EFFECTS OF METRONIDAZOLE AND AMOXICILLIN ON THE PHARMACOKINETICS OF METFORMIN IN TYPE II DIABETIC PATIENTS

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M. A. Garba[1]*, M. T. Bakare-Odunola[2], M. Garba[3], A. Musa[3], A. Haruna[4] and R. Bako[4]

[1]Shehu Idris College of Health Sciences and Technology, Makarfi, Kaduna State, 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
[4]Department of Pharmaceutical and Medicinal Chemistry, Kaduna State University, Nigeria
*Corresponding author: musagarba.abdullahi26@gmail.com

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Abstract: Infection is common in diabetes and in the course of treatment metronidazole and amoxicillin may be co-administered with metformin. The study was designed to evaluate the effects of co-administration of metronidazole tablet and amoxicillin capsule on the pharmacokinetics of metformin in type II diabetic patients. Twelve patients with age ranging from 35 – 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 metronidazole, 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 h post drug administration and stored at – 4^0C 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. High performance liquid chromatography (HPLC) method on a C–8column (4.6 x 150 nm), mobile phase acetonitrile/potassium dihydrogen orthophosphate (21:79) and a ultra violet (UV) detector at 236 nm. When metformin was co-administered with metronidazole, K_a increased to 0.59 ± 0.04 h^{-1}, C_{max} to 1.38 ± 0.35 µg/ml; while AUC and $t_{1/2\beta}$ increased to 536.71 ± 0.02 µgh/ml and 6.2 ± 0.02 h, respectively. These increments were found to be significant ($p < 0.05$). On the other hand, when metformin was co-administered with amoxicillin most of the changes were not significant ($p > 0.05$).

Keywords: Amoxicillin, diabetes, high performance liquid chromatography, metformin, metronidazole

Introduction

Metformin hydrochloride is an oral biguanidine, 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. 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 "flip-flop" type for oral metformin (Pentikainen *et al.*, 2010).

The highly polar compound escapes metabolism almost entirely and is eliminated via renal excretion (Tucker *et al.*, 2010; Denno & Saddle, 2009). As shown below, metformin exists in two tautomeric forms in acidic media. 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 are reported. But most of the methods use either ion pair reagent (Cheng, 2001; Zhang *et al.*, 2002; Zarghi *et al.*, 2003) or cation exchange column (Bonfigli, 2013).

Some methods reported require elaborate sample preparation (Zhouping *et al.*, 2001; Vesterqvist *et al.*, 2014). Though, these methods are sensitive and reproducible, RP-HPLC method for the estimation of metformin in human plasma are found to be more suitable. Previously described methods suffered from several disadvant-ages including use of complex extraction procedures which were tedious and time consuming. Ultra-filtration and column-switching techniques have been suggested to improve specificity and selectivity (Vesterquist *et al.*, 1998). The objectives of the study is to determine the influence of metronidazole on the pharmacokinetics of metformin in type II diabetic Patients using high performance liquid chromatographic method.

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.

Methods

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 informed consent of the Volunteers was obtained. Twelve patients with age ranging between 35 – 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 metronidazole, 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 h post drug administration and stored at – 4^0C before analysis. 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.

Preparation of standard preparation

Stock solution of metformin was prepared 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 extraction method used for this method was adopted and modified from Bhavesh *et al.* (2007). A 100 µL of metformin hydrochloride solution of appropriate concentration and 100 µL of phenformin hydrochloride solution (20 μgmL^{-1}) 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 min, 700 µL of the supernatant was evaporated to dryness at 45^0C under nitrogen. The residue was reconstituted in 100 µL of mobile phase and 20 µL of this was injected to the HPLC system.

