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Evaluating the effect of artesunate on the pharmacokinetics of gliclazide in diabetic subjects

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ABSTRACT

Therapeutic management of diabetic patients frequently involves polypharmacy which is likely to provoke drug-drug interactions (DDIs). This may require appropriate monitoring and most common clinically relevant DDIs that occur with antidiabetic medications often leads to variations in therapeutic response. Due to the wide use of artesunate combination in the treatment of malaria in diabetic patients, drug interactions with gliclazide is a possibility. This study was aimed at determining the effect of artesunate on the pharmacokinetics of gliclazide in diabetic subjects. Six freshly diagnosed diabetics subjects participated in the study. Written informed consent was sought and obtained from the volunteers. The study is a one-way single dose cross-over study in two phases. Phase 1 of the study involved the administration of a single oral dose of 80 mg of gliclazide after an overnight fast. After a wash out period of one week, 80 mg gliclazide and 100 mg artesunate were co-administered. Serial blood samples were collected over a period of 24 h during each phase into an EDTA vacutainer. High Performance Liquid Chromatography method was used in the estimation of plasma glucose concentration; while the glucose oxidase peroxidase method was used in the estimation of plasma glucose concentration. Results showed changes in the pharmacokinetic parameters and blood glucose concentration were not significant ($p > 0.05$). This shows that artesunate does not alter the pharmacokinetics and of gliclazide in diabetic patients after single oral dose administration; and hence can be co-administered without dose modification.

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INTRODUCTION

Diabetes is health problems that occur when blood glucose remains high over a period of time (ADA, 2019). The diseases are classified as: Type 1 (insulin dependent) usually first diagnosed in children, teenagers and young adults; Type 2 (non-insulin-

dependent) often associated with older age; and gestational diabetes associated with pregnancy. Type 2 diabetes mellitus (T2DM) is a complex progressive disorder characterized by impaired insulin sensitivity, reduced insulin secretion and progressive failure of β -cells (Campbell et al., 2009). The

disease develops when the body doesn't make enough insulin or is not able to use insulin effectively, or both. As a result, glucose builds up in the blood instead of being absorbed by cells in the body. The body's cells are then starved of energy despite high blood glucose levels (WHO, 2016; CDC, 2017).

Gliclazide, a sulphonyl urea is a widely used drug for the treatment of type 2 diabetes (Yang et al., 2018). It is administered either alone or in combination with other oral antidiabetic drug. They act by blocking K channels in the pancreatic cells and extra pancreatic activation of voltage-dependent calcium channels and calcium influx, which triggers secretory granule exocytosis mediating insulin secretion (Drain, 2013). It has effect on both the early and late phase of insulin secretion (Ligtenberg and van Haeften, 2001).

Combination of drugs with gliclazide has been known to show varied effect as a result of drug interaction. It is known that hypoglycaemia may be potentiated when a gliclazide is used concurrently with some agents such as long-acting sulfonamides, tuberculostatics, NSAIDs, fibrates, monoamine oxidase inhibitors, salicylates, probenecid, and beta-blockers amongst others. This means that such polypharmacy is a risk factor in the treatment of Type 2 diabetes (Austin, 2006; Pinnamraju et al., 2018).

Due to the widespread use of artesunate combination in the treatment of malaria, drug interaction is a possibility in diabetic patients on gliclazide. Although gliclazide interactions with a number of drugs have been widely studied, there is no such interaction study conducted with artesunate to the best of our knowledge. This study is therefore aimed at determining the effect of artesunate on the pharmacokinetics of gliclazide in diabetic subjects.

MATERIALS AND METHODS

Subjects and ethical clearance

For the purposes of this study, diagnosis of diabetes mellitus was made by the presence of classic symptoms of

hyperglycemia and fasting plasma glucose concentration ≥ 130 mg/dl. The diagnosis was established at the Medical Outpatient Department (MOD) of Barau-Dikko Teaching Hospital, in Kaduna, Nigeria. The study plan was approved by Kaduna State Ministry of Health Ethical Committee (approval number MOH/ADM/744/T/17, dated 28th January, 2010) in accordance with the National Code of Health Research Ethics (2006), Federal Ministry of Health, Nigeria; and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was carried out at Barau-Dikko Teaching Hospital (formerly, Barau-Dikko Specialist Hospital), Kaduna, Nigeria between October and December, 2011. All volunteers gave their written informed consent, which was documented and archived.

