Insulin resistance induced by antiretroviral drugs: Current understanding of molecular mechanisms

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Abstract

The increase in incidence of HIV infection continues to be a major public health problem across the world, but more especially in sub-Saharan Africa. Treatment with highly active antiretroviral therapy (HAART) has improved the prognosis of patients with AIDS, but it has also increased the incidence of various metabolic disorders, in particular insulin resistance accompanied by dyslipidaemia, hyperglycaemia and lipodystrophy. This is often accompanied by frank type 2 diabetes and increased mortality from cardiovascular disease. It is important to understand the mechanistic basis for these side-effects as the incidence of these is likely to increase as the rollout of antiretroviral drugs continues.

Introduction

Twenty-six years after the acquired immune deficiency syndrome (AIDS) was first reported, human immunodeficiency virus (HIV) infection still remains a major global challenge, especially in the southern part of sub-Saharan Africa. In South Africa, there were six million people infected, and more than 400 000 had died from AIDS by 2007.[1-3]

Currently, the most potent treatment for HIV infection is highly active antiretroviral therapy (HAART). This therapy is a combination of two or more antiretroviral drugs. These drugs work as inhibitors which contain five classes including entry inhibitors (EIs), nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleotide reverse transcriptase inhibitors (NNRTIs), integrase inhibitors (IIs) and HIV protease inhibitors (HPIs). This treatment can extend the lifespan of HIV-infected patients by suppressing HIV viral load, increasing CD4+ T-cell[a] counts,[b] and reducing opportunistic infections associated with AIDS.[4, 5]

Unfortunately, besides improving patient prognosis, these drugs also result in metabolic abnormalities, in particular insulin resistance accompanied by dyslipidaemia,[c] hyperglycaemia and lipodystrophy.[6,7] Consequently, patients are also at high risk of developing premature cardiovascular morbidity and type 2 diabetes mellitus.[8-11]

A substantial number of clinical and epidemiological studies have shown that HPIs play a major role in inducing metabolic disorders in HAART-treated patients.[6,12,13] It appears that HPIs impair insulin-mediated metabolism in adipose tissue and result in insulin resistance.[14] However, the mechanisms for these side-effects are largely unknown. It is important to understand the mechanistic basis for these side-effects as the incidence of these is likely to increase as the rollout of antiretroviral drugs continues.

### The insulin signalling pathway (see Figure 1)

Signalling through the insulin receptor pathway is critical for the regulation of blood glucose levels by insulin.[15] In the post-prandial phase, in response to glucose, the pancreas releases insulin into the bloodstream. Insulin then binds to its receptor. The heterotetrameric (2 α- and 2 β-subunits) receptor is a ligand-activated tyrosine kinase. The binding of insulin leads to autophosphorylation of the receptor β-subunit on tyrosine residues. This then activates the intrinsic tyrosine kinase domain and leads to tyrosine phosphorylation of its substrate, insulin receptor substrate-1 (IRS-1). Typically, phosphotyrosine residues interact with a particular type of protein domain, Src homology 2 (SH2) domain with high specificity and affinity. The tyrosine phosphorylated IRS-1 binds to the SH2 domain of phosphoinositide 3-kinase (PI3-K) leading to activation of this enzyme and conversion of phosphatidylinositol 4,5-biphosphate (PIP_2) into phosphatidylinositol 3,4,5-triphosphate (PIP_3). The bound PIP_3 causes translocation of both PIP_3-dependent protein kinase (PDK) and PKB, also known as Akt. This then allows PDK to phosphorylate and then activate (PKB). The activation of PKB/Akt is necessary for the final steps leading to glucose transport. This then results in the migration of glucose transporter 4 (GLUT4) from the cytoplasm to the cell membrane to facilitate the uptake of extracellular glucose. Some of the detailed final steps leading to the translocation of GLUT4 are still unclear and is still the subject of intensive research.

