

In: **55th Annual Meeting of the American College of Veterinary Pathologists (ACVP) & 39th Annual Meeting of the American Society of Clinical Pathology (ASVCP)**, ACVP and ASVCP (Eds.)

Publisher: American College of Veterinary Pathologists & American Society for Veterinary Clinical Pathology, Middleton WI, USA

Internet Publisher: Publisher: International Veterinary Information Service (www.ivis.org), Ithaca, New York, USA.

Proteasomal Toxicity: Pathogenesis of HIV Protease Inhibitor-Induced Dyslipidemia and Lipodystrophy (13-Nov-2004)

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Introduction

HIV infection, if left untreated, produces a chronic disease course characterized by uncontrolled infections. The first classes of antiretroviral drugs developed to fight this disease were relatively toxic, and include nucleoside and non-nucleoside reverse transcriptases, and HIV protease inhibitors (PI). The most effective combinations of these agents, "highly active antiretroviral therapy" (HAART), have consistently been associated with a side-effect profile of likely related symptoms including dyslipidemia (hypertriglyceridemia and hypercholesterolemia), centripetal fat depot redistribution, lipodystrophy (subcutaneous adipose and fibrous tissue masses), and type II diabetes mellitus, the treatment of which has hitherto been only palliative and short lasting. Phase III clinical trials with the recently filed Bristol-Myers Squibb HIV PI, atazanavir, showed a marked reduction in the incidence and severity of these side effects when atazanavir was included as the main protease inhibitor in the HAART cocktail. The objective of these studies was to develop and test an hypothesis explaining the contribution of PIs to HAART dyslipidemia.

Hypothesis - Proteasome Inhibition is Key to HAART Dyslipidemia

To overcome the limitations inherent in using tissue explants obtained from HAART-treated patients, fibroblasts differentiated towards adipocytes and hepatoma cells were exposed to protease inhibitors *in vitro*. A series of analyses were undertaken to evaluate whether *in vitro* biochemical phenotypes recapitulate those effects observed in patients. Initial transcriptomic evaluation by principal components analyses (PCA) were used to demonstrate a clustering of atazanavir with control samples, whereas more toxic compounds including ritonavir and nelfinavir were distinctly separated from controls and atazanavir-exposed cells in chemogenomic space.

Further evaluation of genes contributing to this separation revealed consistently marked changes in transcripts of gene products involved in the synthesis of lipids in both adipocytes and hepatoma cells.

A unifying hypothesis linking these alterations with tissue growth, serum abnormalities and the pharmacology of aspartic acid protease inhibition, was that the proteasomal degradation of transcription factors such as sterol-regulatory element binding protein (SREBP) is directly inhibited by PIs. The inhibition of proteasome-mediated degradation of transcription factors would, according to this hypothesis, result in their sustained activity, and lead to the exaggerated synthesis of the genes and their products controlling lipid synthesis.

Experimental Support for the Proteasome Inhibition Hypothesis

To test this hypothesis, HepG2 cells were exposed to PIs with a range of side-effect potencies. Inhibition of proteasomal substrates by the PIs demonstrated a clear dose-response relationship and sustained elevation of SREBP was observed with more toxic inhibitors. Immunohistochemistry showed that SREBP translocated from the cytoplasm and accumulated in the nucleus. In parallel, the synthesis of lipids by HepG2 cells was stimulated. TG synthesis from [¹⁴C]-acetate was stimulated by ritonavir and nelfinavir at 3 - 30 uM, but atazanavir had no effect up to 100 uM.

Inhibition of the GLUT4 glucose transporter is an added burden for adipocytes

In contrast to hepatocytes, triglyceride synthesis was inhibited in 3T3 adipocytes, indicating the possibility of an additional component to PI toxicity in these cells. Each PI demonstrated a characteristic potency for inhibition of adipocyte

triacylglycerol (TG) accumulation (eg., ritonavir 3 - 10 uM, atazanavir 10 - 30 uM). Gene expression profiles in adipocytes showed that transcription factors, lipogenic enzymes, and other markers of differentiation were suppressed by PIs at levels inhibiting TG. Experiments with cultured cells differentiated towards adipocytes demonstrated clear discrimination in the inhibition of GLUT-4-mediated glucose uptake between PIs. Exceptionally, atazanavir did not inhibit GLUT4 at pharmacologically relevant concentrations.

Human adipose depots vary in their reliance on *de novo* fatty acid synthesis from glucose or fatty acid uptake from circulating triglycerides, regardless of the fatty acid source. Glucose is also required for the synthesis of the glycerol-phosphate precursor for fatty acid esterification into triglycerides. Therefore, PI-inhibition of the GLUT4 transporter and starvation of glucose as a fuel and substrate for triglyceride synthesis creates an additional burden in adipose tissue potentially contributing to lipoatrophy and fat redistribution.

GLUT4 is the main transporter in tissues responsible for most of whole body glucose disposal (muscle, fat). Inhibition of GLUT4 insulin-dependent glucose transporter-mediated glucose uptake was also found in primary cultures of muscle cells and potentially explains insulin resistance and the predisposition to type II diabetes in patients on HAART therapy.

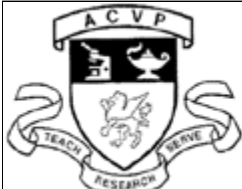
Conclusions

HIV PIs inhibit the proteasomal degradation of transcription factors, enhance lipid biosynthesis and inhibit GLUT-4 mediated glucose uptake. Consistent with clinical studies showing a favorable effect of atazanavir on patient's lipid profiles, atazanavir-mediated effects on all endpoints tested only occurred at concentrations significantly greater than expected clinical exposures while those of other PIs occurred at concentrations comparable to or less than clinically-relevant exposures. These *in vitro* findings are thus directly relevant to the proposed understanding of the improved side-effect profile of atazanavir.

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