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FIELD OF VISION

Additional attention to combination antiretroviral therapy-related lipodystrophy

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Abstract

The occurrence of lipodystrophy in patients taking anti-human immunodeficiency virus (HIV) medications is a serious problem as it is irreversible even after drug withdrawal. Although it was first recognized in patients taking proteinase inhibitors, other types of anti-HIV agents can also cause lipodystrophy. In a recent publication by Jones *et al* entitled "Highly active antiretroviral therapy dysregulates proliferation and differentiation of human pre-adipocytes" in *World Journal of Virology*, it was reported that simultaneous treatment of human subcutaneous adipocytes with anti-HIV drugs with different mechanisms of action synergistically exerted anti-adipogenesis effects *in vitro*, warning us to take utmost care in every case receiving combination antiretroviral therapy (cART). For elucidation of the molecular basis for cART-related lipodystrophy, multi-faceted approaches should be taken, based on a deeper understanding of the development and organization of adipose tissues.

Key words: Combination antiretroviral therapy; Lipodystrophy; Protease inhibitor; Reverse transcriptase inhibitor; Human immunodeficiency virus

Core tip: Development of lipodystrophy in patients receiving combination antiretroviral therapy (cART) has been a serious problem. Although it was first reported in patients taking proteinase inhibitors, other types of anti-human immunodeficiency virus (HIV) agents also cause lipodystrophy. A recent publication in *World Journal of Virology* reported unexpected synergism among anti-HIV drugs with different mechanisms of action in inhibiting adipogenesis *in vitro*. To elucidate the molecular basis for cART-related lipodystrophy, multi-faceted approaches should be taken with a deeper understanding of the development and organization of adipose tissues.

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COMMENTARY ON A RECENT ARTICLE ON COMBINATION ANTIRETROVIRAL THERAPY-RELATED LIPODYSTROPHY

Lipodystrophy is a serious problem in patients receiving combination antiretroviral therapy

The development of combination antiretroviral therapy (cART), where more than two types of anti-human immunodeficiency virus (HIV) agents with different mechanisms of action are used, has greatly improved the prognosis of acquired immune deficiency syndrome (AIDS) by reducing mortality rates and suppressing opportunistic infections. With the extension of the administration period, however, it has becoming increasingly important to take effective measures to control the side effects of cART. The major adverse conditions caused by anti-HIV agents are lipodystrophy, atherosclerosis, eruption, osteoporosis and lactic acidosis. Since the pathophysiology of cART-related lipodystrophy is an irreversible process, elucidation of the mechanism how anti-HIV agents induce lipodystrophy is a matter of particular importance. In the current commentary, possible mechanisms of cART-related lipodystrophy are discussed by referring a recent paper by Jones *et al*[1] in *World Journal of Virology*, which reported an unexpected synergy among anti-HIV drugs with different mechanisms of action in inhibiting proliferation and differentiation of human preadipocytes *in vitro* (Figure 1).

Organization of adipose tissues

Adipose tissues consist of parenchymal cells (*i.e.,* adipocytes and their progenitors) and non-parenchymal cells (*i.e.,* non-adipocyte lineage cells) (Figure 2) and disorders of any of these components may result in the development of lipodystrophy. Caso *et al*[2] previously reported that two proteinase inhibitors, ritonavir (RTV) and atazanavir (ATV), inhibited the proliferation and differentiation of human subcutaneous preadipocytes *in vitro*. In a recent paper, the same group showed that protease inhibitors (RTV, ATV), nucleoside/nucleotide reverse transcriptase inhibitors [emtricitabine (FTC), tenofovir (TDF)] and a non-nucleoside reverse transcriptase inhibitor [efavirenz (EFV)] synergistically inhibited proliferation and differentiation/maturation of human preadipocytes obtained from subcutaneous fat depots[1]. It is surprising that synergism exists among drugs with completely different action mechanisms. Although the molecular basis of this synergism remains unknown, the finding has sounded the alarm about the risk of unexpected occurrence of lipodystrophy in cART-receiving patients.

