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HIV Protease Inhibitors and Insulin Resistance: Lessons from In Vitro, Rodent and Healthy Human Volunteer Models

Paul W Hruz
Department of Pediatrics and Department of Cellular Biology and Physiology, Washington University School of Medicine, St Louis MO

Abstract

Purpose of review—While the use of HIV protease inhibitors (PIs) is linked to the development of insulin resistance and other metabolic changes that greatly increase the risk for cardiovascular disease, the molecular mechanisms responsible remain incompletely understood. This review summarizes recent advances that have been made in understanding the relative contributions of individual PIs to both acute and chronic insulin resistance together with newly identified cellular mediators.

Recent findings—Individual PIs alone and in combination have differing propensities to induce insulin resistance, reflecting relative differences in both affinities for identified molecular targets and pharmacokinetic profiles. Several of the most recent PIs approved for clinical use or in development appear to be less likely to induce insulin resistance. In addition to direct effects on GLUT4 activity, oxidative stress, proteosome inhibition, altered adipokine levels and SOCS-1 signaling have been implicated.

Summary—A better understanding of the propensity of individual PIs to produce insulin resistance will allow the tailoring of individual treatment plans based upon overall risk for diabetes. The elucidation of the molecular mechanisms for alterations in glucose homeostasis will facilitate the development of newer generations of PIs that maintain their clinical efficacy without contributing to the development of diabetes mellitus and other pro-atherogenic effects.

Keywords

Insulin resistance; euglycemic clamp; rodent model; adipocytes; glucose transport; drug toxicity

Introduction

Several review articles discussing the ability of HIV protease inhibitors (PIs) to influence insulin sensitivity have been published within the last year [1–5]. Most of the previous discussions have appropriately considered the full spectrum of metabolic abnormalities encountered in the setting of highly active antiretroviral therapy (HAART). This includes the ability of PIs to directly and/or indirectly alter body fat composition [6,7], lipid profiles [8], adipocytokine levels [9], and mediators of inflammation [10]. In addition, the potential for nucleoside reverse transcriptase inhibitor (NRTI)-induced changes in mitochondrial function to also influence insulin sensitivity has recently gained greater attention [11]. The complexity of antiretroviral regimens employed in clinical practice including frequent changes in therapies have hindered efforts to identify the direct effects of individual PIs on tissue-specific and

Contact Information: Paul W Hruz MD PhD, 660 South Euclid Avenue, Campus Box 8208, St Louis MO 63110, Phone: 314-286-2797, Email: hruz_p@kids.wustl.edu

whole-body glucose homeostasis. However, in the search for safer anti-retroviral agents, increased understanding of the direct molecular targets of PIs offers great potential in ongoing efforts to design and test for newer generations of drugs that in addition to overcoming the problem of viral resistance also have improved safety profiles. This is particularly important given the expectations for long-term PI exposure in aging HIV infected populations. This review will focus primarily upon the most important recent studies that have been able to characterize the direct influence of individual PIs on glucose homeostasis. The concordance of the evidence from various model systems (*in vitro*, animal, and healthy human volunteer) will be evaluated. Where known, the specific cellular targets altered by these drugs will be discussed. This information will then be placed back into the context of antiretroviral treatment regimens in HIV infected patients.

Clinically observed abnormalities in glucose homeostasis in relation to PI use

The clinical use of HIV protease inhibitors in HIV positive humans is clearly associated with the development of measurable changes in insulin sensitivity [12]. In many patients, when combined with underlying genetic risk, environmental factors, and other components of highly active antiretroviral therapy, these changes are sufficient for the manifestation of overt diabetes mellitus [13,14]. While significant efforts to characterize the incidence, progression, and molecular mechanisms underlying this induction of insulin resistance continue, many questions remain. It has become increasingly clear that individual PIs differ in their ability to induce insulin resistance in treated patients. The molecular and/or pharmacokinetic basis for these differences is only now beginning to be understood.

