LIST OF ORIGINAL PUBLICATIONS

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LIST OF ORIGINAL PUBLICATIONS AS ABSTRACTS


LIST OF PRESENTATIONS IN CONFERENCES

Poster Presentations:

I. **Mastan S.K. and Eswar Kumar K.** Studies on the mechanisms of drug interactions of protease inhibitors with gliclazide in animal models. 3rd National Conference on Infectious Diseases, Organized by All India Institute of Medical Sciences at New Delhi, India on 17-18 Apr, 2010. *(Best Research Paper Award)*


Oral Presentations:


II. **Mastan S.K. and Eswar Kumar K.** Study of pharmacodynamic and pharmacokinetic interactions of selected protease inhibitors with gliclazide in animal models. 2nd Annual Biotechnology Conference for Students, Organized by Indian Institute of Information Technology, Pune, India on 13-14 Nov 2010.
Influence of non-nucleoside reverse transcriptase inhibitors (efavirenz and nevirapine) on the pharmacodynamic activity of gliclazide in animal models
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Abstract

Background: Type 2 diabetes may occur as a result of HIV infection and/or its treatment. Gliclazide is a widely used drug for the treatment of type 2 diabetes. Efavirenz and nevirapine are widely used non-nucleoside reverse transcriptase inhibitors for the treatment of HIV infection. The role of Efavirenz and nevirapine on the pharmacodynamic activity of gliclazide is not currently known. The objective of this study was to examine the effect of oral administration of efavirenz and nevirapine on blood glucose and investigate their effect on the activity of gliclazide in rats (normal and diabetic) and rabbits to evaluate the safety and effectiveness of the combination.

Methods: Studies in normal and alloxan induced diabetic rats were conducted with oral doses of 2 mg/kg bd. wt. of gliclazide, 54 mg/kg bd. wt. of efavirenz or 18 mg/kg bd. wt. of nevirapine and their combination with adequate washout periods in between treatments. Studies in normal rabbits were conducted with 5.6 mg/1.5 kg bd. wt. of gliclazide, 42 mg/1.5 kg bd. wt. of efavirenz or 14 mg/1.5 kg bd. wt. of nevirapine and their combination given orally. Blood samples were collected at regular time intervals in rats from retro orbital puncture and by marginal ear vein puncture in rabbits. All the blood samples were analysed for blood glucose by GOD/POD method.

Results: Efavirenz and nevirapine alone have no significant effect on the blood glucose level in rats and rabbits. Gliclazide produced hypoglycaemic/antidiabetic activity in normal and diabetic rats with peak activity at 2 h and 8 h and hypoglycaemic activity in normal rabbits at 3 h. In combination, efavirenz reduced the effect of gliclazide in rats and rabbits, and the reduction was more significant with the single dose administration of efavirenz than multiple dose administration. In combination, nevirapine has no effect on the activity of gliclazide in rats and rabbits.

Conclusion: Thus, it can be concluded that the combination of efavirenz and gliclazide may need dose adjustment and care should be taken when the combination is prescribed for their clinical benefit in diabetic patients. The combination of nevirapine and gliclazide was safe. However, further studies are warranted.
Background

The study of mechanisms of drug interactions is of much value in selecting the drug concentrations to provide rational therapy. The drug interaction studies assume much importance especially for drugs that have narrow margin of safety and where the drugs are used for prolonged period of time. Diabetes mellitus is one such metabolic disorder that needs treatment for prolonged periods and maintenance of normal blood glucose level is very important in this condition, since both hyperglycemia as well as hypoglycemia is an unwanted phenomenon [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]. Type-1 diabetes is due to decrease in the synthesis of insulin and type-2 diabetes is characterized by hyperglycemia in the context of insulin resistance and relative insulin deficiency. There are estimated 143 million people worldwide suffering from diabetes [3] and the number may probably double by the year 2030 [4]. In India the prevalence rate of diabetes is estimated to be 1.5%.

Among the many metabolic perturbations that occur as a result of Human Immuno Deficiency Virus (HIV) infection and its treatment, alterations in normal glucose homeostasis remain a particularly prevalent and alarming clinical change in affected patients [5]. Much of concern is due to the recognition of the long-term complications of insulin resistance and hyperglycemia and understood is the context of the growing worldwide epidemic of type 2 diabetes mellitus [6].

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). HAART generally includes nucleoside reverse transcriptase inhibitors and protease inhibitors. Since many studies have suggested that PI therapy [7] is linked to the development of metabolic complications, it is of importance to propose therapeutic strategies with fewer side effects, such as the use of the non-nucleoside reverse transcriptase inhibitors (NNRTIs) and this approach appear successful to control HIV infection [8].

Efavirenz and nevirapine are NNRTIs used widely in combination with other antiretroviral drugs to treat HIV-infected patients. Recent reports demonstrate that a switch from a protease inhibitor to nevirapine or efavirenz results improvement of metabolic complications in HIV-infected patients [9] and short-term improvement in insulin resistance has been demonstrated with the substitution of nevirapine or efavirenz for the PI component of an antiretroviral regimen [10-12]. However the effect of efavirenz and nevirapine in diabetic condition/oral hypoglycemic agents is unknown.

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 [13-15], antioxidant property [16,17], low incidence of producing severe hypoglycemia [19,20] and other haemobiological effects [21,22]. Gliclazide is known to act mainly by releasing insulin by blocking K+ channels in the pancreatic β cells [23].

Since there is every possibility for the combined use of gliclazide and NNRTIs (efavirenz and nevirapine) in chronic diabetics with associated HIV infection, the study is planned to investigate the effect of efavirenz and nevirapine on blood glucose and their effect on the activity of gliclazide in rats (normal and diabetic) and rabbits to evaluate the safety and effectiveness of the combination with respect to blood glucose level.

Methods

Gliclazide and NNRTIs (efavirenz and nevirapine) are 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.

Albino rats of either sex of 6 to 7 weeks of age, weighing between 250-320 g and normal albino rabbits of either sex of 3 months of age, weighing between 1.35-1.75 Kg were used in the study. They were procured from National Institute of Nutrition, Hyderabad, India. They were maintained under standard laboratory conditions at an ambient temperature of 25 ± 2°C and 50 ± 15% relative humidity with a 12-h light/12-h dark cycle. Animals were fed with 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 Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).
Study design
In clinical practice, NNRTIs and gliclazide in therapeutic dose will be administered orally as antiretroviral and anti-diabetic therapy, respectively. Hence, human oral therapeutic doses of the respective drugs were extrapolated to rat/rabbit based on body surface area [24]. But the dose of gliclazide for rat experiments was selected as 2 mg/kg bd. wt based on the influence of dose-effect relationship of gliclazide on blood glucose in normal rats. Efavirenz and nevirapine were suspended in 0.5% CMC for oral administration [25,26]. Gliclazide solution was prepared by dissolving it in a few drops of 0.1 N NaOH then made up to the volume with distilled water.

The study consists of two phases.

Phase-1: pharmacodynamic interaction study between efavirenz and gliclazide

Phase-2: pharmacodynamic interaction study between nevirapine and gliclazide

Each phase consists of 3 stages.

Stage-1: study in normal rats
Stage-2: study in diabetic rats
Stage-3: study in normal rabbits

Study in normal rats
A group of six rats was administered with 2 mg/kg bd. wt of gliclazide, orally. The same group was administered with interacting drug (efavirenz 54 mg/kg bd. wt. or nevirapine 18 mg/kg bd. wt., orally) and the combination of interacting drug and gliclazide. One week washout period was maintained between treatments. After this single dose interaction study the same group was continued with the daily treatment of interacting drug (efavirenz/nevirapine) for the next eight days with regular feeding. Later after 18 h fasting they were again given the combined treatment on the ninth day.

Collection of blood samples
Blood samples were withdrawn from retro orbital plexus [28] 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 analysed for blood glucose by GOD/POD method [29] using commercial glucose kits.

Data and statistical analysis
Data were expressed as mean ± SEM. The significance was determined by applying Student’s paired ‘t’ test.

Results
Pharmacodynamic interaction study between efavirenz and gliclazide
Gliclazide produced hypoglycemic activity with maximum biphasic reduction of 41.64 ± 0.80% and 38.70 ± 1.43% at 2 h and 8 h respectively in normal rats (Table 1). Gliclazide produced antihyperglycemic activity with maximum biphasic reduction of 42.05 ± 1.73% and 44.05 ± 1.55% at 2 h and 8 h respectively in diabetic rats (Table 2). Gliclazide produced hypoglycemic activity with maximum reduction of 34.25 ± 0.99% at 3 h in normal rabbits (Table 3). Efavirenz alone has not produced any significant effect on the blood glucose level of rats (normal and diabetic) and rabbits (Tables 1, 2, 3). In combination efavirenz has reduced the gliclazide activity in rats and rabbits and the reduction was more significant with the single dose treatment of efavirenz than multiple dose treatment (Tables 1, 2, 3).

Pharmacodynamic interaction study between nevirapine and gliclazide
Gliclazide produced hypoglycemic activity with maximum biphasic reduction of 40.72 ± 0.56% and 37.46 ± 1.18% at 2 h and 8 h respectively in normal rats (Table 4). Gliclazide produced antihyperglycemic activity with maximum biphasic reduction of 42.50 ± 1.40% and 44.46 ± 1.46% at 2 h and 8 h respectively in diabetic rats (Table 5). Gliclazide produced hypoglycemic activity with maximum reduction of 36.18 ± 1.08% at 3 h in normal rabbits (Table 6). Nevirapine alone has not produced any signifi-
cant effect on the blood glucose level of rats (normal and diabetic) and rabbits (Tables 4, 5, 6). In combination nevirapine has no impact on the glimepiride activity in rats and rabbits following single and multiple dose treatments (Tables 4, 5, 6).

Discussion

HIV infected patients are likely to suffer with diabetes mellitus [5] and hence most often antiretroviral drugs are co-administered along with oral antidiabetic drugs. HIV infection and diabetes are both chronic diseases that significantly affect lifestyle. When they intersect, the treatment regimens required for both diseases can be overwhelming for patients. Frequently prescribed antiretroviral drugs belong to the class of non-nucleoside reverse transcriptase inhibitors (NNRTIs) in HIV-infected patients.

Efavirenz and nevirapine are commonly prescribed NNRTIs for the treatment of HIV-infection and known to be improving the metabolic complications in HIV-infected patients [9-12]. However, there is no much evidence on the activity of efavirenz/nevirapine alone in diabetic condition, as well as their effect on the activity of glimepiride. Based on these factors the study was planned to investigate the effect of efavirenz/nevirapine on blood glucose and its effect on the activity of glimepiride in rats (normal and diabetic) and rabbits to evaluate the safety and effectiveness of the combination with respect to blood glucose level. In our study, the multiple dose effect of efavirenz and nevirapine on the glimepiride activity was also studied for the influence of the long term treatment with efavirenz/nevirapine since both are used for chronic period.

Drug interactions are usually seen in clinical practice and the mechanisms of interactions are evaluated usually in animal models (rodents and non-rodents). We studied the influence of efavirenz and nevirapine on the activity of glimepiride in rats (normal and diabetic) and rabbits. 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. Since small amount of blood was required for glucose analysis, the blood samples were collected by retro-orbital puncture as it was reported to be good method when small samples of blood were required [28]. Diabetes was induced with alloxan.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Gliclazide</th>
<th>Efavirenz</th>
<th>Efavirenz + Gliclazide (Single dose treatment)</th>
<th>Efavirenz + Gliclazide (Multiple dose treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31.46 ± 1.39</td>
<td>-06.66 ± 2.95</td>
<td>24.40 ± 1.20***</td>
<td>26.57 ± 1.09*</td>
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<td>2</td>
<td>41.64 ± 0.80</td>
<td>-04.44 ± 1.44</td>
<td>33.77 ± 0.87***</td>
<td>36.26 ± 1.53**</td>
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<tr>
<td>3</td>
<td>28.21 ± 0.95</td>
<td>-02.94 ± 0.96</td>
<td>21.19 ± 1.15***</td>
<td>22.26 ± 0.99***</td>
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<td>4</td>
<td>24.21 ± 1.13</td>
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<td>16.16 ± 0.79***</td>
<td>18.68 ± 0.96***</td>
</tr>
<tr>
<td>6</td>
<td>31.47 ± 1.46</td>
<td>02.14 ± 1.49</td>
<td>23.34 ± 1.09***</td>
<td>28.71 ± 1.85</td>
</tr>
<tr>
<td>8</td>
<td>38.70 ± 1.43</td>
<td>04.29 ± 1.09</td>
<td>28.36 ± 1.70***</td>
<td>31.21 ± 2.03***</td>
</tr>
<tr>
<td>10</td>
<td>26.08 ± 1.02</td>
<td>06.86 ± 1.78</td>
<td>19.00 ± 1.44***</td>
<td>20.45 ± 1.11***</td>
</tr>
<tr>
<td>12</td>
<td>12.27 ± 1.55</td>
<td>06.13 ± 1.59</td>
<td>08.27 ± 0.64</td>
<td>08.98 ± 1.02</td>
</tr>
</tbody>
</table>

Table 1: Mean percent blood glucose reduction in normal rats (N = 6)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Gliclazide</th>
<th>Efavirenz</th>
<th>Efavirenz + Gliclazide (Single dose treatment)</th>
<th>Efavirenz + Gliclazide (Multiple dose treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24.05 ± 1.95</td>
<td>05.08 ± 2.46</td>
<td>30.02 ± 1.59***</td>
<td>28.26 ± 1.62</td>
</tr>
<tr>
<td>2</td>
<td>31.21 ± 1.74</td>
<td>03.21 ± 2.41</td>
<td>20.70 ± 1.31***</td>
<td>25.45 ± 1.93**</td>
</tr>
<tr>
<td>3</td>
<td>25.13 ± 1.77</td>
<td>02.40 ± 2.24</td>
<td>16.78 ± 2.21***</td>
<td>20.78 ± 1.36**</td>
</tr>
<tr>
<td>6</td>
<td>36.14 ± 1.56</td>
<td>03.99 ± 1.97</td>
<td>22.15 ± 1.34***</td>
<td>30.12 ± 1.47*</td>
</tr>
<tr>
<td>8</td>
<td>44.05 ± 1.55</td>
<td>04.45 ± 2.26</td>
<td>32.01 ± 1.39***</td>
<td>38.27 ± 1.42**</td>
</tr>
<tr>
<td>10</td>
<td>28.56 ± 1.87</td>
<td>05.39 ± 1.72</td>
<td>16.25 ± 2.25***</td>
<td>20.89 ± 1.49***</td>
</tr>
<tr>
<td>12</td>
<td>24.66 ± 2.32</td>
<td>04.28 ± 1.53</td>
<td>08.49 ± 2.32***</td>
<td>11.31 ± 2.17***</td>
</tr>
</tbody>
</table>

Table 2: Mean percent blood glucose reduction in diabetic rats (N = 6)
monohydrate, since it was more economical and easily available.

Gliclazide produced biphasic response in rat model when administered alone, which may be due its biliary excretion and entero hepatic cycling [30]. Such effect is not seen in rabbit model. Gliclazide is known to produce hypoglycemic/antihyperglycemic activity by pancreatic [31-33] (stimulating insulin secretion by blocking K+ channels in the pancreatic \( \beta \) cells) and extra pancreatic [34-36] (increasing tissue uptake of glucose) mechanisms.

Our study revealed the safety profile of efavirenz and nevirapine in diabetic condition also, with respect to blood glucose levels. However, contrary to the theoretical expectation, the activity of gliclazide was reduced in the presence of efavirenz in rats (normal and diabetic) and rabbits and it confirms the presence of potential interaction between efavirenz and gliclazide. The impact of efavirenz on the activity of gliclazide was more significant following single dose administration.

