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PHARMACOKINETIC DIFFERENCES OF SOME GENERIC TABLET GLICLAZIDE 80 MG ON PAKISTANI POPULATION

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ABSTRACT

The goal of rational drug therapy is to produce a desired pharmacological response in an acceptable and predictable manner while minimizing the occurrence of undesired events. The Pharmacokinetics of different generics of tablet gliclazide 80 mg was investigated on healthy (10 x 2), Pakistani subjects. For this exploration an open-label, randomized, two-period crossover (Balanced in Complete Block Design) study, was conducted The out come of the said study suggests that all generics were found analogous regarding pharmacokinetic behavior in-spite of having different excipients, concentration of excipients, sources of raw material, manufacturing process, machinery, resources and also inter individual variation of the study. Results of the study also undoubtedly advocate that generics manufactured in different manufacturing units of Pakistan are near to the standard formulation and produce comparable results. No significant differences in pharmacokinetics parameters were observed, however, minor differences might narrate with inter individual variation in human volunteers and in different generic as well as different pharmaceutical unit.

INTRODUCTION

Gliclazide:

It is a second-generation sulfonylurea with hypoglycemic and potentially useful hemobiological properties. Gliclazide is one of the newer (second-generation) sulphonylureas and potential hypoglycemic drug (Kobayashi *et al.,* 1984). It may be preferred in the elderly - because of tablet scoring, sufficiently low doses can be prescribed. It has been shown to reduce platelet adhesiveness and increase fibrinolytic activity, factors thought to be implicated in the long- term pathogenesis of diabetes.

Chemistry:

Gliclazide is chemically described as1-(3-Azabicyclo[3.3.0]oct-3-yl)-3- *p*-tolylsulphonylurea. Its empirical formula is $C_{15}H_{21}N_3O_3S$ and the relative molecular mass is 323.4. It is synthesized by the addition of a **N** containing heterocyclic ring with an endocyclic double bond to the sulfonylurea grouping.

Pharmacokinetic Studies:

This is the first choice of the study in respect of bioavailability or bioequivalence. A specific, selective and sensitive bio-analytical method is mandatory to measure the drugs, its metabolites

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concentration and biochemical marker inside the body fluid which provides:

- Comparative Bioavailability of Locally manufactured drugs formulation against same generic formulation approved by the national or international regulatory agencies for safe use of human.
- Understanding of the bioequivalence problems of same generic drugs and inters individual variation.
- Differentiation of pharmacokinetics from population to population due to different phenotype, dietary patterns, difference in physiological and pharmacokinetic factors.
- Contribution to rationale drug use through the appropriate use of pharmacokinetic techniques, therapeutic drug monitoring and interpretation of the literature.

Pharmacokinetics of Gliclazide:

A consistent and optimal release of Gliclazide from this formulation leads to a low to moderate overall variability of its pharmacokinetics parameters. (Delrat *et al.*, 2002). The pharmacokinetics of oral gliclazide appears more complex than previously thought. Gliclazide. (Davis *et al.,* 2000) The pharmacokinetics parameters did not differ significantly between healthy and diabetic subjects or between single and successive administrations; moreover, they did not differ between the free and total drug level. Although there were inter subject variations, the therapeutic effects of oral administration of Gliclazide on serum glucose and insulin levels were found in four diabetic patients. Study also suggested pharmacokinetics of the total Gliclazide level reflect those of the free Gliclazide in serum. (Kobayashi *et al.,* 1984) Kobayashi (1981) reported that each pharmacokinetic parameter was not changed by differences between the healthy and diabetic subjects, the total and free drug levels, and method of administration of the drug; each mean parameter

Absorption, Distribution, Metabolism and Excretion:

