



HAL
open science

Human immunodeficiency virus protease inhibitors accumulate into cultured human adipocytes and alter expression of adipocytokines.

Cécile Vernochet, Stéphane Azoulay, Daniele Duval, Roger Guedj, Françoise Cottrez, Hubert Vidal, Gérard Ailhaud, Christian Dani

► To cite this version:

Cécile Vernochet, Stéphane Azoulay, Daniele Duval, Roger Guedj, Françoise Cottrez, et al.. Human immunodeficiency virus protease inhibitors accumulate into cultured human adipocytes and alter expression of adipocytokines.. *Journal of Biological Chemistry*, 2005, 280 (3), pp.2238-43. 10.1074/jbc.M408687200 . hal-00322398

HAL Id: hal-00322398

<https://hal.science/hal-00322398>

Submitted on 1 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright

Human Immunodeficiency Virus Protease Inhibitors Accumulate into Cultured Human Adipocytes and Alter Expression of Adipocytokines*

Received for publication, July 30, 2004, and in revised form, September 27, 2004
Published, JBC Papers in Press, November 3, 2004, DOI 10.1074/jbc.M408687200

Cécile Vernochet^{‡§}, Stéphane Azoulay[¶], Daniele Duval[¶], Roger Guedj[¶], Françoise Cottrez^{||},
Hubert Vidal^{**}, Gérard Ailhaud[‡], and Christian Dani^{‡ ††}

From the [‡]Institut Signalisation, Biologie du Développement et Cancer, UMR 6543 CNRS, Centre de Biochimie, 06108 Nice, France, [¶]Laboratoire de Chimie Bio-Organique UMR 6001 CNRS, 06108 Nice, France, ^{||}Unité INSERM 343, Hôpital de l'Archet, 06202 Nice, France, and ^{**}INSERM U.449, Faculté de Médecine R. Laennec, 69372 Lyon, France

Lipodystrophic syndrome is a major side effect of highly active antiviral therapy. Fat tissue redistribution is associated with changes in adipocyte gene expression and in circulating levels of adipocytokines involved in the development of insulin resistance. However, the evidence that HIV drugs accumulate into human adipocytes and have a direct effect on the expression of adipocyte-specific genes is still lacking. To address these questions, we used adipocytes derived from adult stem (hMADS) cells isolated from human adipose tissue. We showed by ELISA that two inhibitors of the HIV protease, lopinavir and ritonavir, accumulated at similar levels during the development of hMADS cells in adipocytes, whereas a non-nucleoside reverse transcriptase inhibitor, the nevirapine, accumulated at lower levels. Two fluorescent protease inhibitors then have been generated to investigate their subcellular localization. The data showed that HIV drugs accumulated into adipocytes and displayed various effects on hMADS cell-derived adipocytes. Indinavir, amprenavir, and nevirapine did not alter differentiation of precursor cells. In contrast, lopinavir, saquinavir, and ritonavir inhibited the development of preadipocytes into adipocytes. In adipocytes, amprenavir increased leptin expression and ritonavir was able to up-regulate tumor necrosis factor- α , interleukin 6, and leptin expression and down-regulate the expression of peroxisome proliferator-activated receptor γ and adiponectin. Intracellular accumulation and localization of HIV drugs into human adipocytes strongly suggest that adipose tissues store these drugs. Because ritonavir can alter the 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, we propose that protease inhibitors participate in insulin resistance through a direct effect on adipocytes.

Highly active antiretroviral therapy (HAART)¹ combines treatment with three classes of anti-HIV drugs: 1) protease

inhibitors (PIs); nucleoside reverse transcriptase inhibitors (NRTIs); and non-nucleoside reverse transcriptase inhibitors (NNRTIs). These drugs have demonstrated their efficiency in improving the lifespan of HIV-infected patients. However, patients under HAART develop metabolic alterations including fat tissue redistribution with peripheral lipoatrophy and increased central adiposity. The lipodystrophic syndrome affects up to 60% treated HIV-infected patients and is emerging as a significant medical concern (1). The adipose tissue of lipodystrophic HIV-infected patients contains clusters of small adipocytes (2) that can result from a defect in differentiation of adipocyte precursors or from metabolic alterations of mature adipocytes. PIs and NNRTIs were detected in adipose tissue of patients, but evidence that HIV drugs accumulate and have a direct effect on the development of human adipocytes are still lacking. It has been recently shown that PIs can enter and accumulate in cultured mouse adipocytes (3, 4) and that PIs, but not all, inhibited differentiation of mouse adipocytes (3, 5, 6). These reports indicate a direct effect of PIs on adipogenesis. However, the effects of PIs were different according to the mouse preadipocyte clonal lines (3), indicating that studies with human adipocytes should be more appropriate to gain better insights into the molecular events that occur in human adipose tissue.

