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EFFECTS OF AMOXICILLIN AND CLOXACILLIN ON THE PHARMACOKINETIC PROFILE OF METFORMIN

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ABSTRACT

Metformin is prescribed with amoxicillin /cloxacillin in the treatment of diabetic disease with infections. However, the effects of these drugs on the pharmacokinetic profile of metformin are still unknown. The study aimed at investigating the pharmacokinetic interactions of amoxicillin and cloxacillin when co-administered with metformin. It is a one-way single dose cross-over study in two phases. The subjects acted as their own control, and each phase was preceded by an overnight fast**.** In phase one, metformin was administered to all the diabetic patients, while phase two was divided into two groups. The first group received a single dose of metformin with amoxicillin while the second group received metformin with cloxacillin. Serial blood samples were collected over a period of 24 h during each phase into an ethylene diaminetetra acetic acid (EDTA) vacutainer and stored at -4 ˚C before analysis. After collection, blood samples were processed. A validated high-performance liquid chromatography (HPLC) method was used in the estimation of serum concentration of metformin. The pharmacokinetic of metformin when co- administered with amoxicillin C_{max} (µg/ml) and AUC_{0-8} (h µgml/h) decreased to 1.104.40 ± 0.04 and 4.25 ± 0.45 respectively. On the other hand, with cloxacillin K_a 0.58 ± 0.04 h^{-1}, C_{max} to 1.28 ±0.35 µg/ml, AUC and $t_{1/2\beta}$ increased to 5.18 ± 0.02 µg.h/m.h, and 6.2 ± 0.02 h respectively. Our findings showed that metformin may be co-administered with amoxicillin to Type 2 diabetic patients without risk of side effects, while co-administration of cloxacillin with metformin need adjustment of the dose to avoid the possible risk of toxicity.

KEYWORDS: **Bioavailability; Drug-drug interactions; HPLC; Toxicity; Therapeutic failure.**

INTRODUCTION

The term diabetes mellitus describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action World Health Organization [1]. Diabetes mellitus has been classified by WHO into four main groups: type I, type II, gestational diabetes and "other types" [2]. The disease is characterized by polyuria, nocturia, decreased visual acuity, frequent thirst, dry mouth, valvular pruritus 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 are complications 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 [3]. Currently, diabetic mellitus is a great threat to the world community with more than 100 million persons suffering from diabetes. According to World Health Organization (WHO) and the American Diabetic Association's (ADA) criteria, an adult is considered diabetic if fasting blood sugar measured exceeds 8mmol/l on more than one occasion or if on two

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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 [4]. Diabetes mellitus is one of the major causes of death in Europe and the United States [5]. From various studies, 5% of the general population of the USA will eventually develop the disease.

The prevalence of diabetes has steadily increased for the past 3 decades and is growing most rapidly in low and middle-income countries [6, 7]. About 1 in 11 adults worldwide have diabetes mellitus, 90% of whom have type 2 diabetes mellitus [8-9]. More than 16 million people were estimated to have diabetes in the AFRO region and the figure is expected to increase to 41 million to by the year 2045 [10]. Polypharmacy is common in clinical practice [11-13]. The chronic treatment with one drug may be supplemented by a further short-term treatment with a second drug, or several drugs may be co-administered on a long-term basis [11, 12]. It is therefore important to consider the possibility of drug interactions [6, 14-16].

Drug interactions occur when two or more compounds are administered simultaneously and one drug affects the pharmacokinetics or the pharmacological activity of the other. These may be complex and influenced by many factors [17, 18]. Among the various hypoglycaemic agents, metformin has been widely used in clinical practice [19 - 22]. Metformin has the advantage of less likely to experienced secondary failure [23, 24], owing to their well-established efficacy and safety profile. It is a drug of choice for the treatment of type 2 diabetes mellitus in the elderly [25, 26], children and adolescents [27].

Many high performance chromatographic (HPLC) methods for the analysis of metformin in plasma were reported but most of the methods used were either ion pair reagent or cation exchange column [28, 29], but some methods reported require elaborate sample preparation [15, 30].

