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CEPHALEXIN EFFECT ON METFORMIN PHARMACOKINETIC PARAMETERSIN FRESHLY DIAGNOSED PATIENTS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Background: Several drugs were often used to obtain a therapeutic objective or to treat a coexisting disease. The use of multiple drugs concomitantly can give rise to drug-drug interactions could diminish therapeutic efficacy or increase the toxicity of one or more of the administered drugs.

Aim: To determine the effects of co-administration of cephalexin on the pharmacokinetics of metformin in freshly diagnosed type 2 diabetic patients.

Methods: Twelve patients with age 25-55 years, weight range 50-70 kg, and height 1.5 -1.75 m took part in the study. A 2 x 2 double-blind randomized cross over study was conducted for the study. Each of the patients received orally either 1 g of metformin with placebo or a combination of 1 g of metformin and 500 mg of cephalexin. Blood samples were collected at an interval of 0, 0.5, 1.5, 3.0, 4.0, 6.0, and 8.0 hours and stored at -4 ^{0}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 an adopted and validated HPLC method on a reversed phase column C-8, 4.6 x 150 nm, mobile phase acetonitrile/potassium dihydrogen orthophosphate (21:79), and a UV detector at 236 nm.

Results: The systemic disposition of metformin was altered by the co-administration of cephalexin, Cephalexin increased C_{max} and AUC by an average of 54% and 19%, respectively, and reduced renal clearance by 17%. The renal clearance of metformin was reduced in a time dependent manner in the presence of *cephalexin.*

Conclusion: It was concluded that co-administration of cephalexin and metformin showed interaction that may lead to adverse effect.

Keywords: Metformin, Cephalexin, Pharmacokinetics, drug interaction, HPLC.

INTRODUCTION

Diabetes mellitus (DM) is a disorder of carbohydrate, protein, and fat metabolism due to decreased insulin secretion, insulin receptor sensitivity, or both and can be identified by hyperglycemia in the patient [1]. In 2015. The International Diabetes Federation rated Indonesia as the country with the seventh greatest number of people with diabetes aged 20–79 years, with 10 million people with the disease. By 2040, the number of people with diabetes in Indonesia is projected rise to 16.3 million. Furthermore,

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90–95% of all cases of diabetes are type 2 DM [2].

Metformin is primarily used for the treatment of type 2 diabetes mellitus, particularly in obese patients. Metformin has been shown to reduce diabetes mortality and complications by thirty percent compared to insulin, glibenclamide and chlorpropamide [3]. Metformin reduces serum glucose level by several different mechanisms, notably through nonpancreatic mechanisms without increasing insulin secretion. It increases the effects of insulin; hence, it is termed "insulin sensitizer". Metformin also suppresses the endogenous glucose production by the liver, which is mainly due to a reduction in the rate of gluconeogenesis and a small effect on glycogenolysis. Moreover, metformin activates the enzyme adenosine monophosphate kinase (AMPK) resulting in the inhibition of key enzymes involved in gluconeogenesis and glycogen synthesis in the liver while stimulating insulin signaling and glucose transport in muscles. AMPK regulates the cellular and organ metabolism and any decrease in hepatic energy, leads to the activation of AMPK. This study to an extent has put forth to explain the mechanism of metformin action on liver gluconeogenesis [4-5].

Furthermore, metformin increases the peripheral glucose disposal that arises largely through increased non-oxidative glucose disposal into skeletal muscle. It usually does not cause hypoglycemia and this cause to be considered as a unique anti-diabetic drug.[6]

Treatment of diabetes with metformin is associated with less weight gain compared with insulin and sulfonylureas. Weight gain helps in better glucose control. In a study it was shown that, over a 10-year treatment period, the patients treated with metformin gained about one kg, the patients treated with glibenclamide gained about three kg, and the patients treated with the insulin gained six kg, where weight. [7].

Cephalexin is primarily indicated in respiratory tract infections, otitis, skin and skin structure infections. It is well absorbed from the gastro-intestinal tract, and absorption may be delayed by food, but the amount absorbed is not affected [8-9].