HIV-infected patients under HAART develop dyslipidemia and systemic insulin resistance. Adipose tissue is thought to play a critical role in insulin resistance, in particular, through the expression of adipocytokines such as leptin, tumor necrosis factor- α (TNF α), interleukin-6 (IL6), and adiponectin. Leptin is an important circulating satiety factor that regulates food intake via its action in the central nervous system (7), and insulin sensitivity correlates negatively with leptin in lipohyperthrophic HIV-infected patients (8). It has also been reported that HIV-infected lipodystrophic patients exhibit 40% reduced plasma adiponectin levels compared with non-lipodystrophy patients leading to the proposal that adiponectin replacement therapy could improve the treatment of HAART-associated syndrome (9). The role of TNF α in insulin sensitivity has been largely documented mainly using murine systems. In human,

* This work was supported in part by a grant from the French National Agency for AIDS Research (ANRS) (to C. D.), a Sidaction grant (to D. D.), and a special grant from the Fondation pour la Recherche Médicale (Imagerie cellulaire-UMR6543 CNRS) and the Association de Recherches contre le Cancer (ARC CR402/7696). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[§] Recipient of an ANRS fellowship.

^{††} To whom correspondence should be addressed. Tel.: 33-4-92076436; Fax: 33-4-92076404; E-mail: dani@unice.fr.

¹ The abbreviations used are: HAART, highly active antiretroviral

therapy; HIV, human immunodeficiency virus; hMADS, human multipotent adipose-derived stem; ELISA, enzyme-linked immunosorbent assay; TNF α , tumor necrosis factor- α ; IL6, interleukin 6; PPAR γ , peroxisome proliferator-activated receptor γ ; PIs, protease inhibitors; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; IDV, indinavir; RTV, ritonavir; SQV, saquinavir; APV, amprenavir; NVP, nevirapine; G3PDH, glycerol-3-phosphate dehydrogenase; MANT, *N*-methylantraniloyl; HPLC, high pressure liquid chromatography; DAPI, 4',6-diamidino-2-phenylindole; MDR, multi-drug resistance.

plify small fragments (80–200 bp) and to hybridize in different exons of the target sequences. The list of the primers and real-time PCR conditions for each mRNA assay is available upon request. Each assay was performed in duplicate, and the validation of the real-time PCR runs was assessed by evaluation of the melting temperature of the products and by the slope and error obtained with the standard curve. The analyses were performed using the Light-Cycler software.

PIs Labeling and Time-lapse Fluorescence Microscopy—Pyridine (0.178 mmol), *N,N*-dimethylaminopyridine (0.0158 mmol), and dansyl chloride (0.297 mmol) were added to amprenavir (0.198 mmol) in anhydrous dichloromethane under nitrogen atmosphere for 8 h at reflux temperature. The solvent then was removed *in vacuo*, and the crude product was purified by flash chromatography on a silica gel (96/4, chloroform/methanol) to obtain the APV-dansyl. *N,N*-Dimethylaminopyridine (0.081 mmol) and *N*-methylisatoic anhydride (0.089 mmol) were added to indinavir (0.081 mmol) in anhydrous *N,N*-dimethylformamide under nitrogen atmosphere for 5 h at room temperature. The solvent then was removed *in vacuo*, and the crude product was purified by TLC (96/4, chloroform/methanol) to obtain the indinavir-*N*-methylanthraniloyl (IDV-MANT). All of the products were fully characterized by nuclear magnetic resonance and mass spectrometry. Details of the chemical procedure are available upon request. Cell images were recorded by using the $\times 40/0.65$ – 1.35 NA oil immersion objective lens. Image acquisition and analysis were performed using the Applied Precision Deltavision system (Applied Precision, Issaquah, WA) built on an Olympus IX-70 base. For APV-dansyl and IDV-MANT, excitation and emission filters were FITC-DAPI and DAPI-DAPI, respectively. The stability of the labeling has been investigated. For that purpose, intracellular APV-dansyl and IDV-MANT have been recovered from adipocytes 2 days after incubation and analyzed by HPLC-UV. No uncoupled PIs and no free fluorophore were detected, indicating that the labeling was stable and that the fluorescence reflected the PI localization.

