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Pharmacokinetics of Amoxicillin/Clavulanic Acid Combination after Oral Administration of New Suspensions Formulation in Human Volunteers

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Abstract: The pharmacokinetic properties of amoxicillin and clavulanic acid when used alone or in combination may show an interaction between these two agents that might decrease the absolute bioavailability of clavulanic acid. In an open, randomized, replicated Latin square under fasting condition, the pharmacokinetics of new formulations of amoxicillin/clavulanic acid were compared with reference formulation after single dose administration in 15 healthy male volunteers. Subjects were given equal molar doses of new suspension formulations of amoxicillin/clavulanic acid or Augmentin® as reference product. After one week wash-out period blood samples were collected exactly before and after drug administration of any formulations at different time points up to 6 h. The concentrations of the antibiotics in plasma were measured by validated high-performance liquid chromatography methods. Three formulations exhibited a similar mean C_{max} and T_{max} either for amoxicillin or for clavulanic acid. The $AUC_{0\text{-}inf}$ for amoxicillin was about 1278±172 μg min mL^{-1} and it was about 354±66 μg min mL^{-1} for clavulanic acid. There were no significant differences in pharmacokinetic parameters between three formulations. The two generic formulations investigated in this study proved to be bioequivalent with brand-name Augmentin® with regard to the pharmacokinetic parameters C_{max}, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$ and t_{max}. We may conclude that two new formulations are bioequivalent with reference suspension and can be considered equally effective in medicinal practice. Moreover, there were no interaction in pharmacokinetic parameters between amoxicillin and clavulanic acid. No serious adverse event was observed with the studied drugs.

Key words: Amoxicillin, clavulanic acid, pharmacokinetics, suspension

INTRODUCTION

The drug combination of amoxicillin/clavulanate is a broad spectrum antibiotic for treatment of a wide range of bacterial infections, including upper and lower respiratory tract infections and infections of the skin and soft tissue structures (Todd *et al*., 1990; Neu *et al*., 1993; Barry *et al*., 1994). It was first introduced into clinical medicine in Europe in 1981 and into United State in 1984. Since its release the drug has been extensively used in patients of all ages including infants, children and adults (Reed, 1996). The highly desirable antibacterial spectrum of activities of the drug combined with its favorable pharmacokinetic and safety profiles underscore its rapid acceptance as one of the most commonly prescribed antibiotics. In many countries the standard regimen for pediatric patients aged ≥ 3 months for the treatment of mild to moderate infections is now amoxicillin/clavulanic acid 25 mg/3.6 mg per kg/day, divided in either two or three doses. For more severe infections such as Acute Otitis Media (AOM) the standard regimen is amoxicillin/clavulanic acid 45 mg/6.4 mg per kg/day divided in two doses (White *et al*., 2004). Reported data support a nonlinear absorption process for amoxicillin, saturable transport mechanisms, limited solubility and the existence of an absorption window are possibly involved in the gastrointestinal absorption of this antibacterial, all leading to a decrease in the pharmacokinetic parameters of this drug. Furthermore a possible interaction between amoxicillin and clavulanic acid that might decrease the absolute bioavailability of clavulanic acid is reported (Navarro, 2005). A new oral suspension of this combination was prepared therefore, its pharmacokinetic characteristics need to be evaluated and compared to the same product produced by the innovator. In this regard pharmacokinetic parameters of active ingredients of these

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products were measured and compared with the reference standard (Augmentin®) available in the market. Then gained data analyzed statistically to determine whether the test and the reference products yield comparable values.

MATERIALS AND METHODS

Amoxicillin/clavulanate potassium (Co-amoxiclav®) for oral suspensions; 312 mg/5 mL (250 mg amoxicillin plus 62.5 mg clavulanic acid, T_1) and 156 mg/5 mL (125 mg amoxicillin plus 62.5 mg clavulanic acid, T_2) were evaluated. These formulations were compared with the reference product (R), Augmentin®, for oral suspension; 312 mg/5 mL (250 mg amoxicillin plus 62.5 mg clavulanic acid).

Solvents used for drug measurement, were of HPLC grade; while other chemicals and reagents were of analytical grade. Amoxicillin and clavulanic acid powder were purchased from Beecham Pharmaceuticals, Brantford, England. Other materials were purchased from local market.

