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Effects of nevirapine and efavirenz on human adipocyte differentiation, gene expression, and release of adipokines and cytokines

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ABSTRACT

The non-nucleoside reverse transcriptase inhibitors (NNRTIs) nevirapine and efavirenz are drugs of choice for initial antiretroviral treatment for HIV-1 infection. Although NNRTIs have not traditionally been associated with the appearance of adipose alterations, recent data suggest that efavirenz may contribute to adipose tissue alterations in antiretroviral-treated patients, consistent with its ability to impair differentiation of adipocytes in cell cultures. No such effects have been reported for nevirapine, the other most commonly used NNRTI. In this study, we determined the effects of nevirapine on differentiation, gene expression and release of regulatory proteins (adipokines and cytokines) in differentiating human adipocytes, and compared them with those of efavirenz. Efavirenz caused a dose-dependent repression of adipocyte differentiation that was associated with down-regulation of the master adipogenesis regulator genes SREBP-1, PPARγ and C/EBPα, and their target genes encoding lipoprotein lipase, leptin and adiponectin, which are key proteins in adipocyte function. In contrast, nevirapine does not affect adipogenesis and causes a modest but significant coordinate increase in the expression of SREBP-1, PPARγ and C/EBPα and their target genes only at a concentration of 20 μM. Whereas efavirenz caused a significant increase in the release of pro-inflammatory cytokines (interleukin [IL]-8, IL-6, monocyte chemoattractant protein-1), plasminogen activator inhibitor type-1 and hepatocyte growth factor (HGF), nevirapine either had no effect on these factors or decreased their release (IL-6 and HGF). Nevirapine significantly increased adiponectin release, whereas efavirenz strongly repressed it. Moreover, nevirapine inhibited preadipocyte endogenous reverse transcriptase activity, whereas efavirenz did not alter it. It is concluded that, in contrast with the profound anti-adipogenic and pro-inflammatory response elicited by efavirenz, nevirapine does not impair adipogenesis.

1. Introduction

Alterations in adipose tissue distribution (lipodystrophy syndrome) and systemic metabolic disturbances (dyslipidemia and insulin resistance) appear frequently in HIV-1-infected patients under highly active antiretroviral treatment (HAART). HAART employs a drug regimen that typically includes nucleoside-analog reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs) and/or non-nucleoside analog inhibitors of reverse transcriptase (NNRTIs). The complex metabolic alterations that arise with these treatment regimens reflect the action of HAART drug combinations on the susceptible status of HIV-1-infected patients. Although individual drug treatments cannot account for the development of the lipodystrophy syndrome, NRTIs, such as stavudine and zidovudine, are thought to predispose toward peripheral lipoatrophy, whereas PIs are considered to favor insulin resistance and dyslipidemia (Villarroya et al., 2005). NNRTIs are drugs of choice for initial antiretroviral treatment of HIV-1-infected patients, in combination with drugs from other families. Unlike NRTIs, NNRTIs do not inhibit DNA polymerase-γ, and therefore are not expected to elicit mitochondrial toxicity, a major suspected cause of adipose tissue alterations in HAART-treated HIV-1-infected patients.

Efavirenz, an NNRTI, is the preferred third agent to include in antiretroviral regimes according to most international antiretroviral treatment guidelines (Hammer et al., 2008; Gazzard et al., 2008). This is because efavirenz has never been surpassed in clinical trials, and, in fact, has shown better antiretroviral efficacy than PIs in pivotal clinical trials (Staszewski et al., 1999; Riddler et al., 2008). Nevirapine, though more restricted in use than efavirenz, is the other NNRTI commonly used in several European countries, especially in developing countries where a compact pill with stavudine and lamivudine is often used for initial antiretroviral therapy (Zhou et al., 2007; Colebunders et al., 2005). The 2NN

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study was originally intended to directly compare efavirenz and nevirapine (van Leth et al., 2004a); unfortunately, however, no conclusion could be drawn from this study in terms of non inferiority between both agents.

NNRTIs have not traditionally been associated with the appearance of lipodystrophy and are often perceived as benign in terms of adipose alterations. However, recent clinical trials have shown that efavirenz could favor lipoatrophy when used as a component of the HAART cocktail (Riddler et al., 2008; Haubrich et al., 2009). In contrast, several reports have indicated that shifting PI- or NRTI-based regimes to one containing nevirapine modestly ameliorated disturbances in the lipid profile of adults (Negredo et al., 1999) or pediatric (Gonzalez-Tome et al., 2008) patients. Switching to nevirapine also caused minor improvements in lipodystrophy (Negredo et al., 1999), but did not attenuate specific events associated with lipoatrophy, such as adipose tissue apoptosis (Domingo et al., 2001). Nevirapine appears to cause a better improvement in the lipid profile of treated patients respect to efavirenz (Fisac et al., 2005; van Leth et al., 2004b), basically by increasing HDL-cholesterol and the total cholesterol/HDL-cholesterol ratio.

