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Original scientific paper

Evaluation of pharmacokinetic and pharmacodynamic interaction between repaglinide and atazanavir in healthy, diabetic and hepatic impaired rats: possible inhibition of CYP3A, OATP, and P-glycoprotein transporters

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Abstract

The metabolic syndrome in HIV infected patients is particularly associated with the use protease inhibitors. Atazanavir is an inhibitor of the cytochrome P 450 (CYP) system, in particular CYP3A4 and CYP2C9 which can affect the metabolism of several drugs. To treat metabolic syndrome in HIV patients repaglinide is used and it is a short acting insulin secretagogues undergoing metabolism with CYP 3A4 and CYP 2C8 enzyme system. The purpose of this study was to assess the possible pharmacokinetic and pharmacodynamic drug interaction of repaglinide and atazanavir in healthy, diabetic and impaired hepatic function rats. Human oral therapeutic doses of atazanavir and repaglinide were extrapolated to rats based on the body surface area. The pharmacokinetic parameters and blood glucose concentrations of repaglinide were determined after oral administration of repaglinide alone (0.5 mg/kg) and in the presence of atazanavir (36 mg/kg) in normal, diabetic and hepatic impaired rats. The pharmacokinetics (PK) and blood glucose concentrations of repaglinide were significantly altered in the presence of atazanavir. The peak plasma concentration (C_{max}), area under the plasma concentration time profile (AUC) and elimination half-life of repaglinide were significantly (P<0.0001) increased. The repaglinide clearance (CL) was significantly (P<0.0001) decreased in the presence of atazanavir treatment. In the presence of atazanavir, repaglinide hypoglycaemic activity was increased significantly (P<0.0001) when compared with the repaglinide control group. The present study demonstrated the significant difference in the PK/PD changes due to the enhanced bioavailability and decreased total body clearance of repaglinide may be due to the inhibition of the CYP P450 metabolic system, OATP and P-gp transporters by atazanavir.

Keywords

AIDS; Drug drug interaction; hypoglycaemic activity; Liver dysfunction; Peak plasma concentration C_{max}; Area under the curve AUC; Clearance; Elimination half life.

Introduction

The prevalence and incidence of metabolic syndrome, particularly diabetes mellitus, was increasing in HIV patients. Treatment of HIV-infected patients with HIV-1 protease inhibitors (PIs) as part of highly active

antiretroviral therapy (HAART) has contributed considerable reductions in HIV viral load and increased in CD_4 lymphocyte numbers, thereby slowing disease progression and improving patient survival. However, despite this clinical success, it is recognized that PI-based therapy is correlated with a number of significant metabolic complications, including lipodystrophy, hyperlipidaemia, and insulin resistance. Around 80 % of patients who receive PIs develop insulin resistance leading to diabetes mellitus [1]. Drugs are primarily eliminated from the body by metabolism and excretion. In these two biologic processes, the liver plays a vital role in the metabolism of parent drugs and formation of metabolites prior to their excretion by the kidneys. The overall capacity of the liver to carry out its metabolic role is primarily dependent on three factors: activity of the metabolizing enzymes within the smooth endoplasmic reticulum and the cytosol of the hepatocytes; the degree of protein binding in the blood, which affects the amount of unbound drugs available for uptake into the hepatocytes; and liver blood flow, which delivers drugs to the hepatocytes via the portal vein for orally administered drugs and via the systemic circulation for all administered drugs. In hepatic impairment, patient-specific factors that affect enzymatic activity, protein binding, or liver blood flow would potentially result in significant alterations in drug disposition and therapeutic response. An understanding of the pharmacokinetic basis of hepatic drug elimination is helpful to conceptualize and quantify altered drug disposition in patients with liver dysfunction [2].

In hepatic dysfunction, the disruption of the liver vascular architecture may lead to increased blood flow resistance, which limits blood flow through the liver and causes portal vein pressure to rise. As a consequence, the formation of portocaval shunts may occur that allow the drug to bypass the first pass in the liver, which increase the systemic bioavailability of drugs. Chronic hepatic disease causes damage to hepatocytes; this in turn may cause a decreased intrinsic clearance of the drug metabolizing liver enzymes. Different CYP may be affected differently by the hepatocytes damage. In addition, the damage may also be different in the different regions of the liver. Cholestasis will impair the elimination of drugs that are excreted in the bile. Liver dysfunction causes a decrease of albumin in serum, which implies variation of the binding of drugs to the circulating proteins and can potentially affect the distribution volume of certain drugs [3].

