

## EFFECT OF RITONAVIR ON THE PHARMACODYNAMICS OF GLICLAZIDE IN ANIMAL MODELS

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*Key words: gliclazide, ritonavir, diabetes, HIV-infection, hypoglycemia*

### SUMMARY

*The present study was performed to investigate the effect of ritonavir on the pharmacodynamics of gliclazide in rats (normal and diabetic) and rabbits to evaluate the safety and effectiveness of the combination. All blood samples were analyzed for blood glucose by the GOD/POD method and insulin in rabbit blood samples by the radioimmunoassay method. Ritonavir alone produced significant elevation in glucose and insulin levels. Gliclazide produced hypoglycemic/antidiabetic activity in normal and diabetic rats with peak activity at 2 h and 8 h and hypoglycemic activity in normal rabbits at 3 h. In combination, ritonavir significantly ( $P < 0.05$ ) enhanced the effect of gliclazide in rats and rabbits and this effect was more significant following multiple dose than single dose administration. Contrary to the theoretical expectation, this interaction between ritonavir and gliclazide appeared to be pharmacokinetic rather than pharmacodynamic. The combina-*

*tion may need dose adjustment and care should be taken when the combination is prescribed for clinical benefit in diabetic patients.*

### INTRODUCTION

The study of the mechanisms of drug interactions is of much value on choosing drug concentrations to provide rational therapy. The drug interaction studies assume much importance, especially for drugs that have narrow safety margins and where drugs are used for a prolonged period of time. Diabetes mellitus is a metabolic disorder that needs treatment for prolonged periods and maintenance of normal blood glucose level is very important in this condition, since both hyperglycemia and hypoglycemia are unwanted phenomena (1).

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels and disturbances in carbohydrate, fat and protein metabolism and an increased risk of complications from vascular disease (2). Diabetes may be due to a decrease in the synthesis of insulin (type 1) or decrease in the secretion of insulin (type 2) from  $\beta$ -cells of the pancreatic islets of Langerhans. It is estimated that 143 million people worldwide suffer from diabetes (3) and

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the number may probably double by the year 2030 (4). In India, the prevalence of diabetes is estimated to 1%-5%.

Among the many metabolic perturbations that occur as the result of the human immunodeficiency virus (HIV) infection and its treatment, alterations in normal glucose homeostasis remain a highly prevalent and alarming clinical change in affected patients (5). Insulin resistance, impaired glucose tolerance and type 2 diabetes are conditions that are increasingly described in HIV-1 infected subjects receiving highly active antiretroviral therapy (HAART), especially with protease inhibitors (PIs) (6). Much of concern is due to the recognition of the long-term complications of insulin resistance and hyperglycemia in the context of the growing epidemic of type 2 diabetes mellitus worldwide (7). Ritonavir was the second agent in the HIV-1 protease inhibitor class that ushered in the era of HAART for HIV infection more than a decade ago. Today, ritonavir is almost exclusively used at low, subtherapeutic doses to 'enhance or boost' the exposure of concomitantly administered HIV PIs, and has become a cornerstone of HIV therapy, thus exploiting what was originally thought to be one of the greatest limitations of ritonavir (8). The dose of ritonavir administered in boosted PI regimens is generally considered subtherapeutic (100-200 mg) (9). However, the effect of ritonavir alone on oral antidiabetic therapy is not known.

Oral hypoglycemic agents are used in the treatment of type 2 diabetes, among which gliclazide, a second generation sulphonylurea derivative, is preferred in therapy because of its selective inhibitory activity towards pancreatic  $K^+$  ATP channels (10), antioxidant property (11), low incidence of producing severe hypoglycemia (12), and other hemobiological effects (10).

Since there is every possibility for the combined use of gliclazide and ritonavir in chronic diabetics with associated HIV infection, the study was designed to investigate the effect of ritonavir on the activity of gliclazide in rats (normal and diabetic) and rabbits to evaluate the safety and effectiveness of the combination with respect to glucose and insulin levels.