There are few studies on the effects of NNRTIs on adipocytes. Using murine cell models, we reported (Rodríguez de la Concepción et al., 2005) that nevirapine favors differentiation and expression of marker genes of adipocyte function, such as peroxisome proliferator-activated receptor gamma (PPARγ), in primary brown adipocytes. This was in contrast to the anti-adipogenic effect of efavirenz in these cells. Efavirenz has been reported to decrease the capacity of mouse 3T3-L1 preadipocytes to accumulate triglycerides due to impaired lipogenesis (El Hadri et al., 2004), whereas no effects were observed for nevirapine in the murine adipogenic cell line 3T3-F422A (Caron et al., 2004). Similarly, studies of differentiating human adipocytes, which have been limited to reports on morphological changes, have shown reduced adipogenesis with efavirenz treatment (El Hadri et al., 2004) and no adipogenic effects of a single concentration (10 μg/ml) of nevirapine (Vernochet et al., 2005).

One of the main physiopathogenic components of the interplay between adipose tissue disturbances and systemic metabolic derangements in response to viral and pharmacological insults is interference with the secretory functions of adipose tissue. Adipose tissue is not only a site of fat storage, but is also responsible for releasing regulatory factors such as adipokines and pro-inflammatory cytokines that act both locally and on distant organs (e.g., liver, muscle, heart, pancreas) to influence overall metabolism. For instance, adipose tissue releases the insulin-sensitizing hormone adiponectin and other factors that affect insulin resistance, such as resistin, as well as inflammatory cytokines such as tumor necrosis factor α (TNFα) and interleukin (IL)-6 (Hauner, 2005). Thus, the potential action of drugs on adipose tissue may not only affect adipose tissue development, it can also alter adipose tissue secretory functions, and thereby alter systemic metabolism.

The effects of the NNRTIs nevirapine and efavirenz on the secretory functions of adipocytes have not been investigated to date. In the present study, we conducted a comparative analysis of nevirapine and efavirenz action on adipocyte differentiation, gene expression and release of adipokines and cytokines by human adipocyte cells in culture.

2. Methods

2.1. Preadipocyte differentiation and culture

Human adipocyte precursor cells from healthy individuals, obtained from Advancell (Barcelona, Spain), were cultured as previously reported (Schlüter et al., 2002). Differentiation was induced by treating cells at 80% confluence with Dulbecco's modified Eagle's (DMEM)/F12 medium containing 33 μM biotin, 17 μM sodium pantothenate, 200 nM insulin, 25 nM dexamethasone, 0.5 mM IBMX (3-isobutyl-1-methylxanthine), 2 μM rosiglitazone, and 0.2 nM triiodothyronine. After 4 days, the medium was replaced with culture medium with the same composition but without IBMX, rosiglitazone or dexamethasone; thereafter, medium was replaced every 5 days. In untreated cells, maximal differentiation, estimated from the maximal percentage of cells showing lipid droplet accumulation, was attained 15 days after induction of differentiation. Treatment with nevirapine or efavirenz was initiated on day 0 and was maintained throughout the differentiation process. Fresh drugs (dissolved in DMSO) were included with each change of medium. An equal amount of DMSO (<0.1%) was added to all control cell cultures.

2.2. Assessment of cytotoxicity

Potential cytotoxic effects of drugs on differentiating human preadipocytes were determined using a CytoTox96 kit (Promega, Madison, WI) following the Manufacturer's instructions.

2.3. Assessment of preadipocyte differentiation

The extent of morphological differentiation was quantified by measuring the percentage of the cell culture surface occupied by adipocytes relative to controls (defined as 100%). Adipocyte differentiation was also quantified after 15 days by measuring the intracellular lipid accumulation after Oil Red O staining, as reported elsewhere (Laughton, 1986).

2.4. Assessment of gene expression

RNA was extracted from cells using an RNeasy mini kit (Qiagen, Hilden, Germany). Reverse transcription was performed in a total volume of 20 μl using random hexamer primers (Applied Biosystems, Foster City, CA) and 0.5 μg total RNA. mRNA targets were amplified from cDNA by polymerase chain reaction (PCR) using an ABI/Prism 7700 Sequence Detector System. Each 25 μl reaction mixture contained 1 μl of cDNA, 12.5 μl of TaqMan Universal PCR Master Mix, 250 nM probes and 900 nM primers from the Assays-on-Demand Gene Expression Assay Mix (TaqMan, Applied Biosystems). The Assay-on-Demand probes used were cytochrome *c* oxidase subunit IV (COX4I1; Hs00266371), CEBPα (Hs00269972), lipoprotein lipase (Hs00173425), PPARγ (Hs00234592), adiponectin (Hs00605917), leptin (Hs00174877), adipocyte fatty-acid binding protein-4 (aFABP4/aP2; Hs00609791), sterol regulatory element-binding protein-1 (SREBP-1; Hs00231674) and 18S rRNA (Hs99999901). The sequences of primers and probe for the detection of cytochrome *c* oxidase subunit II (COII) and assessment of mitochondrial DNA (mtDNA) abundance, designed using the Assay-by-Design system (Custom TaqMan Gene Expression Assays, Applied Biosystems), were 5′-CAA ACC ACT TTC ACC GCT ACA C-3′ (forward primer), 5′-GGA CGA TGG GCA TGA AAC TGT-3′ (reverse) and 5′-AAA TCT GTG GAG CAA ACC-3′ (FAM-labeled probe). mtDNA was quantified using this primer/probe set and expressed relative to nuclear DNA, determined by amplification of the intronless gene C/EBPα. Controls containing no RNA, primers, or reverse transcriptase were included in each set of experiments. Each sample was run in duplicate, and the mean value of the duplicates was used to calculate the relative amount of each target mRNA. The mean value for each target was normalized to that of the 18S rRNA gene using the comparative ($2^{-\Delta CT}$) method following the Manufacturer's instructions.