Pharmacotherapy in patients with impaired hepatic function is an important and difficult aspect of medical practice. The elimination of many drugs takes place by inactive, active, or toxic metabolites may be significantly altered by hepatic dysfunction and dosage must be evaluated carefully. The presence of liver dysfunction that leads to increased plasma drug or metabolite concentrations may increase the prospect of toxicity which results in more complex drug-drug interaction. Hence, the preferred approach to prescribing medications for hepatic impaired patients is to select drugs that have predictable pharmacokinetic properties, are minimally affected by hepatic impairment and have a low potential for drug interactions with other drugs [4]. Safe pharmacological treatment of these complications requires an understanding of the drug-drug interactions between antiretroviral drugs and the drugs used in the treatment of diabetes [5].

Repaglinide is short acting anti diabetic drug used to normalize postprandial glucose concentrations in patients with type II diabetes mellitus [6]. Repaglinide is a substrate of the influx transporter OATP1B1 and metabolized mainly by CYP3A4 and CYP2C8. Pharmacokinetic drug interactions were observed in repaglinide with drugs which are inhibitors of CYP3A4, CYP2C8 and OATP1B1. Recently, a number of DDIs caused by the inhibition and induction of drug transporters have also been reported. Among these transporters, OATP1B1 is responsible for the influx of many therapeutic drugs in the liver; there have been a large number of reports on DDI caused by inhibition and induction of this transporter [7-8]. Protease

inhibitors are the potent inhibitors of CYP 3A4 and OATP transporters. Several DDIs were noticed when protease inhibitors (PIs) co-administered with drugs metabolized by CYP 3A4. Repaglinide also has an affinity for P-gp and it can contribute significantly to potential drug-drug interactions with other P-gp substrates or inhibitors, hence the co-administration of repaglinide with the known P-gp inhibitor, cyclosporine A resulted in a significant increase in the plasma concentrations of repaglinide in human [9]. The protease inhibitors (PIs) are also potent mechanism based inhibitors, of which atazanavir is most potent substrate and potent inhibitor of the cytochrome P450 (CYP) system, in particular CYP3A4 and CYP2C9 and affect the metabolism of several drugs [10].

Atazanavir is an azapeptide HIV-I protease inhibitor approved by FDA for the combination of HIV-I infection. Atazanavir is metabolized by CYP3A4, as a substrate; however atazanavir interacts with drug transporter proteins. The study by Mothanje Barabara Lucia *et al* indicated that Atazanavir is a possible inhibitor for P-glycoprotein and competitively inhibits their efflux activity in a dose dependent manner [11].

In the present study, we hypothesize that the repaglinide elimination would be effected by OATP and P-gp transporters, which makes available for the metabolism by CYP3A4. Atazanavir can inhibit CYP 3A4, CYP2C9 enzymes and it is OATP, P-gp inhibitor. Hence, the inhibition of transporter mediated hepatic uptake and metabolism may change the pharmacokinetics of repaglinide. However, there seem to be no published studies reported so far, so in the current study we have evaluated the influence of atazanavir on the pharmacokinetics and pharmacodyamics of repaglinide in normal, diabetic and hepatic impaired rats.

Experimental

Drugs and chemicals

Repaglinide a gift sample obtained from Dr. Reddy's Lab (Hyderabad, India) and atazanavir from Aurobindo Labs (Hyderabad, India). Alloxan monohydrate was purchased from Sigma Aldrich, Bangalore (Bangalore, India). Glucose kits purchased from Agappe diagnostics, (Mumbai, India). Acetonitrile HPLC grade and formic acid were obtained from Merck Chemicals (Mumbai, India). HPLC system: Agilent Technologies (California, USA) and MS/MS API-3200 mass spectrometer Sciex technologies (Foster City, CA, USA), HPLC column: Hypersil GOLD C18, 5µ column 4.6 * 100 mm internal diameter Thermo scientific, (North America). Vortex mixer and centrifuge were obtained from Remi laboratory instruments (Mumbai, India). Hematocrit heparinized rat bleeding capillaries was purchased from Top-Tech lab equipments Pvt Ltd (Hyderabad).

Pharmacokinetics and Pharmacodynamic interaction study in normal rats

Male albino Wistar rats weighing 200-250 gm were obtained from the Mahaveera enterprises (Hyderabad, India). They were housed under standard conditions and were maintained under a 12 hour light/dark cycle in the laboratory animal recourses facilities, Creative Educational Society's college of pharmacy, Kurnool, India. Rats were fasted overnight before dosing and until 4 hours after dosing. Water was allowed ad libitum during the fasting period. The 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 the committee for the purpose of control and supervision of experiments and animals (CPCSEA). Rats were divided into 5 groups (n=6, each):

Group-1: Repaglinide oral control group administered 0.5 mg/kg [12],
Group-2: Atazanavir oral control group administered 36 mg/kg [13],

Group-3: Atazanavir (36 mg/kg) administered, followed by repaglinide (0.5 mg/kg) to normal rats,
Group-4: Atazanavir (36 mg/kg) administered, followed by repaglinide (0.5 mg/kg) to diabetic induced rats,
Group-5: Atazanavir (36 mg/kg) administered, followed by repaglinide (0.5 mg/kg) to a hepatic impaired rats.

