

Influence of Metronidazole on the Pharmacokinetics of Metformin in Type II Diabetic Patients

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ABSTRACT

Infection is common in diabetes and metronidazole is often co-administered with metformin. This study is therefore to examine the influence of concomitant administration 400 mg dose metronidazole tablet with (2x500mg) metformin on pharmacokinetic parameters of metformin in type II diabetic Patients. The approval for the research was granted by the Ethical Committee of Ahmadu Bello University Teaching Hospital Zaria, Nigeria. Six Type II diabetic patients with age ranging from 25-55 years, weight from 50-70 kg took part in the study. Each of the six patients received the following treatment at two weeks intervals; Metformin tablet (1gm alone and concomitantly with 400 mg metronidazole tablets. Blood samples were collected at interval of 0 to 8 hours and stored at -4°C before analysis. High performance liquid chromatography (HPLC) was adopted, modified and validated to measure the plasma levels of metformin in the samples. Samples were chromatographed on Agilent Technologies 1120 Compact LG model of HPLC, on column Eclipse x BD C-8,4.6 x 150 nm for metformin with mobile phase acetonitrile: potassium dihydrogen orthophosphate (79:21) with flow rate of 1.50 mL/min, using UV detector. The results obtained altered the pharmacokinetic parameters of metformin significantly. The highest plasma concentration increased from 1140.43±0.52 to 1326.67±0.4 ng/ml at T_{max} 3.0 hours, with increase in area under the plasma concentration – time curve from 4388.81±0.52 to 5361.84±0.80 ng/ml/h. Significant decrease in volume of distribution from 333852.19±0.87 to 313, 061.43 ± 0.02ml was also observed. The results showed that metronidazole has potentiation effect on the disposition of metformin when concomitant administered to type II diabetic patients. Diabetic patients who require metronidazole needs adjustment of dosage regimen to avoid the risk of toxicity.

Keywords: Diabetes, HPLC, Metformin, Metronidazole, Pharmacokinetics.

1.0 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, 1995).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, 1992; Hermann, 1997). Metformin is safe and not teratogenic (Denno and Saddle, 1994) 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.*, 1981; Denno and Saddle 1994). 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 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, 1999). Some methods reported require elaborate sample preparation (Vesterqvist *et al.*, 1998; Zhouping *et al.*, 2001). 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 disadvantages 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 (Vesterqvist *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.

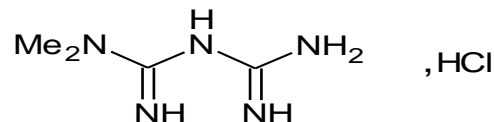


Figure 1: Structure of Metformin

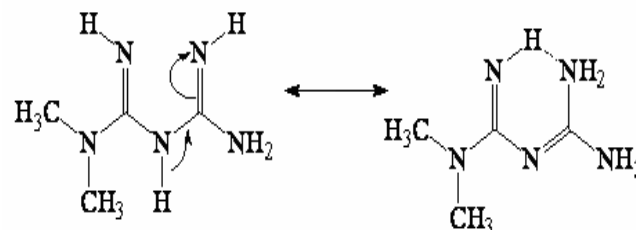


Figure 2: Tautomeric forms of Metformin in acidic media

2.0 MATERIAL AND METHODS

2.1 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.

2.2. Methods

2.2.1. 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. Six Type II diabetic patients with age ranging from 25-55 years, weight from 50-70 kg with

height range 1.5-1.75m (mean \pm S.D, 1.65 0.1M) took part in the study. Each of the six patients received the following treatment at two weeks intervals. Metformin tablets (1gm) alone and concomitantly with 400 mg metronidazole tablets. Blood samples were collected at interval of 0, 0.5, 1.5, 3.0, 4.0, 6.0, 0.8 hours. The blood samples collected were centrifuged for ten minutes at 100 revolutions per minutes (rpm) and the plasma transferred into a bijoux bottle with a Pasteur pipette. These stoppered bottles were then stored in the freezer at -4°C before analysis.

