



Relative potency of protease inhibitors on glucose-insulin homeostasis, hemoglobin and glycosylated hemoglobin in normal rats

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Abstract

The present study has been carried out to investigate the relative potency of protease inhibitors (indinavir, ritonavir and atazanavir) on glucose-insulin homeostasis, hemoglobin and glycosylated hemoglobin in normal rats following oral administration for a period of 30 days. The effects of protease inhibitors were compared with normal control rats. Blood samples were collected from retro orbital puncture and the parameters observed are blood glucose, insulin, total hemoglobin, glycosylated hemoglobin and body weight. The insulin resistance index and percent beta cell function were determined by homeostasis model assessment (HOMA-1 and HOMA-2) models. Percent insulin sensitivity was determined by HOMA-2 model. Indinavir and ritonavir were significantly ($p < 0.05$) elevated the blood glucose, insulin, insulin resistance index and glycosylated hemoglobin values and decreased the total hemoglobin, beta cell function, insulin sensitivity and body weights when compared to control rats. The alterations associated with indinavir are more compared to ritonavir treated rats. Atazanavir has not shown any significant effect on any parameter when compared to control rats, except increase in body weight. From this study we conclude that glucose-insulin homeostasis disorders associated with protease inhibitors are not a class specific, but are drug specific. Thus, it can be concluded that atazanavir is having safe profile compared to indinavir and ritonavir with respect to glucose-insulin homeostasis. Indinavir and ritonavir are having potent tendency to alter the glucose homeostasis and insulin profile to produce the events related to type 2 diabetes. So care should be taken when the indinavir and ritonavir are prescribed for their clinical benefit in diabetic patients.

Keywords: Protease inhibitors, indinavir, ritonavir, atazanavir, insulin

Introduction

Highly active antiretroviral therapy (HAART) is a combination of at least three antiretroviral agents, two nucleoside reverse transcriptase inhibitors (NRTIs) plus a third agent, a protease inhibitor (PI), a non-nucleoside reverse transcriptase inhibitor (NNRTI) or possibly a third NRTI [1]. HIV PIs have contributed greatly to reductions in HIV-associated morbidity and mortality over the last decade and remain a cornerstone of HAART [2]. Despite their success, however, it is now clear that the use of these drugs is associated with the development of disturbing metabolic changes that greatly increase the lifetime risk for cardiovascular disease and other complications [3, 4]. Among the many metabolic perturbations that occur as a result of Human Immuno Deficiency Virus (HIV) infection and its treatment, alterations in normal glucose-insulin homeostasis remain a particularly prevalent and alarming clinical change in affected patients. Insulin resistance, impaired glucose tolerance and type 2 diabetes are conditions that are increasingly described in HIV-1 infected subjects receiving highly active antiretroviral therapy, especially with protease inhibitors [5]. Much of concern is due to the recognition of the long-term complications of insulin resistance and hyperglycemia and understood is the context of the growing worldwide epidemic of type 2 diabetes mellitus and other macro vascular complications.

The use of protease inhibitors have been associated with alterations in glucose homeostasis, the percentage of patients affected and the severity of the perturbations in insulin sensitivity and/or insulin resistance differ among the PIs [6]. It thus remains unclear whether PIs act in a class-specific manner or whether there are effects that are specific to individual drugs. From a clinical standpoint, understanding the degree of induction/impact on glucose homeostasis and/or insulin homeostasis of a given PI relative to other PIs is advantageous in weighing the benefits of efficacy versus the adverse effects on glucose metabolism. There are few prospective studies of PIs and glucose homeostasis or insulin sensitivity, and differences in design and methodology make direct comparison of single PIs among such studies difficult. So the design of the study is an important consideration in the comparative assessment of the effects of PIs on glucose-insulin homeostasis and hematological parameters. Furthermore, HIV infection and/or diabetes itself, therapy-induced restoration to health, immune reconstitution, and changes in body composition may contribute to alterations in insulin sensitivity. The use of normal rodent model in this study allows for the isolation of the direct effects of PIs. So the present study was planned to investigate the relative potency of protease inhibitors (indinavir, ritonavir and atazanavir) on blood glucose, insulin, insulin resistance index, insulin sensitivity index, β -cell function, total hemoglobin, glycosylated hemoglobin and body weight in normal rats.

Materials and methods***Drugs and chemicals***

Protease inhibitors (indinavir, ritonavir and atazanavir) are the gift samples from Aurobindo Pharma Ltd (Hyderabad, India). Glucose kits (Span diagnostics) were purchased from local pharmacy. All other reagents/chemicals used were of analytical grade.

