PHARMACOKINETICS INTERACTION STUDY OF METFORMIN AND METHYLDOPA IN TYPE II DIABETIC PATIENTS WITH HYPERTENSION.

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Abstract

Pharmaceutical drug interaction may result in the alterations of the absorption, distribution, metabolism and excretion of a drug and this may affect its pharmaceutical actions. Diabetes and high blood pressure tend to occur together because they share certain physiological traits. Metformin is widely used in Nigeria to manage type II diabetes in hypertensive patients. Metformin (1 g) was administered alone and with 250 mg of Methyldopa to patients diagnosed for diabetes with hypertension. HPLC method (Agilent Technologies, 1120 LC series, USA) was used to analysed the serum samples of the patients using Hypersil C18 column at a wavelength of 238 nm. Solvent system was acetonitrile with methanol and buffer (13:7:80). The maximum concentration of Metformin insignificantly decreased from C_{max} 1890±0.22 ng/ml to 1752.17±0.5 ng/ml when alone and when co-administered with Methyldopa at maximum absorption time of 3 hrs. Area under curve (AUC) $_{0\text{-}8h}$ also insignificantly increased from $AUC_{0\text{-}8}$ 8882.10 ng/ml/h to 8895.30 ng/ml/h metformin alone and when interacted with methyldopa. AUC $_{0\text{-}\infty}$ also decreased from AUC $_{0\text{-}\infty}$ 12106.87ng.h/ml metformin alone to $AUC_{0\text{-}\infty}$ 12061.80 ng .h/ml when interacted with Methyldopa. Elimination rate constant, K_{el}, decreased from K_{el}, $0.116h^{-1}$ for metformin alone to 0.105 h^{-1} when interacted with Methyldopa. This result show the significant increase observed in the elimination half-life when Methyldopa was co- administered with metformin. This has also increased clearance (Cl) of the drugs, though, clinically insignificant. Administration of metformin with Methyldopa was clinically acceptable for therapeutic management of Type 2 Diabetic with Hypertension.

***Keywords*:** metformin, methyldopa, HPLC, Pharmacokinetics

Introduction

Type II Diabetes mellitus (formerly called non-insulin-dependent diabetes mellitus (NIDDM), or adult-onset diabetes), is a disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency (Robbins and Cotran, 2007). Traditionally considered a disease of adults, type II diabetes is increasingly diagnosed in children in parallel to rising obesity rates due to alterations in dietary patterns as well as in life styles during childhood (Vahidi *et al.*, 2010). In Nigeria, Studies conducted revealed the prevalence rate of less than 1 % for diabetes in Nigeria from 1960 to 2000

with most patients having type II diabetes (Ogbera and Ekpebeh, 2014). Diabetes and hypertension tend to occur together because they share certain physiological traits, that is, the effects caused by each disease tend to make the other disease more likely to occur (HDS, 1993).

The record of type II diabetes in patients with hypertension attending diabetic clinic of Ahmadu Bello University Teaching hospital, Zaria (ABUTH) indicated that 63 % of them were prescribed metformin along with antihypertensive drugs (e. g methyldopa). This co-administration of drugs would likely lead to drug-drug interactions due to their metabolic link, hence the need to investigate.

Chemistry of metformin

One of the most widely used drugs for the treatment of type 2 diabetes is the biguanide, metformin. It works primarily by reducing liver release of blood glucose from glycogen stores and secondarily by provoking some increase in cellular uptake of glucose in body tissues (Bailey and Turner, 1996).

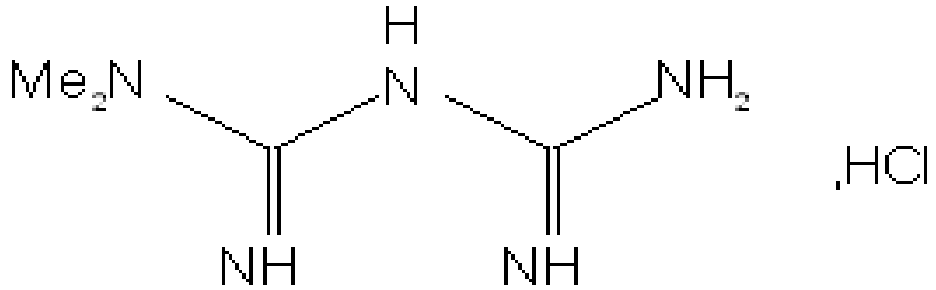

Figure 1: Structure of metformin

A systematic review of randomized controlled trials found that metformin and second-generation sulfonylureas are the preferred choices for most patients with type 2 diabetes mellitus, especially at early in the course of the disease (Bolen, 2007)**.** For patients who also have heart failure, metformin may be the best tolerated drug (Eurich *et al*., 2007).

