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RESEARCH ARTICLE

Effect of Food on the Pharmacokinetics of Gliclazide 60 mg Modified Release Tablet in Healthy Caucasian Volunteers

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Objective: To evaluate the food effect on glicazide disposition in clinical trials conducted on healthy Caucasian volunteers who were given a new modified release oral formulation of Gliclazide 60 mg developed by Sun Pharmaceutical Industries, India. **Methods**: The studies were designed as open-label, randomized, single-dose, crossover studies that consisted of two periods. During each study, venous blood samples were taken before and after drug administration up to 96 hours. Subsequently, individual plasma profiles were determined and non-compartmental method was employed for the assessment of food effect on the pharmacokinetic profile of gliclazide. The statistical significance of differences for the main pharmacokinetic parameters was evaluated by ANOVA test, for $p < 0.05$ statistical significance was decided. The relative profiles of absorption of gliclazide were obtained by mathematical deconvolution. All calculation were performed by Phoenix WinNonlin®. **Results**: High-fat, high-calorie meal decreased gliclazide exposure. The mean maximum plasma concentration decreased with 14%, while the mean total area under the plasma concentration-time profile registered a 17% decrease. The elimination half-lives under fasted and fed conditions were comparable and the time to maximum plasma concentration was shortened under fed condition. Safety evaluation showed that overall gliclazide was well tolerated under both fasted and fed condition. **Conclusions**: The statistical analysis revealed the lack of food effect on the new modified release tablets of Gliclazide 60 mg. However, before stating a definite conclusion regarding the food effect on gliclazide pharmacokinetic profile, additional studies on patients with type 2 diabetes mellitus should be conducted.

Keywords: gliclazide, food effect, pharmacokinetics, clinical trial, healthy Caucasian subjects

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Introduction

Diabetes mellitus (DM) is a major health concern worldwide, which has reached epidemic proportions by currently affecting more than 422 million people around the world, with a prevalence among adults that has risen to 8.5% in 2014 [1,2]. Only in 2015, DM directly caused an estimated 1.6 million deaths, and World Health Organization (WHO) presumes that by 2030 DM will become the seventh leading cause of death worldwide, while the International Diabetes Federation (IDF) forecasts that by 2035 the total number of people suffering of DM will rise to 592 million [1,3]. Type 2 diabetes mellitus (T2DM) is non-insulin-dependent DM accounting for approximately 90 to 95% of all diagnosed cases of DM, and is characterized by insulin resistance in tissues and/or in pancreatic β-cell dysfunction (defects in insulin secretion) [4,5]. Eventually, this leads to progressive hyperglycemia and reduced sensitivity to insulin in tissues such as liver and muscle [6]. The insulin resistance developed in the liver contributes to elevated hepatic gluconeogenesis, while in muscle the glucose uptake is limited [7]. Furthermore, the adipose tissue may exacerbate the development of insulin resistance by secreting inflammatory cytokines (i.e., IL-6 and TNF-α) into the bloodstream and thus lead to the progression of T2DM [8,9]. Long-term hyperglycemia can lead to complications at microvascular level (retinopathy, neuropathy and nephropathy) and macrovascular level (cerebrovascular, cardiovascular and peripheral vascular disease), thus increasing the mortality, morbidity, and healthcare costs and, on the other hand, reducing the quality of life and life span [6,8]. The risk factors for T2DM include obesity, physical inactivity, impaired glucose metabolism, family history of diabetes, tobacco use, race/ethnicity, and recent data suggest the genetic predisposition (more precisely genes CAPN10, PPARG, TCF7L2, KCNJI1, FTO, HHEXIDE, KCNQ1 and MC4R) as a key to susceptibility often correlated with rapid environment changes (for instance, lifestyle factors) [4].

The main objective of T2DM therapy is to reduce hyperglycemia, reach target blood glucose level and maintain a glycosylated hemoglobin (HbA1c) concentration of ≤ 7%, as recommended by The American Diabetes Association, or even ≤ 6.5 % if it can be achieved in an affordable and safe manner [6,10]. These objectives are attained by elevating plasma insulin levels (by administering oral agents that promote insulin secretion or by direct insulin administra-

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tion), improving insulin sensitivity of tissues, and reducing the extent of carbohydrate absorption from the gastrointestinal tract [5]. These therapeutic effects can be achieved through insulin secretaogues (for instance, sulphonylureas such as gliclazide, or meglitinides), insulin sensitisers (e.g. thiazolidinediones), and external insulin delivery (insulin analogues) [4,6].