2.5. Assessment of adipokine and cytokine secretion

Adipokines and cytokines released by adipocytes were quantified using 25 μl of medium collected from adipocyte cultures corresponding to the last 5 days before harvest. Adiponectin, leptin, monocyte chemoattractant protein-1 (CCL-2), IL-6, IL-8, total plasminogen activator inhibitor type-1 (PAI-1), hepatocyte growth factor (HGF), and nerve growth factor (NGF) were detected using an antibody-linked, fluorescently labeled microsphere bead-based multiplex analysis system (Linco Research/Millipore, Billerica, MA) and quantified using Luminex100ISv2 equipment. Although the multiplex system used (HADCYT-61K, Millipore) also allowed for the quantification of IL-1β, resistin and tumor necrosis factor-α, the levels of these factors in adipocyte culture medium were below detection limits under all conditions tested. Lactate in the medium was measured spectrophotometrically (Roche, Sant Cugat del Vallés, Spain).

2.6. Assessment of endogenous reverse transcriptase activity

Cellular reverse transcriptase (RT) activity assay was performed as previously reported (Mangiacasale et al., 2003). Briefly, cells were lysed in ice-cold lysis buffer (10 mM Tris–HCl, pH 7.5, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM PMSF, 5 mM β-mercaptoethanol, 0.5% CHAPS, 10% glycerol). After three freeze-and-thaw cycles, cells were incubated for 30 min on ice and centrifuged for 30 min at 14,000 rpm at 4 °C. The supernatant containing the RT activity was aliquoted, quickly frozen in dry ice and stored at −80 °C. The protein concentration was determined by Bradford analysis. RT activity was tested using a MultiScribe TM Reverse Transcriptase (Applied Biosystems) in 20 μL reactions containing 10 ng of MS2 phage RNA (Roche Diagnostics), 30 pmol of MS2 reverse primer (see below) and substituting commercial RT with cell-free extract (12 μg total protein). Reaction mixtures were incubated at 25 °C for 10 min, 48 °C for 30 min followed by 5 min at 95 °C. A 2 μL volume from each reaction with forward (5′-TCCTGCTCAA-CTTCCTGTCGAG-3′) and reverse (5′-CATAGGTC AAACCTCCTAGGAATG-3′) MS2 primers were analyzed by quantitative PCR using SYBR green fluorescent dye (Applied Biosystems).

HT-29 adenocarcinoma cell line was used as positive control cells of RT activity (Mangiacasale et al., 2003). RT activity was measured in differentiating human adipocytes after 2- or 15-days induction of differentiation. The effects of antiretroviral drugs were assessed in 48-h treated differentiating human adipocytes.

2.7. Statistics

Where appropriate, statistical analyses were performed using Student's *t*-test. Differences with *P*-values <0.05 were considered statistically significant.

3. Results

3.1. Effects of nevirapine and efavirenz on human adipocyte viability and differentiation

Nevirapine was not cytotoxic to human adipose cells at any of the concentrations tested. At low concentrations (0.5 and 4 μM), efavirenz was not significantly cytotoxic, but at 20 μM caused extensive cell death, precluding further analysis of differentiation effects at higher efavirenz concentrations (data not shown).

A quantitative assessment of the effect of drug treatment on the extent of differentiation of human adipocytes compared to untreated controls (defined as 100%) is shown in Fig. 1A and B. Representative microscopic images depicting the effects of drug treatment on morphological changes associated with the acquisition of adipocyte morphology during *in vitro* differentiation are shown in Fig. 1C. Nevirapine at 0.5, 2, 4 and 10 μM did not affect morphological adipocyte differentiation; in contrast, efavirenz at a concentration of 1 μM (data not shown), 2 and 4 μM (the highest non-toxic concentration) significantly impaired differentiation (Fig. 1A). Exposure of differentiating cells to 20 μM nevirapine significantly increased adipocyte differentiation. Increasing the nevirapine concentration to 30 and 40 μM did not further modify adipogenesis. Measurement of lipid accumulation by quantification of eluted Oil Red O stain also revealed an inhibition of differentiation by efavirenz (1 (data not shown), 2 and 4 μM) whereas nevirapine did not significantly affect lipid accumulation at any of the concentrations tested (Fig. 1B).

