Journal of Applied Pharmaceutical Sciences and Research

Original Article

E-ISSN: 2581-5520

http://www.japsr.in

JAPSR, 2(3):14-22

INFLUENCE OF NEVIRAPINE ON THE PHARMACODYNAMICS AND PHARMACOKINETICS OF REPAGLINIDE IN RATS AND RABBITS

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ABSTRACT

Introduction: Management of HIV/AIDS is gradually expanding to include the chronic and metabolic complications and the adverse effects associated with its treatments like Type II diabetes mellitus. Repaglinide is a novel oral hypoglycemic agent chemically unrelated to sulphonylureas, metformin or acarbose used for the treatment of type II diabetes. Nevirapine is widely used non-nucleoside reverse transcriptase inhibitors for the treatment of HIV infection. **Objective:** The objective of this study was to examine the effect of oral administration of nevirapine on blood glucose and investigate their effect on the activity of repaglinide and to evaluate the safety and effectiveness of the combination. **Materials and Methods:** Studies in normal, diabetic rats and normal rabbits were conducted with oral doses of repaglinide, nevirapine and their combination. All the animals were fasted for 18 h prior to experimentation; during this period the animals were fed with water *ad libitum*. The blood samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, and 8hours in rats by retro orbital puncture and by marginal ear vein puncture in rabbits at different time intervals. Further, the samples were analyzed for glucose by glucose oxidase/peroxidase (GOD/POD) method. The rabbit blood samples were analyzed by HPLC for serum repaglinide concentration. The serum repaglinide levels and pharmacokinetic parameters of repaglinide were evaluated with multiple dose treatments of nevirapine in rabbits. **Result and Discussion:** Nevirapine alone have no significant effect on the blood glucose level in rats and rabbits. Repaglinide produced hypoglycemic and antihyperglycemic activity in normal and diabetic rats with peak activity at 2 h and hypoglycemic activity in normal rabbits at 1.5 h. In combination, nevirapine reduced the effect of repaglinide in rats and rabbits. The interaction was found to be significant at both pharmacodynamic as well as at pharmacokinetic levels. **Conclusion:** Thus, it can be concluded that the combination of nevirapine and repaglinide may need dose adjustment and care should be taken when the combination is prescribed for their clinical benefit in diabetic patients. However, further studies are warranted.

Keywords: Repaglinide, Nevirapine, Diabetes mellitus, Drug interaction

Article info:

Received: June 02, 2019

Revised: July 12, 2019

Published Online: October, 2019

DOI: https://doi.org/10.31069/japsr.v2i3.3

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INTRODUCTION

Any two drugs when administered together, the risk for drug interaction has been reported to be 6% [1] [2] increasing exponentially with the number of co administered drugs.[3] [4] Drug interactions are an important aspect of clinical drug application in patients receiving multiple drug regimens. In drug interactions, the combined effects of interacting drugs are greater or less than the arithmetic sum of their individual actions, usually due to pharmacokinetic or pharmacodynamic interference. The underlying mechanism of pharmacokinetic drug interactions is usually a change in the systemic clearance or distribution of one drug by another drug. In pharmacodynamic drug interactions the co-administered drugs have overlapping pharmacologic mechanisms or similar target systems. Drug interactions can lead to therapeutic failure (due to lack of effect) or drug overdose (exaggerated pharmacological response and/or drug toxicity), but drug interactions may also be modest and lack clinical significance. [5] [6] Of all adverse drug reactions, up to 20 to 30% are assumed to be caused by drug interactions. [7] [8] [9]

Diabetes is a metabolic disease that results from defects in the secretion or activity of insulin within a person's body. Diabetes has many potential health complications, including coronary heart disease, stroke, peripheral vascular disease, blindness, kidney disease, and lower-extremity amputation. Although diabetes is still more common in developed countries, it is rapidly increasing in developing countries. [10]

Repaglinide is a novel oral hypoglycemic agent which stimulates the release of insulin from pancreatic beta-cells by inhibition of potassium efflux resulting in closure of ATP regulated K^+ channels.[11] [12] This results in depolarization of the cell and subsequent opening of calcium channels, leading to influx of calcium into the cell which causes release of insulin.[11] The cytochrome P-450 enzyme system, specifically 2C8 and 3A4, have been shown to be involved in the N-dealkylation of repaglinide to an oxidized dicarboxylic acid (M2) and then further oxidation to

the aromatic amine (M1). Metabolites do not contribute to the glucose-lowering effect of repaglinide. [13]

