

Ritonavir Accumulates into Cultured Human Adipocytes and Alters Insulin Resistance Related Adipocytokines Levels

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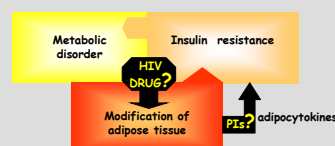
INTRODUCTION

HIV therapy includes three classes of drugs : nucleoside (NRTIs) and non nucleoside (NNRTIs) reverse transcriptase inhibitors, and protease inhibitors (PIs). Lipodystrophic syndrome is a major side effect of HIV antiretroviral therapy, and fat tissue redistribution is associated with the development of insulin resistance (IR). Reports from clinical studies indicate several changes in circulating levels of cytokines (adiponectin decrease, IL6 and TNF α increase) secreted from adipose tissue which are implicated in the outbreak of insulin resistance. Yet there is no evidence that HIV drugs accumulate in human adipocytes and affect secretion of adipocytokines.

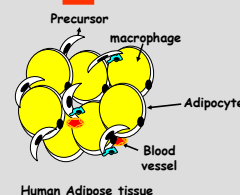
METHODS

Accumulation of fluorescently labelled amprenavir and indinavir were visualised by time-lapse images in living cells established from human adipose tissue (hMADS cells). Intracellular accumulation of ritonavir, lopinavir, and nevirapin was quantified by ELISA. Expression of adipocyte-specific genes was analysed to determine the effects of chronic exposure of differentiating hMADS cells to HIV drugs. Regulation of the expression of various adipocytokines (leptin, adiponectin, IL6 and TNF α) by PIs was studied in differentiated cells by Real Time PCR.

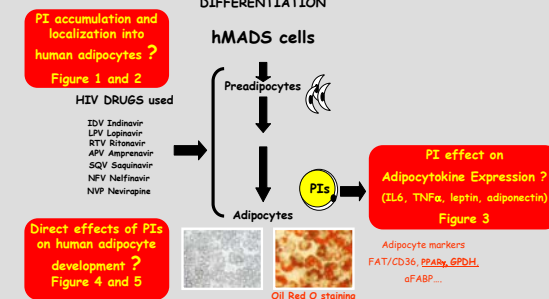
Lipodystrophic syndrome



A NEW HUMAN ADIPOCYTE CELL MODEL TO STUDY DIRECT EFFECT OF HIV DRUGS



DIFFERENT STAGES OF ADIPOCYTE DIFFERENTIATION



LOCALISATION OF PIs INTO ADIPOCYTES



HIV DRUGS ACCUMULATE INTO HUMAN ADIPOCYTES

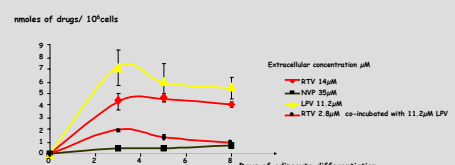


Figure 1 : Confluent hMADS cells were differentiated in adipogenic medium (transferin, triiodo-L-thyronine, insulin, isobutyl-methylxanthine, dexamethasone, the PPAR γ agonist (BRL-49653) and HIV drugs as indicated for the first 3 days. Thereafter isobutyl-methylxanthine and dexamethasone were omitted. ELISAs were performed at indicated time points.

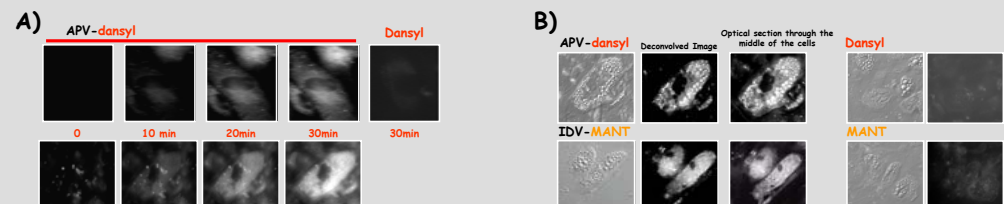


Figure 2 : We labeled two PIs, APV and IDV. Non differentiated and differentiated hMADS cells were cultivated in adipogenic medium. Then this new fluorescent probes were added in adipogenic medium. Image acquisition and analysis were performed using the Applied Precision DeltaVision system (Applied Precision, Issaquah, WA) built on an Olympus IX-70 base. (A) Living cells from the same field are shown at various times after starting observation by Time-Lapse. (B) Left panel : Intracellular images of APV-dansyl and IDV-MANT in human adipocytes after 30 min incubation. Right panel : Images of Dansyl or MANT alone in human adipocytes were performed after 30 min of incubation.

