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Clinical Pharmacokinetics of Clavulanic Acid, a Novel β -Lactamase Isolated from *Streptomyces clavuligerus* and Its Variability

Anab Fatima, Mohammad Jiyad Shaikh, Hina Zahid,
Ishart Younus, Sheikh Abdul Khaliq and
Farah Khalid

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Abstract

The clavulanic acid derived by fermentation of *Streptomyces clavuligerus* and possessed the capability to inactivate a broad range of β -lactamase enzymes. A complex physico-chemical process involves the binding of clavulanic acid to β -lactamases in which clavulanic acid itself deplete irreversibly along with β -lactamase enzyme rendering amoxicillin spared which otherwise would hydrolyze by an enzyme. It is therefore termed as 'suicide' inhibitor for β -lactamases. We discussed here pharmacokinetic parameters and identified factors responsible for the variability of absorption of clavulanic acid. The results based on individual plasma concentration-time curve of amoxicillin and clavulanic acid in an open, randomized, two-way crossover study involving 10 healthy male subjects administered with two amoxiclav formulations.

Keywords: clinical, clavulanic acid, pharmacokinetics, variable absorption, AUC total, pharmacokinetics, β -lactamase

1. Introduction

Clavulanic acid derived by fermentation of *Streptomyces clavuligerus* and possessed the capability to inactivate a broad range of β -lactamase enzymes. The molecular formula of clavulanate potassium is $C_8H_8KNO_5$ with the molecular weight of 237.25. Chemically it is potassium

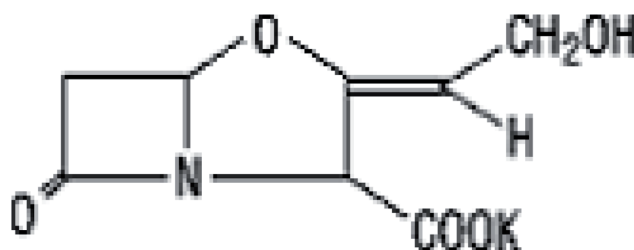


Figure 1. Structure of clavulanate potassium.

(Z)-(2R,5R)-3-(2-hydroxyethylidene)-7-oxo-4-1-azabicyclo[3.2.0]-heptane-2-carboxylate, represented as in **Figure 1**. Mainly it combines with amoxicillin to broaden its antibacterial spectrum [1]. A complex physicochemical process involve in binding of clavulanic acid to β -lactamases in which clavulanic acid itself deplete irreversibly along with β -lactamase enzyme rendering amoxicillin spared which otherwise would hydrolyzed by enzyme. It is therefore termed as 'suicide' inhibitor for β -lactamases. A very low plasma concentration of clavulanic acid is required for this target action. After oral administration the pharmacokinetic parameters of both components i.e. amoxicillin and clavulanic acid were similar and they did not affect pharmacokinetic parameters of each other [2–6]. This could be one of reason for their antimicrobial combination. In combination clavulanic acid and amoxicillin used in different composition i.e. 250/125, 500/125 and 850/125 respectively. In this amount of amoxicillin varies but that of clavulanic acid remain constant i.e. 125 mg. It would suggest that there is no significant amount required for clavulanic acid to inhibit β lactamase enzymes [3]. We discussed here pharmacokinetic parameters and identified factors responsible for variability of absorption of clavulanic acid and compared it with previous reported data. The results based on individual plasma concentration-time curve of amoxicillin and clavulanic acid in an open, randomized, two-way crossover study involving 12 healthy male subjects administered with two amoxiclav formulations.