Metabolic and endocrine abnormalities can also occur as a complication of therapy with antiretroviral drugs and chemotherapeutic agents used for the treatment or prevention of opportunistic infections.[14] [15] [16] [17] [18] [19] For instance, insulin resistance, dislipidemia, and fat redistribution have been reported in HIV-infected patients, particularly in those treated with effective antiretroviral drugs.[14] [15] [19] [20] Abnormalities of glucose homeostasis, including insulin resistance and related metabolic abnormalities (hypertriglyceridemia, low high-density lipoprotein [HDL] cholesterol level, or an atherogenic lipid profile) occur frequently in association with changes in body composition among HIV-infected patients receiving HAART (highly active antiretroviral therapy.) [21] [22] [23]

Nevirapine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) of HIV-1, which interrupts the reverse transcription of viral RNA to DNA, a crucial step for HIV replication, by a mechanism of action different from nucleoside analogues. Nevirapine binds directly to the HIV-1 RT enzyme and blocks the RNA-dependent and DNA-dependent DNA polymerase activities by causing a disruption of the enzyme catalytic site. Nevirapine is an inducer of hepatic cytochrome P450 (CYP) metabolic enzymes 3A4 and 2B6. [24] As a result, drugs that are metabolized by these enzyme systems may have lower than expected plasma levels when co-administered with nevirapine.

Diabetes mellitus is not only a chronic illness associated with substantial morbidity and mortality but also a major public health problem of epidemic proportions. HIV/AIDS patients, therefore, frequently present with diabetes and metabolic complaints. As treatment of HIV develops, and access to therapy improves, the incidence of HIV-associated diabetes is bound to grow. People undergoing HAART therapy have 5-fold increased risk of developing diabetes. [25] The study of safety of drug combination (Repaglinide + Nevirapine) is required since both are used for the treatment of chronic diabetes associated with HIV-infection; and both are metabolized by same enzyme system. As the need for monitoring drug therapy in poly pharmacy is to gain better therapeutic effect with lower rate of risk, the combination of nevirapine and repaglinide may need dose adjustment. However, further studies are warranted for the above combination for the clinical benefit in diabetic patients associated with HIV/AIDS.

MATERIAL AND METHODS

Drugs and Chemicals

Repaglinide and Nevirapine are the gift samples from Strides Arcrolabs (Bangalore, India) and Hetero Drugs (Hyderabad, India) respectively. Gliclazide (IS), pure sample was gifted by Sun pharmaceuticals (Mumbai, India). Alloxan monohydrate was purchased from LOBA chemicals Pvt. Ltd, Mumbai, India. Blood glucose kits (Auto span) manufactured by Span diagnostics Ltd (Surat, India) were purchased from a local pharmacy. Acetonitrile and methanol (HPLC grade) were obtained from Qualigens chemicals (Mumbai, India). TBHS (AR grade) were purchased from SD fine chemicals (Mumbai, India). Triple distilled water prepared in the laboratory was used during the entire HPLC procedure. All other reagents/chemicals used were of analytical grade.

Experimental Animals

Albino wistar rats of either sex of 6 to 7 weeks of age, weighing between 150- 260 g and normal albino rabbits of either sex of 3 months of age, weighing between 1.35-1.75 kg were obtained from Ghosh Enterprises (Kolkata, India) were maintained at a constant temperatureof 26 ± 2°C and humidity 30-40% with 12 h light/dark cycle, throughout the experiments. The animals were fed with standard cereal grain base diet animal pellet for rat (12mm diameter pellets) and rabbit (4 mm diameter pellet, 4 - 10mm long)manufactured by Rayan's Biotechnologies Pvt. Ltd (Hyderabad, India) and sterile water was given *ad libitum*. The rats were housed in polypropylene cages and in clean rabbit cages in an air-conditioned animal house. 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. 926/ab/06/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).

Experimental Study Design

Dose and Drug administration: In clinical practice, Repaglinide and Nevirapinein therapeutic doses are administered orally. Hence, human therapeutic doses extrapolated to rats and rabbits based on the body surface area were used and administered. [26] The therapeutic dose (TD) of repaglinide was selected and used for studying the interaction in all sets of experiment based on the influence of dose-effect relationship of repaglinide on blood glucose with 1/2 TD, TD and 2TD in normal rats (Table 1 and Figure 1).

