

Effects of Ampiclox and Amoxicillin on the Pharmacokinetics of Metformin in Type II Diabetic Patients

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Abstract: Infection is common in diabetes and in the course of treatment, ampiclox and amoxicillin may be co-administered with metformin. The study was designed to evaluate the effects of co-administration of ampiclox and amoxicillin on the pharmacokinetics of metformin in type II diabetic patients. Twelve patients with age ranging from 25 – 55 years, weight 50 – 70 kg and height 1.5 – 1.75 m participated in the study. The study was divided into two phases with a washout period of seven days between the phases. In phase one, metformin alone was administered to all the subjects with 150 ml of water after an overnight fasting. In phase two, the subjects were divided into two groups, with six subjects in each group. The first group received a single dose of metformin with ampiclox, while the second group received metformin co-administered with Amoxicillin. Blood samples were collected at 0, 0.5, 1.5, 3.0, 4.0, 6.0 and 8.0 hours post drug administration and stored at -4°C before analysis. Plasma was obtained from the blood and the drug was extracted from the plasma using three times its volume of acetonitrile. The samples were analyzed for metformin using a reversed phase HPLC method on a C-8 column (4.6 x 150 nm), mobile phase acetonitrile/potassium dihydrogen orthophosphate (21:79) and a UV detector at 236 nm. When metformin was co-administered with ampiclox, K_a increased to $0.58 \pm 0.04 \text{ hr}^{-1}$, C_{max} to $1.28 \pm 0.35 \mu\text{g/ml}$, while AUC and $t_{1/2\beta}$ increased to $518.71 \pm 0.02 \mu\text{g.h/ml}$ and $6.2 \pm 0.02 \text{ hr}$ respectively. These increments were found to be significant ($p < 0.05$). On the other hand, when metformin was co-administered with amoxicillin no significant changes ($p < 0.05$) were observed.

Keywords: Ampiclox, Amoxicillin, Diabetes, HPLC, Metformin

Date of Submission: 19-01-2018

Date of acceptance: 01-02-2018

I. Introduction

Diabetes mellitus is regarded as a complex disease characterized by a grossly abnormal pattern of full usage or over utilization of glucose by the liver and underutilization by other organs (WHO, 2014). Diabetes is refers to the excessive urination and mellitus is from Latin meaning sweetened with honey, and also refer to the high level of glucose in the urine. Mellitus distinguishes the disease from diabetes insipidus which is caused by impaired renal reabsorption of water (Makaryus and McFarlane, 2013). When blood glucose level falls below the renal threshold (10 mmol/l or 180mg/100ml) glycosuria ceases in diabetes as does the osmotic diuresis of water and electrolytes. Polyuria with dehydration and excessive thirst are thus alleviated. If the blood-glucose falls much below normal levels, appetite is stimulated. Diabetes mellitus is classified by World Health Organization into four main groups: Type I, type II, gestational diabetes and “other types” (Gardner and Shoback, 2011). The disease is characterized with polyuria, nocturia, decreased visual acuity, frequent thirst, dry mouth, vulvularpruritis in women, severe skin infection, impotence and dark-spot due to skin necrosis. Gangrene of the feet and other extremities, coronary artery disease, retinal diseases and parathesis due to neuropathy are complication of diabetes mellitus. Others include urinary tract infection due to favorable media, pulmonary or respiratory diseases due to depressed body immune system, and skin infections with Staphylococcus organisms as a result of skin abrasion (Walker *et al.*, 2014). According to World Health Organization and the American Diabetic Association’s criteria, an adult is considered diabetic if fasting blood sugar measured exceeds 8mmol/l on more than one occasion or if on two occasions, the concentration of glucose exceeds 11mmol/l 30, 60, 90 minutes after ingesting about 75 g glucose and remains about this levels two hours after ingestion (ADA, 2013). Diabetes mellitus is one of the major causes of death in Europe and the United States (Fowler, 2007). From various studies, 5% of the general population of the USA will eventually develop the disease. The prevalence is similar to other countries. About 382,000,000 people of the World population have been reported to be afflicted with diabetes mellitus (Melmed *et al.*, 2012; Shi and Hu, 2014).

II. Materials And Methods

Materials

Digital weighing balance OHAUS model EP 64 BY Ohaus corporation, Switzerland, U.V. detector T80 + U.V/Vis spectrometer by PG instrument Ltd U.K, High Performance Liquid Chromatography; Agilent Technologies, 1120LC series, USA, Centrifuge: Heroes (labafuge300) D-37520 ostence mated: 2003, serial No40267581, BN: 75003230, Methanol: Sigma – Aldrich \geq 99.9% U.K , Mntd: Sept 14, 2011, Acetonitrile: Sigma – Aldrich \geq 99.9%, U.K ,Mntd: Sept 14, 2011, Potassium Dihydrogen phosphate (Buffer) by J.T Baker 99.5% USA, Metformin HCL (reference standard), Sulphadoxine : internal standard –Ranbaxy pharmaceutical Ltd., Lagos, PK Functions or Microsoft Excel software by Joel.

