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Original Article

Effect of Candesartan on Pharmacokinetics and Pharmacodynamics of Sitagliptin in Diabetic Rats

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ABSTRACT

Objective: The aim of the present study was to evaluate the effect of candesartan (antihypertensive drug) on pharmacokinetic and pharmacodynamics of sitagliptin (antidiabetic drug) in diabetic rats.

Methods: Alloxan-induced Diabetic model in rats has been used in this study. After induction of diabetes, sitagliptin (10mg/kg/p.o) and candesartan (5mg/kg/p.o) were administered orally for 7days.The Pharmacokinetic parameters like $t_{1/2}$, AUC, Clearance, T_{max} and $C_{max\ of}$ sitagliptin with and without combination of candesartan treatment were determined. The blood glucose levels were estimated using Glucose Oxidase-Peroxidase (GOD-POD) method, creatinine by alkaline picrate method and albumin by BCG-dye method.

Results: The pharmacokinetic results showed similar sitagliptin plasma concentration when used in combination with candesartan and no change in the pharmacokinetic parameters were observed and no change in the blood glucose levels of sitagliptin was observed in the presence of candesartan indicates the no significant ($p>0.05$) interaction. The renoprotective effect of candesartan in the presence and absence of sitagliptin were evaluated. The creatinine levels were increased in combination when compared to sitagliptin and candesartan alone ($P<0.05$). The albumin levels were also increased in combination of sitagliptin and candesartan groups when compared to individual groups which indicates the potentiating effect of candesartan on renoprotective activity in presence of sitagliptin.

Keywords: Sitagliptin, Candesartan, Pharmacokinetics, Pharmacodynamics, Diabetes and Diabetic nephropathy.

INTRODUCTION

Drug interaction is the modification of the effect of one drug (object drug) by the prior or concomitant administration of another drug (precipitant drug). Drug interaction may either enhance or diminish the intended effect of one or both drugs. It may modify the diagnostic, preventive or therapeutic activity of either drug[1]. In poly pharmacy, it is important to determine the incidence and frequency of occurrence of drug interactions, which serious implications, in hospitalized patients. In addition, it is also important to find out agents that are most likely to produce hazardous interactions[2]. As per survey, the incidence of drug-drug interaction ranges from 3 to 5 % in patients taking a few drugs to 20% in patients receiving many drugs. According to a report that, the drug interaction may be fourth to sixth leading cause for death in United States[3]. Diabetes mellitus - a metabolic disorder characterized by elevated blood glucose levels requires lifelong treatment. Diabetic patients may also be affected with many other diseases like peptic ulcer, hypertension and fungal infections, which require prolong treatment. Diabetic nephropathy is a major long-term complication of diabetes mellitus. Clinically there is development of microalbuminuria with progression to overt proteinuria, increased in blood pressure and reduced renal function[4]. Excessive deposition of extracellular matrix protein in the glomeruli and subsequent mesangial expansion are the main structural alterations in diabetic nephropathy[5]. Therefore the present study was conducted on diabetic rats to assess the effect of candesartan on the pharmacokinetics and pharmacodynamics of sitagliptin.

MATERIALS AND METHODS

Animals

Study was conducted on diabetic rats (wistar strain) of either sex, weight range 180-220 g. The animals were procured from Mahaveer Enterprises, Hyderabad. They were housed under standard conditions (temperature of 28 ± 2ºC and 50 ± 2% relative humidity with 12 hr light / dark cycle) and provided with water *ad libitum*. The experiments were planned after the approval of Institutional Animal Ethical Committee (IAEC No: 10/VCP/DP/2013/06 dated 09/03/2013).

Drugs

Sitagliptin was obtained from Matrix pharmaceuticals, Hyderabad. Candesartan was obtained from Macs Biopharma, Hyderabad; Candesartan suspension was prepared using 0.5 % w/v sodium CMC as suspending agent.