Table 1: Percent blood glucose reduction with different doses of repaglinide (1/2TD, TD, 2TD) in normal rats

Time (in Hr)	½ TD	TD	2 TD
0.5	9.91±0.83	22.64±2.27	25.17±1.55
1	25.96±1.94	40.45±2.31	44.79±1.50
1.5	38.06±2.05	52.93±2.31	62.425±1.21
2	44.14±1.82	56.00±1.78	42.63±2.51
3	32.55±1.30	43.16±1.48	32.34±1.81
4	21.84±1.10	31.09±2.14	22.21±0.87
6	12.97±1.01	24.64±1.30	12.85±0.89
8	5.22±0.59	16.33±1.82	5.85±0.54

***TD** = Therapeutic dose

Repaglinide and nevirapine suspension was prepared by dispersing it in a 5% acacia and made up to volume with distilled water. The study consists of two phases:

Phase-1: Pharmacodynamic interaction study between Nevirapine and Repaglinidein normal and diabetic rats.

Phase-2: Pharmacodynamic and Pharmacokinetic interaction study between Nevirapine and Repaglinide in normal rabbits.

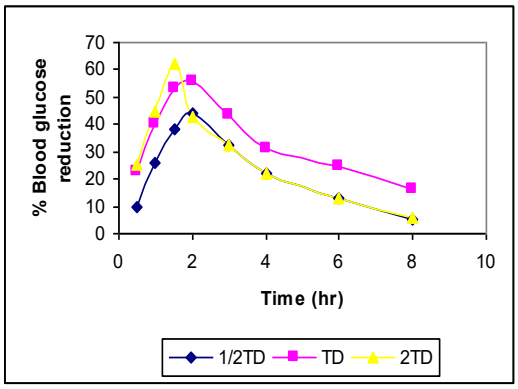


Figure 1: Percent blood glucose reduction with different doses of repaglinide in normal rats ***TD** = Therapeutic dose

Pharmacodynamic study in normal rats

Adult wistar rats were used in the study. The animals were fasted for a period of 18 h prior to the experimentation and water was supplied *ad libitum.*[27] A group of six rats was administered with TD of repaglinide, orally. The same group was administered with interacting drug nevirapine (TD), orally. One-week washout period was maintained between treatments.

After the above study the same group was continued with the daily treatment of interacting drug (nevirapine) for two week with regular feeding. Later on, 15th day after 18 h fasting they were again given the combined treatment i.e. nevirapine (TD) was administered, 30 minutes prior to the administration of repaglinide (TD). The blood samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6 and 8hr by retro orbital puncture method. The samples were centrifuged (Remi Centrifuge) for serum separation at 4,000 rpm for 10 min. The serum samples were analyzed for glucose by GOD/POD method. [28]

Pharmacodynamic study in diabetic rats

Induction of Diabetes: Albino rats of either sex were fasted over night before injection with alloxan. Experimental diabetes in rats was induced by injecting alloxan monohydrate intraperitonially at a dose of 150 mg/kg in ice-cold normal saline. After 72 h, samples were collected by retro orbital puncture from all surviving rats, and the serum analyzed for glucose levels. Rats with blood glucose levels of 200 mg/dL and above were considered as diabetic and selected for the study. [29] [30] [31] The same treatment as described in the study in normal rats was performed with a group of six alloxan-induced diabetic rats.

Pharmacodynamic and Pharmacokinetic study in normal rabbits

The rabbits were fasted for 18 h prior to the experiment with water *ad libitum*. During the experiment water was also withdrawn. A group of six rabbits was administered with repaglinide (TD), orally. The same group was administered with interacting drug nevirapine (TD), orally. After this study, the same group was continued with the daily treatment of interacting drug nevirapine for the next one week with regular feeding. Later on, 15th day after 18 h fasting they were again given the combined treatment i.e. nevirapine (TD) was administered, 30 minutes prior to the administration of repaglinide (TD). One-week washout period was maintained between treatments. Blood samples were withdrawn from the marginal ear vein of each rabbit at 0, 0.5, 1, 1.5, 2, 3, 4, 6 and 8hr and analyzed for glucose by GOD/POD and for serum repaglinide by high performance liquid chromatography (HPLC).

Serum extraction

To 200 µl of serum, 100 µl of gliclazide (internal standard) and 200 µl working solution were added, after vortex mixing for 10s, 500µl of acetonitrile was added and the mixture was shaken vigorously for 1 min. The mixture was then centrifuged for 5 min at 8000 rpm. Aliquot of 800 µl the upper organic layer containing repaglinide and IS was transferred to a clean test tube. Further 25 µl aliquot was injected onto the HPLC column. The eluent was detected by UV detector at 230 nm, and the data was acquired, stored and analyzed with the software Class-VP series version 6.14SP1 (Shimadzu).

Analytical Method

Instrument: A gradient High Pressure Liquid Chromatography (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps, variable wavelength programmable UV/VIS Detector SPD-10A VP, (Shimadzu), SCL-10A VP system controller (Shimadzu) and RP C-18 column (LiChroCART,250 mm x 4 mm I.D.; particle size 5 µm; YMC Inc., USA) was used. The HPLC system was equipped with the software "Class-VP series version 6.14SP1 (Shimadzu).