Competitive ELISA—RTV, NVP, and LPV were quantified by competitive ELISA as previously described (15, 16). Results were expressed as nanomole of HIV drugs/ 10^6 cells.

RESULTS

HIV Drugs Enter and Accumulate into Human-cultured Adipocytes—We first investigated by ELISA the ability of two PIs (RTV and LPV) and one NNRTI (NVP) to enter and accumulate in human adipocytes. The amounts of HIV drugs we used are in the range of those detected in the sera of HIV-infected patients under HAART (17–20). As shown in Fig. 1A, HIV drugs accumulated at different levels during adipocyte development. Whereas RTV and LPV accumulated at similar levels (4.1 ± 0.3 and 5.4 ± 0.8 nmol/ 10^6 cells, respectively), the intracellular level of NVP was much lower (0.7 ± 0.03 nmol/ 10^6 cells) despite a higher extracellular concentration. Two concentrations of RTV were used, and as shown in Fig. 1A, the intracellular accumulation was dose-dependent. The involvement of the multi-drug resistance (MDR) and MDR-associated protein transporters in preadipocyte accumulation of RTV has been investigated. The addition of $20 \mu\text{M}$ reserpin, a pharmacological inhibitor of the MDR1 and of MDR-associated protein transporter 1, for 3 h led to a higher accumulation of RTV (12 ± 0.8 nmol/ 10^6 cell compared with 4.1 ± 0.3 nmol/ 10^6 cell in the absence of reserpin, $n = 3$). This result strongly suggested that accumulation of the drugs into preadipose cells could be regulated via MDR transporters. We have previously shown that the antibodies used in the ELISAs are specific for the native form of the drugs and do not recognize metabolic forms (15, 16). In addition, HPLC-UV analysis indicated that the PIs stored into adipose cells displayed a chemical profile identical to the native form of the PIs (not shown). Altogether, these data strongly suggest that, even if we could not rule out the presence of minor oxidized or chemically modified forms of the drugs, preadipocytes and adipocytes stored the native form of the HIV drugs. The generation of fluorescent APV (APV-dansyl) and IDV (IDV-MANT) using a straightforward chemical synthesis then allowed us to investigate their apparent uptake and subcellular localization in live cells imaged by time-lapse digital fluorescence microscopy. APV-dansyl was detected in undiffer-