Method

Ethical Clearance For The Study

The ethical clearance for the present study was obtained by the proper representation and discussion of various ethical issues with human ethics committee of Ahmadu Bello University Zaria, Nigeria with reference number of F-MED/COMM/19.

Pharmacokinetic Studies

The criteria for selecting the participants were based on the National Diabetes Data group's recommendation of 1989 and the selection was done by the practicing clinician, none of participants was below the age of 35 years. The approval for the research was granted by the Ethical Committee of Ahmadu Bello University Teaching Hospital Zaria, Nigeria. Twelve patients with age ranging from 25 – 55 years, weight 50 – 70 kg, and height 1.5 – 1.75 m participated in the study. The study was divided into two phases with a washout period of seven days between the phases. In phase one, metformin alone was administered to all the subjects after an overnight fasting. In phase two, the subjects were divided into two groups, with six subjects in each group. The first group received a single dose of metformin with ampiclox, while the second group received metformin co-administered with amoxicillin. Blood samples were collected at 0, 0.5, 1.5, 3.0, 4.0, 6.0 and 8.0 hours post drug administration and stored at -4°C before analysis. The samples were analyzed for metformin using a reversed phase HPLC method on a C-8column (4.6 x 150 nm), mobile phase acetonitrile/potassium dihydrogen orthophosphate (21:79) and a UV detector at 236 nm.

Preparation Of Standard Solution

Stock solution of metformin was made by dissolving 0.1 g of metformin standard powder in 100 ml of methanol to give 1 mg/mL Serial dilutions of working concentrations of 300 - 4000 ng/ml were prepared from the stock. Stock solution of internal standard (Sulfadoxine) was also prepared in a similar manner.

Extraction

The method of extraction of drug free plasma was adopted and modified (Bhavesh *et al.*, 2007). A 100 μL of metformin hydrochloride solution of appropriate concentration and 100 μL of phenformin hydrochloride solution (20 $\mu\text{g}/\text{mL}$) were added to 900 μL of drug free plasma contained in a clean 5 ml Ria vial and was properly mixed. To this 50 μL of protein precipitating agent (perchloric acid: acetonitrile 50 % v/v each) was added and was vortexed for 30 seconds. After centrifugation at 3000 rpm for 10 minutes, 700 μL of the supernatant was evaporated to dryness at 45°C under nitrogen. The residue was reconstituted in 100 μL of mobile phase and 20 μL of this was injected to the HPLC system.

Hplc Conditions

Mobile	:	Acetonitrile	:	0.01M KH_2P_04
		21		79
Pressure	:	120-245 psi		
Column	:	Eclipse X BD C-8 4.6 x150mn		
Flow rate	:	1.50 mL/min.		
Injection volume	:	20 μL		
Wave length	:	236 nm		
pH	:	5.4 (adjusted with phosphoric acid)		
Column	:	ambient temperature		

Chromatogram

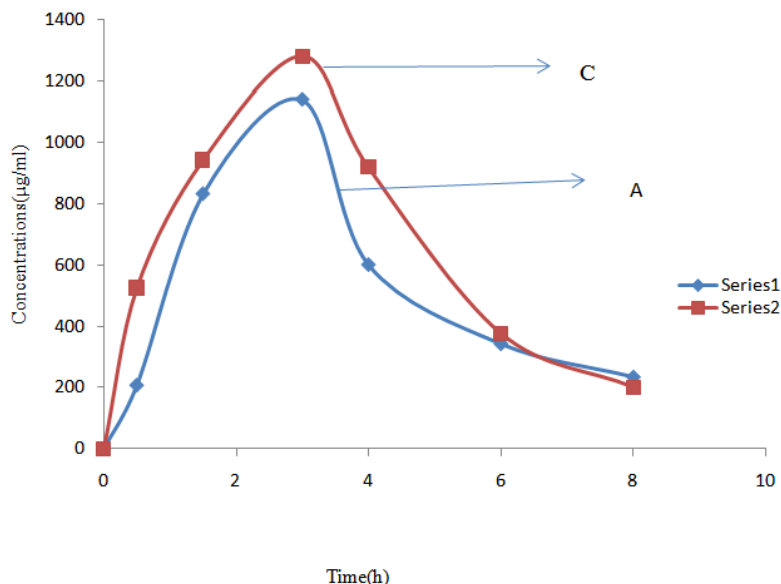


Figure 4: Comparison of plasma concentrations curve ($\mu\text{g/ml}$) of metformin alone (A) and co-administered with ampiclox (C)
 Key: Curve A (Series1) = Metformin, Curve C (Series 2) = Metformin co-administered with ampiclox