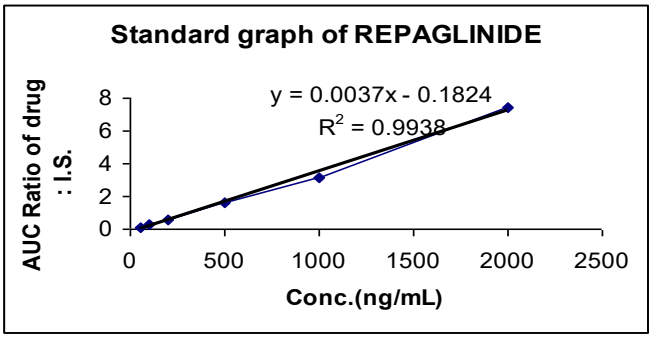
Chromatographic conditions: The mobile phase consisted of acetonitrile, methanol (HPLC grade; supplied by M/s. Qualigens, Mumbai, India) and 10mM TBHS (Tetrabutylammonium hydrogensulfate) in triple distilled water. The mobile phase components were filtered before use through 0.45µm membrane filter and pumped in the ratio of 30:30:40 {Acetonitrile:methanol:TBHS (10mM)} from the solvent reservoirs.

The flow rate of the mobile phase was maintained at 1.0 ml/min which yield column back pressure of 140-150 kgf and the temperature was maintained at 4-35^{0}C. The elute was monitored at 230 nm wavelength; sensitivity was set at 0.0001 to 2.56 AU/FS (Absorbance Units Full Scale).

Standard solutions: Primary stock solution of 1 mg/ml of repaglinide and IS (Internal Standard) were prepared in mobile phase and stored. Appropriate dilutions of repaglinide were made in mobile phase to produce concentrations of 10, 1 µg/ml and 500, 200, 100, 50 ng/ml. These dilutions were used to spike serum in the preparation of calibration curves. The IS working stock solution (20µg/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 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 (50, 100,200, 500 and 1000 ng/ml). A linear relationship was found between the amounts of repaglinide added to the serum samples and the corresponding ratios of AUC (area under curve) of repaglinide to IS (Table 5). The graphical representation of the repaglinide concentration vs. the corresponding AUC ratio value is shown in the Figure 5.

Table 5: Construction of standard graph

CONC. OF REPAGLINIDE (ng/ml)	RATIO OF REPAGLINIDE/ INTERNAL STANDARD			
	SET - I	SET - II	SET - III	AVERAGE±SEM
50	0.1423	0.1466	0.1359	0.1416 ± 0.0025
100	0.2850	0.2952	0.2990	0.2930 ± 0.0034
200	0.5618	0.6164	0.6035	0.5949 ± 0.0134
500	1.7593	1.5692	1.4562	1.5949 ± 0.0722
1000	2.9698	3.2365	3.1968	3.1343 ± 0.0678



Where Y= ratio of AUC of repaglinide to IS, X= amount of repaglinide added to blood samples in ng.
From this the repaglinide concentration per ml was calculated.

Figure 5: Standard Curve of Repaglinide

Data Analysis

The hypoglycemic activity of repaglinide at anytime, **t** in rats and rabbits was calculated as the percentage blood glucose change at that time with respect to initial blood glucose level according to the formula given below: [32]

Percentage blood glucose reduction at time **t**= [(a-b)/a] ×100

a – Initial blood glucose level

b – Blood glucose level at time t.

The various pharmacokinetic parameters of repaglinide were analyzed by using following equations. The parameters obtained thereby are:

- C_0 (blood concentration at time 0 hr.), Kel (elimination rate constant) & Ka (absorption rate constant) were used in calculating the other pharmacokinetic parameters.
- The elimination half life ($t_{1/2}$) was calculated using the relationship t1/2 = 0.693/Kel.
- Volume of distribution was calculated using the relationship, Vd = F X Dose/C_0.
- The area under the blood repaglinide v/s time curve, from 0 to 24 hrs was calculated by the trapezoidal rule.
- Total body clearance was determined by C_L = F x Dose/ AUC 0-α
- The parameter T_{max} was calculated using the relationship, T_{max}. = [2.303 X log (Ka – Kel)] / Ka – Kel
- Cmax. was calculated using the formula; Cmax. = (F X Dose / Vd) e–Kel x T_{max}

Statistical Analysis

The significance of the observed differences in pharmacokinetic parameters and percent blood glucose reduction of repaglinide between the drug treated and control rats (percent glucose reduction in both normal and diabetic rats) and rabbits (percent glucose reduction and pharmacokinetic parameters in normal rabbits) were assessed by student's paired t-test. Value of $P < 0.05$ is considered for statistical significance to find out the difference between the parameters of comparison. The results are expressed as mean ± SEM. Statistical significance was set accordingly.