Animals

Albino rats of either sex of 6 to 8 weeks of age, weighing between 300-350 g were used in the study. They were procured from National Institute of Nutrition, Hyderabad, India. They were maintained under standard laboratory conditions at an ambient temperature of $25 \pm 2^\circ\text{C}$ and $50 \pm 15\%$ relative humidity with a 12-h light/12-h dark cycle. Animals were fed with a commercial pellet diet (Rayan's Biotechnologies Pvt Ltd., Hyderabad, India) and water *ad libitum*. 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. 516/01/A/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).

Selection of doses and preparation of oral test suspensions

In clinical practice, protease inhibitors in therapeutic dose will be administered orally as antiretroviral therapy. Hence, human therapeutic doses were extrapolated to rat based on body surface area [7] were used and administered orally. Protease inhibitor suspension was prepared by suspending each PI in 3% CMC-Na [8] for oral administration to rats.

Experimental design

Animal were divided into four groups of six animals each.

- | | | |
|-----------|---|--|
| Group I | : | Control |
| Group II | : | Treated with indinavir-72 mg/kg bd. wt., orally |
| Group III | : | Treated with ritonavir-18 mg/kg bd. wt., orally |
| Group IV | : | Treated with atazanavir-36 mg/kg bd. wt., orally |

Each respective group was treated successively for 30 days.

Collection of blood samples

After the completion of experimental regimen (30 days), blood samples were withdrawn from retro orbital plexus of each fasted rat to analyze the various biochemical parameters including glucose.

Estimation of blood glucose

These blood samples were analyzed for blood glucose by GOD/POD method [9] using commercial glucose kits.

Estimation of plasma insulin

Plasma insulin concentrations were determined by radioimmunoassay kit (Pharmacia, Uppsala, Sweden) with a betamatic counter (Cronex, Dupont, France). The kit included human insulin as standard and ^{125}I labeled human insulin antibody, which cross-reacts similarly with rat insulin.

Estimation of total hemoglobin and glycosylated hemoglobin (HbA1C)

Total hemoglobin was estimated by the cyanomethaemoglobin method [10] and glycosylated hemoglobin (HbA1C) was estimated by the modified method [11, 12].

Determination of insulin resistance index, β -cell function and insulin sensitivity index by homeostasis model assessment (HOMA)

HOMA-1: the original HOMA model

The insulin resistance index and β -cell function were assessed by the homeostasis model assessment were calculated as follows [13]

$$\begin{aligned}\text{Insulin resistance} &= (\text{FPI} \times \text{FPG})/22.5 \\ \%\text{Beta function} &= (20 \times \text{FPI})/ (\text{FPG} - 3.5)\end{aligned}$$

Where FPI is fasting plasma insulin concentration ($\mu\text{u/ml}$) and FPG is fasting plasma glucose (mmol/l).

HOMA-2: model the updated HOMA model (i.e., the computer model)

The insulin resistance index, β -cell function and insulin sensitivity were obtained by the program HOMA Calculator v 2.2.2 [14].

Data and statistical analysis

Data were expressed as mean \pm SEM. The data was subjected to one way ANOVA followed by student's 't' test to determine the statistical significance.

Results and Discussion

Effect of protease inhibitors on blood glucose

Indinavir and ritonavir produced statistically significant ($p < 0.05$) hyperglycemia when compared to control group and the elevation of glucose levels is more with indinavir compared to ritonavir treated group. Atazanavir has not shown any significant difference in glucose level when compared to control group. Results were shown in table 1.

Table 1. Effect of protease inhibitors on blood glucose and insulin in normal rats

Treatment	Glucose (mg/dL)	Insulin ($\mu\text{u/mL}$)
Normal control	84.67 \pm 0.42	15.63 \pm 0.32
Diabetic control	247.46 \pm 2.32	06.94 \pm 0.87
Normal + Indinavir	141.00 \pm 2.35*	20.17 \pm 0.37*
Normal + Ritonavir	120.33 \pm 0.95*	19.65 \pm 0.26*
Normal + Atazanavir	83.50 \pm 0.34	15.25 \pm 0.16

Values were given as mean \pm SEM (N=6)

*Statistically significant from normal control, $p < 0.05$

Effect of protease inhibitors on insulin

Indinavir and ritonavir produced statistically significant ($p < 0.05$) elevation in insulin levels when compared to control group and the elevation is more with indinavir compared to ritonavir treated group. Atazanavir has not shown any significant difference in insulin level when compared to control group. Results were shown in table 1.