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[a] T cell or T helper cell is a subgroup of lymphocytes; a type of white cell which functions to activate other immune cells and express surface protein CD4
[b] This method is to detect HIV infection as HIV targets cells that express CD4+ T cells and reduces the circulating levels
[c] Dyslipidaemia usually manifests as elevated triglycerides and cholesterol

Figure 1: Insulin signalling pathway
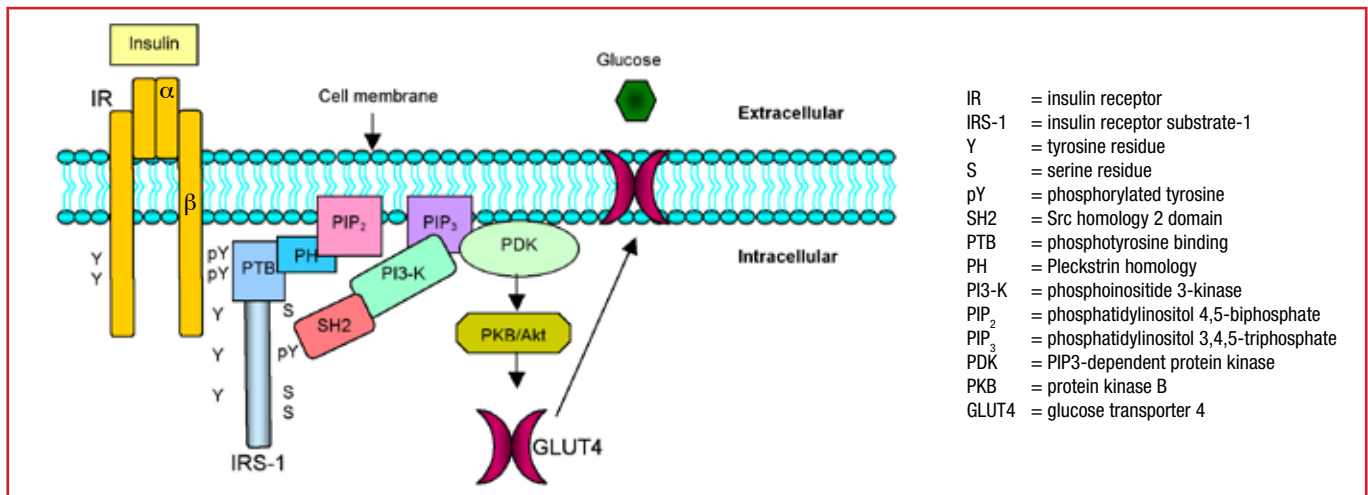
Insulin resistance and HIV protease inhibitors (HPIs)

The mechanism of insulin resistance induced by antiretroviral drugs is not well-understood. Insulin resistance occurs when normal insulin levels are inadequate to stimulate glucose uptake in the insulin signalling pathway in insulin-sensitive tissues such as liver, muscle and subcutaneous adipose tissue. The insulin resistance manifests as hyperinsulinaemia, hyperglycaemia and dyslipidaemia (hypertriglyceridaemia).

It has emerged that one class of the antiretroviral drugs, the HPIs, plays a critical role in inducing the side-effects. HPIs are potent competitive inhibitors of HIV aspartyl endopeptidase, an enzyme required for producing a mature HIV virion, such as saquinavir, indinavir, ritonavir, nelfinavir, amprenavir, lopinavir/ritonavir (Kaletra), fosamprenavir, atazanavir, tipranavir and darunavir.[16]

A clinical report revealed glucose intolerance and insulin resistance in HIV-positive patients treated with HPIs.[17] Moreover, the incidence of insulin resistance in HIV-positive patients is significantly higher in HPI-treated patients than in patients treated with NRTIs or NNRTIs,[18] even when one considers related factors such as demography and virology.[19] Furthermore, HPIs have been shown to induce insulin resistance in seronegative patients and in animal models,[20-23] implying a clear link between HPI use and insulin resistance. However, the underlying precise mechanisms have yet to be elucidated. The purpose of this review is to analyse the possible molecular mechanisms for the effects of the HPIs on the insulin signalling pathway.