To the best of our knowledge, there is very little work done on possible interactions of metformin with amoxicillin / cloxacillin. This study, therefore, is aimed at investigating the effect of amoxicillin and colxacillin on the pharmacokinetic profile of metformin in freshly diagnosed diabetic volunteers

MATERIALS AND METHOD

Subjects and ethical clearance

The subjects were diagnosed of diabetes mellitus at the Medical Outpatient Department Gambo Sawaba General Hospital Zaria, Kaduna State Nigeria. For the purposes of this study, diagnosis of diabetes mellitus was made by the presence of classic symptoms of hyperglycemia and fasting plasma glucose concentration ≥ 130 mg/dL. The ethical clearance for the present study was obtained by the proper representation and discussion of various ethical issues with the human ethics committee of Ahmadu Bello University Zaria, Nigeria with the reference number of FMED/COMM/19. All volunteers gave their written informed consent, which was documented and archived.

Inclusion/exclusion criteria

At baseline, a structured questionnaire was completed for each volunteer that included medical history, prior hospital admissions, and clinical and laboratory data. For inclusion, volunteers for the study are patients freshly diagnosed and were on lifestyle modification, willingness to fill an informed consent form, nonsmokers, non-alcohol drinking, and willingness to abstain from heavy exercise. They were not on other medications and caffeine during the study, and have a Body Mass Index (BMI) of less than 30 kg/m^2. Pregnancy was excluded, volunteers were not undergoing any medication or planned treatment during the study period.

Study design and blood sampling

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. Twelve freshly diagnosed diabetic patients with age ranging from 29.0 ± 4.9 years, weight 66.1 ± 10.5 kg, and height 162.8 ± 10.6 cm participated in the study.

The protocol adopted was a one-way single dose cross-over study in two periods. Each phase was preceded by an overnight fast. The subjects act as their own control. The study was divided into two phases with a washout period of one week between the phases. In phase one, metformin (1 g) alone was administered to all the subjects after overnight fasting. In phase two, the subjects were divided into two groups, with six subjects in each group. Group one received a single dose of metformin with amoxicillin (1 g) with 150 ml of water [1, 4 &31], while group 2 received metformin co-administered with cloxacillin (1 g) in the same manner. Blood samples were collected at different time intervals of 0, 0.5, 1.5, 3.0, 4.0, 6.0, 8.0,12, 16 and 24 h post drug administration and stored in an EDTA vacutainer at – 4 °C before analysis. The concentration of

Metformin hydrochloride was estimated by injecting 20 µL of deproteinized supernatant liquid into the HPLC 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.

Blood sample processing

After collection, blood samples centrifuged at 3000 rpm and plasma kept frozen in a freezer maintained at -4 °C prior to analysis. The extraction method used for thus study was adopted and modified from [32]. 100 µl of metformin hydrochloride solution of appropriate concentration and 100µl of sulfadoxine solution (20 µg ml^{-1}) were added to 900 µl of drug free plasma contained in a clean 5 ml Ria Vial and were properly mixed. To this 50 µl of protein precipitating agent (perchloric acid: acetonitrile 50% v/v each) was added and was vortex for 30 seconds. After centrifugation at 3000 rpm for 10 minutes, 700 µl of the supernatant was evaporated to dryness at 45°C. The residue was reconstituted in 100 µl of mobile phase and 20 µl of this was injected in to the HPLC system.

Determination of plasma metformin concentration

A validated high performance liquid chromatography (HPLC) method [16] was used in the estimation serum metformin concentration using a HPLC instrument (Agilent Technologies 1120 compact model LC Series, USA) The data was validated for range, accuracy, repeatability, intermediate precision, coefficient correlation, sensitivity and system suitability parameters were calculated. The system used (Agilent Technologies 1120 compact model LC Series USA) consist of pump type L-7100 with U.V absorbance detector (VWD) part NO-G1314 and with thermostat column at ambient temperature with auto sampler, and automatic injection system of 10-20µl volume with different column of each test drug, prominence with the following accessories: SIL-20AC auto-sampler; DGU-20A3 degasser; SPDM20A UV-diode array detector; 5 µm VP-ODS Eclipse X BD C-8 4.6 x150mn column oven, CBM-20Alite system controller and Windows LC solution software. The chromatographic conditions were made up of a mobile phase: solvent A: 0.01M KH_2P04 (pH 5.4) 79 %; solvent B: acetonitrile 21%; mode: isocratic; flow rate 1.5 ml/min; injection volume 20 µl detection UV 236 nm column oven temperature was ambient. Sulphadoxine was used as an internal standard.