In vitro studies have shown that efavirenz inhibits CYP2C9, CYP2C19 and CYP3A4 with inhibition constant (\( K_i \)) values (8.5-17 \( \mu M \)) in the range observed efavirenz plasma concentrations [37,38]. In vitro and in vivo studies also demonstrated that efavirenz induces CYP3A4 activity in a concentration- and time-dependent manner [25,39,40]. Clinical drug-drug interaction studies showed that efavirenz decreased the systemic exposure of several CYP3A4 substrates, such as amproenavir, indinavir and methadone [41-43] in addition to the CYP2C9 and CYP2C19 substrates [44]. Gliclazide is known to be metabolized by hepatic microsomal enzymes CYP2C9 primarily and partly by CYP3A4 [33,45]. So the decreased activity of gliclazide in the presence of efavirenz may be due to its increased metabolism by hepatic microsomal enzymes. During initial preclinical studies, chronic administration of efavirenz was shown to induce its own metabolism and to increase activities of CYP3A4 in rats, rhesus monkeys, and humans [39,46,47]. So this autoinduction may be the reason behind the less impact of the efavirenz on the activity of gliclazide following multiple dose administration in rats and rabbits. Overall the interaction between gliclazide and efavirenz appears to be due to pharmacokinetic rather than pharmacodynamic in nature. The present study is limited to describe the exact mechanism of action(s) behind this interaction and it has to be confirmed by conducting pharmacokinetic interaction studies in different species.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Gliclazide</th>
<th>Efavirenz</th>
<th>Efavirenz + Gliclazide (Single dose treatment)</th>
<th>Efavirenz + Gliclazide (Multiple dose treatment)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>18.30 ± 1.88</td>
<td>-04.36 ± 1.49</td>
<td>09.25 ± 1.37***</td>
<td>11.54 ± 1.24***</td>
</tr>
<tr>
<td>2</td>
<td>24.50 ± 1.24</td>
<td>-06.16 ± 1.93</td>
<td>14.96 ± 1.12***</td>
<td>16.61 ± 1.21***</td>
</tr>
<tr>
<td>3</td>
<td>34.25 ± 0.99</td>
<td>-03.63 ± 1.24</td>
<td>24.97 ± 0.87***</td>
<td>27.14 ± 0.67***</td>
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<tr>
<td>4</td>
<td>25.92 ± 1.30</td>
<td>01.48 ± 1.22</td>
<td>14.61 ± 1.22***</td>
<td>16.97 ± 1.40***</td>
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<tr>
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<td>24.50 ± 0.96</td>
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<td>12.44 ± 1.59***</td>
<td>14.05 ± 1.53***</td>
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<td>18.96 ± 2.85</td>
<td>02.09 ± 1.94</td>
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<td>12.28 ± 0.80***</td>
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<td>16</td>
<td>05.71 ± 1.13</td>
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</tr>
<tr>
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<td>04.60 ± 1.78</td>
<td>01.42 ± 0.45</td>
<td>02.85 ± 0.90</td>
</tr>
</tbody>
</table>

***Significant at \( P < 0.001 \) compared to gliclazide control

<table>
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<tr>
<th>Time (h)</th>
<th>Gliclazide</th>
<th>Nevirapine</th>
<th>Nevirapine + Gliclazide* (Single dose treatment)</th>
<th>Nevirapine + Gliclazide* (Multiple dose treatment)</th>
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</thead>
<tbody>
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<td>1</td>
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<td>30.83 ± 0.94</td>
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<tr>
<td>2</td>
<td>40.72 ± 0.56</td>
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<td>38.35 ± 0.72</td>
<td>40.49 ± 0.84</td>
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<tr>
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<td>28.34 ± 0.82</td>
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<td>26.84 ± 0.79</td>
<td>28.30 ± 0.68</td>
</tr>
<tr>
<td>4</td>
<td>23.97 ± 0.79</td>
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<td>22.90 ± 0.99</td>
<td>23.63 ± 1.13</td>
</tr>
<tr>
<td>6</td>
<td>30.90 ± 0.46</td>
<td>02.51 ± 0.66</td>
<td>30.42 ± 1.00</td>
<td>31.53 ± 0.67</td>
</tr>
<tr>
<td>8</td>
<td>37.46 ± 1.18</td>
<td>01.43 ± 0.71</td>
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<td>37.62 ± 1.44</td>
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<tr>
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<td>10.52 ± 1.14</td>
<td>00.34 ± 1.40</td>
<td>10.33 ± 1.30</td>
<td>11.80 ± 1.16</td>
</tr>
</tbody>
</table>

*Statistically no significance compared to gliclazide control
On other side, nevirapine is known to be an inducer of CYP3A4 and CYP2B6 [48,49]. There is a minor and non-significant reduction in gliclazide activity following nevirapine administration in rats and rabbits. The minor and non-significant decreased activity of gliclazide in the presence of nevirapine may be due to its increased metabolism by hepatic microsomal enzyme CYP3A4. Even though the reduction in gliclazide activity in the presence of nevirapine is minor and non-significant, comparatively the reduction is more with single dose treatment of nevirapine than multiple dose treatment. Just like efavirenz, nevirapine is also known to be undergoing autoinduction of CYP3A4 and CYP2B6 mediated metabolism following multiple dose administration [50]. So the effect associated with the multiple dose treatment of nevirapine may be due to autoinduction of nevirapine. However, either the single dose or multiple dose treatment of nevirapine has no significant impact on the gliclazide activity in rats (normal and diabetic) and rabbits and it confirms the combination was safe with respect to blood glucose level.

**Conclusion**

Since the interaction between efavirenz and gliclazide was seen in two dissimilar species, it is likely to occur in humans also leading to decreased 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. Since there is no interaction between nevirapine and gliclazide in any species, it is likely to be safe combination in humans also. However the present study warrants further studies to find out the relevance of these interactions in human beings and to know the exact mechanism of action behind this interaction(s) if any.

**List of abbreviations**

CMC: Carboxymethyl cellulose; CYP: Cytochrome P-450; GOD/POD: Glucose oxidase peroxidase; HIV: Human immuno deficiency virus; K+ ATP: Potassium adenosine triphosphate; NNRTIs: Non-nucleoside reverse transcriptase inhibitors; PIs: Protease inhibitors.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

SKM Participated in the design, carried out the study and drafted the manuscript.

**Table 5: Mean percent blood glucose reduction in diabetic rats (N = 6)**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Gliclazide</th>
<th>Nevirapine</th>
<th>Nevirapine + Gliclazide* (Single dose treatment)</th>
<th>Nevirapine + Gliclazide* (Multiple dose treatment)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>33.45 ± 1.76</td>
<td>02.08 ± 0.38</td>
<td>32.42 ± 1.47</td>
<td>33.23 ± 1.63</td>
</tr>
<tr>
<td>2</td>
<td>42.50 ± 1.40</td>
<td>04.34 ± 1.40</td>
<td>41.68 ± 1.27</td>
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</tr>
<tr>
<td>6</td>
<td>36.89 ± 1.30</td>
<td>06.28 ± 1.53</td>
<td>36.25 ± 1.48</td>
<td>36.78 ± 1.38</td>
</tr>
<tr>
<td>8</td>
<td>44.46 ± 1.46</td>
<td>03.08 ± 0.54</td>
<td>43.25 ± 1.35</td>
<td>44.37 ± 1.33</td>
</tr>
<tr>
<td>10</td>
<td>28.72 ± 1.76</td>
<td>01.67 ± 0.67</td>
<td>27.93 ± 1.55</td>
<td>28.92 ± 1.68</td>
</tr>
<tr>
<td>12</td>
<td>24.39 ± 1.81</td>
<td>01.64 ± 0.71</td>
<td>23.81 ± 1.80</td>
<td>24.98 ± 1.83</td>
</tr>
</tbody>
</table>

*Statistically no significance compared to gliclazide control

**Table 6: Mean percent blood glucose reduction in normal rabbits (N = 6)**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Gliclazide</th>
<th>Nevirapine</th>
<th>Nevirapine + Gliclazide* (Single dose treatment)</th>
<th>Nevirapine + Gliclazide* (Multiple dose treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19.44 ± 2.25</td>
<td>02.08 ± 2.38</td>
<td>21.09 ± 2.93</td>
<td>20.39 ± 2.00</td>
</tr>
<tr>
<td>2</td>
<td>26.73 ± 2.57</td>
<td>03.57 ± 2.48</td>
<td>25.38 ± 2.40</td>
<td>26.56 ± 0.90</td>
</tr>
<tr>
<td>3</td>
<td>36.18 ± 1.08</td>
<td>05.72 ± 2.23</td>
<td>34.38 ± 0.69</td>
<td>35.18 ± 1.39</td>
</tr>
<tr>
<td>4</td>
<td>28.90 ± 3.23</td>
<td>04.59 ± 2.66</td>
<td>26.48 ± 1.47</td>
<td>27.61 ± 1.83</td>
</tr>
<tr>
<td>6</td>
<td>24.62 ± 2.00</td>
<td>02.53 ± 1.89</td>
<td>23.21 ± 2.51</td>
<td>25.49 ± 1.05</td>
</tr>
<tr>
<td>8</td>
<td>19.13 ± 2.46</td>
<td>02.50 ± 1.42</td>
<td>18.59 ± 1.80</td>
<td>19.63 ± 2.79</td>
</tr>
<tr>
<td>10</td>
<td>14.46 ± 1.76</td>
<td>02.82 ± 2.51</td>
<td>12.87 ± 0.86</td>
<td>12.49 ± 1.91</td>
</tr>
<tr>
<td>12</td>
<td>08.23 ± 1.85</td>
<td>01.00 ± 2.29</td>
<td>06.36 ± 2.31</td>
<td>07.14 ± 0.99</td>
</tr>
<tr>
<td>20</td>
<td>04.99 ± 1.49</td>
<td>01.04 ± 1.54</td>
<td>03.92 ± 1.52</td>
<td>05.33 ± 1.18</td>
</tr>
<tr>
<td>24</td>
<td>02.14 ± 1.09</td>
<td>00.63 ± 1.94</td>
<td>01.37 ± 1.43</td>
<td>03.87 ± 1.91</td>
</tr>
</tbody>
</table>

*Statistically no significance compared to gliclazide control
KEK Conceived of the study, participated in the design of the study and performed the statistical analysis and interpretation of the data.

Both the authors read and approved the final manuscript.

Acknowledgements
The authors are thankful to M/s. Aurobindo Pharma Ltd, Hyderabad and M/s. Micro Labs, Bangalore for supplying gift samples of NNRTIs (efavirenz and nevirapine) and gliclazide, respectively.

References


Brief Communication

Influence of atazanavir on the pharmacodynamics and pharmacokinetics of gliclazide in animal models

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ARTICLE INFO

Article history:
Received 20 July 2009
Accepted 5 October 2009

Keywords:
Gliclazide
Atazanavir
Diabetes
HIV infection
Hypoglycemia
Pharmacokinetics

ABSTRACT

Background: The objective of this study was to investigate the effect of atazanavir on the pharmacodynamics and pharmacokinetics of gliclazide in rats (normal and diabetic) and rabbits to evaluate the safety and effectiveness of the combination.

Methods: Blood samples were analysed for blood glucose by GOD/POD method, serum gliclazide levels by HPLC method and insulin by Radio Immuno Assay method.

Results: In combination, atazanavir significantly enhanced the pharmacodynamic activity and altered the pharmacokinetic parameters of gliclazide in animal models.

Conclusions: The interaction between atazanavir and gliclazide appears to be pharmacokinetic interaction at metabolic level in animal models.

1. Introduction

The study of mechanisms of drug interaction is of much value in selecting drug concentrations to provide rational therapy. Drug interaction studies assume much importance, especially for drugs that have a narrow margin of safety, and where the drugs are used for a prolonged period of time. Diabetes mellitus is one such metabolic disorder that needs treatment for prolonged periods, and maintenance of normal blood glucose level is very important in this condition, since both hyperglycemia, as well as hypoglycemia, is unwanted phenomenon [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. Diabetes may be due to a decrease in the synthesis of insulin (type-1) or a decrease in the secretion of insulin (type-2) from the β-cells of islets of Langerhans of the pancreas. There are an estimated 143 million people world wide suffers from diabetes [2] and the number may well double by the year 2030 [3]. In India, the prevalence rate of diabetes is estimated to be 1–5%.

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) [4,5]. Atazanavir is a commonly prescribed protease inhibitor, due to its once-daily dosing regimen, favorable metabolic profile and low frequency of adverse effects [6]. However, its effect 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 [7], antioxidant property [8], low incidence of producing severe hypoglycemia [9] and other haemobiological effects. Gliclazide is known to act mainly by releasing insulin by blocking K+ channels in the pancreatic β-cells [10].

Atazanavir is a substrate and potent inhibitor of the cytochrome P450 (CYP) system, in particular CYP3A4 and CYP2C9 and affect the metabolism of several drugs [11]. Because atazanavir can inhibit CYP3A4 and CYP2C9-mediated drug metabolism and gliclazide is reported to be metabolized by CYP2C9 primarily and partly by CYP3A4 [10,12], it is important to study the possible effects of atazanavir on the pharmacokinetics and pharmacodynamics of gliclazide. However, there seem to be no published studies on the effects of enzyme inhibition on the pharmacokinetics of gliclazide.

Since there is every possibility for the combined use of gliclazide and atazanavir in chronic diabetics with associated HIV infection, the study is planned to investigate the effect of atazanavir on the activity of gliclazide in normal and diabetic rats, to evaluate the
safety and effectiveness of the combination. Also the study is planned to find the pharmacodynamics and pharmacokinetics of gliclazide in the presence of atazanavir in rabbits, to evaluate the mechanisms of interaction if they occur.

2. Material and methods

2.1. Drugs and chemicals

Gliclazide and atazanavir are gift samples from Micro Labs (Bangalore, India) and Aurobindo Pharma Ltd. (Hyderabad, India), respectively. Alloxan monohydrate was purchased from LOBA Chemicals Pvt. Ltd., Mumbai, India. Orthophosphoric acid (AR grade) and dichloromethane (AR grade) were purchased from a local pharmacy. Acetonitrile (HPLC grade) was obtained from Qualigens chemicals, Mumbai, India. Glucose kits (span diagnostics) were purchased from SD Fine Chemicals, Mumbai, India and Loba Chemie Pvt. Ltd., Mumbai, India, respectively. All other reagents used were of an analytical grade.

2.2. Animals

Albino rats of either sex, 6–7 weeks of age, weighing between 250 to 320 g, and normal albino rabbits of either sex of 3 months of age, weighing between 1.35 to 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 ± 2°C and 50 ± 15% relative humidity, with a 12-h light/12-h dark cycle. Animals were fed with 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, the food and water were withdrawn. 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).

2.3. Selection of doses and preparation of oral test solution/suspension

In clinical practice, atazanavir and gliclazide in a therapeutic dose will be administered orally as antiretroviral and antidiabetic therapy, respectively. Human oral therapeutic doses of the respective drugs were extrapolated to rat/rabbit based on body surface area [13]. But the dose of gliclazide for rat experiments was selected as 2 mg/kg bd. wt. based on the influence of dose effect-relationship of gliclazide on blood glucose in normal rats. Atazanavir was suspended in 2% CMC-Na for oral administration [14]. Gliclazide solution was prepared by dissolving it in a few drops of 0.1 N NaOH then made up to the volume with distilled water. All the drugs were administered to the respective groups by oral gavage.

2.4. Pharmacodynamic interaction study in normal and diabetic rats

A group of six normal rats was administered with 2 mg/kg bd. wt. of gliclazide, orally. The same group was administered with atazanavir 36 mg/kg bd. wt., orally and the combination of atazanavir and gliclazide. One week washout period was maintained between treatments. After this single dose interaction study, the same group was continued with the daily treatment of interacting drug (atazanavir) for the next 8 days with regular feeding. Later after 18 h fasting, they were again given the combined treatment on the 9th day.

The same treatment (single dose followed by multiple dose interaction study) was repeated in a group of six alloxan-induced diabetic rats. Diabetes was induced in rats by the administration of alloxan monohydrate in two doses, i.e. 100 mg and 50 mg/kg bd. wt. intraperitoneally for two consecutive days [15]. After 72 h, samples were collected from rats by orbital puncture of all surviving rats, and the serum was analysed for glucose levels. Rats with blood glucose levels of 200 mg/dl and above were considered as diabetic and selected for the study.

Blood samples were withdrawn from retro orbital plexus [16] of each rat at 0, 1, 2, 3, 4, 6, 8 and 12 h. These blood samples were analysed for blood glucose by GOD/POD method [17] using commercial glucose kits.

2.5. Pharmacodynamic and pharmacokinetic interaction study in rabbits

A group of six rabbits was administered with 5.6 mg/1.5 kg bd. wt. of gliclazide, orally. The same group was administered with atazanavir 28 mg/1.5 kg bd. wt., orally and the combination of atazanavir and gliclazide. One week washout period was maintained between treatments. After this single dose interaction study the same group was continued with the daily treatment of interacting drug (atazanavir) for the next 8 days with regular feeding. Later after 18 h fasting they were again given the combined treatment on the 9th day.

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 analysed for blood glucose by GOD/POD method using commercial glucose kits. Plasma insulin was measured by Radio Immuno Assay method using a commercially available kit (human insulin as standard; Insik-5, Sorin Biomedica, Saluggia, Italy) as per the instructions provided by the manufacturers at 3 and 24 h. The serum gliclazide concentrations were determined by HPLC method [18]. The pharmacokinetic parameters of gliclazide were determined on subjecting the concentration-time data to non-compartmental analysis using WinNonlin (version 5.0.1) software.

2.6. Data and statistical analysis

Data were expressed as mean ± SEM. The significance was determined by applying Student’s paired ‘t’ test.