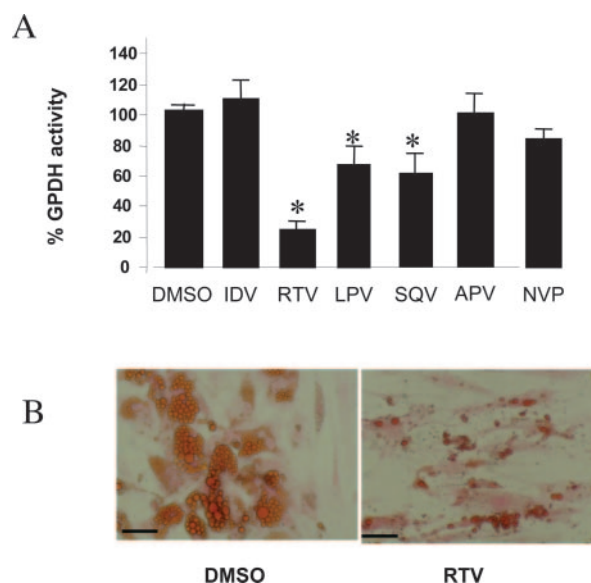


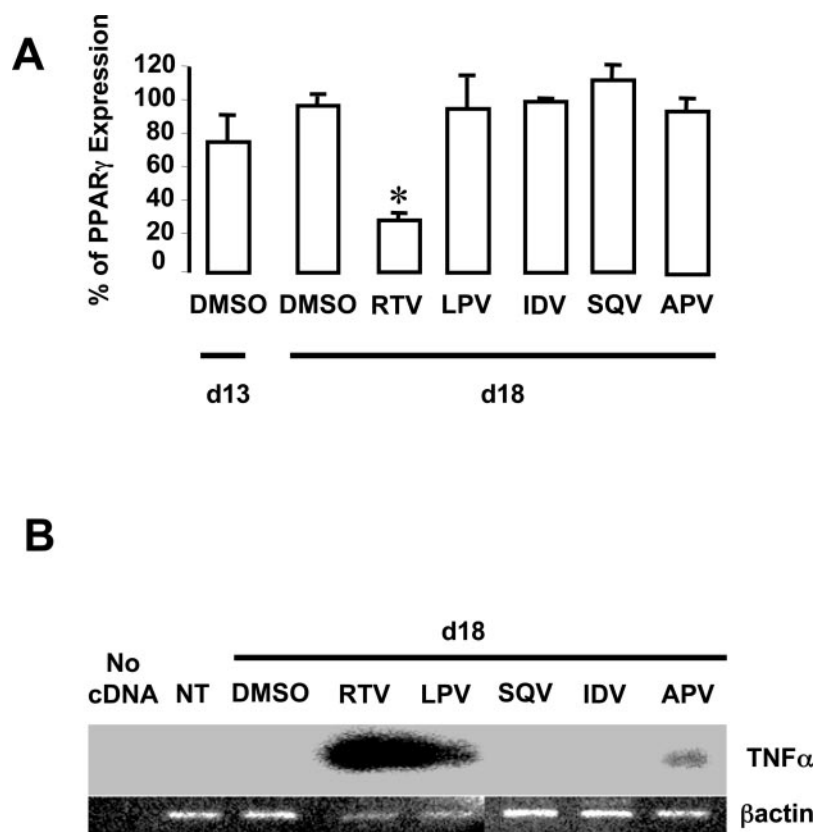
FIG. 2. Effects of HIV drugs on differentiating hMADS cells. A, undifferentiated cells were induced to differentiate into adipocytes in the presence of $10 \mu\text{g/ml}$ IDV, RTV, LPV, APV, and NVP or $4 \mu\text{g/ml}$ SQV. G3PDH activities were determined 10 days after treatment. Data are reported by taking as 100% the G3PDH activity of untreated cells Me_2SO (DMSO). The means \pm S.D. are representative data from three independent experiments. Significant differences ($p < 0.05$, Mann-Whitney *U* test) between control (Me_2SO) and treated cells are denoted by asterisk. B, photo-micrographic records of hMADS cells induced to undergo differentiation for 10 days in the presence of 0.1% Me_2SO or $14 \mu\text{M}$ RTV. Cells were stained with Oil Red O for lipid droplets and counterstained with eosin. The bar represents $50 \mu\text{m}$.

entiated and in fully differentiated hMADS cells within 10 min following the addition to the culture medium and became distributed throughout the cytoplasm within 30 min (Fig. 1B). We treated images by deconvolution to analyze more precisely the intracellular localization of both labeled PIs after a 1-h incubation in living adipocytes (Fig. 1C). In differentiated hMADS cells, APV-dansyl accumulated at a high rate into lipid droplets. In contrast, IDV-MANT accumulated more outside the lipid droplets.

Effect of PIs on Differentiating hMADS Cells—*In vitro* models are required to investigate the deleterious effects of individual PI and other HIV drugs. We have analyzed the effects of chronic addition of pharmacological concentrations of HIV drugs on differentiating hMADS cells. With the exception of nelfinavir ($4 \mu\text{g/ml}$), no cytotoxicity of HIV drugs was observed at the used concentrations. The induction of adipocyte differentiation of hMADS cells in the presence of $10 \mu\text{g/ml}$ IDV, APV, or NVP did not modify the differentiation process as indicated by the neutral lipid accumulation (data not shown) and the G3PDH activities, a marker of mature adipocytes, determined 10 days after the addition of the drugs (Fig. 2A). In contrast, when induced to differentiate in the presence of SQV ($4 \mu\text{g/ml}$) or LPV ($10 \mu\text{g/ml}$), the inhibition of G3PDH activity was observed. RTV ($10 \mu\text{g/ml}$) elicited the highest inhibitory effect on G3PDH activity, and RTV-treated human adipocytes displayed a fibroblast-like morphology and accumulated less cytoplasmic triacylglycerol droplets (Fig. 2B). These data indicated that, when used in the range of concentrations determined in serum of HIV-infected patients under HAART, some HIV drugs but not all could inhibit adipocyte differentiation of precursor cells.

