

Study of Pharmacokinetic Drug-Drug Interaction between Glimepiride and Gemfibrozil in Healthy Subjects

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ABSTRACT

Objective: Our objective was to study the effects of gemfibrozil on the pharmacokinetics of glimepiride, a sulfonylurea oral anti-diabetic drug and a substrate of cytochrome P450 2C9 (CYP2C9). **Introduction:** Some of disorders like diabetes, hypertension and hyperlipidemia co-exist lifelong. In this case, there will be high incidence of drug-drug interaction. Diabetes and, dyslipidemia exists with increased triglycerides, LDL and low HDL levels. So, oral hypoglycaemic drug with addition of lipid lowering drug is necessary to manage dyslipidemia in diabetic patients. There is a possibility for drug-drug-interaction between anti-hyperlipidemic drugs like gemfibrozil and oral anti-diabetic drugs like glimepiride, which are commonly prescribed with each other. **Methods:** In a randomized, crossover study, 10 healthy volunteers were received 600 mg oral gemfibrozil or placebo twice daily for 2 days. On day 3, they received a single dose of 600 mg gemfibrozil or placebo and 1 hour later a single dose of 3 mg glimepiride orally was received by both groups. Plasma glimepiride levels were measured up to 12 hours. **Results:** Gemfibrozil increased the mean calculated area under the plasma concentration-time curve (AUC) of glimepiride by 2.41 fold ($P \leq 0.05$) compared to glimepiride only. The mean calculated mean resistance time (MRT) was increased from 4.1 ± 0.096 to 4.88 ± 0.14 hr. The Cl/F was significantly decreased from 2003 ± 28.64 to 829 ± 13 ml/hr. The mean elimination half-life of glimepiride was prolonged from 2.6 to 4.1 hours ($P < 0.05$). **Conclusions:** Gemfibrozil significantly increases the plasma concentrations of glimepiride. This may be induced by decreasing the rate of glimepiride hepatic metabolism through inhibition of CYP2C9.

Key words: Gemfibrozil, CYP2C9, drug-drug interaction, glimepiride, LDL, pharmacokinetics, type 2 diabetes mellitus

Introduction

Some of disorders like type 2 diabetes mellitus, hypertension and hyperlipidemia are co-exist in the same patient. In this case the possibility of drug -drug interaction may occur. Diabetes and, dyslipidemia exists with increased triglycerides, LDL and low HDL levels. Hence oral hypoglycaemic drugs are usually prescribed in addition to lipid lowering drugs. This co administration of both drugs becomes necessary to control dyslipidemia associated with diabetes. In such a situation the patient will be at high risk of drug-drug-interaction between anti-hyperlipidemic like gemfibrozil and oral hypoglycemic drugs like glimepiride (Bosse *et al.*, 2002; Wang *et al.*, 2002).

Gemfibrozil is a fibric acid derivative that is used for the treatment of certain dyslipidemias. Gemfibrozil increases the activity of extra-hepatic lipoprotein lipase (Bosse *et al.*, 2002; Wang *et al.*, 2002), thereby increasing lipoprotein triglyceride lipolysis. It does so by activating Peroxisome proliferator-activated receptor-alpha (PPAR α) 'transcription factor ligand', a receptor that is involved in metabolism of carbohydrates and fats, as well as adipose tissue differentiation. This increase in the synthesis of lipoprotein lipase thereby increases the clearance of triglycerides. Chylomicrons are degraded, VLDLs are converted to LDLs, and LDLs are converted to HDL. This is accompanied by a slight increase in secretion of lipids into the bile and ultimately the intestine. Gemfibrozil also inhibits the synthesis and increases the clearance of apolipoprotein B, a carrier molecule for VLDL (Bosse *et al.*, 2002; Rizvi *et al.*, 2003; Hinton *et al.*, 2008).

Gemfibrozil inhibits CYP2C9 in vitro even more potently than it inhibits CYP2C8. Usually it concomitantly administered with glimepiride in diabetic type 2 DM (Diabetes Mellitus) patients to control dyslipidemia in those patients (Wen *et al.*, 2001).