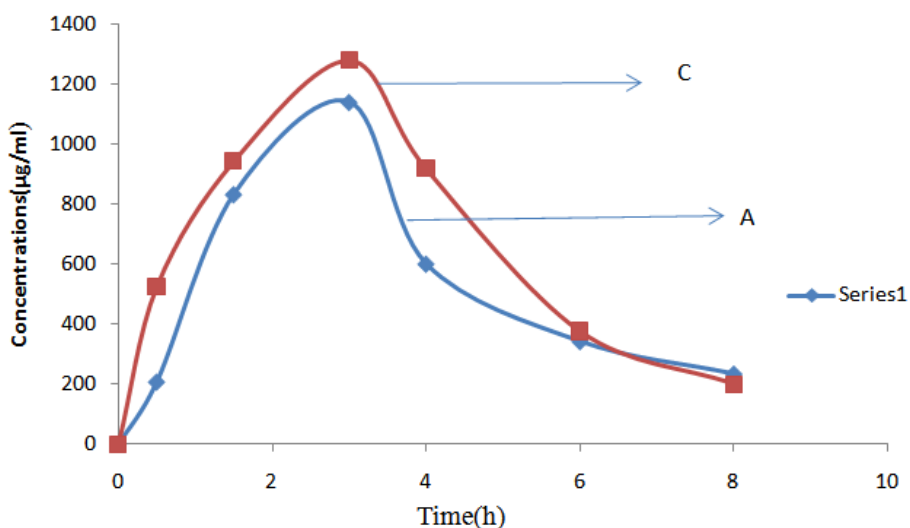


Figure 5: Comparison of plasma concentrations curve ($\mu\text{g/ml}$) of metformin alone (A) and co-administered with amoxicillin (D)
 Key: Curve A (Series1) = Metformin, Curve C (Series 2) = Metformin co-administered with amoxicillin.

Linearity

The linearity of the peak area ratios of metformin to sulphadoxine against their corresponding concentrations was found to be in the range of 0.03 – 4.0 $\mu\text{g/ml}$. The linear regression of equation from the plot is $y = 343.94x + 161.11$; where y is the peak area ratios, x is the concentration, 343.94 is the slope while 161.11 is the intercept and a correlation coefficient (r) of 0.983.

TABLE 4: Pharmacokinetic parameters of Metformin alone and Metformin co-administered with amoxicillin (Mean \pm S.D, N=6)

	Metformin alone	Metformin + Amoxicillin	Paired sample T- test value
$K_e(\text{hr}^{-1})$	0.43 \pm 0.04	0.17 \pm 0.01	S
C_{max} ($\mu\text{g/ml}$)	1.24 \pm 0.52	1104.40 \pm 0.04	NS
AUC_{0-8} (hr $\mu\text{g/ml/hr}$)	4.35 \pm 0.71	4250.25 \pm 0.45	NS
Vd (ml)	334852.19 \pm 0.27	3497352.06 \pm 0.11	NS
CL(ml/hr)	58013.39 \pm 0.41	61196.88 \pm 0.39	NS

$p < 0.05^*$ = Significant(s) $p > 0.05$ = Not significant (NS)

Table 5: Pharmacokinetic parameters of Metformin alone and Metformin co-administered with Ampiclox (Mean \pm S.D., N=6)

	Metformin alone	Metformin + Ampiclox	Paired sample T-TEST
K_a (hr ⁻¹)	0.46 \pm 0.03	0.58 \pm 0.04	S
C_{max} (μ g/ml)	1.14 \pm 0.52	1.28 \pm 0.35	NS
AUC ₀₋₈ (hr g/ml/hr)	4.39 \pm 0.71	5.18 \pm 1.02	NS
Vd (ml)	337852.19 \pm 0.27	303061.43 \pm 0.40	NS

p < 0.05* = Significant(S) p > 0.05 = Not significant (NS)