3.2. Effects of nevirapine and efavirenz on gene expression in human adipocytes differentiating in vitro

An analysis of marker genes of adipocyte differentiation and metabolism showed that treatment of human adipocytes with nevirapine during differentiation did not modify gene expression except at the 20 μM concentration, which caused a significant increase in the mRNA levels of the adipogenesis master regulator genes SREBP-1, PPARγ and C/EBPα as well as adiponectin, leptin and lipoprotein lipase, marker genes of adipogenic differentiation and metabolism (Fig. 2). In contrast, efavirenz exerted the opposite effect, causing a dose-dependent reduction in the expression of adipogenesis marker genes, first evidenced as a significant decrease in SREBP-1 mRNA, PPARγ mRNA and leptin mRNA at 0.5 μM efavirenz. At higher doses of efavirenz, the reduction in the expression of these genes was more pronounced and there was also a significant decrease in adiponectin and lipoprotein lipase mRNA levels beginning at 2 μM, and also in C/EBPα and aFABP4/aP2 mRNA levels at 4 μM. Neither drug caused significant mitochondrial toxicity at any of the concentrations analyzed, as evidenced by the absence of changes in COIV (nuclear DNA-encoded) or COII (mtDNA-encoded) mRNA levels, or the relative levels of mtDNA.

3.3. Effects of nevirapine and efavirenz on the release of adipokines, cytokines and lactate by differentiating human adipocytes

The effects of nevirapine and efavirenz at concentrations representative of their opposing effects on human adipocytes (20 μM nevirapine and 4 μM efavirenz) were studied for their effects on the release of regulatory proteins and lactate by adipocytes (Fig. 3). Efavirenz caused a dramatic decrease in adiponectin and leptin release into the medium. In contrast, nevirapine did not alter leptin release and significantly increased adiponectin levels in adipose cell culture medium. Moreover, efavirenz profoundly increased the expression of the pro-inflammatory cytokines IL-6, CCL-2 and IL-8, whereas nevirapine did not significantly alter either CCL-2 or IL-8 levels, and significantly decreased the levels of IL-6.

With respect to other regulatory proteins released by adipocytes, nevirapine had no effect on total PAI-1 levels whereas efavirenz significantly increased it, and nevirapine significantly reduced HGF levels in adipocyte culture medium whereas efavirenz increased it. Neither drug had any effect on NGF release, and there were no differences among controls, nevirapine-treated and efavirenz-treated adipocyte cell cultures with respect to release of lactate into the medium.

3.4. Effects of nevirapine and efavirenz on human adipocyte endogenous reverse transcriptase activity

Mainly nevirapine but also efavirenz have been reported to exhibit anti-proliferative and pro-differentiating effects on several cell types, including tumor cells, through inhibition of endogenous