Materials and methods

Materials, drugs, reagents and equipment

Table 1; Drugs details

Drug	Brand	Manufactured Date	Batch No	Expiry Date	Manufactured by
1. Metformin	Diabetmin® 500 mg	June 2012	Bco6597	June 2015	Hovid Bhd, 21 Malaysia
2. Methyldopa	Dopatab® 250 mg	Aug 2012	Bco8572	Aug 2015	Hovid Bhd, Malaysia

Equipment;

* 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, 1120 LC series, USA.
* Centrifuge: Heraeus (labafuge 300) D-37520 ostence mated: 2003, serial No 40267581, BN: 75003230.

Reagents;

- Methanol (≥ 99.9%): Sigma – Aldrich, U.K.
- Acetnitrile (≥ 99.9%,): Sigma – Aldrich, U.K
- Potassium Dihydrogen phosphate (99.5%) (Buffer) obtained from J.T Baker, USA
- Metformin HCl reference Standard
- Sulfadoxine: (Internal standard) obtained from Rambax Pharmaceutical Ltd, Lagos.

Methodology

Quality Control, in-vitro analysis, of methyldopa and metformin were carried out (Identification, Assay and Disintegration) and the result was within acceptable range of B.P 2002. In-vivo studies were conducted using inclusion criteria. 6 patients were screened to participate in the study. Their ages ranged between 28-45 years free from liver and kidney diseases and the fasting blood sugar (FBS) test and blood pressure (B.P) were taken before and after the study.

Drug Study Profile

Free drug blood samples at fasting state were taken from the patients, after which, 1 g of metformin tablets (1 g) were administered with (200 ml) water. The patients were given food after 30 minutes of the drug administration to avoid hypoglycemia in the patients. Blood samples (3 ml) were withdrawn at 0.0, 0.5, 1.5, 3, 4, 5, 6, 8, hours. Thereafter, the blood sugar levels and blood pressure of each patient were concomitantly determined. Blood were collected inside anticoagulant bottles, centrifuged and stored in a refrigerator at -4 ^{0}C.

After washout period of a week, six (6) patients were co-administered with metformin (1 g) and methyldopa (250 mg) with water (200 ml) each. Blood samples (3 ml) were withdrawn at 0, 0.5. 1. 5, 3, 4, 5, 6 and 8 hours for blood sugar level and metformin concentration determination.

Extraction method

Bhavesh, (2007) extraction method adopted and modified as follows; 100 µl metformin hydrochloride solution of 20 µg ml^{-1} and 100 µl of sulfadoxine solution (20 µg ml^{-1}) were added to 900 µl of blank 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) was added, vortex for 30 seconds centrifuged at 3000 rpm for 10 minutes and the supernatant was evaporated to dryness at 45^0C. The residue was reconstituted in 100 µl of mobile phase and 20 µl of this was injected on to the HPLC system.

Hplc chromatographic condition

Mobile phase; Acetonitrile: 25 mM KH_2P0_4: Methanol: 13807
Column : ODS Hypersil –C8 4. 6 x 125 mm, 5 um Wavelength: 238 nm
Temperature: 30 ^{0}C
Flow rate: 1.00 ml/min
Run time: 7 minute
Injection volume:20 µl
pH : 5.8 (adjusted with acetic acid)
Chromatogram; Metformin
sulfadoxine Retention time (min): 1.111
4.999

Optimization of the method

Precision of the method was determined by selecting 200 ng/ml, 500 ng/ml and 1000 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 Height Ratio obtained were calculated followed by coefficient of variation in percentage

Calibration curve

Calibration curve based on peak-height ratio were prepared by spiking drug-free plasma with standard solution of metformin to give concentration range 100 ng – 3 µg/ml and 200 ng/ml of sulfodoxine as internal standard. Coefficient of Variation and correlation coefficient R^2 (0.994) 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.