Effect of PIs on Human Adipocytes Derived from hMADS Cells—We studied the effects of PIs on fully differentiated adipocytes at day 13 after induction of adipogenesis. hMADS cell-derived adipocytes were treated for 5 days with different PIs, and the expression of both $\text{PPAR}\gamma$ and various adipocyto-

FIG. 3. RTV inhibits expression of PPAR γ and induces expression of TNF α in mature adipocytes. Undifferentiated hMADS cells were maintained in adipocyte medium for 13 days with no addition of HIV drugs. At that stage, cells were differentiated and expressed PPAR γ (d13, day 13). Subsequently, adipocytes were treated with 10 μ g/ml IDV, RTV, LPV, and APV or 4 μ g/ml SQV for 5 days (d18, day 18) and RNAs were prepared. **A**, the expression of PPAR γ was analyzed by Northern blotting. Quantification of the signals was performed and standardized as described under "Experimental Procedures." Data are expressed by taking as 100% the signal obtained in 0.1% Me₂SO (DMSO) treatment used as negative control. The means \pm S.D. are representative data from three independent experiments. Significant differences ($p < 0.05$, Mann-Whitney U test) between control (Me₂SO) and treated cells are denoted by asterisk. **B**, expression of TNF α was investigated by RT-PCR. Blotting was carried out in the same experiment and is representative of two independent experiments. Primers used for PCR amplification of TNF α were 5'-ATCTTCTCGAAC-CCCGAGTGA-3' and 5'-GGAGCTGCCCTCAGCTT-3', and primer used for hybridization was 5'-CTGATGATGAAA-ACTACT-3'. NT, not treated.



kines was analyzed. As shown in Fig. 3A, exposure to RTV but not to SQV, LPV, IDV, or APV inhibited PPAR γ expression in mature adipocytes. TNF α was undetectable in untreated hMADS adipocytes. However, the expression of TNF α was reproducibly induced after treatment with RTV (Fig. 3B) and, at a less extent, after treatment with LPV and APV. The expression profile of leptin, IL6, and adiponectin has been investigated by quantitative RT-PCR. As shown in the Table I, a 5-day exposure of adipocytes to RTV decreased adiponectin expression and induced both leptin and IL6 gene expression. APV and LPV did not display any significant effect on adiponectin and IL6 expression. In contrast, leptin expression was dramatically induced upon the addition of APV. These data point out that PIs have a direct effect in human adipocytes on the expression of cytokines that are tightly associated with the development of insulin resistance. Fig. 4 summarizes the effects of the various HIV drugs on adipocyte differentiation of precursor cells and on the alteration of gene expression in adipocytes.

DISCUSSION

We have shown that APV and IDV, which belong to the same class of drugs, displayed different intracellular localization. It is well documented that, in peripheral blood mononuclear cells, the intracellular accumulation of HIV drugs depends of several parameters such as their chemical structures, their half-lives, or their affinity to drug transporters. Intracellular sequestration due to protein binding or ion trapping has also been suggested (21). APV was stored preferentially into lipid droplets, whereas IDV accumulated outside the lipid droplets. The lipophilic properties of APV and IDV could be responsible for their differential distribution, but active mechanisms cannot be ruled out. This observation indicates that adipose tissues can store PIs in treated patients. The capacity of adipocytes to mobilize and to release PIs remains to be investigated. It is interesting to note that the two labeled PIs were not detected into the nucleus. Even if we cannot exclude a role of the flu-

TABLE I
PIs alter expression of adipocytokines in human cultured adipocytes

Adipocytes derived from hMADS cells were treated with 10 μ g/ml indicated PIs or with 0.1% Me₂SO as negative control for 5 days as indicated in Fig. 3. RNAs were prepared, and adipocytokine expression was determined by real-time RT-PCR as described under "Experimental Procedures." Data were normalized to cyclophilin A signals and expressed by taking as reference the expression of each adipocytokine in Me₂SO-treated cells.

	Leptin	IL6	Adiponectin
Me ₂ SO	1	1	1
LPV	1.1 \pm 0.4	1.1 \pm 0.2	1.1 \pm 0.3
APV	34 \pm 15	0.9 \pm 0.1	1.1 \pm 0.3
RTV	4.1 \pm 0.7	3.3 \pm 0.1	0.5 \pm 0.1

orophore on the localization of the drugs, these results indicate that nuclear proteins are not direct targets of PIs. This hypothesis does not exclude the effects of PIs in the cytoplasm on maturation of nuclear proteins. Altogether, these data strongly suggest that HIV drugs could have intracellular targets and direct effects on the development of human adipose cells.

