Review

Anti-HIV drugs and the mitochondria

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Abstract

Several drugs are currently used that can significantly prolong the course of the infection with the human immunodeficiency virus (HIV), the cause of the acquired immunodeficiency syndrome (AIDS). Among these drugs, the nucleosidic inhibitors of viral reverse transcriptase can alter mitochondrial (mt) function by inhibiting the mitochondrial DNA polymerase gamma (the enzyme responsible for the replication of mtDNA). Decreased mtDNA content provokes a diminished synthesis of respiratory chain enzymes, leading to alterations in mt function. These are in turn responsible for a variety of side effects frequently observed in HIV+ patients, that range from hyperlactatemia and lactic acidosis to lipodystrophy, a pathology characterized by accumulation of visceral fat, breast adiposity, cervical fat-pads, hyperlipidemia, insulin resistance and fat wasting in face and limbs. In this paper, data concerning the effects of different compounds on mitochondria, their role in the pathogenesis of lipodystrophy, and problems related to studies on the mt toxicity of antiviral drugs are reviewed and thoroughly discussed.

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1. Introduction

Potent antiretroviral drugs can prolong for extremely long periods the course of the infection with the human immunodeficiency virus (HIV), the cause of the acquired immunodeficiency syndrome (AIDS). According to the Joint United Nations Programme on HIV/AIDS (UNAIDS), AIDS has caused the death of more than 25 million people since it was first recognized in 1981 [1]. It has been estimated that in 2005 alone, deaths due to AIDS were more than 3 million, among which more than half a million were children. The total number of people infected with the human immunodeficiency virus (HIV) reaches now its highest level, with an estimated number of more than 40 million persons. Close to 5 million people have been newly infected by the virus in 2005, and there is no evidence that dissemination of the infection is decreasing.

Before the use of antiviral drugs, infection with HIV caused an inexorable decline in immune functions, leading to fatal consequences. Until 10 years ago, the only drugs that had some effects on HIV infection were a few compounds of the category of nucleosidic reverse transcriptase inhibitors (NRTIs), present in the pharmacopoeia for decades, that had a weak antineoplastic action and some antiretroviral efficacy [2]. Other drugs capable of inhibiting HIV protease (instead of the viral reverse transcriptase) were designed in the mid-1990s that have been capable first of delaying the onset of AIDS, then to stop the progression of the infection, even for extremely long periods [3]. The ability to inhibit viral replication is currently achieved in most patients with the so-called highly active antiretroviral therapy (HAART), a combination of viral protease inhibitors (PIs) and nucleosidic, non-nucleosidic or nucleotidic reverse transcriptase inhibitors. Another category of drugs is now available, such as the inhibitors of viral entry into the cell [4]. HIV+ patients assuming HAART survive by keeping the infection dormant, but not by eliminating the virus altogether. This therapy has indeed increased the quality of life in HIV+ individuals. Nonetheless, most infected persons live in countries where treatment is not available or is absolutely unaffordable at its current price. Furthermore, HAART has serious side effects, of social, economical and clinical importance. First of all, most infected persons live in countries where treatment is not available or has an unaffordable price. Second, the
efficacy of HAART has resulted in a relaxation of appropriate health measures that threaten a recrudescence of epidemic infection, especially in western countries. Third, antiretroviral drugs have several side effects, with different incidences and severities, including myopathy, migraine, dementia, ataxia, stroke-like episodes, hypertrophic cardiomyopathy, gastrointestinal complaints, increased bilirubin serum levels, nephrolithiasis, perioral parasthesias, diabetes mellitus, hypothryroidism, hypogonadism, infertility, dysphagia, nausea, vomiting, intestinal pseudo obstruction, anemia, pancytopenia, pancreatitis, depression, among others. Finally, it has been observed that HAART is responsible for a frequent and devastating side effect such as lipodystrophy, a syndrome characterized by peripheral fat wasting in face and limbs, accumulation of visceral fat, breast adiposity, cervical fat pads, hyperlipidemia, and insulin resistance [5,6]. Several mechanisms are likely involved in the pathogenesis of lipodystrophy, and some of them are poorly known. However, there is a general agreement that drugs of the category of nucleosidic reverse transcriptase inhibitors (NRTIs) are the main responsible for fat loss, since they can alter mitochondrial function, provoke apoptosis, and induce the elimination of precursors and eventually stem cells in different tissues [7–9].