Required amount of drugs (repaglinide and atazanavir) were weighed and placed in two separate motor's triturated with pestle, to this required amount of tween 80 was added as a wetting agent and triturated well until the whole compound was wet, then 0.5 % of methyl cellulose was added in gravimetric dilution method and triturated uniformly. This suspension was administered to the respective group of animals through oral gavage [14]. Blood samples collected retro-orbital puncture at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 h [15]. Plasma was separated by centrifugation using Remi research centrifuge at 4000 rpm for 10-20 min. Blood glucose levels were determined using Glucose oxidase peroxidase (GOD-POD) method by measuring optical density spectrophotometrically at 510 nm [16] and the remaining samples were stored in vials at -80 °C until LC/MSMS analysis.

Pharmacokinetics and pharmacodynamic interaction study in diabetic rats

Diabetes was induced in rats by the administration of alloxan monohydrate in ice cold normal saline, in two doses, that is 100mg and 50 mg/kg body wt intraperitoneally for two consecutive days [17]. After 72 h, the samples were collected from rats by retro-orbital puncture of all surviving animals and the plasma was analyzed for glucose levels. Rats with blood glucose levels of 200 mg/dl and above were considered as diabetic and selected for the study. Similarly a separate set of one group (n=6) was used for the pharmacodynamic interaction study with pretreated atazanavir (36 mg/kg) followed by 0.5 mg/kg repaglinide in diabetic rats.

Pharmacokinetics and Pharmacodynamic interaction study in hepatic impaired rats

Hepatic impairment was induced in rats by administration of carbon tetrachloride in olive oil (2ml/kg) intraperitoneally for one day [18]. After 24 h, blood samples were collected from rats by retro-orbital puncture of all surviving animals and the serum was analysed for total bilirubin (>2mg/dl), alanine transaminase (>150 mg/dl), aspartate transaminase (>200mg/dl) and albumin (<3 mg/dl) were selected for the study [19]. Similarly, a separate set of one group (n=6) was used for the pharmacodynamic interaction study with pretreated atazanavir (36 mg/kg) followed by 0.5 mg/kg repaglinide in hepatic impaired rats.

Liquid chromatography-mass spectrometry analysis

In vivo samples were prepared by protein precipitation with the addition of 200 µL acetonitrile containing an internal standard (rosuvastatin) to 50 µL sample volume. Samples were vortexed and centrifuged at 4000rpm for 10 min prior to evaporation of the supernatant. Atazanavir and repaglinide concentrations from plasma samples were measured using validated liquid chromatography/mass spectrometry (LC-MS/MS) method. Positive-ion multiple reaction monitoring was used for the tandem mass spectrometric detection of repaglinide, atazanavir and rosuvastatin. The selected $[M+H]^+$ precursor ions were m/z 453.2 for repaglinide, m/z 705.5 for atazanavir, and m/z 482.3 for rosuvastatin, and the product ions monitored were at m/z 230.3, 168.2 and 258.15 for repaglinide, atazanavir and rosuvastatin (internal standard), respectively. The high-performance liquid chromatography column was a Hypersil GOLD C18 (4.6x100mm,5µ) maintained at 40 °C with a flow rate of 1.3 mL/min. Mobile phase consisted of 0.1 % formic acid in milliQ water (A) and acetonitrile (B). A gradient elution was used. The overall run time was 6.0 min. An aliquot of 5 µl was injected at a mobile phase flow rate of 1300 µl/min. The liquid

chromatography was interfaced to API 3200 (Absciex, CA) ion-trap mass spectrometer operated in the positive ion electrospray and full tandem mass spectrometry mode. Before analyzing plasma, atazanavir and repaglinide standard solutions prepared in acetonitrile were run on LC to optimize the analysis and standardize calibration. Standard concentrations were chosen on the basis of levels found in plasma samples. The peak area ratio of analyte (repaglinide, atazanavir) to internal standard was plotted against analyte concentrations, and standard curves were fitted by weighted ($1/x^2$) least-squares linear regression in the concentration of 9-20000 ng/mL for repaglinide and 19.5–20000 ng/mL for atazanavir. A correlation of 0.994 was desirable for all the calibration curves. The limit of quantitation for the purposes of this assay was 9 ng/mL and 19.5 ng/mL for repaglinide and atazanavir, respectively [20-21].