EXPERIMENTAL PROCEDURES

Experimental Induction of Diabetes in Rats

A cohort of Wistar rats was fasted overnight for at least 8 hours. Diabetes was induced in each fasted rat by administering alloxan monohydrate (120 mg/ Kg body weight; intraperitoneal) in normal saline. The control cohort was administered normal saline intraperitoneally. At 3 days post-induction of hyperglycemia, blood glucose was assayed by the glucose oxidase method, using a glucose kit. Only those rats with established hyperglycemia (blood glucose >200 mg/dl) were included for subsequent treatment.

Experimental Study Design

The study was conducted in albino wistar rats of either sex weight range 180-

220gms were suitably divided in 5 groups of 5 rats in each.

Group I: Normal control group treated with vehicle (0.5%Sod CMC)
Group II: Diabetic control group treated with vehicle.
Group III: Diabetic rats treated with Sitagliptin (10mg/kg/p.o)[6]
Group IV: Diabetic rats treated with Candesartan (5 mg/kg/p.o)[7]
Group V: Diabetic rats treated with Sitagliptin (10mg/kg/p.o) and Candesartan (5mg/kg/p.o) Treatment was continued for 7 days and on the last day blood samples were collected through retro orbital puncture and centrifuged to separate plasma and stored at -20^{o}c.

Collection of blood samples

Blood samples were collected from all the group of rats in different intervals at 0min, 30min, 1hr, 2hr, 4hr and 6hr by retro orbital puncture of rat's eye using a capillary tube of size 3 inches. Sodium citrate (3.8% w/v) is used as an anticoagulant to collect blood. Animals were fasted for 12 hrs before the collection of blood. Blood samples were then centrifuged for 10 min at 3000 rpm to collect the plasma and were stored at -20^{0}C until analysis. The plasma samples were analyzed for blood glucose levels and also for plasma Sitagliptin concentration to study the effect of Candesartan on Pharmaco-dynamics and pharmacokinetic of Sitagliptin respectively.

Analysis of plasma sitagliptin concentrations

All the collected blood samples in single and multiple day study were analyzed for sitagliptin concentrations using High Pressure Liquid Chromatography using the reported method[8].

Preparation of standard solution and calibration standards

Stock solution of Sitagliptin was prepared in methanol at a concentration of 1mg/ml and was kept at -20^{0}C.This stock solution was diluted with methanol to obtain the concentration required for preparation of standard working solutions. Sitagliptin working solutions were in the range of 0.1µg/ml-10µg/ml.

HPLC Conditions

Phenomenex (250×4.6mm) C18 5µm reverse phase analytical column was used. The mobile phase consisted of Buffer and Acetonitrile (30Mm: pH 5.8) in the ratios of 55:45 v/v. Before Use, the mobile phase was filtered by using it through a 0.45µm filter and the filtrate is degassed by using bath sonicator. The mobile phase was pumped at an isocratic flow of 1ml/min at room temperature. The peaks were determined using a detector set at a wavelength of 254nm. All the procedures were performed at ambient temperature.

Extraction procedure

The plasma samples were collected from all groups of rats. To the sample add 2ml of mobile phase and allow it for 15min sonication and centrifuged for 10 min at 3000 rpm. After centrifugation, the supernatant was transferred into a new tube, to the 2ml of supernatant liquid add 2ml of mobile phase from this 20 µl was injected into the HPLC.

Statistical Analysis

All the experimental Values are expressed as mean ± SD. The Blood Glucose levels were compared with diabetic control value at each time point using One-Way ANOVA followed by post dunnet test. The statistical significance was judged at the 0.05 probability level. Statistical analysis was carried out using demo version of Graph Pad Prism.

RESULTS

Diabetes mellitus was successfully induced in the rats by the administration of alloxan monohydrate (120mg/kg body weight, i.p) and was confirmed by the elevation of blood glucose levels from 77.8 to 230.5 mg/dl.

Effect of Candesartan on pharmacodynamics of Sitagliptin

The blood samples from all the groups of rats on 1st and 7th day of treatment at different intervals and were analyzed for fasting glucose levels to assess the effect of Candesartan on pharmacodynamics of Sitagliptin diabetic rats and the results were showed in tables 1-2.