In addition to affecting parenchymal cells, anti-HIV agents may impair the functions of non-parenchymal cells, which consist of mesenchymal stem cells (MSCs), vascular endothelial cells (VEC), pericytes/vascular smooth muscle cells, resident macrophages (M2) and inflammatory macrophages (M1) (Figure 2). Among these, VECs may be of most interest for the following three reasons: First, anti-HIV agents reportedly induce oxidative damage to VECs[3], which is considered to be one of the causes of development of cART-related atherosclerosis. Atherosclerosis in adipose tissue vasculatures within specific regions of subcutaneous fat depots may cause local ischemia, resulting in degeneration/loss of adipose tissues in specific areas such as the face and limbs. However, the reason why the face and limbs are commonly affected by cART-related lipodystrophy remains unknown. Secondly, VECs in adipose tissues (A-VEC) is known as one of the ancestors of adipocytes[4,5]. Recurrent damage to A-VECs by anti-HIV agents may reduce the population size of adipocyte precursors, which might, in turn, result in the occurrence of lipodystrophy after a time. Lastly, it is accepted that A-VECs have distinctive characteristics compared with VECs of other tissues; for example, VECs of white adipose tissues exhibit specific antigenicity[6]. Moreover, it was recently reported that poly(2-methacryloyloxyethyl phosphorylcholine-co-n-butyl methacrylate (PMB)-coated carbon nanotubes specifically accumulated in the capillary endothelial cells of adipose tissues[7]. Even under conditions where anti-HIV drugs do not cause oxidative damage to A-VEC, they might possibly affect specific functions of A-VEC, thus promoting the development of lipodystrophy.

Other non-parenchymal cells could also be the targets of cART. The MSCs can be precursors of preadipocytes, and pericytes/vascular smooth muscle cells are recognized as an equivalent of MSCs[8], although a recent paper by Guimarães-Camboa *et al*[9] has challenged this idea. Similarly to A-VECs, pericytes/vascular smooth muscle cells in adipose tissues are considered as one of the ancestors of adipocytes[5]. It is known that white adipose tissues are particularly rich in M2 macrophages, which provide a niche for preadipocytes[10]. On the other hand, M1 inflammatory macrophages are recruited into adipose tissues from the bone marrow when adipocytes undergo degeneration. Defects in the clearance of degenerative adipocytes may create pathological states of adipose tissues, which possibly lead to the development of lipodystrophy.

Although the critical target cells remain undetermined, the etiology of cART-related lipodystrophy should be investigated from multiple perspectives.

Possible mechanisms of cART-lipodystrophy

The incidence of lipodystrophy was first recognized in patients receiving protease inhibitors[11,12] and more than half of affected patients reportedly take these drugs[13]. In addition to suppressing growth and differentiation of preadipocytes[1], protease inhibitors reportedly exert multifactorial effects on adipocytes including impairment of mitochondrial functions[14], changes in the gene expression

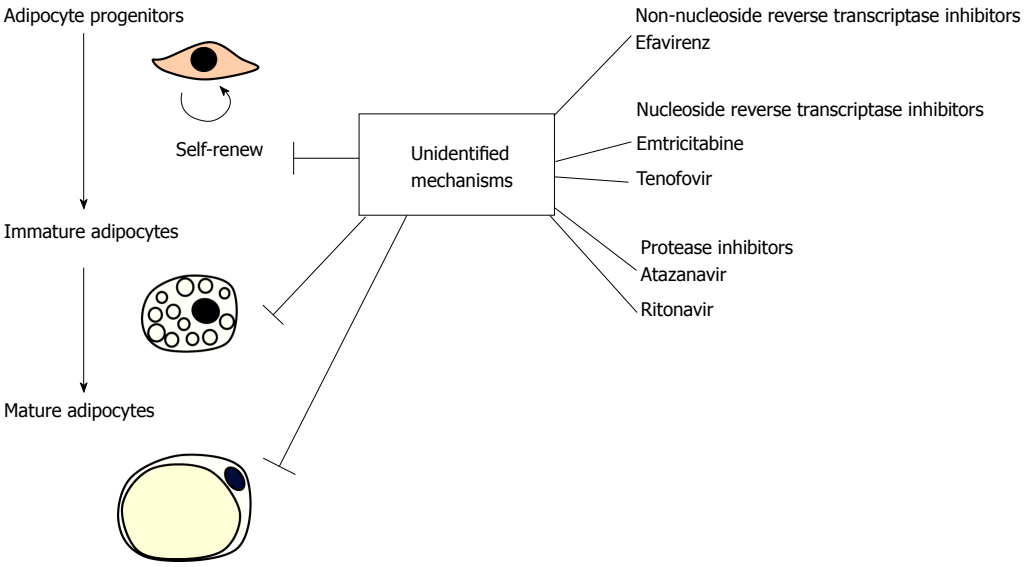

Figure 1 Diverse anti-human immunodeficiency virus agents synergistically inhibit adipogenesis *in vitro*. Although detailed processes remain elusive, anti-human immunodeficiency virus agents of different action mechanisms synergistically inhibit proliferation and differentiation of preadipocytes that are prepared from human subcutaneous fat depots as reported by Jones *et al*[1].