HPIs impair the distal steps in the insulin signalling pathway

Initial studies examined the effects of antiretrovirals on both glucose transport and translocation of the intracellular glucose transporter, GLUT4. An early study of insulin resistance induced by HPIs demonstrated that the drug inhibits glucose uptake (measured by radioactive glucose uptake) without affecting GLUT4 translocation (assessed by immunostaining).[24] GLUT4 is considered to be the major transporter responsible for insulin-stimulated glucose disposal into adipose tissue, cardiac and skeletal muscle, and plays a critical role in whole-body glucose homeostasis.[25] In this particular study, it was reported that 3T3-L1 adipocytes treated with indinavir, an HPI, at 100 μM for 4 h displayed decreased glucose uptake. There

was no evidence for any effects on early insulin signalling events such as insulin receptor and IRS-1 tyrosine phosphorylation or on the translocation of GLUT4 to the cell surface.[24] In addition, indinavir did not have any effect on the activation of PKB/Akt. The effect of indinavir on glucose uptake was rapid and did not change even when indinavir was added 20 min after insulin stimulation indicating that the drug acted at a site distal to GLUT4 translocation.[24] Similar effects on glucose uptake were reported in rats and in HIV-negative patients treated with HPIs.[20,21,23]

However, in a subsequent study, it was reported that prolonged treatment (18 h) of 3T3-L1 adipocytes with nelfinavir, an HPI, at plasma concentrations (10 μM) impaired both GLUT4 translocation and glucose uptake.[26] Nelfinavir appeared to induce insulin resistance by inhibiting the stimulation of PKB/Akt serine 473 phosphorylation without any effect on the tyrosine phosphorylation of IRS-1. Nelfinavir also decreased expression of the lipolysis regulator, perilipin.[26] These effects of nelfinavir to impair PKB/Akt activation, GLUT4 translocation and glucose uptake were confirmed in a subsequent study. In this particular study, nelfinavir at 30 μM induced insulin resistance in 3T3-L1 adipocytes by inhibiting recruitment and activation of PI3-K, leading to impaired GLUT4 translocation and thus preventing insulin-stimulated glucose uptake.[27,28] These effects were not accompanied by changes in insulin receptor expression or insulin receptor tyrosine phosphorylation.[27] More recently, the effects of nelfinavir were studied in 3T3-L1 adipocytes expressing a GLUT4-green fluorescent protein (GFP) fusion protein to analyse transporter movement. Fusion of GLUT4 to GFP allows real time visualisation of glucose transporter movement under a fluorescent microscope. The authors of this study maintained that PKB/Akt activation was affected (i.e. inhibited) after insulin-stimulated generation of PIP$_3$, leading to impaired GLUT4 translocation because even when cellular levels of PIP$_3$ were raised artificially by using a constitutively activated PI3-K, thus bypassing the requirement for normal insulin stimulation, nelfinavir inhibited GLUT4 translocation.[29] When a membrane targeted constitutively active PKB is expressed, in the presence of nelfinavir, PKB is phosphorylated normally by PDK and is able to induce GLUT4 translocation. The authors concluded that nelfinavir interfered with the "sensing" of PIP$_3$ by PKB/Akt or the PIP$_3$-induced translocation of PKB/Akt based on the fact that even in cells expressing a constitutively activated PI3-K, nelfinavir was still able to block the activation of PKB/Akt.

Taken together, the results imply that nelfinavir has more profound effects on insulin signalling than indinavir. Indinavir inhibits glucose transport without affecting translocation, while nelfinavir inhibits both translocation and uptake, possibly at the level of PKB/Akt. The differences in the studies reviewed above may therefore have arisen from differences in the HPIs used or from the experimental methodology used to quantify GLUT4 translocation, which is often semi-quantitative and subjective, as compared to the measurement of glucose uptake.

The notion that nelfinavir has more profound effects is supported by studies on adipocytes that show that it induces oxidative stress.[30] Oxidative stress induces insulin resistance by activating serine kinases that lead to serine phosphorylation of IRS-1 and also by disrupting the subcellular distribution of PI3 kinase (i.e. decreased membrane localisation). This would in turn lead to decreased tyrosine phosphorylation of IRS-1 and consequently reduced PKB/Akt activation and hence decreased GLUT4 translocation.

Do HPIs impair the proximal steps of the insulin signalling pathway?