## MATERIALS AND METHODS

### *Drugs and chemicals*

Gliclazide and ritonavir were the gift samples from Micro Labs (Bangalore, India) and Aurobindo Pharma Ltd. (Hyderabad, India), respectively. Alloxan monohydrate was purchased from LOBA Chemie (Mumbai, India). Glucose kits (Span Diagnostics) were purchased from local pharmacy. All other reagents/chemicals used were of analytical grade.

### *Animals*

Albino rats of either sex of 6 to 7 weeks of age, weighing 250-320 g, and normal albino rabbits of either sex of 3 months of age, weighing 1.35-1.75 kg were used in the study. They were procured from the National Institute of Nutrition, Hyderabad, India. They were maintained under standard laboratory conditions at an ambient temperature of  $25 \pm 2$  °C and  $50 \pm 15$ % relative humidity with a 12-h light/12-h dark cycle. Animals were fed a commercial pellet diet (Rayan's Biotechnologies Pvt Ltd., Hyderabad, India) and water *ad libitum*. They were fasted for 18 h prior to the experiment and during the experiment they were withdrawn from food and water. The animal experiments were performed after prior approval of the study protocol by the institutional Animal Ethics Committee and by the government regulatory body for animal research (Reg. No. 516/01/A/CPCSEA). The study was conducted in accordance with the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

### *Selection of doses and preparation of oral test solution/suspension*

In clinical practice, ritonavir administered in boosted PI regimens is 100-200 mg and gliclazide 80 mg orally as antiretroviral and antidiabetic therapy, respectively. For our study we selected 200 mg dose of ritonavir by considering its highly variable plasma concentrations (13). Hence, human therapeutic doses extrapolated to rat/rabbit based on body surface area (14) were used

and administered orally. For rat experiments, the selected dose of gliclazide was 2 mg/kg body weight (b.w.), based on the influence of dose-effect relationship of gliclazide on blood glucose in normal rats. Ritonavir was suspended in 3% CMC-Na for oral administration (15). Gliclazide solution was prepared by dissolving it in a few drops of 0.1N NaOH and then made up to the volume with distilled water.

#### *Dose-effect relationship of gliclazide in rats*

A group of six normal rats were administered 1 mg/kg b.w. gliclazide orally. The same group of animals were administered gliclazide 2 mg/kg b.w. orally and 4 mg/kg b.w. gliclazide orally. One week washout period was maintained between treatments.

#### *Pharmacodynamic study in normal rats*

A group of six rats were administered 2 mg/kg b.w. gliclazide orally. The same group of animals were administered ritonavir 18 mg/kg b.w. orally and the combination of ritonavir and gliclazide. One week washout period was maintained between treatments. After this single dose interaction study, daily treatment with the interacting drug (ritonavir) was continued in this group for the next eight days with regular feeding. Later, after 18-h fasting, they were given the combined treatment on day 9 again.

#### *Pharmacodynamic study in diabetic rats*

Diabetes was induced in rats by the administration of alloxan monohydrate in two doses, i.e. 100 mg and 50 mg/kg b.w. intraperitoneally for two consecutive days (16). After 72 h, samples were collected from rats by orbital puncture of all surviving rats and serum was analyzed for glucose levels. Rats with blood glucose levels of 200 mg/dL and above were considered as diabetic and selected for the study. The same treatment as described in the study in normal rats was performed in the group of six alloxan-induced diabetic rats.

#### *Pharmacodynamic study in normal rabbits*

A group of six rabbits were administered 5.6 mg/1.5 kg b.w. gliclazide orally. The same group were administered ritonavir 14 mg/1.5 kg b.w. orally and the combination of ritonavir and gliclazide. One week washout period was maintained between treatments. After this single dose interaction study, the same group continued with daily treatment with interacting drug (ritonavir) for the next eight days with regular feeding. Later, after 18-h fasting, they were given the combined treatment on day 9 again.