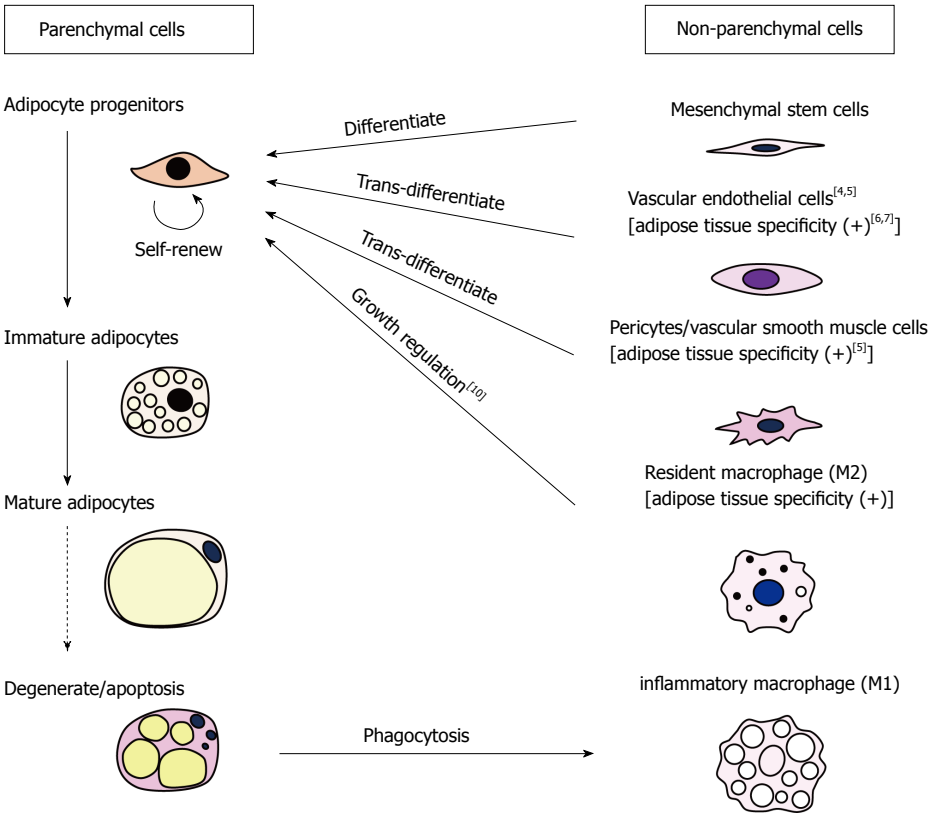

Figure 2 Organization of adipose tissues. Adipose tissues consist of parenchymal cells and non-parenchymal cells. Coordinated interactions of these two components are important for adipose tissue performance.

related to ER-stress and lipid droplet formation[14] and even an induction of mitophagy[15]. cART-related dystrophy is not restricted to protease inhibitor-receiving cases[16]. Patients with cART-related dystrophy are relatively healthy[13], although hypertriglyceridemia and insulin resistance may be present[11-13,16-19]. Nevertheless, cART-related dystrophy is a crucial issue to be addressed, since loss of fat depots frequently occurs in the face and limbs and fat wasting of the face considerably reduces quality of life. The reason why adipose tissues in the distal parts of the body are commonly affected by cART-related dystrophy remains elusive. In some cases,

subcutaneous fat depots are reciprocally accumulated in the central parts of body including the abdomen, trunk and neck. Since plasma cortisol values are reportedly within the normal range, central fat accumulation cannot be attributed to hypercortisolism[16]. Interestingly, adipose tissue-specific Dicer knockout (ADicerKO) mice reportedly exhibit a phenotype that bears a close resemblance to cART-related lipodystrophy[20]. They even show buffalo hump-like phenotypes, in which brown adipose tissues (BAT) are substantially enlarged and degenerated into white adipose tissue accumulations[20]. Since BATs are mainly located in the neck and upper back areas in mice and humans, central fat accumulation in cART-related lipodystrophy might possibly be associated with abnormal lipid accumulation in BATs.

In summary, the etiology of cART-related lipodystrophy remains largely unknown and further investigations will be required to elucidate its developmental mechanisms.

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ORIGINAL ARTICLE

Basic Study

Highly active antiretroviral therapy dysregulates proliferation and differentiation of human pre-adipocytes

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Author contributions: Jones E contributed to data collection; McNurlan MA contributed to study design; Darras F recruited study subjects, collected surgical samples, and contributed to drafting the manuscript; Gelato MC contributed to overall study design; Caso G contributed to study design, performed the experiments; Jones E, Mazirka P, McNurlan MA and Caso G contributed to data analysis; Jones E, Mazirka P, McNurlan MA, Gelato MC and Caso G writing of the manuscript; Jones E, McNurlan MA, Gelato MC and Caso G contributed to data interpretation; all authors approved the final version of the manuscript.

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Abstract

AIM

To investigate the mechanism(s) by which potential effects of multi-drug highly-active antiretroviral therapy contributes to lipodystrophy syndrome.