In diabetic rats after single dose treatment with sitagliptin, the plasma blood glucose levels were calculated at various time intervals. The blood glucose levels were reduced maximum at 4th hr and were found to be 110.25 mg/dl. In the single dose treatment of candesartan the plasma blood glucose levels were similar with those of diabetic control ($P > 0.05$), whereas in the combination of drugs sitagliptin and candesartan the blood glucose levels were reduced maximum at 4th hr and was found to be 123.3 mg/dl and were statistically significant ($P < 0.05$) when compared with sitagliptin alone (table 1).

In diabetic rats after multiple dose treatment with sitagliptin, the plasma blood glucose levels were calculated at different time intervals. The blood glucose levels were reduced maximum at 4th hr and was found to be 71.20 mg/dl and the blood glucose levels of candesartan alone group at 4th hr was 250.63 mg/dl. Whereas in combination with candesartan (sitagliptin 10 mg/kg and candesartan (5mg/kg) it was 75.47 mg/dl and were statistically significant ($P < 0.05$) when compared with sitagliptin alone (table 2).

In multiple dose interaction study, treatment with Sitagliptin at a dose of 10mg/kg the serum Creatinine and albumin levels were reduced to 1.56 mg/dl and 3.3 mg/dl, whereas with candesartan, those were found as 1.38 and 3.8 mg/dl and were in combination with sitagliptin and candesartan the plasma Creatinine levels were 1.26 and 4.0 mg/dl respectively. (Table 3).

Effect of Candesartan on pharmacokinetics of Sitagliptin

The effect of candesartan on pharmacokinetics of sitagliptin was studied in single and multiple day interaction. The blood samples from all the groups of rats on 1st and 7th day of treatment at different intervals and were analyzed for sitagliptin concentrations and the results of estimated pharmacokinetic parameters were showed in tables 4-5.

Single dose interaction study

The plasma sitagliptin concentrations in the groups of sitagliptin alone and in combination with candesartan were found to be similar and no statistically significant difference was observed ($P > 0.05$) (table 4).

Multiple dose interaction study

The plasma sitagliptin concentrations in the groups of sitagliptin alone and in combination with candesartan were found to be similar and no statistically significant difference was observed ($P > 0.05$) (table 5).

DISCUSSION

Diabetes is a syndrome characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins and increases the risk in cardiovascular diseases The increasing prevalence of type-2 diabetes itself may confer 75-90% of the excess risk of enhancing micro vascular complications like diabetic retinopathy, nephropathy, neuropathy and macro vascular complications like coronary diseases, hypertension, cardiac myopathy, cerebro vascular diseases and peripheral vascular diseases. Both

antihypertensive and oral hypoglycemic drugs are being increasingly used in many therapeutic areas. These drugs are important for controlling hypertention, heart failure and diabetic nephropathy and are being administered increasingly at earlier stages in these conditions.

The study was conducted to assess the single and multiple dose drug interaction between the sitagliptin and candesartan in alloxan induced diabetic rats. The present study was aimed at evaluating the effect of candesartan on pharmacokinetics and pharmacodynamics of sitagliptin in alloxan induced diabetic rats.

Pharmacodynamic interaction

In diabetic rats after single dose treatment with sitagliptin, the plasma blood glucose levels were calculated at various time intervals. The blood glucose levels were reduced maximum at 4^{th} hr and were found to be 110.25 mg/dl. In the single dose treatment of candesartan the plasma blood glucose levels were similar with those of diabetic control ($P > 0.05$), whereas in the combination of drugs sitagliptin and candesartan the blood glucose levels were reduced maximum at 4^{th} hr and was found to be 123.3 mg/dl and were statistically significant ($P < 0.05$) when compared with sitagliptin alone.

Pharmacokinetic interaction

The plasma sitagliptin concentration in the groups of sitagliptin alone and in combination with candesartan were found to be similar and no statistically significant difference was observed ($P > 0.05$).After single dose treatment of Sitagliptin (10mg/kg) in presence of candesartan (5mg/kg) the pharmacokinetic parameters AUC and AUMC were increased when compared to Sitagliptin (10mg/kg) in presence of candesartan (5mg/kg) and no statistically significant difference was observed ($P > 0.05$).

