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Effect of Atorvastatin on Pharmacology of Sitagliptin in Streptozotocin-Nicotinamide Induced Type-II Diabetes in Rats

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Abstract

Prolonged type 2 diabetes mellitus (Type2DM) may lead to high risk of cardiovascular disease (CVD) requiring a global therapeutic approach. Statin therapy has proven its efficacy in reducing CVD events in Type2 DM patients. Dipeptidyl peptidase-4 inhibitors (gliptins), which are increasingly used to target hyperglycemia. In the present study type 2 DM in rats by i.p. Administration of Streptozotocin (STZ); (60 mg/kg) -Nicotinamide (120 mg/kg). Diabetes induced rats were divided into groups and treated with Sitagliptin alone and in combination with atorvastatin for 7 days. Blood samples were collected by retro orbital puncture. Mean glucose concentration was measured by GOD-POD method using commercial glucose kits and sitagliptin in plasma was estimated by RP-HPLC method using methanol: water (60:40 v/v, containing 10 mM Tris and 10 mM Triethylamine) was adjusted to pH 9.0 using 1 mol/L hydrochloric acid. The blood glucose lowering activity of sitagliptin was increased by the presence of atorvastatin in diabetic rats. The pharmacokinetic (PK) and pharmacodynamic (PD) parameters of sitagliptin in diabetic rats were significantly changed in the presence of atorvastatin. The present study concludes that co-administration of atorvastatin with sitagliptin significantly improved the responses of sitagliptin in diabetic rats. Improved glucose metabolism and increased sitagliptin levels due to competitive inhibition of CYP 3A4 enzyme by atorvastatin may be responsible for the improved anti-hyperglycemic activity of sitagliptin.

Keywords: Sitagliptin; atorvastatin; Type2 diabetes mellitus; DPP-4 inhibitor; HPLC; PK/PD parameters

Introduction

Diabetes mellitus is a complex metabolic disease affecting about 5% of people all over the world. According to the classification proposed by the American Diabetes Association, diabetes is divided into different types [1]. Type 1 diabetes accounts for 5–10% of all diabetic cases and results from the autoimmune destruction of the pancreatic β-cells. This type of diabetes usually develops rapidly because of the grave destruction of the insulin-secreting cells and patients are dependent on exogenous insulin. Type-II diabetes is more frequent, accounts for 90–95% of all diabetic cases characterized by insulin resistance and relative insulin deficiency. Frequently accompanied by overweight or obesity. Hyperglycemia usually develops slowly and at earlier stages, blood glucose is moderately elevated. In Type-II diabetes, insulin resistance is initially compensated by increased secretion of insulin; however, this prolonged over stimulation of insulin secretion leads over time to progressive exhaustion and degradation of β-cells [2,3]. Dyslipidemia is common in patients with Type-II diabetes. Dyslipidemia is an abnormal amount of lipids (e.g. cholesterol and/or fat) in the blood.

Sitagliptin is an orally-active, potent and highly selective inhibitor of the Dipeptidyl peptidase 4 (DPP-4) enzyme, used in the treatment of Type-II diabetes. The DPP-4 inhibitors are class of agents that act as incretin enhancers. By inhibiting the DPP-4 enzyme, sitagliptin increases the levels of two known active incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) [4]. Incretins are part of an endogenous system involved in the physiologic regulation of glucose homeostasis. When blood glucose concentrations are normal or elevated, GLP-1 and GIP increase insulin synthesis and release from pancreatic β cells. GLP-1 also lowers glucagon secretion from pancreatic α cells, leading to reduced hepatic glucose production. This mechanism is unlike the mechanism seen with sulfonylureas; sulfonylureas cause insulin release even when glucose levels are low, which can lead to sulfonylurea-induced hypoglycaemia in patients with type 2 diabetes and in normal subjects [5]. Sitagliptin is a potent, highly selective inhibitor of the enzyme DPP-4 and does not inhibit the closely-related enzymes DPP-8 or DPP-9 at therapeutic concentrations [6,7]. Sitagliptin differs in chemical structure and pharmacological action from GLP-1 analogues, insulin, sulfonylureas or meglitinides, biguanides, peroxisome proliferator-activated receptor gamma (PPARγ) agonists, alpha-glucosidase inhibitors and amylin analogues.