2. Parameter which is associated to show variability of absorption

Mainly C_t (concentration at time t) is responsible for clinical effects of any drug. When C_t is higher the AUC (Area under Curve) and C_p (plasma concentration) will also show higher values. Thus they all are co-related to show any clinical effect but question arises that which pharmacokinetic parameter or any other factor is most likely to show variability in absorption of any drug which would ultimately affect its clinical effect. Clavulanic acid along with amoxicillin is well absorbed from stomach after oral administration without having any impact of fasting and fed state on the pharmacokinetics of amoxicillin. While relative bioavailability of clavulanic acid becomes reduced when administered after 30 and 150 min of high fat breakfast. The logic behind reduced bioavailability of clavulanic acid after ingestion with the meal was due to prolong residence time of clavulanic acid in GI due to intragastric tablet deposition in the proximal stomach. The half-life of clavulanic acid is 1.0 h and 25–40% of it is excreted unchanged in urine following first 6 h of administration. Clavulanic acid is difficult to extract out from plasma as it has been bound approximately 25% to human serum and therefore required double extraction procedure to observe by liquid chromatography. After absorption clavulanic acid is well distributed in body tissues [7].

2.1. Content assay for co-amoxiclav tablets by HPLC

The content assay for co-amoxiclav tablets was carried out by validated HPLC method. The method validation was carried out according to USP guidelines. For good and accurate resolution and reproducibility of the presented method various suitability considerations including tailing factor, retention time, resolution, RSD% of retention time and peak areas were determined and were found within acceptable range. The method was found to be specific for the determination of particular analyte. Specificity was determined by injecting the analytical placebo(containing all excipient of tablet except amoxicillin and clavulanic acid). The interference by these excipients were determined by evaluating mixture of all excipient(placebo),standard solutions and commercial pharmaceutical preparations contained amoxicillin and clavulanic acid within the same chromatographic condition.

The linearity of HPLC method was determined for amoxicillin and clavulanic acid. Ten dilutions of amoxicillin and eight dilutions clavulanic acid of different concentrations were prepared in mobile phase then sample size of 20 μ l of each concentration was injected into HPLC. The detector response was measured at 235 nm and the calibration plots(concentration versus peak area) were obtained using the linear regression method. The linearity data showed linearity over a concentration range of 0.03–31.25 μ g/ml for amoxicillin and 0.24–15.6 μ g/ml for clavulanic acid. Repeatable and intermediate precision of method was determined. During the same day with the same experimental condition repeatability of four determination ($n = 4$) at the same concentration was calculated. The results of assay were compared and evaluated on three different days by different analyst for intermediate precision. The precision values for amoxicillin and clavulanic acid were found to be 0.91 and 0.35% for intraday and 0.89 and 0.34% for inter-day respectively.

The recovery studies were carried out for the assurance of reliability and accuracy of the proposed method. A known quantity of the drug added with preanalyzed sample and then reanalyzed by the proposed method. The recovery studies for amoxicillin and clavulanic acid were performed at three different concentrations corresponded to 80, 100 and 120% of active ingredients. For each concentration mean %recovery were from 99.7 to 101.4 for amoxicillin and 100 to 101.4% for clavulanic acid.

Small variation in the method parameters was created to measure its reliability during routine usage and the robustness. Not any significant effect on the method performance was observed by changing the flow rate of mobile phase, column temperature and ratio of organic content in mobile phase indicated that the test method was robust for all variable conditions (**Table 1**).

2.2. Pharmacokinetic evaluation of co-amoxiclav tablet

In Pakistan Co-amoxiclav tablet of 375 mg marketed by a multinational company Code #1 Pakistan and a local company Code #2 (**Table 2**). Physico-chemical and potency determination

Medium	Parameters	Amoxicillin	Clavulanic acid
In mobile phase/plasma	Limit of Quantification {LOQ(μ g/ml)}	0.030/0.0075	0.243/0.12
	Limit of Detection {LOD(μ g/ml)}	0.015/0.0037	0.12/0.06

Table 1. Validation parameters of amoxicillin and clavulanic acid in mobile phase.

Name of product	Strength (mg)	Date of mfg.	Date of exp.	Retail price (Rs)	Industrial supplier
(AMCL1)	250/125	02-12	08-13	75.00	Code#1
(AMCL2)	250/125	04-12	04-14	82.00	Code#2

Note: This was a 2 year study and during this period we have taken products from different lots available in the market.