Single oral administration of a 30 mg tablet, gliclazide was completely absorbed both under fasted and fed conditions (Delrat *et al*., 2002). Gliclazide belongs to the group of intermediate acting hypoglycaemic agents. It is rapidly absorbed from the GIT, reaching peak serum concentrations within 4 to 6 hours. This results in reductions of blood glucose levels by approximately 23% five hours after a single dose was administered. There is extensive binding of Gliclazide to plasma proteins. The half-life of gliclazide is approximately 12 hours. Gliclazide is distributed in the extracellular fluid, leading to high concentrations in the liver, kidneys, skin, lungs, skeletal muscle, intestinal and cardiac tissue when administered to animals. There appears to be negligible penetration into the central nervous system. Gliclazide also crosses the placental barrier and circulates in the foetal blood system. A low apparent volume of distribution is probably reflected in the high degree of gliclazide binding to proteins (approximately 94% at a plasma concentration of 8mcg/mL). Metabolism of gliclazide occurs in the liver, being metabolized into at least eight metabolites, which have been identified using thin layer and gas-liquid chromatography. The identity of only one metabolite is known (ρ-toluene sulphonamide). None of the metabolites have any recorded hypoglycaemic activity. Approximately 70% of the administered dose is excreted slowly in the urine, reaching a peak 7 to 10 hours after administration. Metabolites are detectable in the urine 120 hours after administration. Faecal elimination accounts for approximately 11 % of the administered dose. Gliclazide is usually completely eliminated within 144 hours post dose.

The drug is metabolized extensively in the liver but unchanged drug is the predominant constituent in plasma. From 60 to 80% of a dose is excreted in the urine, primarily as metabolites (Davis *et al*., 2000).

Bioavailability of Gliclazide:

Systemic bioavailability of an oral dose varies considerably leading to slow or fast absorbers (Campbell DB et al 1980). This may be attributed to differences in first pass metabolism than absorption rate. The oral bioavailability of gliclazide is at least 80% (Davis *et al.,* 2000) Peak serum concentrations of gliclazide usually occur 2 to 4 hours following oral doses, although a delay of up to 8 hours has been reported; considerable variation in peak levels has been observed. (Davis *et al.,* 2000) 2.8 h was reported by Kobayashi K et al 1984 and 1981 Mean-10hrs (range: 6-14 hrs) (Campbell *et al*., 1980 and Kobayashi, 1984). The elimination half-life of gliclazide is 8 to 12 hours. (Davis *et al.,* 2000). Vd: 17-25 L. (15-40% of body weight), 17.4 L and 16.4 L as reported by Kobayashi *et al.* (1984), (Kobayashi *et al*., 1981) respectvely 12.3 h. Elimination rate (ke) 0.07 hr-1hrs elimination half life has reported by Kobayashi *et al*. (1984) (Kobayashi *et al.,* 1981).

MATERIALS AND METHODS

Table-1
Label information of Tab gliclazide 80 mg different brands
used in the pharmacokinetics and pharmacodynamics study

ID	Batch No	Mfg. Date	Exp. Date	Status
1/A	RIPSGT1	Dec 1999	Dec 2002	T_1
2/B	BE001	Sep 1999	Sep 2002	T_2
2/C	9E001	Sep 1999	Sep 2002	T_3
4/D	GL01	Nov 1999	Nov 2001	T_4
5/E	E0700	June 1999	June 2002	R_1

T= Test R= Reference

Study Design:

Study was designed according to described objectives and conducted on a triple occasion, (F.D.A. 1997, Draft Guidance for industry) with an interval of one week between the first and second occasion. In both occasion allotted brands were administered according to study designed (Table-2). The exactly same diet in quantity and quality was provided to the volunteers on both occasions. Water consumption was restricted two hours prior to and up to half an hour after the dosing.

Crossover Design:

In this design each subject is successfully exposed to two or more treatments in random order. As the same subject is exposed to different treatments, each subject's acts as his own control, but before getting exposed to the next treatment, each subject should be brought back to the status prior to exposure of treatment. The period with out exposure to any treatment is called a "washout" period. Introduction of a washout period between two successive treatments prevents the influence of treatment given in the first period from having any impact on the response to treatment given in the second period.