3. Results

3.1. Pharmacodynamic interaction study in normal and diabetic rats

Gliclazide produced hypoglycemic activity with maximum biphasic reduction of 40.88 ± 0.57% and 39.01 ± 0.73% in normal rats, and 42.95 ± 1.74% and 44.14 ± 1.78% in diabetic rats at 2 h and 8 h, respectively. Atazanavir has no significant effect on the blood glucose levels in normal and diabetic rats. In combination, atazanavir produced enhanced hypoglycemic effect of gliclazide with maximum blood glucose reduction of 48.27 ± 1.04% & 45.69 ± 1.53% at 2 h and 8 h, following single dose and multiple dose administration of atazanavir, respectively, in normal rats (Table 1). In combination, atazanavir produced enhanced hypoglycemic effect of gliclazide with maximum blood glucose reduction of 44.99 ± 1.13% & 48.00 ± 1.26% and 48.99 ± 1.18% & 50.25 ± 0.87% at 2 h and 8 h, following single dose and multiple dose administration of atazanavir, respectively, in diabetic rats (Ta-
3.3. Pharmacokinetic interaction study in normal rabbits

The enhancement in gliclazide effect is more with the multiple dose treatment of atazanavir than single dose treatment.

3.2. Pharmacodynamic interaction study in normal rabbits

Gliclazide produced hypoglycemic activity with maximum reduction of 35.22 ± 1.09% at 3 h in normal rabbits. Atazanavir has no significant effect on the blood glucose levels in normal rabbits. Atazanavir produced enhanced hypoglycemic effect of gliclazide with maximum reduction of 43.66 ± 0.39% and 45.72 ± 0.94% in the blood glucose in normal rabbits at 3 h following single dose and multiple dose treatment of atazanavir, respectively (Table 2). The enhancement in gliclazide effect is more with the multiple dose treatment of atazanavir than the single dose treatment. The serum insulin levels were increased with atazanavir treatment in normal rabbits (Fig. 1).

3.3. Pharmacokinetic interaction study in normal rabbits

The serum gliclazide levels were increased, and pharmacokinetic parameters of gliclazide like $C_{\text{max}}$, $T_{\text{max}}$, AUC, AUMC, $K_{\text{el}}$ and $T_{1/2}$ were altered significantly with single- and multiple-dose treatments of atazanavir in normal rabbits (Table 3). The percent increase of serum gliclazide level is 22.73% and 24.26% following single dose and multiple dose administration of atazanavir, respectively.

4. Discussion

Drug interactions are usually seen in clinical practice, and the mechanisms of interactions are evaluated usually in animal models (rodent and non-rodent). We studied the influence of atazanavir on the pharmacodynamics and pharmacokinetics of gliclazide in rats (rodents) and rabbits (non-rodent). The normal rat model served to quickly identify the interaction and the 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. The multiple dose effect of atazanavir on gliclazide activity was also studied for the influence of long term treatment with atazanavir, since both are used for chronic period.

Rats are known to be more sensitive to gliclazide response. So we have conducted the dose effect-relationship study of gliclazide to select the oral dose, which produces approximately 35% of blood

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Normal rats</th>
<th>Gliclazide (5.6 mg/1.5 kg bd. wt.)</th>
<th>Atazanavir (28 mg/1.5 kg bd. wt.)</th>
<th>Atazanavir + gliclazide (single dose treatment)</th>
<th>Atazanavir + gliclazide (multiple dose treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.43 ± 1.80</td>
<td>20.17 ± 2.05</td>
<td>24.04 ± 2.48</td>
<td>36.53 ± 1.27</td>
<td>38.77 ± 1.76</td>
</tr>
<tr>
<td>2</td>
<td>26.59 ± 0.66</td>
<td>05.30 ± 1.40</td>
<td>34.15 ± 0.52</td>
<td>36.87 ± 0.51</td>
<td>38.77 ± 2.08</td>
</tr>
<tr>
<td>3</td>
<td>35.22 ± 1.09</td>
<td>04.59 ± 1.47</td>
<td>43.66 ± 0.39</td>
<td>45.72 ± 0.94</td>
<td>40.07 ± 1.44</td>
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<tr>
<td>4</td>
<td>27.63 ± 1.76</td>
<td>04.57 ± 1.24</td>
<td>35.90 ± 0.64</td>
<td>37.55 ± 1.28</td>
<td>42.59 ± 1.15</td>
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<tr>
<td>5</td>
<td>25.52 ± 0.75</td>
<td>03.17 ± 1.77</td>
<td>34.85 ± 0.80</td>
<td>36.53 ± 1.27</td>
<td>31.75 ± 1.47</td>
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<td>6</td>
<td>19.70 ± 2.44</td>
<td>08.47 ± 1.43</td>
<td>28.28 ± 2.08</td>
<td>30.07 ± 1.31</td>
<td>32.74 ± 0.94</td>
</tr>
<tr>
<td>12</td>
<td>12.54 ± 2.14</td>
<td>06.38 ± 1.81</td>
<td>20.74 ± 2.29</td>
<td>30.07 ± 1.31</td>
<td>32.74 ± 0.94</td>
</tr>
<tr>
<td>16</td>
<td>07.16 ± 4.01</td>
<td>04.85 ± 1.42</td>
<td>16.90 ± 1.72</td>
<td>20.17 ± 2.05</td>
<td>32.54 ± 1.27</td>
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<tr>
<td>20</td>
<td>06.79 ± 1.34</td>
<td>04.93 ± 1.76</td>
<td>12.32 ± 0.99</td>
<td>15.92 ± 1.35</td>
<td>12.75 ± 1.08</td>
</tr>
<tr>
<td>24</td>
<td>03.92 ± 1.41</td>
<td>03.18 ± 0.90</td>
<td>10.20 ± 0.83</td>
<td>50.23 ± 0.87</td>
<td>28.56 ± 1.77</td>
</tr>
</tbody>
</table>

* Significant at $P < 0.05$ compared to gliclazide control.
The drug atazanavir 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. Hence, the interaction at hepatic metabolism with reduced gliclazide metabolism by atazanavir, leading to raised serum levels, remains possible.

5. Conclusions

The interaction appears to be pharmacokinetic interaction at metabolic level. 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 clinical benefit in diabetic patients. However, the present study warrants further studies to find out the relevance of this interaction in human beings.

Acknowledgements

The authors are grateful to M/s. Aurobindo Pharma Ltd., Hyderabad and M/s. Micro Labs, Bangalore for supplying gift samples of atazanavir and gliclazide, respectively.

References

[6] Brien RC, Luo M, Balazs N, Mercuri J. Therapy with protease inhibitors effects were drug specific but not class specific, as other protease inhibitors have a significant impact on glucose homeostasis. Interestingly, however, the gliclazide hypoglycemic and antidiabetic activity was significantly enhanced by atazanavir, following a single and multiple dose treatment in rat and rabbit models, and this confirmed the presence of potential interaction between gliclazide and atazanavir. Further, the presence of interaction was supported by an increase in serum insulin levels with atazanavir treatment. It is clear that since atazanavir did not alter blood glucose levels on its own, the increase in the effect of gliclazide on blood glucose may be due to improved blood gliclazide level in the presence of atazanavir, as it was confirmed by pharmacokinetic interaction study in rabbits.

There was a significant rise in serum gliclazide levels and an alteration in pharmacokinetic parameters like Cmax, tmax, AUC, AUMC, Kd and T1/2 of gliclazide with single- and multiple-dose treatments of atazanavir. The increase in AUC and AUMC indicates improved availability of gliclazide in presence of atazanavir. There might be interaction at absorption level, since oral absorption of atazanavir is not high. Gliclazide is a highly protein bound drug (85%–99%) [22], whereas atazanavir is bound to proteins to the extent of 86%–89% [23]. Hence, the possibility of displacing gliclazide from protein bound sites by atazanavir was low. Moreover, the rise of gliclazide blood levels in the presence of atazanavir might be other than improved absorption and altered distribution.

The altered Kd and T1/2 indicates alteration either in metabolism or the excretion process. Atazanavir is reported to be a potent inhibitor of CYP3A4 and CYP2C9 [11], and there is more possibility of atazanavir for inhibition of metabolism of gliclazide, which is also metabolized by both CYP2C9 and CYP3A4 [10,12]. Further gliclazide is eliminated through renal (80%) and biliary (20%) routes [22,24]. The major elimination pathway of atazanavir is the biliary route. Atazanavir is eliminated by 13% being in urine and 79% in fecal matter [23]. Hence, there is also a possibility for interaction between atazanavir and gliclazide at biliary excretion. However, Table 3

Mean pharmacokinetic parameters of gliclazide before and after atazanavir administration in rabbits.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Gliclazide</th>
<th>Atazanavir + Gliclazide (single dose treatment)</th>
<th>Atazanavir + Gliclazide (multiple dose treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/ml)</td>
<td>346.79 ± 4.21</td>
<td>428.21 ± 8.68*</td>
<td>431.63 ± 8.61*</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>3.00 ± 0.00</td>
<td>3.00 ± 0.00</td>
<td>3.00 ± 0.00</td>
</tr>
<tr>
<td>AUC0–24 (ng/ml/h)</td>
<td>4013.84 ± 147.00</td>
<td>4913.80 ± 112.05*</td>
<td>4969.46 ± 11.53*</td>
</tr>
<tr>
<td>AUCinf (ng/ml/h)</td>
<td>4777.04 ± 206.58</td>
<td>6048.95 ± 176.75*</td>
<td>6143.66 ± 174.7*</td>
</tr>
<tr>
<td>AUCExtrapolation</td>
<td>15.82 ± 1.20</td>
<td>18.62 ± 1.51*</td>
<td>18.98 ± 1.45*</td>
</tr>
<tr>
<td>AUMC0–24 (ng/ml/h)</td>
<td>38787.60 ± 809.59</td>
<td>46863.51 ± 1440.81*</td>
<td>47517.88 ± 1426.66*</td>
</tr>
<tr>
<td>AUMCExtrapolation</td>
<td>66332.61 ± 4484.81</td>
<td>89966.53 ± 5680.50*</td>
<td>92221.81 ± 5612.05*</td>
</tr>
<tr>
<td>MRT0–inf (h)</td>
<td>9.64 ± 0.13</td>
<td>9.53 ± 0.09</td>
<td>9.55 ± 0.09</td>
</tr>
<tr>
<td>MRTinf (h)</td>
<td>13.82 ± 0.49</td>
<td>14.81 ± 0.62*</td>
<td>14.95 ± 0.60*</td>
</tr>
<tr>
<td>Kd (h⁻¹)</td>
<td>0.08 ± 0.00</td>
<td>0.07 ± 0.00</td>
<td>0.07 ± 0.00</td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>8.15 ± 0.53</td>
<td>9.41 ± 0.58*</td>
<td>9.50 ± 0.56*</td>
</tr>
</tbody>
</table>

* Significant at P < 0.05 compared to gliclazide control.
Effect of antiretroviral drugs on the pharmacodynamics of gliclazide with respect to glucose–insulin homeostasis in animal models

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Abstract: The objective of this study was to investigate the effect of oral administration of antiretroviral drugs (indinavir, ritonavir, atazanavir, efavirenz and nevirapine) on the pharmacodynamics of gliclazide in rats (normal and diabetic) and rabbits with respect to glucose–insulin homeostasis to evaluate the safety and effectiveness of the combinations. Blood samples were collected at regular time intervals in rats from retro orbital puncture and by marginal ear vein puncture in rabbits. All the blood samples were analyzed for blood glucose by glucose oxidase–peroxidase method and insulin by a radio immuno assay method. The insulin resistance index and β-cell function were determined by a homeostasis model assessment. Indinavir and ritonavir alone had significant impact on glucose–insulin homeostasis in animal models among the antiretroviral drugs used in our study. In combination, indinavir and efavirenz significantly reduced the activity of gliclazide, while ritonavir and atazanavir significantly increased the activity of gliclazide. However, nevirapine had no significant effect on the activity of gliclazide. From this study we conclude that glucose–insulin homeostasis disorders associated with antiretroviral drugs are not class-specific, but are drug-specific. So care should be taken when indinavir, ritonavir, atazanavir and efavirenz are prescribed for diabetic patients.

Keywords: protease inhibitors, efavirenz, nevirapine, gliclazide, homeostasis model assessment, diabetes

Introduction
The study of mechanisms of drug interactions is valuable when selecting the drug concentrations that provide rational therapy. Drug interaction studies assume greater importance for drugs that have a narrow margin of safety or where the drugs are used for prolonged periods of time. Diabetes mellitus is one such metabolic disorder that requires drug treatment for prolonged periods and the maintenance of normal blood glucose levels are particularly important in this condition, since both hyperglycemia as well as hypoglycemia are unwanted phenomenon.¹

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels and disturbances in carbohydrate, fat and protein metabolism with both micro- and macrovascular complications that result in a significant morbidity and mortality.² Type 1 diabetes occurs as a result of a decrease in the synthesis of insulin and type 2 diabetes is characterized by hyperglycemia in the context of insulin resistance and relative insulin deficiency. The number of people suffering from diabetes mellitus worldwide is increasing at an alarming rate with a projected 366 million people likely to be diabetic by the year 2030, (as against 191 million estimated in 2000).³
Type 2 diabetes may occur as a result of HIV infection and/or its treatment. Highly active antiretroviral therapy (HAART), a combination of nonnucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside reverse transcriptase inhibitors (NRTIs), and protease inhibitors (PIs) are widely used to control HIV-infection and the development of AIDS. Among the many metabolic perturbations that occur as a result of HIV-infection and its treatment, alterations in normal glucose homeostasis remain particularly prevalent and alarming clinical changes in affected patients. HAART has been associated with a spectrum of metabolic abnormalities that range from insulin resistance to impaired glucose tolerance, diabetes mellitus, dyslipidemia, and alterations in body fat distribution, especially with PIs. Of much concern is the recognition of long-term complications of insulin resistance and hyperglycemia especially in the context of the growing worldwide epidemic of type 2 diabetes mellitus. Since many studies have suggested that PI therapy is linked to the development of metabolic complications, it is important to introduce therapeutic strategies with fewer side effects, for example the use of NNRTIs an approach which appears successful for the control of HIV infection.

The widely used PIs include ritonavir, atazanavir and indinavir, and NNRTIs include efavirenz and nevirapine. Oral hypoglycemic agents are used in the treatment of type 2 diabetes, and gliclazide; a second generation sulphonylurea derivative, is preferred in such therapy because not only for its antidiabetic activity, but also for the lower occurrence of severe hypoglycemia, antioxidant properties and other hemobiological effects.

Regulation of glucose metabolism is a key aspect of metabolic homeostasis and insulin is the predominant hormone influencing this regulatory system. Insulin plays a key role in the maintenance of glucose homeostasis and is the major modulator of glucose storage and utilization. In this study Glucose was measured as a metabolic control of insulin action. The impairment of glucose homeostasis and increase in plasma glucose levels are associated with diabetes. Insulin resistance is a state where normal or elevated insulin level produces a reduced biological response and refers to impaired sensitivity to insulin mediated glucose disposal. Therefore it is of the utmost importance to study glucose–insulin homeostasis, in order to better understand the pathological process of insulin resistance to evaluate the safety and effectiveness of drug combinations. The homeostasis model assessment (HOMA) is a more reliable and validated method to measure insulin resistance and β-cell function from fasting glucose and insulin.

In our previous studies, we investigated the effect of antiretroviral drugs (indinavir, ritonavir, atazanavir, efavirenz and nevirapine) on the pharmacodynamics of gliclazide in rats (normal and diabetic) and rabbits with respect to blood glucose levels. However, there is no evidence on the effect of antiretroviral drugs on the activity of gliclazide with respect to glucose–insulin homeostasis.

Therefore in this study we investigated the effect of antiretroviral drugs on the activity of gliclazide with respect to glucose, insulin, insulin resistance and β-cell function in rats (normal and diabetic) and rabbits to evaluate the safety and effectiveness of the combination.

Material and methods

Gliclazide and antiretroviral drugs were 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 Udhna, India) were purchased from a local pharmacy. All other reagents/chemicals used were of analytical grade.

Animals

The rats were of either sex, albino, 6 to 7 weeks of age and weighed between 250 to 320 g. The rabbits were also of either sex, albino, –3 months of age and weighed between 1.35 to 1.75 Kg. Both rats and rabbits were procured from the National Institute of Nutrition, Hyderabad, India. They were maintained under standard laboratory conditions at an ambient temperature of 25 ± 2 °C and 50% ± 15% relative humidity with a cycle of 12 hours light and 12 hours dark. The animals were fed with a commercial pellet diet (Rayan’s Biotechnologies Pvt Ltd., Hyderabad, India) and water ad libitum. The animals were fasted for 18 hours prior to the experiment and during the experiment both food and water were withdrawn. The animal experiments were performed after the study protocol had approval from 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 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, antiretroviral drugs and gliclazide in therapeutic doses are administered orally. Hence, human oral therapeutic doses of the respective drugs were extrapolated to rat/rabbit based on body surface area. But the dose of gliclazide for rat experiments was selected as 2 mg/kg body...
weight based on the influence of the dose-effect relationship of gliclazide on blood glucose in normal rats. Indinavir, ritonavir, atazanavir, efavirenz and nevirapine were orally administered in the dose of 72, 18, 36, 54 and 18 mg/kg body weight to both normal and diabetic rats and 56, 14, 28, 42 and 14 mg/1.5 kg body weight to normal rabbits, respectively. Gliclazide was orally administered to normal rabbits in the dose of 5.6 mg/1.5 kg body weight. Antiretroviral drugs were suspended in carboxy-methylcellulose (CMC) for oral administration.17–20 A gliclazide solution was prepared by dissolving it in a few drops of 0.1N sodium hydroxide which was then made up to the required volume with distilled water. All the drugs were administered to the respective groups by oral gavage.

