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Research Article

A Study of the influence of Bendrofluazide on Metformin in Type II diabetic patients with hypertension

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ABSTRACT

Hypertension is more prevalent in patients with Type 2 diabetes than in the Non-Diabetic Population. Diabetes and high blood pressure tend to occur together because they share certain physiological traits. Most patients with diabetes will require combination therapy with multiple antihypertensive drugs to achieve good control. Metformin is widely used in Nigeria to manage Type II diabetes. 1000mg of metformin was administered alone and with 5mg of bendrofluazide to patients newly diagnosed for diabetes with hypertension. The wash out period was one week. HPLC method developed and validated was used to analyse the plasma samples of the patients using Hypersil C18 column at a wavelength of 238nm. Solvent system was acetonitrile with methanol and buffer. Plasma glucose levels were determined using the standard glucose oxidase method. The pharmacokinetic parameters changes were not statistically significant with the administration of metformin alone or with bendrofluazide ($p>0.05$). The maximum absorption concentration of metformin decreased from1890.67±0.22 ng/ml when administered alone to 1865.67±0.5ng/ml when co-administered with bendrofluazide at maximum absorption time of 3 hrs. Area under serum drug concentration time curve (AUC) $_{0\text{-}8h}$increased from 8882.10ng/ml/h alone to 8985.83 ng/ml/h when interacted with bendrofluazide. All other pharmacokinetic parameters in exception of the elimination half-life ($T_{1/2el}$) were not significantly altered. $T_{1/2el}$ significantly ($p<0.05$) increased from 6.0 to 7.48 h. The significant increase observed in the elimination half –life did not affect the rate of elimination significantly and may not result in toxicity.Diabetic patients on metformin can be prescribed bendrofluazide without risk of side effects.

Key words: Metformin, Bendrofluazide, HPLC and pharmacokinetics.

INTODUCTION

Metformin is a widely used and effective drug for the treatment of type 2 diabetes (UKPDS Group, 1998). Thiazide diuretics are recommended as first-line treatment for hypertension (ALLHAT, 2002). People with hypertension associated with diabetes are usually treated with combination antihypertensive treatment, which often includes thiazides (Mogensen, 2003).

Therefore, the interaction study of co-administration of this combination therapy is necessary to ascertain it clinical safety and efficacy.

MATERIALS AND METHODS

Some of materials used;

Major equipment used for this research were the followings

* Digital weighing balance OHAUS model EP 64 BY Ohauscorporation, 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: Heraeus (labafuge 300) D-37520 ostence mated: 2003, serial No40267581, BN: 75003230.

Similarly, Solvents and reagents used are were of analytical grade

- Methanol: Sigma – Aldrich ≥ 99.9% U.K , Mntd: Sept 14, 2011
- Acetnitrile: 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
- Sulfadoxine: Internal standard source: Rambax Pharmaceutical Ltd, Lagos.

METHODOLOGY

Quality Control of Bendroflazide and metformin were carried out and the result was within acceptable range. Thereafter, 6 patients were screened to participate in the study. Their ages ranged between 28-45 years. They were also 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.

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

The six (6) patients were co-administered with 1 g metformin and 5 mg bendrofluazide with 200 ml of water after 7 days washout period. 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

The extraction method used for thus study was adopted and modified from (Bhavesh*et al.*,2002).100 µl of metformin hydrochloride solution of appropriate concentration and 100µl of sulfadoxine solution (20 µg ml^{-1}) were 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 (perchloricacid : acetonitrile 50 %v/v each) was added and was vortex for 30seconds. After centrifugation at 3000 rpm for 10 minutes, 700 µl of the supernatant was evaporated to dryness at 45^{0}C. 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 : 25mMKH_2P0_4 : Methanol
13 80 7

Column : ODS Hypersil –C8 4. 6 x 125 mm, 5um
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	sulfadixine
Retention time (min):	1.111	4.999

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 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

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 0.1 µg – 3 µg/ml and 0.2 µg/ml of sulfodoxine as internal standard. Coefficient of Variation and correlation coefficient R^2 (0.994) were computed with a statistical data package. The results showed good response of the detector at the concentration used.