Balance Incomplete Block Design (BIBD):

In case there are more than three formulations, the Latin Square Design is not ethically advisable, mainly because each volunteer is subject to frequent pricking for drawing too many

blood samples. As per this design five possible pairs of subjected formulations were made and each volunteer received two different formulation on both occasion in such a way that every formulation were given to two different volunteers per occasion. Test E was manufactured in research lab for completion of BIBD study only and not incorporated in results.

Dosing:

Five different formulations (Table-1) make ten possible pairs in two periods (AB, BC, CD, DE, EA, AC, CE, EB, BD and DA). These pairs were assigned randomly to ten volunteers. Each volunteer took the first brand of the pair on first blood level trial and second brand on the second blood level trial. This way study was balanced over occasions. On each occasion each formulation was tested on two subjects. The scheme of brands administered to the volunteers is reported in Table II. Each volunteer was given orally one randomly selected branded tablet of 80 mg Gliclazide with one glass (240 ml) of drinking water. Zero time blood samples were collected before the dosing. Breakfast was served half an hour of the dosing and lunch was given five hours after.

Table-2

Scheme of dosing of five different formulations of Tab gliclazide 80 mg used in the pharmacokinetics study

Volunteer no	Volunteer ID	1st Occasions	2nd Occasions
01	AM	A	B
02	AR	A	C
03	AT	B	C
04	KA	B	D
05	MA	C	D
06	AH	C	E
07	MS	D	A
08	US	D	E
09	OB	E	B
10	ZA	E	A

Volunteers:

The panel of human subjects consisted of ten human, healthy, adults, male volunteers weighing between 48-85 kg, with a height range of 62-70 cm and of age between 29-49 years. Detail of volunteers is given in Table-3.

Health/Safety Evaluation:

The panel members were given a general medical examination to establish good health. The following selection criteria were used for this purpose.

- No congenital disease
- No underline hypertension
- No diabetic
- History in family

Response of the following were checked and got normal:

- Hepatic
- Gastrointestinal tract
- Cardiovascular
- Respiratory tract
- Hematological
- Neurological
- Psychiatric

Table-3

Description of volunteers participated in the pharmacokinetics study

Volunteer		Age	Weight	Height	Blood Pressure mm Hg		Body Temp	BSA*
No	**ID**	**Years**	**Kg**	**Cm**	**Systolic**	**Dia-stolic**	**F°**	**m^2**
1	VI	23	51	157.5	120	80	98.5	1.49
2	V2	24	57	162.6	110	80	98.0	1.60
3	V3	21	55	157.5	110	75	98.0	1.55
4	V4	24	68	165.1	125	90	98.9	1.76
5	V5	21	54	165.1	120	85	98.1	1.57
6	V6	24	75	177.8	125	90	98.0	1.92
7	V7	23	55	162.5	120	80	98.3	1.57
8	V8	20	48	162.5	115	75	98.2	1.47
9	V9	28	49	165.1	105	65	98.0	1.49
10	V0	49	85	171.4	130	90	98.2	2.01
Mean ±SEM		25.7	59.7	164.7	118	81	98.2	1.64
		2.55	3.70	1.85	2.39	2.45	0.09	0.06

*Body Surface Area.

No history of hypersensitivity to sulfonylurea, Gliclazide and to any other ingredient used in the formulation of test or reference drug. Neither treatment taken nor any drug used for at least a month prior to the study. Pre and post study biochemical/hematological tests other then physical examination by a consulting physician were performed in order to assure the safety/selection based on this criteria. Each subject was observed throughout the study for possible adverse event and shortly monitored the blood glucose level. The consulting physician continued physical examination during the study regarding vital signs.

Exclusion Criteria:

Subjects with any current or past medical condition that might significantly affect their pharmacokinetics and pharmacodynamics response to the administered drug were the limiting factors in the study. All volunteers were thoroughly informed about the aims and objectives of the study, the drug to be tested, and the hazards/ side effects of the drug Gliclazide and also their rights to separate themselves from the study at any stage without mentioning any reason. Informed consent was also obtained from each subject to participate in the study.