There were reported cases of drug interaction between amodiaquine and gliclazide, in which amodiaquine affects the rate of absorption of gliclazide, it does not affect the bioavailability and overall disposition of gliclazide after a single oral dose [9], also the interaction of ciprofloxacin and metformin was reported same as metformin increased in K_a, AUC and $t_{1/2\beta}$.These increments were found to be significant ($p < 0.05$) [10]. These aforementioned interactions conclusively indicated that, they could be concurrently administered together with our fear of loss activity or risk of toxicity [9-13]. *The study aimed to determine the* cephalexin effect on metformin pharmacokinetic parameters in freshly diagnosed patients using high performance liquid chromatography.

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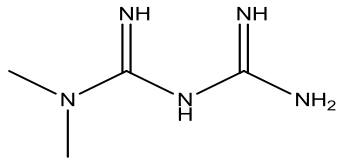

HCl

Figure 1: Molecular Structure of Metformin hydrochloride

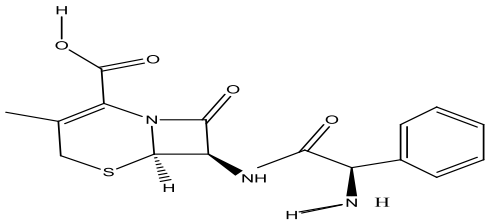

Figure 2: Molecular Structure of cephalexin

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 ≥ 99.9% U.K , Mntd: Sept 14, 2011, Acetonitrile: Sigma – Aldrich ≥ 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.

METHODOLOGY

Subjects and ethical clearance

The subjects were diagnosed with diabetes mellitus at the Medical Outpatient Department of Yusuf Dantsoho General Hospital, Tudun- wada, Kaduna, Kaduna State, Nigeria. In this study, the diagnosis of diabetes mellitus was made by the presence of classic symptoms of hyperglycemia and a 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.

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 made by the practising

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clinicians. Twelve freshly diagnosed diabetic patients with age ranging from 25-55 years, weight range 50-70 kg, and height 1.5 -1.75 m took part 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 control. The study was divided into two phases, with a washout period of one week between the phases. In phase one, metformin (1 g) was administered to all the subjects after overnight fasting. In phase one, subjects received a single dose of metformin (1 g) with 150 ml of water [5, 14, 15], while in phase two, subjects received metformin co-administered with cephalexin (500 mg) in the same manner. 3 ml of the blood samples were collected at different time intervals of 0, 0.5, 1.5, 3.0, 4.0, 6.0, 8.0, 12 h, 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.

Sample Extraction

The extraction method used for this study was adopted and modified from [16]. A 100µl solution of metformin hydrochloride and a 100µl solution of sulfadoxine (20 g ml^{-1}) were added to 900 µl of drug-free plasma in a clean 5 ml Vial and thoroughly mixed. This was mixed with 50µl of protein precipitating agent (perchloric acid: acetonitrile 50% v/v) and vortexed 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 into the HPLC system.

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Table 1: HPLC Chromatographic Condition

Mobile phase	Acetonitrile: 21	0.01M KH_2P0_4 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	
Run time	7.2 min	
pH	5.4 (adjusted with phosphoric acid)	
Column Temperature	Ambient temperature	
Chromatogram	Metformin	Sulphadoxine
Retention time(min)s	1.06 min	2.25min

Precision and Accuracy

The precision of the method was determined by selecting 200 ng/ml, 500 ng/ml, and 1000 ng/ml concentrations from prepared serial dilutions and were used to determine within-day and day-to-day variations. For within day variation, three concentrations were run six times in the morning and afternoon of the same day. The same concentrations were run 6 times a day after that to get the inter-day variations. The standard deviations of the Peak Area Ratio obtained were calculated, followed by the coefficient of variation in percentage.