In much of the published clinical literature, “insulin resistance” is considered as the net sum of alterations in each of the individual insulin responsive tissues (primarily liver, adipose tissue and skeletal muscle) and changes in insulin secretion from pancreatic beta cells. This is the result of the practicality of assessing glucose homeostasis via fasting blood sugar levels (which primarily reflect hepatic insulin sensitivity) and responses to an oral glucose challenge (which reflect both insulin secretion and peripheral glucose disposal). Many studies have relied upon indirect estimation of insulin sensitivity with tools such as homeostasis model assessment (HOMA) or quantitative insulin sensitivity check index (QUICKI). A growing number of studies have used the hyperinsulinemic euglycemic clamp technique, the established gold standard measure of insulin sensitivity. Euglycemic clamps, by their intrinsic design, do not assess beta cell function. Only a few studies have directly assessed pancreatic function *in vivo* using hyperglycemic clamps [15,16]. The use of tracer dilution experiments to assess hepatic glucose production has also been reported in only limited number of studies [16,17]. The ability to identify differences in the abilities of individual PIs to affect each of these tissues may allow tailoring of PI-based treatment regimens based upon the molecular defects present.

Comparison of *in vitro* effects of individual PIs on insulin sensitivity

The use of an increasing number of *in vitro* model systems has greatly assisted efforts to characterize the effects of individual PIs on insulin signaling and glucose transport. These studies have allowed study of cell-specific effects under static drug concentrations independent of systemic changes in homeostatic mechanisms. However, in the interpretation of these experiments, proper distinction needs to be made between acute versus chronic changes. Acute experiments (i.e. those occurring within minutes of drug exposure) are more likely to aid in the identification of direct molecular targets, whereas chronic treatment studies are more likely to mimic cellular changes that are observed in treated patient populations. Both are important for the development of means to prevent and/or treat PI-mediated insulin resistance.

The first studies to establish that PIs (specifically indinavir, ritonavir and amprenavir) are capable of acutely inducing insulin resistance were performed in cultured 3T3-L1 adipocytes and Xenopus oocytes, where GLUT4 was identified as a direct target of indinavir [18]. These findings were later confirmed at physiologically relevant drug levels in primary rat adipocytes [19]. Additional tissues that have also shown PI-mediated insulin resistance *in vitro* include rat skeletal muscle (indinavir) [20], L6 myocytes (nelfinavir, indinavir, saquinavir)[21], HepG2 cells (nelfinavir and indinavir) [22–24], human SBG adipocytes (ritonavir) [25], and human subcutaneous adipocytes (lopinavir/ritonavir) [26].

Even accounting for the varying concentrations of PIs that were used in these studies, significant differences have been observed in the magnitude of the effects of individual drugs on glucose transport. Atazanavir (even with boosting levels of ritonavir) appears to have little to no effect on glucose uptake [26]. Studies in primary rat adipocytes have provided insights into the structural basis for these differences [27]. There is also evidence that some of the newer PIs in development also have little effect on glucose uptake. For example, in cultured adipocytes, the novel diethyl-phosphonate containing PI GS-8374 does not acutely inhibit glucose uptake [28].

There have been conflicting reports on the ability of PIs to affect intracellular signaling pathways. Alterations in insulin signaling (such as IRS-1 [25] and AKT [22]) have been observed most frequently following chronic drug exposure (i.e. after hours to days) as opposed acute exposure (i.e. within minutes) which produces impaired glucose uptake despite normal signaling and GLUT4 translocation [18]. The differences that have been reported in the effects of PIs on β-cell function can also be understood in consideration of the length of drug exposure [15,29–32]. Many of these studies also further reflect inherent differences between individual PIs which may be due either to effects on distinct molecular targets or differing affinities for the same cellular proteins.