METHODS

Preadipocytes from healthy donors were assessed for proliferation and differentiation in the presence of nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs) individually and in combination. Effects on proliferation were assessed with a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide assay and effects on differentiation were assessed from glycerol-3-phosphate dehydrogenase (GPDH) activity and quantitation of Oil Red O staining for intracellular lipid. Data were analyzed with a randomized block ANOVA with post-hoc Fisher's Least Significant

Difference test.

RESULTS

Preadipocyte proliferation was inhibited by a combination of NNRTI + NRTI (14% at 48 h, $P < 0.001$) and PI + NRTI (19% at 48 h, $P < 0.001$) with additional suppression when ritonavir (RTV) was added (26% at 48 h). The drug combination of atazanavir (ATV) + RTV + emtricitabine (FTC) + tenofovir (TDF) had the greatest inhibitory effect on proliferation at 48 h. Preadipocyte differentiation was most significantly reduced by the efavirenz + FTC + TDF assessed either by GPDH activity (64%) or lipid accumulation (39%), $P < 0.001$. Combining NRTIs with a PI (ATV + FTC + TDF) significantly suppressed differentiation (GPDH activity reduced 29%, lipid accumulation reduced by 19%, $P < 0.01$). This effect was slightly greater when a boosting amount of RTV was added (ATV + FTC + TDF + RTV, $P < 0.001$).

CONCLUSION

Although combination antiretroviral therapy is clinically more efficacious than single drug regimens, it also has a much greater inhibitory effect on preadipocyte proliferation and differentiation.

Key words: Nucleoside reverse transcriptase inhibitors; Non-nucleoside reverse transcriptase inhibitors; Protease inhibitors; Pre-adipocytes; Highly active antiretroviral therapy; Lipodystrophy

Core tip: We demonstrated an *in vitro* system for evaluating potential antiretroviral regimens for adipose tissue toxicity. In general, combination regimens resulted in greater preadipocyte proliferation and differentiation inhibition than single therapies. The drug combination of atazanavir + emtricitabine + tenofovir had inhibitory effects on preadipocytes and adding ritonavir at levels equivalent to clinical boosting, increased toxicity still further.

Jones E, Mazirka P, McNurlan MA, Darras F, Gelato MC, Caso G. Highly active antiretroviral therapy dysregulates proliferation and differentiation of human pre-adipocytes. *World J Virol* 2017; 6(3): 53-58 Available from: URL: http://www.wjgnet.com/2220-3249/full/v6/i3/53.htm DOI: http://dx.doi.org/10.5501/wjv.v6.i3.53

INTRODUCTION

A link between highly active antiretroviral therapy (HAART) and HAART-associated lipodystrophy (HALS) has been recognized for well over a decade. HALS is associated with abnormal changes in fat distribution throughout the body, insulin resistance and altered levels of triglycerides, cholesterol and lipoproteins[1]. These changes impact the health of an individual as well as their quality of life and have reduced the impact of anti-HIV therapy development[2,3]. HAART regimens with various combinations of protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI) and nonnucleoside reverse transcriptase inhibitors (NNRTI) have been associated with HALS (*e.g.*[4-6]). Previously we reported on the *in-vitro* effects of two PIs, ritonavir (RTV) and atazanavir (ATV) on preadipocyte proliferation and adipogenesis[7]. In the present study, we report the effects of common first-line combination regimens used in HIV treatment; efavirenz (EFV) + emtricitabine (FTC) + tenofovir (TDF), ATV + FTC + TDF and ATV + RTV + FTC + TDF, as well as their individual components, on *in-vitro* preadipocyte proliferation and differentiation.

MATERIALS AND METHODS

Patients and study design

Preadipocytes were obtained from abdominal subcutaneous fat tissue from healthy kidney donors undergoing nephrectomy. The samples were collected from 10 kidney donors (6 females, 4 males) who gave a written informed consent. All participants were HIV-seronegative, had an average age of 37 ± 4 years and a BMI of 29 ± 1 kg/m^2. Subjects were placed under standard general anesthesia and subcutaneous fat tissue was removed from the peri-umbilical area during nephrectomy. The specimens were then immediately placed in a sterile Hank's Buffered Salt Solution (HBSS) at pH 7.4 containing antibiotics and amphotericin. All fat samples were processed within one hour.