Pharmacokinetic and statistical analysis

The pharmacokinetic parameters were obtained by fitting the plasma concentration-time data to non-compartmental model by using (Phoenix -v6.3.0.395; Pharsight, Mountain view, CA). The maximum plasma concentration (C_{max}) and the time to reach the same (T_{max}), the half-life of plasma drug elimination ($t_{1/2}$) was the ratio of 0.693 to the slope obtained by log-linear regression of the terminal phase of the drug plasma profile and the area under the concentration-time curve to terminal time (AUC_{0-t}),area under the concentration-time curve to infinite time ($AUC_{0-\infty}$) was calculated by the linear/log trapezoidal rule and the total plasma clearance (CL) were calculated by phoenix software. Data were expressed as a mean±standard deviation (SD). The significance was determined by applying one way ANOVA analysis. The results were considered to be statistically significant when $p<0.0001$.

Glucose reduction calculations

Percentage Reduction in BGL = {(IBGL – FBGL) / IBGL} x 100

where BGL = blood glucose level; IBGL = initial blood glucose level; FBGL = final blood glucose level [22].

Results

LC-MS/MS analytical method validation

The analysis of plasma samples was completed after thorough validation of LC-MS/MS method. No interfering peaks were observed in blank plasma chromatograms at atazanavir and repaglinide retention time indicating the selectivity of the present method. The calibration curves and linearity of repaglinide (19.5-20000 ng/ml) and atazanavir (19-20000 ng/ml) samples was determined using weighted ($1/x^2$) linear regression analysis. The correlation coefficients were 0.994 for calibration curves. The retention times for atazanavir, repaglinide and internal standard were 3.0, 3.5 and 3.3 min, respectively. Quality control (QC) samples were prepared at low, medium, and high concentrations to assess accuracy, precision and recovery. Intraday precision from a measurement of a minimum of six replicates was <7.1 % in rat plasma. The inter-day precision was determined from analysis of standard samples on three consecutive days and the relative standard deviation was found to be <6.9 % and <9 % for atazanavir and repaglinide, respectively. The overall accuracy of QC samples was in the range of 90-110 %. Percentage recovery was calculated as a ratio of the peak areas of drug in the matrix and the corresponding peak area of drug in acetonitrile. In plasma samples, greater than 85 % drug recovery was accomplished for both atazanavir and repaglinide. The lower limit of quantification (LOQ) was determined based on intra-run accuracy of LOQ replicates. The tested LOQ values were 9 ng/mL and 19.5 ng/mL for repaglinide and atazanavir, respectively, in rat plasma.

Effect of atazanavir on the pharmacokinetics and pharmacodynamics of repaglinide in normal rat

Mean plasma concentration of repaglinide in the absence and presence of atazanavir in normal rats is shown in Figure 1. Mean pharmacokinetic parameters of repaglinide alone and in the presence of atazanavir in normal rats were shown in Table 1 (Column I and II). The pharmacokinetic parameters of repaglinide like AUC, C_{max}, and clearance were altered significantly with single dose treatment of atazanavir in normal rats when compared to repaglinide treated group. Compared to the repaglinide control group (repaglinide alone) atazanavir increased 2-fold peak plasma concentration (C_{max}), 11-fold area under the plasma concentration time curve (AUC_{0-t} and $AUC_{0-\infty}$) of repaglinide and atazanavir relative decrease in the total body clearance by 6 folds. Repaglinide produced a hypoglycemic effect which was indicated by percentage reduction levels in normal rats. The hypoglycemic effect of repaglinide enhanced when given in combination with atazanavir. It was indicated by a significant increase in percentage glucose reduction in comparison to repaglinide alone treated group in normal rats were shown in Table 2 and Figure 4.