The studies reviewed above suggest that the effects of HPI occur downstream in the insulin signalling pathway in 3T3-L1 adipocytes, in rats and humans,[21,22,24] possibly at the level of PKB/Akt activation and beyond. However, there is evidence that HPIs possibly act at a more proximal level of the insulin signalling pathway. For example, in HepG2 hepatoma cells exposed to 100 µM indinavir for 48 h there was a 30–60% decrease in the insulin-stimulated tyrosine phosphorylation of IRS-1 and this was associated with decreased PI3-K activation and no change in insulin binding.[31] In a subsequent study, it was found that ritonavir at 10 µM decreased insulin receptor numbers in 3T3-L1 pre-adipocytes without any effect on binding affinity. This was accompanied by decreased IRS-1 tyrosine phosphorylation in response to insulin. [32]

A more recent study suggested that lopinavir, an HPI, inhibited IRS-1 phosphorylation in human adipocytes and resulted in a concentration-dependent decrease in glucose uptake.[33] It was proposed that the drug decreased the phosphorylation of IRS-1 directly since there was no effect observed on the phosphorylation of the insulin receptor β-subunit i.e. the decrease in IRS-1 phosphorylation was primary, rather than secondary to a change in receptor activation. Taken together, these data imply that some of the HPIs may impair the earlier steps in the insulin signalling pathway.

The differences in the studies reviewed above are likely to be due to differences in experimental design, cell types, types of HPIs used and durations of exposure. It is possible that, differences between the various drugs on protein and gene expression in cells are more likely to be unmasked after prolonged exposure, hence resulting in different final observations in the various studies. These differences will be exaggerated further when different cell lines are used. Overall, however, it appears that the final common pathway is an effect on glucose transport and that all of the HPIs reviewed above display some effect at one or more steps in the insulin signalling pathway.

Other factors involved in the insulin resistance induced by HPIs: oxidative stress, adapter proteins and adipokines

As mentioned above, some of the HPIs may increase oxidative stress.[30,34] HPIs may also alter chemokines, cytokines or adiponectin

production in human adipocytes and macrophages.[35] The induction of oxidative stress is known to cause insulin resistance.

Changes in the expression of regulatory proteins could also potentially explain the effects of HPIs. Suppressor of cytokine signalling-1 (SOCS-1) is one example of a known inducer of insulin resistance. SOCS-1 and the related protein suppressor of cytokine signalling-3 (SOCS-3) bind to the insulin receptor following activation and impair the ability of the insulin receptor to phosphorylate downstream substrates. In addition, SOCS proteins are able to target IRS-1 for degradation. In essence, increased expression of SOCS proteins will interfere with the signalling functions of the insulin receptor and IRS-1. In addition to the previously described effects on the signalling proteins in the early part of the insulin signalling pathway, it has also been shown that the expression of SOCS-1 was increased in rats exposed to indinavir for seven weeks.[36] In this study, chronic HPI exposure induced SOCS-1 expression in muscle, liver and adipose tissue. This was associated with an increase in the expression of tumour necrosis factor-α (TNF-α) and the downstream target sterol regulatory element-binding protein-1 (SREBP-1) along with decreased expression of insulin receptor substrate-2 (IRS-2). IRS-2 is another major substrate that mediates insulin action in the insulin signalling pathway.

TNF-α induces insulin resistance primarily by affecting the function of IRS proteins. It can also induce the expression of SOCS-1. As discussed above, the association of increased TNF-α in animals exposed to indinavir may have arisen from the induction by TNF-α or by another unknown mechanism.

In relation to IRS-1, TNF-α induces the activation of several serine kinases (c-Jun N-terminal kinase (JNK); inhibitor κB kinase (IKK)) leading to increased serine phosphorylation of IRS-1. Increased serine phosphorylation of IRS-1 converts it into an inhibitory protein of the insulin receptor, leading to insulin resistance. Therefore, in summary, TNF-α can induce insulin resistance by affecting IRS-1 function directly or by inducing the expression of SOCS-1.

HPIs have also been found to alter adipose tissue gene expression *in vivo*.[37] The mRNA expression levels of the CCAAT/enhancer-binding protein α, leptin, and adiponectin in HIV-positive patients treated with HPIs have been found to be significantly lower than in HPI-naive patients.[37] Since adiponectin levels are important in the whole body response to insulin, a lowering of adiponectin would affect glucose homeostasis adversely and oppose the actions of insulin.