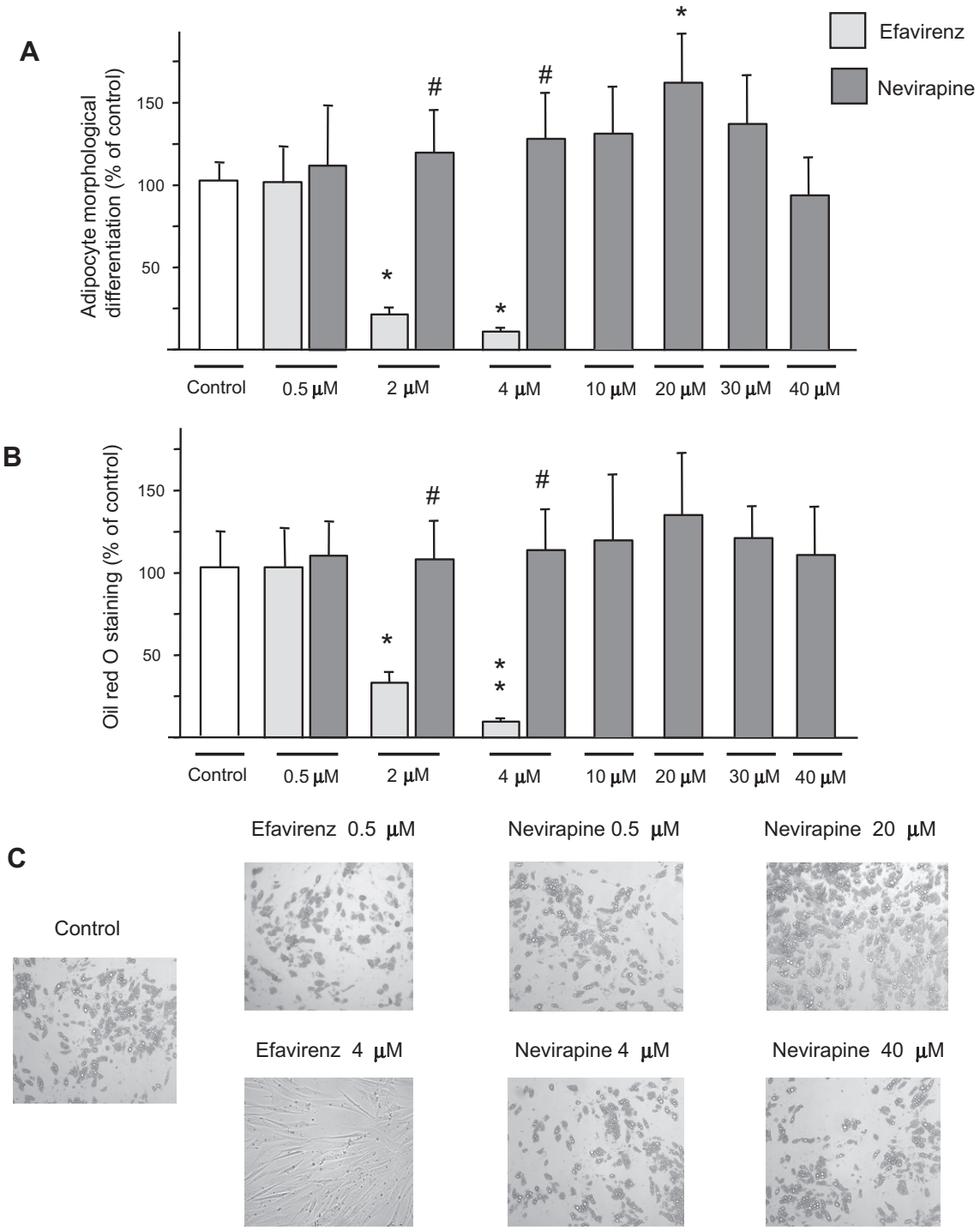

Fig. 1. Effects of nevirapine and efavirenz on human preadipocytes differentiating in culture. Human preadipocytes were differentiated in culture in the presence of the indicated concentrations of drugs. Bars are means ± SEM of the extent of morphological adipocyte differentiation (A) or of the Oil Red O staining of lipid accumulation (B) of 4–5 independent cultures at each of the indicated concentrations, and are expressed relative to values from untreated control cells, defined as 100% (see Section 2). ($^{*}P < 0.05$ and $^{**}P < 0.01$ for each drug treatment vs control; $^{\#}P < 0.05$ for nevirapine vs efavirenz treatment at the same concentration). (C) Representative microphotographs of adipocyte cell cultures differentiating in the presence of the indicated concentrations of drugs.

cellular reverse transcriptase (RT) activity (Spadafora, 2004). As depicted in Fig. 4A, quantitative assessment of endogenous RT activity showed higher levels in HT-29 adenocarcinoma cells (6-fold) and human preadipocytes (2-fold) than in human adipocytes, in agreement with more elevated endogenous RT activity in proliferating respect quiescent/differentiated cells in other cell types. When the effects of nevirapine and efavirenz were tested in human differentiating adipocytes, a different behavior was observed (Fig. 4B). Efavirenz had no effect on endogenous RT activity whereas nevirapine significantly reduced it, to levels similar to those found in fully differentiated human adipocytes.

4. Discussion

The present study establishes that nevirapine, in contrast to the other most widely used NNRTI, efavirenz, does not impair