Some HIV drugs, but not all, could inhibit differentiation of precursor cells isolated from human adipose tissue. Similar conclusions have been previously reported with precursor cells isolated from human bone marrow (22). In regard to the lipodystrophy associated with the use of PI, alternative therapies substituting NNRTIs (efavirenz or nevirapine) for protease inhibitors have been proposed (23). NNRTIs represent a promising approach to combine antiviral efficiency and limited adverse effects. However, recent papers have reported that efavirenz could accumulate in adipose tissue (24) and inhibit triacylglycerol accumulation in mouse preadipose clonal lines (25). We show in this paper that, despite a high extracellular concentration of nevirapine, the intracellular accumulation of this NNRTI was low compared with PIs. More interestingly, nevirapine did not interfere with lipid accumulation during adipocyte development of hMADS cells.

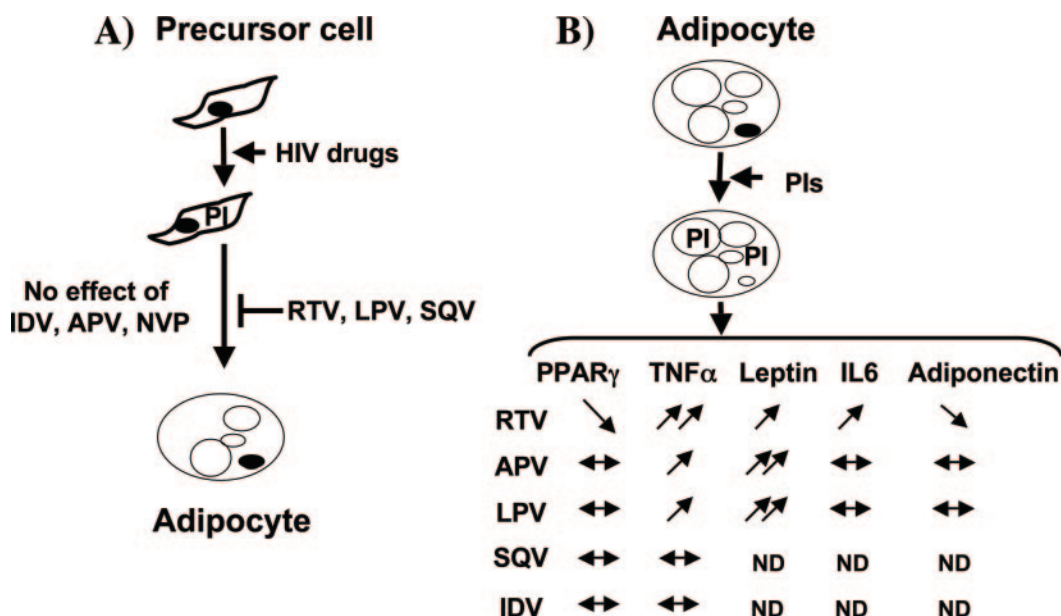


FIG. 4. **A summary of the effects of HIV drugs on human-cultured adipose cells.** A, HIV drugs enter into hMADS cells, and some of them inhibit adipocyte differentiation. B, PIs enter into mature adipocytes and display various effects on PPAR γ and adipocytokine gene expression. ND, not determined. Lipid droplets are open circles, and the nucleus is in black.

hMADS cells accumulated less triacylglycerol droplets when exposed to RTV in agreement with its inhibitory effects previously reported on adipocyte differentiation of various mouse preadipocyte clonal lines (3, 5). We observed that lipid accumulation was inhibited, but the number of differentiated cells seemed to be similar in untreated and RTV-treated cultures. This is reminiscent of a previous report showing morphological changes in lipotrophic adipose tissue of HIV-infected patients where the clusters of adipocytes with few lipid droplets were observed. As suggested by the authors, these small adipocytes could be young regenerative cells replacing adipocytes lost by apoptosis (26). The inhibition of adipocyte formation induced by RTV could also be linked to a decreased expression of PPAR γ known to be essential both for adipocyte differentiation and adipocyte survival (27).