Effect of protease inhibitors on insulin resistance index

Indinavir and ritonavir produced statistically significant ($p < 0.05$) insulin resistance index (IRI) when compared to control group and the IRI is more with indinavir compared to ritonavir treated group. Atazanavir has not shown any significant difference in IRI when compared to control group. Results were shown in table 2. The IRI of protease inhibitors determined by HOMA-1 and HOMA-2 models confirmed the potent insulin resistance associated with indinavir and ritonavir.

Table 2. Effect of protease inhibitors on insulin resistance index in normal rats

Treatment	Insulin resistance index	
	HOMA-1	HOMA-2
Normal control	3.27 ± 0.07	01.98 ± 0.04
Normal + Indinavir	7.02 ± 0.17*	04.82 ± 0.06*
Normal + Ritonavir	5.54 ± 0.11*	03.84 ± 0.04*
Normal + Atazanavir	3.22 ± 0.04	01.50 ± 0.07

Values were given as mean ± SEM (N=6)

HOMA-1: homeostasis model assessment by calculation method

HOMA-2: homeostasis model assessment by program HOMA Calculator v 2.2.2

*Statistically significant from normal control, $p < 0.05$

Effect of protease inhibitors on % beta-cell function

The percent beta-cell function was significantly decreased with indinavir and ritonavir treated groups compared to control group ($p < 0.05$), and it is more with indinavir treated group compared to ritonavir group. But atazanavir treated group has not shown any significant effect on percent beta-cell function compared to control group. HOMA-1 and HOMA-2 models confirmed the same result as indinavir and ritonavir are associated with decrease in beta cell function. Results were shown in table 3.

Table 3. Effect of protease inhibitors on %beta-cell function and %insulin sensitivity index in normal rats

Treatment	% Beta-cell function		% Insulin sensitivity index (HOMA-2)
	HOMA-1	HOMA-2	
Normal control	260.50 ± 0.02	173.65 ± 2.81	50.97 ± 0.98
Normal + Indinavir	93.16 ± 0.12*	79.13 ± 2.45*	35.33 ± 0.63*
Normal + Ritonavir	116.93 ± 0.05*	99.82 ± 0.94*	39.38 ± 0.53*
Normal + Atazanavir	262.54 ± 0.02	174.55 ± 1.40	52.28 ± 0.51

Values were given as mean ± SEM (N=6)

HOMA-1: homeostasis model assessment by calculation method

HOMA-2: homeostasis model assessment by program HOMA Calculator v 2.2.2

*Statistically significant from normal control, p<0.05

Effect of protease inhibitors on insulin sensitivity index (ISI)

The ISI was determined by HOMA-2 model. The ISI was significantly decreased with indinavir and ritonavir treated groups compared to control group (p<0.05), and it is more with indinavir treated group compared to ritonavir group. But atazanavir treated group has not shown any significant effect on ISI compared to control group. Results were shown in table 3.

Effect of protease inhibitors on total hemoglobin, glycosylated hemoglobin and body weight

The total hemoglobin and body weight were significantly decreased with indinavir and ritonavir treated groups compared to control group (p<0.05), and it is more with indinavir treated group compared to ritonavir group. The glycosylated hemoglobin was significantly increased with indinavir and ritonavir treated groups compared to control group (p<0.05). But atazanavir treated group has shown increase in total hemoglobin (not significant) and body weight (significant, p<0.05), and decrease in glycosylated hemoglobin (not significant) compared to control group. Results were shown in table 4. Disorders of glucose metabolism have been reported in individuals infected with HIV [15-17]. Several studies have reported a prevalence of diabetes of 2% to 7% among HIV-infected patients receiving protease inhibitors [17-19] and an additional 16% having impaired glucose tolerance [17]. The incidence of diabetes mellitus in HIV-infected patients has been estimated to range from 1% to 10% in various studies [20-22]. However, the contribution and/or potency of each specific protease inhibitor to promote the diabetes mellitus were unknown. But it is essential from the clinical point of view to understand the relative potency of protease inhibitors on glucose-insulin homeostasis for the better and rational therapy of HIV-infected patients.