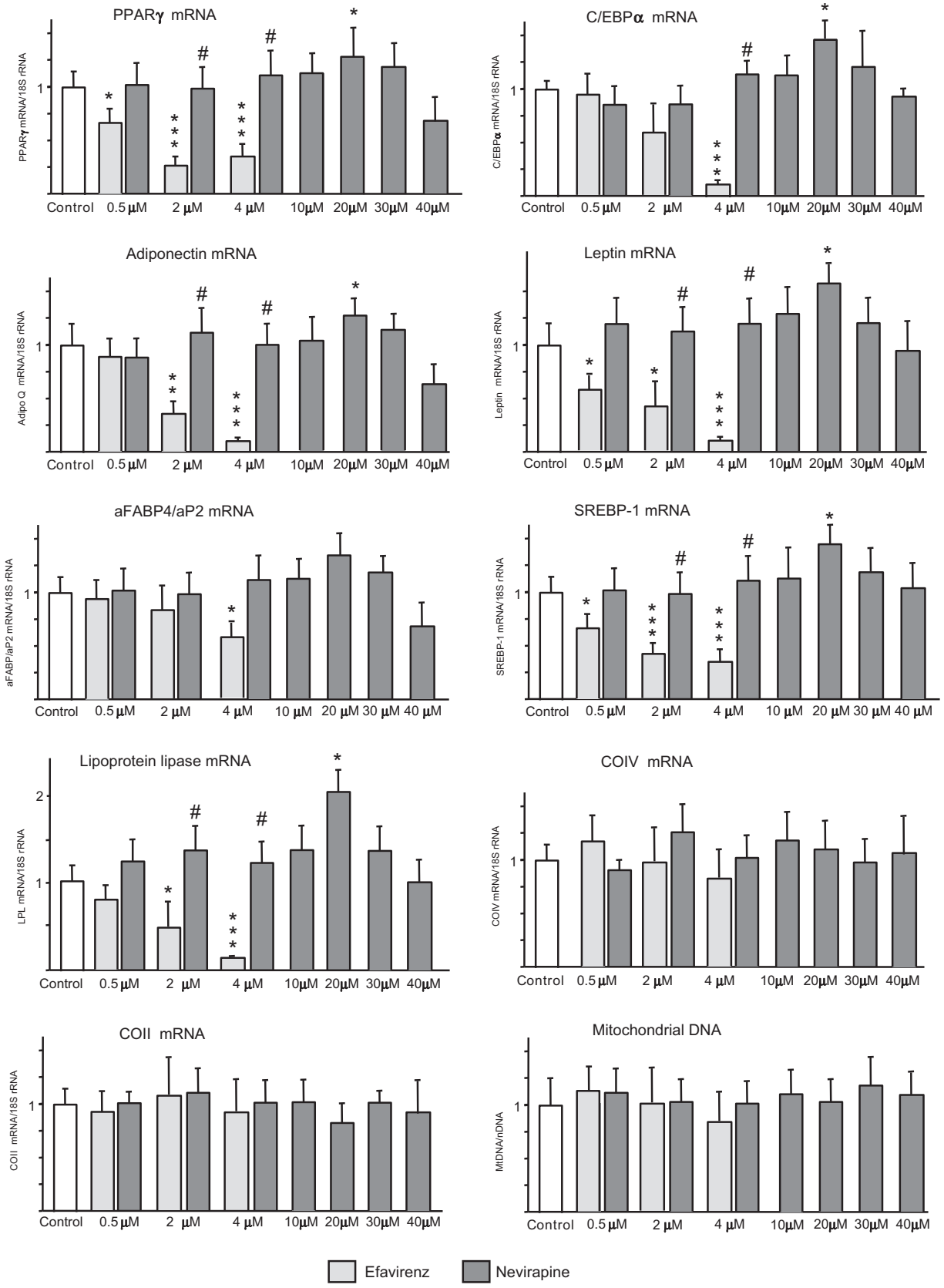

Fig. 2. Effects of nevirapine and efavirenz on mtDNA levels, and expression of genes related to mitochondrial function and adipogenic function in human adipocytes differentiating in culture. Human preadipocytes were differentiated in culture in the presence of the indicated concentrations of efavirenz (light gray bars) or nevirapine (dark gray bars). Data are presented as means ± SEM from 4 to 5 independent experiments, and are expressed relative to values from untreated control cells (defined as 1). ($^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ for each drug treatment vs control; $^{\#}P < 0.05$ for nevirapine vs efavirenz treatment at the same concentration).

