Nigerian Journal of Pharmaceutical and Applied Science Research, 9(1):41-45 January 2020 (ISSN 1485-8059. Available at www.nijophasr.com)

Interaction Studies of Ciprofloxacin and Metformin in Type 11 Diabetic Patients.

[1]*Garba, Musa Abdullahi, Anas, Haruna[1], Danbaba, Abduljalal[1], Bakare-Odunola, Moji Thaibet[2], Magaji. Garba[3,]

[1]Department of Pharmaceutical and Medicinal Chemistry, Kaduna State University, Kaduna, Nigeria
[2]Department of Pharmaceutical and Medicinal Chemistry, University of Ilorin, Kwara State, Nigeria
[3]Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Nigeria

ABSTRACT

Metformin is mostly prescribed with ciprofloxacin in the treatment of diabetic disease with infections. However, the effect of this drug on the pharmacokinetic profile of metformin is yet to be established. The study was aimed at investigating the influence of ciprofloxacin when co-administered with metformin to freshly diagnosed patients. It is a one-way single dose cross-over study in two phases. The subjects acted as their control, and each phase was preceded by an overnight fast. In phase one, metformin was administered to all the diabetic patients, while in phase two metformin was co-administered with 500 mg ciprofloxacin. Plasma glucose levels were determined using the standard glucose oxidase method. Blood samples were collected at 0, 0.5, 1.5, 3.0, 4.0, 6.0, 8.0 and 24 h 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. The K_a, C_{mx} $t_{1/2}$ and AUC of metformin when administered alone were 0.46 ±0.04 h^{-1}, 1.14 ±0.52 µg/ml, AUC 4.39 hµg/ml/h respectively, When co-admistered with ciprofloxacin K_a increased to 0.68 ± 0.04 h^{-1}, C_{max} to 1.43 ± 0.35 µg/ml; while AUC and $t_{1/2\beta}$ increased to 5.72 ± 0.02 µgh/ml and 6.8 ± 0.02 h, respectively. These increments were found to be significant ($p < 0.05$).Our findings showed that metformin may be co-administered with ciprofloxacin to Type 2 diabetic Patients with cautions to avoid the possible risk of toxicity or therapeutic failure.

Keywords: Bioavailability; Ciprofloxacin Metformin; HPLC; Pharmacokinetics.

INTRODUCTION

Metformin hydrochloride is an oral biguanide, which reduces the elevated blood glucose concentration in patients with diabetes but does not increase insulin secretion. It does not lower the blood glucose in nondiabetic subjects (Hermann, 2010). Augmentation of muscular glucose uptake and utilization, and reduction of increased hepatic glucose production through an antigluconergic action explain the blood glucose lowering effect (Bailey, 2012; Hermann, 2013). Metformin is safe and not teratogenic (Denno and Saddle, 2009) in many of the species studied. The oral bioavailability of metformin is about 50 – 60% and fecal recovery is about 30%. The rate of absorption was slower than that of elimination, which resulted in a plasma concentration profile of the "flip-flop" type for oral metformin (Pentikainen *et al*., 2010). The highly polar compound escapes metabolism almost entirely and is eliminated via renal excretion (Denno and Saddle, 2009, Tucker *et al.,* 2010). Metformin exists in two tautomeric forms in acidic medium (Figure1). Metformin is practically insoluble in most organic solvents (Pentikainen *et al.,* 2010) which renders its extraction from the aqueous complex plasma matrix difficult (Zhang *et al.,* 2002; Zarghi *et al.,* 2003). 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 (Cheng and Chou, 2001; Zhang *et al*., 2002 and Zarghi *et al.,* 2003) or cation exchange column (Bonfigli, 2013). Metformin is a widely used and effective drug for the treatment of type 2 diabetes (UKPDS Group, 1998). To the best of our knowledge, there is very little work done on possible interactions of metformin with ciprofloxacin. This study, therefore, is aimed at investigating the effect of ciprofloxacin on the pharmacokinetic profile of metformin in freshly diagnosed diabetic volunteers