The mechanism of action of glimepiride in lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cells, and increasing sensitivity of peripheral tissues to insulin. Glimepiride likely binds to ATP-sensitive potassium channel receptors on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the membrane. Membrane depolarization stimulates calcium ion influx through voltage-sensitive calcium channels. This increase in

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intracellular calcium ion concentration induces the secretion of insulin (Schneider, 1996; Rosenstock et al., 1996)

Glimepiride is completely metabolized by oxidative biotransformation to a major metabolite, cyclohexyl hydroxymethyl derivative, via the hepatic cytochrome P450 2C9 (Renwick *et al.*, 2000; Kita *et al.*, 1981; Kyrklund *et al.*, 2003; Hinton *et al.*, 2008).

The aim of this study was to investigate possible pharmacokinetics changes as a result of concomitant administration gemfibrozil with the glimepiride.

Because gemfibrozil can inhibit CYP2C9-mediated drug metabolism and glimepiride is a substrate of CYP2C9 (Hinton *et al.*, 2008). It is important to study the possible effects of gemfibrozil on some pharmacokinetics parameters of glimepiride.

Subjects and Methods

Subjects

10 healthy volunteers were enrolled in this study. The volunteers were informed about the objectives of the study and all the procedures were explained to them. A written consent form was signed by each agreement to participate in this study.

Inclusion criteria

Ten healthy male adult volunteers with age ranging from 18 up to 27 years, and their weight ranging from 60 to 87 kg (non-obese) were enrolled. Physical examination showed that all the volunteers had no clinical evidences of chronic diseases. For each volunteer the liver function was assessed by measuring ALT and AST (Table 1) by using Transaminases - Colorimetric End-Point Method (Reitman, and, Frankel, 1957). The volunteers were instructed not to take any drug for at least 72 hours prior to and throughout the study period.

Exclusion criteria

Volunteers with liver diseases, smokers, regular prescription medication, chronic diseases (hypertension, diabetes, ischemic heart diseases), over and underweight volunteers were excluded.

Drugs

Glimepiride 2 mg (Amaryl^R) produced by Sanofi Aventis, El Sawah El Amiriya Egypt.

Gemfibrozil 600mg (Lopoid^R) produced by Pfizer, Egypt.

Instrumentation

A UV-visible spectrophotometer, model UV 1800 (Shimadzu) with 2ml matched quartz cell was used to measure absorbance of the resulting solutions. All the reagents and chemicals used were of analytical grade.

Study Design

10 volunteers were randomly divided into two groups, each 5 volunteers. First group (control group) was received placebo (twice daily orally) for 2 days, and at the same time the second group (gemfibrozil group) was received 600 mg oral gemfibrozil twice daily for 2 days. On day 3, they received a single dose of 600 mg gemfibrozil or placebo, and 1 hour later they received a single dose of 3 mg glimepiride orally. Plasma glimepiride was measured at definite time intervals up to 12 hours.

Before blood sampling, each volunteer drinks sweaty juice to guard against hypoglycemia that may occur.

Determination of working wave length:

In order to determine the wave length of maximum absorption (λ_{\max}) of glimepiride, different solutions of glimepiride with methanol were scanned using spectrophotometer within the wave length region of 200-400 nm. against methanol as blank, and the λ_{\max} was found at 229 nm.

Analytical methods used in the study:-

Standard Solutions:

Primary stock solution of 180 ug/ml of glimepiride (3 tablets of amaryl 3mg were extracted by 50 ml methanol), and stored at 4°C. Appropriate dilutions of glimepiride were made in methanol to prepare 1200, 1000, 500, 400, 144, 126, 108, 90, 72, 54, ng/ml. standard curve was made, its R^2 is 0.999.

Blood Sampling

On the days of administration of glimepiride, a forearm vein of each volunteer was cannulated with a plastic cannula. Timed blood samples were drawn just after glimepiride administration and 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, and 12 hr later. All samples were collected in heparinized tubes. Plasma was separated by centrifugation for 15 minutes at 4000 rpm and then stored at -20 C° until analysis.