Results

Result of Precision

Table 2: Intra and Inter-day precision

Sample	Concentration ng/ml	CV %	n
Intraday	200	3.4 ± 0.56	6
(Metformin)	500	1.8 ± 0.87	6
	1000	0.5 ± 0.64	6
Inter-day			
(Metformin)	200	4.2 ±0.23	6
	500	3.5 ±0.41	6
	1000	1.2 ±0.04	6

CV = Coefficient of Variation, N= Number of samples

Percentage extraction recovery

The percentage extraction recovery are shown on Table 3

Table 3: % Recovery of Metformin

Sample	Concentration ng/ml	Recovery % ± S.D	n
Metformin	300	97.47 ± 4.2	6
	500	97.58 ± 6.7	6

Calibration curve of metformin standard solution

The calibration curve obtained from the dilution ratio of standard metformin concentrations 100 ng-3 µg/ml was linear with a correlation coefficient of 0.994

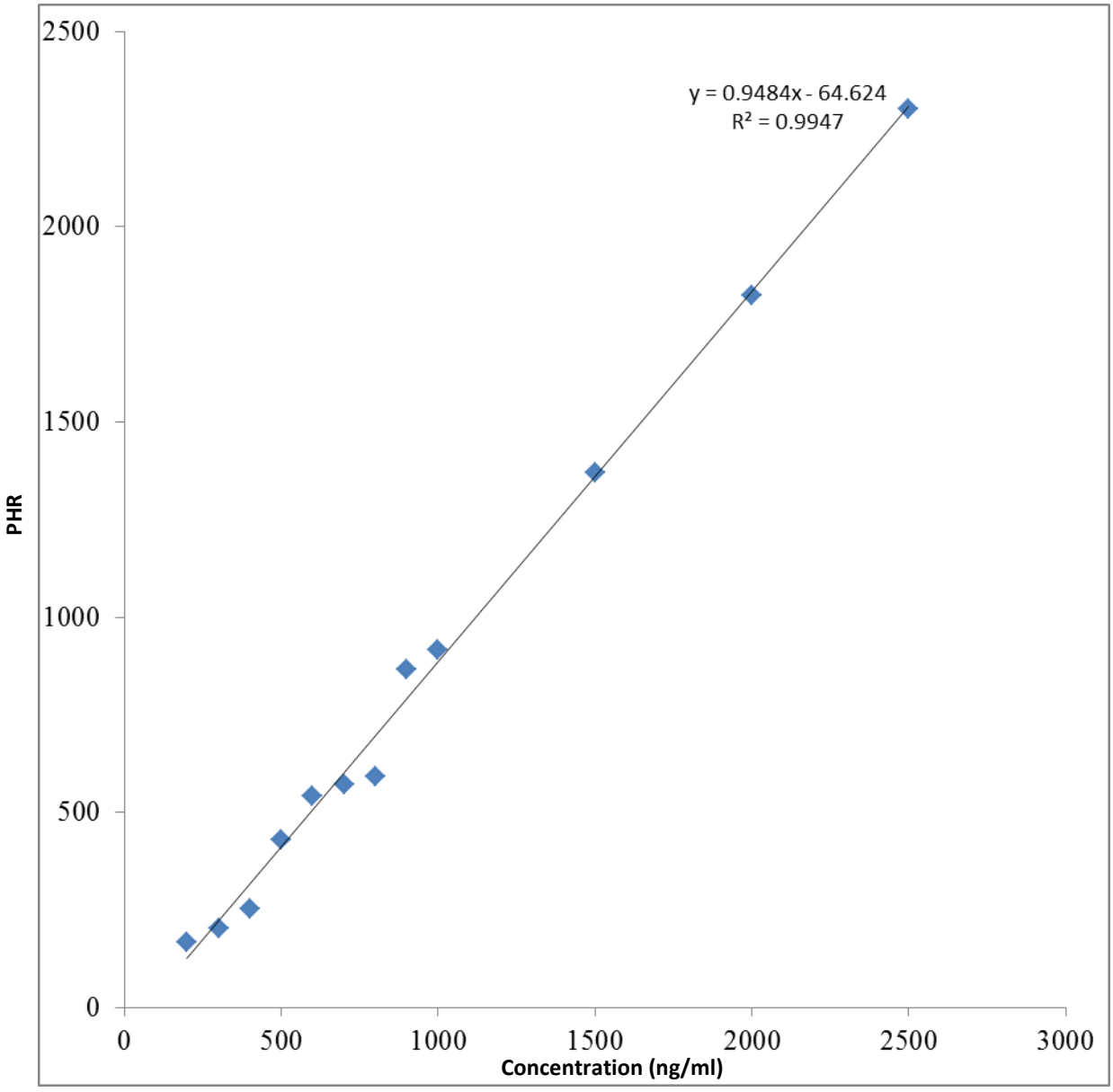

Figure 2. Linear calibration curve of Metformin

Co-administration of metformin and methyldopa

The result of administration of metformin alone also in type 2 diabetic patients and that of co-administration of metformin tablet (1 g) with methyldopa (250 mg) in type 2 diabetic patients

were shown in figure 3. The various concentrations detected were plotted against their corresponding sampling times. The Cmax and Tmax of co-administered of metformin (1 g) with methyldopa (250 mg) were 1752.17 ng/ml and 3 hrs respectively. The Cmax has reduced from 1890.67 ng/ml, which is statistically insignificant (P > 0.05) as shown in table 4