RTV alters the expression of adipocytokines in mature hMADS adipocytes in a way parallel to the situation observed in lipodystrophic HIV patients developing insulin resistance. Since it has been shown that TNF α inhibits adiponectin gene expression on human adipose tissue fragments (28), it is tempting to postulate that the inhibition of adiponectin upon the addition of RTV on hMADS adipocytes was mediated by RTV-induced TNF α . It has also been reported that TNF α inhibits PPAR γ expression in adipocytes (29) and that IL6 inhibits adiponectin (30). When subjected to an expression profile analysis, we have confirmed that adipocytes derived from hMADS cells express IL6 receptor, the gp130 signal transducer, and the two TNF α receptors, *i.e.* TNF receptors 1 and 2.² Therefore, we could postulate that the up-regulation of IL6 and TNF α by RTV leads in adipose tissue to an autocrine/paracrine mechanism that could participate in the insulin resistance observed in lipodystrophic patients under HAART.

In conclusion, our data point out that HIV drugs display various effects on human adipocytes and that their storage alters the expression of adipocytokines involved in insulin resistance. Identification of molecular mechanisms implicated in adipocyte gene regulation by PIs might improve the treatment of HAART-associated syndrome.

Acknowledgment—We thank Cedric Matthews (UMR 6543 CNRS) for the time-lapse microscopy analysis.

REFERENCES

- Carr, A., Samaras, K., Thorisdottir, A., Kaufmann, G. R., Chisholm, D. J., and Cooper, D. A. (1999) *Lancet* **353**, 2093–2099
- Bastard, J. P., Caron, M., Vidal, H., Jan, V., Auclair, M., Vigouroux, C., Lubinski, J., Laville, M., Maachi, M., Girard, P. M., Rozenbaum, W., Levan, P., and Capeau, J. (2002) *Lancet* **359**, 1026–1031
- Vernochet, C., Azoulay, S., Duval, D., Guedj, R., Ailhaud, G., and Dani, C. (2003) *AIDS* **17**, 2177–2180
- Janneh, O., Hoggard, P. G., Tjia, J. F., Jones, S. P., Khoo, S. H., Maher, B., Back, D. J., and Pirmohamed, M. (2003) *Antiviral Ther.* **8**, 417–426
- Zhang, B., MacNaull, K., Szalkowski, D., Li, Z., Berger, J., and Moller, D. E. (1999) *J. Clin. Endocrinol. Metab.* **84**, 4274–4277
- Roche, R., Poizat-Martin, I., Yazidi, C. M., Compe, E., Gastaut, J. A., Torresani, J., and Planells, R. (2002) *AIDS* **16**, 13–20
- Friedman, J. M. (2002) *Nutr. Rev.* **64**, 85–87
- Vigouroux, C., Maachi, M., Nguyen, T. H., Coussieu, C., Gharakhanian, S., Funahashi, T., Matsuzawa, Y., Shimomura, I., Rozenbaum, W., Capeau, J., and Bastard, J. P. (2003) *AIDS* **17**, 1503–1511
- Kinlaw, W. B., and Marsh, B. (2004) *Endocrinology* **145**, 484–486
- Hotamisligil, G. S. (2000) *Int. J. Obes. Relat. Metab. Disord.* **24**, Suppl. 4, S23–S27
- Rodriguez, A., Elabd, C., Delteil, F., Astier, J., Vernochet, C., Saint-Marc, P., Guesnet, J., Guezennec, A., Ez-Z., A., Dani, C., and Ailhaud, G. (2004) *Biochem. Biophys. Res. Commun.* **315**, 255–263
- Dani, C. (2002) *Methods Mol. Biol.* **185**, 107–116
- Vassaux, G., Negrel, R., Ailhaud, G., and Gaillard, D. (1994) *J. Cell. Physiol.* **161**, 249–256
- Dani, C., Smith, A. G., Dessolin, S., Leroy, P., Staccini, L., Villageois, P., Darimont, C., and Ailhaud, G. (1997) *J. Cell Sci.* **110**, 1279–1285
- Azoulay, S., Nevers, M. C., Creminon, C., Heripret, L., Durant, J., Dellamonica, P., Grassi, J., Guedj, R., and Duval, D. (2004) *Antimicrob. Agents Chemother.* **48**, 104–109
- Akeb, F., Ferrua, B., Creminon, C., Roptin, C., Grassi, J., Nevers, M. C., Guedj, R., Garraffo, R., and Duval, D. (2002) *J. Immunol. Methods* **263**, 1–9
- Balani, S. K., Woolf, E. J., Hoagland, V. L., Sturgill, M. G., Deutsch, P. J., Yeh, K. C., and Lin, J. H. (1996) *Drug Metab. Dispos.* **24**, 1389–1394
- Hsu, A., Granneman, G. R., Witt, G., Locke, C., Denissen, J., Molla, A., Valdes, J., Smith, J., Erdman, K., Lyons, N., Niu, P., Decourt, J. P., Fourtillan, J. B., Girault, J., and Leonard, J. M. (1997) *Antimicrob. Agents Chemother.* **41**, 898–905
- Pai, V. B., and Nahata, M. C. (1999) *Ann. Pharmacother.* **33**, 325–339
- Boffito, M., Dickinson, L., Hill, A., Back, D., Moyle, G., Nelson, M., Higgs, C., Fletcher, C., Mandalia, S., Gazzard, B., and Pozniak, A. (2004) *Antiviral Ther.* **9**, 423–429
- Koo, S. H., Hoggard, P. G., Williams, I., Meaden, E. R., Newton, P., Wilkins, E. G., Smith, A., Tjia, J. F., Lloyd, J., Jones, K., Beeching, N., Carey, P., Peters, B., and Back, D. J. (2002) *Antimicrob. Agents Chemother.* **46**, 3228–3235
- Jain, R. G., and Lenhard, J. M. (2002) *J. Biol. Chem.* **277**, 19247–19250
- Barreiro, P., Soriano, V., Blanco, F., Casimiro, C., de la Cruz, J. J., and Gonzalez-Lahoz, J. (2000) *AIDS* **14**, 807–812
- Dupin, N., Buffet, M., Marcelin, A. G., Lamotte, C., Gorin, I., Ait-Arkoub, Z.,