#### *Blood sampling and determination of blood glucose and insulin*

Blood samples were withdrawn from retro-orbital plexus (17) of each rat at 0, 1, 2, 3, 4, 6, 8 and 12 h. Blood samples were withdrawn from the marginal ear vein of each rabbit at 0, 1, 2, 3, 4, 6, 8, 12, 16, 20 and 24 h. These blood samples were analyzed for blood glucose by the GOD/POD method (18) using commercial glucose kits. Plasma insulin in rabbit blood samples was measured at 3 and 24 h by radioimmunoassay (RIA) method (19) using a commercially available kit (human insulin as standard; Insik-5, Sorin Biomedica, Saluggia, Italy) as *per* instructions provided by the manufacturers.

#### *Statistical analysis*

Data were expressed as mean  $\pm$  SEM. The significance was determined by use of Student's paired t-test.

## **RESULTS**

#### *Dose-effect relationship of gliclazide in rats*

Dose dependent response was observed with the three oral doses tried with gliclazide. The 2 mg/kg b.w. gliclazide was selected based on the ideal blood glucose reduction, which is about 35%. Gliclazide produced hypoglycemic activity with maximum biphasic reduction of  $26.77 \pm 1.13\%$  and  $28.91 \pm 2.53\%$ ,  $38.59 \pm 1.58\%$  and  $40.50 \pm 1.40\%$ , and  $46.28 \pm 1.67\%$

and  $50.65 \pm 1.46\%$  at 2 h and 8 h with 1 mg/kg b.w., 2 mg/kg b.w. and 4 mg/kg b.w. dose, respectively (Fig. 1).

Figure 1. Dose effect relationship of gliclazide in normal rats (n=6).

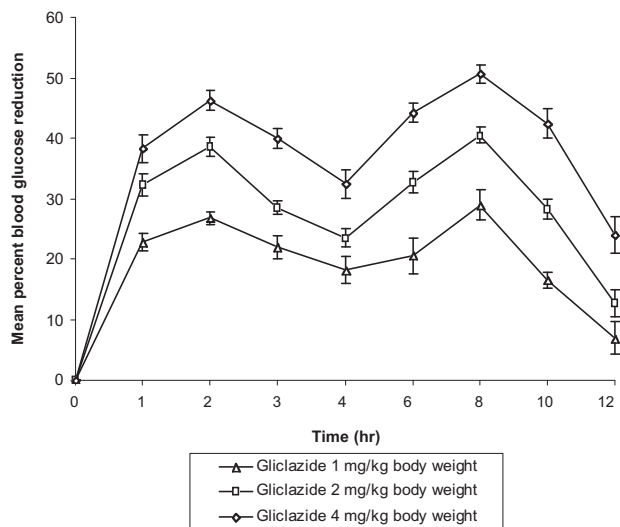
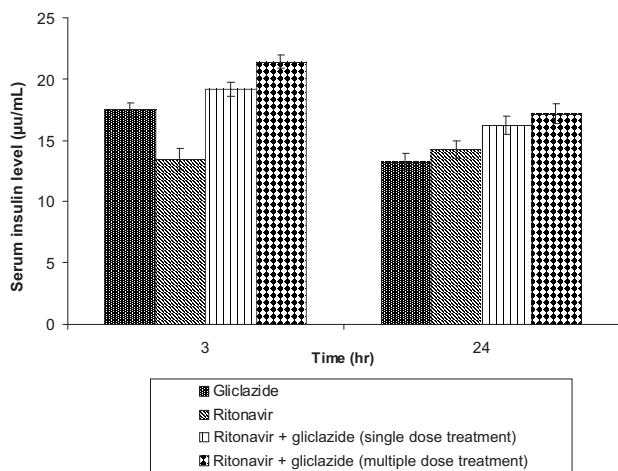


Figure 2. Effect of ritonavir/gliclazide and their combination on serum insulin levels in normal rabbits (n=6).



*Pharmacodynamic interaction study in normal rats*

In normal rats, gliclazide produced hypoglycemic activity with maximum biphasic reduction of  $41.05 \pm 1.02\%$  and  $39.21 \pm 0.89\%$  at 2 h and 8 h,

respectively (Table 1). Ritonavir alone produced minor hyperglycemia in normal rats (Tables 1). In combination, ritonavir significantly ( $P < 0.05$ ) enhanced the hypoglycemic effect of gliclazide with maximum blood glucose reduction of  $46.05 \pm 0.70\%$  and  $44.14 \pm 1.37\%$ , and  $48.68 \pm 1.54\%$  and  $46.05 \pm 0.88\%$  at 2 h and 8 h following single dose and multiple dose administration of ritonavir, respectively (Table 1). The enhancement in gliclazide effect was higher with multiple dose than with single dose treatment with ritonavir.