Gliclazide is an oral hypoglycemic agent indicated for the treatment of T2DM. It binds to β-cell sulfonylurea receptor and blocks K_{ATP} channels, leading to depolarization of β-cells and decreased potassium efflux, thus opening the voltage-dependent Ca^{2+} channels resulting in calmodulin activation, and eventually leading to release of insulin-containing secretory granules [11]. As gliclazide belongs to the second generation class of sulphonylureas, it is widely used as the second-line recommended treatment of hyperglycemia after metformin, or in combination with it when metformin alone does not suffice to control blood glucose level [6,12,13]. It displays affinity only for sulphonylurea receptors localized in β-cell type (SUR1), smooth muscle and adipose tissue (SUR2B) [6]. Studies have reported that sulphonylureas can reduce the Hb1Ac levels by around 1.51% if used as monotherapy, or by approximately 1.62% when used in combination with oral diabetes treatment [6]. Considering the advantages of the second-generation sulphonylureas, it is very likely that gliclazide is commonly prescribed for the treatment of T2DM worldwide.

Gliclazide displays rapid and complete absorption after oral administration, with inter-individual variability regarding the time to reach the peak plasma concentration (t_{max}). Also, age related differences were observed for this parameter and for peak plasma concentration (C_{max}), which is attained within 4-6 h after oral administration [11]. Steady-state concentration of gliclazide is attained after 2 days administration of 40 to 120 mg, with increased C_{max} and t_{max} after multiple doses. A low volume of distribution (13 to 24 L) due to high plasma protein binding affinity (85 to 97%) characterizes gliclazide [11]. It is extensively metabolized to 7 metabolites mainly in the liver, primarily by CYP2C9 and to a lesser extent by CYP2C19 and CYP2C18 [14]. The kidneys (60-70%) and feces (10-20%) eliminate its metabolites and conjugates. The hypoglycemic effect of gliclazide can display individual differences because of CYP2C9 polymorphism in addition to pharmacodynamics factors [14,15]. The half-life of elimination ($t_{½}$) varies from 8.1 h to 20.5 h after administration of 40 to 120 mg p.o. The initial dosage of gliclazide is 40 mg per day and can be increased to 320 mg daily, depending on the severity of glycaemia and disease state [11]. Modified release pharmaceutical formulations which exist already on the market release gliclazide through a gel layer which is formed as a consequence of the hydration of the tablet, thus assuring a prolonged release of the active substance for a better glycaemia control, lesser tablet administrations per day, high compliance and adherence to treatment of T2DM patients [16,17].

In Romania, approximately 12.4% of the population was diagnosed with diabetes, according to the 8th Edition of International Diabetes Federation (2017), many of them currently being under medication with hypoglycemic agents such as gliclazide [18]. A new modified release oral tablet of *Gliclazide 60 mg* was developed *by* Ranbaxy Laboratories Limited, now Sun Pharmaceutical Industries, India. The formulation was proved bioequivalent with Diamicron® MR (Servier, France) in a previous study conducted at the Clinical Pharmacology and Pharmacokinetics Department of Terapia SA, Romania [19]. The aim of the study presented in this article was to evaluate the food effect on gliclazide pharmacokinetics in healthy Caucasian volunteers by comparing the data obtained in two bioequivalence study of the newly developed tablet with Diamicron® MR (Servier, France), conducted under fasted and fed condition of subjects.

Materials and methods

Subjects

The clinical trials were conducted in accordance with US 21 CFR Part 320, the ICH E6 (R1), Good Clinical Practice guidelines and the principles of Helsinki (1964) and its amendments (Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996, Edinburgh 2000, Washington DC 2002, Tokyo 2004, Seoul 2008 and Brazil 2013). The study protocols were approved by the Ethics Committee of the University of Medicine and Pharmacy "Iuliu Hatieganu", from Cluj-Napoca (Romania) and by the National Agency for Medicines and Medical Devices, Romania. A written informed consent was obtained from each volunteer prior to performing any procedures related to the clinical studies.

The studies took place at the Clinical Pharmacology and Pharmacokinetics Department of Terapia SA, Romania.