Experimental design
The study consists of four phases. Phase I: dose-effect relationship of gliclazide in normal rats. Phase II: interaction study between antiretroviral drugs and gliclazide in normal rats. Phase III: interaction study between antiretroviral drugs and gliclazide in diabetic rats. and Phase IV: interaction study between antiretroviral drugs and gliclazide in normal rabbits. Each phase from II to IV consists of five groups (N = 6), for the interaction study of the five antiretroviral drugs with gliclazide (single dose study followed by multiple dose study).

Dose-effect relationship of gliclazide in rats
To a group of six normal rats gliclazide was administered orally (1 mg/kg body weight). At the conclusion of this, following a one week wash out period, the same group was given gliclazide at 2 mg/kg body weight and finally at 4 mg/kg body weight. A one-week washout period was maintained between all changes in treatments.

Interaction study between antiretroviral drugs and gliclazide in normal rats
Each group of six rats was administered with gliclazide, orally. The same group was administered with interacting drug (Indinavir or ritonavir or atazanavir or efavirenz or nevirapine) and the combination of respective interacting drug and gliclazide. One week washout period was maintained between treatments. After this single dose interaction study the same group was continued with the daily treatment of respective interacting drug for the next eight days with regular feeding. Later after 18 h fasting they were again given the combined treatment on the ninth day.

Interaction study between antiretroviral drugs and gliclazide in diabetic rats
Diabetes was induced in rats by the administration of two intraperitoneal doses of alloxan monohydrate, the first being 100 mg/kg body weight followed 24 hours later by a second dose of 50 mg/kg body weight.21 After 72 hours samples were collected from all surviving rats by orbital puncture before the serum glucose concentration was determined. Rats with blood glucose levels of 200 mg/dL and above were considered as diabetic and selected for the study. The same protocol (single dose interaction study followed by multiple dose interaction study) as described in the study in normal rats was performed with a group of six alloxan-induced diabetic rats.

Interaction study between antiretroviral drugs and gliclazide in normal rabbits
Each group of six rabbits was administered with gliclazide, orally. The same group was administered with interacting drug (Indinavir or ritonavir or atazanavir or efavirenz or nevirapine) and the combination of respective interacting drug and gliclazide. One week washout period was maintained between treatments. After this single dose interaction study the same group was continued with the daily treatment of respective interacting drug for the next eight days with regular feeding. Later after 18 h fasting they were again given the combined treatment on the ninth day.

Blood sampling, determination of blood glucose and insulin
Blood samples were withdrawn from the retro orbital plexus22 of each rat at 2 hours and then 8 hours and for each rabbit from the marginal ear vein of at 3 hours. These blood samples were analyzed for blood glucose by the glucose oxidase–peroxidase (GOD/POD) method23 using commercial glucose kits and plasma insulin was measured by Radio Immuno Assay method.

Determination of insulin resistance index and β-cell function
The insulin resistance index and β-cell function were assessed by the HOMA protocol and were calculated as follows:24,25

\[
\text{Insulin resistance} = \frac{\text{FPI} \times \text{FPG}}{22.5} \\
\text{and } \beta\text{-cell function} = \frac{20 \times \text{FPI}}{\text{FPG} - 3.5}
\]

Where FPI is fasting plasma insulin concentration (mU/mL) and FPG is fasting plasma glucose (mmol/L).

Data and statistical analysis
Data were expressed as mean ± standard error of mean (SEM). The significance was determined by applying paired Student’s t-test.
Results
Dose-effect relationship of gliclazide in rats
A dose dependent response was observed with the three oral doses undertaken with gliclazide. The 2 mg/kg body weight of gliclazide was selected based on an ideal blood glucose reduction which is about 35%. The gliclazide produced hypoglycemic activity with maximum biphasic reduction of 26.77% ± 1.13% and 28.91% ± 2.53%, 38.59% ± 1.58% and 40.50% ± 1.40% and 46.28% ± 1.67% and 50.65% ± 1.46% at 2 hours and 8 hours with 1 mg/kg body weight, 2 mg/kg body weight and 4 mg/kg body weight of gliclazide, respectively (Figure 1).

Effect of indinavir on the activity of gliclazide
The levels of blood glucose, insulin, insulin resistance and β-cell function following gliclazide, indinavir and their combination (single dose and multiple doses) were represented in Table 1 and Table 6. Indinavir alone produced a significant increase in glucose, insulin, insulin resistance index and decrease in β-cell function. When given in combination indinavir significantly (P < 0.05) altered the pharmacodynamics of gliclazide in both rats (normal and diabetic) and rabbits following both single and multiple dose treatments which is reflected by increases in glucose, insulin and insulin resistance together with a decrease in β-cell function for the diabetic rats. The reduction in gliclazide effect is more marked with the single dose treatment of indinavir rather than with the multiple dose treatment.

Effect of ritonavir on the activity of gliclazide
The levels of blood glucose, insulin, insulin resistance and β-cell function following gliclazide, ritonavir and their combination (single dose and multiple doses) are represented in Table 2 and Table 6. Ritonavir alone produced a significant increase in glucose, insulin and insulin resistance index and decrease in β-cell function. When given in combination ritonavir significantly (P < 0.05) altered the pharmacodynamics of gliclazide in both rats (normal and diabetic) and rabbits following both single and multiple dose treatments of ritonavir, which is reflected by a significant decrease in glucose, with increases in insulin, insulin resistance and β-cell function. The reduction in gliclazide effect was

![Figure 1 Dose effect relationship of gliclazide in normal rats (N = 6).](image-url)
### Table 1: Effect of indinavir on the activity of gliclazide in normal and diabetic rats (N = 6)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gliclazide 2 hours</th>
<th>Gliclazide 8 hours</th>
<th>Indinavir 2 hours</th>
<th>Indinavir 8 hours</th>
<th>Indinavir + Gliclazide (SDA) 2 hours</th>
<th>Indinavir + Gliclazide (MDA) 2 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>53.83 ± 0.54</td>
<td>55.67 ± 0.61</td>
<td>106.67 ± 0.67</td>
<td>83.33 ± 1.84</td>
<td>71.00 ± 1.91*</td>
<td>76.67 ± 0.42*</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>12.04 ± 0.26</td>
<td>11.89 ± 0.38</td>
<td>19.05 ± 0.27</td>
<td>21.58 ± 0.33</td>
<td>23.07 ± 0.44*</td>
<td>23.14 ± 0.43*</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>0.60 ± 0.04</td>
<td>0.63 ± 0.05</td>
<td>0.50 ± 0.08</td>
<td>0.43 ± 0.06</td>
<td>0.04 ± 0.12*</td>
<td>0.04 ± 0.07*</td>
</tr>
<tr>
<td>β-cell function</td>
<td>483.52 ± 34.68</td>
<td>602.01 ± 42.99</td>
<td>157.22 ± 2.95</td>
<td>398.49 ± 33.74</td>
<td>1504.49 ± 388.86*</td>
<td>613.31 ± 23.31*</td>
</tr>
</tbody>
</table>

**Diabetic rats**

| Glucose (mg/dL)    | 141.67 ± 0.95     | 135.67 ± 0.61     | 307.17 ± 1.94    | 258.00 ± 0.89    | 207.33 ± 8.09*                       | 190.67 ± 4.28*                      |
| Insulin (µU/mL)    | 11.19 ± 0.32      | 11.64 ± 0.16      | 15.46 ± 0.50     | 13.84 ± 0.23     | 16.31 ± 0.44*                        | 16.70 ± 0.26*                       |
| Insulin resistance | 0.91 ± 0.10       | 0.90 ± 0.06       | 8.72 ± 0.34      | 8.82 ± 0.13      | 8.38 ± 0.51*                         | 0.78 ± 0.27*                        |
| β-cell function    | 51.30 ± 1.67      | 57.66 ± 0.66      | 22.82 ± 0.85     | 25.57 ± 0.51     | 41.17 ± 1.85*                        | 47.28 ± 1.25*                       |

**Notes:** Data was expressed as Mean ± SEM; †Calculated by homeostasis model assessment method; *Significant at P < 0.05 compared to gliclazide control.

**Abbreviations:** SDA, single dose administration; MDA, multiple dose administration.

### Table 2: Effect of ritonavir on the activity of gliclazide in normal and diabetic rats (N = 6)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gliclazide 2 hours</th>
<th>Gliclazide 8 hours</th>
<th>Ritonavir 2 hours</th>
<th>Ritonavir 8 hours</th>
<th>Ritonavir + Gliclazide (SDA) 2 hours</th>
<th>Ritonavir + Gliclazide (MDA) 2 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>53.00 ± 0.45</td>
<td>54.67 ± 0.42</td>
<td>97.33 ± 1.52</td>
<td>96.00 ± 0.89</td>
<td>46.33 ± 1.58*</td>
<td>51.67 ± 0.95*</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>12.35 ± 0.24</td>
<td>12.55 ± 0.12</td>
<td>18.01 ± 0.20</td>
<td>18.11 ± 0.23</td>
<td>21.14 ± 0.24*</td>
<td>20.66 ± 0.81*</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>1.62 ± 0.04</td>
<td>1.69 ± 0.01</td>
<td>4.23 ± 0.09</td>
<td>4.29 ± 0.09</td>
<td>2.42 ± 0.09*</td>
<td>2.63 ± 0.11*</td>
</tr>
<tr>
<td>β-cell function</td>
<td>449.85 ± 22.85</td>
<td>548.63 ± 23.52</td>
<td>190.65 ± 8.12</td>
<td>197.93 ± 2.92</td>
<td>491.59 ± 68.07*</td>
<td>683.76 ± 61.61*</td>
</tr>
</tbody>
</table>

**Diabetic rats**

| Glucose (mg/dL)    | 142.67 ± 1.23     | 140.00 ± 1.03     | 274.00 ± 3.14    | 279.00 ± 2.52    | 135.67 ± 1.20*                       | 130.33 ± 0.95*                      |
| Insulin (µU/mL)    | 11.79 ± 0.21      | 12.13 ± 0.28      | 15.56 ± 0.46     | 15.36 ± 0.24     | 20.99 ± 0.24*                        | 21.94 ± 0.50*                       |
| Insulin resistance | 0.45 ± 0.06       | 0.49 ± 0.10       | 10.54 ± 0.37     | 10.59 ± 0.22     | 0.07 ± 0.07                          | 0.07 ± 0.14                         |
| β-cell function    | 53.40 ± 1.46      | 56.76 ± 1.42      | 26.55 ± 0.70     | 25.61 ± 0.36     | 104.19 ± 2.41*                       | 117.55 ± 3.57*                      |

**Notes:** Data was expressed as Mean ± SEM; †Calculated by homeostasis model assessment method; *Significant at P < 0.05 compared to gliclazide control.

**Abbreviations:** SDA, single dose administration; MDA, multiple dose administration.
greater with the multiple dose treatment of ritonavir than the single dose treatment.

**Effect of atazanavir on the activity of gliclazide**

The levels of blood glucose, insulin, insulin resistance and β-cell function following gliclazide, atazanavir and their combination (single dose and multiple doses) are represented in Table 3 and Table 7. Atazanavir alone had no significant effect in glucose, insulin, insulin resistance index and β-cell function. When given in combination, atazanavir significantly ($P < 0.05$) altered the pharmacodynamics of gliclazide in both rats (normal and diabetic) and rabbits following both single and multiple dose treatments of atazanavir. This is reflected by a significant decrease in glucose, and an increase in insulin and β-cell function. The reduction in gliclazide effect is greater with the multiple dose treatment of atazanavir than the single dose treatment.

**Effect of efavirenz on the activity of gliclazide**

The levels of blood glucose, insulin, insulin resistance and β-cell function following gliclazide, efavirenz and their combination (single dose and multiple doses) are represented in Table 4 and Table 7. Efavirenz alone had no significant effect on glucose levels, insulin, insulin resistance index and β-cell function. When given in combination, efavirenz significantly ($P < 0.05$) altered the pharmacodynamics of gliclazide in both rats (normal and diabetic) and rabbits following both the single and multiple dose treatments of efavirenz, this is reflected by a significant increase in glucose and decrease in insulin levels. The β-cell function of gliclazide was significantly decreased in diabetic rats with this combination. The reduction in gliclazide effect is greater with the single dose treatment of efavirenz than the multiple dose treatments.

**Effect of nevirapine on the activity of gliclazide**

The levels of blood glucose, insulin, insulin resistance and β-cell function following gliclazide, nevirapine and their combination (single dose and multiple doses) are represented in Tables 5 and 8. Nevirapine alone had no significant effect in glucose, insulin, insulin resistance index and β-cell function. When given in combination nevirapine had no significant effect on the pharmacodynamics of gliclazide in both rats and rabbits following a single and multiple dose treatments...
### Table 4 Effect of efavirenz on the activity of gliclazide in normal and diabetic rats (N = 6)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gliclazide (Normal)</th>
<th>Efavirenz (Normal)</th>
<th>Efavirenz + Gliclazide (SDA) (Normal)</th>
<th>Efavirenz + Gliclazide (MDA) (Normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hours</td>
<td>8 hours</td>
<td>2 hours</td>
<td>8 hours</td>
<td>2 hours</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>53.67 ± 0.61</td>
<td>56.33 ± 0.95</td>
<td>96.00 ± 0.73</td>
<td>88.00 ± 0.89</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>12.16 ± 0.15</td>
<td>12.11 ± 0.14</td>
<td>08.37 ± 0.08</td>
<td>08.90 ± 0.23</td>
</tr>
<tr>
<td>Insulin resistance†</td>
<td>01.61 ± 0.03</td>
<td>01.69 ± 0.04</td>
<td>01.98 ± 0.02</td>
<td>01.93 ± 0.06</td>
</tr>
<tr>
<td>β-cell function†</td>
<td>481.58 ± 35.25</td>
<td>758.07 ± 139.46</td>
<td>91.56 ± 2.27</td>
<td>128.87 ± 5.02</td>
</tr>
<tr>
<td>Diabetic rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>145.67 ± 0.80</td>
<td>140.67 ± 0.84</td>
<td>263.33 ± 0.67</td>
<td>239.67 ± 4.77</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>11.49 ± 0.11</td>
<td>12.94 ± 0.12</td>
<td>04.59 ± 0.17</td>
<td>04.70 ± 0.17</td>
</tr>
<tr>
<td>Insulin resistance†</td>
<td>04.13 ± 0.04</td>
<td>04.50 ± 0.04</td>
<td>02.98 ± 0.11</td>
<td>02.79 ± 0.12</td>
</tr>
<tr>
<td>β-cell function†</td>
<td>50.06 ± 0.77</td>
<td>60.04 ± 0.90</td>
<td>08.25 ± 0.31</td>
<td>09.60 ± 0.36</td>
</tr>
</tbody>
</table>

**Notes:** Data was expressed as Mean ± SEM; †Calculated by homeostasis model assessment method; *Significant at P < 0.05 compared to gliclazide control.  
**Abbreviations:** SDA, single dose administration; MDA, multiple dose administration.

### Table 5 Effect of nevirapine on the activity of gliclazide in normal and diabetic rats (N = 6)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gliclazide (Normal)</th>
<th>Nevirapine (Normal)</th>
<th>Nevirapine + Gliclazide (SDA) (Normal)</th>
<th>Nevirapine + Gliclazide (MDA) (Normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hours</td>
<td>8 hours</td>
<td>2 hours</td>
<td>8 hours</td>
<td>2 hours</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>54.33 ± 0.61</td>
<td>57.33 ± 1.23</td>
<td>89.67 ± 0.61</td>
<td>91.00 ± 0.45</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>12.41 ± 0.10</td>
<td>12.16 ± 0.07</td>
<td>8.51 ± 0.08</td>
<td>8.60 ± 0.12</td>
</tr>
<tr>
<td>Insulin resistance†</td>
<td>1.67 ± 0.03</td>
<td>1.72 ± 0.04</td>
<td>1.88 ± 0.02</td>
<td>1.93 ± 0.03</td>
</tr>
<tr>
<td>β-cell function†</td>
<td>529.15 ± 35.00</td>
<td>995.12 ± 189.01</td>
<td>115.11 ± 2.28</td>
<td>110.63 ± 1.34</td>
</tr>
<tr>
<td>Diabetic rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>146.00 ± 0.00</td>
<td>141.00 ± 0.45</td>
<td>244.33 ± 4.83</td>
<td>247.67 ± 4.96</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>11.24 ± 0.07</td>
<td>12.54 ± 0.08</td>
<td>05.09 ± 0.15</td>
<td>04.17 ± 0.04</td>
</tr>
<tr>
<td>Insulin resistance†</td>
<td>04.05 ± 0.03</td>
<td>04.37 ± 0.03</td>
<td>03.07 ± 0.11</td>
<td>02.55 ± 0.03</td>
</tr>
<tr>
<td>β-cell function†</td>
<td>48.75 ± 0.28</td>
<td>57.89 ± 0.44</td>
<td>10.14 ± 0.35</td>
<td>08.18 ± 0.28</td>
</tr>
</tbody>
</table>

**Notes:** Data was expressed as Mean ± SEM; †Calculated by homeostasis model assessment method; *Significant at P < 0.05 compared to gliclazide control.  
**Abbreviations:** SDA, single dose administration; MDA, multiple dose administration.
of nevirapine, with respect to glucose, insulin and insulin resistance index.