RESULTS

Pharmacodynamic interaction study between nevirapine and repaglinide

Table 2: Percent Blood glucose reduction with repaglinide (TD) with and without nevirapine (TD) in normal rats

Time	REP	NVR	REP + NVR
0.5	22.64±2.27	3.78±0.30	*13.16±1.06
1	40.45±2.31	8.07±0.92	*31.76±2.31
1.5	52.93±2.31	10.12±0.64	*44.10±2.26
2	56.00±1.78	10.44±0.73	*48.81±1.94
3	43.16±1.48	12.13±0.84	*34.08±1.64
4	31.09±2.14	13.55±1.34	*20.40±2.61
6	24.64±1.30	13.60±1.37	*13.53±0.62
8	16.33±1.82	15.80±1.06	*5.79±1.1

* **TD** = Therapeutic dose, **REP** = Repaglinide, **NVR** = Nevirapine

Table 3: Percent blood glucose reduction with REPAGLINIDE (TD) in combination with NEVIRAPINE (TD) in diabetic rats

Time	REP	NVR	REP + NVR
0.5	25.79±4.01	4.87±0.49	*17.27±3.27
1	35.78±3.40	9.25±1.13	*29.22±3.07
1.5	52.86±3.50	11.06±0.86	*45.25±3.30
2	56.80±2.21	11.57±0.74	*49.90±2.46
3	41.04±3.03	13.62±0.32	*34.37±2.86
4	27.59±1.70	14.48±1.04	*22.73±3.61
6	24.80±4.73	16.07±0.99	*17.33±4.38
8	15.42±1.40	16.76±1.08	*6.911±0.85

* **TD** = Therapeutic dose, **REP** = Repaglinide, **NVR** = Nevirapine

Table 4: Percent Blood glucose reduction with repaglinide (TD) with and without nevirapine (TD) in normal rabbits (n=6).

Time	REP	NVR	REP + NVR
0.5	13.88±0.53	2.04±0.28	*10.24±0.67
1	30.48±2.33	5.95±1.02	*26.2±1.45
1.5	44.73±3.84	6.76±0.46	*40.03±1.61
2	47.22±2.78	7.90±0.82	*43.63±1.86
3	36.05±2.42	9.80±1.02	*31.55±1.10
4	26.09±2.07	13.21±0.73	*22.24±0.52
6	16.49±1.13	14.80±0.35	*13.39±0.53
8	8.03±0.92	14.96±0.45	*7.02±0.46

* **TD** = Therapeutic dose, **REP** = Repaglinide, **NVR** = Nevirapine

Repaglinide produced hypoglycemic activity with maximum reduction of 56.00 ± 1.78% at 2 h in normal rats (Table 2). Repaglinide produced antihyperglycemic activity with maximum reduction of 56.80 ± 2.21% at 2 h in diabetic rats (Table 3). Repaglinide produced hypoglycemic activity with maximum reduction of 47.22 ± 2.78% at 2 h in normal rabbits (Table 4).

Nevirapine alone has not produced any significant effect on the blood glucose level of rats (normal and diabetic) and rabbits (Tables 2, 3, 4 respectively). In combination the multiple dose treatment of nevirapine has reduced the repaglinide activity in rats (normal and diabetic) and rabbits. (Tables 2, 3 and 4 respectively) The percent blood glucose reduction with repaglinide (TD) with and without nevirapine (TD) in rats (normal & diabetic) and in normal rabbits is shown in Figure 2, 3 and 4.

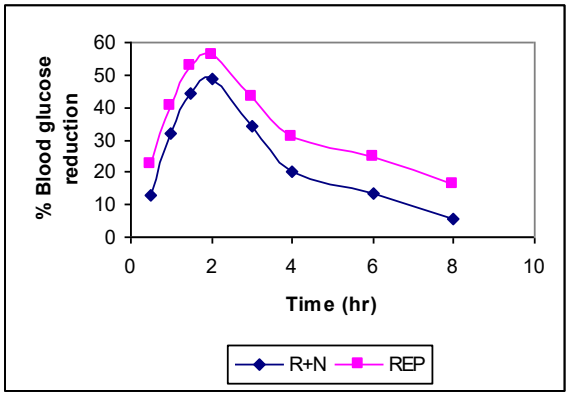


Figure 2: Percent Blood glucose reduction with repaglinide (TD) with and without nevirapine (TD) in normal rats.