Restrictions:

No volunteer was allowed to take any prescription or OTC drug one week prior to dosing and during the study period, in order to avoid interference with the kinetic behavior of Gliclazide in

the body or in the determination of drug in the blood. The volunteers were instructed to report the investigator about the illness/side effects and the treatment undertaken. No volunteer took any drug for at least one month prior to and during the study.

Blood Collection, Handling and Bio-analysis:

Before drug administration, a control/blank venous blood sample was collected from each volunteer through sterile disposable 5cc syringe (BD) aseptically inserted in the vein of left/right arms. Blood was immediately transferred to heparinzed centrifuge tubes. Serial blood samples were drawn and 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 and 10.0 hours after the dose through disposable syringes as mentioned above. The blood samples in heparinzed centrifuge tubes were chilled in a refrigerator until centrifuge (not later than 30 minutes from collection) for 15 minutes at approximately 2000 rpm. Extraction of drugs from samples and their bio-analysis is carried out by using High performance liquid chromatography according to the method reported Roohi Obaid *et al.* (2002).

Data Quality Assurance:

Data obtained throughout the study was recorded on the subjects' in-process control sheet for bio-analysis of Gliclazide. Steps taken to assure accurate and reliable data included selection of research associate review of protocol procedure and report with the principle investigator and associated personnel at an investigator meeting. Labels of different color were used for differentiation of samples.

Pharmacokinetic Calculation:

Microsoft 1997 program was utilized for the calculation of pharmacokinetic parameters.

RESULTS AND DISCUSSION

Plasma Levels:

The plasma level profiles along with SEM bars are shown in Fig. 1. To evaluate any significance difference in blood level patterns of test product and reference product, ANOVA (analysis of variance) was performed. No significance difference could be detected in all the five formulation at the level (Fig. 2) of $p>0.05$. ANOVA is given in Table-4. It reveals that the all five formulation are almost similar to each other in producing the blood levels and consequently in rate and extent of availability of the drug to the systemic circulation.

Bioavailability, Bioequivalence and Pharmacokinetic:

The mean value for area under the curve AUC, peak plasma levels; C_{max}, time to peak; T_{max}, absorption rate constant; ka and mean residence time MRT are reported in Tables-4 and 7. Fig. 2 reveals the blood level profile of the test products with the kinetic analysis. For evaluation of any significance difference among the test products and reference product the main bioavailability parameter and the therapeutic efficacy meter; extent of hypoglycemia was analyzed for analysis of variance. The analysis results are given in Table-5. The ANOVA Tables reveal that among test products and the reference brand no significance difference exists at $p>0.05$ in the bioavailability and therapeutic efficacy. Time to peak concentration, and maximum concentration and AUC (mean N=20) of gliclazide tablet 80 mg was observed at 3 hours, 5.761 ± 2.435 microgram/liter and 28.986 ± 4.645 ng. hr/ml respectively after drug administration to 20 human healthy volunteers, while, inter formulation difference was observed in this study which is not significant enough. Our data is in accordance with the study reporting 2-4 hour T_{max} (Davis *et al.,* 2000). There have been several studies of the pharmacokinetics and efficacy of gliclazide in diabetic patients (Campbell *et al*., 1980; Kobayashi *et al*., 1981; Forette *et al*., 1982 and Shiba *et al.,* 1986). The reason for the

discrepancy between the half-life and duration of action of sulfonylurea is not clear (Lebovitz and Feinglos, 1983). As not unexpectedly, sulfonylurea may cause hypoglycemic reaction including coma (Ferner and Neil, 1988; Seltzer, 1989). Sulfonylurea can be ranked in order of decreasing risk of causing hypoglycemia based on the half-life.