Inclusion/exclusion criteria

Structured questionnaire was completed for each volunteer that included medical history, prior hospital admissions, and clinical and laboratory data. For inclusion, volunteers for the study are patients freshly diagnosed and were on lifestyle modification, willingness to fill an informed consent form, non-smokers, non-alcohol drinking, and willingness to abstain from heavy exercise. They were not on other medications and caffeine during the study, and have a Body Mass Index (BMI) less than 30 kg/m². Pregnancy and currently undergoing any medication or planned treatment during the study period were excluded.

Study design and blood sampling

One-way single dose cross-over study protocol in two periods was adopted for the study. Each Phase was preceded by an overnight fast. The subjects act as their own control. Phase 1 of the study involved the administration of a single oral dose of 80 mg of gliclazide after an overnight fast. After a wash out period of one week, 80 mg gliclazide and 100 mg artesunate were co-administered. Serial blood samples (5 ml) were collected at intervals of 0, 0.5, 1, 2, 4, 6, 8, 12, 16 and 24 h during each phase into an EDTA vacutainer.

Blood sample processing

Blood samples collected were centrifuged at 2000 rpm and plasma kept frozen in a freezer maintained at -20 °C prior to analysis. For the extraction of gliclazide from the plasma, the frozen plasma was thawed and to 1 ml of plasma were added 0.1ml of glipizide (internal standard, 20 µg/ml), 0.2 ml of 0.4 mol/l HCl, 5.0 ml of benzene- isopropanol (98:2, v/v) and was vortex-mixed for 2 min. Then mixed samples were centrifuged at 2000 rpm for 5 min. 4.0 ml of the upper layer was transferred into another tube. The extraction was dried in a hot air oven (Memmert 854 Schwalbach, Germany) at 40 °C. The residue was resolved with 0.15 ml methanol and 20 µl of solution was injected into the liquid chromatograph.

Determination of plasma gliclazide concentration

High Performance Liquid Chromatography (HPLC) method (Yang et al., 2004) was used in the estimation serum gliclazide concentration using a HPLC instrument (Shimadzu® chromatograph-LC-10 series, Japan). The system used (Shimadzu Corporation, Kyoto Japan) consist of Ultra-Fast LC-20AB prominence with the following accessories: SIL-20AC auto-sampler; DGU-20A3 degasser; SPD-M20A UV-diode array detector; 5µm VP-ODS C₁₈ and dimensions (4.6 x 150 mm); CTO-20AC column oven, CBM-20Alite system controller and Windows LCsolution software. The chromatographic conditions were made up of a mobile phase: solvent A: water (pH 2.8) 51%; solvent B: acetonitrile 49%; mode: isocratic; flow rate 1 ml/min; injection volume 20 µl detection UV 229 nm Column oven temperature was 40 °C. Glipizide was used as an internal standard. The total run time was 7.5 min.

Determination of glucose concentration

Glucose oxidase peroxidase method (Trinder, 1969) was used for this study. Plasma glucose concentration was measured over a period of 24 h at 9 time points-interval.

Ten µl of the plasma sample (A_{sample}) or standard (A_{standard}) was pipetted into a 1.5 ml microcentrifuge tube containing 1000 µl of the glucose reagent (Randox) mixed well and incubated for 10 min at 20±5°C. The slightly pink mixture was then transferred to 1cm path length cuvette and the absorbance of the standard and sample were measured at λ 500 nm against the 1000 µl reagent blank within 30 min. Glucose concentrations were determined according to the following equation: Glucose Concentration (mmol/L) = $\frac{A(\text{sample})}{A(\text{standard})} \times 5.5$. The plasma glucose concentrations for all time points between the two phases of the study were compared.