Precision and accuracy

Precision of the method was determined by selecting 500, 1000 and 4000 ng/ml concentrations from prepared serial dilution 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 and Discussion

Following the concomitant administration of a single dose of 1 g of metformin with 400 mg metronidazole to Type II diabetic patients, significant increase ($p < 0.05$) in the absorption rate constant (k_a), peak plasma concentration (C_{max}) and area under the curve (AUC) of metronidazole were observed. The greater the amount of drug absorbed, the greater the AUC and the greater the bioavailability. A decrease in elimination rate constant k_e (hr^{-1}) from 0.18 ± 0.12 to 0.11 ± 0.02 hr^{-1} was bserved on co-administration of metformin with metronidazole to Type II diabetic patients, with an increase in elimination half-life 3.8 ± 0.07 to 6.2 ± 0.02 hr^{-1} and decrease clearance from 59013.39 ± 0.41 to 42435.56 ± 0.21 ml/h which were significant ($p < 0.05$). In a similar study, the influence of 400 mg dose of metronidazole on the pharmacokinetic profile of 250 mg of Chlorpropamide was examined in Type II diabetic patients. Metronidazole significantly reduced the rate, extent and the maximum amount of chlorpropamide absorbed. However, despite the reduction, the hypoglycemic responses were not significantly affected (Garba *et al.*, 1999). In a similar study, ampiclox was administered with chlorpropamide; ampiclox caused significant increase in absorption which also resulted in increase in hypoglycemic effect (Bakare-Odunola *et al.*, 2001). Thus, there could be possibility that both drugs compete for the same renal-tubular secretion. When this happens, there is every tendency that metronidazole known to be rapidly eliminated from the system will be secreted first in preference to chlorpropamide when they are co-administered. They indicated that both drugs may be competing for the same renal tubular secretion.

On the other hand, the changes in pharmacokinetic parameters were not statistically significant when metformin was administered alone and with amoxicillin (Eileen *et al*., 2007). Despite the fact that, amoxicillin does not affect most of the pharmacokinetics parameters of metformin, there were changes observed, peak plasma concentration decreased from 1.114 ± 0.52 to 1.104 ± 0.04. This could be due to the fact that metformin oral decreases effects by opposing drug effects.

HPLC Conditions

Mobile : Acetonitrile: 0.01M KH_2P04
21 79
Pressure : 120-245 psi
Column : Eclipse X BD C-8 4.6 x 150 mn
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
Retention time (min)
MetforminSulfadoxine (I.S)
1.06 2.25

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 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 were computed with a statistical data package SPSS 16.0 and Excel 2007. The results showed good response of the detector at the concentration used.

Pharmacokinetic parameters calculation

The pharmacokinetic parameters were calculated from the concentrations derived from the corresponding Peak Height Ratio observed in HPLC machine. Residual method and a software package, PKF Microsoft excel were used to compute the pharmacokinetic parameters as shown in Tables 3 and 4.

Table 3: Pharmacokinetic parameters of Metformin alone and co -administered with Metronidazole (Mean ±S.D, N=6)

	Metformin alone	Metformin + Metro.	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	0.59±0.04	S
C_{max} (µg/ml)	1.14±0.52	1.38±0.35	S
T_{max}(min)	3.0±0.19	3.0±0.19	NS
AUC_{0-8}(h µg/ml/h)	4.39±0.71	5.36±0.02	S
Vd (ml)	337,852.19 ±0.87	313,061.43 ±0.02	NS
CL(ml/h)	59013.39 ±0.41	42435.56 ±0.21	S
$t_{1/2\beta}$(h)	3.8±0.07	6.2± 0.02	S
Ke(h^{-1})	0.18±0.12	0.11±0.02	S

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

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

	Metformin alone	Metformin + Amoxicillin	Paired sample T- test value
$K_e(h^{-1})$	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 µgml/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)

Conclusion

The HPLC method in 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 metronidazole and amoxicillin. Potentiation effects on metformin were only observed with concomitant administration of a single dose of 1 g metformin tablets with 400 mg metronidazole. The amoxicillin showed insignificant interactions ($p > 0.05$). These effects were followed with significantly higher postprandial glucose level but non- significant higher glucose level at T_{max}. It is therefore, recommended that metformin can be co-administered with amoxicillin by Type II diabetic patients without risk of side effects. On the other hand, diabetic patients who may require metronidazole with metformin need adjustment of dose regimen to avoid the possible risk of toxicity or therapeutic failure.