Summary (see Table I)

The individual differences in the effects of HPIs observed in the different studies may be attributed to the different HPI drugs used in the studies and in experimental design. HPIs induce different effects on different cell lines.[38,39] Additionally, several HPIs demonstrate different results *in vitro* and *in vivo*.[40] The most consistent phenomenon observed has been the effect on glucose uptake, the most obvious physiological end result of insulin. Whether these phenomena arise primarily from effects on early steps in the insulin signalling pathway is not entirely clear. Recent studies[29,33] support the idea that HPIs affect a more proximal level of the insulin signalling pathway, in particular tyrosine phosphorylation of the insulin receptor and its substrate, IRS-1. This then leads to the secondary inhibition of downstream events in the insulin signalling

Table I: Summary of the effect of HPIs in the insulin signalling pathway

HPI Effects	References	Drug	Experimental model	Observations
1) The distal steps in the insulin signalling pathway	Murata et al (2000)[24]	Indinavir (100 µM) for 4 h	3T3-L1 adipocytes	Decreased glucose uptake
	Rudich et al (2001)[26]	Nelfinavir (10 µM) for 18 h	3T3-L1 adipocytes	Impaired GLUT4 translocation and glucose uptake by inhibiting stimulation of PKB serine 473 phosphorylation
	Ben-Romano et al (2004)[27] and Rudich et al (2005)[28]	Nelfinavir (30 µM) for 18 h	3T3-L1 adipocytes	Inhibited recruitment and activation of PI3-K, impaired GLUT4 translocation and glucose uptake
	Kachko et al (2009)[29]	Nelfinavir (30 µM) for 18 h	3T3-L1 adipocytes used a GLUT4-GFP chimeric construct	Interfered with the sensing of PIP3 by PKB/Akt and inhibited GLUT4 translocation
2) The proximal steps in the insulin signalling pathway	Schutt et al (2000)[31]	Indinavir (100 µM) for 48 h	HepG2 hepatoma cells	Decreased tyrosine phosphorylation of IRS-1 and PI3-K activation
	Cammalleri and Germinario (2003)[32]	Ritonavir (10 µM) for 11 days	3T3-L1 pre-adipocytes	Decreased insulin receptor expression and tyrosine phosphorylation of IRS-1
	Djedaini et al (2009)[33]	Lopinavir (10 µg/ml) for 48 h	Human adipocytes	Decreased tyrosine phosphorylation of IRS-1 and glucose uptake
3) Other factors	Ben-Romano et al (2006)[30]	Nelfinavir (30 µM) for 18 h	3T3-L1 adipocytes	Induced oxidative stress
	Lagathu et al (2007)[35]	Indinavir, amprenavir, lopinavir, ritonavir (10 µmol/l), atazanavir (4 µmol/l), nelfinavir (5 µmol/l) for 24 and 48 h	Human adipocytes and macrophages	Induced oxidative stress and altered chemokines, cytokines or adiponectin production
	Carper et al (2008)[36]	Indinavir (20 µM) for 7 weeks	Rats	Increased SOCS-1, TNF-α and SREBP-1 expression
	Chapparo et al (2005)[37]	Indinavir, nelfinavir, liponavir/ritonavir, ritonavir and saquinavir	Human adipose tissue biopsies	Altered adipose tissue gene expression

cascade, in particular, extracellular glucose uptake by GLUT4. With the exception of the studies cited, there is a paucity of studies that have examined the proximal steps in the insulin receptor signalling cascade, particularly at the early steps in the activation of the insulin receptor tyrosine kinase.

Conclusion

Insulin resistance is a major complication of treatment with antiretroviral drugs. With the increased longevity of patients following antiretroviral treatment, the incidence of these complications is likely to increase. An understanding of the molecular basis of these complications could lead to the development of diagnostic tests to predict the onset of the side-effects or alternatively to rational drug design. In conclusion, the studies reviewed above suggest that the manifestation of insulin resistance due to HPI treatment is likely to be associated with dysregulation of several cellular factors, specifically in the early steps in the pathway of insulin receptor signalling, in addition to alterations in GLUT4 transporter function. Alternatively, the perturbation of glucose transport may be secondary to inhibition of critical steps early in the signalling pathway.

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