Study subjects: Fifteen healthy adult male subjects were enrolled in the fasting study. One subject refused to continue the study before completion so leaving 14 subjects completed three phases of the study. Subjects ranged in ages from 21 to 38 years, in body weight from 57 to 85 kg and in height from 163 to 185 cm. All subjects were in good health as indicated by medical history (history or evidence of hepatic, renal, gastrointestinal and hematological disorders, acute or chronic diseases or allergy to beta-lactam antibiotics), physical examination and clinical laboratory tests (hematology and blood biochemistry). Also subjects were not permitted to smoke, to take any drug and to do hard physical activity from two weeks before to the end of study and not to have beverages and foods containing caffeine during the study.

The volunteers were informed about the risk and the aim of the study and signed the written informed consent statement before entering the study.

Drug administration and sample collection: The study was designed based on a single-dose, replicated Latin square under fasting condition. After an overnight fasting (10 h), subjects were given equal molar doses (10 mL of T_1, 20 mL of T_2 or 10 mL of R) of drug followed by 250 mL water. They were fasted over 2 h post-dose then they received the same breakfast and lunch according to the time scheduled. Therefore, all subjects received equivalent 500 mg amoxicillin and 125 mg clavulanic acid on three occasions separated by a 7 days wash out period.

To determine amoxicillin and clavulanic acid concentrations, samples of venous blood (8 mL) were collected at 0 h pre-dose and at 0.25, 0.50, 0.75, 1, 1.5, 2, 3, 4 and 6 h post-dose and transported to the laboratory on dry ice to be analyzed.

Chromatographic conditions: Serum samples were analyzed to measure amoxicillin and clavulanic acid concentrations. The validated HPLC chromatographic method for each active ingredient (Foulston *et al.*, 1982) was used. The HPLC system was consisted of 515 isocratic pump Waters® equipped with 717 plus auto sampler with heater/cooler system and dual-absorbance UV-visible detector connected to a millennium® 32 software data integrator. Chromatographic separation was performed on a reversed 2.1×150 mm i.d., stainless steel C_{18} μ-bondapack (3.5 μm particle size) column connected to a C_{18} guard column. The mobile phase composed of potassium dihydrogen phosphate 0.05 M (pH = 2.75), methanol and acetonitrile (94:3.5:2.5%V/V).

Calibration curve: Standard curve generated by preparing nine plasma standards over the range of 0.2-20 or 0.05-10 μg mL^{-1} for either amoxicillin or clavulanic acid, respectively. Standards were analyzed in triplicates (n = 9).

Pharmacokinetic data analysis: Pharmacokinetic analysis was performed by model independent method using SPSS® and MS Excel® softwares. The maximum amoxicillin concentrations (C_{max}) and the corresponding T_{max} were determined by the inspection of the individual drug plasma concentration-time profiles. The elimination rate constant (K_{el}) was obtained as the slope of the linear regression of the log-transformed plasma concentration values versus time data in the terminal phase. $T_{1/2}$ was calculated as $0.693/K_{el}$. AUC to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. AUC extrapolated to infinity ($AUC_{0-\infty}$) was calculated by equation $AUC_{0-t} + C_t/K_{el}$ where C_t is the last measurable concentration. Oral clearance ($Cl_{p.o.}$) was calculated as $D/AUC_{0-\infty}$ and volume of distribution (Vd/F) was calculated by dividing corresponding $Cl_{p.o.}$ by β. The relative bioavailability was calculated by dividing $AUC_{0-\infty}$ values of the active ingredient of new formulations over the same values after administration of standard solution.

Sample preparation for HPLC injection

Amoxicillin: Each serum sample was transferred to filter tube for ultra-centrifugation at 5°C and 5000 g for 40 min.

Twenty microliter from filtrated liquid was injected to chromatograph by auto-sampler. Wavelength of UV detection was set at 229 nm. The mobile phase was pumped at a flow rate of 0.2 mL min^{-1} and the run time was regulated at 12 min.

Clavulanic acid: Each serum sample was transferred to filter tube for ultra-centrifugation at 5°C and 5000 g for 40 min. Hundred microliter of imidazole buffer was added to 400 μL of filtrated sample. Obtained solutions were vortxed and kept in 30°C for 13 min. Then, 10 μL was injected into the column by auto sampler. The wavelength of UV detection was set at 320 nm and flow rate of mobile phase was 0.1 mL min^{-1}. Run time was regulated at 10 min.

Statistical analysis: For the purpose of pharmacokinetic analysis for each active ingredient, AUC_{0-t}, AUC_{0-inf}, C_{max}, T_{max}, $t_{1/2}$, $Cl_{p.o.}$ V_d was compared as the pharmacokinetic variables. The difference between two related parameters was considered statistically significant for a p-value≤0.05. After logarithmic transformation C_{max}, AUC_{0-t} and AUC_{0-8} were analyzed as per current Code of Federal Regulation (1998) and FDA Guidelines (2000).