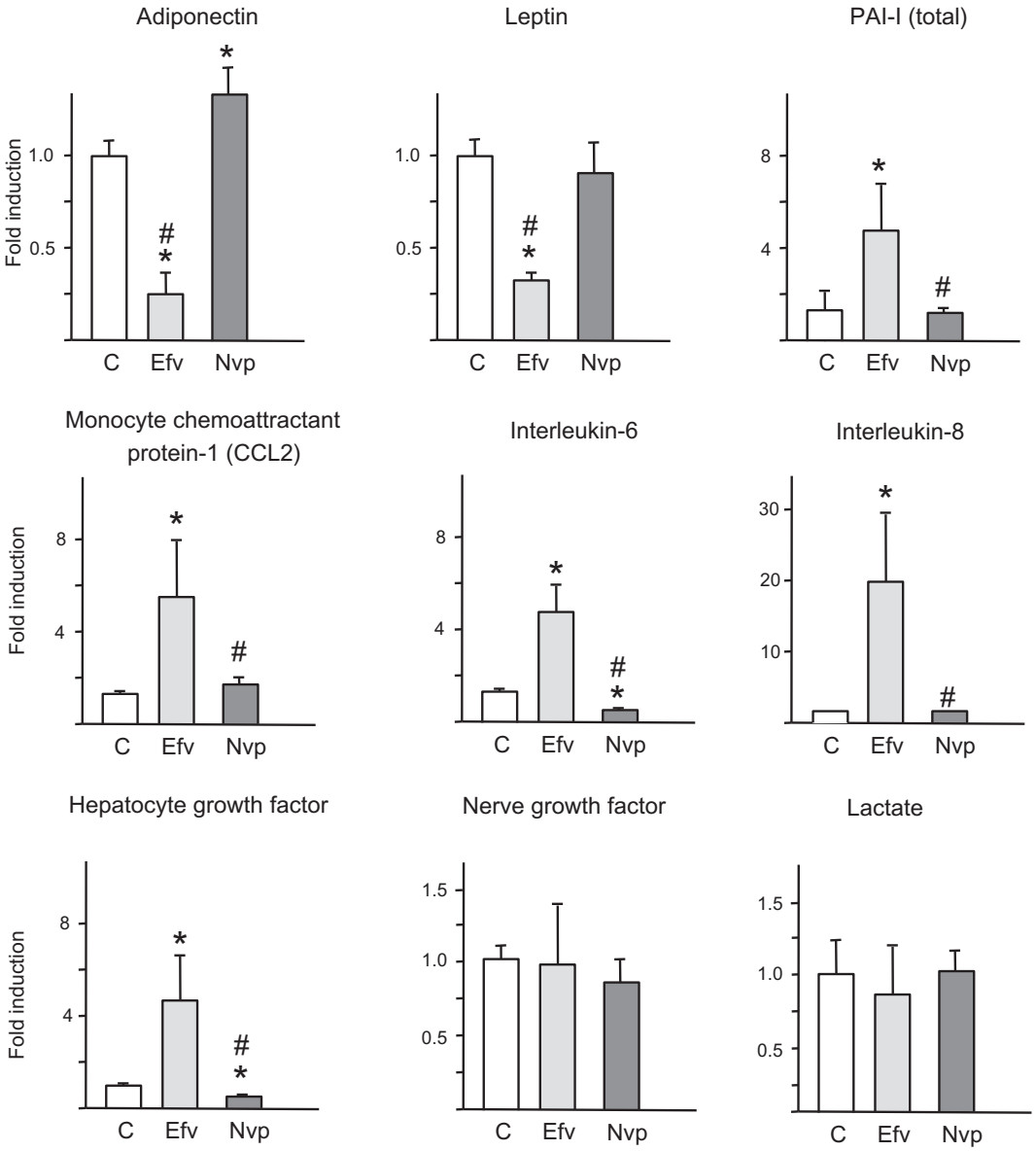

Fig. 3. Effects of nevirapine and efavirenz on the release of adipokines, cytokines and lactate by human adipocytes in culture. Human preadipocytes were differentiated in culture in the presence of 4 μM efavirenz (Efv) or 20 μM nevirapine (Nvp), and release of adipokines, cytokines and lactate was measured. Values represent concentrations in cell culture medium, presented as means ± SEM from 4 to 5 independent experiments and expressed relative to values from untreated control cells (defined as 1). Mean values of cytokines, adipokines and lactate in the medium from control untreated cells were as follows: adiponectin, 0.24 ± 0.3 μg/ml; leptin, 9.0 ± 0.8 ng/ml; total PAI-I, 0.41 ± 0.06 ng/ml; CCL-2, 3.2 ± 0.4 ng/ml; IL-6, 66 ± 9 pg/ml; IL-8, 29 ± 4 pg/ml; HGF, 85 ± 10 pg/ml; NGF 8.1 ± 1.1 pg/ml; lactate, 2.1 ± 0.5 mM. ($^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ for each drug treatment vs control; $^{\#}P < 0.05$ for nevirapine vs efavirenz treatment).

adipogenesis and even causes mild positive effects at a single concentration. Negative effects of efavirenz on human adipocyte differentiation have been reported (El Hadri et al., 2004) and recent data from clinical trials indicate potentially negative effects of efavirenz when used as part of some antiretroviral drug combinations (Haubrich et al., 2009). In the present study, we confirmed that these detrimental effects of efavirenz are associated with a profound dose-dependent repression of the master transcriptional regulators of adipogenesis, SREBP-1, PPARγ and C/EBPα, and a reduction in the expression of genes involved in fat accretion in the adipose cells, such as lipoprotein lipase. These effects are in strong contrast to the effects of nevirapine, which did not cause inhibition of adipogenesis at any concentration tested, in agreement with lack of nevirapine effects on murine 3T3-F442A adipocyte differentiation (Caron et al., 2004). Present data also indicate that, in fact, at 20 μM nevirapine even tended to have the opposite effect, significantly increasing the morphological differentiation of adipocytes, and enhancing the expression of PPARγ and C/EBPα, and genes associated with the adipocyte phenotype, such as lipoprotein lipase and adiponectin.

Neither the lack or mild positive effects of nevirapine nor the negative effects of efavirenz on human adipocytes involved mitochondrial alterations. The absence of changes in mtDNA levels, expression of mtDNA-encoded or nuclear DNA-encoded transcripts for mitochondrial proteins, or lactate release into the medium indicates that NNRTIs may alter adipocyte biology without causing mitochondrial toxicity. This is in agreement with studies on adipose tissue from patients, which have shown that the most marked mitochondrial toxicity is attributable to the inclusion of NRTI thymidine-analogs as part of treatment, rather than to the

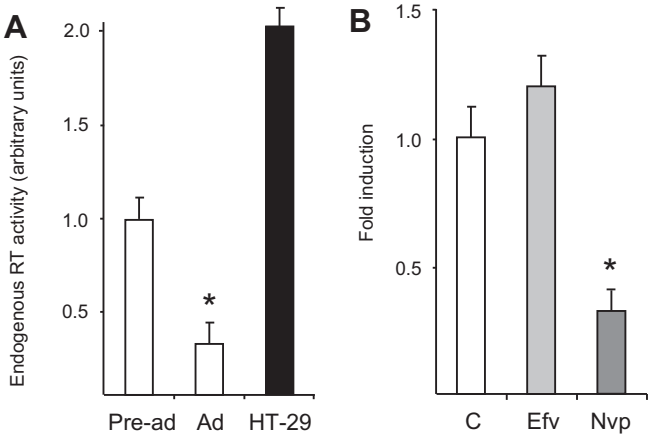

Fig. 4. Effects of nevirapine and efavirenz on human adipocyte endogenous reverse transcriptase activity. (A) Functional RT activity assay after incubation of MS2 RNA with lysates from human preadipocytes (Pre-ad), human adipocytes (Ad) and HT-29 adenocarcinoma cells. (*$P < 0.05$, for comparisons between Pre-ad vs Ad). (B) Human preadipocytes differentiating in culture were treated for 48 h in the presence of 4 μM efavirenz (Efv) or 20 μM nevirapine (Nvp), and endogenous RT activity was measured. (*$P < 0.05$, for drug treatment vs control).

presence of NNRTIs efavirenz or nevirapine (Villarroya et al., 2007b).