2. Mitochondria damage in HIV+ patients

The interactions between HIV, drugs and mitochondria have been under investigation for several years. In vitro studies on 3′-azido-3′-deoxythymidine and other dideoxynucleoside analogues first showed that these molecules can alter mitochondrial (mt) DNA content by inhibiting polymerase gamma, the enzyme responsible for the replication of mtDNA [10,11]. Ex vivo studies were then performed by electron microscopy on portions of cardiac tissue from patients who died from AIDS, searching for the presence of the virus [12]. Unexpectedly, it was observed that cardiac myocytes were characterized by the presence of large numbers of proliferating multilamellated membrane bodies, predominantly associated with mitochondria. Subsequently, it was reported that in zidovudine-treated patients with myopathy reduced amounts of mtDNA were present in muscle biopsy specimens. In contrast, muscle mtDNA content was not affected in HIV+ patients who had not received zidovudine [13]. Depletion of mtDNA seemed to be reversible, and it was hypothesized that it was due to zidovudine-induced inhibition of mtDNA replication by DNA polymerase gamma, and was not a secondary effect of HIV infection.

In vivo studies were performed by 31P magnetic resonance spectra from the calf muscles of zidovudine-treated patients and age-matched controls, at rest and during exercise [14]. The recovery of phosphocreatine following exercise reflects mitochondrial oxidative function, and was significantly delayed in treated patients. This supported the hypothesis that the myopathy associated with chronic zidovudine treatment could result from the inhibitory effects on mtDNA synthesis and, secondarily, on the inhibition of mitochondrial oxidative metabolism. Concerning other drugs of the NRTIs category, analysis of mitochondrial function using several in vitro or ex vivo systems or transgenic animal models revealed that almost all NRTIs had such a side effect [15–17]. Drugs of the so-called D category, i.e., dideoxynucleoside analogs such as zalcitabine (ddC), didanosine (ddI), stavudine (d4T) are the most potent inhibitors of DNA polymerase gamma, as uniformly reported by a large number of basic and clinical studies [17–24].

NRTIs inhibit this enzyme through four different mechanisms encompassing their effects as: (i) mtDNA chain terminators (once incorporated into a growing strand, DNA replication is abruptly halted); (ii) competitive inhibitors (competing with natural nucleotides to be incorporated into growing DNA chains by polymerase gamma); (iii) inducers of errors in the fidelity of mtDNA replication inhibiting the exonucleolytic proofreading function of polymerase gamma; and (iv) contributors to the decrease of mtDNA reparatory exonuclease activity (resisting exonucleolytic removal by exonuclease activity of polymerase gamma because of the lack of the group 3OH in NRTIs) [25].

Several experimental systems have shown a strong correlation between micromolar concentrations of D-NRTIs triphosphates and decreased mtDNA or mtRNA content, decreased production of mitochondrial polypeptides, and defective ultrastructure of the organelle [10,11,26–47]. Inhibition of adenylate kinase, adenine nucleotide translocator, NADH oxidase, protein glycosylation and a “bystander” effect have also been shown, along depletion of reduced glutathione [48–53].

At the clinical level, a diminished mitochondrial function is supposed to be responsible for several adverse events occurring in individuals with HIV infection who receive a therapy based upon NRTIs. As previously observed in myocytes [13], mitochondrial damage is reversible also in other cell types, since changing treatment or suspending NRTIs can result in a consistent increase in mtDNA content and improvement in other mt parameters [54,55].

Drugs of the category of PIs, whose advent has consistently improved the survival of infected patients [56], do not seem to have such a toxic effect. They are able to inhibit apoptosis of both infected and uninfected T cells, and it was hypothesized that the mechanisms underlying this effect are associated with a specific activity against mitochondrial modifications occurring in the execution phase of apoptosis [57]. The protective effects of PIs on mitochondria has been further demonstrated by using uninfected human T lymphocytes sensitized to CD95/Fas-induced physiological apoptosis via pre-exposure to HIV envelope protein gp120. PIs were capable of hindering early morphogenetic changes bolstering T cell apoptosis, such as cell polarization and mitochondrial hyperpolarization, and acted as boosters of mitochondrial defense mechanisms, including modulation of endogenous uncouplers [58].