Once isolated, preadipocytes were tested *in vitro* for their ability to replicate and differentiate in the presence of different classes of antiretroviral drugs, which were applied individually or in combination. Fat samples from each donor were processed individually and each of the test conditions (drug combinations) were repeated with each donor sample. The selected drug combinations are recommended antiretroviral regimens for (naïve) HIV patients[8]. These included a NNRTI-based regimen consisting of a NNRTI (EFV) and 2 NRTIs (TDF and FTC) (*i.e.*, EFV + TDF + FTC); and a PI-based regimen consisting of a PI (ATV) and 2 NRTIs (TDF and FTC) (*i.e.*, ATV + TDF + FTC). The PI-based combination was tested with or without the addition of another PI, RTV (*i.e.*, ATV + RTV+ TDF + FTC), since this regimen is often recommended to boost the effects of other protease inhibitors[9]. The following drug concentrations were used in all the experiments: EFV, 20 μmol/L; FTC, 15 μmol/L; TDF, 1 μmol/L; ATV, 10 μmol/L; RTV, 2 μmol/L. These concentrations are in the range of those observed in the plasma of patients treated with the specific antiretroviral combination regimens[10-12]. The effects of antiretroviral medications were compared with control samples in which preadipocytes were cultured and stimulated to differentiate in the absence of antiretroviral medications.

Pre-adipocyte isolation and culture

Preadipocyte isolation and culture from subcutaneous fat biopsies were previously described[7]. In brief, fat tissue was digested with collagenase (3 mg/mL, type Ⅱ, Worthington, Lakewood, NJ) to obtain stromal cells that were then separated from mature adipocytes by centrifugation and incubated in erythrocyte lysing buffer (154 mmol/L NH_4Cl, 10 mmol/L K_2HPO_4, 1 mmol/L EDTA, pH 7.4) for 10 min at room temperature to eliminate red cells. Remaining debris was then removed by filtering cell suspension through a 70 μm nylon filter and then centrifuged. Pelleted preadipocytes were plated in a basal medium consisting of DMEM/F-12 (Gibco, Carlsbad, CA) supplemented with 10% Fetal Calf Serum (FCS), 2 mmol/L glutamine, 100 IU/mL penicillin and 100 μg/mL streptomycin (Gibco) and incubated for 16-18 h. After incubation, attached cells were extensively washed with warm PBS, removed from the plates with trypsin, suspended and counted. Preadipocytes were then seeded at a density of 5 × $10^3/cm^2$.

To assess cell replication, cultures were incubated in untreated growth medium for 48 h after which the medium was exchanged for fresh medium (control) or medium containing drugs. Cell numbers were assessed over a 72 h period. For the assessment of differentiation, cultures were grown to confluence and differentiation was induced with serum-free medium (control) or the same medium containing antiretroviral drugs as previously described[7,13]. Since some drugs (*i.e.*, EFV) are tightly bound to plasma albumin, 2 g/L bovine serum albumin (Sigma, St. Loius, MO) was added to differentiation medium in all control and treated groups. Medium was replaced every 72 h and differentiation assessed after 12 d.

Assessment of preadipocyte proliferation

The effect of antiretroviral drugs on preadipocyte proliferation was assessed by measuring the cell number in cultures exposed to the drugs for 48 and 72 h. Viable cell number was assessed with a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide based assay, as previously described[7,14]. Since all cultures were plated at the same initial cell number, an increase or decrease in viable cells at 48 and 72 h was assumed to represents a change in the potential of cells to increase in number, *i.e.*, proliferate.

Assessment of preadipocyte differentiation

The differentiation of preadipocytes into mature adipocytes was estimated after 12 d by measuring the activity of the lipogenic marker enzyme glycerol-3-phosphate dehydrogenase (GPDH) and by quantifying the intracellular lipid accumulation after staining with Oil Red O.

GPDH assay: Determination of GPDH activity was based on the oxidation of NADH and reported as mU where 1 mU corresponded to the amount of enzyme needed to oxidize 1 nmol of NADH/min as reported previously[7].

Oil Red O staining: Cells fixed in a 10% formaldehyde solution were stained with Oil Red O, extracted with isopropanol and assessed for absorbance at 500 nm[7].

Statistical analysis

Results are expressed as means ± SEM. Differences between control and treated groups and among the treated groups were analyzed with a randomized block ANOVA with post-hoc Fisher's Least Significant Difference (LSD) test. P values < 0.05 were considered statistically significant.

RESULTS

The effect of anti-retroviral drugs, individually and in combination, was assessed for two distinct aspects of preadipocyte metabolism; namely the proliferation of preadipocytes and the ability of preadipocytes to differentiate into adipocytes.

Preadipocyte proliferation in the presence of NRTIs, NNRTIs and PIs

All individual antiretroviral drugs inhibited the proliferation of preadipocytes incubated in concentrations of the drugs comparable to the levels seen in the plasma of treated patients compared with untreated cells (control). This effect was statistically significant at 72 h (Figure 1; P < 0.02). The inhibition of proliferation in FTC-treated cells was apparent even at 48 h of incubation (P < 0.01).

