et al.*, 1993; Lode, 1991). It is therefore possible that rapid release of azithromycin from capsules and shorter residence in the gastrointestinal tract might be responsible for the higher drug concentration in the plasma, which was sufficient to drive its concentration-dependent (first-order) distribution extravascularly.

With regards to tablet dosage forms, however, the slower release, which would cause distribution rate to be faster than absorption rate, may not support accumulation in the plasma to the extent that the drug could be distributed significantly outside of the plasma after single dose. This observation suggests careful model consideration in the treatment of bioavailability data for bioequivalence evaluation in order to determine all the possible contributors to the observed differences in pharmacokinetic parameters and to accurately differentiate between drug specific and dosage form effects.

CONCLUSIONS

Model dependent evaluation of azithromycin capsule against proprietary tablet shows no statistically significant difference between their bioavailability parameters as they generally fell within the -20 to + 25 % range accepted by the FDA. Model evaluation with Akaike Information Criterion (AIC) and Schwarz Criterion (SC) indicated two-compartment and one-compartment open model as the best for describing pharmacokinetics of azithromycin from capsules and tablets dosage forms respectively. Since drug absorption and systemic distribution of azithromycin (a BCS class II drug) are both first-order processes, the relative rapidity of drug release from capsule may be responsible for the observed two-compartment behavior compared with tablets which demonstrated slower and polyphasic dissolution profile. However, statistical analysis showed no significance between the respective pharmacokinetic parameters of capsules and tablets (except with absorption rate constants and volume of distribution) in fasted black male subjects. The differences between other pharmacokinetic

parameters of tablets and capsules fell generally within the US FDA acceptance range -20% to plus +25 %.Study suggests that incorporation of surfactant may increase the absorption rate of azithromycin capsule from the stomach thereby decreasing the potential effect of co-administered food on azithromycin bioavailability. Further studies on capsules and tablets of other drug in different BCS classes may be useful for verifying the dosage form dependent effect on pharmacokinetics parameters of drugs.

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How to cite this article:

Amusa S. Adebayo and Noel McFarlane. Model-Based Bioequivalence assessment of a commercial Azithromycin Capsule against Pfizer Zithromax® Tablet marketed in Jamaica. J App Pharm Sci, 2014; 4 (10): 062-068.