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Influence of Fenofibrate on the pharmacodynamic activity of Glimepiride in rats and rabbits

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ABSTRACT

Fenofibrate, a lipid lowering agent was commonly prescribed in diabetic patients with high lipid profile. As it was reported to influence the blood glucose levels, the present study was designed to report the effect of fenofibrate on hypoglycemic activity of glimepride. The influence of fenofibrate on the hypoglycemic activity of glimepiride was studied in rats and rabbits. Fenofibrate and glimepiride were studied at doses of 18 and 0.09mg/kg in rats and 9.33, 0.047mg/kg in rabbits, respectively. The blood samples collected at pre-determined time intervals were analyzed for glucose levels using a glucometer. Glimepiride exhibited a maximum reduction in blood glucose levels at the 4th hour in rats and rabbits. Fenofibrate showed significant effect on the hypoglycemic activity of glimepiride in both single and multiple dose interaction studies in rats and rabbits. The study indicates that fenofibrate pretreatment elevates the pharmacodynamic activity of glimepiride by a possible rise in insulin sensitivity and improving insulin homeostasis or may be due to the inhibition of CYP2C9. The study also suggests that caution may be recommended concerning combined use of fenofibrate and an oral hypoglycemic agent, glimepiride.

KEYWORDS: Glimepiride, Fenofibrate, Hypoglycemia, Drug Interactions.

INTRODUCTION

Type 2 diabetes mellitus is a progressive and complex metabolic disorder in which a person has high blood sugar, either because the body does not produce enough insulin or because the cells do not respond to the insulin that is produced (Bastaki, 2005). A majority of the patients are overweight or obese at diagnosis and will be unable to achieve normoglycemia without an oral anti diabetic agent (Krentz, 2005). The goals of pharmacologic therapy are to achieve adequate glycemic control while avoiding hypoglycemia and weight gain and to minimize the risk of future micro and macro vascular complications. Glimepiride is an effective well tolerated oral sulfonyl urea derivative with less incidence of hypoglycemia (Rosenstock, 1996). Fenofibrate, a peroxisome proliferator activated receptor α (PPAR- α) has shown beneficial effects on lipid profile, glucose control and insulin resistance in type II diabetic patients (Damci *et al*. 2003) it has shown significant protective effects on micro vascular complications (Chen *et al.* 2013). Glimepiride is metabolized by cytochrome P-450 CYP2C9 (Maekawa, 2009). Fenofibrate is metabolized by CYP 3A4 and inhibits CYP 2C9 (Kim, 2003). Based on the existing metabolic profile it was hypothesized that it enhances the hypoglycemic activity of glimepiride. Hence the study was designed to investigate the effect of fenofibrate, a PPAR-*α* activator on the pharmacodynamic activity of glimepiride.

MATERIALS AND METHODS

Drugs and chemicals: Glimepiride and fenofibrate were obtained from Zydus Cadila, Ahmedabad and Radiant Research Pvt. Ltd, Bangalore, India, respectively. Alloxan monohydrate was obtained from Sigma Chemicals, India. Glucometer from Roche Diagnostics was used for blood glucose estimation.

Animals: As the physiology of rats resemble to that of the humans, they were selected for pharmacodynamics study. Rabbits were also selected establish the observed change in pharmacodynamic data. Adult wistar rats of either sex, weighing150–250gm and albino rabbits of either sex weighing 1.3-1.5 kg, obtained from the animal house of Bapatla College of Pharmacy (1032/ac/07/CPCSEA), Bapatla, were maintained at a constant temperature of 22 ± 3°C and humidity 60–70% with 12 h light/dark cycles throughout the study. The animals were fed with commercial rat feed (Rayan's Biotechnologies Pvt Ltd, Hyderabad, India) and sterile water was given *ad libitum*. The protocol was approved by the Institutional Animal Ethics Committee (IAEC/IV/03/BCOP/2012), all the procedures were performed in accordance with the guidelines of the CPCSEA.

Dosage and drug administration: In clinical practice, fenofibrate and glimepiride are administered orally. Hence, their human therapeutic doses were extrapolated to rats and rabbits based on their body weight and administered orally for the study (Ramachandra, 2005).

Pharmacodynamic interaction studies in normal rats:

Effect of fenofibrate and glimepiride on blood glucose levels in normal rats: This study was conducted to evaluate the onset, duration and maximum hypoglycemic activity of the individual drugs. Adult wistar rats were divided into three groups of six animals each. The animals were fasted for a period of 18 h prior to the experimentation and water was supplied *ad libitum* (Rosenstock, 1996). Group I served as control and received distilled water, group II received glimepiride 0.09 mg/kg, and group III received fenofibrate 18 mg/kg. The blood samples were collected by tail tip removal method at 0, 1, 2, 3, 4, 6, 8, 10, 12 and 16 h after drug treatment and were analyzed for blood glucose levels using a glucometer (Shim Y*et al*. 2003).