Pharmacokinetic parameters and statistical analysis

The pharmacokinetic parameters were determined for the two phases of the study. The highest plasma concentration observed and the corresponding time was defined as the C_{max} and T_{max} values, respectively. The elimination rate constant (K_e) was obtained by linear regression from the best-fit slope of the terminal log-linear decay in plasma concentrations versus time profile. The half-life ($t_{1/2}$) was obtained as $0.693/K_e$. The area under the plasma concentration curve to the last quantifiable concentration (Ct) at time t (AUC_{0-t}) was determined by linear trapezoidal integration. The AUC extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t} + Ct/K_e$ [25]. Pharmacokinetic parameters such as maximum plasma concentration (C_{max}), time to maximum plasma concentration (t_{max}), total body clearance (Cl), volume of distribution (VD), area under the curve from zero hours to last measurable concentration (AUC_{0-t}), Area under the curve (from zero hours to infinity ($AUC_{0-\infty}$), area under the moment curve from zero were generated with the aid of the Software – Pharm PK software [6,33,34]. Data were expressed as mean ± SEM. Graph Pad Prism Version 7.02 software for Windows (San Diego California, USA) was used for data analysis using Wilcoxon (matched-pairs) signed rank test with $p<0.05$ considered significant as shown in (Tables 2 & 3). 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 µg/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. Coefficient of variation and a correlation coefficient (r) of 0.983. The results showed a good response of the detector at the concentration used.

Precision and accuracy

Serial dilutions were prepared (500, 1000 and 4000 ng/mL concentrations) from the stock solution for intraday variations. The same concentrations were run 6 times in the morning and afternoon of the same day while the same concentrations were run 6 times a day after to get the inter-day variations. The standard deviations of peak area ratio obtained were

calculated followed by the coefficient of variation in % (Table1).

RESULTS

Twelve freshly diagnosed diabetic subjects (4 males and 8 females) on dietary and lifestyle modification met the inclusion criteria. Their mean age (years) were 55.0±5.7, and mean Body Mass Index (BMI) of 24.8 ±1.9 are shown in Table 1. All subjects were included in the pharmacokinetic analysis. The accuracy, precision, percentage recovery, as well as detection and quantitation limits for the developed method are shown in (Tables 2 & 3). Satisfactory percentage recovery, detection and quantitation limits were achieved for metformin in the adopted method. Table 4 showed the mean pharmacokinetic parameters generated after a single oral dose of 1 g of metformin alone and when co administered with 1 g of cloxacillin to the volunteers. Table 5 showed the mean pharmacokinetic parameters generated after a single oral dose of 1 g of metformin alone and when co administered with 1 g of amoxicillin to the volunteers.

DISCUSSION

Polypharmacy is common in diabetic patients [35]. Despite this growing phenomenon, the influence of diabetes on drug metabolism in polypharmacy has not been fully investigated [36]. This study evaluated the effect of 500 mg amoxicillin / 500 mg cloxacillin co-administered with 500 mg metformin in freshly diagnosed diabetes subjects which were not yet placed on treatment. This is to establish the need for concomitant drug intake during the course of the study. As shown in the result, all the pharmacokinetic parameters generated, the interactions of amoxicillin and cloxacillin in the pharmacokinetic profile of metformin revealed significant pharmacokinetic changes ($P < 0.05$). An increase in the absorption rate constant k_a from 0.46±0.04 to 0.58±0.04 h^{-1} was observed when co-administered with cloxacillin these changes were found to be significant ($p < 0.05$, using student's t-test for paired data). Differences in the $C_{max,}$ AUC, Vd,, Cl, lag- time were found to be significant, while other parameters were not significantly different in their values. There was an increase in peak plasma concentration on co-administration of metformin with cloxacillin (C_{max}) from 1.14 ± 0.52 to 1.28 ±0.35 µg/mL while area under the curve (AUC) increased from 4.39 ± 0.71 to 5.18 ±0.02 µg/mL/h which was statistically insignificant at ($P>0.05$). This is in agreement with the finding of [37-38], that the high AUC value of metformin in the presence of cloxacillin capsules is most likely responsible for the decreased plasma glucose concentration following treatment with the two drugs [33]. It could be because of both drugs bound to plasma protein at the same binding sites. Competition for binding sites when both drugs were administered concomitantly may result in the displacement of metformin [39]. This may be the most likely reason for the high bioavailability of metformin observed which resulted in an increased of AUC and C_{max}. Competition for binding sites when both drugs are concurrently administered may lead to the displacement of metformin from its binding sites. In this study, there was an insignificant decrease ($P > 0.05$) in the volume of distribution (Vd) of metformin from 337852.19 ± 0.27 to 303061.43 ± 0.40 ml in the presence of cloxacillin capsules which agreed with the result of the study carried out by[40] on the influence of cloxacillin on the pharmacokinetics of chlorpropamide in type 2 diabetic patients. The observed significant decrease ($p<0.05$) in clearance from 59013.39 ± 0.41 to 41028.98 ± 0.37 mL/h with a reduction in the volume of distribution when metformin 1 g was co-administered with 500 mg cloxacillin to type 2 diabetic patients, maybe due to the decrease in elimination rate constant [41].The result of concomitant administration of a single dose of 1 g of metformin with 1 g amoxicillin capsules to type 2 diabetic patients, revealed changes in the pharmacokinetics of amoxicillin. Absorption rate constant decreased significantly ($p<0.05$) from 0.46 ± 0.04 to 0.19 ±0.1 h^{-1}. A statistically significant decrease ($p<0.05$) in C_{max} of metformin from 1.14±0.52 to 1.104 ± 0.04 µg/ml accompanied by decrease area under the curve from 4.39 ±0.71 to 4.25 ± 0.45 µg/ml/h when administered concomitantly with amoxicillin was recorded.