Table 2. Details of different amoxicillin/clavulanic acid products available in Pakistan.

from both sources of different batch were analyzed. This analysis was carried out just to compare the two different brands available in Pakistan as an added parameter. The main objective of study is to conduct pharmacokinetic study of Co-amoxiclav tablet from a multinational company in local population and to find out difference in the pharmacokinetic parameters with the previous reported data due to racial inconsistency. It was preferred to compare only multinational brand because this has been most commonly prescribed by the prescriber to treat infections and the local brand did not proved to be as efficacious as that of multinational brand.

The study was two treatments, two sequences, single dose and cross over design in 12 normal healthy volunteers. An equal number of volunteers were assigned to each sequence. The study covers to determine the pharmacokinetic parameters i.e. C_{max}, T_{max}, AUC, rate constant, V_d, total clearance and T_{1/2} of Co-amoxiclav in local population. The subjects engaged in the study were member of community at large and full-fill all of criteria to be included in the study. This criterion includes healthy males with normal vital signs, blood hematology and chemistry, non-smoker, able to consent and swallow. The study design was endorsed by the National Bioethics Committee, Ministry of Health, Government of Pakistan, Islamabad after critical ethical review and a written informed duly signed by volunteers has been taken. Four volunteers withdraw during study.

2.3. Bioanalytical validation

Plasma amoxicillin and clavulanic acid concentrations were determined using validated methods such as LC/MS/MS analysis (GTF) [8] (**Table 3**). The method was also validated according to International Council for Harmonization (ICH) guidelines (**Figures 2 and 3**).

The fundamental parameters of validation were Specificity, linearity, accuracy, precision, sensitivity, reproducibility, stability and robustness. All these parameters were determined and validated.

Product name	% labeled strength	Product name	% labeled strength
AMCL1	106.1	CODE #1	113.38
AMCL2	119.8	CODE #2	109.9
Mean	112.9		111.64
S.D	9.6		2.46

>> Amoxicillin and clavulanate potassium tablets contain equivalent of not less than 90.0% and not more than 120.0% of the labeled amount of amoxicillin and clavulanic acid (USP 28).

Table 3. HPLC assay of different brands of amoxicillin/clavulanic acid tablet (250/125 mg) available in Pakistan.

Linearity/Accuracy/Precision

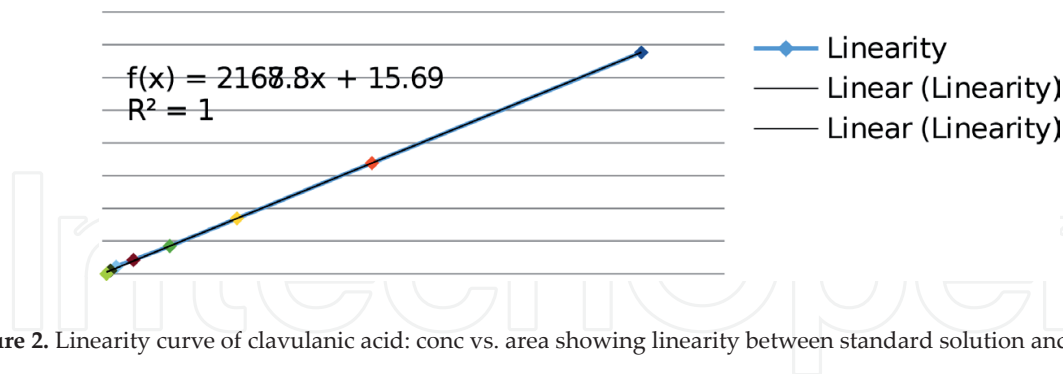


Figure 2. Linearity curve of clavulanic acid: conc vs. area showing linearity between standard solution and peak area.

Mobile Phase containing methanol (10 volume) and 0.02 M disodium hydrogen phosphate buffer (90 volume). The pH was adjusted to 3.0 by phosphoric acid. The mobile phase was filtered and degassed. An HPLC isocratic pump with UV-VIS detector was attached with RP 18e column (Hibar, 250 × 4.6 cm).

In a glass stoppered 15 ml centrifuge tube 0.75 ml of acetonitrile was added to 0.5 ml of plasma. After mixing(30s) the mixture centrifuged for 10 min at 5000 × g. Then 2.5 ml of dichloromethane was added to 300 μ l of supernatant. After mixing (30 s) the mixture centrifuge for 10 min at 5000 × g. Then 20 μ l of supernatant was injected into liquid chromatograph at 235 nm detection wave length.