RESULTS AND DISCUSSION

New formulations were tolerated well by the volunteers. Unexpected incidents that could have influenced the outcomes of the study did not occur. All volunteers who had started the study and continued to the end were discharged in good health. All formulations were readily absorbed from the gastrointestinal tract and active ingredients were measurable at the first sampling time (15 min) in all volunteers. The mean concentration-time profiles of the three formulations for amoxicillin and clavulanic acid are shown in the Fig. 1 and 2. As would be expected a sharp peak in plasma amoxicillin concentrations at ~1 to 1.5 h after administration, with a sharp decline thereafter was observed indicating a prompt distribution to the peripheral compartment. The terminal

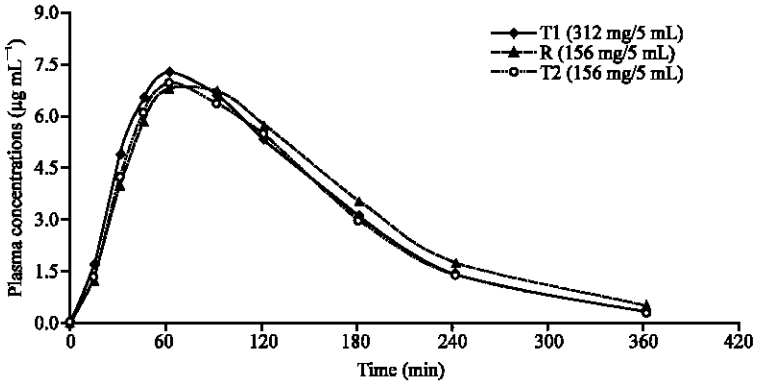

Fig. 1: Mean concentration-time profiles of the amoxicillin in three formulations

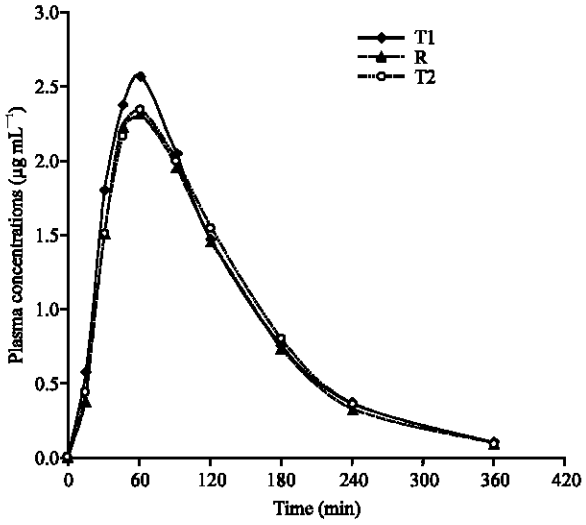

Fig. 2: Mean concentration-time profiles of the clavulanic acid in three formulations

Table 1: Pharmacokinetic parameters of amoxicillin for three formulations (mean±SD)

Formulations	C_{max} (mg L^{-1})	T_{max} (h)	$T_{1/2}$ (h)	$Cl_{P.O}$ (L h^{-1})	AUC_{0-8} (mg h L^{-1})
T1	7.59±1.45	1.2±0.4	1.166±0.16	26.34±3.68	19.32±2.66
T2	7.32±1.68	1.26±0.45	1.286±0.14	25.65±4.26	18.57±3.11
R	7.30±1.72	1.23±0.35	1.140±0.1	27.66±4.77	20.0±3.37

Table 2: Pharmacokinetic parameters of clavulanic acid for three formulations (mean±SD)

Formulations	C_{max} (mg L^{-1})	T_{max} (h)	$T_{1/2}$ (h)	$Cl_{P.O}$ (L h^{-1})	AUC_{0-8} (mg h L^{-1})
T1	2.60±0.68	1±0.18	1.03±0.122	22.2±5.9	6.11±1.38
T2	2.43±0.50	1±0.23	1.02±0.15	22.98±3.68	5.91±1.2
R	2.44±0.53	1.05±0.33	1.06±0.07	22.73±5.59	5.68±0.72

elimination half life of amoxicillin of about 1.2 h was similar in all formulations. The reduced terminal elimination half life (1-2 h in all formulations) indicates that amoxicillin, like other penicillins rapidly eliminated from the body and no accumulation occurs after repeated dosed in subjects with normal renal function.