Discussion
HIV infected patients are likely to suffer with diabetes mellitus and hence most often antiretroviral drugs are co-administered along with oral antidiabetic drugs. HIV infection and diabetes are both chronic diseases that significantly affect lifestyle. When they intersect, the treatment regimens required for both diseases can be overwhelming for patients. Several studies have reported a prevalence of diabetes of 2% to 7% among HIV-infected patients receiving protease inhibitors and an additional 16% having impaired glucose tolerance. The incidence of diabetes mellitus in HIV-infected patients has been estimated to range from 1% to 10% in various studies. In our study we have investigated the effect of widely used antiretroviral drugs from both PIs and NNRTIs on the activity of the widely used antidiabetic drug, gliclazide, as PIs are reported to have higher tendency to promote disorders of glucose–insulin homeostasis while NNRTIs are known to have a safer profile with respect to metabolic complications in HIV-infected patients.

Previously we have reported the effect of these antiretroviral drugs on the activity of gliclazide with respect to glucose levels in animal models. However, in our present study we investigated the effect of antiretroviral drugs on the activity of gliclazide with respect to glucose, insulin, insulin resistance and β-cell function using HOMA, which is believed to be a more reliable and validated surrogate measure. In this study, the multiple dose effect of antiretroviral drugs on gliclazide activity was studied to determine the influence of the long term treatment with antiretroviral drugs since both are used for chronic conditions.

Drug interactions are often seen in clinical practice and the mechanisms of such interactions are often evaluated in animal models both rodent and nonrodent. We studied the influence of antiretroviral drugs on the pharmacodynamics of gliclazide in rats (rodents) and rabbits (nonrodent). The normal rat model served to quickly identify the hypothesized interaction and the diabetic rat model served to validate the response in conditions that mirror the clinical application of these drugs. The rabbit model, another quite dissimilar species also validated the occurrence of such an interaction. Usually, if the interaction is observed in both rodent and non-rodent species, it is likely to occur in humans. Although animal models can never replace the need for comprehensive human trials, the use of animal models can provide important insights in understanding and evaluation of potent drug interactions. Since such a small amount of blood was required for this study, the blood samples were collected by retro-orbital puncture and marginal ear vein as they were reported to be suitable 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 a dose effect relationship study of gliclazide to select the oral dose which produces approximately 35% of blood glucose reduction in rats. Consistent with our previous studies and literature, gliclazide produced a biphasic response (at 2 hours and 8 hours) in the rat model when administered alone, which may be due its biliary excretion and entero hepatic cycling. Gliclazide is known to produce hypoglycemic (antihyperglycemic) activity by pancreatic β cells) and extra pancreatic (increasing the tissue uptake of glucose) mechanisms. Such an effect was

Table 6 Effect of indinavir and ritonavir on the activity of gliclazide in normal rabbits (N = 6)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Glucose (mg/dL)</th>
<th>Insulin (µU/mL)</th>
<th>Insulin resistance</th>
<th>β-cell function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indinavir vs gliclazide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gliclazide</td>
<td>59.33 ± 0.84</td>
<td>06.90 ± 0.47</td>
<td>1.01 ± 0.07</td>
<td>1190.88 ± 415.26</td>
</tr>
<tr>
<td>Indinavir</td>
<td>110.67 ± 0.99</td>
<td>17.55 ± 0.29</td>
<td>4.80 ± 0.09</td>
<td>132.85 ± 3.25</td>
</tr>
<tr>
<td>Indinavir + gliclazide (SDA)</td>
<td>72.33 ± 1.82*</td>
<td>22.97 ± 0.44*</td>
<td>4.11 ± 0.14*</td>
<td>1201.76 ± 313.82*</td>
</tr>
<tr>
<td>Indinavir + gliclazide (MDA)</td>
<td>70.00 ± 1.71*</td>
<td>23.15 ± 0.30*</td>
<td>04.00 ± 0.06*</td>
<td>2395.80 ± 1129.14*</td>
</tr>
<tr>
<td>Ritonavir vs gliclazide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gliclazide</td>
<td>53.33 ± 0.42</td>
<td>11.87 ± 0.26</td>
<td>01.56 ± 0.33</td>
<td>444.97 ± 13.16</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>104.00 ± 0.52</td>
<td>17.19 ± 0.10</td>
<td>04.41 ± 0.04</td>
<td>151.00 ± 1.51</td>
</tr>
<tr>
<td>Ritonavir + gliclazide (SDA)</td>
<td>59.00 ± 0.45*</td>
<td>21.40 ± 0.22*</td>
<td>03.12 ± 0.05</td>
<td>2059.28 ± 219.07*</td>
</tr>
<tr>
<td>Ritonavir + gliclazide (MDA)</td>
<td>56.00 ± 0.52*</td>
<td>22.30 ± 0.27*</td>
<td>03.09 ± 0.06</td>
<td>1184.14 ± 93.83*</td>
</tr>
</tbody>
</table>

Notes: Data was expressed as Mean ± SEM; *Calculated by homeostasis model assessment method; †Significant at P < 0.05 compared to gliclazide control.
Abbreviations: SDA, single dose administration; MDA, multiple dose administration.
Table 7 Effect of atazanavir and efavirenz on the activity of gliclazide in normal rabbits (N = 6)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Glucose (mg/dL)</th>
<th>Insulin (µU/mL)</th>
<th>Insulin resistance&lt;sup&gt;†&lt;/sup&gt;</th>
<th>β-cell function&lt;sup&gt;†&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atazanavir vs gliclazide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gliclazide</td>
<td>60.00 ± 0.73</td>
<td>11.89 ± 0.06</td>
<td>1.76 ± 0.02</td>
<td>585.04 ± 608.53</td>
</tr>
<tr>
<td>Atazanavir</td>
<td>98.67 ± 1.61</td>
<td>08.03 ± 0.11</td>
<td>1.96 ± 0.05</td>
<td>81.71 ± 2.75</td>
</tr>
<tr>
<td>Atazanavir + gliclazide (SDA)</td>
<td>53.33 ± 0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.44 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.56 ± 0.04</td>
<td>730.36 ± 29.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Atazanavir + gliclazide (MDA)</td>
<td>51.00 ± 0.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.10 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.78 ± 0.02</td>
<td>674.14 ± 36.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Efavirenz vs gliclazide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gliclazide</td>
<td>60.67 ± 0.67</td>
<td>11.83 ± 0.20</td>
<td>1.77 ± 0.04</td>
<td>2747.44 ± 625.78</td>
</tr>
<tr>
<td>Efavirenz</td>
<td>96.33 ± 0.61</td>
<td>08.21 ± 0.13</td>
<td>1.95 ± 0.02</td>
<td>88.89 ± 2.77</td>
</tr>
<tr>
<td>Efavirenz + gliclazide (SDA)</td>
<td>70.00 ± 0.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>07.86 ± 0.30&lt;sup&gt;†&lt;/sup&gt;</td>
<td>1.38 ± 0.05</td>
<td>414.09 ± 27.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Efavirenz + gliclazide (MDA)</td>
<td>67.00 ± 0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>07.73 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.28 ± 0.01</td>
<td>749.04 ± 87.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Notes: Data was expressed as Mean ± SEM; <sup>†</sup>Calculated by homeostasis model assessment method; <sup>a</sup>Significant at P < 0.05 compared to gliclazide control.
Abbreviations: SDA, single dose administration; MDA, multiple dose administration.

not observed in our previous studies in rabbits (maximum response at 3 hours), which is consistent with the available literature. Based on this background we selected 2 hours and 8 hours for the rat experiments and 3 hours for the rabbit experiments as blood sampling time points to measure the glucose–insulin homeostasis.

The elevated insulin levels together with increased glucose levels suggests an insulin resistant state.<sup>10,11</sup> Diabetes related glucose intolerance is characterized by an increase in insulin resistance and alterations in insulin clearance, insulin sensitivity of hepatic and peripheral tissues. In this study indinavir and ritonavir produced a significant impact on glucose–insulin homeostasis concomitant with insulin resistance and impaired in β-cell function in both rats and rabbits. These effects were augmented in the diabetic animals in comparison to the normal controls indicating the potency of these drugs towards exacerbation of existing diabetes mellitus. Comparatively indinavir showed a potent impact on glucose–insulin homeostasis in our study. Atazanavir, efavirenz and nevirapine alone did not have any significant effect on glucose–insulin homeostasis disorders associated with antiretroviral drugs are drug specific, but not a class-specific. All these observations are consistent with our previous studies.<sup>12–15</sup>

In combination, the pharmacodynamics of gliclazide was significantly reduced in the presence of indinavir following both single and multiple dose treatments in the rat (normal and diabetic) and rabbit models and it confirmed the presence of a potential interaction between gliclazide and indinavir. The possible mechanism of this pharmacodynamic interaction appears to be due to the opposing effects of gliclazide and indinavir on insulin resistance, insulin release or tissue uptake of glucose as reflected in our study.

In contrast to the theoretical expectation and consistent with our previous study,<sup>13</sup> the pharmacodynamics of gliclazide was enhanced by ritonavir following single and multiple dose administration in rats and rabbits, even though ritonavir alone has shown significant alterations in glucose–insulin homeostasis. Gliclazide is known to be metabolized by the hepatic microsomal enzymes CYP3A4 primarily and partly by CYP3A4.<sup>9,31</sup> Ritonavir is a well known potent inhibitor of CYP3A4<sup>16</sup> and used to enhance the pharmacokinetic and anti-HIV activity profiles of the concomitantly administered PIs.<sup>37</sup> Since ritonavir increased blood glucose and insulin

Table 8 Effect of nevirapine on the activity of gliclazide in normal rabbits (N = 6)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Glucose (mg/dL)</th>
<th>Insulin (µU/mL)</th>
<th>Insulin resistance&lt;sup&gt;†&lt;/sup&gt;</th>
<th>β-cell function&lt;sup&gt;†&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nevirapine vs gliclazide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gliclazide</td>
<td>60.00 ± 0.73</td>
<td>11.03 ± 0.12</td>
<td>1.63 ± 0.03</td>
<td>2023.527 ± 562.45</td>
</tr>
<tr>
<td>Nevirapine</td>
<td>86.33 ± 1.31</td>
<td>8.76 ± 0.37</td>
<td>1.87 ± 0.09</td>
<td>136.90 ± 8.30</td>
</tr>
<tr>
<td>Nevirapine + gliclazide (SDA)</td>
<td>58.67 ± 0.67</td>
<td>11.03 ± 0.18</td>
<td>1.60 ± 0.04</td>
<td>1028.46 ± 136.28&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nevirapine + gliclazide (MDA)</td>
<td>61.00 ± 0.45</td>
<td>11.22 ± 0.17</td>
<td>1.69 ± 0.02</td>
<td>2675.20 ± 536.40&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Notes: Data was expressed as Mean ± SEM; <sup>†</sup>Calculated by homeostasis model assessment method; <sup>a</sup>Significant at P < 0.05 compared to gliclazide control.
Abbreviations: SDA, single dose administration; MDA, multiple dose administration.
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 a pharmacokinetic interaction at a metabolic level rather than a pharmacodynamic interaction. However, it would need to be confirmed by conducting further pharmacokinetic studies in animal models.

In combination, atazanavir has enhanced the pharmacodynamics of gliclazide with respect to glucose–insulin homeostasis and consistent with our previous study it confirms the pharmacokinetic interaction, at a metabolic level, as per our previous study, as atazanavir inhibited CYP3A4 and CYP2C9-mediated drug metabolism that leads to raised serum levels of gliclazide.14

This study revealed additional information regarding the safety profile of efavirenz and nevirapine in both the normal and diabetic condition. However, contrary to the theorized expectation, the activity of gliclazide was significantly reduced in the presence of efavirenz in rats (normal and diabetic) and rabbits with respect to glucose–insulin homeostasis. Additionally it confirms the presence of a potential interaction between efavirenz and gliclazide. The possible mechanism of such an interaction between efavirenz and gliclazide may be due to the increased metabolism of hepatic microsomal enzymes by efavirenz, as it is known to be a potent CYP3A4 inducer.15,18,39 However this would need to be confirmed by further pharmacokinetic interaction studies.

With respect to the safety profile of nevirapine with gliclazide and glucose–insulin homeostasis the results of this study are consistent with the findings of our previous study.15 However, the metabolic complications arising from HIV-infection and/or antiretroviral therapy are multifactorial and complex therefore the study of other possible factors and mechanism(s) behind these interactions can’t be ruled out.

Conclusion
This study has confirmed that glucose–insulin homeostasis disorders associated with antiretroviral drugs are not a class specific, but are drug specific. Since the interaction between antiretroviral drugs (indinavir, ritonavir, atazanavir and efavirenz) and gliclazide was seen in two dissimilar species, it is likely to occur in humans, leading to increased/decreased activity of gliclazide, which may need dosage adjustment. Hence care should be taken when the combinations of these two drug types prescribed for the treatment of diabetic patients. Since there is no interaction between nevirapine and gliclazide in any species, this will probably be a safe combination of drugs in humans too. However the present study indicates the need for further studies to determine the relevance of these interactions in human beings and to understand the exact pharmacodynamic mechanisms of such interactions.

Disclosures
SK Mastan participated in the design, carried out the study and drafted the manuscript. K Eswar Kumar conceived of the study, participated in the design of the study and performed the statistical analysis and interpretation of the data. The authors report no conflicts of interest in this work.

Acknowledgements
The authors are thankful to Aurobindo Pharma Ltd, Hyderabad and Micro Labs, Bangalore for supplying gift samples of the antiretroviral drugs and gliclazide, respectively. The authors are grateful to Dr Katherine Samaras, Head, Diabetes and Obesity Clinical Group, Garvan Institute of Medical Research, New South Wales 2010, Australia for her kind help and support during the literature survey.

References


Effect of protease inhibitors (indinavir and ritonavir) on the pharmacokinetics of gliclazide in rabbits

Kilari Eswar Kumar1
Shaik Mastan2,3

1Pharmacology Division, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India; 2Research and Development Cell, Jawaharlal Nehru Technological University, Hyderabad, Andhra Pradesh, India; 3Cytel Statistical Software and Services Pvt Ltd, Pune, Maharashtra, India

Abstract: The objective of this study was to investigate the effect of protease inhibitors (indinavir and ritonavir) on the pharmacokinetics of gliclazide in rabbits and to evaluate the mechanism of interaction of the combination. Studies in rabbits were conducted with oral doses of gliclazide, selected protease inhibitor, and their combination with a 1-week washout period between each treatment (single dose followed by multiple dose treatment). Blood samples were collected at regular time intervals by marginal ear vein puncture and serum gliclazide levels were analyzed by high-pressure liquid chromatography. Pharmacokinetic analysis was performed by noncompartmental analysis using WinNonlin Software. In combination, ritonavir significantly increased serum gliclazide levels and altered the pharmacokinetic parameters of gliclazide in rabbits while indinavir had no significant effect. The percentage increase of serum gliclazide level was 22.34% and 27.78% following single-dose and multiple-dose treatment of ritonavir, respectively. The interaction of ritonavir with gliclazide is pharmacokinetic at a metabolic level (by CYP3A4 inhibition) in normal rabbits, while the interaction of indinavir with gliclazide is pharmacodynamic, which needs dose adjustment, and care should be taken when these combinations are prescribed for their clinical benefit in diabetic patients.

Keywords: gliclazide, indinavir, ritonavir, diabetes, HIV infection, pharmacokinetics

Introduction
Diabetes mellitus and HIV infection are major current health concerns worldwide, which need chronic and rational therapy. There is a strong relationship between HIV infection therapy and diabetes, especially with protease inhibitors (PIs).1 Insulin resistance, impaired glucose tolerance, and type 2 diabetes are conditions that are increasingly described in HIV-1-infected subjects receiving antiretroviral therapy.2 Among the many metabolic perturbations that occur as a result of HIV infection and its treatment, alterations in normal glucose–insulin homeostasis remain a particularly prevalent and alarming clinical change in affected patients.3 Much of the concern is due to the recognition of the long-term complications of insulin resistance and hyperglycemia in the context of the growing worldwide epidemic of type 2 diabetes mellitus.4 In this context there will be more chances of co-administration of antiretroviral drugs with oral hypoglycemic drugs in diabetes patients suffering with HIV infection which may lead to drug–drug interactions.