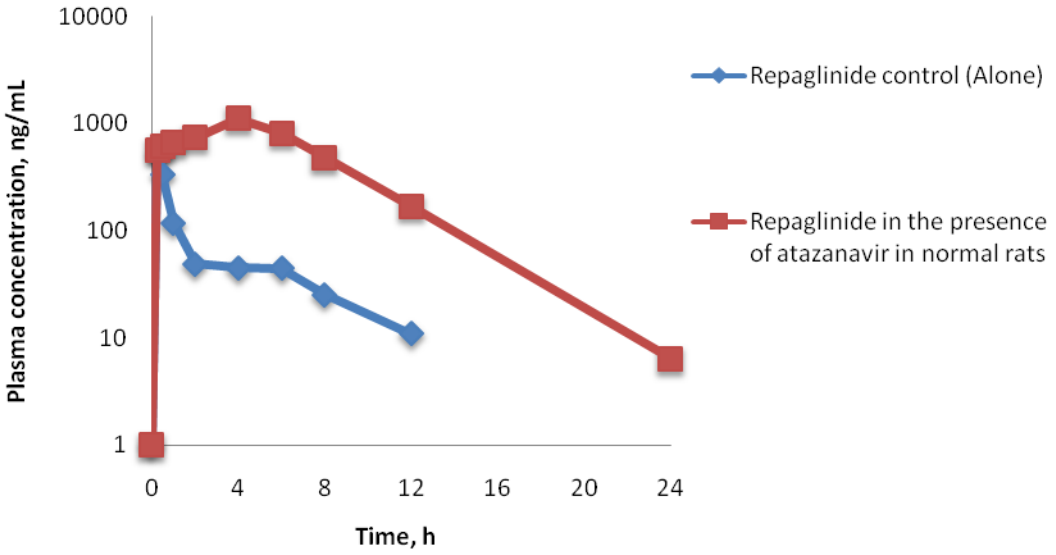

Figure 1. Plasma concentration-time curves of repaglinide following its oral administration (0.5 mg/kg) in control and atazanavir (36 mg/kg) pretreated normal rats. Data are expressed as mean ±SD in six rats.

Table 1. Plasma concentration time profiles of repaglinide following its oral administration at 0.5 mg/kg in control and atazanavir (36 mg/kg) pretreated normal, diabetic and hepatic impaired rats. Data are expressed as mean ±S.D. in (n=6) rats.

Pharmacokinetic Parameters	Repaglinide Alone (Control)	Repaglinide in the presence of atazanavir in normal rats	Repaglinide in the presence of atazanavir in Diabetic rats	Repaglinide in the presence of atazanavir in Hepatic impaired rats
C_{max} (ng/mL)	536 ± 30	1089 ± 123***	2753 ± 7 ***	3410 ± 64 ***
T_{max} (h)	0.25 ± 0.00	4.0 ± 0.0 **	4.0 ± 0.0 **	0.5 ± 0.0
AUC_{0-t} (ng/mL.h)	678 ± 51	8031 ± 274***	12602 ± 227***	18301 ± 151***
AUC_{total}(ng/mL.h)	726 ± 55	8044 ± 265***	12722 ± 209 ***	19101 ± 184***
Oral $T_{1/2}$ (h)	3.0 ± 0.1	2.6 ± 0.1	3.5± 0.1	6.2 ± 0.3 **
Oral CL (mL/min/kg)	11 ± 1	1.03 ± 0.03***	0.44 ± 0.01***	0.65 ± 0.01 ***

*** Significant at P<0.0001 compared to repaglinide control (alone) group.
**Significant at P<0.0001 compared to repaglinide control (alone) group

Effect of atazanavir on the pharmacokinetics and pharmacodynamics of repaglinide in diabetic rats

Mean plasma concentration of repaglinide in the absence and presence of atazanavir in diabetic rats is shown in Figures 2 & 3. Mean pharmacokinetic parameters of repaglinide and atazanavir in diabetic rats are shown in Table 1 (column I and III). The pharmacokinetic parameters of repaglinide like AUC, C_{max}, and clearance were altered significantly with single dose treatment of atazanavir in diabetic rats when compared to repaglinide treated group. Compared to the repaglinide control group (repaglinide alone) atazanavir increased 5-fold peak plasma concentration (C_{max}), 18-fold area under the plasma concentration time curve ($AUC_{0-\infty}$) of repaglinide and atazanavir relatively decreases in the total body clearance by 25-fold. Repaglinide produced a hypoglycemic effect indicated by percentage reduction levels in diabetic rats. The hypoglycemic effect of repaglinide exaggerated when given in combination with atazanavir, it was indicated by a significant increase in percentage glucose reduction in comparison to repaglinide alone treated group in normal rats were shown in Table 2 and Figure 4.

Table 2. Mean percentage blood glucose reduction of repaglinide following its oral administration at 0.5mg/kg in control and atazanavir (36 mg/kg) pretreated normal, diabetic and hepatic impaired rats. Data are expressed as mean ±S.D. in (n=6) rats.

	Repaglinide alone (Control)	**Repaglinide in the presence of atazanavir in normal rats**	**Repaglinide in the presence of atazanavir in Diabetic rats**	**Repaglinide in the presence of atazanavir in Hepatic impaired rats**
Time, h	Mean±SD	Mean±SD	Mean±SD	Mean±SD
0.25	37.07±3.80	17.70±5.91	15.76±4.77***	43.95±2.32***
0.50	25.12±5.84	37.21±22.51*	33.27±24.79***	64.62±3.31***
1	19.76±5.99	43.12±15.56**	28.25±2.38***	57.15±2.04***
2	7.03±5.02	44.50±6.84**	53.88±2.93***	46.54±1.72***
4	9.63±3.65	59.22±7.72**	65.89±1.27***	38.45±5.03***
6	12.57±5.11	47.72±5.50**	31.13±3.25***	23.54±4.18***
8	3.47±3.67	38.38±3.03**	16.86±2.29***	14.47±15.70***
12	2.28±6.66	27.75±6.10**	10.69±3.81***	9.14±10.03***
24	0.34±5.34	12.24±3.84*	4.82±3.24***	3.71±11.59***

*** Significant at P<0.0001 compared to repaglinide control (alone) group.
** Significant at P<0.005 compared to repaglinide (alone) control group.