Forty-eight Caucasian males and females were enrolled in the fasted study, the Reference data of the assessment, while twenty-six healthy volunteers were enrolled in the fed study, the Test data of the assessment. Inclusion criteria required the study population to be healthy, nonsmoking Caucasian females or males, aged between 18 and 55 years. Their health status was evaluated according to their medical history, physical examination, electrocardiogram (ECG) and routine laboratory investigations (biochemistry, hematology, urine and serological tests). In case of female volunteers, a pregnancy test was carried out at screening, prior to admission in each study period, as well as at the end of the clinical trial. The volunteers were also tested for hepatitis B surface antigen, anti-HIV, anti-hepatitis C antibody, and in case of positive results they were further excluded from the trial. Also, subjects were excluded from the study if they were smokers, they had a history of drug or alcohol abuse, a history of documented allergy, a hypersensitivity to gliclazide, or if they were under regular medication. Exclusion criteria were also considered any medical

condition, dietary products or lifestyle factors that could influence drug response. Drug intake was evaluated for 28 days prior to studies in order to avoid any drug-drug interaction or enzymatic induction/inhibition.

The number of subjects for each study period was assessed based on: test/reference ratio in the range of 95%-105% for fasting conditions and respectively 90%-110% for fed conditions, an approximately 30% expected coefficient of variation in fasting condition and respectively 15% in fed conditions, power of 80%, level of significance of 0.05, bioequivalence interval 0.8 -1.25 using SAS software version 9.1.3 and the possible withdrawals and/or dropouts were also taken into consideration.

Study design and drug administration

The data were collected from two bioequivalence studies; each consisted in gliclazide administration with or without food [19]. Each study was designed as an open-label, randomized, single-dose, crossover study that consisted of two periods during which the subjects were given the test product developed by Ranbaxy Laboratories Limited, now Sun Pharmaceutical Industries, India, and reference product Diamicron® MR, Servier, France [19]. For the current assessment of the food effect on the pharmacokinetics of gliclazide, the Reference data was considered the study conducted under fasted condition, while Test data was elected the study conducted under fed state of subjects; in each study the new formulation Gliclazide 60 mg developed by Sun Pharmaceutical Industries, India, was administered to every subject.

During the fasted study, Reference data, the test product was administered to all volunteers after a fasting period of at least 10 h. The drug (gliclazide 60 mg) was taken as a single-dose under medical supervision and trained study personnel with 240 mL of 20% glucose solution. After drug administration, 60 mL of 20% glucose solution were given to each volunteers every 15 min for up to 4 h post-dose in order to avoid hypoglycemia. Moreover, post-dose no food intake was allowed for at least 4 hr. Furthermore, subjects were allowed to drink water only with at least 1 h pre-dose and starting 2 h post-dosing. During their 46 h confinement at the Clinical Pharmacology and Pharmacokinetics Department of Terapia SA, Romania, they were provided with identical standardized meals at the same hours in each study period. All healthy volunteers were given gliclazide while seated and were carefully instructed to remain seated or in semi-supine position for 2 h post-dose.

The study protocol and drug administration for the fed study (Test data) was similar to the Reference period, with the following difference: after a 10 h fasting period, all subjects were given a high-fat, high-calorie standard meal, 30 minutes before drug administration and the confinement was of 46.5 h. Thus, during the Test period, all volunteers were under fed condition, while during the Reference period, the pharmacokinetic profile of gliclazide 60 mg was obtained under fasting condition.

The pharmaceutical product used was Gliclazide 60 mg, a new modified release oral tablet developed by Ranbaxy Laboratories Limited, now Sun Pharmaceutical Industries, India.

Blood plasma samples collection and bioanalytical methods

During the Reference period, venous blood samples (4 ml) were drawn prior to drug administration and at 1, 2, 3, 4, 5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 15, 16, 20, 24, 36, 48, 72 and 96 hours after drug administration. For the Test period, the sampling design was the following: pre-dosing and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 20, 24, 36, 48, 72 and 96 hours post-dose. The blood samples were collected in K_3-EDTA vacutainers, centrifuged for 15 min at 4000 rpm under refrigeration and the separated plasma was recovered and kept at -50°C until analysis.