Pharmacokinetic analysis

Non-compartment method was used for the pharmacokinetic analysis. The pharmacokinetic parameters were determined for the two phases of the study. The Pharmacokinetic Software - PharmPK software (Joel et al., 2005) was used to generate the following Pharmacokinetic parameters: Maximum plasma concentration (C_{max}); Time to 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 hours to last measurable concentration ($AUMC_{0-t}$); Area under the Moment curve from zero hours to infinity ($AUMC_{0-\infty}$), Elimination half- life ($t_{1/2\text{eli}}$); and Elimination rate constant (K_{eli}). The method of residual was used to generate Absorption half- life ($t_{1/2\text{abs}}$), Absorption rate constant (K_{abs}) using Microsoft excel. Total body clearance (Cl), volume of distribution (VD) and Mean residence time (MRT).

Statistical analysis

Data were expressed as mean±SEM. GraphPad Prism Version 7.01 software for Windows (San Diego California, USA) was used for data Analysis using Student's T-test with $p < 0.05$ considered significant.

RESULTS

Subjects

Six subjects (2 males and 4 females) freshly diagnosed with diabetes mellitus on dietary and lifestyle modification met the inclusion criteria. Their mean age (years) were 54 ± 1 , and mean Body Mass Index (BMI) of 28.11 ± 0.381 . All subjects completed the treatment periods and were included in the pharmacokinetic analysis.

Plasma gliclazide concentration

Table 1 showed the mean pharmacokinetic parameter of gliclazide generated. The result showed large inter-patient variability from 37% - 75% of the pharmacokinetic parameter. The range of observed pharmacokinetic parameters are C_{max} 2.418-5.072 g/ml, T_{max} of 2-8 h, K_{eli} of 0.083-0.1524/h, $T_{1/2eli}$ of 4.550-8.3747, AUC_{0-t} of 22.915-36.252 $\mu\text{g}\cdot\text{h}/\text{ml}$, AUC_{0-inf} of 24.360- 41.234 $\mu\text{g}\cdot\text{h}/\text{ml}$, $AUMC_{0-t}$ of 182.477-261.096 $\mu\text{g}\cdot\text{h}/\text{ml}$, $AUMC_{0-inf}$ of 186.193-314.655 $\mu\text{g}\cdot\text{h}/\text{ml}$, MRT of 6.573-10.570 h, Cl of 1940.130-3284.028 ml/h, VD of 14977.240-.38392.667ml/h, $T_{1/2abs}$ of 0.4741-2.1359 h, and K_{abs} of 0.325-1.146 h.

Figure 1 shows the concentration-time profile of gliclazide when give alone and after co-administration with artesunate. The curve is monophasic with a maximal at

approximately 4 h for both phases of the study.

Glucose concentrations

Table 2 compares the mean glucose concentration from time zero to 24 h measured at 9 different time points. There was a 5% decrease in plasma glucose concentration at the T_{max} . All the changes on co-administration were not statistically significant from zero to 24 h with $p > 0.05$. For all time points, there were insignificant changes in the blood glucose concentration on co-administration of gliclazide with artesunate.

Figure 2 shows the mean plasma glucose concentration for both phases of the study. Maximum reduction in glucose concentration is seen to occur at time corresponding to the T_{max} . There was gradual increase in plasma glucose concentration after the point of maximal reduction. Gliclazide produced maximal hypoglycaemic activity at peak value at about 4 h. There was a mono phasic decline in blood glucose concentration corresponded with the concentration at T_{max} . A gradual decline in plasma glucose concentration was observed from zero hours to 4 h for both phases of the studies and thereafter, a gradual increase. None of the subject blood glucose level fell beyond the hypoglycaemic range during the study.

Table 1: Mean pharmacokinetic parameters of gliclazide ($n = 6$) when given alone and when co-administered with artesunate.