Effect of atazanavir on the pharmacokinetics and pharmacodynamics of repaglinide in hepatic rats

Mean plasma concentration of repaglinide in the absence and presence of atazanavir in hepatic impaired rats has shown in Figures 2 & 3. Mean pharmacokinetic parameters of repaglinide in the presence of atazanavir in hepatic impaired rats were shown in Table 1 (column I and IV). The pharmacokinetic parameters of repaglinide like AUC, C_{max}, $t_{1/2}$, clearance were altered significantly by a single treatment of atazanavir in normal rats when compared to repaglinide treated group. In a single dose study with atazanavir, increases in pharmacokinetic parameters of repaglinide are as follows: AUC (26-fold), C_{max} (6-

fold) with a relative decrease in total body clearance of 16-fold. The hypoglycemic effect of repaglinide enhanced when given in combination with atazanavir, it was indicated by a significant increase in percentage glucose reduction in comparison to repaglinide alone treated group in normal rats (see Table 2 and Figure 4).

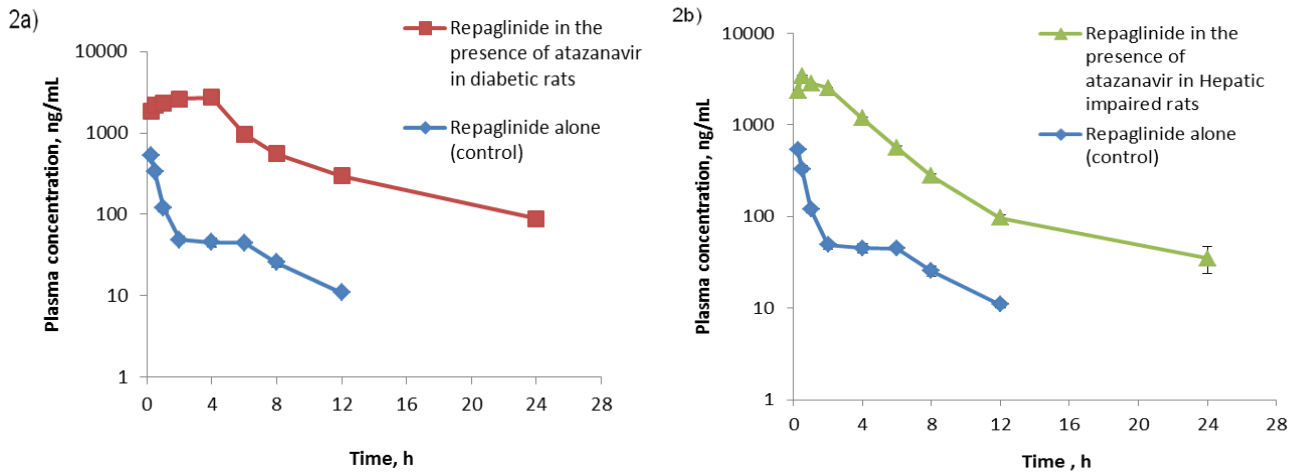

Figure 2. Plasma concentration - time curves of repaglinide following its oral administration at 0.5 mg/kg in control and atazanavir (36 mg/kg) pretreated a) diabetic and b) hepatic impaired rats. Data are expressed as mean ±S.D. in (n=6) rats.