Multiple dose drug interaction study in diabetic rats

Pharmacodynamic interaction

In diabetic rats after multiple dose treatment with sitagliptin, the plasma blood glucose levels were calculated at different time intervals. The blood glucose levels were reduced maximum at 4th hr and was found to be 71.20 mg/dl and the blood glucose levels of candesartan alone group at 4th hr was 250.63 mg/dl. Where as in combination with candesartan (sitagliptin 10 mg/kg and candesartan (5mg/kg) it was 75.47 mg/dl and were statistically significant ($P < 0.05$) when compared with sitagliptin alone.

Pharmacokinetic interaction

The plasma sitagliptin concentration in the groups of sitagliptin alone and in combination with candesartan were found to be similar and no statistically significant difference was observed ($P > 0.05$).After multiple dose treatment of Sitagliptin (10mg/kg) in presence of candesartan (5mg/kg) the pharmacokinetic parameters AUC and AUMC were increased when compared to Sitagliptin (10mg/kg) in presence of candesartan (5mg/kg) and no statistically significant difference was observed ($P > 0.05$).

Renoprotective activity of angiotensin II receptor antagonist (candesartan)

In diabetic rats after multiple dose treatment with sitagliptin, the serum Creatinine and albumin levels were 1.56 mg/dl and 3.3 mg/dl and were 1.38 and 3.8 mg/dl with candesartan whereas those were changed to 1.26 and 4.0 mg/dl respectively in the presence of both sitagliptin and candesartan. It indicates the potentiation of

renoprotective effect of candesartan in sitagliptin.

The present study demonstrated that combination of an angiotensin II receptor antagonist (candesartan) and DPP4 inhibitor (sitagliptin) showed no statistical significant change in pharmacokinetic parameters and the plasma glucose reductions were also similar in sitagliptin alone and in combination with candesartan, but attenuated the increase in albuminuria of experimental diabetes. The combination of sitagliptin and candesartan showed significant increase in albumin levels and decrease in creatinine levels. Angiotensin converting enzyme inhibitors or angiotensin II receptor antagonist has potent renoprotective actions.

The results of this study demonstrated that the combination of sitagliptin and candesartan have significant effect on reduction of Creatinine and improved plasma concentration of albumin.

CONCLUSION

The present study results suggest that the pharmacokinetic interactions were not observed after single and multiple dose treatment with sitagliptin and candesartan. The combination of sitagliptin with candesartan resulted in equal antidiabetic potential of sitagliptin as compared to sitagliptin alone. On pharmacokinetic interaction of sitagliptin with candesartan has shown similar effect on bioavailability of sitagliptin as a single drug.

The creatinine levels were decreased in combination of sitagliptin and candesartan group when compared to alone sitagliptin and candesartan groups. The albumin levels were increased in combination of sitagliptin and candesartan group when compared to alone sitagliptin and candesartan groups.

In conclusion, the results of the present study showed that the combination of sitagliptin and candesartan compared to mono therapy have better renoprotective effects in diabetic groups. The combination also attenuated the progression of diabetic nephropathy by slowing the microalbuminuria. Further studies have to be done for dosage adjustment of sitagliptin in patients receiving sitagliptin and candesartan.

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Table 1. The blood glucose levels in rats treated with Sitagliptin alone and in presence of Candesartan Single Dose study

Group	0 hr	30 min	1 hr	2 hr	4 hr	6 hr
Normal Group	77.8±0.45	77.71±1.25	78.7±2.56	81.9±1.89	81.4±3.61	82.0±2.98
Diabetic control	229.3±6.8	230.5±6.60	233.5±6.5	238.0±6.62	243.0±7.00	245.0±7.00
Sitagliptin (10mg/kg)	219.0±0.12	210.01±0.70**	192.3±1.78**	167.6±4.33 ***	110.25±3.43 ***	138.51±4.10 ***
Candesartan (5mg/kg)	230.4±5.80	229.6±6.32	231.7±5.81	226.7±4.76	225.8±1.57	226.9±6.9
Candesartan (5mg/kg) Sitagliptin (10mg/kg)	223.3±1.77	215.7±1.32 **	200.5±1.40 **	175.4±0.63 ***	123.84±0.69 ***	145.68±0.69 ***

All the values are expressed as mean ±S.D (n=5) **p<0.01, ***p<0.001 vs diabetic control.