	Control		Gliclazide+		T-Test	
	(Gliclazide alone)		Artesunate		(paired value)	
	Mean	SEM(\pm)	Mean	SEM(\pm)	(P-Value)	
C_{max} ($\mu\text{g}/\text{ml}$)	3.616	0.180	2.542	0.188	$p > 0.05$	($p = 0.2446$)
T_{max} (h)	4.667	0.276	4.333	0.234	$p > 0.05$	($p = 0.8222$)
K_{eli} (h^{-1})	0.117	0.028	0.078	0.030	$p > 0.05$	($p = 0.0801$)
$T_{1/2eli}$ (h)	6.212	0.204	10.923	0.426	$p > 0.05$	($p = 0.1409$)
AUC_{0-t} ($\mu\text{g}\cdot\text{h}/\text{ml}$)	28.934	0.409	30.031	0.485	$p > 0.05$	($p = 0.8361$)

AUC _{0-inf} (µg.h/ml)	31.154	0.439	31.073	0.477	<i>p</i> >0.05	(<i>p</i> =0.9876)
AUMC _{0-t} (µg.h ² /ml)	212.529	0.964	281.596	1.419	<i>p</i> >0.05	(<i>p</i> =0.1529)
AUMC _{0-inf} (µg.h ² /ml)	243.523	1.146	481.053	2.339	<i>p</i> >0.05	(<i>p</i> =0.0368)
MRT (h)	7.950	0.201	17.969	0.750	<i>p</i> >0.05	(<i>p</i> =0.1420)
Cl (mlh ⁻¹)	2669.541	4.699	2758.267	5.894	<i>p</i> >0.05	(<i>p</i> =0.854)
VD (ml)	23966.619	18.079	49652.692	44.397	<i>p</i> >0.05	(<i>p</i> =0.2602)
T _{1/2 abs} (h)	1.447	0.164	1.183	0.149	<i>p</i> >0.05	(<i>p</i> =0.5765)
K _{abs} (h ⁻¹)	0.587	0.115	1.186	0.223	<i>p</i> >0.05	(<i>p</i> =0.4533)