CONCLUSIONS

The foregoing showed that the amoxicillin and cloxacillin tablets may have influenced the rate of absorption of metformin, it does not affect the bioavailability and overall disposition of metformin after a single oral dose the pharmacokinetics of metformin. The findings also indicated that type 2 diabetic patients on metformin who may require cloxacillin may require adjustment of dose regimen to avoid the possible risk of toxicity or therapeutic failure but on the other hand, metformin may be co-administered with amoxicillin without any possible risk of toxicity or therapeutic failure.

Table 1: Demographic characteristics of freshly diagnosed diabetic volunteered subjects

Characteristics	Sample Size (n=26)
Men	60.0%
Women	40%
Age (years)	29.0 ± 4.9
Weight (kg)	66.1 ± 10.5
Waist (cm)	78.6 ± 8.0
Hip (cm)	91.5 ± 6.1
Height (cm)	162.8 ± 10.6
BMI (Kg/m^2)	2.4±1.9

Table 2: Intra and inter-day precision

Sample	Concentration	C.V %	N (ng/mL)
Intra-day run			
(Metformin)	500	3.4±0.58	6
	1000	2.8±0.89	6
	4000	1.2±0.68	6
Inter- day run			
(Metformin)	500	4.2±0.34	6
	1000	3.1±0.42	6
	4000	2.3±0.03	6

CV = Coefficient of Variation, n = Number of samples

Table 3: Percentage recovery of metformin

Sample	Concentration (ng/mL)	Recovery % ± S.D	N
Metformin	20 0.00	96.52± 6.7	6
	400.0	98.43± 7.0	6

SD= Standard Deviation.

Table 4: Pharmacokinetic parameters of Metformin alone and metformin co-administered with cloxacillin (Mean ±S.D, N=6)

	Metformin alone	Metformin plus cloxacillin	Paired sample T-test value
$t_{1/2\alpha}$ (h)	1.5±0.03	1.2±0.05	S
$K_a(h^{-1})$	0.46±0.03	0.58±0.04	S
C_{max} (µg/ml)	1.14±0.52	1.28±0.35	NS
AUC_{0-8}(h µg/m l/h)	337,852.19±0.27	303,061.43±0.40	NS
CL(ml/hr)	59013.39±0.41	41028.98±0.37	S
Vd (ml)	337,852.19±0.27	303,061.43±0.40	NS
$t_{1/2\beta}(h\text{-}_1)$	3.8±0.07	6.2 ± 0.03	S
$Ke(h^{-1})$	0.18±0.12	0.14±0.01	S

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

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

	Metformin alone	Metformin+ amoxicillin	Paired sample T-test Value
Ke(h-1)	0.18 ± 0.12	0.19 ± 0.01	S
$t_{1/2\alpha}$(h)	1.5±0.02	0.75±0.02	S
$K_a(h^{-1})$	0.46±0.04	0.19±0.01	S
C_{max} (µg/ml)	1.114±0.52	1.104.40±0.04	NS
T_{max}(min)	3.0±0.19	3.0±0.19	NS
AUC_{0-8}(h µg ml/h)	4.39±0.71	4.25±0.45	NS
Vd (ml)	337852.19±0.27	3497352.06±0.11	NS
CL(ml/h)	59013.39±0.41	62196.88±0. 39	NS
$t_{1/2\beta}$(h)	3.80±0.07	3.70 ± 0.02	S

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

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