The plasma samples were prepared for analysis as it follows: over 150 µL of plasma were added approximately 50 µL of internal standard dilution (Gliclazide D4, concentration 1500 ng/mL) and 150 µL of water and the mixture was vortex mixed. Afterwards, the solid-phase extraction of the samples was carried out in cartridges (type Cleanert PEP-H 30 mg/mL), which were previously conditioned with 1 mL of water and 1 mL of methanol. Consequently, gliclazide was extracted by evaporation under nitrogen steam by using methanol and the obtained residue was reconstituted with 200 µL of mobile phase. All samples were processed in the previously presented manner under low light conditions.

Table I. Performance of the 2 tests

Center	Moment	Specificity	CI95%	PPV	CI95%	P
Târgu Mureș	Clonidine 30 minutes	6.82%	1.43-18.46%	57.29%	46.78-67.34%	<0.08
	Clonidine 60 minutes	86.36%	72.66-94.82%	90.16%	79.81-96.31%	<0.0001
	Clonidine 90 minutes	61.36%	45.44-75.65%	76.39%	64.9-85.63%	<0.0001
	Clonidine 120 minutes	25%	13.19-40.29%	62.5%	51.56-72.56%	<0.0001
	Insulin 30 minutes	75%	19.42-99.37%	95.83%	78.86-99.89%	0.0014
	Insulin 60 minutes	50%	6.76-93.24%	92%	73.96-99.02%	0.0171
	Insulin 90 minutes	50%	6.76-93.24%	92%	73.96-99.02%	0.0171
	Insulin 120 minutes	25%	0.63-80.58%	88.46%	69.86-97.55%	0.1481
Bucharest	Clonidine 60 minutes	89.1%	77.7-95.9	95.16	89.7-98.2	<0.0001
	Clonidine 120 minutes	18.2%	9.1-30.9	72.4	64.9-79.1	<0.0001
	Insulin 30 minutes	68.4%	43.4-87.4	92.3	84.01-97.1	<0.0001
	Insulin 60 minutes	42.1	20.2-66.1	85.7	75.9-92.6	<0.0001

A validated high-throughput liquid chromatography tandem mass spectrometry method (LC-MS/MS) using Gliclazide D4 as internal standard was employed to determine the plasma concentrations of gliclazide. The chromatographic system was an Agilent 1200 series (binary pump, autosampler, thermostat, from Agilent Technologies®, Santa Clara, CA, USA) coupled with a triple quadrupole mass spectrometer API 3200 (from Applied Biosystem MDS SCIEX®, Framingham, MA, USA). The chromatographic column used was a BDS HYPERSIL C18 (50 mm × 4.6 mm, 3 µm, from Thermo Fisher Scientific Inc., USA) and the mobile phase consisted of a mixture of water/methanol/formic acid 98%– 100% [100:900:0.01 v/v/v]. The flow rate was 0.8 mL/min, the thermostat temperature was set at 35°C, the injection volume was 10 µL, and the run time was 2.5 min. The mass spectrometry detection was in multiple reaction monitoring mode, positive ions, using Turbo Ion Spray as ionization source. The monitored ions transitions were m/z of 324.10→127.10 for gliclazide and 328.10→127.10 for Gliclazide D4 as internal standard. The retention times for gliclazide and internal standard were between 0.3-2 min. Gliclazide concentrations for each sample were determined from peak area ratios of gliclazide and internal standard and the calculations were performed by the Analyst software version 1.4.2. The quantification limit was 5.00 ng/mL. The analytical method was validated in terms of specificity, linearity, between- and within-run precision and accuracy, and analyte recovery. The calibration curve of gliclazide and internal standard were linear between 5-5016.48 ng/mL. The between-run accuracy was in the interval 90.53%-110.14%, the within-run accuracy was from 89.10% to 114.98%, the between-run precision was 1.83%-4.69%, and the within-run precision was 0.96%-4.38% [19].

