Research Article

Assessment of Non Nucleoside Reverse Transcriptase Inhibitor (Efavirenz) on the Pharmacokinetics of Sitagliptin in Diabetic Rabbits

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ABSTRACT

Treatment of HIV infection is a multi-drug issue. Not only are there drugs for the treatment of HIV but also concomitant drugs for opportunistic infections, complications arising from the anti-retroviral therapy and other conditions related to a chronic disease. HIV infection and treatment has been associated with the development of insulin resistance, glucose intolerance and diabetes. Besides, there can be co-morbid situations of HIV infection and diabetes. 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. The aim of the present study was to evaluate the influence of efavirenz (anti retroviral drug) on pharmacokinetics of sitagliptin (antidiabetic drug) in diabetic rabbits. The blood samples were collected at different intervals, the blood plasma levels were measured by validated HPLC method. The pharmacokinetic parameters like $t_{1/2}$, AUC, clearance, T_{max}, C_{max} of Sitagliptin with and without combination of efavirenz treatment was determined. There was no change in the pharmacokinetic parameters in presence of Efavirenz indicates the absence of pharmacokinetic interaction.

Keywords: Efavirenz, Sitagliptin, Diabetes, HIV infection.

INTRODUCTION

Polypharmacy is very common practice for the patients suffering with chronic diseases such as diabetes mellitus and HIV infection, and thus leads to the undesirable potent. Drug-drug interactions (pharmacodynamic and/or pharmacokinetic) which can alter the safety and efficacy profile of a drug in many ways. Recent reports reveals that drug interactions played a vital role in reported adverse events and that majority of the drugs withdrawn for safety reasons from the US market were related with significant drug-drug interactions.[1,2] The importance of this fact is further emphasized by increased post marketing adverse event reports by 240% over the last decade.[3] Diabetes mellitus is a metabolic disorder that needs treatment for prolonged periods and maintenance of normal blood glucose level is very important in this condition, since both hyperglycemia as well as hypoglycemia is unwanted phenomenon.[4,5] Since many studies suggested that PI therapy[6,7] is linked to the development of diabetic complications, it is of importance to propose therapeutic strategies with fewer side effects. Frequently prescribed antiretroviral drugs belong to the class of non nucleoside reverse transcriptase inhibitors (NNRTIs) in HIV-infected patients. Efavirenz is commonly used NNRTIs for the treatment of HIV- infection. Non Nucleoside reverse transcriptase inhibitors are to be improving the metabolic complications in HIV-infected patients.[8,9] In this contest, there are more chances of co administration of non nucleoside reverse transcriptase inhibitors with oral hypoglycemic drugs in patients with concurrent type 2 diabetes mellitus and HIV infection which may leads to potent drug-drug interactions. Based on this background, formerly we have conducted a preliminary study to investigate the effect of Efavirenz on the pharmacodynamic activity of sitagliptin in rats (normal and diabetic) with respect to blood glucose levels only. However, determination of insulin along with blood glucose levels would be a more precious and dependent approach to conclude a clear pharmacodynamic interaction scenario in the view of clinical and scientific stand-point.

MATERIALS AND METHODS

Drugs and Chemicals

Sitagliptin and Efavirenz were obtained as gift samples from Mylan Pharmaceuticals, Hyderabad and Aurobindo pharma Ltd. Hyderabad. Alloxan monohydrate was purchased from LOBA Chemie (Mumbai, India).

Animals

Study was conducted on healthy Albino Wistar rats of either sex, weight range 200-250 g .The animals were procured from Mahaveer enterprises, Hyderabad. All rats were kept for acclimatization for seven days prior to start the study. Animals were subjected to a constant daily cycle of 12 hours of light and 12 hours of darkness (06:00-18:00), constant temperature (21 ± 3 ºC) and relative humidity of 55 ± 15 %. Rats had access to commercial pelleted non-sterilised chow and normal tap water ad libitum, except during fasting access to food was restricted. Diabetes was induced in rats by the administration of alloxan monohydrate in two doses, i.e. 100 mg and 50 mg/kg bd. wt. intraperitoneally for two

consecutive days[10]. After 72 h, samples were collected from rats by orbital puncture of all surviving rats, and the serum 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. All the experiments were carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Environment and Forest, Government of India and the experimental protocol has been approved by the IAEC.

Drug administration

Route of administration: Per oral

Vehicle: Antiretroviral drugs were suspended in sodium CMC for oral administration. Sitagliptin solution was prepared by dissolving it in 5% gum acacia. All drugs were administered to respective groups by oral gavage method.