RESULTS

Result of precision

Percentage extraction recovery

The percentage extraction recovery are shown on Table 4

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

In-vivo results

The result of co-administration metformin (1 g) with bendrofluazide (5 mg) and metformin alone in patients studied was also compared to see the effect of the co-administration. The profile obtained is as shown in figure 2

Table 1
Intra and Inter-day Assay variation of Metformin

Sample	Concentration ng/ml	CV %	N
Intraday run	200	3.4 ± 0.56	6
(Metformin)	500	1.8 ± 0.87	6
	1000	0.5 ± 0.64	6
Inter-day run			
(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

Table 2.
% Recovery of Metformin

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

Table 3
Comparison of pharmacokinetics of metformin (mean, n = 6) alone and when co-administered with bendrofuazide in type 2 diabetic patients

Pharmacokinetic parameter	Met. Alone	Met.+bendro.	P-value
Lag time(h)	0.12 ± 001	0.12 ± 0.002	P > 0.05
t ½ abs (h)	1.45 ± 0.016	1.23 ± 0.052	P > 0.05
K abs (/h)	0.478 ± 0.002	0.563 ± 0.067	P > 0.05
Cmax (ng/ml)	1890.67± 0.107	1865.67 ± 0.002	P > 0.05
AUC_{0-8} (ng/ml/h)	8882.10 ± 0.205	8985.83 ± 0.041	P > 0.05
$AUC_{0-\infty}$ (ng/ml/h)	12106.87± 0.061	13626.77 ± 0.006	P > 0.05
Vd (L)	112.59 ± 0.062	111.29 ± 0.120	P > 0.05
t ½ el (h)	6.0 ± 0.000	7.48 ± 0.038	P > 0.05
K el (/h)	0.116 ± 0.000	0.093 ± 0.073	P > 0.05
T max (h)	3.0 ± 0.000	3.0 ± 0.000	P > 0.05
CL(ml/h)	970.7 ± 0.330	1200.7 ± 0.010	P > 0.05

Met + bendro = metformin co-administered with bendrofluazide

Table 4
Comparison of mean sugar level in group treated with metformin alone and metformin when co-administered with bendrofluazide

Time (h)	Metformin alone (mmol/l)(mean)	Met.+bendro (mmol/l) (mean)	P– value
0	6.3 ± 0.02	6.4 ± 0.14	P > 0.05
2	7.9 ± 0.73	10.8 ± 0.8	P < 0.05
3	6.4 ± 0.31	7.9 ± 0.1	P > 0.05
5	8.0 ± 0.43	4.0 ± 0.4	P < 0.05
8	8.0 ± 0.57	2.7 ± 0.2	P < 0.05