*Pharmacodynamic interaction study in diabetic rats*

In diabetic rats, gliclazide produced antihyperglycemic activity with maximum biphasic reduction of  $42.92 \pm 1.54\%$  and  $44.01 \pm 1.36\%$  at 2 h and 8 h, respectively (Table 2). Gliclazide mediated biphasic hypoglycemic effect in diabetic rats was comparatively higher than in normal rats and the maximum hypoglycemic effect of gliclazide was observed at 8 h in diabetic rats *versus* 2 h in normal rats. Ritonavir alone produced minor hyperglycemia in diabetic rats and this effect was higher in normal rats (Table 2). In combination, ritonavir significantly ( $P < 0.05$ ) enhanced the hypoglycemic effect of gliclazide with maximum blood glucose reduction of  $46.05 \pm 1.22\%$  and  $48.19 \pm 1.03\%$ , and  $47.69 \pm 1.19\%$  and  $49.16 \pm 1.14\%$  at 2 h and 8 h following single dose and multiple dose administration of ritonavir, respectively (Table 2). The enhancement of gliclazide effect was higher with multiple dose than with single dose treatment with ritonavir.

*Pharmacodynamic interaction study in normal rabbits*

Gliclazide produced hypoglycemic activity with maximum reduction of  $34.60 \pm 1.09\%$  at 3 h in normal rabbits (Table 3). Ritonavir alone produced minor hyperglycemia in rabbits (Table 3). Ritonavir significantly ( $P < 0.05$ ) enhanced the hypoglycemic effect of gliclazide with maximum reduction of  $40.40 \pm 0.83\%$  and  $42.18 \pm 1.04\%$  in blood glucose in normal rabbits at 3 h following single dose and multiple dose treatment

with ritonavir, respectively (Table 3). The enhancement in gliclazide effect was higher with multiple dose than with single dose treatment with ritonavir. Serum insulin levels increased with ritonavir treatment in normal rabbits (Fig. 2).

## DISCUSSION

HIV infected patients are likely to suffer from diabetes mellitus (5) and hence antiretroviral drugs are usually co-administered with oral antidiabetic drugs. HIV infection and diabetes are both chronic diseases that significantly affect lifestyle. When they interact, the treatment regimens required for both diseases can

Table 1. Mean percent blood glucose reduction in normal rats (n=6)

Time (h)	Gliclazide	Ritonavir	Ritonavir + gliclazide (single dose treatment)	Ritonavir + gliclazide (multiple dose treatment)
1	32.15 ± 1.20	-04.13 ± 0.73	34.86 ± 2.03	36.45 ± 1.69*
2	41.05 ± 1.02	-08.25 ± 1.32	46.05 ± 0.70*	48.68 ± 1.54*
3	28.83 ± 0.91	-12.17 ± 3.20	33.33 ± 2.49*	36.40 ± 1.70*
4	24.02 ± 0.91	-16.60 ± 2.99	28.28 ± 1.45*	29.75 ± 1.44*
6	30.68 ± 1.14	-10.58 ± 2.22	36.70 ± 2.06*	37.87 ± 1.68*
8	39.21 ± 0.89	-06.86 ± 2.01	44.14 ± 1.37*	46.05 ± 0.88*
10	26.95 ± 1.46	-03.83 ± 1.42	30.81 ± 1.89*	33.13 ± 0.99*
12	13.99 ± 1.32	-01.16 ± 1.08	18.23 ± 2.15*	23.54 ± 1.11*

Gliclazide: 2 mg/kg b.w. treated group

Ritonavir: 18 mg/kg b.w. treated group

\*Significant compared to gliclazide control ( $P < 0.05$ )

Table 2. Mean percent blood glucose reduction in diabetic rats (n=6)