Pharmacokinetic analysis

The pharmacokinetic parameters of gliclazide, when given under fast or fed condition of subjects, were estimated by a non-compartmental analysis method, performed by using Phoenix WinNonlin® PK version 6.3 (Pharsight Co., Mountain View, Calif., USA). The following pharmacokinetic (PK) parameters were determined: the maximum plasma concentration (C_{max}, ng/mL) and the time to reach C_{max} (t_{max}, h) were obtained directly from evaluating the values obtained experimentally, according to the non-compartmental analysis. The area under the plasma concentration–time curve from time zero to the last quantifiable concentration (AUC_{0-t}, ng*h/mL) was calculated by using the linear trapezoidal rule. The total area under the curve ($AUC_{0-\infty}$, ng*h/mL) was obtained by adding to AUC_{0-t} the report value of the last measurable concentration divided by the elimination rate constant (C_t/k_{el}). The constant of elimination (k_{el}, h^{-1}) was estimated by log-linear regression analysis of terminal portion of the plasma concentration-time profile. The half-life time of gliclazide ($t_{½}$, h) was calculated using the formula $t_{½} = 0.693/k_{el}$. Other determined pharmacokinetic parameters were the apparent clearance (Cl_F, mL/h) and the apparent volume of distribution (Vd_F, mL), estimated by taking into account the values of k_{el}. Moreover, with the purpose of obtaining the absorption profile of gliclazide from the site of administration (gastrointestinal tract is the main site of absorption after oral administration of the novel modified release tablet), the data obtained from plasma samples were further analyzed by mathematical deconvolution, thus obtaining the relative fraction of absorbed gliclazide over time.

Statistical analysis

The statistical analysis was performed by using Phoenix WinNonlin® PK version 6.3 (Pharsight Co., Mountain View, Calif., USA). For the comparison of gliclazide's plasma profiles under fasted and fed conditions, the analysis used Type III sum of squares from analysis of variance (ANOVA). A p value less than 0.05 determined for the differences of the PK parameters in-between study periods was considered statistically significant, whilst for t_{max} the non-parametric Friedman test was elected for assessment of differences.

Safety evaluation

Safety evaluation was conducted throughout both clinical trials and consisted of monitoring any adverse event or change in the volunteers' health condition which could be attributed to the given medication, gliclazide respectively. For safety reasons, the glycaemia levels were measured before drug administration and post-dose at 1, 2, 3, 6 and 7 h in both studies. The vital signs (axillary body temperature, sitting blood pressure, and radial pulse) were measured predosing and after drug intake at 4, 8, 12, 24, 36, 48, 72, and 96 h during both studies. For at least 24 h following gliclazide administration in each study period, either the principal investigator or a subinvestigator was available at the Clinical Pharmacology and Pharmacokinetics Department of Terapia SA, Romania, the site of investigation. Moreover, basic clinical examinations including hematological tests, biochemistry and urine analysis were carried out at the end of the study. Likewise, for female subjects the pregnancy test was repeated after the end of the clinical trial.

Results

Subjects

The demographic data of the healthy volunteers who were selected for the clinical trial are shown in Table I. For the Reference period, a number of 48 subjects were enrolled of which 41 completed the study. For the Test period, 26 subjects were selected of which a number of 23 finalized the trial without any protocol deviations.

Pharmacokinetic analysis

Mean plasma concentration-time profiles of gliclazide, when given under fasted or fed state, are presented in Figure 1.

Table I. Demographic characteristics of the subjects included in the study

Characteristic	Reference period (fasted state)	Test period (fed state)
Number of subjects	41	23
Gender (number) – Men	31	23
-Women	10	0
Age (years) – mean (SD)	23.1 (4.5)	24.7 (5)
Range	19-40	19-35
BMI** (kg/m2) – mean (SD)	23.5 (3.1)	22.9 (2.9)
Range	18.67-28.90	18.6-28.7

*SD – standard deviation; **BMI – body mass index

Following the graphical representation of plasma concentration profiles for gliclazide, the mean pharmacokinetic parameters, when given in fasted state of subjects or when the subjects were under fed state are shown in Table II.

The food effect findings reported in this study indicate that gliclazide exposure was lower after food intake. The values of C_{max} were by ~14% lower under fed condition (2066.11 ± 925.43 ng/mL vs 1800.17 ± 405.20 ng/mL) and $AUC_{0-\infty}$ registered a ~17% decrease during the fed state study period (38849.79 ± 20018.59 ng*h/mL vs 33041.37 ± 11075.75 ng*h/mL). The time to reach the peak plasma concentration (t_{max}) was shorten from 9.15 ± 2.41 h under fasted condition to 8.95 ± 2.10 h under fed state of the subjects. Likewise, the time from the administration to the beginning of absorption (t_{lag}) recorded a 3.3% decrease after food intake, from 2.51 ± 1.40 h to 2.43 ± 1.16 h. On the other hand, the half-life time of gliclazide ($t_{½}$) increased with ~5% during Test period, from 14.42 ± 4.63 h (fasted state) to 15.27 ± 4.60 h (fed state), even though the constant of elimination did not display any modification during the study (k_{el}=0.05 h^{-1}).