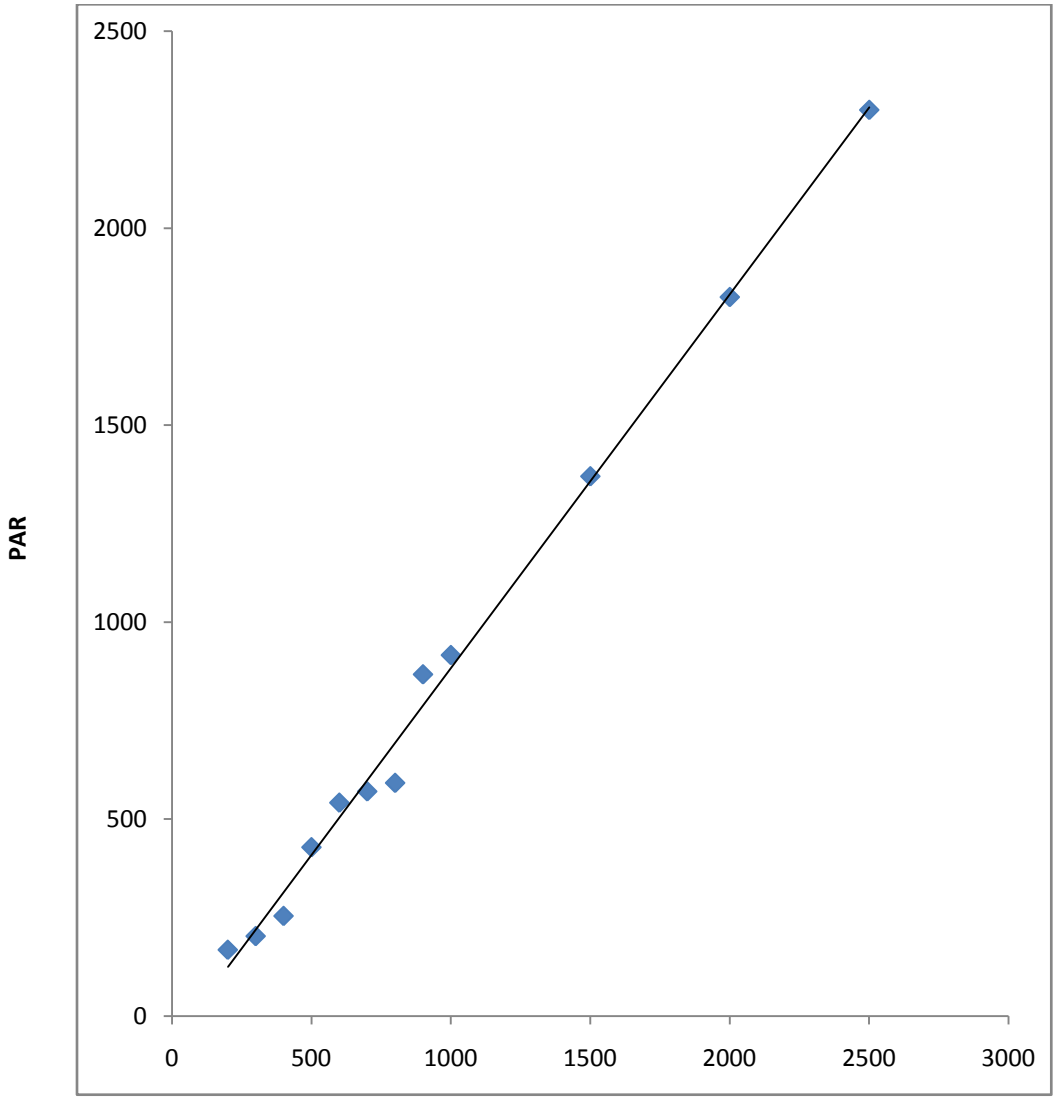

Figure 1.
Linear calibration curve of Metformin

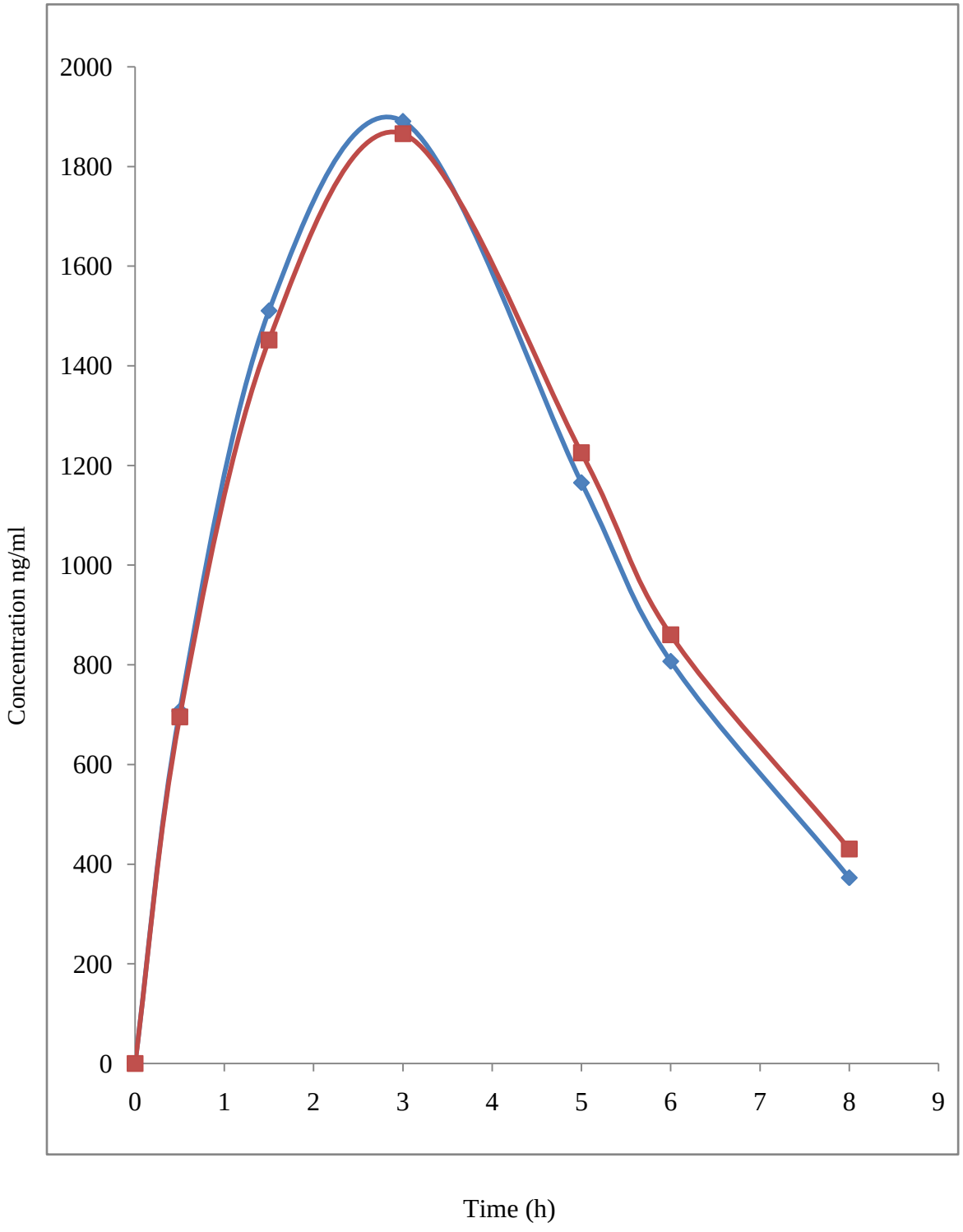

Figure 2
Comparison of mean metformin concentration following administration of metformin (1 g) alone (A) and co-administered with bendrofuazide (5 mg) in type 2 diabetic patients (B)

DISCUSSION

The changes in pharmacokinetic parameters were not statistically significant when metformin was administered alone and with bendrofluazide. (Eileen *et al*., 2007)also reported insignificant changes in pharmacokinetics of metformin when co-administered with a thiazide, diuretics like bendrofluazide. Despite the fact that, bendrofluazide does not affect most the pharmacokinetics parameters of metformin, there were changes observed. Cmax decreased from 1890.67± 0.107 to 1865.67 ± 0.002. This could be due to the fact that bendroflumethiazide oral decreases effects of metformin oral by opposing drug effects. Thiazide diuretics, in doses over 50mg/day, and similar drugs increase glucose levels, reducing the effect of drugs used for treating diabetes: (RxList, 2017)

Although, a clear theoretical mechanism is not obvious, and metformin and the thiazides interact with different transporters, thiazides could affect transporter activity through altered ionic balance (ALLHAT, 2002).

CONCLUSION

The results of metformin interaction with bendrofluazide 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 bendrofluazide in Type 2 diabetic patients with hypertension.

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