Sample preparation

To 1 mL of each standard plasma samples in test tubes, 400 µL of 10 % tri-chloroacetic acid were added, for protein precipitation. The test tubes were shaken well for 2 minutes using vortex mixer, and then centrifuged for 15 minutes at 4000 rpm. The supernatant was transferred to clean dry test tube for extraction of glimepiride. To each tube 3mL of chloroform were added then the test tubes were shaken well for 2 minutes using vortex mixer, and centrifuged for 15 minutes at 4000 rpm. Then 2.5 ml of the chloroform extract was transferred to clean dry test tube, and the extraction process was repeated again with another 3 ml of chloroform. Then the two chloroform extracts were combined and then evaporated at room temperature over night until dryness. The residue was reconstituted in 2 mL of methanol by shaking well for 10 seconds using vortex mixer, and then absorbance was measured against blank at λ_{max} 229 nm.

Table 1: Lists the demographic information of the enrolled volunteers participated in the study

	Volunteer no.	1	2	3	4	5	6	7	8	9	10	Mean ± SD
	Age (year)	21	19	18	26	24	20	25	19	22	25	21.9 ± 2.77
	Weight (kg)	70	83	67	74	67	64	83	87	71	82	74.8 ± 7.8
Liver Function	ALT* (IU / L)	23	17	26	19	31	12	19	24	28	33	23.2 ± 6.22
	AST** (IU / L)	35	31	23	22	27	34	18	23	29	30	27.2 ± 5.28

* ALT is Alanine transaminase (normal range 5- 40 IU/L)

** AST is aspartate transaminase (normal range 7-56 IU/L)

Statistical analysis

The pharmacokinetic parameters used for comparison were C_{max} , t_{max} , $t_{1/2}$, k , $AUC_{0 \rightarrow \infty}$, MRT, and, Cl/F. The pharmacokinetic parameters were calculated for each volunteer and the results were presented as the mean ± standard deviation (±SD) and were analyzed by Statistical Package for Social Science (SPSS) version 20. The Paired t-test was used. The level of significance was set at P value of 0.05 or less. The mean is the arithmetic average and can be calculated by

$$\bar{X} = \frac{\sum X}{n} \text{ Where } \sum x \text{ is the sum of all observations and } n \text{ is the number of observations}$$

Results

In this study, ten healthy adult male volunteers were enrolled. The volunteers received 3 mg glimepiride orally with placebo (control group) and 3 mg glimepiride with 600 mg gemfibrozil orally (gemfibrozil group), our results were as follows:-

The mean plasma concentration-time curves of control group and gemfibrozil group are shown in figure (1). After administration of 3mg glimepiride as single oral dose, the concentration of glimepiride in plasma starts to increase rapidly after oral administration to maximum concentration (327.1 ± 3.4 ng/ml) after 1.5 hour from the time of administration as shown in table (2), and then plasma concentration starts to decline by first order elimination. Whereas, in gemfibrozil group after administration of single oral 3 mg glimepiride with 600mg gemfibrozil, our results showed that, the mean plasma concentrations of glimepiride starts to increase rapidly to mean maximum concentration of 1108.5 ± 44.52 ng/ml., ($P \leq 0.05$), and this increase in C_{max} of gemfibrozil group is more than 3.4 fold of that in control group, as shown in table (2) and figure (1).

Our results showed that prolongation in half-life of glimepiride in gemfibrozil group is increased by more than 1.5 fold that in control group (2.6 versus 4.1 hr). (Table 2)

In table (2), the calculated area under plasma concentration time curve ($AUC_{0-\infty}$) for glimepiride in control group is 1498 ± 21.6 (ng.hr/ml). Whereas, the calculated mean $AUC_{0-\infty}$ for glimepiride in gemfibrozil group is significantly increased by 2.41 fold (3619.124 ± 58.0) ($P \leq 0.05$) compared to glimepiride in control group as shown in table (2) and figure (3).