While GLUT4 inactivation is an established primary mechanism by which some PIs acutely alter glucose uptake *in vitro* [18], the relationship between this effect and changes in other pathways is less clear. Reports that some PIs also inhibit the proteosome [1] have provided a potential link to the development of lipodystrophy and dyslipidemia which are indirect contributors to insulin resistance. In addition, Bashan and colleagues have demonstrated in the 3T3-L1 model system that PIs (nelfinavir) induce oxidative stress [33]. Capeau and colleagues have also provided evidence that the generation of reactive oxygen species (ROS) following 24–48 hour exposure of cultured adipocytes to ritonavir contribute to changes in inflammatory mediators and adipocytokine levels [34]. In general, these studies all show a remarkable consistency in the differing effects of individual PIs on insulin sensitivity. Specifically, indinavir and ritonavir appear to be the most toxic whereas atazanavir is without effect.

Insights from Rodent model systems

Correlation between these *in vitro* effects and those observed *in vivo* have been provided by a number of studies in healthy lean rodents given PIs acutely by intravenous infusion during hyperinsulinemic euglycemic clamps. In addition to precise titration of steady state serum drug levels, this model allows the investigation of PI-mediated effects in a genetically and environmentally homogeneous background. The degree of insulin resistance induced in this model is in general agreement with the observations that have been made regarding the degree of impaired glucose uptake *in vitro* and insulin resistance observed in human patients. Specifically, indinavir, lopinavir and ritonavir have each been shown to produce the highest reductions in glucose disposal [35,36]. In contrast, peripheral glucose disposal is not altered by either atazanavir or tipranavir under these same conditions [37]. Due to the acute nature of

these experiments, compensatory changes in serum adipokine or lipid levels did not occur. In agreement with *in vitro* data, GS-8374 also does not alter glucose disposal in this model [38].

Underscoring the importance of underlying genetic risk, Carper et al have shown that in susceptible rodent (the Zucker rat), chronic administration of ritonavir resulted in an acceleration in the progression to overt diabetes [39]. This was accompanied by detectable changes in TNF-α, SREBP-1, and IRS-2. Increased SOCS-1 expression was implicated in the pathogenesis of these effects.

The use of rodent models has also assisted in the evaluation of consequences of PI-mediated glucose transport blockade in insulin-responsive tissues. For instance, we have recently reported that ritonavir, but not atazanavir, acutely blocks glucose transport into rodent myocardium and that in the setting of heart failure this is accompanied by significant changes in contractile function [40]. In the future, the use of animal models with specific disruption and/or augmentation of cellular pathways implicated in the pathogenesis of PI-mediated insulin resistance is likely to provide more definitive evidence for the specific contribution of individual molecular targets.

Healthy human volunteer studies

The relevance of the *in vitro* and animal model studies discussed above to human disease has been clearly demonstrated in the investigation of acute PI-mediated changes in healthy HIV negative human volunteers. Specifically, some but not all PIs have been shown to alter peripheral glucose disposal under hyperinsulinemic euglycemic clamp conditions. The earlier studies by Noor and colleagues which examined both 4 week [41] and single dose [42] treatment with indinavir convincingly demonstrated significant reductions in glucose disposal. These studies have now been extended to look at additional PIs that are currently in widespread clinical use. These experiments have demonstrated that the effects of PIs on insulin sensitivity are not the same for all drugs within this class. While full dose ritonavir [43,44] and lopinavir [45] are able to acutely induce insulin resistance in this model system, amprenavir [44] and atazanavir [46] are without effect. Again, attention to the duration of drug exposure is important in understanding the molecular basis for these observations. While single dose [45] and 5 days of treatment [46] with lopinavir significantly reduced glucose disposal, no effect was found with 4 weeks of exposure to this PI [47]. These differences may be due to compensatory changes in adiponectin levels in the chronically treated subjects [48]. This contrasts with the observation in HIV-positive patients with lipodystrophy that adiponectin levels are decreased [49].

Based upon *in vitro* dose response profiles of individual drugs, the differing propensities for the induction of peripheral insulin resistance with individual PIs may be related at least in part by the serum drug levels achieved during treatment relative to the binding affinity of cellular targets such as GLUT4 [36]. PIs in which the K_d for GLUT4 and C_{max} are similar will have significant effects on insulin sensitivity. With K_d/C_{max} ratios greater than 5–10, drug-induced changes in glucose disposal are likely to be minimal.