Time (h)	Gliclazide	Ritonavir	Ritonavir + gliclazide (single dose treatment)	Ritonavir + gliclazide (multiple dose treatment)
1	34.17 ± 1.63	-08.08 ± 2.03	38.53 ± 1.00*	40.76 ± 1.25*
2	42.92 ± 1.54	-10.33 ± 2.03	46.05 ± 1.22*	47.69 ± 1.19*
3	30.95 ± 1.52	-14.46 ± 1.50	34.56 ± 1.11*	37.85 ± 1.13*
4	24.70 ± 1.55	-21.20 ± 1.91	30.89 ± 0.98*	32.91 ± 1.22*
6	36.86 ± 1.03	-15.20 ± 0.77	40.11 ± 1.33*	41.96 ± 1.31*
8	44.01 ± 1.36	-12.36 ± 2.14	48.19 ± 1.03*	49.16 ± 1.14*
10	28.24 ± 2.01	-10.60 ± 1.95	32.15 ± 1.31*	36.76 ± 1.43*
12	24.65 ± 1.97	-06.86 ± 1.81	28.42 ± 1.71*	31.56 ± 1.35*

Gliclazide: 2 mg/kg b.w. treated group

Ritonavir: 18 mg/kg b.w. treated group

\*Significant compared to gliclazide control ( $P < 0.05$ )

Table 3. Mean percent blood glucose reduction in normal rabbits (n=6)

Time (h)	Gliclazide	Ritonavir	Ritonavir + gliclazide (single dose treatment)	Ritonavir + gliclazide (multiple dose treatment)
1	18.68 ± 1.77	-04.31 ± 0.53	20.91 ± 1.71	22.00 ± 0.90*
2	25.22 ± 0.92	-08.69 ± 1.32	31.21 ± 1.52*	34.60 ± 1.09*
3	34.60 ± 1.09	-12.31 ± 1.57	40.40 ± 0.83*	42.18 ± 1.04*
4	26.63 ± 1.64	-18.03 ± 1.05	32.60 ± 1.12*	33.14 ± 1.29*
6	23.04 ± 1.34	-11.21 ± 1.29	29.06 ± 0.72*	30.63 ± 1.23*
8	18.31 ± 2.72	-08.00 ± 1.99	24.50 ± 1.87*	27.36 ± 1.34*
12	09.69 ± 1.11	-04.31 ± 1.22	12.39 ± 0.57*	14.43 ± 0.86*
16	04.97 ± 1.29	-02.88 ± 0.71	10.62 ± 1.32*	12.61 ± 1.52*
20	02.11 ± 0.97	-01.07 ± 0.48	08.86 ± 0.63*	10.42 ± 1.89*
24	01.03 ± 1.18	01.42 ± 0.91	06.01 ± 0.61*	08.63 ± 1.58*

Gliclazide: 5.6 mg/1.5 kg b.w. treated group

Ritonavir: 14 mg/1.5 kg b.w. treated group

\*Significant compared to gliclazide control ( $P < 0.05$ )

be overwhelming for patients. In our study, the effect of multiple dose ritonavir on gliclazide activity was also studied for the influence of long term treatment with ritonavir since both drugs are used as chronic medication.

Drug interactions are usually seen in clinical practice and the mechanisms of interactions are mostly evaluated in animal models (rodent and non-rodent). We studied the influence of ritonavir on the pharmacodynamics of gliclazide in rats (rodent) and rabbits (non-rodent). The normal rat model served to quickly identify the interaction and diabetic rat model served to validate the same response in the actually used condition of the drug. The rabbit model is another dissimilar species to validate the occurrence of the interaction. Usually, if the interaction is observed in rodent and non-rodent species, it is likely to occur in humans too, with due consideration of their representative variability in humans. Although animal models can never replace the need for comprehensive studies in humans, their use can provide important insights to understand and to evaluate the potent interactions between drugs. Since a small amount of blood was required for glucose analysis, blood samples were collected by retro-orbital puncture and marginal ear vein as they were reported to be good methods when small samples of blood were required. Diabetes was induced with alloxan monohydrate, since it was more economical and easily available.