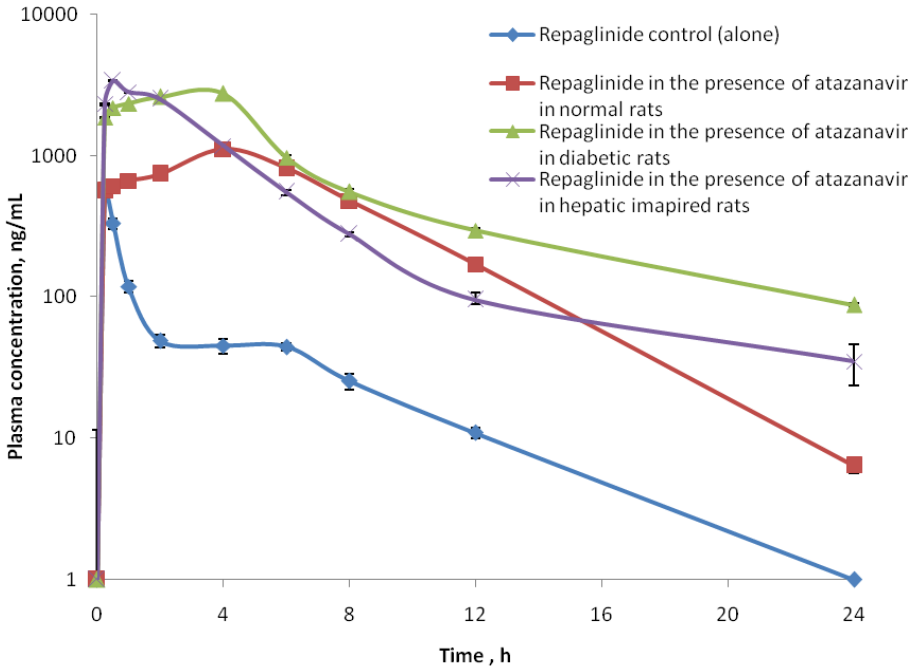

Figure 3. Plasma concentration - time curves of repaglinide following its oral administration at 0.5 mg/kg in control and atazanavir (36 mg/kg) pretreated normal, diabetic and hepatic impaired rats. Data are expressed as mean ±S.D. in (n=6) rats.

Discussion

The most challenging aspect in treating HIV patients are to give the safe combination of drugs during the therapy because the HIV therapy regimen contains one nucleoside reverse transcriptase inhibitor, one non-nucleoside reverse transcriptase inhibitor and two protease inhibitors to reduce the viral load in the patients. The HIV patients will also have several other co-morbid conditions like Kaposi's sarcoma, lymphoma, and tuberculosis. Apart from this the highly active antiretroviral is encountered with the

increase in prevalence of diabetes mellitus includes insulin resistance and glucose tolerance. The development of these metabolic syndromes like glucose and lipid disturbances presents a pharmacological challenge because of the possible pharmacokinetic drug-drug interactions associated with oral hypoglycemics and antiretroviral drugs. There are limited published data on drug interactions between antidiabetic medications and antiretroviral agents. In this investigation we studied the influence of atazanavir on the pharmacokinetics and pharmacodynamics of repaglinide at therapeutic doses in healthy, diabetic and hepatic impaired rats. The healthy rat model served to quickly identify the interaction and the diabetic and hepatic impaired model to validate the same response in the disease condition.

Repaglinide is extensively metabolized by the hepatic cytochrome P450 enzyme system, particularly with CYP3A4; CYP2C8 with less than 2 % of an oral dose excreted unchanged in humans. Among this CYP3A4 has been identified as an important enzyme in the in-vitro metabolism of repaglinide [23]. The repaglinide pharmacokinetics was further complicated because of active hepatic uptake of repaglinide by OATP uptake transporter and it is a substrate for the OATP transporter [24]. Repaglinide also act as a substrate for P-glycoprotein which can significantly contribute to potential drug-drug interactions with other P-gp substrate or inhibitors [25].