***REP** = Repaglinide, **R+N** = Repaglinide + Nevirapine

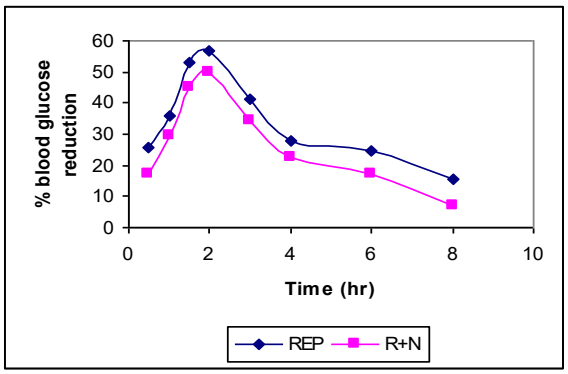


Figure 3: Percent Blood glucose reduction with repaglinide (TD) with and without nevirapine (TD) in diabetic rats. * **REP** = Repaglinide, **R+N** = Repaglinide+Nevirapine

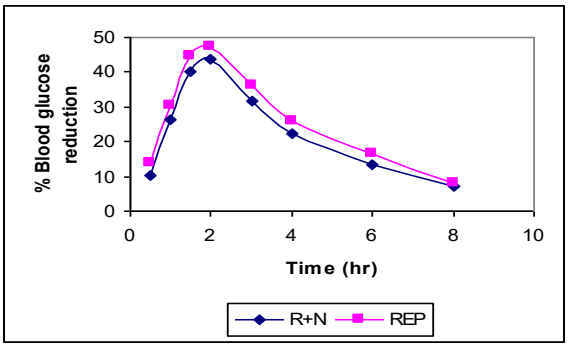


Figure 4: Percent Blood glucose reduction with repaglinide (TD) with and without nevirapine (TD) in normal rabbits

* **REP** = Repaglinide, **R+N** = Repaglinide+Nevirapine

Pharmacokinetic interaction studies (normal rabbits)

In the case of pharmacokinetic interaction study, the serum concentrations of repaglinide alone, and in the case of interaction studies with nevirapine, were determined (Table 6). The mean values of different pharmacokinetic parameters of repaglinide (TD) before and after nevirapine (TD) treatment were computed and subjected to statistical interference (Table 7). The AUC of repaglinide were decreased in combination group from 2201.86 ng/mL *h to 1001.74 ng/ml*h, compared to repaglinide treated group indicating less availability of repaglinide in the presence of nevirapine. The Vd was slightly changed from 26.58 to 26.73L. The Cmax was changed from 282.11 to 182.30 ng/ml. The T_{max} remained unchanged. The absorption half life ($t^{1/2}_{(a)}$) and absorption rate constant (Ka) remained unchanged, indicating that the absorption was not altered. The elimination half life ($t^{1/2}$), elimination rate constant (Kel) and clearance (C_L) were altered of repaglinide in the presence of nevirapine. Std. Chromatogram corresponding to Repaglinide (1µg) and IS (2µg) is shown in Figure 6. Chromatograms corresponding to Repaglinide alone and along with Nevirapine in normal rabbits are shown in Figure 7 and 8 respectively.

Table 6: Serum repaglinide levels (ng/ml) with and without nevirapine (TD) in normal rabbits

Time	REP	REP + NVR
0.5	203.25 ± 1.75	139.07 ± 2.84
1	281.26 ± 4.06	181.46 ± 1.51
1.5	338.58 ± 1.95	210.8 ± 2.11
2	242.88 ±1.07	189.46 ± 2.07
3	220.14 ± 2.21	152.33 ± 1.21
4	176.02 ± 1.04	133.16 ± 1.34
6	162.49 ± 1.01	118.20 ± 1.29
8	132.37 ± 1.52	100.18 ± 0.93

* **REP** = Repaglinide, **REP + NVR** = Repaglinide + Nevirapine

Table 7: Significance of mean pharmacokinetic parameters of repaglinide (TD) with and without nevirapine (TD) in normal rabbits

PHARMACOKINETIC PARAMETER	REP	REP + NVR	SIGNIFICANT AT P<0.05
AUC 0-t(24hrs) (ng/ml/h)	2201.86	1001.74	*Significant
Kel (h^{-1})	0.51	0.82	*Significant
Ka (h^{-1})	1.45	1.26	Not significant
t(1/2 (h)	1.38	0.89	*Significant
Vd SS (L)	26.58	26.73	Not significant
Cmax (ng/ml)	282.11	182.30	*significant
Tmax(h)	1.15	1.25	Not significant
t1/2(a) (h)	0.50	0.56	Not significant
CL (L/h)	34.96	76.19	*Significant