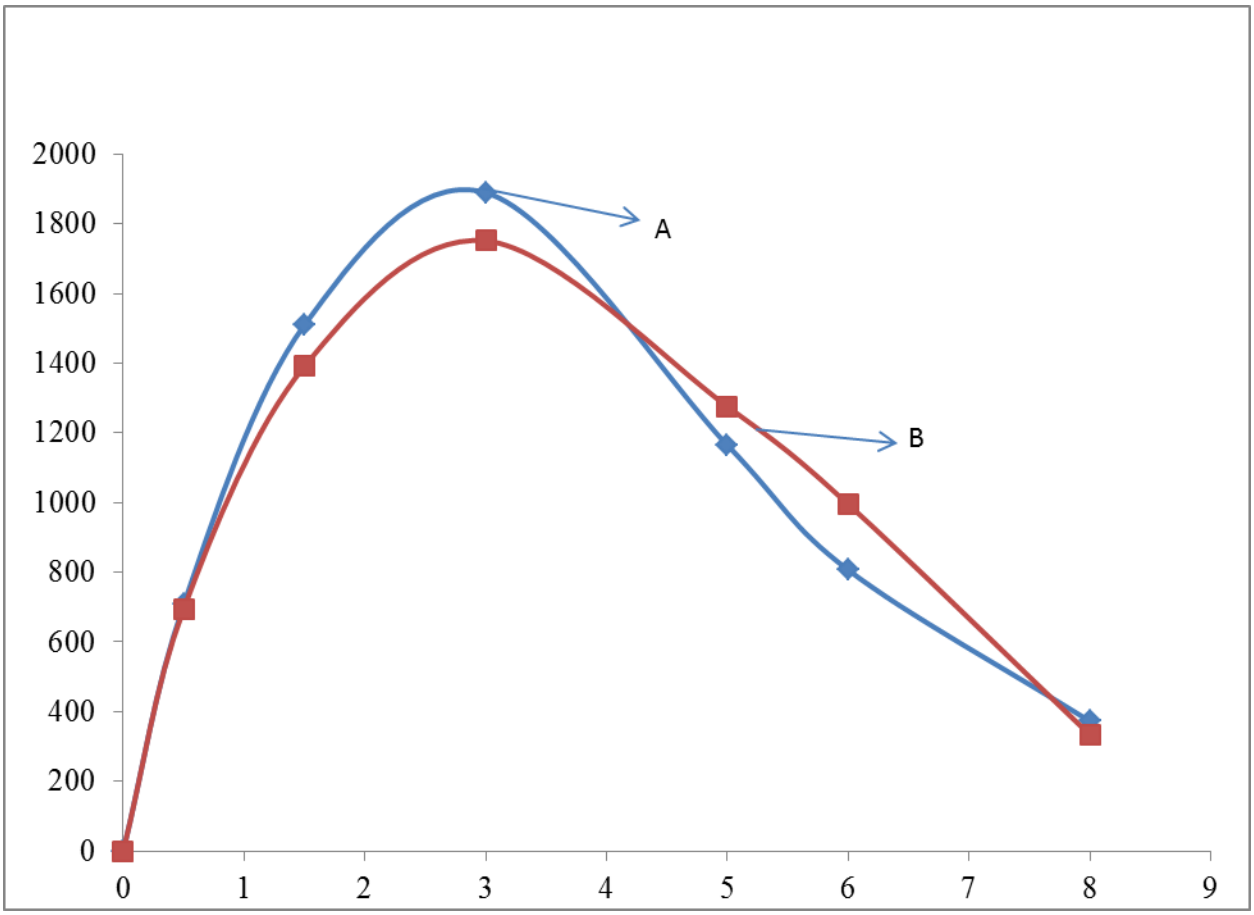

Figure 3: Comparison of mean concentration of metformin following administration of metformin (1 g) alone (A) and co- administered with methyldopa (250 mg) in type II diabetic patients (B)

Table 4 below show so pharmacokinetic parameters of metformin when administered alone and when co-administered with 250 mg of methyldopa to type 2 diabetic patients with hypertension.

Table 4: Comparison of pharmacokinetics of metformin (1 g) (mean, n = 6) alone and co-administered with methyldopa (250 mg) in type 2 diabetic patients.

Pharmacokinetic parameter	Met. Alone	Met.+ methyldopa	P-value
Lag time (h)	0.12 ± 001	0.12 ± 0.001	P > 0.05
t ½ abs (h)	1.45 ± 0.016	1.38 ± 0.046	"
K abs (/h)	0.478 ± 0.002	0.502 ± 0.001	"
Cmax (ng/ml)	1890.67 ± 0.107	1752.17 ± 0.232	"
AUC_{0-8} (ng/ml/h)	8882.10 ±0.205	8895.30 ± 0.223	"
$AUC_{0-\infty}$ (ng/ml/h)	12106.87 ±0.061	12061.80 ± 0.043	"
Vd (L)	112.59 ± 0.062	112.50 ± 0.061	"
t ½ el (h)	6.0 ± 0.000	6.58 ± 0.014	"
K el (/h)	0.116 ± 0.000	0.105 ± 0.020	"
T max (h)	3.0 ± 0.000	3.0 ± 0.000	"
CL(ml/h)	970.7 ± 0.330	1022.7 ± 0.316	"

Table 5: Comparison of mean sugar level in group treated with metformin alone and metformin when co-administered with methyldopa

Time (h)	Met.(alone) (mmol/l) (mean)	Met.+methyldopa(co-adm)(mmol/l) (mean)
0	6.3 ± 0.02	8.0 ± 0.67*
2	7.9 ± 0.73	13.1 ± 0.12*
3	6.4 ± 0.31	9.2 ± 0.22*
5	8.0 ± 0.43	9.2 ± 0.06
8	8.0 ± 0.57	7.1 ± 0.38

* Significant difference, (P < 0.05)

Discussions

The changes observed in pharmacokinetic parameters were not statistically significant ($P < 0.05$). It was reported that pharmacokinAetic 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 (Tatro, (2000). However, methyldopa would, in non-acid drugs, compete for the same pathway through the kidney (RxList, 2014). Methyldopa (oral) will increase the level or effect of metformin (oral) by basic (cationic) drug competition for renal tubular clearance (Chobanian, *et al.*, 2003).

However, co-administration of metformin (1 g) with cephelaxin (0.5 g) orally, increased Cmax and AUCs by 34 % and 24 % respectively and reduced clearance by 14 %. This is due to inhibition of renal tabular secretion of metformin which resulted in high cieculation of metformin concentration (Jayasagar, *et al.*, 2002).

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

Conclusion

The results of metformin interaction with methyldopa showed insignificant interactions ($P>0.05$). Therefore, type 2 diabetic patients with hypertension complication can be co-administered with metformin and methyldopa with no or little clinical implications.

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