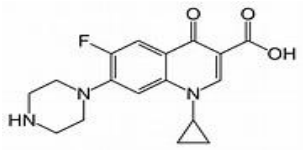

Figure 1:- Molecular Structure of Ciprofloxacin

Corresponding Author:*musagarba.abdullahi26@gmail.com.+2348034509999

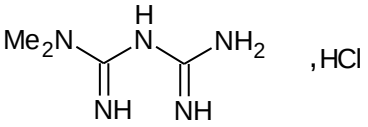

Figure 2:- Molecular Structure of Metformin

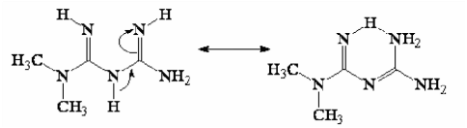
Figure. 3: Tautomeric forms of metformin in acidic medium.

MATERIALS AND METHODS

Subjects and ethical clearance

The subjects were diagnosed with diabetes mellitus at the Medical Outpatient Department Gambo Sawaba General Hospital Zaria, Kaduna State Nigeria. For this study, the 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.

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 Clinicians. 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 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 one subjects received a single dose of metformin (1 g) with 150 ml of water (ADA, 2013, Marathe *et al.*, 2000 and PattanaSripalakit *et al.*, 2006), while in phase two, subjects received metformin co-administered with ciprofloxacin (500 mg) 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

The extraction method used for this study was adopted and modified from (Bhavesh *et al.,* 2007). A100 µl of metformin hydrochloride solution of appropriate concentration and 100µl of sulfadoxine solution (20 µg ml^{-1}) was 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 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.

Determination of plasma metformin concentration

A validated High Performance Liquid Chromatography (HPLC) method (Rowland and Tozer ,1995) was used in the estimation of serum metformin concentration using a HPLC instrument (Agilent Technologies 1120 compact model LC Series, USA). consisting of pump type L-7100 with the following accessories: SIL-20AC auto-sampler; DGU-20A3 degasser; SPDM20A UV-diode array detector; Eclipse X BD C-8 4.6 x150mn column oven, CBM-20 Alite system controller and Windows LC solution software. The chromatographic conditions were made up of a mobile phase: solvent A: 0.01M KH_2P0_4 (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. The data was validated for range, accuracy, repeatability, intermediate precision, coefficient correlation, sensitivity and system suitability parameters were calculated.

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 $AUC_{0-t} + Ct/K_e$ 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 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 (Joel *et al.*, 2012, Melmed, *et al.*, 2012, Sambo, *et al.*, 2019). Data were expressed as mean ± SEM. Graph Pad Prism Version 7.02 software 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 (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 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

Precision of the method was determined by selecting 200 ng/ml, 500 ng/ml and 1000 ng/ml concentrations from prepared serial dilution and were used to determine within-day and day-to-day variations. For within day variation, three concentrations were run 6 times in the morning and afternoon of same day. 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 coefficient of variation in percentage.

RESULTS

Table 1: Comparison of pharmacokinetics of metformin (mean, n = 6) alone and when co-administered with ciprofloxacin in type 2 diabetic patients (Mean ±S.D, N=6)

	Metformin alone	Metformin +Cipro	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±0.04	0.68±0.03	S
C_{max} (µg/ml)	1.14±0.52	1.35±0.42	S
T_{max}(min)	3.0±0.19	3.0±0.19	NS
AUC_{0-8}(h µg/ml/h)	4.39±0.71	5.72±0.80	S
Vd (ml)	337,852.19±0.87	303,061.43±0.02	NS
CL(ml/h)	59,013.39±0.41	42,435.56±0.21	S
$T_{1/2\beta}$(h)	3.8±0.07	6.8± 0.02	S
Ke(h^{-1})	0.18±0.12	0.15±0.02	S