After the mathematical deconvolution of the plasma concentrations of gliclazide, the relative fraction absorbed from the site of administration over time was obtained and is depicted in Figure 2.

The relative profile of gliclazide's systemic absorption confirmed the t_{lag} of approximately 2.5 h after drug administration. Afterwards, plasma levels increase progressively, from 3 to 24 hours post-dose 80% of gliclazide is systemically absorbed. Its absorption continues with almost constant rate up to 96 hours post-dosing resulting in a plateau shaped curve, when the entire given dose is completely absorbed in the general circulation from the small intestine.

Statistical analysis

The statistical evaluation by ANOVA test was performed with the purpose of investigating the existence of a statistically significant difference between the mean values of the main pharmacokinetic parameters of gliclazide within study periods and the results are summarized in Table III. For t_{max} differences evaluation, the non-parametric Friedman test concluded the lack of statistical significance of the registered differences under fasted versus fed state of the subjects.

The statistical evaluation by ANOVA test did not conclude any statistically significant difference for the evaluated pharmacokinetic parameters between studies, therefore food intake did not have a major impact on gliclazide's disposition in the body.

Safety evaluation

A summary of the adverse event monitored during both study periods, after administration of 60 mg gliclazide p.o., is given in Table IV.

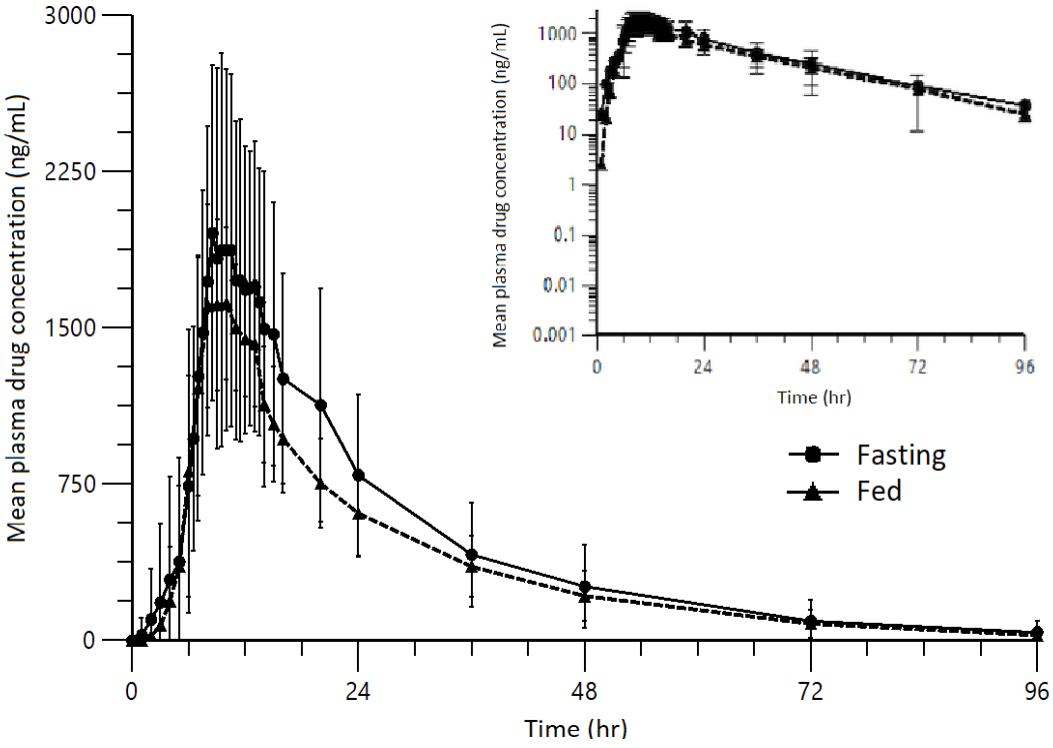

Fig.1. Mean ± standard deviation (SD) plasma concentration-time curves of gliclazide (60 mg, p.o.) administered in fasting state (n=41) or fed state (n=23). Insert: semi-logarithmic presentation