* **REP** = Repaglinide, **REP + NVR** = Repaglinide + Nevirapine

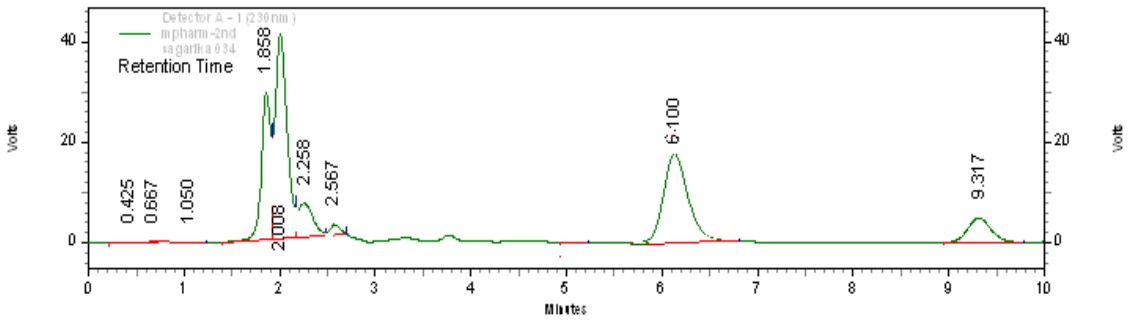


Figure 6: Std. Chromatograms corresponding to Repaglinide (1µg) and IS (2µg)

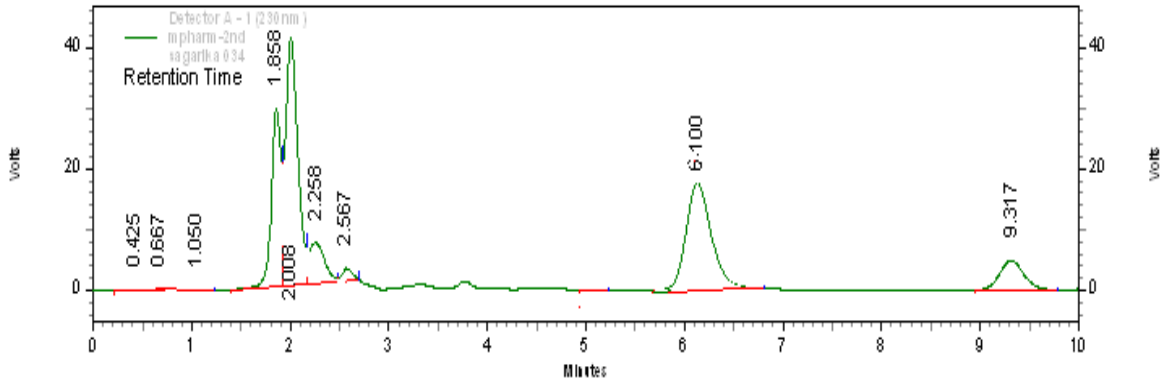


Figure 7: Chromatogram corresponding to Repaglinide in normal rabbits

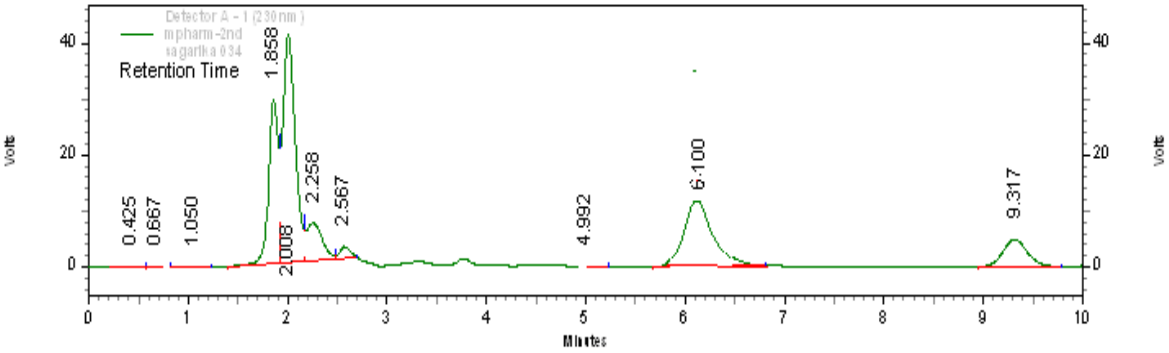


Figure 8: Chromatogram corresponding to Repaglinide along with Nevirapine in normal rabbits

