HIV protease inhibitors block human preadipocyte differentiation, but not via the PPARγ/RXR heterodimer

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Abstract

A recent prospective clinical study has shown that antiviral therapy with HIV protease inhibitors (PIs) is associated with a syndrome of peripheral fat wasting (lipodystrophy) and disordered glucose and lipid metabolism (Carr et al. 1999). We have studied the effects of indinavir and saquinavir, two HIV protease inhibitors, on cultured primary human preadipocytes and report that these compounds inhibit their differentiation. However, we find that these agents do not inhibit either transcriptional activation or adipocyte P2 gene induction by the PPARγ/RXR nuclear receptor heterodimer. Together, our findings suggest that impaired adipogenesis is the basis of PI-associated lipodystrophy, but that this occurs via a PPARγ/RXR-independent mechanism.

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Introduction

The novel HIV protease inhibitors (PIs) are highly effective antiviral agents for the treatment of HIV infection (Danner et al. 1995, Hammer et al. 1997). However, a syndrome of peripheral fat wasting (lipodystrophy) in association with PI therapy has recently been described (Carr et al. 1998a, Carr et al. 1999). This syndrome is quite common (over 80%), progressive and associated with hyperlipidaemia and glucose intolerance. These metabolic complications are also seen in other forms of lipodystrophy (Jackson et al. 1997) and in mice with targeted ablation of adipose tissue (Moitra et al. 1998), suggesting that PIs may exert direct toxic effects on adipose tissue in vivo.

Adipocytes are derived from fibroblast-like preadipocyte precursor cells (Tontonoz et al. 1995). A key mediator of preadipocyte differentiation is the peroxisome proliferator-activated receptor-gamma (PPARγ) (Tontonoz et al. 1994a), a ligand-dependent transcription factor which is a member of the nuclear receptor superfamily. PPARγ enhances target gene transcription in preadipocytes and other contexts by binding to specific DNA response elements as a heterodimer with retinoid-X-receptor (RXR) (Tontonoz et al. 1994b). It has therefore been hypothesised that the function of the PPARγ/RXR heterodimer is inhibited by protease inhibitors, leading to lipodystrophy (Carr et al. 1998b).

In this study, we have examined the effects of the HIV protease inhibitors indinavir and saquinavir on the differentiation of primary human preadipocytes. We show that these antiviral agents inhibit both basal and ligand (BRL49653, LG100268)-stimulated preadipocyte differentiation, yet do not impair PPARγ/RXR-mediated induction of the adipocyte P2 target gene in preadipocytes or transcriptional activation by the receptor heterodimer. PIs might therefore cause lipodystrophy by inhibiting preadipocyte differentiation in vivo, but via a mechanism independent of the PPARγ/RXR heterodimer.

Materials and Methods

Indinavir and saquinavir were supplied by Merck and Roche respectively and dissolved in ethanol. Tissue culture reagents were obtained from Sigma unless otherwise stated. Preadipocytes were isolated from human breast adipose tissue by dissection into 1-2 mm³ pieces and digestion in Hanks Buffered Salts Solution supplemented with 2% w/v bovine serum albumin and 3 mg/mL type II collagenase. Cells were cultured to confluence in SC medium (DMEM-F12 supplemented with 1% v/v P/S, 2 mM L-glutamine, 33 µM biotin, 17 µM pantotenic acid, 10 µg/mL human apotransferrin, 0.2 nM T3, 100 nM dexamethasone and 500 nM bovine insulin) ± 0.1 µM BRL49653 or 0.1 µM LG100268 (Adams et al. 1997a). Glycerol-3-phosphate dehydrogenase enzyme activity, an established marker of adipogenesis (Tontonoz et al. 1994a), was assessed between 14 to 21 days post induction of differentiation as previously described (Adams et al. 1997a), and normalised to total lysate protein. Transient transfection assays were performed using the calcium phosphate technique in 24-well cultures of 293EBNA cells as
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Figure 1 Indinavir and saquinavir inhibit human preadipocyte differentiation. Confluent cells cultured in 6-well plates were treated with SF medium supplemented with vehicle or protease inhibitor in the presence of PPARγ (BRL49653) or RXR (LG100268)–specific ligands as shown. G3PDH enzyme activity, normalised to total protein concentration, is expressed relative to vehicle-treated cells. Enzyme activity was induced 10-fold and 8-fold by BRL49653 and LG100268 respectively, consistent with the known adipogenic activity of these ligands. The results shown represent the mean±S.E.M. of three independent estimations.

Cells were then treated with thiazolidinedione (BRL49653) or rexinoid (LG100268), which are PPARγ- and RXR-specific ligands respectively and known to enhance differentiation. Similar inhibitory effects of indinavir and saquinavir were observed following 14 days culture in SF medium supplemented with either 0.1 μM BRL49653 or 0.1 μM LG100268 (Fig. 1).

Protease inhibitors do not impair PPARγ/RXR signalling

The observation that indinavir and saquinavir inhibit preadipocyte differentiation in response to PPARγ- and RXR-specific ligands raised the possibility that PIs might impair PPARγ/RXR-dependent transcription. These antiviral agents were tested in transient transfection assays with the human PPARγ2 isoform and a reporter gene construct. Our results (Fig. 2a) indicate that the basal transcriptional activity of transfected PPARγ2 and endogenous RXR is not altered by saquinavir or indinavir. Furthermore, PPARγ2/RXR activation by BRL49653 and LG100268 was also unaffected by the presence of HIV protease inhibitors. Similar results were obtained when the human PPARγ1 isoform was tested (data not shown). Finally, we examined the effect of protease inhibitors on adiopocyte P2 (aP2) expression – a well recognised PPARγ/RXR target gene (Tontonoz et al. 1994c). Saquinavir and indinavir had no effect on either basal or BRL49653-induced aP2 mRNA levels in human preadipocytes (Fig. 2b).

Discussion

In this study, we show for the first time that HIV protease inhibitors can inhibit human preadipocyte differentiation (Fig. 1), with saquinavir having a greater inhibitory effect than indinavir. Whilst the rank order of potency of these two agents has not been directly compared in clinical studies, patients treated with a ritonavir/saquinavir combination are more prone to develop lipodystrophy than those treated with indinavir alone (Carr et al. 1998b). Together, these observations suggest that impaired adipogenesis may account for lipodystrophy associated with these antiviral agents in vivo. Given the role of adipose tissue in lipid and glucose metabolism (Tontonoz et al. 1995), it is possible that the hyperlipidaemia and glucose intolerance observed in PI-associated lipodystrophy are secondary to such impaired adipogenesis.

We find no evidence to support the hypothesis that this effect is mediated by a direct action of PIs on the PPARγ/RXR heterodimer, as neither indinavir nor saquinavir inhibited PPARγ/RXR reporter gene activation in transient transfection assays or aP2 mRNA induction in human preadipocytes (Fig. 2). These findings suggest that the antiadipogenic activity of protease inhibitors we have observed may result from perturbation of additional signalling pathways involved in preadipocyte differentiation. Moreover, the lack of effect on these compounds on PPARγ/RXR action predicts that the insulin-sensitising thiazolidinediones (the ‘glitazones’)
may even be effective in the treatment of HIV protease inhibitor-associated diabetes.

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