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

DISCUSSION

This study evaluated the effect of 500 mg ciprofloxacin co-administered with 1 g metformin in freshly diagnosed diabetic subjects which were not yet placed on treatment. This is to establish the need for concomitant drug intake during the study. An increase in the absorption rate constant k_a from 0.46±0.04 to 0.68±0.04 h^{-1} was observed when co-administered with ciprofloxacin, 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 ciprofloxacin (C_{max}) from 1.14 ± 0.52 to 1.43 ±0.35 µg/mL while the area under the curve (AUC) increased from 4.39 ± 0.71 to 5.72 ±0.02 µg/mL/h which was statistically insignificant at ($P<0.05$). This is in agreement with the finding of (Hills, 1987, Paxton, 1989) , that the high AUC value of metformin in the presence of ciprofloxacin tablet is most likely responsible for the decreased plasma glucose concentration following treatment with the two drugs (Garba *et al.*, 2018). It could be because both drugs are 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 (Ptalsky, 1989). This may be the most likely reason for the high bioavailability of metformin observed which resulted in an increased of AUC and C_{max}. Also since

absorption rate is significantly increased, this also account for the significant increase in AUC. Elimination rate constant is reduced significantly 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 in the presence of ciprofloxacin which agreed with the result of the study carried out by (Bakare-Odunola, *et al*., 2001) on the influence of ciprofloxacin 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 42,435.56±0.21 mL/h with a reduction in the volume of distribution when metformin 1 g was co-administered with 500 mg ciprofloxacin to type 2 diabetic patients, maybe due to the decrease in elimination rate constant (Charles *et al.*, 2009).

CONCLUSION

The foregoing showed that ciprofloxacin 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 findings also indicated that type 2 diabetic patients on metformin who may require ciprofloxacin may require adjustment of dose regimen to avoid the possible risk of toxicity or therapeutic failure and careful monitoring of blood glucose is recommended.

Acknowledgement

The authors want to thank the volunteers for participating in the study. They are grateful to Mr Mustapha (Chief Laboratory Technologist) of the Department of Pharmaceutical and Medicinal Chemistry, University of Lagos. . We are grateful to Dr. Ado and Dr. Maryam and the Matron of Gambo Sawaba General Hospital Zaria for their immense contr]xzibutions towards this study. We are thankful to Mallam A.M.Shitu of Department of Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. We also thank Mallam Ismaila (Technician) with Barau Dikko Teaching Hospital for his immense contribution.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

ADA (American Diabetes Association) (2013). Nutritional recommendations and principles for an individual with diabetes Mellilus. *Diabetes care .1*0:126 – 132.

Bailey CJ 2012. Biguanides and NIDDM. *Diabetes Care.*, 15(6): 755-772.

Bakare-Odunola MT, Garba M., Enemali SI., Okeniyi SO. and Gebi J (2001).The influence of ampicillin/cloxacillin on the pharmacokinetics of chlorpropamide in Type 2 diabetic patients.*West African Journal of Parmacology. Drug Research 17: (1and 2) 74-76.*

Bhavesh D, Chetan,G , Bhat KM. and Shivprakash, "Estimation and pharmacokinetics of Metformin in human volunteers"*Indian Journal of Pharmarceutial Education Research*, 2007;41(2):135-139.

Bonfigli AR, Manfrini S, Gregorio F, Testa R, Testa I, Sio G and Coppa G.(2013). Determination of plasma Metformin by new cation exchange HPLC technique. 21(3):330-334.

Charles BG , Precechagoom, Y, Lee TC ,Sheer PA , Flennady VJ and Debuse, N (2009). Population pharmacokinetics of intravenous amoxicillin in very low birth weight infants. *Journal of Pharmaceutical Sciences.* 86(22):1288-12292.

Cheng CL and Chou CH. (2001). Determination of Metformin in human plasma by High performance liquid chromatography with spectrophotometric determination. *J. Chromatogr B. Biomed Sci. Appl*., 762(1): 51-58.