2.2.2 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

HPLC Conditions

Mobile	:	Acetonitrile:	0.01M $\text{KH}_2\text{P04}$
		21	79
Pressure	:	120-245 psi	
Column	:	Eclipse X BD C-8 4.6 x150mm	
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)
Metformin	1.06
Sulfadoxine (Internal standard)	2.25

internal standard (Sulfadoxine) was also prepared in a similar manner.

2.2.3. Extraction

The method of extraction of drug free plasma was adopted and modified(Bhavesh *et al.*,2007), 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.

3.0. RESULTS AND DISCUSSION

3.1 Results

Table 1:Percentage Recovery of Metformin (n=6)

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

SD= Standard Deviation.

The precision of the analytical method

Table 2: Intra and Inter-day Assay Variation of Metformin (n=6)

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

CV = Coefficient of Variation, n = Number of samples

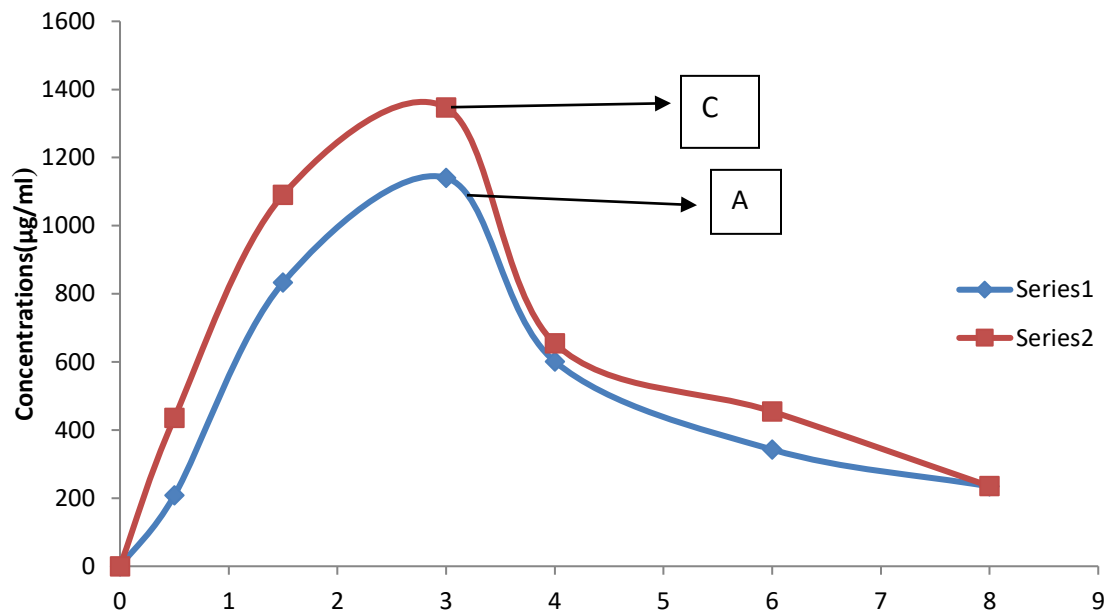


Figure 4:Comparison of plasma concentrations curve (µg/ml) of metformin alone (A) and co-administered with metronidazole (C)

Key:Curve A(Series1) = Metformin, Curve C(Series 2) = Metformin co administered with Metronidazole

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 and a correlation coefficient (r) of 0.983.

Table 4: Pharmacokinetic parameters of Metformin alone and Metformin coadministered with Metronidazole ((Mean ±S.D, N=6)

	Metformin alone	Metformin+Metro.	Paired sample T- test value
Lag time (h)	0.12±0.02	0.11±0.05	NS
$t_{1/2\alpha}$ (h)	1.5±0.03	1.2±0.05	S
K_a (h ⁻¹)	0.46±0.04	0.59±0.03	S
C_{max} (ng/ml)	1140.0±0.52	1350.0±0.42	S
T_{max} (min)	3.0±0.19	3.0±0.19	NS
AUC ₀₋₈ (hng/ml/h)	4390.0±0.71	5360.0±0.80	S
Vd (ml)	337852.19±0.87	313061.43±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
K_e (h ⁻¹)	0.18±0.12	0.11±0.02	S

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

3.2 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 (h⁻¹) from 0.18±0.12 to 0.11±0.02 h⁻¹ was observed 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 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.