Dyslipidemia is common in patients with type 2 diabetes [8] and statins remain as the first choice of drugs in the treatment of diabetic dyslipidemia [9]. Atorvastatin represents a first line treatment option, alongside other hydroxyl methyl glutaryl coenzyme A (HMG-CoA) reductase, dose dependently lowers both cholesterol [10,11] and triglyceride [12] levels in hyperlipidemic patients. Atorvastatin produces larger reductions of cholesterol and triglycerides compared with other drugs of the same class [13].

The study of mechanisms of drug interaction is of much value in diabetes mellitus therapy, because this is one of the metabolic disorders that needs treatment for prolonged periods and maintenance of normal

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blood glucose level is very important in this condition.Since both hyperglycemia and hypoglycemia are unwanted phenomena, there is every possibility for the combined use of sitagliptin and atorvastatin in diabetic dyslidemia [14]. Hence the present study was aimed at investigating the effect of atorvastatin on the activity of sitagliptin in diabetes induced rats. To evaluate the safety and effectiveness of the combination with respect to blood glucose levels.

Materials and Methods

Wistar rats of either sex of 8-9 weeks of age, weighing between 190 ± 20g were used for the study. They were procured from Mahaveer agencies, Hyderabad, India. Animals were maintained under standard laboratory conditions at an ambient temperature of 25 ± 2^{0}C and 50 ± 15% relatively humidity with a 12h light/dark cycle. Animals were fed with commercial standard pellet diet (Rayan's Biotechnologies Pvt Ltd., Hyderabad, India) and water *ad libitum*. Animals were fasted for 18h prior to the experiment and during the experiment they were withdrawn from food and water. The animal experimental procedures and protocols were carried out according to the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Study Design

In clinical practice, both Sitagliptin and Atorvastatin were administered orally [15,16]. Hence, human oral therapeutic doses of the respective drugs were extrapolated to rat, based on body surface area [17]. The dose of Sitagliptin and Atorvastatin for rat experiments selected were 10.4 & 20.0 mg/kg body weights respectively. Based on the dose-effect relationship of sitagliptin on blood glucose in normal rats. Sitagliptin and Atorvastatin were suspended in 0.5% CMC, for oral administration [18].

Induction of diabetes in rats

Experimental diabetes (NIDDM) was induced in overnight fasted adult Wistar strain albino rats weighing 190±20g by a single *i.p.* injection of 60 mg/kg Streptozotocin (in citrate buffer, pH 4.5), 15min after the i.p. administration of 120 mg/kg of nicotinamide (in normal saline). Hyperglycemia was confirmed by the elevated glucose levels in plasma determined at 72h and then on day 7 after injection. Plasma was collected by centrifuging the blood samples at a speed of 4000rpm for 15min. 10µl of plasma and 1ml of working reagent (GOD-POD) were mixed and incubated for 15min at 37^{0}C. The absorbance of sample and standard (provided by manufacturer) was measured against blank at 505 nm. Rats showing fasting plasma glucose levels≥220mg/dl (diabetic) were selected for study [19].

Diabetic albino Wistar rats were selected randomly and divided into 5 groups of 6 animals in each. Before the experiment, all animals were fasted for 18hr and water provided at *ad libitum*. Water was withdrawn during the experiment. After collection of initial blood samples, drugs were administered in the following order.

Group I: Diabetic control (Treated with 0.1ml of 0.5% of CMC) per 7days

Group II: Treated with sitagliptin (10.4 mg/kg; p.o.) per 7 days

Group III: Treated with atorvastatin (20 mg/kg; p.o.) per 7 days

Group IV: Treated with atorvastatin (10.4 mg/kg; p.o.) after 1h followed by sitagliptin (20 mg/kg; p.o.), (Single dose interaction studies; SDIs) per one day.

Group V: Pre-treated with atorvastatin (20 mg/kg; p.o.) for 7 days, on the 8th day after 1 hr administration of atorvastatin (20 mg/kg; p.o.), sitagliptin (10.4 mg/kg; p.o.) was given.