Rats are known to be more sensitive to gliclazide response. So, we conducted the dose effect-relationship study of gliclazide to select the oral dose producing approximately 35% of blood glucose reduction in rats. Gliclazide produced biphasic response in rat model when administered alone, which may be due to its biliary excretion and enterohepatic cycling. Such effect is not seen in rabbit model. The enterohepatic recirculation observed in our study was consistent with the previous reports in animal models (1,20,21) and humans (22). As *per* a recent clinical study (23), the extent of mean enterohepatic recirculation observed in humans was consistent with data recorded in animals. This consistency will address the probable correlation of preclinical studies to human subjects, and their use might provide important

insights into the mechanisms of drug interactions for better understanding and rational therapy. Gliclazide is known to exert hypoglycemic/antihyperglycemic activity by pancreatic (24) (stimulating insulin secretion by blocking  $K^+$  channels in the pancreatic  $\beta$ -cells) and extrapancreatic (25) (increasing tissue uptake of glucose) mechanisms.

In our study, the boosting dose of ritonavir alone produced significant hyperglycemic activity in rats (normal and diabetic) and rabbits, and increase in insulin levels in rabbits. The elevated insulin levels in face of increased glucose levels suggest an insulin resistant state (26). Insulin resistance is accepted as the underlying fundamental defect that predates and ultimately leads to the development of type 2 diabetes mellitus (27). Our results were consistent with the earlier reports, as ritonavir in full-dose or combination form is reported to produce hyperglycemia, new-onset diabetes mellitus, exacerbation of existing diabetes mellitus (28), insulin resistance (29) and reduction in insulin-mediated glucose disposal (30). The hyperglycemic effect of ritonavir is comparatively higher than normal in diabetic rats, clearly indicating the potency of ritonavir to exacerbate the existing diabetes mellitus (28). So, based on the hyperglycemic effect of ritonavir, theoretically we may expect that gliclazide activity may be decreased in the presence of ritonavir. However, in contrast to this expectation, the gliclazide hypoglycemic and antidiabetic activity was significantly enhanced by ritonavir following single and multiple dose treatment in rat and rabbit models and it confirmed the presence of potential interaction between gliclazide and ritonavir. Furthermore, the presence of interaction was supported by the increase in serum insulin levels with ritonavir treatment. The enhancement was higher with the multiple dose than with single dose ritonavir treatment. Literature data suggest that an increase in adiponectin levels during chronic administration of ritonavir may contribute to the amelioration in the acute induction of insulin resistance (31).

It is also clear that, since ritonavir increased blood glucose and insulin levels on its own, the increase in the effect of gliclazide on blood glucose might be due to improved blood gliclazide level in the presence of

ritonavir, as there is a possibility of pharmacokinetic interaction at the metabolic level. Ritonavir is a well known potent CYP3A4 inhibitor (32) and is used to enhance the pharmacokinetic and anti-HIV activity profiles of the concomitantly administered PIs (33-35). Gliclazide is known to be metabolized by hepatic microsomal enzymes CYP2C9 primarily and partly by CYP450-3A4 (10,21). However, the drug ritonavir did not change the pattern of biphasic response of gliclazide indicating that it did not interfere with the reabsorption of gliclazide in its enterohepatic circulation in rats. So, the increased activity of gliclazide in the presence of ritonavir may be due to its reduced metabolism by hepatic microsomal enzyme CYP3A4. This process might have dominated the overall interaction between gliclazide and ritonavir in the pharmacokinetic rather than pharmacodynamic pattern. It has to be confirmed by conducting pharmacokinetic interaction studies. Even though our study demonstrated that the hyperglycemic activity of ritonavir was due to insulin resistance, the other

possible mechanisms of ritonavir influencing the glucose-insulin homeostasis could not be ruled out because the glucose-insulin homeostasis is a multifactorial process.

## CONCLUSION

Since the interaction was seen in two dissimilar species, it is likely to occur in humans also leading to increased activity of gliclazide, which may need dosage adjustment. Hence, care should be taken when the combination is prescribed for their clinical benefit in diabetic patients. However, the present study warrants further studies to find out the relevance of this interaction in humans and to know the exact mechanism of action behind this interaction.

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