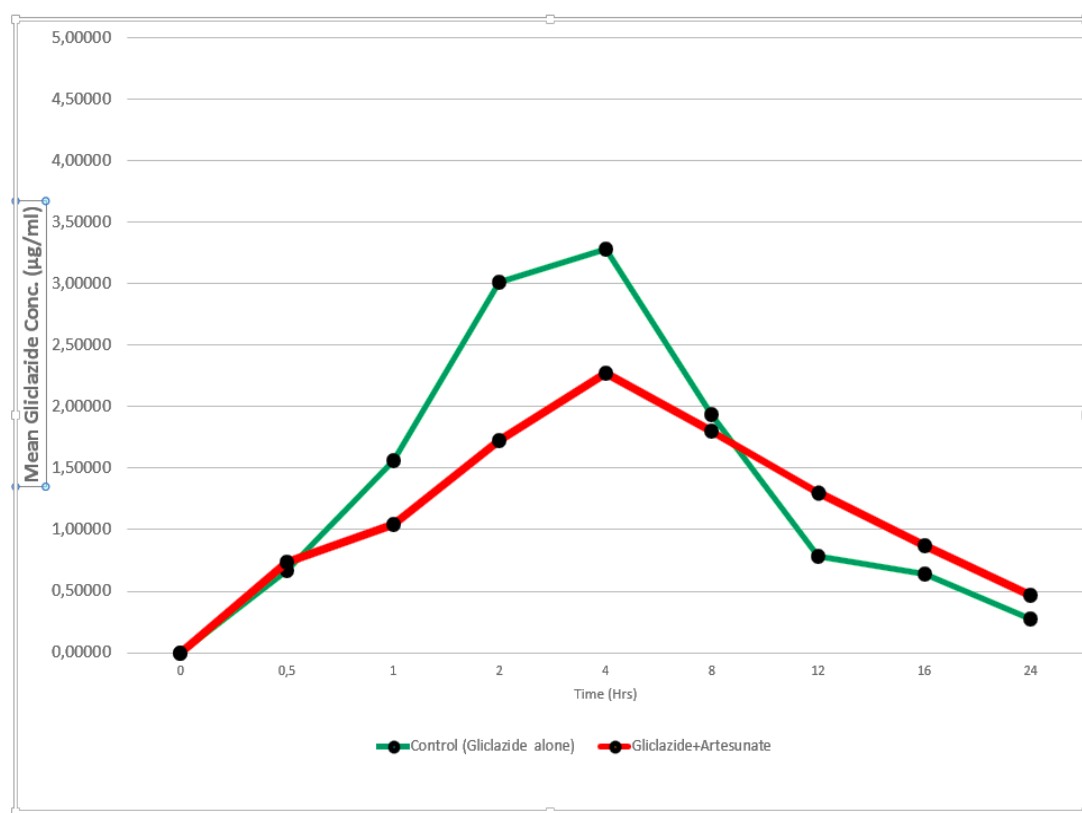


Figure 1: Mean concentration-time profile of glizalide when given alone and after co-administration with artesunate (n=6).

Table 2: Mean glucose concentration from time zero to 24 h measured at 9 different time points.

TIME (h)	Control (Gliclazide alone)		T-Test (paired value)		
	Mean	SEM(±)	Mean 2	SEM(±)	(P-Value)
0	8.22089	0.26116	8.94236	0.31007	$p>0.05$ ($p=0.7557$)
0.5	6.91550	0.29397	8.33132	0.28971	$p>0.05$ ($p=0.5498$)
1	7.32522	0.22222	7.90909	0.28752	$p>0.05$ ($p=0.7417$)
2	6.86758	0.20856	7.20107	0.29366	$p>0.05$ ($p=0.8585$)
4	6.28293	0.24982	5.94290	0.27228	$p>0.05$ ($p=0.8433$)
8	8.90112	0.19671	8.95882	0.30483	$p>0.05$ ($p=0.9701$)
12	9.66697	0.18039	9.64981	0.30910	$p>0.05$ ($p=0.9913$)
16	9.32272	0.22321	10.65855	0.27501	$p>0.05$ ($p=0.4439$)
24	9.84226	0.25802	11.33894	0.26378	$p>0.05$ ($p=0.4347$)