Blood samples (1ml) were collected by retro-orbital plexus at time intervals 0, 1st , 2nd , 4th , 8th , 12th and 24th h using heparinized capillaries into a micro centrifugation tube containing anticoagulant (sodium citrate). Plasma was separated by centrifugation and stored at -20^{0}C until further analysis. These plasma samples were analyzed for blood glucose by GOD/POD method [20] and for plasma sitagliptin by HPLC method [21,22] (A gradient High Pressure Liquid Chromatography (Shimadzu Japan) equipped with C18 reversed-phase column). Hydrocortisone was used as internal standard and the mobile phase consist of methanol:water (60:40, v/v, containing 10 mMTris and 10 mMTriethylamine) was titrated to pH 9.0 using 1 mol/L hydrochloric acid. The mobile phase was eluted at a flow rate 1 ml/min C18 reversed-phased column at 30°C and the effluent was monitored at a wavelength of 267nm. The ratio of peak areas of Sitagliptin to that of internal standard was used for the quantification of Sitagliptin in plasma samples. The HPLC method was validated in terms of reproducibility, system suitability, recovery, accuracy, and precision and then applied for the estimation of sitagliptin in rat plasma. Maximum plasma concentration (C_{max}) was the peak plasma concentration of a drug after administration, Time of peak plasma concentration (T_{max}) was time to reach peak plasma concentration of a drug after administration, The area under the plasma drug concentration-time curve (AUC) reflects the actual body exposure to drug after administration of a dose of the drug, Half-life ($t_{1/2}$) was the time required for the concentration of the drug to reach half of its original value, Mean residence time (MRT) was the time required for 63.2% of the dose to be eliminated from the body, Clearance (Cl) was the volume of plasma cleared of the drug per unit time and Volume of distribution (V_d) was theoretical volume in which the total amount of drug would need to be uniformly distributed to produce the desired blood concentration of a drug.

Data and statistical analysis

Data were expressed as mean ± SD. The significance was determined by applying the Student's paired t test. Significance values were found to be $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to diabetic control. The plasma concentration–time data were analyzed by non-compartmental analysis using the Kinetica Software (version 5.0 from Thermo Fisher scientific Inc, USA).

Results

Sitagliptin produced antihyperglycemic activity with maximum percentage reduction (42.75 ± 1.24%) at 2 hr in diabetic rats. The average percentage blood glucose reduction with sitagliptin, atorvastatin and their combination in diabetic rats is shown in Table 1. Atorvastatin alone has no significant effect on the blood glucose level of diabetic rats (Table 1). When administered in combination with sitagliptin, atorvastatin significantly increased the effect of sitagliptin in diabetic rats (42.75 vs 51.80; $P<0.01$). The average plasma sitagliptin concentrations alone and in combination with atorvastatin in diabetic rats were shown in (Table 2). The mean PK parameters of sitagliptin alone and in combination with atorvastatin in diabetic rats are shown in Table 3. The plasma sitagliptin levels at 1, 2, 4, 8, 12, and 24hrs intervals were increased in the presence of atorvastatin and the PK parameters, such as C_{max}, T_{max}, $t_{1/2}$, Cl, $V_{d,}$ AUC and MRT were altered significantly in the presence of atorvastatin in diabetic rats.

Time	Diabetic control	Sitagliptin	Atorvastatin	Sitagliptin + Atorvastatin (SDI)	Sitagliptin + Atorvastatin (MDI)
0	00.00	00.00	00.00	00.00	00.00
1	0.92 ± 0.04	27.28 ± 0.85*	12.15 ± 0.35	31.38 ± 1.50*	37.62 ± 1.47*
2	1.56 ± 0.12	42.75 ± 1.24**	21.69 ± 0.89	45.94 ± 1.20**	51.80 ± 2.58**
4	1.66 ± 0.20	49.57 ± 1.98**	27.24 ± 1.02	64.46 ± 3.47**	64.10 ± 2.98**
8	2.03 ± 0.35	35.45 ± 1.24*	17.35 ± 0.25	47.12 ± 2.51*	41.49 ± 1.87*
12	2.29 ± 0.42	24.29 ± 1.5*	8.99 ± 0.27	42.32 ± 2.05*	29.85 ± 0.98*
24	2.46 ± 0.35	17.82 ± 1.68*	4.54 ± 0.05	26.94 ± 0.98*	34.40 ± 0.41*

Data were expressed as mean ± SD. The significance was determined by applying the Student's paired t test. Significance values were found to be *P<0.05, **P<0.01, ***P<0.001 compared to diabetic control. (SDI-Single Dose Interaction, MDI-Multi Dose Interaction).

Table 1: Percentage of glucose reduction in diabetic rats after oral administration of Sitagliptin, Atorvastatin alone and in combination (SDI & MDI).