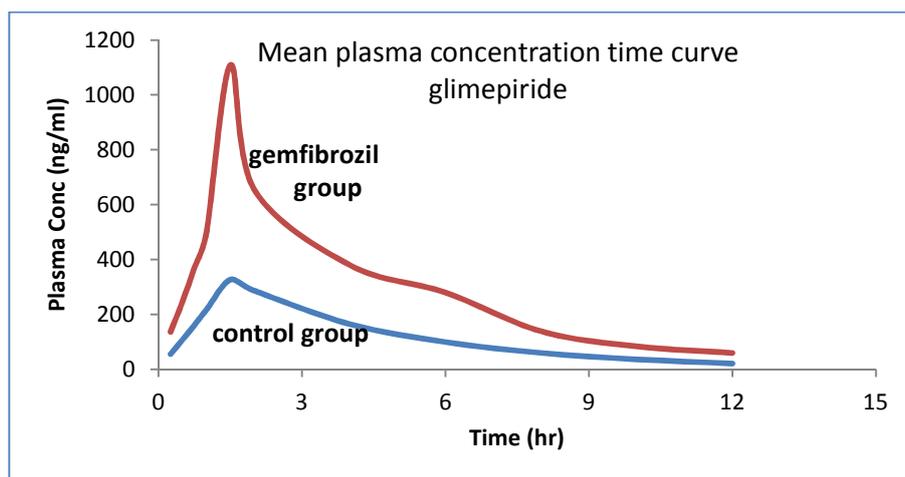


Fig. 1: Mean (\pm SD) plasma concentration-time curve (ng/ml) observed after administration of a single oral dose 3mg of glimepiride with placebo(control group) and glimepiride with 600 mg gemfibrozil (gemfibrozil group) orally to 10 volunteers in cross over study.

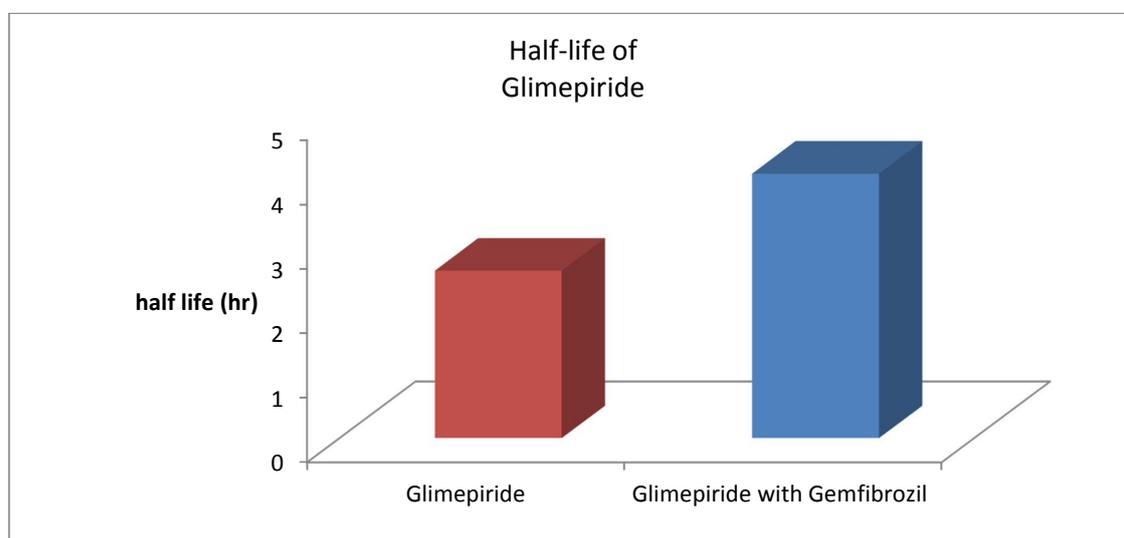


Fig. 2: Mean (\pm SD) half-life (hr) observed after administration of a single oral dose 3mg of glimepiride with placebo(control group) and glimepiride with 600 mg gemfibrozil (gemfibrozil group) orally to 10 volunteers in cross over study.

Table 2: The pharmacokinetic parameters of Glimepiride 3mg oral in 10 healthy subjects after pretreatment with placebo or 600 mg Gemfibrozil once daily for 2 days.