² C. Vernochet and C. Dani, unpublished data.

- Treluyer, J. M., Bui, P., Calvez, V., and Peytavin, G. (2002) *AIDS* **16**, 2419–2424
25. El Hadri, K., Glorian, M., Monsempes, C., Dieudonne, M. N., Pecquery, R., Giudicelli, Y., Andreani, M., Dugail, I., and Feve, B. M. (2004) *J. Biol. Chem.* **279**, 15130–15141
26. Domingo, P., Matias-Guiu, X., Pujol, R. M., Francia, E., Lagarda, E., Sambeat, M. A., and Vazquez, G. (1999) *AIDS* **13**, 2261–2267
27. Imai, T., Takakuwa, R., Marchand, S., Dentz, E., Bornert, J. M., Messaddeq, N., Wendling, O., Mark, M., Desvergne, B., Wahli, W., Chambon, P., and Metzger, D. (2004) *Proc. Natl. Acad. Sci. U. S. A.* **101**, 4543–4547
28. Lihn, A. S., Richelsen, B., Pedersen, S. B., Haugaard, S. B., Rathje, G. S., Madsbad, S., Andersen, O. (2003) *Am. J. Physiol.* **285**, E1072–E1080
29. Perrey, S., Ishibashi, S., Yahagi, N., Osuga, J., Tozawa, R., Yagyu, H., Ohashi, K., Gotoda, T., Harada, K., Chen, Z., Iizuka, Y., Shionoiri, F., and Yamada, N. (2001) *Metabolism* **50**, 36–40
30. Fasshauer, M., Kralisch, S., Klier, M., Lossner, U., Bluher, M., Klein, J., and Paschke, R. (2003) *Biochem. Biophys. Res. Commun.* **301**, 1045–1050

Human Immunodeficiency Virus Protease Inhibitors Accumulate into Cultured Human Adipocytes and Alter Expression of Adipocytokines
Cécile Vernochet, Stéphane Azoulay, Daniele Duval, Roger Guedj, Françoise Cottrez, Hubert Vidal, Gérard Ailhaud and Christian Dani

J. Biol. Chem. 2005, 280:2238-2243.

doi: 10.1074/jbc.M408687200 originally published online November 3, 2004

Access the most updated version of this article at doi: [10.1074/jbc.M408687200](https://doi.org/10.1074/jbc.M408687200)

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

This article cites 29 references, 8 of which can be accessed free at <http://www.jbc.org/content/280/3/2238.full.html#ref-list-1>