Single-dose interaction study in normal rats: Single-dose interaction studies were carried out on group II animals to evaluate the effect of a single dose of fenofibrate on the hypoglycemic activity of glimepiride after a brief washout period of 1week, as it is necessary to consider that the elimination period should be at least five times the terminal half-life (of the active ingredient or its metabolites, or of the acute pharmacological effect, etc.). The animals were fasted for 18 h prior to experimentation and water supplied *ad libitum*. The interacting drug, fenofibrate 18 mg/kg was given to animals followed by glimepiride 0.09 mg/kg after 30 minutes. The blood samples were collected at predetermined time intervals for glucose estimation as mentioned previously.

Multiple-dose interaction study in normal rats. As single-dose interaction study results can't be extrapolated to chronic use effects, the study was extended to include a multiple-dose interaction study to evaluate the effect of chronic use of fenofibrate on the hypoglycemic activity of glimepiride. The group II animals were given fenofibrate 18 mg/kg, for the following 7 consecutive days after the single-dose interaction study. During this period, the animals had free access to food and water. On the 7th day of the study food was withdrawn 6 h after the fenofibrate administration, but water was supplied *ad libitum*. On the 8th day, glimepiride 0.09 mg/kg was given 30 minutes after fenofibrate administration and the blood samples collected at predetermined intervals were analyzed for blood glucose levels.

Pharmacodynamic interaction studies in diabetic rats

Induction of diabetes: Experimental diabetes in rats was induced by injecting alloxan monohydrate intraperitoneally at a dose of 150 mg/kg in ice-cold normal saline. Blood samples collected after 72 hours were analyzed for blood glucose levels. Rats with blood glucose levels above 200 mg/dl were considered as diabetic and selected for the study (Ghosh, 2005; Murthy and Mayuren, 2008).

Single & multiple-dose interaction studies in diabetic rats: The study was designed to evaluate the effect of single and multiple doses of fenofibrate on the anti-hyperglycemic activity of glimepiride. The diabetic rats were divided into two groups of six animals each. The animals were fasted for a period of 18 h prior to experimentation and water supplied *ad libitum.* Group I was treated with the vehicle and group II was given glimepiride 0.09 mg/kg and blood glucose levels were analyzed at predetermined intervals. After a brief washout period of 1week, the animals of group II were used for the interaction study. An experimental protocol similar to that followed in normal rats for the single and multiple-dose interaction studies was employed in diabetic rats.

Pharmacodynamic interaction Studies in Normal Rabbits

Single & multiple-dose Interaction Study in Normal Rabbits: Albino rabbits of either sex weighing between 1.3-1.5 kg were divided into 3 groups of 5 animals each. The animals were fasted for a period of 18 h prior to experimentation and water supplied *ad libitum*. Group I was treated with the vehicle, Group II was treated with glimepiride 0.047 mg/kg and group III was treated with fenofibrate 9.33 mg/kg. Blood samples were collected at 0, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24 h of drug treatment from the marginal ear vein of the rabbits and analyzed for blood glucose levels. After a brief washout period of one week, the animals of group II were used for interaction study. An experimental protocol similar to that followed for normal rats was employed for the single and multiple-dose interaction studies in normal rabbits. The blood samples collected from the marginal ear vein at 0, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24 h were analyzed for blood glucose levels.

Statistical analysis: The hypoglycemic activity of glimepiride at any time *t* was calculated as the percentage blood glucose change at that time with respect to initial blood glucose level according to the formula (Satyanarayana, 1998). Percentage blood glucose reduction at time $t = ((a - b)/a) \times 100$ where '*a'* is the initial blood glucose level and '*b'* is the blood glucose level at time *t*.

The significance of the observed difference in the pharmacodynamic parameters of glimepiride was assessed by one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison tests using GraphPad Prism software. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Pharmacodynamic interaction studies in normal rats: Glimepiride at the dose of 0.09 mg/kg was studied in normal rats. The onset of action was observed at the first hour and was observed to last until the 16thhr of the study. Glimepiride produced hypoglycemia in normal rats, with peak activity at the4th hour (Blood glucose 65.173 ± 0.7mg/dl, percentage decrease in blood glucose 31.63 ± 0.88), which may be due to the stimulation of insulin release from pancreas (Rosenstock, 1996) and due to the ability of glimepiride to increase the sensitivity of pancreatic β cells to glucose. Fenofibrate at a dose of 18 mg/kg exhibited a peak hypoglycemic effect at the 4th hour (Blood glucose 77.33 ± 0.88 mg/dl, percentage decrease in blood glucose 16.98 ± 0.67).