Drug interactions may occur when two drugs are concurrently administered and one drug (or both) may influence the time course of the other in the body. Drug interaction studies assume much importance especially for drugs that have a narrow margin of
safety and where the drugs are used for prolonged periods of time. Diabetes mellitus is one such metabolic disorder that needs treatment for prolonged periods. Maintenance of normal blood glucose level is very important in this condition, since both hyperglycemia and hypoglycemia are undesirable. However, there is little information that describes the mechanisms of drug interactions of PIs with oral hypoglycemic drugs, information that is needed to provide rational therapy.

Oral hypoglycemic agents are used in the treatment of type 2 diabetes, among which gliclazide, a second-generation sulfonylurea derivative, is preferred in therapy because of its selective inhibitory activity towards pancreatic K⁺ adenosine triphosphate (ATP) channels, antioxidant properties, low incidence of severe hypoglycemia, and other hemobiological effects. Indinavir and ritonavir are widely used PIs to treat HIV-infected patients.

We have previously studied the effect of indinavir and ritonavir on the pharmacodynamics (glucose, insulin, insulin resistance, β-cell function) of gliclazide in rats (normal and diabetic) and rabbits in terms of safety and effectiveness of the combination. These studies showed that both indinavir and ritonavir alone have a tendency to produce hyperglycemia and alterations in insulin-glucose homeostasis, and accelerated the diabetic condition in animal models. In combination, indinavir significantly reduced the effect of gliclazide while ritonavir increased the effect of gliclazide (contrary to its individual potency to exacerbate diabetes) and confirmed the existence of significant interactions. But the pharmacodynamic or pharmacokinetic mechanism of these interactions in nature is not known. Therefore the present study was investigated the effect of indinavir and ritonavir on the pharmacokinetics of gliclazide in rabbits and evaluated the mechanism of the interaction of the combination.

Materials and methods

Drugs and chemicals

Gliclazide, PIs, and nicorandil are the gift samples from Micro Labs (Bangalore, India), Aurobindo Pharma Ltd (Hyderabad, India), and Sun Pharmaceuticals Industries Ltd (Ahmedabad, India), respectively. Acetonitrile (HPLC grade), orthophosphoric acid (AR grade), and dichloromethane (AR grade) were obtained from Qualigens Chemicals (Mumbai, India), SD Fine Chemicals (Mumbai, India), and Loba Chemie Pvt Ltd. (Mumbai, India), respectively. All other reagents or chemicals used were of analytical grade.

Animals

Normal albino rabbits of either sex of 3 months of age, weighing 1.25–1.75 kg were procured from the National Institute of Nutrition, Hyderabad, India. They were maintained under standard laboratory conditions at an ambient temperature of 25 ± 2°C and 50% ± 15% relative humidity with a 12/12 light/dark cycle. Animals were fed with a commercial pellet diet (Rayan’s Biotechnologies Pvt Ltd. Hyderabad, India) and water ad libitum. They were fasted for 18 hours prior to the experiment and during the experiment food and water were withdrawn as described in Drug administration section. The animal experiments were performed after prior approval of the study protocol by the Institutional Animal Ethics Committee. The study was conducted in accordance with the guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Drug administration and blood samples collection

In clinical practice, PIs and gliclazide in therapeutic dose are administered orally as antiretroviral and antidiabetic therapy, respectively. Hence, human therapeutic doses were extrapolated to rabbit based on body surface area and administered orally. Indinavir (56 mg/1.5 kg body weight) and ritonavir (14 mg/1.5 kg body weight) were suspended in 3% CMC-Na for oral administration. Gliclazide (5.6 mg/1.5 kg body weight) solution was prepared by dissolving it in a few drops of 0.1N NaOH then made up to the volume with distilled water.

Two groups of 6 rabbits each were administered with 5.6 mg/1.5 kg body weight of gliclazide orally. The same group was administered with interacting drug (indinavir or ritonavir) and the combination of PI and gliclazide. A 1-week washout period was maintained between treatments. After this single dose interaction study the same group was continued for multiple-dose treatment with the daily treatment of interacting drug (indinavir or ritonavir) for the next 8 days with regular feeding. Later, after 18 hours fasting, they were again given the combined treatment on the ninth day. 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 hours. The blood samples collected from the rabbits were centrifuged at 3000 rpm for 15 minutes for obtaining the serum for analysis.

Chromatography

A gradient high pressure liquid chromatograph (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps,
variable wavelength programmable UV/VIS detector SPD-10A VP, CTO-10AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu), and RP C-18 column (250 mm × 4.6 mm I.D.; particle size 5 µm; YMC Inc., USA) was used. The HPLC system was equipped with the software Class-VP series version 6.12 SP2 (Shimadzu). The mobile phase consisted of acetonitrile and triple distilled water. The mobile phase components were filtered before use through a 0.22-µm membrane filter and pumped in the ratio of 30:70 (acetonitrile:triple distilled water containing 0.5% triethylamine) from the respective solvent reservoirs. The pH of the mobile phase was adjusted to 3.5 using orthophosphoric acid and column temperature was maintained at 30°C. The mobile phase was eluted at a flow rate 0.8 mL/min and the effluent was monitored at a wavelength of 230 nm. The ratio of peak area of gliclazide to that of internal standard was used for the quantification of gliclazide in serum samples.

Standard solutions
Primary stock solution of 1 mg/mL of gliclazide and nicorandil (internal standard) were prepared in methanol and stored at 4°C. Appropriate dilutions of gliclazide were made in mobile phase to produce concentrations of 10, 1 µg/mL and 500, 200, 100, 50, and 20 ng/mL. These dilutions were used to spike serum in the preparation of calibration curves. The internal standard working stock solution (10 µg/mL) was made from primary stock solution using mobile phase for dilution. Calibration samples were prepared by spiking 200 µL of individual blank serum with an appropriate amount of drug on the day of analysis. Samples for the determination of recovery, precision, and accuracy were prepared by spiking control rabbit serum in bulk of appropriate concentrations (20, 100, 500, and 1000 ng/mL) and stored at −4°C.

Extraction from the serum
To 200 µL of serum, 100 µL of nicorandil working solution and 0.1 mL of 0.07 M phosphate buffer (pH 4.5) were added. After vortex mixing for 10 seconds, 4 mL of dichloromethane was added and the mixture was shaken vigorously for 1 minute. The mixture was then centrifuged for 5 minutes at 8000 rpm. A 3 mL aliquot of the upper organic layer containing gliclazide and internal standard was transferred to a clean test tube and evaporated to dryness at 50°C. The residue was reconstituted into 100 µL of mobile phase and a 25 µL aliquot was injected onto the HPLC column. The eluent was detected at 230 nm by UV detector, and the data were acquired, stored, and analyzed by software Class-VP series version 6.12 SP2 (Shimadzu). Quantification was achieved by measuring the peak area ratio of the drug against the internal standard. The HPLC method was validated in terms of reproducibility, system suitability, recovery, accuracy, and precision and then applied for the estimation of gliclazide in rabbit serum.

Pharmacokinetic analysis
The peak concentration in plasma (C max) and concentration peak time (T max) were directly read from the concentration-time data. Other pharmacokinetic parameters were determined on subjecting the concentration-time data to noncompartmental analysis using WinNonlin (version 5.0.1; Pharsight, Sunnyvale, CA) software. The elimination rate constant (K e) was determined by linear regression analysis of the log-linear part of the plasma drug concentration-time curve. A minimum of 3 data points was used to calculate the terminal half-life (T 1/2 = ln2/K e). Area under the concentration-time curve (AUC) was calculated by use of the linear trapezoidal rule with extrapolation to infinity by dividing the last measured concentration by K e. The mean residence time (MRT) was calculated using the formula MRT = AUMC 0-Inf/AUC 0-Inf.

Statistical analysis
Data were expressed as mean ± SEM. Student’s paired t-test was performed to test the effect of PIs on the pharmacokinetics of gliclazide, and P < 0.05 was considered to be significant.

Results

Chromatography
The extraction procedure and the chromatographic conditions yielded a clear chromatogram for gliclazide. Recoveries of gliclazide from the spiked plasma samples (QC samples) estimated at 20, 100, 500, and 1000 ng/mL concentrations, ranged from 97% to 99%, and the limit of quantification is 20 ng/mL. The intra-day precision of the assay was determined by analyzing 3 spiked serum samples at each concentration on the same day. For the determination of inter-day precision, the samples were analyzed on 4 different days. The intra-day %accuracy (%CV) for 20, 100, 500, and 1000 ng/mL of gliclazide was 100.20 (0.20), 99.92 (1.72), 99.25 (1.25), and 99.29 (1.26), respectively. The inter-day %accuracy (%CV) for 20, 100, 500, and 1000 ng/mL of gliclazide was 100.55 (0.15), 99.86 (1.32), 99.25 (1.20), and 98.92 (1.28), respectively. The
system suitability parameters of gliclazide were determined as Limit of quantification (ng/mL) 20, theoretical plates 12248, tailing factor 1.14, retention time of gliclazide 7.82–8.32 minutes, retention time of internal standard (IS) 6.02–6.44 minutes, and resolution between drug peak and IS peak 2.316. The calibration curve (Figure 1) in the rabbit serum for gliclazide was linear within a concentration range of 20–1000 ng/mL, and the calibration regression equation was \( y = mx + c \), where \( y \) represents the peak area ratio of gliclazide to internal standard, \( x \) represents the concentration of gliclazide, \( m \) is slope of the curve, and \( c \) is the intercept. The typical regression equation was \( y = 0.0059x - 0.0167 \); \( r^2 = 0.9987 \). The typical chromatogram of gliclazide and internal standard is shown in Figure 2.

**Pharmacokinetics**

The mean concentration versus time curves after oral administration of gliclazide in the presence of indinavir and ritonavir are shown in Figures 3 and 4, respectively. The pharmacokinetic parameters of gliclazide alone, and in the presence of indinavir and ritonavir following single- and multiple-dose treatment in rabbits, are shown in Tables 1 and 2, respectively. The serum gliclazide levels as well as pharmacokinetic parameters were not significantly altered following single- and multiple-dose administration of indinavir. The serum gliclazide levels were increased and pharmacokinetic parameters of gliclazide such as \( C_{max} \), AUC, AUMC, \( K_{el} \), and clearance were altered significantly following single- and multiple-dose treatments of ritonavir. The percentage increase in serum gliclazide level was 22.34% and 27.78% following single- and multiple-dose administration of ritonavir, respectively.

**Discussion**

HIV-infected patients are likely to suffer from diabetes mellitus and hence most often antiretroviral drugs are co-administered along with oral antidiabetes drugs. HIV-infection and diabetes are both chronic diseases that significantly affect lifestyle. When they intersect, the treatment regimens required for both diseases can be overwhelming for patients. Drug interactions are usually seen in clinical practice and the pharmacokinetic interactions are usually evaluated in animal models. Although animal models can never replace the need for comprehensive studies in human subjects, their use can provide important insights to help understand and evaluate the mechanism of potent interactions between drugs. Although scientists are continually searching for an animal species in which all of the pharmacokinetics of drugs is consistently the same as in humans, it is very unlikely that such an animal species will ever be found.

Cytochromes P450 are a superfamily of hemoproteins that play a central role in the metabolism of endogenous compounds and xenobiotics. Cytochrome P4503A isoforms (especially
CYP3A4) are the most abundant human isoforms that metabolize over one-half of clinically used drugs. However, it is well known that the metabolism of drugs may differ significantly among species, both qualitatively and quantitatively. Nevertheless, with a careful selection of animal species and appropriate experimental conditions, it is possible to make a reasonably good prediction of the pharmacokinetics of drugs in humans. It is worth noting that several findings have confirmed the functional similarity of CYP3A forms in rabbits and humans, suggesting that the rabbit is a valuable in vivo model for the assessment of drug interaction occurring among species, both qualitatively and quantitatively.

The metabolism of drugs in humans is well known that the metabolism of drugs may differ significantly among species, both qualitatively and quantitatively. Moreover, studies performed on rabbits, evaluating the pharmacokinetics of other drugs metabolized in humans via CYP3A4 pathway have confirmed the usefulness of the rabbit model for such investigations. Based on these findings, and apart from convenience of serial blood sampling, we preferred rabbit as an animal model to perform the pharmacokinetic interaction studies. The multiple-dose effect of indinavir and ritonavir on the pharmacokinetics of gliclazide in rabbits was also studied since both are used for long-term treatment.

Gliclazide is known to produce hypoglycemic/antihyperglycemic activity by pancreatic25 (stimulating insulin secretion by blocking K+ channels in the pancreatic β cells) and extra pancreatic26 (increasing tissue uptake of glucose) mechanisms. Gliclazide is rapidly absorbed in all species (man, monkey, beagle, rabbit, and rat) with similar excretion in all species and inter-species variation in half-life.5,27 Our results in rabbits showed that gliclazide produced peak concentration at 3 hours with no second peak, while in rat models5,8–10,20,21 and humans28 a second peak is reported to be common due to the presence biliary excretion and enterohepatic cycling of gliclazide. According to Davis et al29 the extent of mean enterohepatic recirculation observed in humans was consistent with animal data. This consistency addresses the probable correlation of preclinical animal studies with studies on human subjects, and their use might provide important insights into the mechanisms of drug interactions which would improve their understanding and provide the basis for rational therapy. Thus our results indicating gliclazide peak concentration at 3 hours and absence of biliary excretion and enterohepatic cycling in rabbits are consistent with our former pharmacodynamic studies as well as the literature.5,20,21

Table 1 Mean pharmacokinetic parameters of gliclazide before and after indinavir administration in rabbits (n = 6)

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Gliclazide</th>
<th>Indinavir + gliclazide* (single-dose treatment)</th>
<th>Indinavir + gliclazide* (multiple-dose treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/mL)</td>
<td>360.68 ± 2.32</td>
<td>360.86 ± 1.83</td>
<td>364.62 ± 2.14</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>3.00 ± 0.00</td>
<td>3.00 ± 0.00</td>
<td>3.00 ± 0.00</td>
</tr>
<tr>
<td>Vd (L)</td>
<td>17.66 ± 1.22</td>
<td>17.84 ± 1.08</td>
<td>17.56 ± 1.10</td>
</tr>
<tr>
<td>AUC0-24 (ng/mL/h)</td>
<td>4006.24 ± 20.55</td>
<td>3969.46 ± 14.12</td>
<td>3975.08 ± 21.12</td>
</tr>
<tr>
<td>AUCinf (ng/mL/h)</td>
<td>4856.02 ± 90.60</td>
<td>4789.34 ± 110.61</td>
<td>4845.16 ± 113.27</td>
</tr>
<tr>
<td>AUC%Extrapolation</td>
<td>17.37 ± 1.48</td>
<td>20.09 ± 1.56</td>
<td>20.64 ± 1.45</td>
</tr>
<tr>
<td>AUCC0-24 (ng/L/h)</td>
<td>38945.25 ± 303.75</td>
<td>37655.96 ± 213.90</td>
<td>38533.66 ± 371.77</td>
</tr>
<tr>
<td>AUCCinf (ng/L/h)</td>
<td>70368.15 ± 4192.72</td>
<td>70014.70 ± 5529.87</td>
<td>70439.43 ± 6035.51</td>
</tr>
<tr>
<td>AUCC%Extrapolation</td>
<td>43.76 ± 3.08</td>
<td>46.73 ± 2.90</td>
<td>47.52 ± 2.46</td>
</tr>
<tr>
<td>MRT0-24 (h)</td>
<td>9.72 ± 0.03</td>
<td>9.66 ± 0.03</td>
<td>9.69 ± 0.04</td>
</tr>
<tr>
<td>MRTinf (h)</td>
<td>14.44 ± 0.58</td>
<td>14.18 ± 0.73</td>
<td>14.41 ± 0.74</td>
</tr>
<tr>
<td>CL (L/h)</td>
<td>1.14 ± 0.04</td>
<td>1.13 ± 0.06</td>
<td>1.13 ± 0.02</td>
</tr>
<tr>
<td>Kcl (h⁻¹)</td>
<td>0.08 ± 0.01</td>
<td>0.08 ± 0.00</td>
<td>0.07 ± 0.00</td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>8.6 ± 0.60</td>
<td>8.8 ± 0.80</td>
<td>8.9 ± 0.90</td>
</tr>
</tbody>
</table>

Notes: Data are expressed as mean ± SEM. *No statistical significance vs gliclazide.
Indinavir is reported to be metabolized by CYP3A4 and an inhibitor of the cytochrome P450 isoform CYP3A4.30,31 As per the FDA label,30 co-administration of indinavir and drugs primarily metabolized by CYP3A4 results in increased plasma concentrations of the other drug, which could increase or prolong its therapeutic and adverse effects. Gliclazide is known to be metabolized by hepatic microsomal enzymes CYP2C9 primarily and partly by CYP3A4.5,6,20,21 Gliclazide is intensively metabolized into at least 5 metabolites (7α-hydroxy gliclazide, 6β-hydroxy gliclazide, 7β-hydroxy gliclazide, hydroxymethyl gliclazide, and carboxy gliclazide) and only small amounts of unchanged compound are excreted in the urine essentially as oxidized and hydroxylated derivatives, the majority of which undergo glucuroconjugation.5,27 Therefore, theoretically we may expect that gliclazide concentrations will be increased in the presence of indinavir (by CYP3A4 inhibition). But in contrast to this theoretical expectation, indinavir in combination with gliclazide did not show any significant effect on concentrations or pharmacokinetic parameters, and there was no pharmacokinetic interaction. The absence of pharmacokinetic interactions and changes in the concentrations of gliclazide in the presence of indinavir might be because of the partial contribution of CYP3A4 enzyme in gliclazide metabolism, or because indinavir is not a potent CYP3A4 inhibitor,31 or has lower absorption32 and protein binding5,6,20,21 than gliclazide in animal models. Thus this study confirmed that the interaction of indinavir with gliclazide is pharmacodynamic not pharmacokinetic due to the opposing effects of gliclazide and indinavir on glucose–insulin homeostasis, insulin resistance, and tissue uptake of glucose. These results are consistent with recent clinical studies on diabetes-inducing capacity of indinavir.33,34

Ritonavir has been reported to cause new-onset diabetes mellitus, exacerbation of pre-existing diabetes mellitus, and hyperglycemia during postmarketing surveillance in HIV-infected patients.35 Our previous pharmacodynamic studies also confirmed these findings and thus, in combination, ritonavir has to decrease the pharmacodynamic activity of gliclazide. But there was a significant rise in serum gliclazide levels and alteration in pharmacokinetic parameters such as C_{max}, AUC, AUMC, K_{e} and T_{1/2} of gliclazide with single- and multiple-dose treatments of ritonavir. The increase in C_{max}, AUC, and AUMC indicates improved availability of gliclazide in the presence of ritonavir. There might not be an interaction during absorption since oral absorption of ritonavir is not high and absorption rate constant (K_{a}) remained unchanged. Gliclazide and ritonavir are highly protein-bound drugs (gliclazide: 85%–99%; ritonavir: 98%–99.5%) and there is every possibility for displacement of gliclazide from the protein binding sites by ritonavir which may lead to increased gliclazide levels. However there was no significant change in V_{d} and T_{1/2} of gliclazide in the presence of ritonavir. Hence its protein displacement in the presence of ritonavir was unlikely and not significant. Hence, the rise of gliclazide blood levels in the presence of ritonavir might be through other than improved absorption and altered distribution. The altered K_{e}, T_{1/2}, and clearance indicates alteration either in the process of metabolism or excretion.