Experimental protocol: Rabbits weighing 1.5 - 2.0 kg were employed in the present study. The rabbits were fasted for 18 hr prior to the experiment with water ad libitum. A group of five rabbits were employed. The study was planned and designed in four groups.

Group –I: Diabetic rabbits treated with sitagliptin (7 mg/kg/po)

Group-II: Diabetic rabbits treated with efavirenz (42 mg/1.5kg/po)

Group-III: Diabetic rabbits treated with efavirenz (42 mg/1.5kg/po) and sitagliptin

(7 mg/kg/po)

Group-IV: Diabetic rabbits treated with efavirenz (42mg/1.5 kg/po) for 7 days and on 8th day received sitagliptin (7 mg/kg/po).

The same group of rabbits was repeated with a washout period of one week after every treatment.Blood was collected from each animal at time intervals of 0.0, 1.0, 2.0, 3.0, 4.0, 8.0, 16.0 and 24.0 hours. The time points for the pharmacokinetic studies were carefully selected to get a comprehensive picture of the pharmacokinetics of test product in rabbits. Plasma was separated after centrifugation for 15 minutes at 4000 rpm. The collected plasma was used to quantify sitagliptin after extraction and to determine sitagliptin concentration levels by validated HPLC method.

RESULTS

The effect of efavirenz on pharmacokinetics of sitagliptin was studied with single dose and multiple dose treatment. The blood samples were collected at different time intervals and analysed for sitagliptin concentration and the results of estimated pharmacokinetic parameters was represented in Table 1. The mean plasma concentration profile of sitagliptin was represented in Table 2 and Figure 1.

Table 1: Pharmacokinetic parameters (Mean ± SD) of Sitagliptin alone and in combination with single dose and multiple dose treatment of Efavirenz in diabetic rabbits

PK parameters	Sitagliptin (µg/ml)	Efavirenz + Sitagliptin (SDT) (µg/ml)	Efavirenz + Sitagliptin (MDT) (µg/ml)
Tmax(hr)	3.0 ± 0.63	3.28 ± 0.41	3.0 ±0.63
Cmax (ug/ml)	3.98 ± 1.06	3.36± 0.38	3.75± 0.58
AUC_{0-t} (ug.hr/ml)	13.87 ± 2.91	14.74 ± 4.73	13.89 ± 4.27
$AUC_{0-\infty}$(ug.hr/ml)	14.18 ± 3.35	14.99± 4.72	14.06± 4.39
MRT hr	5.02 ± 1.07	5.55± 0.81	5.26± 0.31
K_{el} hr^{-1}	0.23 ± 0.07	0.21 ± 0.07	0.22 ± 0.05
$t_{1/2}$ hr	3.41 ± 1.20	3.6 ± 1.35	3.51 ± 0.90
Vd L/kg	6.28 ± 1.47	7.34± 2.55	8.21± 1.79
Cl L/hr	1.50 ± 0.29	1.67 ± 1.12	1.77 ± 0.57

SDT, Single dose treatment; MDT, Multiple dose treatment

The plasma concentration-time profiles of sitagliptin and combination with single dose and multiple dose treatment of efavirenz were found to be similar. The mean Cmax was found to be 3.98 ± 1.06, 3.36 ± 0.38 and 3.75 ± 0.58 µg/ml and the Tmax was found to be 3.0, 3.28 and 3.0 hr for sitagliptin and with combination of single dose and multiple dose treatment of efavirenz respectively. This shows that the rate and extent of absorption were found to be comparable between the sitagliptin alone and in combination with single dose and multiple dose treatment of efavirenz. The mean $AUC_{0-\infty}$ was found to be 14.18 ± 3.35, 14.99 ± 4.72 and 14.06 ± 4.39 µg.hr/ml, mean half-life was found to be 3.41± 1.20, 3.60 ±1.35 and 3.51 ± 0.90 hr and mean clearance was found be 1.50 ± 0.29, 1.67 ± 1.12, 1.77 ± 0.57 L/hr in sitagliptin alone and in combination with single dose and multiple dose treatment of efavirenz respectively and no statistically significant difference was observed at (P>0.05).