4.0 CONCLUSION

It has been established in the course of this study, that metronidazole tablets may have influenced the pharmacokinetics of metformin. The method of high performance liquid chromatography (HPLC) in the monitoring of metformin in the plasma was very effective and efficient. The findings also indicated that type II diabetic patients on metformin who require metronidazole may require adjustment of dose regimen to avoid possible risk of toxicity or therapeutic failure.

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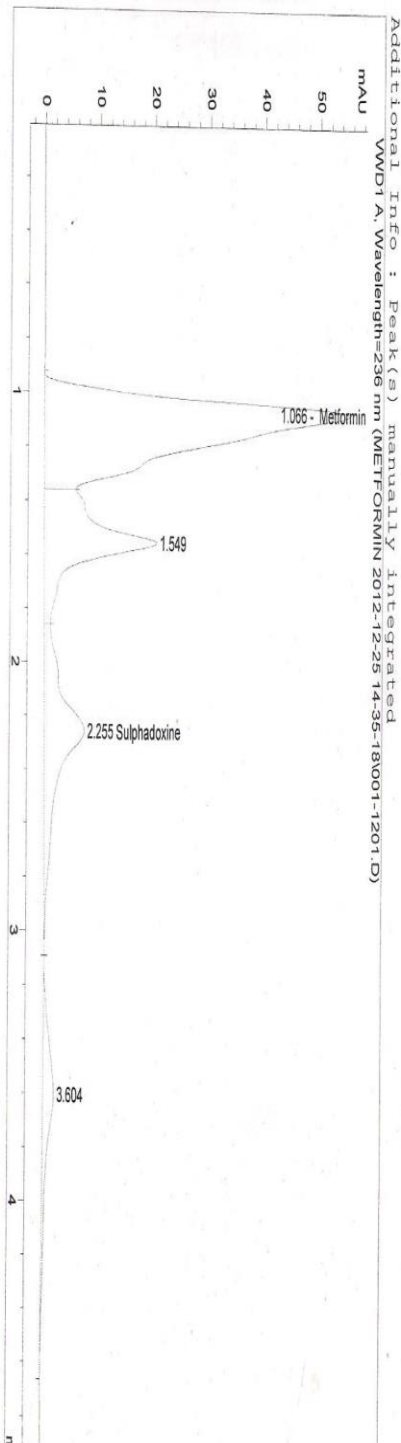
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Chromatogram

Instrument 1 12/28/2012 10:26:04 AM OLAJIDE .M/Garba Musa

Sorted By : Signal
 Calib. Data Modified : 12/28/2012 10:24:49 AM
 Multiplier : 1.0000
 Dilution : 1.0000
 Use Multiplier & Dilution Factor with ISTDs

Area Percent Report



Sample Name: Metformin in Plasma

Acq. Operator : OLAJIDE .M/Garba Musa
 Acq. Instrument : Instrument 1
 Injection Date : 12/25/2012 4:47:23 PM
 Inj Volume : 20.000 µl
 Location : Vial 1
 Inj : 1

Acq. Method : C:\CHEM32\1\DATA\METFORMIN 2012-12-25 14-35-18\METFORMIN.M
 Last changed : 12/25/2012 4:34:41 PM by OLAJIDE .M/Garba Musa
 Analysis Method : C:\CHEM32\1\TEST\METHODS\METFORMIN.M
 Last changed : 12/28/2012 10:25:28 AM by OLAJIDE .M/Garba Musa
 (modified after loading)

Sample Info : Sample 13
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 Mobile Phase: 10mM KH2PO4/ACETONITRILE (79 : 21) %
 Wavelength: 236nm
 TEMP: 30 °C
 Flow Rate: 1.500ml/min
 Pharmaceutical Chemistry Laboratory
 UNILAG

Data File C:\CHEM32\1\DATA\METFORMIN 2012-12-25 14-35-18\001-1201.D
 Sample Name: Metformin in Plasma

Figure 3: Chromatogram of Metformin and Sulfadoxine