2.4. Pattern of variable absorption of clavulanic acid from different oral formulations of co-amoxiclav in healthy subjects

There has been no study to compare the difference of blood concentration time curve of different formulations of co-amoxiclav in local population of Pakistan. Therefore author tried to focus on the Pharmacokinetic pattern especially of clavulanic acid. It is due to fact that absorption of clavulanic acid, after oral administration, is highly variable and may vary over a five-fold range between patients [9]. Based on the plasma amoxicillin and clavulanic acid concentrations of individual subjects, were calculated by applying both compartmental and non-compartmental method of analysis. As best fitted pharmacokinetic model one compartmental

Linearity/Accuracy/Precision

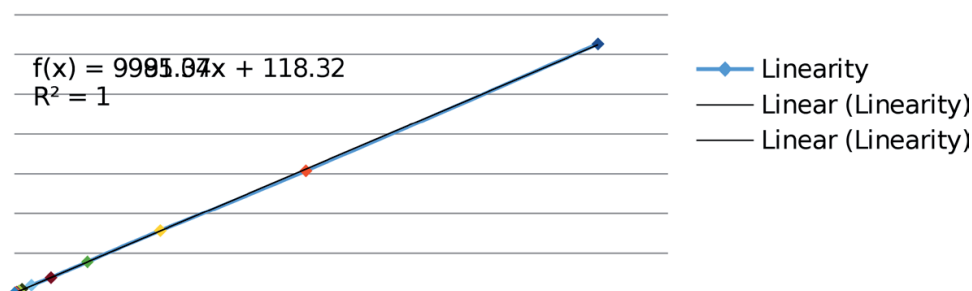


Figure 3. Linearity curve of amoxicillin: conc vs. area showing linearity between standard solution and peak area.

model with lag time, first order absorption and first order elimination was selected for both amoxicillin and clavulanate potassium.

The software Kinetica™ Ver 4.4.1. (Thermo Electron Corporation, USA) used to determine all parameters including both compartmental and non-compartmental analysis and interrelated for any variation in AUCt and demographic facts. The parameters determined were:

C_{max} , T_{max} (observed and calculated), K_a , K_{el} , $T_{1/2\ ka}$, $T_{1/2\ \alpha}$, AUC_{0-t} , $AUC_{0-\alpha}$, λ_z , V_z , V_{ss} , AUC_{last} , $AUC_{extrapolated}$, AUC_{total} , $\%AUC_{extrapolated}$, $AUMC$ and MRT .

where C_{max} is maximum plasma concentration of drug(mg/L), T_{max} is time required to achieve C_{max} (h), K_a is absorption rate constant, AUC_{0-t} , $AUC_{0-\alpha}$, AUC_{last} with the help of linear trapezoidal method to find area under plasma-concentration time curve up to last measurable concentration (mg.h./L), K_{el} is elimination half-life (h), λ_z is terminal rate constant, V_z is apparent volume of distribution during terminal phase (L/kg), V_{ss} is apparent volume of distribution at steady state (L/kg), $AUMC$ is area under the first moment of concentration-time curve from time zero to infinity (amount.(time)²/volume) and MRT is mean residence time (h).

The mean \pm plasma concentration time-curve of co-amoxiclav (250/125 mg) tablet of formulation 1 is shown in **Figure 4** in healthy volunteers (n = 8). The other formulation showed similar results. The half-life of all both formulation was 1.34 ± 0.06 h for amoxicillin and 1.20 ± 0.03 h for clavulanic acid.

The area under the concentration-time curve of clavulanic acid is the best measure of the absorption and beneficial effects in the recipient. Calculating the area under the curve using trough and peak blood levels versus using isolated readings for either of these levels alone is the most effective method of monitoring.