RTV MODULATES EXPRESSION OF ADIPOCYTOKINES IN MATURE HUMAN ADIPOCYTES

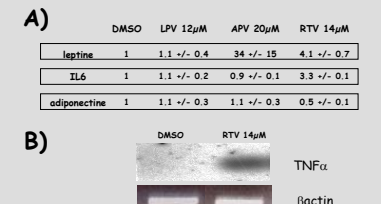


Figure 3 : (A) Differentiated hMADS cells were cultivated in adipogenic medium. Then HIV drugs were added in adipogenic medium for the last 5 days and adipocytokines were quantified by Real-Time PCR. Results are expressed as fold induction compared to control treatment (DMSO). (B) TNF α expression detected by RT-PCR on hMADS adipocyte after 5 days treatment by RTV.

HIV DRUGS INHIBIT ADIPOCYTE DIFFERENTIATION OF HUMAN PRECURSOR CELLS

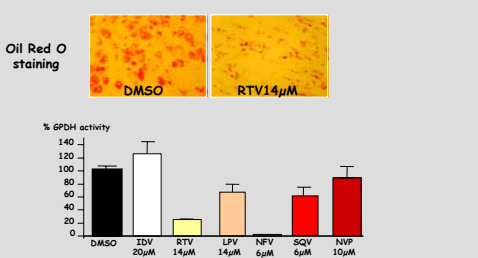


Figure 4 : Confluent hMADS cells were induced to differentiate with an adipogenic medium (transferin, triiodo-L-thyronine, insulin, isobutyl-methylxanthine, dexamethasone, the PPAR γ agonist (BRL-49653) for the first 3 days. Thereafter isobutyl-methylxanthine and dexamethasone were omitted. After 18 days of chronic exposure to HIV drugs Oil Red O staining and GPDH activity were performed.

RTV INHIBITS EXPRESSION OF ADIPOCYTE MARKERS IN MATURE HUMAN ADIPOCYTES

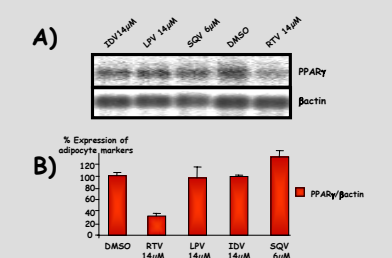


Figure 5: (A) Differentiated hMADS cells were cultivated in adipogenic medium. Then HIV drugs were added in adipogenic medium for the last 5 days. (A) mRNA were prepared and adipocyte markers were analysed by Northern blot. (B) Quantification of signal was normalised using β actin as internal control.

HYPOTHETIC MECHANISM LEADING TO LIPODYSTROPHIC SYNDROME AND INSULIN RESISTANCE

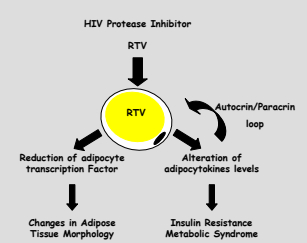


Figure 6

Results

Amprenavir accumulated in the cytosol of hMADS cells and in lipid droplets in mature adipocytes. Lopinavir and ritonavir accumulated during development of hMADS cells in adipocytes (5.4 \pm 0.8 nmoles/10⁶ cells and 4.1 \pm 0.3 nmoles/10⁶ cells respectively). The reverse transcriptase inhibitor nevirapin accumulated at a lower level (0.7 \pm 0.03 nmoles/10⁶ cells). Chronic treatment with indinavir, amprenavir or nevirapin did not alter differentiation of precursor cells into adipocytes, whereas lopinavir, saquinavir and ritonavir reduced G3PDH activity and triglyceride accumulation. In adipocytes, lopinavir did not alter expression of cytokines. In contrast, amprenavir increased leptin expression and ritonavir was able to up-regulate TNF α , IL6 and leptin expression and to down-regulate expression of PPAR γ and adiponectin.

Conclusion (Figure 6)

Intracellular accumulation of HIV drugs into human adipocytes occurs at different levels and PIs display varying effects on the development of adipose cells. Ritonavir can alter expression of insulin resistance-related cytokines in human adipocytes in a way parallel to the situation observed *in vivo* upon treatment of HIV-infected patients developing insulin resistance. Thus, it is proposed that protease inhibitors participate in insulin resistance through a direct effect on adipocytes.