IV. Discussion

Before the commencement of the in vivo study, metformin tablets, ampiclox capsules were subjected to quality control tests and they all passed in accordance to the standard compedia. The World Health Organization (WHO) have always maintained the rationale for such tests and the necessity for such tests was strongly emphasized after the Bangladesh epidemic a reported case of fatal renal failure caused by propylene glycol in paracetamol elixir. (Hannif *et al.*, 1995). Following the concomitant administration of 1 g of metformin with 1 g ampiclox to type II diabetic patients, the influence of ampiclox capsules on the pharmacokinetics of metformin clearly revealed significant pharmacokinetic changes ($P < 0.05$) (Table 4.9). An increase in the absorption rate constant k_a from 0.46 \pm 0.04 to 0.58 \pm 0.04 h⁻¹ was observed, these changes were found to be significant ($p < 0.05$, using student t-test for paired data) differences in the C_{max} , AUC, V_d , Cl, lag- time while other parameters were not significantly different in their values, ($p > 0.05$). There was an increase in peak plasma concentration (C_{max}) from 1.14 \pm 0.52 to 1.28 \pm 0.35 μ g/ml while area under the curve (AUC) increased from 4.39 \pm 0.71 to 5.18 \pm 0.02 μ g/ml/h they were statistically insignificant at ($P > 0.05$), which is also in agreement with the finding of Paxton, 1989 that the high AUC value of metformin in the presence of ampiclox capsules is most likely responsible for the decreased plasma glucose concentration following treatment with the two drugs. It could be due to the fact that both drugs bound to plasma protein and the same binding sites. Competition for binding sites when both drugs were administered concomitantly may result in displacement of metformin. This may be the most likely reason for high bioavailability of metformin observed which resulted in increased of AUC and C_{max} . Both drugs are tightly bound to plasma protein, and there exist the possibility of common binding sites (Ptalsky, 1980, Hills, 1987). Competition for binding sites when both drugs are concurrently administered may lead to the displacement or metformin from its binding sites. Activity of any drug is usually a function of the fraction of the unbound drug, high level of metformin and thus, an enhanced activity in the presence of ampiclox capsules must have accounted for the significant decrease in plasma glucose level observed following their concomitant administration. Greater percentage of metformin and ampiclox are excreted by the renal – tubular secretion mechanism (Burger and Mitchell 1985). Thus, there is a possibility that both drugs compete for the same renal – tubular secretion. When this happens, there is every tendency that ampicillin/cloxacillin known to be rapidly eliminated from the system will be secreted first in preference to metformin when they are co-administered. By such mechanism, the clearance rate of metformin will be reduced as well as its volume of distribution as shown in Table 4.6. In this study, there was an insignificant decrease ($P > 0.05$) in the volume of distribution (Vd) of metformin from 337852.19 \pm 0.27 to 303061.43 \pm 0.40 ml in the presence of ampiclox capsules which agreed with the result of the study carried out by Bakare *et al.*, (2001) on the influence of ampiclox on the pharmacokinetics of chlorpropamide in type 2 diabetic patients. The observed significant decrease ($p < 0.05$) in clearance from 59013.39 \pm 0.41 to 41028.98 \pm 0.37 ml/hr with a reduction in the volume of distribution when metformin 1 g was co-administered with 1g ampiclox to type II diabetic patients, may be due to the decrease in elimination rate constant (Charles *et al.*, 2009). The result of concomitant administration of a single dose of 1 g of metformin with 1 g amoxicillin capsules to type II diabetic patients, revealed significant changes in the pharmacokinetic of amoxicillin. Absorption rate constant decreased significantly ($p < 0.05$) from 0.46 \pm 0.04 h⁻¹. A statistically significant decrease ($p < 0.05$) in C_{max} of metformin from 1.14 \pm 0.52 to 1.10 \pm 0.04 μ g/ml with also decrease area under the curve from 4.39 \pm 0.71 to 4.25 \pm 0.45 μ g/ml/h when administered concomitantly with amoxicillin was recorded.

V. conclusion

The method of high performance liquid chromatography, in the monitoring of metformin in the plasma was very effective and efficient. The results of the findings indicated pharmacokinetics changes when metformin was administered alone and co-administered with ampiclox and amoxicillin. Potentiation effects on metformin was only was observed with concomitant administration of a single dose of 1 g metformin tablets with 1g ampiclox capsules by increasing the peak plasma concentration of metformin while concomitant administration of 1 g metformin with 1 g of amoxicillin had no significant effect.

Acknowledgements

The authors acknowledged the contributions of Professor (Mrs) Odunola Bakare, Professor M. Garba and Professor Yakasai Adamu, Dr. MA. Usman and Dr. Aminu Musa for their contributions towards this publication. We also thank all the Staff of Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Nigeria particularly Chief Laboratory Technologist, Mallam Iliya and his dedicated staff and the staff of Gambo Sawaba Memorial General Hospital particularly Dr. Ado Zakari and his wife Dr. Maryam for counseling the patients used in the study. I must also acknowledge the immense contribution of the Ethical Committee of Ahmadu Bello University Teaching Hospital for the approval of this study.

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Garba, M.A., "Effects of Ampiclox And Amoxicillin on The Pharmacokinetics of Metformin In Type II Diabetic Patients." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)* 13.1 (2018): 39-44.