Ritonavir is primarily metabolized by the CYP3A subfamily through N-demethylation, hydroxylation of the isopropyl side chain, and oxidation and cleavage of the
terminal isopropylthiazole group. Ritonavir is a well-known potent CYP3A4 inhibitor and is used to enhance the pharmacokinetic and anti-HIV activity profiles of the concomitantly administered PIs, and ritonavir is generally considered to have similar CYP3A4-inhibitory potency compared with ketoconazole, a most potent CYP3A4 inhibitor. Interestingly, some studies reported that tolbutamide-4-hydroxylated was inhibited by CYP2C9 inhibition and thus ritonavir may also affect CYP2C9 and CYP2C19 activity apart from CYP3A4 inhibition. Further, gliclazide is eliminated through renal (80%) and biliary (20%) routes and the major elimination pathway of ritonavir is hepatic clearance (<95%). Hence the raised serum concentrations of gliclazide in the presence of ritonavir may be due to its reduced metabolism by CYP3A4 inhibition and thus decreased the elimination process as it was supported by a significant decrease in elimination and clearance of gliclazide from the pharmacokinetic parameters.

In animals and in man, among the CYP group of drug-metabolizing enzymes, CYP3A4 is the major phase I drug metabolizing enzyme. It is present in the liver, jejunum, colon, and pancreas. It has broad substrate specificity and is responsible for metabolism of more than 50% of administered drugs. However, the liver (300 pmol of total CYPs/mg microsomal protein) and the intestinal epithelia (20 pmol of total CYPs/mg microsomal protein) are the predominant sites for P450-mediated drug elimination, while the other tissues contribute to a much smaller extent to drug elimination. In vivo drug interactions with ritonavir (PIs) are most likely due to mechanism-based inhibition of CYP3A and thus pronounced and sustained elevation of the plasma levels of other PIs as well as pharmacoenhancement of other drugs. This pharmacoenhancement reflects the fact that the currently available HIV PIs are both substrates and inhibitors of CYP3A and thus compete for the same metabolic enzyme at both hepatic and intestinal sites and thus the site of drug interaction of ritonavir with gliclazide was expected to be both at hepatic and intestinal sites. Biochemical and structural studies have shown that ritonavir is an irreversible type II inhibitor that inactivates CYP3A4 not only by displacing substrates from the active site and tightly binding to the heme iron via the thiazole nitrogen but also by decreasing the protein redox potential and precluding reduction by cytochrome P450 reductase. Additional possible mechanisms of inhibition by ritonavir which cannot be ruled out include potential to inactivate CYP3A enzymes by the formation of a metabolic intermediate complex, a competitive or mixed competitive–noncompetitive CYP3A4 inactivation.

The interaction of ritonavir with gliclazide is a pharmacokinetic interaction at a metabolic level (by CYP3A4 inhibition) in normal rabbits while the interaction of indinavir with gliclazide is pharmacodynamic, which needs dose adjustment, and care should be taken when these combinations are prescribed for their clinical benefit in diabetic patients.

**Conclusion**

Our study confirmed that the interaction of ritonavir with gliclazide is a pharmacokinetic interaction at a metabolic level by CYP3A4 inhibition in normal rabbits while the interaction of indinavir with gliclazide was pharmacodynamic not pharmacokinetic, which needs dose adjustment, and care should be taken when these combinations are prescribed for diabetes patients. However the present study shows that further studies are warranted to determine the relevance of these interactions in human beings and the exact mechanism of action(s) behind this interaction(s), if any.

**Acknowledgments**

The authors are thankful to M/s Aurobindo Pharma Ltd, Hyderabad and M/s Micro Labs, Bangalore for supplying gift samples of PIs and gliclazide respectively. The authors are grateful to Cytel Management, Pune for kind help and support during the pharmacokinetic analysis.

**Disclosure**

K Eswar Kumar designed the research and data interpretation; SK Mastan designed the research, performed the research, analyzed data, and drafted the manuscript. Both the authors read and approved the final manuscript. The author(s) declare that they have no competing interests.

**References**


Influence of Efavirenz and Nevirapine on the Pharmacodynamics and Pharmacokinetics of Gliclazide in Rabbits

Kilari Eswar Kumara, Shaik Mastanb, c, d

Abstract

Background: Since polypharmacy is very common practice in diabetic patients, the study of drug-drug interactions is an imperative rational approach with respect to safety and efficacy determination. Gliclazide is a widely used drug for the treatment of type 2 diabetes. Efavirenz and nevirapine are widely used non-nucleoside reverse transcriptase inhibitors (NNRTIs) with fewer side effects concerning diabetic complications. The objective of the study is to investigate the effect of selected NNRTIs on the pharmacodynamics and pharmacokinetics of gliclazide in rabbits with respect to safety and efficacy of the combination.

Methods: Influence of selected NNRTIs on the activity of gliclazide was determined by conducting a single dose interaction followed by multiple dose interaction study with two groups consisting of 6 normal rabbits each. Each group was treated with an oral dose of 5.6 mg/1.5 kg bd. wt. of gliclazide, interacting drug treatment (42 mg/1.5 kg bd. wt. of efavirenz or 14 mg/1.5 kg bd. wt. of nevirapine) and their combination with a one week washout period between each treatment. One week washout after this single dose interaction study, each group was continued with the daily treatment of interacting drug for the next eight days and then the combined treatment on the ninth day. Blood samples were collected at regular intervals by marginal ear vein puncture in rabbits and were analyzed for blood glucose by GOD/POD method and insulin by radioimmunoassay method. The serum gliclazide levels were estimated by HPLC method and pharmacokinetic analysis was conducted by noncompartmental analysis using WinNonlin software.

Results: In combination, efavirenz significantly (P < 0.05) reduced the pharmacodynamic activity and serum levels of gliclazide. The pharmacokinetic parameters of gliclazide were significantly (P < 0.05) altered. The percent decrease of serum gliclazide concentration is 24.23% and 15.20% following single dose and multiple dose administration of efavirenz, respectively. In combination, nevirapine has no significant effect on the pharmacodynamics and pharmacokinetics of gliclazide in rabbits.

Conclusions: The significant pharmacokinetic interaction of efavirenz at metabolic level by CYP3A4 induction results in decreased serum gliclazide levels and pharmacodynamic activity of gliclazide. The combination of nevirapine and gliclazide was proved to be safe.

Keywords: Gliclazide; Efavirenz; Nevirapine; Diabetes; HIV infection; Pharmacokinetics

Introduction

Polypharmacy is very common practice for the patients suffering from chronic diseases such as diabetes mellitus and HIV infection, and thus leads to the undesirable potent drug-drug interactions (pharmacodynamic and/or pharmacokinetic) which can alter the safety and efficacy profile of a drug in many ways. Recent reports [1, 2] reveals that drug interactions played a vital role in reported adverse events and that majority of the drugs withdrawn for safety reasons from the US market were related with significant drug-drug interactions. The importance of this fact is further emphasized by increased post marketing adverse event reports by 240% over the last decade [3].

There is a propensity for drug-drug interactions in patients with concurrent type 2 diabetes mellitus and HIV infection that are likely to be treated with antiretroviral and antidiabetic therapy. Diabetes mellitus is a metabolic disorder that needs treatment for prolonged periods and maintenance of normal blood glucose level is very important in this con-
dition, since both hyperglycemia as well as hypoglycemia is unwanted phenomenon [4, 5]. Since many studies suggested that PI therapy [6, 7] is linked to the development of diabetic complications, it is of importance to propose therapeutic strategies with fewer side effects, such as the use of the non-nucleoside reverse transcriptase inhibitors (NNRTIs) and this approach appear successful to control HIV infection [8-10]. In this context, there are more chances of co-administration of NNRTIs with oral hypoglycemic drugs in patients with concurrent type 2 diabetes mellitus and HIV infection which may leads to potent drug-drug interactions. However, there is no much information available to elucidate the mechanisms of drug interactions between NNRTIs and oral hypoglycemic drugs which are essential to the clinicians to prescribe the rational drug combinations with respect to safety and efficacy.

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 antidiabetic activity and other beneficial effects include antioxidant property, low incidence of hypoglycemia and other haemobiological effects [11-14]. Efavirenz and nevirapine are widely used NNRTIs to treat HIV infection. Based on this background, formerly we have conducted a preliminary study [4] to investigate the effect of efavirenz and nevirapine on the pharmacodynamic activity of gliclazide in rats (normal and diabetic) and rabbits with respect to blood glucose levels only. However, determination of insulin along with blood glucose levels would be a more precious and dependent approach to conclude a clear pharmacodynamic interaction scenario in the view of clinical and scientific stand-point. Since the pharmacodynamic (PD) activity of a drug depends on its pharmacokinetics (PK), the PK-PD interaction should be concurrently performed in same group in order to undoubtedly conclude the effect/relation of PK interaction on the pharmacodynamics as a consequence. So the present study was planned to investigate the effect of selected NNRTIs (efavirenz and nevirapine) on the pharmacodynamics (glucose and insulin levels) and pharmacokinetics of gliclazide in rabbits with respect to safety and efficacy of the combination.

Materials and Methods

Drugs and chemicals

Gliclazide and NNRTIs are the gift samples from Micro Labs (Bangalore, India) and Aurobindo Pharma Ltd (Hyderabad, India), respectively. Glucose kits (Span diagnostics) were purchased from local pharmacy. Acetonitrile (HPLC grade), orthophosphoric acid (AR grade) and dichloromethane (AR grade) were obtained from Qualigens Chemicals (Mumbai, India), SD Fine Chemicals (Mumbai, India) and Loba Chemie Pvt. Ltd., (Mumbai, India), respectively. All other reagents or chemicals used were of analytical grade.

Animals

Normal albino rabbits of either sex of 3 months of age, weighing between 1.5-2 Kg were procured from National Institute of Nutrition, Hyderabad, India. Animals were fed with a commercial pellet diet (Rayan’s Biotechnologies Pvt Ltd., Hyderabad, India) and water ad libitum. The animal experiments were performed after prior approval of the study protocol by the Institutional Animal Ethics Committee. The
animals were maintained under standard laboratory conditions and the study was conducted in accordance with the guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Study design

Concerning the use of NNRTIs and gliclazide in clinical practice, human therapeutic oral doses were extrapolated to rabbit based on body surface area [15], and these doses were administered orally [4, 16, 17]. The study design is shown in Figure 1 for more information.

This study consists of two groups:

Group 1: Interaction study of efavirenz and gliclazide in rabbits (n = 6);

Group 2: Interaction study of nevirapine and gliclazide in rabbits (n = 6).

Each group of six rabbits was administered with 5.6 mg/1.5 kg bd. wt. of gliclazide, orally. The same group was administered with interacting drug (efavirenz 42 mg/1.5 kg bd. wt. or nevirapine 14 mg/1.5 kg bd. wt., orally) and the combination of interacting drug and gliclazide. One week washout period was maintained between each treatment. One week washout after this single dose interaction study,

Figure 2. Standard graph for the estimation of gliclazide levels in rabbit serum.

Figure 3. The typical HPLC chromatogram of gliclazide and internal standard.
the respective group was continued with the daily treatment of interacting drug (efavirenz/nevirapine) for the next eight days with regular feeding. Later after 18 h fasting they were again given the combined treatment on the ninth day.

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

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 GOD/POD method [18] using commercial glucose kits and insulin by Radioimmunoassay method [19] using a commercially available kit (Biomedica, Saluggia, Italy) as per the instructions provided by the manufacturers.

**Chromatography**

The serum gliclazide concentrations were determined by HPLC method [20], briefly, a gradient High Pressure Liquid Chromatography method was used for the measurement of gliclazide levels.

### Table 1. Mean Percent Blood Glucose Reduction of Gliclazide in Presence and Absence of Efavirenz in Rabbits (n = 6)

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Gliclazide</th>
<th>Efavirenz</th>
<th>Efavirenz + Gliclazide (Single dose treatment)</th>
<th>Efavirenz + Gliclazide (Multiple dose treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.68 ± 5.13</td>
<td>-2.65 ± 1.86</td>
<td>5.44 ± 5.47*</td>
<td>10.56 ± 3.59*</td>
</tr>
<tr>
<td>2</td>
<td>22.84 ± 6.61</td>
<td>-4.40 ± 2.35</td>
<td>11.43 ± 3.77*</td>
<td>15.69 ± 3.42*</td>
</tr>
<tr>
<td>3</td>
<td>32.82 ± 5.34</td>
<td>-1.98 ± 2.93</td>
<td>21.86 ± 3.11*</td>
<td>26.33 ± 2.55*</td>
</tr>
<tr>
<td>4</td>
<td>24.41 ± 4.65</td>
<td>0.14 ± 3.04</td>
<td>11.07 ± 3.97*</td>
<td>16.05 ± 3.74*</td>
</tr>
<tr>
<td>6</td>
<td>22.85 ± 6.14</td>
<td>1.56 ± 2.29</td>
<td>8.89 ± 2.25*</td>
<td>13.15 ± 2.60*</td>
</tr>
<tr>
<td>8</td>
<td>17.56 ± 3.84</td>
<td>3.67 ± 4.28</td>
<td>6.99 ± 3.09*</td>
<td>11.30 ± 3.00*</td>
</tr>
<tr>
<td>12</td>
<td>8.51 ± 6.13</td>
<td>5.79 ± 4.77</td>
<td>5.14 ± 3.17</td>
<td>9.89 ± 1.84</td>
</tr>
<tr>
<td>16</td>
<td>3.74 ± 6.04</td>
<td>7.55 ± 3.86</td>
<td>0.69 ± 2.24</td>
<td>5.43 ± 4.01</td>
</tr>
<tr>
<td>20</td>
<td>2.28 ± 6.01</td>
<td>6.85 ± 4.97</td>
<td>-0.80 ± 2.70</td>
<td>2.90 ± 3.60</td>
</tr>
<tr>
<td>24</td>
<td>0.03 ± 6.63</td>
<td>6.18 ± 2.20</td>
<td>-2.71 ± 4.44</td>
<td>1.79 ± 2.56</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD; *Significant at P<0.05 compared to gliclazide control.

**Figure 4.** Mean insulin levels (µU/mL) of gliclazide before and after treatment with efavirenz in normal rabbits (n = 6) (Data are expressed as mean ± SD; * Significant difference at P < 0.05 compared to gliclazide control).
Chromatograph (Shimadzu HPLC Class VP series) equipped with the software “Class-VP series version 6.12 SP2 (Shimadzu) was used. Nicorandil was used as internal standard and the mobile phase consisted of acetonitrile and triple distilled water in the ratio of 30:70 (Acetonitrile: triple distilled water containing 0.5% triethylamine). The mobile phase was eluted at a flow rate 0.8 mL/min and the effluent was monitored at a wavelength of 230 nm. The ratio of peak area of gliclazide to that of internal standard was used for the quantification of gliclazide in serum samples. The HPLC method was validated in terms of reproducibility, system suitability, recovery, accuracy and precision and then applied for the estimation of gliclazide in rabbit serum.