All drug combinations tested significantly suppressed preadipocyte proliferation. In the presence of EFV + FTC + TDF (NNRTI + NRTIs) preadipocyte proliferation was inhibited by 14% and 26% respectively after 48 and 72 h compared to controls. Similarly, therapeutic combinations with ATV + FTC + TDF (PI + NRTIs) showed a reduction in preadipocyte growth of 19 % at 48 h, and of 30% at 72 h (Figure 1, P < 0.001). When RTV was added to ATV + FTC + TDF, as it is clinically known to boost other PIs, the inhibitory effect was more noticeable, with a suppression in proliferation of 26% and 37% respectively after 48 and 72 h compared to controls (Figure 1, P < 0.001).

The inhibition of proliferative activity of preadipocytes in multi-drug combinations was more severe than the inhibition of proliferation observed with the individual component drugs in some cases, but not all. In the combination EFV + FTC + TDF, suppression of proliferation was greater than the suppression observed when EFV and TDF were used individually (P < 0.02), but there was not an additional effect when EFV + FTC + TDF combination was compared to FTC treatment alone (P = NS). Combining ATV + FTC + TDF or ATV + RTV + FTC + TDF increased the inhibition of adipocyte proliferation to a greater extent than treatment with the same concentration of the individual drugs both at 48 (P < 0.05) and 72 h (P < 0.01).

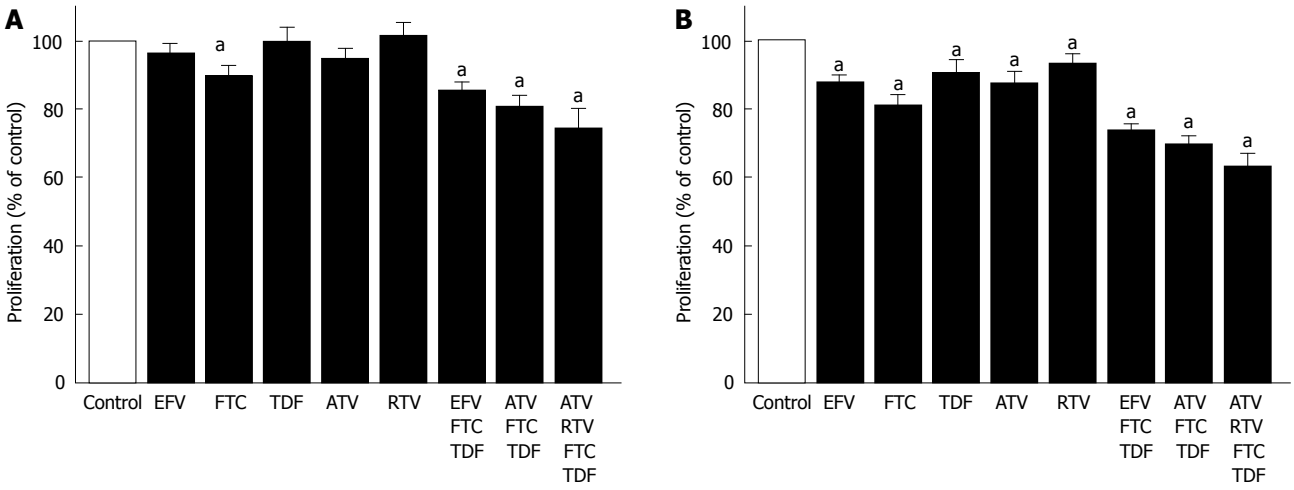

Figure 1 Pre-adipocyte proliferation in the presence of individual drugs or drug combinations for 48 h (A) and 72 h (B) (MTT test). The results are expressed as percent values of control cultures. Mean ± SEM, n = 7. Significantly different from control, $^{a}P \leq 0.05$. EFV: Efavirenz; FTC: Emtricitabine; TDF: Tenofovir; ATV: Atazanavir.