Table 2: Mean plasma concentration levels of Sitagliptin alone and in combination with single dose and multiple dose treatment of Efavirenz in diabetic rabbits

Time (hr)	Sitagliptin (µg/ml)	Efavirenz + Sitagliptin (SDT) (µg/ml)	Efavirenz + Sitagliptin (MDT) (µg/ml)
0	0.000	0.000	0.000
1	0.713	0.701	0.719
2	2.336	1.958	1.832
3	3.432	3.314	2.944
4	1.183	1.478	1.462
8	0.584	0.569	0.638
16	0.105	0.208	0.099
24	0.034	0.019	0.029

SDT: Single dose treatment; MDT: Multiple dose treatment.

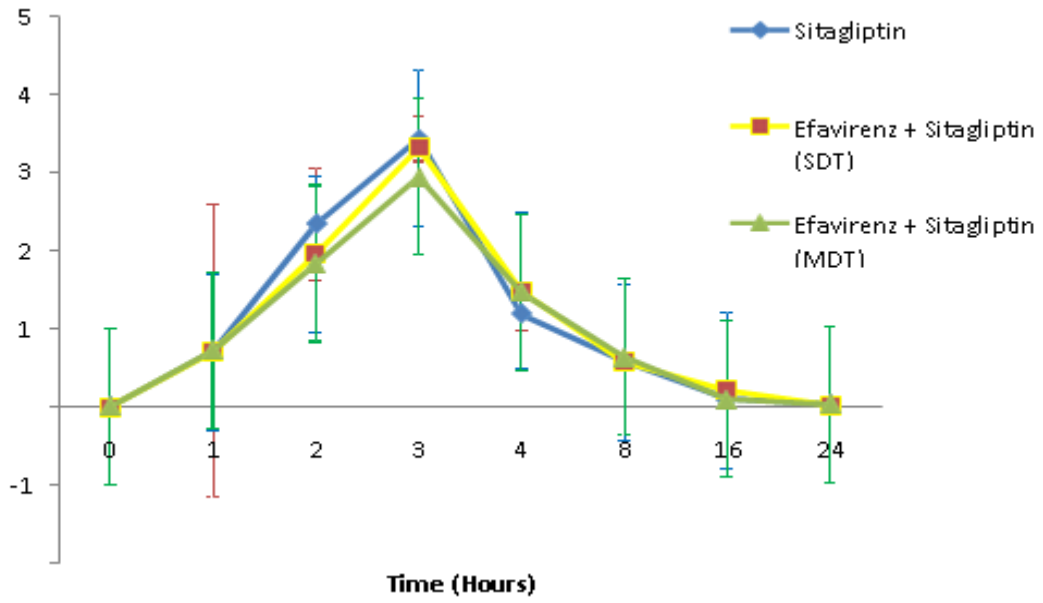

Figure 1: Mean plasma concentraion levels of Sitagliptin alone and in combination with single dose and multiple dose treatment of Efavirenz in diabetic rabbits

DISCUSSION

To validate the effects of efavirenz in non-rodent model, rabbits were selected to carry out pharmacokinetic interaction studies, since this model is suitable for collection of sufficient quantities of blood for adequate number of samples.

In vitro studies have shown that efavirenz inhibits CYP2C9, CYP2C19 and CYP3A4 with inhibition constant (ki) values (8.5-17 µM) in the range observed efavirenz plasma concentrations.[11] *In vitro* and *in vivo* studies demonstrated that efavirenz induces CYP3A4 activity in a concentration and time dependent manner.[12,13] Clinical drug-drug interaction studies showed that efavirenz decreased the systemic exposure of several CYP3A4 substrates, such as amprenavir, indinavir and methadone[14] in addition to the CYP2C9 and CYP2C19 substrates.[15] *In vitro* studies indicated that the primary enzyme responsible for the limited metabolism of sitagliptin was CYP3A4, with contribution from CYP2C8.[16]

The pharmacokinetic data clearly indicate that efavirenz has not altered the onset of action (Tmax), and the overall plasma exposure (AUC) and peak concentration (Cmax) of sitagliptin and $t_{1/2}$ indicating no significant pharmacokinetic interaction. The plasma concentration of sitagliptin in the groups of sitagliptin alone and in combination with single dose and multiple dose of efavirenz were found to be similar and no statistically significant difference was observed (P>0.05). Maximum plasma concentration was achieved at 3 hr representing the consistency between pharmacodynamic and

pharmacokinetic results. Therefore, these experimental findings explicitly convince that there is no significant pharmacokinetic interaction between efavirenz and sitagliptin. The results indicated the absence of pharmacokinetic interaction between sitagliptin and efavirenz in diabetic rabbits, which indicates no occurrence in humans.

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