Table 1 and 2 show the pharmacokinetic parameters for the three test products for amoxicillin and clavulanic acid, respectively. All calculated pharmacokinetic values were in good agreement with previously reported studies that contain the same unit dose of drug (Wardrop *et al*., 1997; Schaad *et al*., 1983). Drug clearance is more than the average glumerular filtration due to tubular secretion, thus explaining why constant administration of probenecid decreases the urinary excretion of amoxicillin leading to a slower elimination rate. After oral administration, amoxicillin is rapidly and well absorbed, a high fraction of the dose reaching the systemic circulation and with a short time to reach maximum concentration, (with t_{max} <76 min) to give (a C_{max} of about 7.5 mg L^{-1}) under fasting and non fasting conditions (Malinaro *et al*., 1997).

The disposition of clavulanic acid is also characterized by the initial rapid phase, indicating easy distribution to the peripheral compartment. The short half life (≈ 1 h) is the consequence of the rapid elimination from the body produced by metabolism and renal excretion. Distribution studies reported for amoxicillin/clavulanic acid have shown that the access of clavulamic acid to ascetic fluid, synovial fluid, bone tissues, gynecological tissues and sputum is similar to that reported for amoxicillin. However, the distribution of clavulamic acid is slightly lower than that established for amoxicillin which may be contributed to lower lipid solubility of clavulanic acid.

To measure the relative bioavailability of new formulations the 90% confidence intervals for the natural log-transformed data were also calculated according to the FDA guidelines (2000) and the results are shown in Table 3 and 4. The mean and standard deviations of AUC_{0-t}, AUC_{0-inf}, C_{max}, $t_{1/2}$, $Cl_{p.o.}$ and T_{max} of the two test products in comparison to reference product did not show any significant differences for either amoxicillin or clavulanic acid, suggesting that the plasma profiles

Table 3: Statistical parameters for pharmacokinetic parameters of amoxicillin after two treatments

		ANOVA		90%
		----------		Confidence
Formulations	Parameter	$F_{calculated}$	$F_{critical}$	interval
T1	C_{max}	0.22	4.23	95-116%
	T_{max}	0.07	4.23	80-116%
	AUC_{0-6}	0.35	4.23	90-104%
	AUC_{0-8}	1.48	4.23	86-100%
T2	C_{max}	0.01	4.23	92-109%
	T_{max}	0.07	4.23	86-119%
	AUC_{0-6}	1.37	4.23	88-98%
	AUC_{0-8}	3.35	4.23	84-94%

Table 4: Statistical parameters for pharmacokinetic parameters of clavulanic acid after two treatments

		ANOVA		90%
		----------		Confidence
Formulations	Parameter	$F_{calculated}$	$F_{critical}$	interval
T1	C_{max}	0.45	4.23	93-118%
	T_{max}	0.21	4.23	82-111%
	AUC_{0-6}	0.98	4.23	93-119%
	AUC_{0-8}	1.14	4.23	94-119%
T2	C_{max}	0.01	4.23	87-113%
	T_{max}	0.11	4.23	84-114%
	AUC_{0-6}	0.35	4.23	92-115%
	AUC_{0-8}	0.38	4.23	92-114%

generated by Co-Amoxiclav® suspensions are comparable to those produced by Augmentin®. Statistical analysis for these parameters, did not differ significantly between each test with reference formulations having a p-value>0.05. The pharmacokinetic behavior of clavulanate in these formulations also showed no differences from those of existing formulation of Augmentin®.

The 90% confidence intervals also demonstrated that the ratios of AUC_{0-t}, AUC_{0-inf}, C_{max} and T_{max} of formulations and for the three periods Lay within the FDA acceptable range of 80-125% for both ingredients.

On the bases of the plasma levels of the 14 volunteers completed the study, the mean relative bioavailability of amoxicillin for T_1 and T_2 were 96.61 and 92.83% for AUC_{0-t}, 92.93 and 89.32% for AUC_{0-8}, 103.89 and 100.21% for C_{max}, 97.10 and 102.90% for T_{max} clearly indicated no significant difference between tests and reference products in any of the calculated pharmacokinetic parameters. The confidence intervals (CIs) for the ratios of mean AUC_{0-t}, AUC_{0-8}, C_{max} and T_{max} indicated that these values are entirely within the

bioequivalence acceptable range of 80-125% (using log transformed values). Similar results were observed for clavulanic acid as well (Table 4).

CONCLUSIONS

Pharmacokinetic parameters of amoxicillin and clavulanic acid in formulations used in this study were similar to previously published data (Adam *et al*., 1982). Furthermore, the new formulations of Co-Amoxiclav® (312 and 156 mg/5 mL) suspensions is bioequivalent to reference formulation (Augmentin® 312 mg/5 mL) manufactured by Beecham, England. Therefore the three products evaluated in this study may be considered equally effective in medicinal practice by using same molar doses.

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