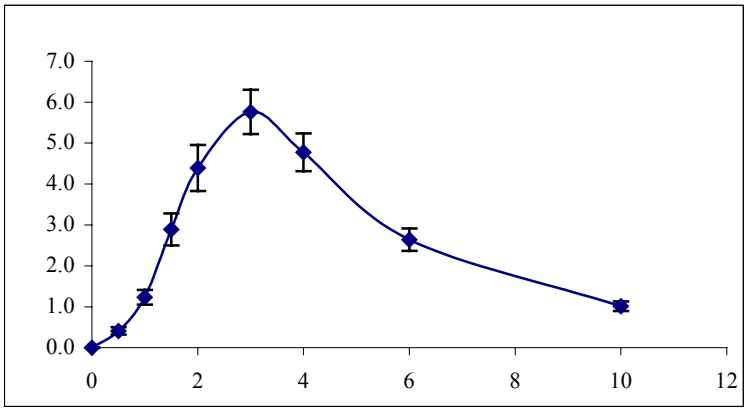

Fig. 1**:** Time (Hours) versus concentration (ug/ ml) plasma profile Plasma concentration of gliclazide Mean (n=20) ± SEM following an oral dose of 80 mg tablet to healthy, human, male, adult volunteers.

Table-4
Mean plasma levels of gliclazide along with ± SEM obtained
after oral administration of five different formulations

Time	Formulation A	Formulation B	Formulation C	Formulation D	Formulation E
0.5	0.226 ± 0.050	0.296 ± 0.152	0.810 ± 0.223	0.176 ± 0.098	0.534 ± 0.282
1.0	0.258 ± 0.272	1.427 ± 0.282	1.901 ± 0.254	1.182 ± 0.116	1.418 ± 0.750
1.5	0.280 ± 0.764	3.431 ± 1.025	4.938 ± 1.105	2.571 ± 0.361	3.236 ± 0.755
2.0	2.430 ± 1.080	4.139 ± 1.472	6.180 ± 1.450	4.075 ± 0.867	5.127 ± 1.287
3.0	6.575 ± 1.282	4.050 ± 1.404	4.911 ± 1.464	6.183 ± 1.223	7.086 ± 1.066
4.0	5.657 ± 1.401	3.968 ± 1.108	3.610 ± 1.181	5.784 ± 0.914	4.849 ± 0.902
6.0	2.398 ± 0.683	3.114 ± 0.567	2.439 ± 0.534	3.097 ± 0.359	2.146 ± 0.491
10.0	0.880 ± 0.228	1.503 ± 0.246	1.089 ± 0.140	1.046 ± 0.165	0.573 ± 0.222

Table-5
Analysis of Variance for Bioavailability of Gliclazide from Five Formulations

Weeks	1.4474	1	1.4474	0.0388
Subjects	1730.1874	9	192.2430	5.1482
Formulation	32.6840	4	8.1710	0.2188^{ns}
Error	186.7086	5	37.3417	--
Total	1951.0274	19	102.6857	2.7499

Table-6
Analysis of Variance for Blood Level Pattern of Gliclazide all over the Sampling Time

Weeks	5.3985	1	5.3985	1.9727
Subjects	127.9385	9	14.2154	5.1945
Formulation	10.2885	4	2.5721	0.9399^{ns}
Error	13.6830	5	2.7366	--
Total	157.3085	19	8.2794	3.0254
Times	5.29.8985	7	75.6998	78.4222
Time*Week	44.5115	7	6.3588	6.5875
Time*Subjects	115.0415	63	1.8261	1.8917
Time*Formul.	70.4835	28	2.5173	2.6078^{ns}
Subplot Error	33.785	35	0.9653	--
Total Subplot	793.7200	140	0.9653	5.8733