Parameters	Control group	Gemfibrozil group
C_{max} (ng/ml)	327.1 \pm 3.4	1108.5 \pm 44.52*
t_{max} (hr)	1.5	1.5
$t_{1/2}$ (hr)	2.6 \pm 0.195	4.1 \pm 0.215*
AUC (ng.hr/ml)	1498 \pm 21.6	3619.124 \pm 58.0*
Ke (hr ⁻¹)	- 0.271 \pm 0.022	- 0.168 \pm 0.009*
Cl/F(ml/hr)	2003 \pm 28.64	829 \pm 13*
MRT (hr)	4.10 \pm 0.096	4.88 \pm 0.14*
AUMC	6151.09 \pm 195.449	17661.57 \pm 569.88*

C_{max} = Maximum Plasma Concentration
 t_{max} = Time at Maximum Plasma Concentration
 $t_{1/2}$ = half life
 AUMC = Area Under the first-order Moment Curve

AUC = Area Under the plasma concentration-time Curve
 Cl/F = total body clearance divided by bio-availability of the drug
 MRT = Mean Residence Time
 * Statistically significant

Discussion

Our study confirms that the method for the determination of glimepiride in human serum samples was simple and accurate and requires relatively small volume of serum (1mL). The standard curve was linear in the concentration range 36 and 1200 ng/ml. so, the method is suitable for conducting pharmacokinetic studies.

There was significant increase in pharmacokinetic parameters like C_{max} , AUC, $t_{1/2}$, MRT, and AUMC of glimepiride in gemfibrozil group. In addition there was a significant decrease in Cl/F of glimepiride in gemfibrozil group. The increase in C_{max} , AUC and AUMC with decrease in Cl/F proved that there is a Pharmacokinetics drug interaction at the elimination site. In the presence of gemfibrozil, the peak serum glimepiride levels were observed at 1.5 hr after co-administration of gemfibrozil, and this peak is increased more than 3.4 fold than that for glimepiride alone.

The above results were in line with Niemi *et al.*, (2001) and Neuvonen *et al.*, (2001). It is reasonable to assume that the glimepiride (sulphonylurea) pharmacokinetics was affected by co-administration with gemfibrozil (Niemi *et al.*, 2001; Kirchheiner *et al.*, 2002).

Our study indicating that the enhanced serum glimepiride levels might be due to inhibition of metabolism of glimepiride by gemfibrozil. The study indicates that, interaction observed is pharmacokinetic drug-drug interaction; this is augmented by our results which proved that the elimination rate constant of glimepiride was decreased upon co-administration of gemfibrozil (- 0.271 versus - 0.168), so the rate of elimination of glimepiride will be significantly decreased which may leads to elevation of glimepiride serum levels, and the incidence of hypoglycemic shock is also increased.

Gemfibrozil is a potent inhibitor of CYP 450 2C9 (Wen *et al.*, 2001). Glimepiride is mainly metabolized by CYP 450 2C9. Hence the interaction may be occurred at the site of the metabolism. Gemfibrozil acts as substrate and a potent inhibitor for CYP 450 2C9 enzyme, and consequently, delay the metabolism of glimepiride leading to enhancement the effect of glimepiride (Niemi *et al.*, 2002; Yin *et al.*, 2005).

We have demonstrated that, using laboratory efficacy and safety data, that clinically significant interactions between glimepiride and gemfibrozil that inhibit CYP2C9 activity so, increasing in the glucose-lowering effect of glimepiride is possible to occur. This may lead to significant risk of hypoglycemia. These results are in line with Scheen *et al.*, (2005).

Our study proved that the elimination of glimepiride was significantly decreased (Cl/F 2003 \pm 28.64 versus 829 \pm 13) so the bioavailability of glimepiride was increased, these results were in line with Olstein *et al.*, (2003).

Conclusion

In conclusion, On the basis of the available evidence, the co-administration of gemfibrozil with glimepiride, pharmacokinetics interaction was found in human plasma, results in alteration of the pharmacokinetics parameters of glimepiride which may lead to hypoglycemic shock.

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