Pharmacokinetic analysis

The peak concentration in plasma (C_{max}) and concentration peak time (T_{max}) were directly read from the concentration-time data. Other pharmacokinetic parameters were determined on subjecting the concentration-time data to non-compartmental analysis using WinNonlin (version 5.0.1) software. The elimination rate constant (K_{el}) was determined by linear regression analysis of the log-linear part of the plasma drug concentration-time curve. A minimum of three data points were used to calculate the terminal half-life (T_{1/2} = ln2/K_{el}). Area under the concentration-time curve (AUC) was calculated by use of the linear trapezoidal rule with extrapolation to infinity by dividing the last measured concentration by K_{el}. The mean residence time (MRT) was calculated using formula, MRT = AUMC_{0-inf}/AUC_{0-inf}.

Data and statistical analysis

Data are presented as mean ± SD. P < 0.05 was considered significant and it was determined by applying Student’s paired t-test.

Results

Chromatography

The calibration curve in the rabbit serum for gliclazide was linear within the concentration range of 20-1000 ng/mL. The intra-day % accuracy (% CV) for 20, 100, 500 and 1000 ng/mL of gliclazide was 100.20 (0.20), 99.92 (1.72), 99.25
Table 2. Mean Pharmacokinetic Parameters of Gliclazide Before and After Efavirenz Administration in Rabbits (n = 6)

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Gliclazide</th>
<th>Efavirenz + Gliclazide (Single dose treatment)</th>
<th>Efavirenz + Gliclazide (Multiple dose treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>391.21 ± 5.50</td>
<td>342.58 ± 13.45*</td>
<td>363.37 ± 14.47*</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>3.00 ± 0.00</td>
<td>3.00 ± 0.00</td>
<td>3.00 ± 0.00</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-24&lt;/sub&gt; (ng/mL/h)</td>
<td>4465.66 ± 168.89</td>
<td>3441.40 ± 278.44*</td>
<td>3841.04 ± 293.26*</td>
</tr>
<tr>
<td>AUMC&lt;sub&gt;0-24&lt;/sub&gt; (ng/mL/h*h)</td>
<td>5520.68 ± 277.21</td>
<td>3907.52 ± 292.87*</td>
<td>4441.83 ± 370.97*</td>
</tr>
<tr>
<td>MRT&lt;sub&gt;0-24&lt;/sub&gt; (h)</td>
<td>9.84 ± 0.09</td>
<td>9.21 ± 0.15*</td>
<td>9.43 ± 0.25*</td>
</tr>
<tr>
<td>MRT&lt;sub&gt;0-inf&lt;/sub&gt; (h)</td>
<td>15.19 ± 0.66</td>
<td>12.24 ± 1.05*</td>
<td>12.94 ± 0.84*</td>
</tr>
<tr>
<td>V&lt;sub&gt;d&lt;/sub&gt; (L)</td>
<td>14.71 ± 0.66</td>
<td>15.15 ± 2.97</td>
<td>14.93 ± 1.60</td>
</tr>
<tr>
<td>CL (L/h)</td>
<td>0.06 ± 0.03</td>
<td>1.51 ± 0.11*</td>
<td>1.33 ± 0.11*</td>
</tr>
<tr>
<td>K&lt;sub&gt;e&lt;/sub&gt; (h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.07 ± 0.00</td>
<td>0.10 ± 0.02*</td>
<td>0.09 ± 0.01*</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>9.62 ± 0.67</td>
<td>7.01 ± 1.45*</td>
<td>7.84 ± 0.93*</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD; *Significant at P < 0.05 compared to gliclazide control.

Pharmacodynamic interaction study of efavirenz with gliclazide

The percent blood glucose reduction and insulin levels of gliclazide in presence and absence of efavirenz are shown in Table 3.

Table 3. Mean Percent Blood Glucose Reduction of Gliclazide in Presence and Absence of Nevirapine in Rabbits (n = 6)

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Gliclazide</th>
<th>Nevirapine</th>
<th>Nevirapine + Gliclazide* (Single dose treatment)</th>
<th>Nevirapine + Gliclazide* (Multiple dose treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18.98 ± 3.65</td>
<td>-0.26 ± 7.67</td>
<td>18.53 ± 5.83</td>
<td>20.33 ± 5.49</td>
</tr>
<tr>
<td>2</td>
<td>25.19 ± 2.65</td>
<td>1.13 ± 9.86</td>
<td>25.89 ± 6.86</td>
<td>24.69 ± 3.34</td>
</tr>
<tr>
<td>3</td>
<td>34.03 ± 2.11</td>
<td>3.48 ± 6.91</td>
<td>35.46 ± 2.97</td>
<td>33.65 ± 2.13</td>
</tr>
<tr>
<td>4</td>
<td>26.34 ± 2.83</td>
<td>2.48 ± 5.10</td>
<td>28.09 ± 8.32</td>
<td>25.70 ± 2.87</td>
</tr>
<tr>
<td>6</td>
<td>24.09 ± 3.43</td>
<td>0.02 ± 9.80</td>
<td>23.82 ± 4.34</td>
<td>22.50 ± 3.71</td>
</tr>
<tr>
<td>8</td>
<td>18.20 ± 6.07</td>
<td>0.11 ± 7.43</td>
<td>18.14 ± 7.46</td>
<td>17.55 ± 3.06</td>
</tr>
<tr>
<td>12</td>
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<td>0.42 ± 9.04</td>
<td>13.43 ± 5.94</td>
<td>11.91 ± 2.35</td>
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<tr>
<td>16</td>
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<td>-1.39 ± 8.01</td>
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<td>5.37 ± 5.07</td>
</tr>
<tr>
<td>20</td>
<td>3.54 ± 4.10</td>
<td>-1.37 ± 7.14</td>
<td>3.92 ± 4.12</td>
<td>2.78 ± 5.92</td>
</tr>
<tr>
<td>24</td>
<td>2.13 ± 3.62</td>
<td>-1.67 ± 5.39</td>
<td>1.04 ± 3.05</td>
<td>0.29 ± 3.68</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD; *No significant difference at P < 0.05 compared to gliclazide control.
Figure 6. Mean insulin levels (µU/mL) of gliclazide before and after treatment with nevirapine in normal rabbits (n = 6) (Data are expressed as mean ± SD; No significant difference at P < 0.05 compared to gliclazide control).

Figure 7. Mean serum gliclazide concentration (ng/mL) before and after treatment with nevirapine in rabbits (n = 6; inset = semi log scale) (Data are expressed as mean ± SD).
in Table 1 and Figure 4, respectively. Gliclazide produced hypoglycemic activity with maximum reduction of 32.82 ± 5.34% and maximum insulin level of 22.32 ± 1.29 at 3 h in normal rabbits. Efavirenz alone has not produced any significant effect on the blood glucose and insulin levels. Whereas in combination, efavirenz significantly (P < 0.05) reduced the gliclazide activity (by decreasing its hypoglycemic activity and insulin levels) and the reduction was more significant with the single dose treatment of efavirenz than multiple dose treatment.

Pharmacokinetic interaction study of efavirenz with gliclazide

The serum concentration-time profiles of gliclazide following single dose and multiple dose administration of efavirenz are shown in Figure 5. Mean pharmacokinetic data of gliclazide in presence and absence of efavirenz is summarized in Table 2. The serum gliclazide concentrations were decreased and pharmacokinetic parameters of gliclazide like C_{max}, T_{max}, AUC, AUMC, MRT, K_{el}, Cl, and T_{1/2} were significantly altered (Table 2) with single- and multiple-dose treatment of efavirenz. Efavirenz significantly decreased the serum gliclazide concentration level by 24.23% and 15.20% following single dose and multiple dose administration of efavirenz, respectively. Efavirenz significantly decreased overall plasma exposure (AUC) and peak concentration (C_{max}) while increasing the clearance and apparent elimination half-life of gliclazide following single- and multiple-dose treatment of efavirenz.

Pharmacodynamic interaction study of nevirapine with gliclazide

The percent blood glucose reduction and insulin levels of gliclazide in presence and absence of nevirapine are shown in Table 3 and Figure 6 respectively. Gliclazide produced hypoglycemic activity with maximum reduction of 34.03 ± 2.11% and maximum insulin level of 23.26 ± 0.65 at 3 h in normal rabbits. Nevirapine alone has not produced any significant effect on the blood glucose and insulin levels. In combination also, nevirapine has no impact on the pharmacodynamic activity of gliclazide.

Pharmacokinetic interaction study of nevirapine with gliclazide

The serum concentration-time profiles of gliclazide following single dose and multiple dose administration of nevirapine are shown in Figure 7. Mean pharmacokinetic data of gliclazide in presence and absence of nevirapine is summarized in Table 4. The serum concentration levels and pharmacokinetic parameters of gliclazide were not altered significantly with nevirapine following single and multiple dose administration.

Discussion

Drug-drug interaction studies are an important aspect of pharmacology research and can be a critical step to opti-
mize the use of selected drugs, especially in the treatment of chronic diseases such as HIV infection and diabetes in which the polypharmacy is very common. This study was planned to evaluate the pharmacodynamic and pharmacokinetic interactions of selected NNRTIs (efavirenz and nevirapine) with gliclazide. It is the first to evaluate efavirenz and nevirapine effects on the pharmacodynamics and pharmacokinetics of gliclazide to explore the possible mechanism of interaction, as well as the first to compare single dose and multiple dose NNRTIs influence on gliclazide disposition.

This study was designed 1) by extrapolating the human therapeutic doses (gliclazide-80 mg; efavirenz-600 mg and nevirapine-200 mg) [21-23] based on body surface area which underscores the clinical relevance of the current investigation 2) to conduct pharmacodynamic and pharmacokinetic studies concurrently in a same group in order to establish a clear association between PD-PK to explore the possible mechanism of drug interaction 3) to determine glucose and insulin levels at regular time intervals up to 24 hours to evaluate an antidiabetic drug (gliclazide) activity in order to emphasize the clinical relevance considering the strong relation between glucose and insulin levels to regulate metabolic homeostasis [24] in diabetic patients 4) to conduct single- and multiple-dose PK/PD interaction studies in order to provide valuable information on the time course and magnitude of NNRTIs interaction with gliclazide with respect to clinical prospective.

Drug interactions are generally evaluated in animal models. Although animal models can never replace the need for comprehensive studies in human subjects, their use can provide important insights to understand and to evaluate the mechanism of potent drug interactions. It is worth noting that several findings have confirmed the functional similarity of CYP forms in rabbits and humans apart from convenience in serial blood sampling design suggesting that the rabbit is a valuable in vivo model for the assessment of drug interactions [5, 19, 25-28].

Gliclazide is known to produce hypoglycaemic/antihyperglycaemic activity by pancreatic (stimulating insulin secretion by blocking K+ channels in the pancreatic β cells) and extra pancreatic (increasing tissue uptake of glucose) mechanisms [11-14]. Gliclazide produced maximum blood glucose reduction, maximum insulin level and maximum serum concentration at 3 h representing the consistency between pharmacodynamic and pharmacokinetic results. Our study revealed the safety profile of efavirenz and nevirapine with respect to glucose-insulin homeostasis. These results are also consistent with the available literature [5, 19, 25, 28] and our former preliminary study [4]. But in combination, efavirenz significantly decreased the pharmacodynamic activity (hypoglycaemic effect and insulin levels) of gliclazide and it confirms the presence of potent interaction between efavirenz and gliclazide. These pharmacodynamic observations are in agreement with our pharmacokinetic findings in which significant decrease in serum concentration levels and alteration in pharmacokinetic parameters of gliclazide in the presence of efavirenz. Therefore, these experimental findings explicitly convince that there is a significant pharmacokinetic interaction between efavirenz and gliclazide which has resulted in decreased serum gliclazide levels and subsequently decreased pharmacodynamic activity.

The pharmacokinetic data clearly suggest that efavirenz has not altered the onset of action (Tmax) of gliclazide, but significantly decreased the overall plasma exposure (AUC and AUMC) and peak concentration (Cmax) of gliclazide indicating overall decreased availability of gliclazide. There might not be an interaction at absorption level since oral bioavailability of efavirenz in animals is 16% [29] while gliclazide is more than 50% [30, 31], further this is emphasized by no alteration in gliclazide Tmax. Gliclazide and efavirenz are highly protein bound drugs (gliclazide: 89%; efavirenz: 99.5-99.75%) [21, 22] and hence there is every possibility for displacement of gliclazide from the protein binding sites by efavirenz and may lead to increased free gliclazide concentration levels. But in fact, the volume of distribution (Vd) of gliclazide was not significantly altered and gliclazide concentration levels were decreased in presence of efavirenz which explicitly convince that the protein displacement is not involving in this interaction. Hence, the decreased gliclazide concentration levels in the presence of efavirenz might be either in metabolism or excretion process as suggested by increased clearance and Kel and decreased MRT and T1/2.

Efavirenz is converted to inactive metabolites by the CYP system, primarily by CYP2B6 and CYP3A4. In vitro and in vivo studies demonstrated that efavirenz is a potent inducer of CYP3A4 in a concentration- and time-dependent manner [22, 32, 33]. Clinical drug-drug interaction studies showed that efavirenz significantly induced CYP enzymes and decreased the concentration levels of several CYP3A4 [34, 35] substrates predominantly and, CYP2C9 and CYP2C19 substrates partly [36]. Enzyme induction has important clinical implications when enhanced drug metabolism results in lower drug concentrations, which leads to a suboptimal efficacious response or, even worse, the development of drug resistance. Enzyme induction can be due to (i) a drug affecting its own metabolism (autoinduction), (ii) co-medication(s) with induction capability, or (iii) both. Gliclazide is known to be metabolized by CYP2C9 primarily and partly by CYP3A4 [5, 25, 28]. Gliclazide is primarily eliminated via renal (60-70%) [21] while efavirenz is primarily eliminated through feces (16-61%) [22]. Based on this context, pharmacokinetic results such as increased CI and Kel and decreased MRT and T1/2 suggest that efavirenz causes time-dependent induction of gliclazide metabolism which in turn results induction of clearance and subsequently decreased gliclazide serum concentrations. This type of efavirenz induced metabolism and clearance was reported in other preclinical [29] and clinical studies [33, 34] also.
In this study, interestingly, the multiple dose treatment of efavirenz on gliclazide activity (pharmacodynamics, serum concentration level and pharmacokinetics) is relatively less compared to single dose treatment. As per the literature, administration of multiple doses of efavirenz results in decreased exposure in humans and animals, suggesting an autoinduction of efavirenz metabolism [29, 32, 33, 36, 37]. The plasma half-life of efavirenz in animals is approximately 0.8 to 1.9 h compared to more than 40 h in humans [38, 39]. In this study, as a part of multiple dose interaction, efavirenz was administered daily up to 9 days which was estimated to be enough time to achieve steady state of efavirenz considering its half life of 0.8 to 1.9 h in animals, representing the clinical relevance. So autoinduction of efavirenz might be the reason behind the less impact of multiple dose treatment of efavirenz on the activity of gliclazide in rabbits.

On other hand, minor and non-significant decrease in gliclazide activity was observed following single and multiple dose administration of nevirapine. Nevirapine is known to be an inducer of CYP3A4 and CYP2B6 [23] and thus leads to this minor and non-significant decreased gliclazide activity. These results are consistent with the literature and our former preliminary study. However, there was no significant interaction (either pharmacodynamic or pharmacokinetic) between nevirapine and gliclazide and thus proved to be a safe combination.

There are recognized potential limitations with this investigation. First, the preclinical results may not be correlated directly to human beings considering inter-species metabolic variations. Second, NNRTIs effects were evaluated in healthy animal models rather than diabetic and HIV-infected validated models. Third, the role of P-glycoprotein or other efflux membrane proteins in this interaction has to be determined in further study. Nevertheless, the consistency observed in the pharmacodynamics, serum concentration levels and pharmacokinetics in this study underscore the clinical relevance of the current investigation.

Conclusion

The results confirmed the presence of pharmacokinetic interaction of efavirenz with gliclazide at metabolic level by CYP3A4 induction and thus result in decrease in pharmacodynamic activity of gliclazide in normal rabbits. This combination needs dose adjustment and care should be taken when this combination is prescribed for the clinical benefit. The combination of nevirapine and gliclazide was proved to be safe.

Acknowledgements

The authors are thankful to M/s. Aurobindo Pharma Ltd, Hyderabad and M/s. Micro Labs, Bangalore for supplying gift samples of NNRTIs and gliclazide, respectively. The authors are grateful to Cytel Management, Pune for the kind help and support during the pharmacokinetic analysis.

Conflict of interests

The authors declare that they have no competing interests.

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