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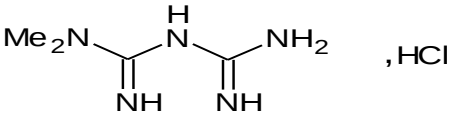


Fig. 1: Molecular structure of metformin

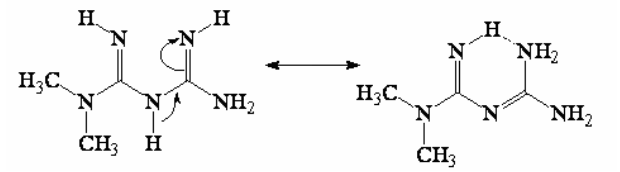


Fig. 2: Tautomeric forms of metformin in acidic media

Table 1: Intra and Inter-day Assay Variation of Metformin

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

CV = Coefficient of Variation; n = Number of samples

Table 2: Recovery of Metformin

Sample	Concentration (ng/mL)	Recovery %± S.D	N
Metformin	200.0	96.52 ±6.7	6
	400.0	98.43 ±7.0	6

SD= Standard Deviation

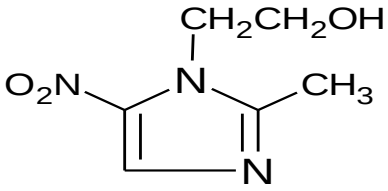


Fig. 3: Structure of metronidazole

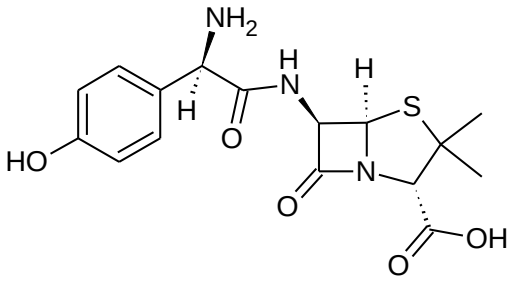
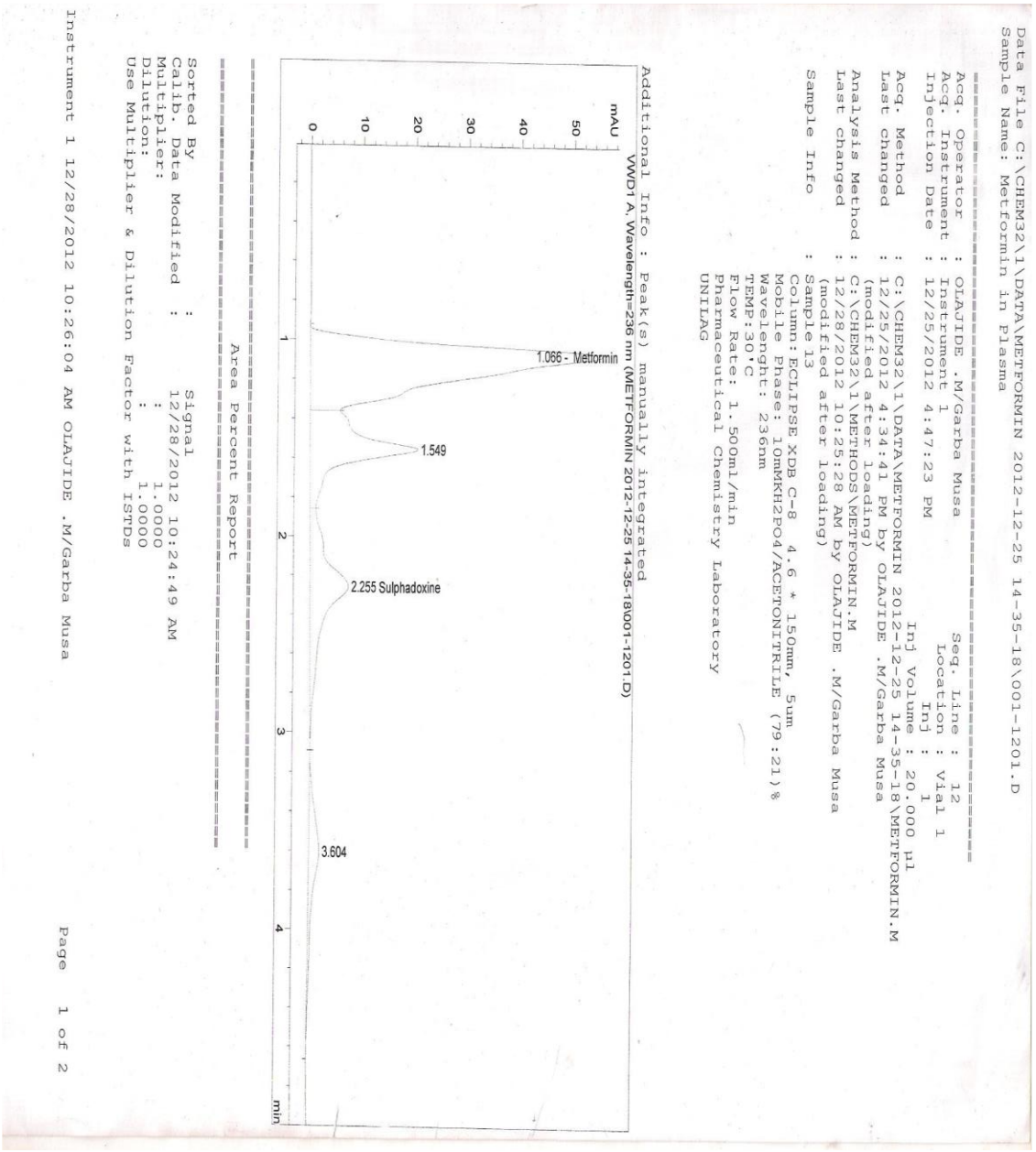
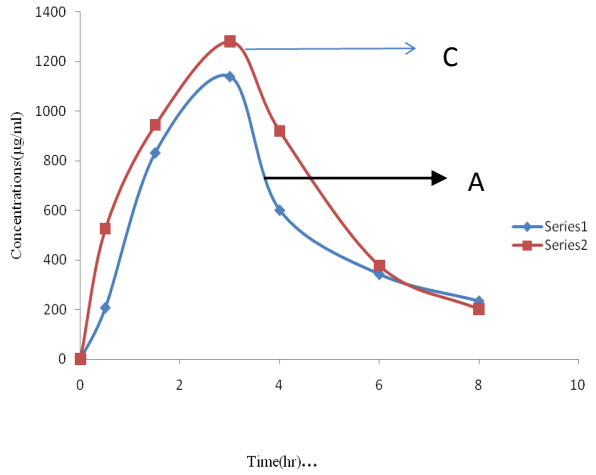