Notably, the present finding that nevirapine may favor the differentiation of adipose cells is consistent with multiple reports indicating positive effects of nevirapine on differentiation and growth arrest in other cell types. Nevirapine has been reported to cause differentiation of normal and multiple types of transformed cells (Mangiacasale et al., 2003), including human prostate carcinoma cells (Landriscina et al., 2009), renal carcinoma cells (Landriscina et al., 2008), cervical carcinoma cells (Stefanidis et al., 2008) and thyroid carcinoma cells (Landriscina et al., 2006; Modoni et al., 2007). This has led to proposals to explore the use of nevirapine in cancer treatment. However, the molecular mechanisms by which nevirapine promotes cell differentiation are currently uncertain, although inhibition of endogenous cellular reverse transcriptase activity has been proposed (Spadafora, 2004; Sciamanna et al., 2005). Present results indicate for the first time that inhibition of endogenous RT activity occurs during human adipocyte differentiation, and that nevirapine is capable to inhibit endogenous RT activity in differentiating preadipocytes. In contrast, although efavirenz was also showed to have inhibitory effects on endogenous reverse transcriptase as well as similar anti-proliferating and pro-differentiating effects than nevirapine on transformed cells (Sciamanna et al., 2005; Landriscina et al., 2008), efavirenz did not affect endogenous RT activity in human preadipocytes, consistently with its negative role on adipocyte differentiation. Thus, differential effects of nevirapine and efavirenz on adipocyte-specific reverse transcriptase activity, together with efavirenz-specific repression of master transcriptional regulators of adipogenesis (El Hadri et al., 2004; Esposito et al., 2009; Gallego-Escuredo et al., 2010, and present results), would constitute an additional mechanism by which nevirapine and efavirenz have differential effects in the context of adipose cells.

The beneficial effects of nevirapine on human adipose cells – from promoting adipogenesis to attenuating the release of pro-inflammatory molecules and stimulating the release of insulin sensitizing, anti-inflammatory molecules such as adiponectin – were evident at only at 20 μM, suggesting a narrow optimal concentration range for these effects. This is similar to our previous findings obtained using the murine brown adipocyte cell culture model (Rodríguez de la Concepción et al., 2005). In addition to its anti-adipogenic effects, efavirenz induced the release of pro-inflammatory cytokines (e.g., CCL-2, IL-6 and IL-8) and other molecules related to the inflammatory processes, such as PAI-1 and HGF. Efavirenz also decreased the release of adiponectin into the medium. In contrast, nevirapine favored the release of adiponectin and repressed the release of IL-6 and HGF. Adiponectin is recognized as a major insulin-sensitizing hormone with anti-inflammatory properties (Kadowaki et al., 2006), and low levels of serum adiponectin are commonly found in HIV-1-infected patients with lipodystrophy and/or metabolic syndrome (Tong et al., 2003). The relevance of these observations in relation to the metabolic status of HAART-treated patients should deserve further attention. Some studies have indicated that switching to nevirapine reduces hyperinsulinemia (Domingo et al., 2001), whereas other reports have found that switching to nevirapine has no effect on adiponectin levels or insulin sensitivity, despite normalization of the lipid profile (Petit et al., 2004). The fact that 20 μM nevirapine decreased the release of IL-6, a pro-inflammatory cytokine that plays a main role in adipose tissue signaling being responsible for eliciting systemic insulin resistance (Kim et al., 2009), is notable, as is the nevirapine-induced decrease in the release of HGF, a powerful angiogenic factor produced by adipose tissue. HGF is increased in the obesity-induced inflammation of adipose tissue (Bell et al., 2008), but data on possible changes in HGF in HIV-1-infected patients undergoing HAART are currently lacking. Collectively, these observations lead us to hypothesize that the presence of nevirapine in HAART cocktails may moderate the local pro-inflammatory environment in adipose tissue elicited by infection-related events and concurrent treatment with other drugs. This might be especially relevant in light of growing evidence that local inflammation in adipose tissue plays a role in mediating systemic metabolic disturbances in patients (Villarroya et al., 2007a), and should drive further attention in clinical studies comparing the side effects of individual drug components of HAART regimes.

In summary, the present results suggest that nevirapine does not inhibit adipogenesis and may even exert mild positive effects on the pattern of inflammation-related signals released by human adipocytes. This is in contrast to the profound anti-adipogenic and pro-inflammatory pattern of response elicited by efavirenz. The present *in vitro* adipocyte study has obvious limitations in terms of extrapolation to the treatment of patients. Notably, however, the average nevirapine plasma concentration in patients is approximately 20 μM (Dupin et al., 2002; Cooper and van Heeswijk, 2007), suggesting that the beneficial effects of nevirapine observed *in vitro* may be relevant *in vivo*. Complicating this interpretation is the fact that serum proteins may bind substantial amounts of drug; thus, the actual free nevirapine concentration in blood may be lower than that in serum-free adipocyte culture medium containing 20 μM nevirapine. However, because nevirapine has been reported to accumulate in adipose tissue from patients, reaching a concentration much higher than that in blood (Dupin et al., 2002), the possibility that adipocytes *in vivo* are exposed to local concentrations of nevirapine that approach the 20 μM range cannot be excluded. The same rationale applies to efavirenz concentrations, as this drug also accumulates at much higher concentrations in adipose tissue than blood of treated patients (Dupin et al., 2002). In any case, because nevirapine is already widely used in certain settings (e.g., some European countries, developing world) and given the potential deleterious metabolic side-effects of antiretroviral treatments, any evidence of neutral, or maybe beneficial, effects of nevirapine should be considered in the context of optimizing drug composition in HAART. However, the fact that nevirapine has been associated with selection of resistant virus as well as with life-threatening hepatic and cutaneous toxicities (de Béthune, 2010) should also be taken into consideration.

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