Single-dose interaction study: Blood glucose levels at various time intervals were subjected to statistical comparison with initial blood glucose of the same group and with that of the glimepiride alone group. There was a significant change in the blood glucose values when compared with their 0 hour blood glucose values and the percentage change (decrease) in blood glucose values were significantly changed when compared with that of glimepiride treatment alone, revealing the significant effect of fenofibrate in normal rats in single dose interaction study. The peak hypoglycemic effect in the single-dose interaction study was observed at the 4thhr with blood glucose level 61.33 ± 0.88 mg/dl and the percentage decrease in blood glucose was found to be 35.44 ± 0.37% (Table 1).

Table.1. Mean percentage blood glucose change in normal rats (n=6) with different treatments

Time	Control	Glimepiride	Fenofibrate	Single Dose Study	Multiple Dose Study
0.5	-2.48±1.46	8.89±1.24	2.85±0.96	8.25±0.51 a^{ns}	13.77±1.64 a^{ns}b*
1	-6.50±1.79	18.19±1.40	6.24±0.94	14.03±0.39 a*	17.86±1.62 a^{ns}b^{ns}
2	-4.49±1.82	20.95±1.13	8.04±0.79	24.19±0.49 a*	29.11±1.57 a*b*
3	-4.10±1.23	26.19±1.15	10.19±0.78	27.39±1.40 a^{ns}	33.21±1.52 a*b*
4	-4.33±2.07	31.63±0.88	16.98±0.67	35.44±0.37 a**	42.05±1.36 a**b**
6	-4.46±1.39	28.13±0.70	13.74±1.94	27.72±0.40 a^{ns}	30.99±1.09 a^{ns}b*
8	-5.53±1.62	16.23±1.72	12.86±0.69	22.09±0.23 a*	25.02±1.02 a*b^{ns}
10	-7.37±1.84	12.03±1.36	8.56±0.72	16.81±0.71 a**	21.96±1.03 a**b*
12	-10.8±1.85	5.76±1.24	8.53±1.11	12.45±0.59 a**	18.04±0.98 a***b**
16	-6.13±1.91	5.07±1.16	6.59±0.88	8.94±0.67 a*	13.95±0.99 a**b*

ns-nonsignificant;*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
a-When compared with glimepiride alone treatment results.
b-When compared with single-dose interaction study results.

Multiple-dose interaction study: There was a significant change in the blood glucose values when compared with their initial 0 hour blood glucose values and the percentage decrease in blood glucose levels were significantly changed when compared with that of glimepiride alone group and with that of values observed in single-dose interaction study, which reveals the significant effect of fenofibrate on normal rats in multiple dose interaction study. This may be due to blood glucose control and increased insulin sensitivity by fenofibrate (Yong, 1999).

Pharmacodynamic interaction studies in diabetic rats:

Single and multiple-dose interaction study: Glimepiride produced anti-hyperglycemic activity in diabetic rats with peak activity at the 4th hour (blood glucose 156.67 ± 1.86 mg/dl, percentage decrease in blood glucose 27.44 ±1.06%) (Table.2). Fenofibrate produced anti-hyperglycemic activity in diabetic rats with peak activity at the 4thhr with blood glucose 183.83 ± 3.1 mg/dl and the percentage decrease in blood glucose was calculated as 13.09 ± 0.98. In single dose interaction study the peak activity of glimepiride was seen at 4thhrwith blood glucose 145.0 ± 3.04mg/dl, percentage decrease in blood glucose was calculated as 32.32 ± 0.87. In single dose interaction study the blood glucose levels decreased significantly when compared with their 0 hour blood glucose values and the percentage decrease in blood glucose levels were significantly changed when compared with that of blood glucose levels observed in rats treated with glimepiride alone, which shows that there was a significant effect of fenofibrate on glimepiride activity in diabetic rats in single-dose study. A multiple-dose interaction study was conducted as in normal rats and a significant difference was observed when compared with single-dose interaction studies. The maximum change in blood glucose at the 4th hour was observed as 131.0 ±1.21 mg/dl and the change was calculated as 37.89 ±1.13%, which may be due to its action on glycemic control and insulin resistance (Damci, 2003) or may be due to inhibition of CYP 2C9 enzyme responsible for glimepiride metabolism. Past studies reveal that fenofibrate has potential anti-oxidant action by preventing endothelial function (Murat *et.al*.2010) which may also be a reason for enhanced hypoglycemic activity of glimepiride when co administered with fenofibrate.

Table.2. Mean percentage blood glucose change in diabetic rats (n=6) with different treatments.