Table II. Summary of pharmacokinetic (PK) parameters of gliclazide after a single-dose of 60 mg p.o. administered under fasted or fed state of the subjects

PK parameter (units)	Study period							
	Reference (fasted state)				Test (fed state)			
	Geometric mean	SD	Median	CV%	Geometric mean	SD	Median	CV%
Cmax (ng/mL)	2066.11	925.43	2093.95	41.59	1800.17	405.2	1885.76	21.97
tmax (ng/mL)	9.15	2.41	8.5	25.52	8.95	2.10	8.00	22.92
AUC0-t (ng*h/mL)	38195.09	20343.49	37148.24	47.94	32479.33	11338.14	32541.45	33.16
AUC0-∞ (ng*h/mL)	38849.79	20018.59	37630.76	46.67	33041.37	11075.75	33162.09	31.96
kel (h-1)	0.05	0.02	0.05	29.91	0.05	0.01	0.05	25.04
t½ (h)	14.42	4.63	14.16	30.69	15.27	4.60	14.54	29.05
tlag (h)	2.51	1.40	3.00	55.67	2.43	1.16	2.00	47.68
MRT (h)	24.73	6.10	23.89	24.01	25.86	6.25	25.77	23.57
Cl_F (mL/h)	1511.12	716.91	1585.08	43.03	1769.72	617.79	1785.92	33.16
Vd_F (mL)	31427.84	7547.70	31502.83	23.39	38994.82	8344.65	37839.01	20.96

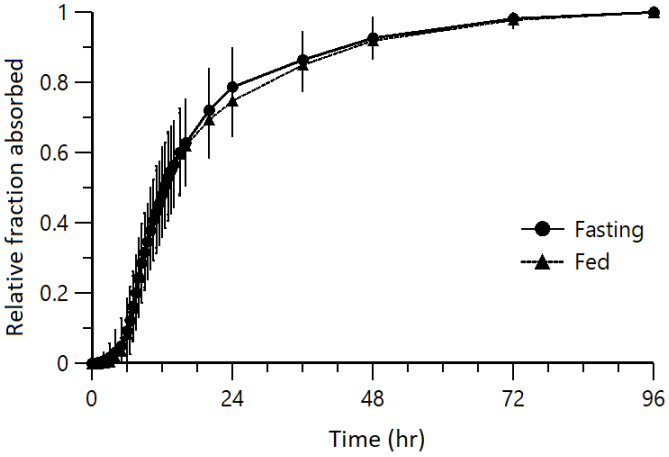

Fig. 2. The relative fraction absorbed of gliclazide (60 mg, p.o.) in the systemic circulation from the site of administration over time in subjects under fasted (n=41) or fed state (n=23)

A total of 15 treatment-emergent adverse events were reported by 18 subjects out of 64 (11 under fasted condition and 6 under fed condition, while 2 side effects were reported in both studies, namely hypoglycemia and increased direct bilirubin). All the reported adverse events were of mild-to-moderate intensity and all subjects recovered without sequelae. The most frequently reported side effect was hypoglycemia, which was expected considering it is widely reported after gliclazide administration in therapy [11]. However, in both trials gliclazide was generally well tolerated by subjects, with no severe adverse events leading to withdrawal from the study.

Table III. Statistical analysis results of the mean pharmacokinetic (PK) parameters comparison between Reference period (fasted state) and Test period (fed state) for gliclazide

PK parameter	Units	F_stat[a]	p_value[b]
C_{max}	ng/mL	2.48	0.1202
AUC_{0-t}	ng*h/mL	2.21	0.1425
$AUC_{0-\infty}$	ng*h/mL	2.36	0.1296
k_{el}	h^{-1}	0.58	0.4484
$t_{½}$	h	0.58	0.4484
t_{lag}	h	0.71	0.4035
MRT	h	0.57	0.4545
Cl_F	mL/h	2.09	0.1530
Vd_F	mL	13.63	0.6550
t_{max}	h	Friedman	NS[c]

[a]F_stat – statistic factor; [b]p < 0.05 statistically significant; [c]NS – statistically non-significant

Discussion

Every time a new modified release formulation is evaluated for market release, apart from the bioequivalence study itself, current guidelines recommend a food intervention study on healthy volunteers to investigate the food-drug interaction, considering that food intake may induce physiological changes in human body related to digestion process [20,21]. The administration of a new drug product with food may alter the drug's absorption and may change the bioavailability of the given active substance by influencing either the drug product or the drug substance (release or dissolution) [20,21]. Consequently, the general recommendations for use after such studies are conducted are listed in the drug product's specification file and may vary from take without food, take with food, or take regardless to food intake (with or without food) [22].