In partial contrast with some of the aforementioned data, using other cell models or other molecular biology techniques it has been shown that mitochondria damage can be caused by the infection with HIV as such. The intracellular distribution of HIV-1 RNA transcripts in infected cells was studied using in situ hybridization detected by electron microscopy and cellular fractionation: viral RNA was found in significantly increased amounts in mitochondria relative to the cytoplasm and nucleus, suggesting that HIV RNA import into mitochondria can compromise mitochondrial function [59]. Untreated HIV+ patients
may have diminished mtDNA levels: muscle and nerve biopsies from untreated individuals with HIV-associated myopathy or neuropathy may have low mtDNA along with abnormalities in mitochondrial structure or in the enzymes of the respiratory chain [60]; these changes are similar to those provoked in vitro by NRTIs. Declines in mtDNA in adipose tissue of untreated individuals have also been described [61]. Changes in mtDNA content in peripheral blood mononuclear cells (PBMCs) have been detected when comparing healthy controls to HIV infected individuals [62,63]. Thus, reductions in mtDNA may be caused by HIV infection alone and precede the use of NRTIs, raising the possibility that HIV directly, or cytokines released in response to HIV infection or during the immune reconstitution may injure mitochondria, potentially making them more vulnerable to the effects of NRTIs. Since a year of treatment may increase mtDNA and mtRNA content in PBMCs, it was suggested that such changes may represent a restorative trend resulting from suppression of HIV-1 infection (independent of the treatment used) [64]. As far as HIV gene products are concerned, it has been demonstrated that viral proteins, and in particular gp120, can induce apoptosis in different cell types [65,66]. Tat and viral protein R (vpr) can damage mitochondria and cause clinical disease [67–69]. Expression of Tat may lead to cardiomyopathy with mitochondrial destruction in mice. Vpr is able to affect the mitochondrial permeability transition pore and may trigger cell apoptosis through a caspase-independent mitochondrial pathway [70]. The HIV-1 protease may also induce cell death by processing procaspase and causing the release of cytochrome-c [71].

Since the beginning of the studies on HIV infection, it has been observed that the virus is able to alter the production and utilization of several cytokines, from those responsible for T cell growth such as interleukin (IL)-2 to those involved in inflammation, such as IL-1, IL-6 or tumour necrosis factor (TNF)-α [72–75]. The production of TNF-α, a pro-apoptotic cytokine that uses mitochondria as targets [76–78], is greatly increased either in vitro or in vivo [79–81]. TNF-α (as well as interferon-γ) can inhibit mitochondrial respiration in smooth muscle and other cells [82]. Interferon-α is a pro-apoptotic cytokine whose effects are mediated through mitochondrial cytochrome-c release and impaired mitochondrial DNA transcription [83]. TNF-α and interferon-α are elevated in untreated HIV infection, especially in the early phases, but may decline with therapy [84–86]. Concerning IL-1, it has been shown that in zidovudine-induced myopathy ragged-red fibers with marked myofibrillar changes expressed IL-1 mRNA, implying that it was produced in muscle cells. Biopsies performed in mitochondrial myopathies of different origin revealed that IL-1 expression was much weaker. This suggested that proinflammatory and destructive effects of the studied cytokines might be responsible for several myopathological changes observed in HIV+ patients [87].

3. Mitochondria, lactic acidosis and lipodystrophy

A generalized impairment of mitochondrial function can cause different effects that have a consistent clinical importance. Such effects range from hyperlactatemia (that can be asymptomatic, in the presence of efficient compensatory mechanisms) to lactic acidosis (which is often fatal). The first description of such syndrome in a HIV+ person taking antiretroviral drugs was that of a 57-year-old patient, assuming zidovudine for 3 years, who showed the concomitant occurrence of muscular and hepatic disturbances and lactic acidosis, along with fatigue, weight, loss and lactic acidosis [88]. The patient became confused and febrile and died 8 days after detection of high blood lactatemia. Liver biopsy showed diffuse macrovacuolar and microvacuolar steatosis; muscle showed mitochondrial abnormalities with ragged-red fibers and lipid droplet accumulation. Southern blot analysis revealed depletion of mtDNA, affecting skeletal muscle and liver tissue, suggesting that zidovudine can induce mitochondrial multisystem disease.