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 were 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 (AUC0-t) was determined by linear trapezoidal integration. The AUC extrapolated to infinity (AUC0-∞) was calculated as AUC0-t + Ct/Ke Pharmacokinetic parameters such as maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (T_{max}), total body clearance (Cl), volume of distribution (VD), area under the curve from

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zero hours to last measurable concentration (AUC0-t), area under the curve from zero hours to infinity (AUC0-∞), and area under the moment curve from zero were generated with the aid of the Software-Pharm PK software[17-19].The data were presented as mean SEM. Graph Pad Prism Version 7.02 software for Windows (San Diego, California, USA) was used for data analysis using the Wilcoxon (matched-pairs) signed rank test with $p<0.05$ considered significant as shown in (Table 1). 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 equation from the plot is y = 343.94x + 161.11; where y is the peak area ratio, x is the concentration, 343.94 is the slope and 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.

RESULTS

The chromatogram of metformin and sulphadoxine and the mean serum concentrations of metformin in the presence or absence of cephalexin are shown in Figure 3 and 4. The mean pharmacokinetic parameters of metformin when given alone and upon coadministration with cephalexin are shown in Table 1. All subjects tolerated both treatments well, with no side effects. However, significant changes in AUC, C_{max} and clearance were found in the presence of cephalexin. The comparison of the mean sugar level in the group treated with metformin alone and metformin when co-administered with cephalexin in Tab. 2. The intra- and inter-day assay variation and percent recovery of metformin are in Tab. 3 and 4 respectively.

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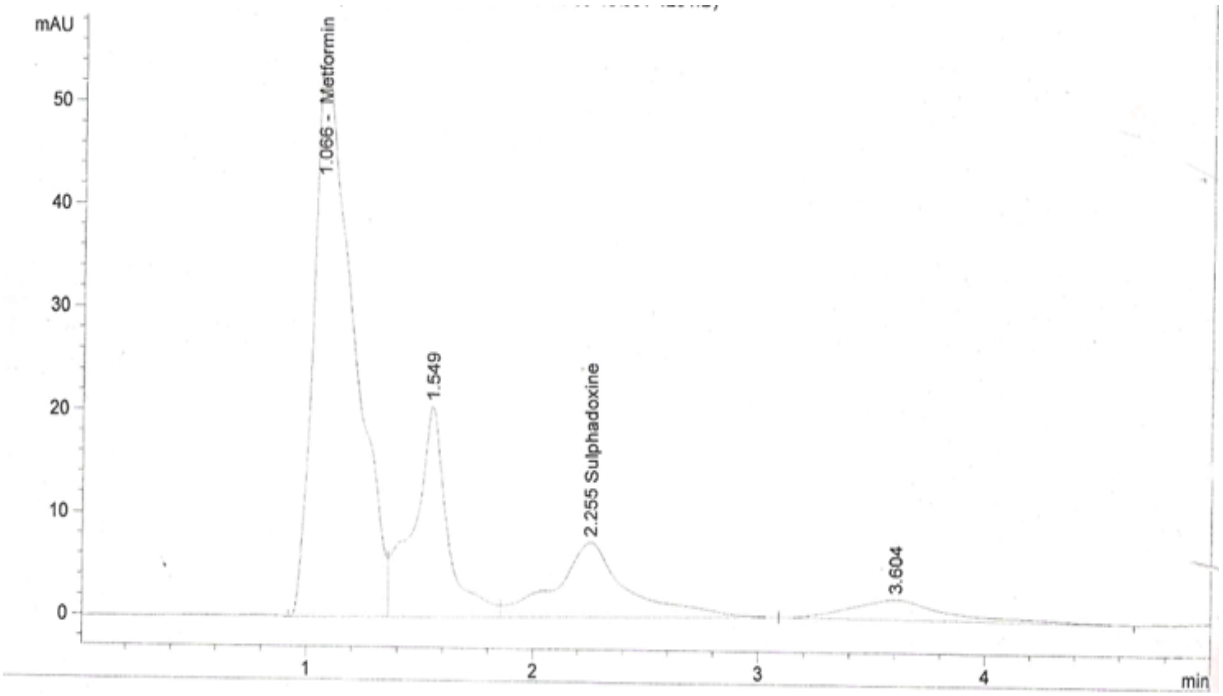