Denno KM and Saddle TW (2009). Effects of biguanidine class of oral hypoglycemic agents on mouse embryogenesis. *Teratology*., 49(4): 260-266.

Garba MA, Bakare-Odunola MT, Garba M,.Haruna A and Bako R (2018). Effects of metronidazole and amoxicillin on the pharmacokinetics of metformin in type ii diabetic patients. *FUW Trends in Science & Technology Journal*, www.ftstjournal.com e-ISSN: 24085162; p-ISSN: 20485170; April, 2018: Vol. 3 No. 1 pp. 309 – 313

Hermann LS (2010). Clinical pharmacology of biguanides. In: Kulhmann I, Plus W editor. Hand Book of Experimental Pharmacology.Hiedelberg: Springer Veelog, pp. 374 - 407.

Hermann LS (2013). Metformin: a review of its pharmacological properties and therapeutic use. *Diabete Metab.*, 5(3): 233-45.

Hills S (1987). Penicillin Antibiotics. In: Antibiotics in Clinical use. Oxford University Press, London. pp 2-4.

Joel IU, Atul D, Diane TL (2012). PK Function on Microsoft excel. Department of Pharmacokinetics and drug Metabolism. Allergen, Irvine, CA 92606. USA

Marathe PH, Arnold ME, Meeker J, Greene DS, Barbhaiya RH (2000). Pharmacokinetics and bioavailability of a metformin/glyburide tablet administered alone and with food. *Journal of Clinical Pharmacology* 40: 1494-1502.

Melmed S, Polonsky KS, Larsen PR and Kronenberg, H.M. (2012). Williams Textbook of Endocrinology, 12th Edn. Philadelphia: Elsevier/Saunders. pp. 1371–1435.

PattanaSripalakit A.B, PenpornNeamhom .B, AurasornSaraphnhotiwitthaya C (2006). High performance liquid chromatographic method for the determination of pioglitazone in human plasma using ultraviolet detection and its application to pharmacokinetic study *Journal of Chromatography* B; 843: 164-169.

Paxton WJ (1989). Drug absorption and distribution. In: Pharmacokinetics in clinical practice *The New Zealand Medical Journal.* 694: 304-306.

Pentikainen PJ, Neuvonen PJ and Pentilla A (2010). Pharmacokinetics of Metformin after Intravenous and oral administration to man. *Eur. J. Clin. Pharmacol*., 16(3): 683- 693.

Ptalsky KM (1980).Disease-induced changes in the plasma binding of basic drugs. *Clinpharmacokin*.5; 246-262.

Rowland M and Tozer TN (1995). "Clinical pharmacokinetics Cocepts and applications"3rd Edition, Lea & Febiger ,Philadelphia, London.

Tucker GT, Casey C, Philips PJ, Con nor H, Ward JD, Wooda HF 2010. Metformin kinetics in healthy subjects and in patients with diabetes mellitus. *British Journal of. Clinical . Pharmacology*. 12(2): 235-246.

Sambo GI, Bakare-Odunola MT, Aminu M, Ibrahim AY, Magaji G and Adzu B (2019). Effect of amodiaquine on the pharmacokinetics of glicliazide in diabetic subjects.*African journal of pharmacy and pharmacology*.13 (11) 139-145.

UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with Metformin on complications in overweight patients with type 2 Diabetes (UKPDS 34) *Lancet*, 1998;352:854–65.

Zarghi A, Foroustan SM, Shafaati A and Khoddam A (2003). Rapid determination of Metformin in human plasma using ion-pair HPLC. *J. Pharm Biomed Anal.*, 31(1): 197-200.

Zhang M, Moore GA, Lever M, Gardiner SJ, Kirkpatric CM and Begg EJ(2002). Rapid and simple High-performance liquid chromatography assay for the determination of Metformin in human plasma and breast milk. *J. Chromatogr B Analyt Technol Biomed Life Sci.*, 766(1): 175-179.