In this clinical trial, the pharmacokinetic parameters of gliclazide (single-dose of 60 mg, new modified release formulation, p.o., developed by Ranbaxy Laboratories Limited, now Sun Pharmaceutical Industries, India) under fasted and fed state were determined, as well as the absorption profile from the gastrointestinal tract.

Considering that assessment of the food effect on the extent and rate of a drug's absorption is part of the development process of a new orally administered drug product, numerical deconvolution of the experimental data was used in order to obtain the relative fraction absorbed by gliclazide at the specified time points [20-22]. Gliclazide is a drug belonging to BCS class II, with high permeabil-

Table IV. Summary of the adverse events reported for both periods of the clinical trial

Reported adverse events after gliclazide 60 mg administration p.o.	
Reference period (fasted state, 41 subjects)	Test period (fed state, 23 subjects)
Hypoglycemia	Hypoglycemia
Diarrheic syndrome	Increased blood urea nitrogen
Nausea	Increased alanine aminotransferase
Flu-like syndrome	Increased direct bilirubin
Headache	Increased total bilirubin
Acute gastroenterocolitis	Leukocyturia
Proteinuria	
Increased lactate dehydrogenase	
Increased direct bilirubin	
Urinary infection	
Increased aspartate aminotransferase	

ity and intermediate solubility that delays the absorption rate and onset of action, but with non-solubility-limited absorption after oral administration [23]. This delay in absorption can be attributed to several factors such as: slow disintegration or even non-disintegration of the modified release tablet in correlation with biphasic gastric emptying or absorption of gliclazide from two distinct sites within the upper gastrointestinal tract [24]. This hypothesis can be supported by the fact that gliclazide is an ampholyte with pH-dependent solubility in the gastrointestinal pH range [23]. The absorption profile of gliclazide displayed almost complete superposition under fasted and fed condition of subjects, thus highlighting the optimal and constant release of gliclazide (60 mg, p.o.) from the new modified release formulation developed by Ranbaxy Laboratories Limited, now Sun Pharmaceutical Industries, India. The outcomes of this research are consisted with those previously reported in the scientific literature [19].

Conclusion

In these clinical trials, food was demonstrated to exhibit no clinically or statistically meaningful effect on the bioavailability of gliclazide, given as a single-dose in healthy volunteers. Food effect was demonstrated to not have a statistically significant influence on the overall (extent and rate) exposure to gliclazide, even though a minor decrease in the peak plasma concentration and total area under the plasma concentration-time curve was observed. The reported adverse events during the study are common in this class of oral hypoglycemic agents and were anticipated, however gliclazide proved a favorable safety profile in healthy subjects under fasted or fed condition. These results suggest that gliclazide 60 mg new modified release tablet developed by Ranbaxy Laboratories Limited, now Sun Pharmaceutical Industries, India, could be safely administered to non-insulin dependent (type 2) diabetic patients without regard to food intake according to individual tolerance and patient preference. However, before stating a definite conclusion regarding the food effect on gliclazide pharmacokinetic profile, additional studies on T2DM patients should be conducted.

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Authors' contribution

DP – Study protocols, study design, data analysis, manuscript writing
AMG – Data analysis, manuscript writing
MO – Data collection, safety monitoring
AM – Study design, data analysis, review the manuscript
SB – Bioanalytical analysis, review the manuscript
AK – Study design, review the manuscript
RK – Study design, review the manuscript
LV – Data analysis, review the manuscript

Conflicts of interest

Diana Pop, Adriana Marcovici, Monica Oroian, Sandeep Bhardwaj, Arshad Khuroo and Ravi Kochhar were employees of the Ranbaxy Laboratories Limited, now Sun Pharmaceutical Industries, India, during the conduct of this study.

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