Fig. 4: Structure of amoxicillin



Chromatogram

Fig. 5: Chromatogram of Metformin and Sulfadoxine



Curve A (Series1) = Metformin, Curve C (Series 2) = Metformin co-administered with Metronidazole
Fig. 6: Comparison of plasma concentrations curve (µg/ml) of metformin alone (A) andco-administered with metronidazole (C)

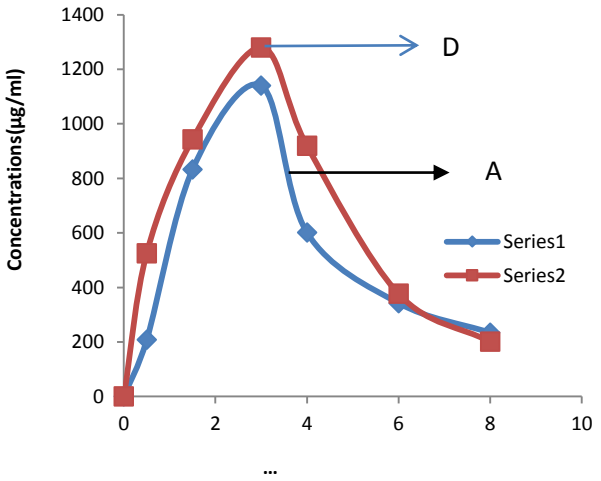


Fig. 7: Comparison of plasma concentrations curve (µg/ml) of metformin alone (A) and co-administered with amoxicillin (D)
Curve A (Series 1) = Metformin, Curve D (Series 2) = Metformin co-administered amoxicillin