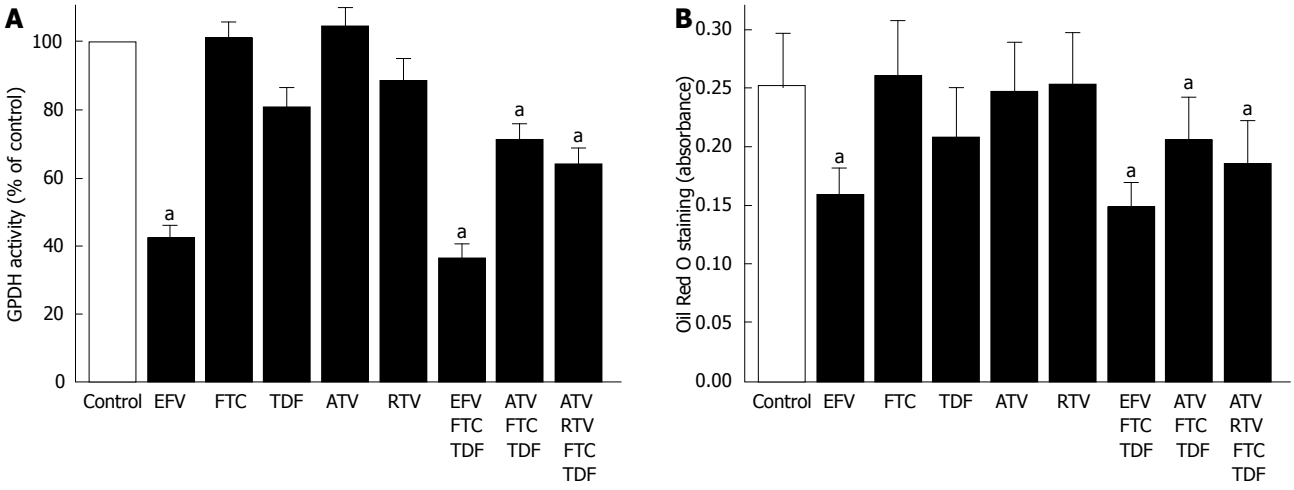

Figure 2 Effect of individual drugs or drug combinations on differentiation of human preadipocytes. Differentiation was assessed after 12 d by measuring the glycerol-3-phosphate dehydrogenase activity (A), and Oil Red O staining (B). All results are expressed as percent values of control cultures. Mean ± SEM, n = 9. Significantly different from control, $^{a}P \leq 0.05$. EFV: Efavirenz; FTC: Emtricitabine; TDF: Tenofovir; ATV: Atazanavir.

Of the three multi-drug regimens, the drug combination ATV + RTV + FTC + TDF had a more suppressive effect on proliferation of preadipocytes than the other multi-drug regimens at 48 h ($P < 0.02$). At 72 h, the combination, ATV + RTV + FTC + TDF, was not statistically significantly different from ATV + FTC + TDF but was significantly different from EFV + FTC + TDF ($P = 0.05$).

Preadipocyte differentiation in the presence of NRTIs, NNRTIs and PIs

Preadipocyte differentiation, in the presence of antiretroviral drugs, was assessed by 2 different techniques, one involved measuring the enzymatic activity of GPDH and the other involved quantifying intracellular lipid accumulation after staining with Oil Red O. The two techniques produced comparable results (Figure 2).

EFV had a profound inhibitory effect on preadipocyte differentiation (Figure 2). Both GPDH activity and lipid accumulation were greatly reduced in cells treated with EFV compared to controls (Figure 2, $P < 0.001$). Of the other anti-retroviral drugs tested, only TDF appeared to have an effect on intracellular lipid accumulation, which tended to be lower when cells were treated with TDF ($P = 0.06$). Preadipocyte differentiation in the presence of remaining individual drugs did not differ from controls (Figure 2).

Figure 2 demonstrates that, preadipocyte differentiation was significantly reduced when the antiretroviral drugs were used in combination compared to untreated cultures. Compared to control cells, the EFV + FTC + TDF (NNRTI + NRTIs) combination showed the most suppressive effect on differentiation with GPDH activity and lipid accumulation 64% and 39% lower respectively (Figure 2, $P < 0.001$). Combining NRTIs with a PI (ATV + FTC + TDF) inhibited GPDH activity by 29% and lipid accumulation by 19% compared to controls (Figure 2, $P < 0.01$). This effect was slightly

greater when a boosting amount of RTV was added (ATV + FTC + TDF + RTV, Figure 2, $P < 0.001$).

The inhibitory effect of a multi-drug combination NNRTI and NRTI (EFV + FTC + TDF) was also compared to the suppression in differentiation observed with the anti-retroviral medications individually. Suppression with the combination resulted in a greater reduction in preadipocyte differentiation than either FTC or TDF alone ($P < 0.001$). However, suppression of differentiation with the combination of EFV + FTC + TDF was comparable in magnitude to treatment with EFV alone suggesting EFV was accountable for most of the reduction in differentiation observed with the combination regimen (Figure 2). The two multi-drug regimens containing PI + NRTI were also examined relative to the incubations with the individual medications. The multi-drug combinations containing PIs had greater inhibitory effects on differentiation than treatment with the same concentration of each individual drug (Figure 2, $P < 0.003$), with the exception of TDF.

The multi-drug regimens have also been compared with each other. The combination with NNRT and NRTI (EFV, FTC, TDF) reduced differentiation to a greater extent than either of the two regimens with PI + NRTI, whether assessed as either GPDH activity ($P < 0.001$) or intracellular lipid accumulation ($P < 0.02$).