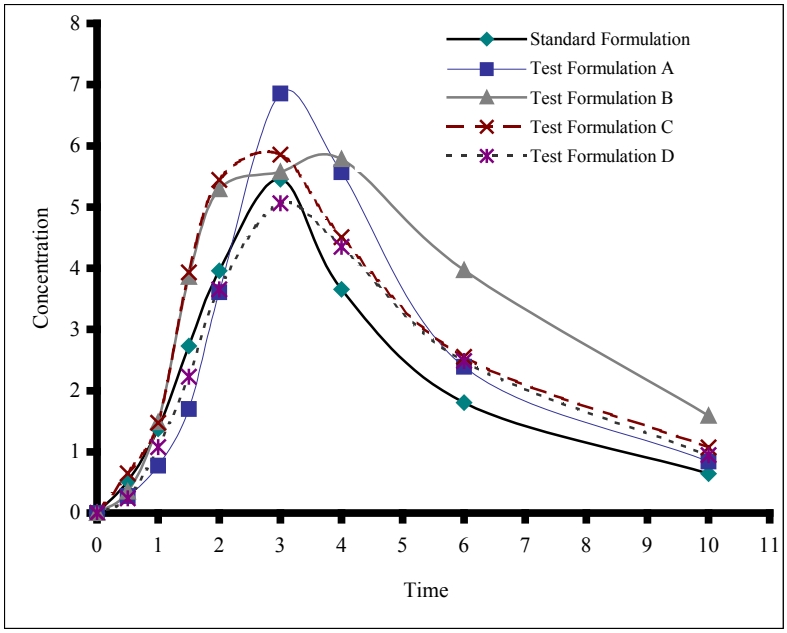

Fig. 2: Mean plasma levels of gliclazide obtained after oral administration of five different formulations of 80 mg tablet.

Results of the study also undoubtedly advocate that formulations manufactured in different manufacturing units of Pakistan are near to the standard formulation and produce comparable results. No significant differences in pharmacokinetics or parameters were observed, however, comparable differences might relate with inter individual variation in human volunteers and in different formulation as well as different pharmaceutical unit. There are reasonable evidences for important changes in both pharmacokinetic and pharmacodynamic parameters due to body composition changes even in the absence of diseases. Although this data assure the ultimate quality of gliclazide tablet manufactured in Pakistan but every formulation should be studied for assurance of safety and efficacy because life of patient is a matter of concern.

Table-7
Pharmacokinetics calculation of different formulations of gliclazide 80 mg after per oral administration to the healthy, human, adult, male Pakistani volunteers

PK Parameter		Std A	Test B	Test C	Test D	Average
		Formulation E	Formulation B	Formulation C	Formulation D	
AUC	Mean	28.3830	28.0310	28.2800	31.2630	28.9893
	±SEM	4.0750	5.0620	5.6860	3.7580	4.6453
C_{max}	Mean	7.0900	4.1400	6.1800	6.1800	5.8975
	±SEM	0.9630	1.4920	1.4280	1.0740	1.2393
T_{max}	Mean	3.0000	2.0000	2.0000	3.0000	2.5000
	±SEM	0.3750	0.6120	0.3750	0.2500	0.4030
K_a	Mean	0.8280	1.2390	0.8280	0.6640	0.8898
	±SEM	0.3740	0.2210	0.4200	0.0690	0.2710
MRT	Mean	3.9600	4.0100	3.9600	4.3400	4.0675
	±SEM	0.1430	0.2310	0.1150	0.1130	0.1505

PK = Pharmacokinetics

CONCLUSION

HPLC technique is playing a vital role in drug screening in clinical/ research laboratories. It provides easiest, effective, accurate, sensitive, precise and validated way to determine minute quantity of drug from different samples. Pharmacokinetics evaluation of any drug may help in assessment of any new molecule as well as formulation. Ultimate quality of any drug can only be assessed by end response of the drug to the body, which can be done by measuring the body behavior toward drugs. Difference in price and quality of same formulations of different companies can be seen and observed but ultimate quality assessment cannot be achieved without pharmacokinetic studies. It is also not necessary that costly drug is more effective, until it is not proved. The out come of the said study suggests that all formulations were found almost equivalent regarding pharmacokinetic evaluation in-spite of having different excipients, concentration of excipients, sources of raw material, manufacturing process, machinery, resources and also inter individual variation of the study.

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