Table 2. The blood glucose levels in rats treated with Sitagliptin alone and in presence of Candesartan Multiple Dose study

Group	0 hr	30 min	1 hr	2 hr	4 hr	6 hr
Normal Group	77.8±0.45	77.71±1.25	78.7±2.56	81.9±1.89	81.4±3.61	82.0±2.98
Diabetic control	255.47±17.49	256.19±17.99	257.65±16.47	259.78±16.90	262.49±16.03	265.19±15.57
Sitagliptin(10mg/kg)	178.24±4.53	168.43±2.27 **	152.47±6.09 **	131.34±5.05 ***	71.20±3.04 ***	100.48±3.40 ***
Candesartan(5mg/kg)	253.64±17.32	254.87±16.37	250.39±17.32	248.43±16.73	250.63±16.53	248.79±15.34
Candesartan(5mg/kg) sitagliptin(10mg/kg)	180.35±0.98	171.45±1.17 **	150.17±2.28 **	139.48±2.63 **	75.96±2.78 ***	110.31±4.40 ***

All the values are expressed as mean ±S.D (n=5) ** p<0.01, *** p<0.001 vs diabetic control.

Table 3. The serum creatinine and albumin levels in diabetic rats treated with Sitagliptin alone and in presence of Candesartan Multiple Dose study

Name of group	Seventh day	
	Creatinine levels	Albumin levels
Normal control	1.2±0.03	4.2±0.38
Diabetic control	3.13±0.03	2.56±0.72
Sitagliptin(10mg/kg)	1.56±0.20 (-39.06) ***	3.3±0.4 (+28.90) **
Candesartan(5mg/kg)	1.38±0.05 (-55.91) ***	3.8±0.51 (+48.43) **
Sitagliptin(10mg/kg) Candesartan(5mg/kg)	1.26±0.06 (-78.19) ***	4.0±0.27 (+56.25) **

All the values are expressed as mean ±S.D (n=5) **p<0.01, ***p<0.001 vs diabetic control. -ve sign indicates reduction in Blood glucose levels, + ve sign indicates increase in Blood glucose levels.

Table 4. The Pharmacokinetics of Sitagliptin in presence & absence of candesartan Single Dose Study

Pk parameters	Sitagliptin (10mg/kg)	Sitagliptin (10mg/kg) + candesartan (5mg/kg)
Vol area (ml)	44.227	46.90
Vol area (ml/kg)	234.50	221.13
$t_{1/2}$(h)	8.87	9.61
CL (ml/hr)	3.66	3.18
CL (ml/hr/kg)	18.30	15.93
T_{max} (h)	4	4
C_{max} (µg/ml)	22.73	23.67
AUC 0-t (µg/ml*h)	170.2	181.78
AUC 0-α (µg-hr/ml)	546.38	627.46
AUMC (0- t)	926.10	1007.71
AUMC (0- α)	8003.20	9865.91
MRT(hr)	14.64	15.72

Table 5. Multiple dose interaction study

Pk parameters	sitagliptin (10mg/kg)	sitagliptin (10mg/kg) + candesartan (5mg/kg)
Vol area (ml)	49.24	49.20
Vol area (ml/kg)	272.63	269.98
$t_{1/2}$(h)	10.91	11.34
CL (ml/hr)	3.12	3.00
CL (ml/hr/kg)	15.62	15.03
T_{max} (h)	4	4
C_{max} (µg/ml)	33.89	33.99
AUC 0-t (µg/ml*h)	169.57	172.95
AUC 0-α (µg-hr/ml)	639.89	665.22
AUMC (0- t)	931.27	937.54
AUMC (0- α)	11163.72	11947.61
MRT(hr)	17.44	17.96