DISCUSSION

There is no doubt that the etiology of HIV/HAART-associated lipodystrophy syndrome is multi-factorial, but antiretroviral medications contribute to the condition. This *in vitro* technique, with primary cultures of pre-adipocytes isolated from healthy subjects, provides a way of assessing effects of single and combination drug regimens on preadipocyte proliferation and differentiation; and consequently, on the potential of drug regimens to contribute to HALS. The antiretroviral medications currently in use have profound effects on both preadipocyte proliferation and differentiation.

All of the antiretroviral agents tested inhibited pre-adipocyte proliferation. Individually, the NRTI, FTC, had a more pronounced effect on preadipocyte proliferation than the NRTI, TDF; the NNRTI, EFV; or the PIs ATV or RTV (Figure 1). While in general combinations of anti-retroviral drugs were more toxic than the individual drugs, this was not true for combinations containing FTC. The addition of TDF (another NRTI) and EFV (a NNRTI) to emtricitabine did not produce any greater toxicity than was observed with EFV alone. However, multidrug regimens containing PIs in combination with NRTI (ATV + FTC + TDF and ATV + RTV + FTC + TDF) resulted in further suppression of proliferation. Having previously demonstrated that RTV does not suppress preadipocyte proliferation at levels comparable to those used for boosting[7], this study indicates that adding RTV to a combination of ATV, TDF and FTC does increase toxicity (Figure 1). Clearly, the toxicity of individual antiretrovirals can be affected by concurrent antiretroviral administration.

In contrast to preadipocyte proliferation, it is the NNRT EFV that has the most profound effect on pre-adipocyte differentiation, an effect that has been reported previously[15,16]. Combining EFV with NRTIs (EFV + FTC + TDF) does not result in any greater suppression. Regimens containing PIs and NRTIs (ATV + FTC + TDF and ATV + RTV + FTC + TDF) are not as toxic as those containing EFV. However, the multi-drug combinations containing PIs suppressed differentiation to a greater extent than the use of any drug individually. This study highlights the importance of assessing both the effects on the proliferation of preadipocytes and the differentiation of preadipocytes into mature adipocytes since multi-drug regimens affect them differently.

In conclusion, antiretroviral medications affect not only the differentiation of preadipocyte into mature adipocytes, these drugs also affect the proliferation of preadipocytes and can, therefore, impact on the number of preadipocytes that are available. While FTC has the most profound effect on preadipocyte proliferation, it is EFV that has the greatest impact on differentiation. Combinations of antiretroviral medications, which have no impact when used individually, increase the toxicity for preadipocytes.

COMMENTS

Background

Highly active antiretroviral therapy (HAART) regimens with various combinations of protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI) and nonnucleoside reverse transcriptase inhibitors (NNRTI) have long been linked to HAART-associated lipodystrophy syndrome (HALS). Once HALS is manifest, it is difficult to reverse. There is a need to develop ways of assessing drug combinations for the potential to contribute to HALS.

Research frontiers

The effect of individual anti-retroviral drugs have been studied *in vitro*, but this is the first report of the effect of drug combinations assessed at clinically relevant concentrations. The differential effects on preadipocyte proliferation and differentiation were also assessed, providing important mechanistic information.

Innovations and breakthroughs

The study demonstrates that replacing ritonavir-based regimens with the clinically more acceptable protease inhibitor, atazanavir, does not eliminate the potential for toxic effects on adipose tissue. In addition, adding ritonavir at "boosting" levels to regimens containing nucleoside reverse transcriptase inhibitors and non-reverse transcriptase inhibitors also increases the lipo-toxic potential of these antiretroviral combinations.

Applications

Although combination antiretroviral therapy is clinically more efficacious than single drug regimens, the combination regimens also have the potential to contribute to adipose tissue toxicity through effects of preadipocyte replication and differentiation. The study also illustrates the value of an *in vitro* system for screening drug combinations for potential adipose tissue toxicity.

Terminology

HALS is a condition that is characterized by loss of subcutaneous fat, particularly in the face, buttocks, arms and legs. Antiretroviral therapy typically includes a combination of drugs with different mechanisms of action including NRTIs, NNRTIs, and PIs. These drug classes have the potential for differential effects on adipose tissue and the effect of combination therapy may be greater than the individual drugs alone. This study also investigated the mechanisms by which antiretroviral drugs can contribute to the loss of adipose tissue;

an inability of preadipocytes to proliferate reduces the potential number of precursor cells for the formation of adipose tissue, the inability of pre-adipocytes to differentiation reduces the formation of adipose tissue by arresting precursor cells in an undifferentiated state.

Peer-review

This manuscript is worth publishing, reporting the synergistic or cooperative effects of anti-HIV agents on inhibiting adipogenesis by performing *in vitro* experiments using human materials. It will help us to recognize the importance to take extra caution in executing HAART.

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