Figure 3: Chromatogram of Metformin and Sulphadoxine

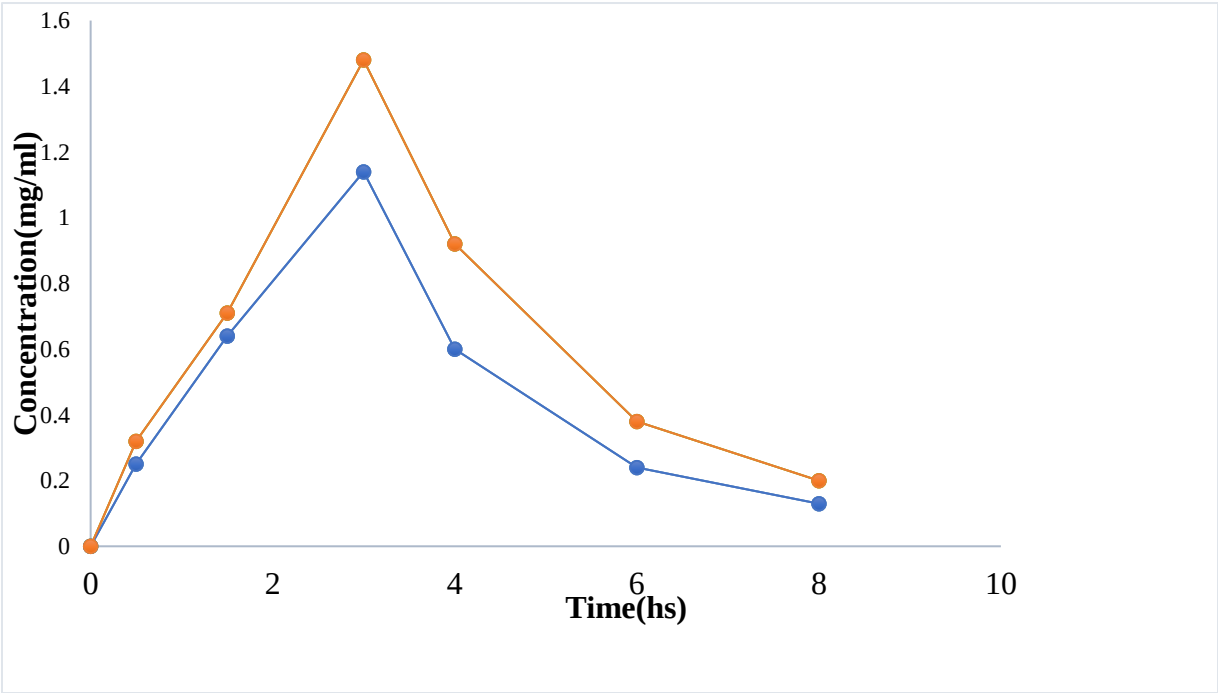

Fig. 4: Mean plasma concentration vs time profiles after metformin alone (M) and metformin with cephalexin (M+C)

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Table 1: Comparison of Pharmacokinetic Parameters of Metformin alone and Metformin Co-administered with Cephalexin in Healthy Volunteers

	Metformin alone	Metformin +Cephalexin
C_{max} (µg/ml)	1.16±0.52	1.79±0.35*
T_{max}(hr)	3.0±0.19	3.0±0.19
$AUC_{0,\alpha}$(h µg/ml/h)	4.39±0.71	5.72±0.80*
$T_{1/2\alpha}$(h)	1.5±0.03	1.2±0.05*
CL(ml/hr)	61,013.34±0.41	52,038.12±0.37*
Ke(h^{-1})	0.18±0.12	0.15±0.02
Vd(ml)	337852.19±0.27	303061.43±0.40

*Significant difference, (P< 0.05)