The mean $AUC_{0-\alpha}$ values calculated through compartmental analysis were 26.81 ± 0.70 $\mu\text{g.h/ml}$ for amoxicillin while for clavulanate potassium 7.90 ± 0.13 $\mu\text{g.h/ml}$. The values of mean AUC_{last} and AUC_{tot} from non-compartmental analysis were 23.33 ± 0.70 and 27.96 ± 0.76 $\mu\text{g.h/ml}$ for amoxicillin. The clavulanate potassium showed the values of AUC_{last} and AUC_{tot} were 7.05 ± 0.11 and 7.70 ± 0.16 $\mu\text{g.h/ml}$.

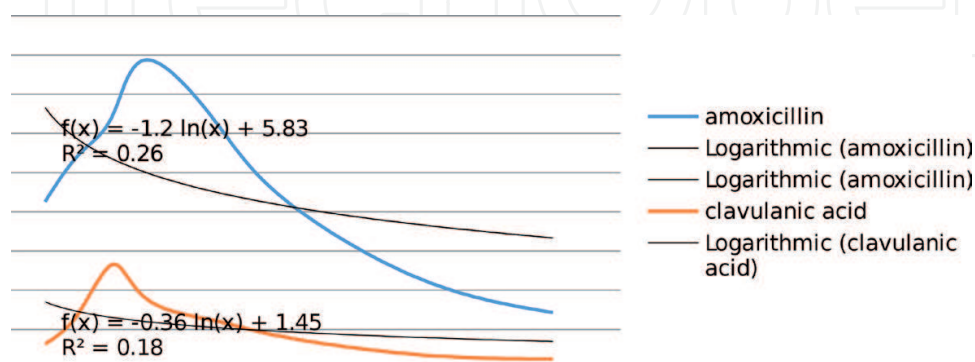


Figure 4. Comparison of mean (\pm S.D) plasma-concentration time profile of amoxicillin and clavulanic acid after oral dose of 250/125 mg co-amoxiclav tablet (n=8: Formulation 1).

The maximum concentration C_{max} of amoxicillin was achieved in 1.85 ± 0.01 h for amoxicillin in compartmental. Similarly C_{max} for clavulanate potassium was achieved in 1.56 ± 0.01 h in compartmental analysis (**Tables 4 and 5**).

The reported values of V_{ss} after IV administration for amoxicillin is 0.28 ± 0.06 L/kg, and the V_{ss} of clavulanic acid as 0.24 ± 0.06 L/kg, showing ratio for the volume of distribution between clavulanic acid and amoxicillin as 0.8571 [14] and therefore on the basis of this the ratio of amoxicillin to clavulanic acid AUCs should be 3.4. when co-amoxiclav is at dose of 250/125. The author observed in this study, the lowest AUCt amoxicillin/clavulanic acid ratio was 2.7 ± 0.50 at the lower doses used. This would assume equal absorption of both amoxicillin and clavulanic acid. But in the same dose amoxicillin/clavulanic acid AUCt ratios was higher that would suggest that with a similar amoxicillin absorption, clavulanic acid absorption must have been reduced. The reported absolute bioavailability of clavulanic acid, when co-administered with amoxicillin has been ranged from 31.4 to 98.8% [10]. Further it is reported that there is no major alteration in the mean AUCt of 125 mg clavulanate when it is administered along 500 mg of amoxicillin, but it creates marked impact on the coefficient of variation for the AUC which alter from 27.6% for clavulanic acid alone to 45.6% when given with amoxicillin [15]. Various other studies showed mean absorption up to 97% when clavulanic acid administered alone with minor inter-patient variability. It indicates interaction between absorption of amoxicillin and clavulanic acid [11, 12]. The author further found that there was no significant variation in the AUC observed for amoxicillin in this study either among subjects, on the basis of demographic data, or between formulations, once corrected for the dose. On the contrary, high variability was seen between subjects in the AUC of clavulanic acid (**Figure 5**). There were all healthy male subjects (with normal renal function), and it is difficult to explain the high variability seen in the clavulanic acid AUC on patient factors. However, it has been reported in other studies [13]. Also study observed broadened T_{max} i.e. increase lag time indicating a rate limiting step in the absorption process. The authors being able to show that two different co-amoxiclav formulations each gave a variation in the absorption, or in the AUCt value, of clavulanic acid for the same 125 mg dose.