Time (H)	Control	Glimepiride	Fenofibrate	Single Dose Study	Multiple Dose Study
0.5	-1.77±0.56	0.17±0.44	2.14±0.81	1.48±0.37 a^{ns}	6.78±0.73a***b**
1	-10.81±0.5	11.69±0.97	3.15±0.5	14.03±1.22 a*	15.68±1.11a*b^{ns}
2	-8.53±1.11	16.26±0.66	5.11±0.71	19.32±1.14 a*	20.22±0.71 a**b^{ns}
3	-8.21±1.12	19.37±0.53	7.1±0.88	22.32±0.64 a*	28.36±0.84 a***b***
4	-0.96±0.41	27.44±1.06	13.09±0.98	32.32±0.87 a*	37.89±1.13 a**b*
6	-1.69±0.49	24.33±1.49	12.99±0.8	28.52±0.82 a*	31.21±1.03 a*b^{ns}
8	5.56±1.11	22.55±1.39	13.07±0.81	24.37±0.82 a^{ns}	28.38±1.06 a*b***
10	9.55±1.18	18.73±0.54	10.96±0.86	22.66±0.61 a*	24.2±1.29 a*b^{ns}
12	9.29±1.36	14.71±0.95	6.28±0.89	19.08±1.34 a*	21.7±1.5 a*b*
16	10.04±1.08	11.77±1.02	4.87±0.76	16.11±1.02 a*	18.45±1.63 a*b^{ns}

ns- nonsignificant;* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
a-When compared with glimepiride alone treatment results.
b-When compared with single-dose interaction study results.

Pharmacodynamic interaction studies in normal rabbits:

Single and multiple-dose interaction study: Glimepiride produced hypoglycemic activity in normal rabbits with peak activity at the 4th hour (blood glucose 51.0 ± 2.02 mg/dl, percentage decrease in blood glucose 45.33 ± 0.66). Fenofibrate has not shown significant blood glucose lowering effect in normal rabbits. In single-dose interaction study the blood glucose levels decreased significantly when compared with their 0 hour blood glucose values and the percentage decrease in blood glucose levels were significantly increased when compared with that of blood glucose levels observed in rats treated with glimepiride alone, which shows that there was a significant effect of fenofibrate on glimepiride in normal rabbits. A multiple dose interaction study was conducted as in normal rats and a significant difference was observed when compared with single-dose interaction studies. The maximum change in blood glucose at the 4th hour was observed as 47.0 ±1.14 mg/dl and the change was calculated as 54.77 ±1.21%, which may be due to its action on glycemic control and insulin resistance (Damci, 2003) or may be due to inhibition of CYP 2C9 enzyme responsible for glimepiride metabolism (Table 3).

Table. 3. Mean percentage blood glucose change in normal rabbits (n=6) with different treatments.

Time (H)	Control	Glimepiride	Fenofibrate	Single Dose Study	Multiple Dose Study
0.5	-3.91±1.62	12.46±0.18	1.07±2.35	15.96±0.94 a*	17.88±1.19 a* b^{ns}
1	-5.28±1.7	20.43±0.36	4.14±2.67	23.65±1.75 a^{ns}	25.32±1.47 a* b^{ns}
2	-11.3±1.15	26.65±0.44	3.74±3.09	33.17±2.08 a*	37.58±2.34 a**b*
3	-7.21±1.11	32.64±0.33	1.89±3.19	39.15±1.49 a**	43.41±2.14 a**b*
4	-3.63±1.51	45.33±0.66	0.34±3.79	49.61±1.5 a*	54.77±1.21 a***b***
6	-3.46±1.36	42.35±0.63	1.19±3.56	46.86±1.41 a*	49.39±1.24 a***b**
8	-2.6±1.1	37.17±0.61	0.2±3.05	42.7±1.29 a**	43.57±1.86 a**b^{ns}
10	1.14±2.2	31.38±0.74	1.0±3.45	37.98±2.02 a*	38.57±2.41 a*b^{ns}
12	3.48±1.02	23.03±0.58	3.49±3.22	30.06±2.23 a*	33.61±2.06 a**b**
16	4.59±0.92	18.09±0.67	4.32±3.19	24.51±1.64 a*	24.75±2.05 a*b^{ns}
20	4.31±1.55	14.41±0.34	2.88±3.01	20.96±2.43 a*	22.47±2.32 a*b^{ns}
24	2.08±0.31	8.59±0.3	2.72±2.69	15.42±2.36 a*	13.75±3.08 $a^{ns}b^{ns}$

ns- nonsignificant;* $p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
a-When compared with glimepiride alone treatment results.
b-When compared with single-dose interaction study results.

CONCLUSION

In conclusion, pharmacodynamics interaction was found in both rat and rabbit models. On the basis of the available evidence, the co-administration of fenofibrate with glimepiride results in alteration of the hypoglycemic activity of glimepiride. Although the combination was well tolerated in both the species and did not induce any hypoglycemic shock this study should be extended to humans to investigate any possible interaction.

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