Table 2: Comparison of the Mean Sugar Level Treated with Metformin alone and when co Administered with Cephalexin

Time (h)	Metformin alone (mmol/l(mean)	Metformin +cephalexin (mmol/l) (mean)
0	6.8 ± 0.02	3.8 ± 0 .14*
2	7.6 ±0.32	7.2 ±0.8*
3	6.1 ±0.25	5.4 ±0.2*
6	8.0 ±0.47	4.2 ±0.3
8	8.2 ±0.26	3±0.2

*Significant difference, (P< 0.05)

Table 3: Percentage Recovery of Metformin

Sample	Concentration (ng/ml)	Recovery % ± S.D	N
Metformin	200	96.52 ±6.7	6
	400	98.43 ±7.0	6

Table 4: Intra and Inter-day Assay Variation of Metformin

Sample	Concentration(ng/ml)	Recovery % ± C.V %	N
Within day run			
Metformin	100	3.4	6
	400	2,8	6
	1000	1.2	6
Between day run			
	100	4.2	6
	400	3.1	6
	1000	2.3	6

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DISCUSSION

In the present study, it was shown that 500 mg of cephalexin oral dose affects the pharmacokinetics of metformin. $C_{max,}$ AUCs and CL significantly increased ($P<0.05$) but the changes in other pharmacokinetic parameters were statistically insignificant. This observation is in agreement with what has been reported [20]. It was reported that pharmacokinetic drug interactions among medications used to treat diabetes are not very common because antidiabetic agents are generally not substrates, inducers, or inhibitors of the major CYP450 enzymes.

Previous reports suggested that high doses of metformin in patients with declining renal function were a risk factor for metformin associated lactic acidosis [4]. Some cationic drugs, such as vancomycin and procainamide also reduce the excretion of metformin through the organic cationic transporter system of the renal proximal tubules [21]. There was a report of an interaction between metformin (a cationic drug) and cephalexin (a zwitterionic drug) that resulted in the elevation of serum concentrations of metformin by reducing tubular secretion [22]. Hence, the co-administration of cephalexin with metformin is considered a risk factor, especially during chronic administration. It is recommended that patients who are prescribed these two drugs concomitantly should have serum metformin levels monitored or an alternative to cephalexin should be considered. In conclusion, cephalexin reduces the renal clearance of metformin by inhibiting tubular secretion via the organic cationic system. The high AUC value of metformin in the presence of cephalexin is most likely responsible for the decreased plasma glucose concentration (Table 1), following treatment with the two drugs [23].

The systemic disposition of metformin was altered by the co-administration of cephalexin, Cephalexin increased C_{max} and AUC by an average of 54% and 19%, respectively, and reduced renal clearance by 17%. This could be due to inhibition of renal tabular secretion of metformin, which resulted in a high circulation of metformin concentration [24-25]. This resulted in elevated blood metformin concentrations, which in turn may lead to adverse effects upon repeated administration.

The mean postprandial glucose level increased significantly at 2 hrs ($P < 0.05$) but was reduced at 3 hrs, 5 hrs and 8 hrs with the administration of metformin alone and in combination with cephalexin. The increment observed in glucose level could be due to an increase in sugar level as a result of food taken. Table 2 shows there was a direct relationship between metformin level alone, in combination with cephalexin and hypoglycemic response in the subjects investigated (Table 2).

CONCLUSION

Conclusion The foregoing result showed that cephalexin may have influenced the pharmacokinetics of metformin by reducing the renal clearance of metformin, thereby inhibiting tubular secretion via the organic cationic system. This resulted in elevated blood metformin concentrations, which in turn may lead to adverse effects upon repeated administration. It is recommended that patients who are prescribed these two drugs concomitantly should have serum metformin levels monitored or an alternative to cephalexin should be considered.

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Conflict of Interests

The authors have not declared any conflict of interests.

Authors' Declaration

The authors hereby declare that the work provided in the article is their own original work, and that they will be held responsible for any claims connected to the contents of the article.

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