Time (hr)	Sitagliptin	Sitagliptin+Atorvastatin (SDI)	Sitagliptin+Atorvastatin (MDI)
0	00.00	00.00	00.00
1	3.38 ± 0.39	5.02 ± 0.81**	4.49 ± 0.85*
2	7.01 ± 0.42	12.31 ± 0.96**	9.22 ± 1.30**
4	3.92 ± 0.26	5.98 ± 0.46**	4.68 ± 0.69*
8	1.19 ± 0.25	2.61 ± 0.51**	2.54 ± 0.72*
12	0.77 ± 0.18	0.92 ± 0.24	1.05 ± 0.41
24	00.00	00.00	00.00

Data were expressed as mean ± SD and analyzed statistically by using one way ANOVA followed by Dunnett's test. Significance values were found to be *P<0.05, **P<0.01,**P<0.001 compared to diabetic control. (SDI-Single Dose Interaction, MDI-Multi Dose Interaction).

Table 2: Concentration of sitagliptin in rat plasma at different time intervals in sitagliptin and combination groups (SDI, MDI) using Kinetica 5.0.

PK parameters	Sitagliptin	Sitagliptin+Atorvastatin (SDI)(Day1)	Sitagliptin+Atorvastatin (MDI) (Day8)
Cmax	6.74 ± 0.42	11.07 ± 0.79**	8.89 ± 1.41**
T_{max}	2 ± 0	2 ± 0	2 ± 0
AUC	33.58 ± 2.49	54.95 ± 2.31**	50.70 ± 1.76**
$t_{1/2}$	3.19 ± 0.45	2.96 ± 0.43	3.97 ± 1.23
MRT	5.17 ± 0.51	5.03 ± 0.53	6.2 ± 1.48
Cl	233.96 ± 14.43	142.90 ± 7.43**	160.88 ± 21.69**
V_d	1072.53 ± 93.78	610.87 ± 75.98**	2524.02 ± 109.82**

Data were expressed as mean ± SD and analyzed statistically by using one way ANOVA followed by Dunnett's test. Significance values were found to be *P<0.05, **P<0.01,**P<0.001 compared to diabetic control. (SDI-Single Dose Interaction, MDI-Multi Dose Interaction).Cmax-Maximum drug concentration, T_{max}-Time of peak plasma concentration, AUC- area under the curve, MRT- Mean Residence Time, Cl- Clearance, V_d-Volume of Distribution.

Table 3: Various pharmacokinetic parameters (Mean& SD) of sitagliptin alone and in combination with atorvastatin (SDI & MDI) in diabetic rats at different time intervals (using Kinetica 5.0.).

Discussion

Drug interactions are usually seen in clinical practice and mechanisms of interactions are evaluated usually in animal models (rodents and no-rodents). The statin derivatives were studied in lipid-lowering action and glucose metabolism effect in hyper-cholesterolemic patients with concurrent diabetes [23] and also canagliflozin versus sitagliptin for type 2 DM [24]. In the present study, influence of atorvastatin on the activity of sitagliptin in diabetic rats was studied. Since small amount of plasma was required for glucose analysis, the blood samples were collected by retro-orbital puncture [25].The present study revealed no or very little glucose-lowering effect of atorvastatin alone in diabetic condition, with respect to pre dose glucose levels. The PD of sitagliptin was increased significantly (P<0.01) in the presence of atorvastatin in diabetic rats. The PK parameters (C_{max},T_{max}, $T_{1/2}$,Cl, V_d, AUC and MRT) of sitagliptin significantly changed in the presence of atorvastatin. This confirms synergistic interaction between atorvastatin and sitagliptin in diabetic rats. The impact of atorvastatin on the activity of sitagliptin was significant following single oral dose administration in diabetic rats. After oral administration the peak anti-hyperglycemic activity was observed at 2h in diabetic rats. Sitagliptin is rapidly absorbed and has a half-life of approximately 8-14h. Sitagliptin is extensively metabolized by the hepatic cytochrome P450 enzyme system (CYP3A4 and CYP2C8) with less than 2% of an oral dose being excreted unchanged in humans [25-27]. Atorvastatin is mainly metabolized by CYP3A4 and meanwhile atorvastatin also having the inhibitory effect against CYP3A4 mediated metabolism. The above literature supports, the presence of atorvastatin significantly increased the PK of sitagliptin in diabetic rats. These results showed that atorvastatin is an effective inhibitor of P-glycoprotein and CYP3A4 mediated metabolism in intestine and/or liver. Therefore, atorvastatin could affect the sitagliptin activity in diabetic rats by increasing plasma levels of the sitagliptin. Overall the interaction of sitagliptin and atorvastatin was mainly due PK in nature and increased blood glucose lowering effect of sitagliptin.

Conclusion

The present study concludes that co-administration of atorvastatin with sitagliptin significantly improves anti-hyperglycemic activity of sitagliptin when compared to sitagliptin alone treated Streptozotocin-Nicotinamide induced diabetic rats.

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