Table 4. Effect of protease inhibitors on total hemoglobin, glycosylated hemoglobin and body weight in normal rats

Treatment	Total hemoglobin (g/dL)	Glycosylated hemoglobin (%)	Body weight (g)
Normal control	260.50 ± 0.02	173.65 ± 2.81	350.97 ± 0.98
Normal + Indinavir	93.16 ± 0.12*	79.13 ± 2.45*	335.33 ± 0.63*
Normal + Ritonavir	116.93 ± 0.05*	99.82 ± 0.94*	339.38 ± 0.53*
Normal + Atazanavir	262.54 ± 0.02	170.55 ± 1.40	356.28 ± 0.51*

Values were given as mean ± SEM (N=6)

*Statistically significant from normal control, $p < 0.05$

Regulation of glucose metabolism is a key aspect of metabolic homeostasis and insulin is the predominant hormone influencing this regulatory system. Insulin plays a key role in the maintenance of glucose homeostasis and provides the major modulator of glucose storage and utilization [23]. Glucose was measured as a metabolic control of insulin action. The impairment of glucose homeostasis and increase in plasma glucose level are associated with diabetes. Insulin resistance refers to the reduced action of circulating insulin to induce uptake of glucose into cells, where glucose then serves as a major substrate for cellular function. Insulin resistance is accepted as the underlying fundamental defect that predates and ultimately leads to the development of type 2 diabetes mellitus [24]. Insulin resistance is recognized as the core component of the metabolic syndrome, having been described by Reaven [25] as the 'Common Soil' from which all metabolic diseases develop. Based on these concepts the present study was planned to investigate the relative potency of protease inhibitors (indinavir, ritonavir and atazanavir) on blood glucose, insulin, insulin resistance index, insulin sensitivity index, β -cell function, total hemoglobin, glycosylated hemoglobin and body weight in normal rats.

The homeostatic model assessment (HOMA) is a validated method to measure insulin resistance and insulin sensitivity from fasting glucose and insulin. The original model HOMA1-IR, has been widely used, especially in epidemiological and clinical studies. Recently, the model was updated with some physiological adjustments to a computer version (HOMA2-IR) providing a more accurate index [26]. The HOMA2-IR is a more accurate representation of the metabolic process because it models the feedback relationship between insulin and glucose in the various organs in the body [27]. In our study we have used these HOMA models, which are more reliable and validated methods with respect to insulin resistance index, insulin sensitivity index and β -cell function.

The elevated insulin levels in the face of increased glucose levels suggest an insulin resistant state [28]. Diabetes related glucose intolerance is characterized by an increase in insulin resistance and alterations in insulin clearance, insulin sensitivity of hepatic and peripheral tissues. In the present study, we also observed the increased level of insulin, glucose and insulin resistance index and decreased level of beta cell function and insulin sensitivity index in indinavir and ritonavir treated rats, indicating the potency of these PIs to promote the diabetes and to exacerbate the existing diabetes, if any. Our results are consistent with the earlier reports that indinavir and ritonavir are associated with hyperglycemia, insulin resistance and alterations in insulin sensitivity in pre-clinical and clinical studies [29-34]. Decreased total hemoglobin content observed in indinavir and ritonavir treated rats might be due to increased formation of glycosylated hemoglobin. Generally total hemoglobin level is much below the normal level in diabetic condition [35] and glycosylated hemoglobin level has been reported to be increased in patients with diabetes mellitus [36]. It was reported that during diabetes mellitus, the excess of glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin [37]. The level of glycosylated hemoglobin is always monitored as a reliable index of glycemic control in diabetes [38]. Elevated levels of glycosylated hemoglobin and reduced levels of total hemoglobin observed in indinavir and ritonavir treated rats reveal that these animals resemble the diabetes condition compared to control group. In addition, ritonavir and indinavir treated animals shown decreased in body weights when compared to control group further indicating the alteration in metabolic pathways. But in our study, atazanavir has not shown any significant effect on any parameter when compared to control rats, except increase in body weight, indicating it is safe drug with respect to glucose-insulin homeostasis.

Conclusion

In conclusion this study demonstrated that the glucose-insulin homeostasis disorders associated with protease inhibitors are drug specific, but not a class-specific manner. Indinavir is the most potent among the protease inhibitors tested, followed by ritonavir, which have potency to promote the development of diabetes mellitus and/or exacerbation of existing diabetes mellitus. Atazanavir was found to be a safe protease inhibitor with respect to glucose-insulin homeostasis. So care should be taken when the indinavir and ritonavir are prescribed for their clinical benefit, especially in diabetic patients. However the present study warrants further studies to find out the relevance of these findings in human beings and in diabetic condition(s).

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