DISCUSSION

Type II diabetes mellitus (DM) is a common disorder associated with high morbidity and mortality.[33] Among the antidiabetic drugs repaglinide is widely used drug because of its high potency, prolonged action and lower incidence of side effects. Nevirapine is known to induce liver microsomal iso-enzyme CYP 450-3A4 and the same iso-enzyme is responsible for the metabolism of repaglinide.[34] [35] Basing on the above reasons, there is every possibility for interaction on concomitant administration of nevirapine and repaglinide. So, the present study was planned to find out the influence of nevirapine on pharmacodynamics and pharmacokinetics of repaglinide in rats (rodent) and rabbits (non rodent). Since rat and rabbit are two dissimilar species, if the interaction occurs in both the species, then there is more probability of its occurrence in humans also.

A preliminary study was conducted in normal rats. Dose dependent response was observed with ½ TD, TD & 2TD of repaglinide in normal rats, therapeutic dose of repaglinide and therapeutic dose of nevirapine (TD) were selected for the interaction study. Nevirapine*per se* did not alter the normal blood glucose level, but when given in combination with the selected dose (TD) of repaglinide, significantly decreased the blood glucose reduction produced by repaglinide. The decrease in repaglinide induced hypoglycemic effect by nevirapine was statistically significant at all the time intervals of the study. To validate the existence of the interaction between nevirapine and repaglinide in diabetic condition, the selected doses of repaglinide (TD) and nevirapine(TD) were administered to alloxan induced diabetic rats. Influence of nevirapine on antihyperglycemic effect of repaglinide (TD) in diabetic rats was found to be similar to that of the normal rats. The decrease in repaglinide induced antihyperglycemic effect by nevirapine was statistically significant at all the time intervals of the study.

To understand the mechanisms of the interaction of nevirapine with repaglinide, pharmacokinetic and pharmacodynamic studies were carried in normal rabbits, since the model was suitable for getting sufficient quantity of blood for adequate number of samples. Rabbit is the animal model for insulin bioassay and insulin release is mainly responsible for repaglinide blood glucose lowering activity. [36]

Nevirapine*per se* has negligible effect on blood glucose level in normal rabbits also. The decrease in hypoglycemic effect of repaglinide by nevirapine was statistically significant at all the time intervals of study and statistical difference was observed in

$AUC_{o\text{-}24}$, t1/2, Kel, Cmax., and C_L of repaglinide with and without combination of nevirapine. The decrease in AUC indicates decreased availability of repaglinide in presence of nevirapine. The decreased availability can't be due to improved absorption since absorption rate or absorption half life was not altered.

Literature reveals that nevirapine induces hepatic CYP450-3A4 iso-enzyme. It also reveals that the repaglinide is also metabolized by the same iso-enzyme. Hence it appears that nevirapine induces the hepatic metabolism of repaglinide and decreases systemic availability of repaglinide. The decreased availability of repaglinide might be responsible for its decreased hypoglycemic activity in the presence of interacting drug.

Hence the induction of repaglinide metabolism by nevirapine with consequent decrease in its activity appears to be main mechanism for the observed pharmacodynamic and pharmacokinetic changes. Since the interaction was seen in two dissimilar species of animals (rat- rodent; rabbit- non rodent) it is most likely to occur in humans also. Finally, since interaction of inducers of CYP3A4 (nevirapine) with repaglinide are likely to be clinically significant, and blood glucose concentrations should be closely monitored as these drugs are administered chronically in diabetes associated with HIV infection, the health care professionals should caution the diabetic patients about this drug interaction and advise for adequate dosage adjustment. Health care professionals should caution diabetic patients when such combination is prescribed.

CONCLUSION

Results of the present study indicate the existence of interaction between nevirapine and repaglinide in two dissimilar species i.e. rat (normal & diabetic) and rabbit (normal). Since it occurred in two dissimilar species, it is likely to occur in humans also. Further, to find out the existence of such interaction in humans, clinical studies in human diabetic patients are required. Such studies will confirm the interaction in human. Studies are required to establish the safety of drug combination, since both the drugs are used daily and its influence on the long-term treatment of chronic diabetes associated with HIV infection.

ACKNOWLEDGEMENT

Repaglinide and Nevirapine are the gift samples from Strides Arcrolabs (Bangalore, India) and Hetero Drugs (Hyderabad, India) respectively. Gliclazide (IS), pure sample was gifted by Sun pharmaceuticals (Mumbai, India). We thank all of them for providing gift sample that has greatly supported the research work.

CONFLICT OF INTEREST

No conflict of interest.

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