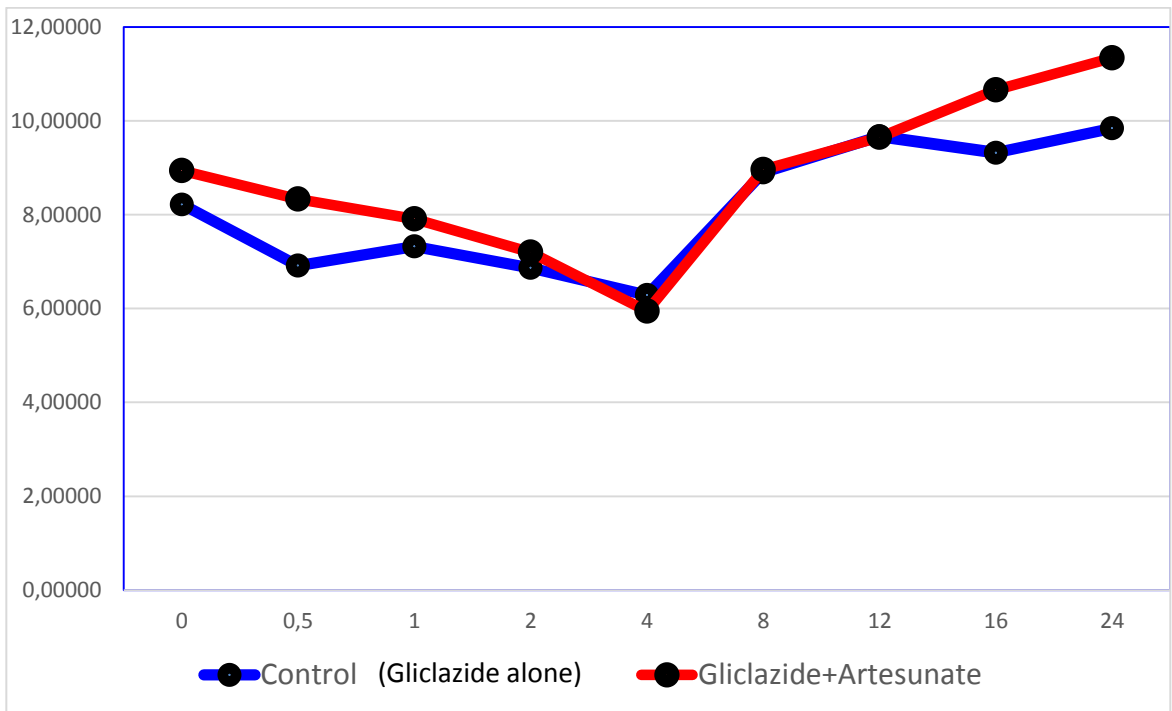


Figure 2: Mean Glucose concentration-time profile (n=6).

DISCUSSION

Diabetes is group of syndromes which require multiple drug therapy to manage and this might represent a frequent reason for additional complications related to pharmacotherapy (Ng et al., 2014). The importance of drug interaction studies belies in the fact that a number of drugs have been withdrawn from the market as a result of drug-drug interactions that were only discovered post-marketing due to partial or complete abolishment of treatment (CHMP, 2012). The fact that drug interaction has not yet been reported between gliclazide and artesunate this does not mean that it does or may not exist. With the high prevalence of malaria possibility of interaction of antimalarial drugs with gliclazide exists.

As seen in the result, all the pharmacokinetic parameters were not significant ($p>0.05$). Statistically, insignificant changes in primary absorption parameters denotes lack of effect of artesunate on the rate of absorption of gliclazide while changes in the values of AUC and AUMC showed that artesunate did not have a significant on the extent of absorption of gliclazide. The level of circulating gliclazide has not been significantly altered on co-administration with artesunate. Very large changes were observed in the measured values $T_{1/2_{eli}}$, K_{eli} and MRT. But these were not statistically significant. This might be due to high interpatient variability for these parameters. Interactions affecting distribution include interactions through modulation of active uptake or efflux transport of the drug, as well as displacement interactions (Uddin et al., 2016). Gliclazide is known to be highly protein bound (Sakar et al., 2011) but non-significant changes in VD clearly shows lack of displacement of gliclazide from the protein binding sites by artesunate. With non-significant changes in, Cl , $T_{1/2_{eli}}$, K_{eli} and MRT ($p>0.05$), the duration of action of gliclazide has not been affected by co-administration with artesunate. Lack of significant changes in clearance might mean that a decrease or increase in therapeutic effect might not have been affected as an increase in drug clearance results in the lesser

concentration of the active drug being present in while a decrease might result to toxicity (Uddin et al., 2016). These results suggest that observed changes in the blood gliclazide concentration might not be as result of metabolism or excretion.

The single dose study may not give information about the level of inhibition or induction, if any. The linear correlation between dose and gliclazide's minimum steady state concentration proved its linear pharmacokinetics. This again is seen when Figures 2 and 4 are compared with the correlation of C_{max} with greatest reduction of blood glucose concentration thus confirming their pharmacokinetic-pharmacodynamic relationship.

Conclusion

Conclusively, the studies showed that though artesunate does not affect the rate of absorption of gliclazide, it also does not affect the bioavailability and overall disposition of gliclazide after a single oral dose. This indicates that any interactions might not be harmful with little changes in the blood glucose concentration.

COMPETING INTERESTS

The authors declared that they have no competing interest.

AUTHORS' CONTRIBUTIONS

SGI, MTB, AM, IAY MG designed the study as part of SGI's PhD dissertation; SGI collected samples; SGI and BA performed experiment, analysed result and wrote draft of manuscript. All authors read and made input to final draft.

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