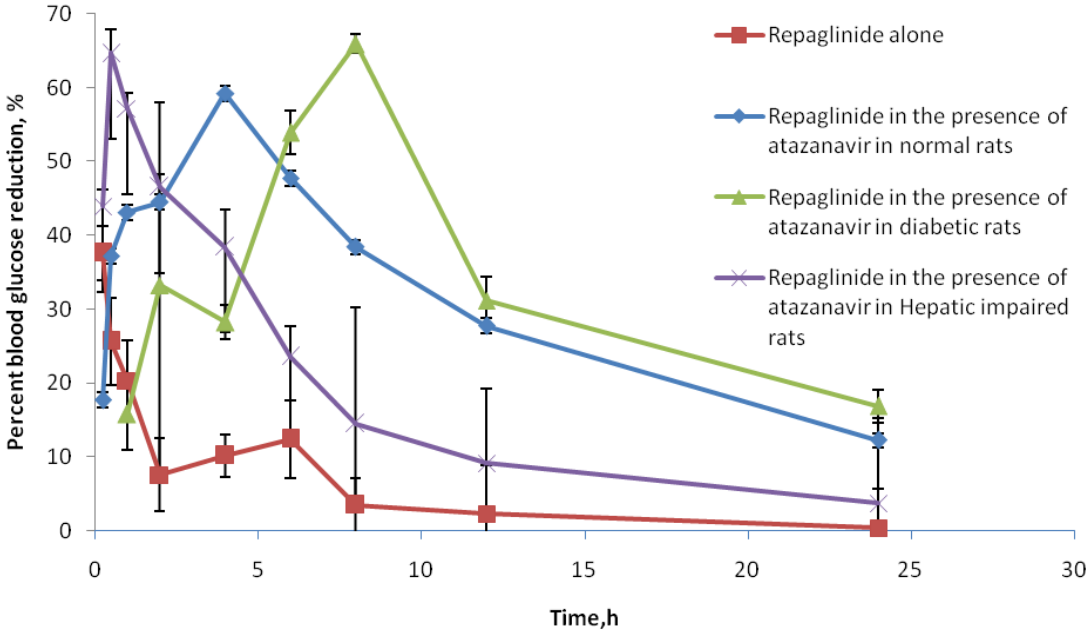

Figure 4. Mean percentage blood glucose reduction curves of repaglinide following its oral administration at 0.5 mg/kg in control and atazanavir (36 mg/kg) pretreated normal, diabetic and hepatic impaired rats. Data are expressed as mean ±S.D. in (n=6) rats.

HIV protease inhibitors have identified as a substrates, inhibitors or inducers of CYP3A4, OATP and P-gp. Atazanavir is an extensively metabolized mainly by Cytochrome P450 enzyme system which have capable of altering both P-gp and CYP 3A4 activity in-vitro [26]. Atazanavir inhibits P-gp mediated transport and acts as a potent mechanism based inhibitor of CYP3A4 and it also inhibit the OATP transport particularly OATP1B1, OATP1B3 and OATP2B1 mediated transport in liver [27].

The pharmacokinetics (C_{max}, AUC_{0-t}, $AUC_{t-\infty}$, $t_{1/2}$, clearance) and pharmacodynamics (percent blood glucose reduction) of repaglinide were significantly altered in the atazanavir treated normal, diabetic and hepatic impaired rats. The significant improvement of C_{max} and AUC of repaglinide in healthy, diabetic and hepatic impaired rats could be due to CYP enzyme and P-gp mediated transport inhibition during the first pass metabolism. Compared to the control group the atazanavir significantly decreased the clearance of

repaglinide in healthy, diabetic and hepatic impaired rats, this could be due to the inhibition of CYP enzyme and OATP transporter in the liver.

The magnitude change in the exposure of repaglinide is more in hepatic impaired rats could be due to the synergistic effect of OATP inhibition by atazanavir and liver dysfunction

The clearance of repaglinide was significantly decreased in normal and diabetic rats, which was due to the inhibition of CYP enzyme and OATP, P-gp transport by atazanavir. However, in hepatic impaired rats the magnitude change in the exposure of repaglinide was more, which could be due to the synergistic effect of OATP inhibition by atazanavir and liver dysfunction. In hepatic impaired rats $t_{1/2}$ of repaglinide was increased significantly when compared to the repaglinide alone control group due to decrease in clearance of repaglinide.

The blood glucose levels were decreased significantly when repaglinide is given in combination with atazanavir in normal, diabetic and hepatic impaired groups. The increased bioavailability (AUC and C_{max}) of repaglinide when administered with atazanavir increases the plasma levels of repaglinide and decreases elimination which results in enhanced mean blood glucose percentage which may further precipitate hypoglycemic action.

Our findings were consistent with the report by Shitara, *et al* reported gemfibrozil increased the AUC of repaglinide approximately 8-fold and prolonged its $t_{1/2}$ from 1.3 to 3.7 in healthy subjects [28]. Gemfibrozil is a strong inhibitor of CYP2C8 in vivo mainly due to its 1-O-ß-glucuronide metabolite. Gemfibrozil and its glucuronide inhibit the OATP1B1 *in vitro*, suggesting that the involvement of drug-drug interaction between gemfibrozil and repaglinide [28]. Kajossari *et al* reported that the co-administration of repaglinide along with the known P-glycoprotein inhibitor cyclosporine A raised the plasma concentrations of repaglinide significantly, probably by inhibiting its CYP3A4-catalyzed biotransformation and OATP1B1-mediated hepatic uptake. Cyclosporine may enhance the blood glucose-lowering effect of repaglinide and increase the risk of hypoglycemia in humans [29].

Conclusions

In this study, atazanavir enhanced the bioavailability of repaglinide after oral administration, this enhanced bioavailability of repaglinide might be mainly due to the possible inhibition of CYP enzyme mediated metabolism and P-glycoprotein effect in the intestine and in the liver the reduced the total body clearance of repaglinide is due to CYP enzyme and OATP mediated transport inhibition. The current study has raised awareness of potential drug-drug interaction between the repaglinide and atazanavir in normal, diabetic and hepatic impaired groups. The clinical significance of this study should be further evaluated in clinical studies.

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