HIV infected patients

A number of recent studies in HIV-infected patients have provided additional insight into the drug-specific effects of PIs on insulin sensitivity and have added to the understanding that some, but not all, of the changes are reversible with changes in antiretroviral therapy. For example, in the Lipostop study, adipose tissue inflammation improved without detectable changes in fat mass within 6 months after PI withdrawal [50]. Prospective analysis of treatment naïve patients started on HAART revealed that insulin resistance (as determined by HOMA) was significantly associated with the use of indinavir [51]. In this study, hepatitis C co-infection was also associated independently with the development of insulin resistance. Confirming the

evidence from the previously discussed model systems that atazanavir has a more favorable metabolic profile, improvement in insulin sensitivity (as determined by euglycemic clamps) was demonstrated in HIV positive men with dyslipidemia following the switch from stable PI containing antiretroviral regimens to atazanavir/ritonavir [52].

Validation of the role of implicated cellular mediators of reduced insulin sensitivity in HIV infected patients is also continuing to emerge. For example, PIassociated changes in markers of oxidative stress have been recently been reported in HIV infected patients [53]. In a recent long-term study comparing 3 different antiretroviral regimens (PI, NNRTI, and PI + NNRTI), insulin resistance was detected within one month only in the PI-based group. However, after 5 years, the same degree of insulin resistance was also found in all three groups [7]. These latter effects, which were gradual and progressive, are likely mechanistically distinct from those observed with shorter-term PI exposure.

Since compensatory hyperinsulinemia allows the maintenance of euglycemia in the setting of peripheral insulin resistance, the progression to overt diabetes mellitus reflects the development of relative β-cell failure. The degree to which PIs directly contribute to altered β-cell function in treated patients, as has been demonstrated in both *in vitro* and rodent models, remains unclear. In the Swiss Cohort Study, current PI use both alone and in combination with NRTIs was associated with an increased prevalence of diabetes whereas cumulative exposure to these drugs was not [14]. Examination of individual PIs revealed that indinavir was most directly associated with diabetes. As with many of the clinical studies to date, power was not sufficient to determine significant changes with other individual PIs, although many did have a positive trend toward increased diabetes risk.

While the utility of diagnosing patients with the “metabolic syndrome” versus consideration of the individual components of this syndrome has been called into question both in the general population [54] and in HIV infected patients [5], the prospective INITIO trial has demonstrated that HIV infected patients on antiretroviral therapy who fulfill the criteria for having metabolic syndrome are at a significantly higher risk for progression to overt diabetes [55]. Although in this study no association between PI use and the metabolic syndrome was found, significant correlations with PI treatment have been reported by others [56]. The study by Mondy and colleagues also failed to show a clear link between PI use and “metabolic syndrome” [57]. As noted above, due to the number of different antiretroviral regimens used, limited statistical power with individual PIs likely contributes to some of these differences. In addition, since insulin resistance does not necessarily coexist with the other components of the “HIV-associated metabolic syndrome” [58], showing definitive associations between individual PIs and insulin resistance in heterogeneous clinical populations is challenging. Nevertheless, these studies emphasize that traditional risk factors play a predominant role in the progression to impaired glucose tolerance.

Conclusion

In vitro, animal model, and healthy human volunteer studies have greatly assisted efforts to understand the contribution of individual PIs to the emergence of insulin resistance and/or overt diabetes mellitus in patients receiving PIs as part of HAART. While these changes have been validated in many of the recent clinical studies, when compared to traditional risk factors and the influence of disease-related cellular changes (such as HIV infection itself or hepatitis C co-infection), the direct PI-mediated effects on glucose homeostasis have been more difficult to indentify and characterize. Thus, the ongoing use of *in vitro*, animal, and healthy human volunteer studies will continue to aid efforts to prevent and/or treat PI-induced effects on insulin sensitivity.

Acknowledgements

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