In a study reported broadened T_{max} with high dose of amoxicillin (875 mg) indicate a rate limiting step in the absorption and support previous other studies [16].

In this study the authors tried to show variation in the absorption or in AUCt value of clavulanic acid at 125 mg dose with two formulations. Although we did not find any report of therapy failure among the patients due to this variation and its clinical efficacy has been maintained. It would suggest that it is more important to focus on the absolute or fixed amount of clavulanic acid rather than on its plasma concentration.

Formulation	Dose (mg)	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (μ g/L)	AUC (μ g.h/L)
AMCL1	125	1.20 ± 0.02	1.56 ± 0.01	2.60 ± 0.03	8.30 ± 0.06
AMCL2	125	1.21 ± 0.03	1.54 ± 0.02	1.98 ± 0.70	7.90 ± 0.13

Table 4. Pharmacokinetic parameters of clavulanic acid.

Formulation	Dose (mg)	T _{1/2} (h)	T _{max} (h)	C _{max} (µg/L)	AUC (µg.h/L)
AMCL1	250	1.34 ± 0.06	1.85 ± 0.01	2.98 ± 0.27	26.81 ± 0.70
AMCL2	250	1.32 ± 0.05	1.83 ± 0.02	3.3 ± 1.12	26.98 ± 0.83

Table 5. Pharmacokinetic parameters of amoxicillin.

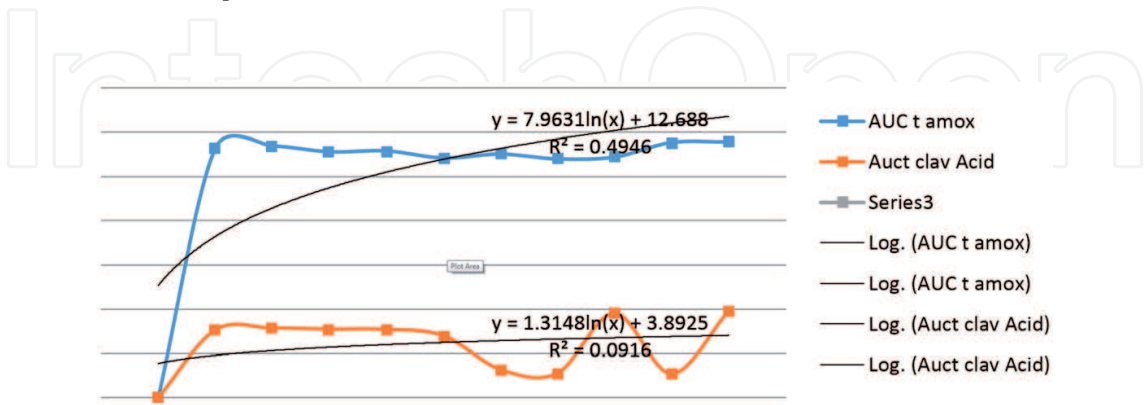


Figure 5. Individual AUCts of amoxicillin plotted versus the AUCts of clavulanic acid. It can be seen that there is a variation in the AUCts of clavulanic acid, with little variation in those of amoxicillin (95% confidence interval).

3. Conclusions

In conclusion, variable absorption nature of clavulanic acid has been highlighted with alteration in AUCt ratio of co-amoxiclav without any known cause. However, it is evident from clinical data that there is not any variability in the efficacy of co-amoxiclav and that the current dosage ratio of 4:1 holds a traditional value.

The study requires further evaluation to find out the reason for this variation.

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Conflict of interest

There is no actual or potential conflict of interest between authors relative to this activity including financial relationship.

Author details

Anab Fatima^{1*}, Mohammad Jiyad Shaikh², Hina Zahid¹, Ishart Younus³,
Sheikh Abdul Khaliq³ and Farah Khalid¹

*Address all correspondence to: anabfatima@gmail.com

1 Faculty of Pharmacy, Dow University of Health Sciences, Karachi, Pakistan

2 Unilever Karachi, Pakistan

3 Faculty of Pharmacy, Hamdard University, Karachi, Pakistan

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