Other studies confirmed the presence of mitochondrial alterations in the liver of patients with AIDS assuming zidovudine [89]. Deletions in sperm mtDNA have been found in patients treated with highly active antiretroviral therapy (HAART) [90], and mtDNA content alterations in peripheral blood cells have been observed in NRTIs-treated HIV+ patients with lactic acidosis [24].

Patients taking HAART can develop lipodystrophy [6,91,92] that has a striking similarity with multiple symmetric lipomatosis (MSL) type 1, a sporadic or familial disorder characterized by the development, during adulthood, of nonencapsulated lipomas around the neck and shoulders which may extend to the back, arms and mediastinum [93,94]. The hypothesis has been put forward that drug-induced damages to mtDNA were able to alter mitochondria function to a similar extent to what occurs in MSL, where genetic defects impair the function of the organelle. It has been demonstrated that this hypothesis is correct, since HIV+ patients with lipodystrophy have a diminished mtDNA content in adipose cells or in skeletal muscle [61,95]. A recent study conducted on a cohort of 11 HIV+ lipodystrophic patients showed that adipocytes collected from different anatomical locations had alterations also in mtRNA content [96]. Three different transcripts (ND1, CYTB and ND6 gene products) were studied, and mtRNA content was normalized versus the housekeeping transcript L13. All 3 gene products were significantly reduced in HIV+ lipodystrophic patients; men and women did not differ in a statistically significant way regarding the levels of ND1 and ND6, whereas the opposite occurred for CYTB.

Studies on the pathogenesis of mitochondrial alterations are crucial to better understand the mechanisms of damage and design possible intervention or protective strategies. However, they are quite complex because a different sensitivity to antiretroviral drugs may exist not only among different cell types, but also among cells of the same type in different anatomic sites [97]. Adipocytes are a source of paracrine and endocrine signals that influence not only adipocyte biology, but also systemic metabolism. HAART-induced mitochondrial damage results in an abnormal perception of the bioenergetic status by adipocytes, thus leading to enhancement of catalytic pathways and apoptosis in peripheral adipose tissue, alterations in the differentiation of brown versus white adipocytes, and the release of hormonal signals that lead to systemic metabolic disturbances [98–100].
However, depot-related differences exist in adipocyte responses to lipolytic and lipogenic stimuli, in adipocyte apoptosis, and in the potential for preadipocyte replication and differentiation [101]. Furthermore, besides the obvious problems related to the collection of the biological material, another limitation exists, i.e., that related to the technology used to measure different mitochondrial parameters, that can account, at least in part, for the heterogeneity of the results present in the literature.

4. Correlations between in vitro studies and patient’s treatment

Several molecular and cellular techniques and different in vitro models are currently available to analyze mitochondria, and a variety of methods have been used by researchers in the field of HIV to investigate cells treated with different compounds, or cells collected from patients taking different therapeutic regimens [90,95–100,102–106].

Studies on the effects of antiretroviral drugs on mitochondria in cell lines are relatively easy, but it is necessary to pay attention to potential discrepancies between data obtained in vitro or ex vivo, namely from continuously growing, cultured cells (of tumour origin) treated with a given drug, and data obtained from a patient treated with the same drug. The evaluation of drug-induced mitochondrial damages in patients is extremely complex because of a variety of problems that include, among others:

(a) The possibility to study adequate and representative cells. Liver is clearly a main target for drugs that have mitochondrial toxicity, and its dysfunction is likely the main etiological factor for the onset of lactic acidosis. It is logical that the best cellular model for the ex vivo detection of the hepatotoxicity of a drug comes from the liver itself, but biopsies are not always feasible either for ethical or practical reasons. It is instead relatively easy to obtain blood from a patient, but not always blood cells can provide useful information. As an example, there is growing interest in the evaluation of the mtDNA copy number in white blood cells from patients treated with different antiretrovirals, as a possible marker of drug toxicity. The first study on mtDNA content in peripheral blood lymphocytes analyzed mitochondrial function and apoptosis in cells of HIV-infected children, with or without lipodystrophy, who were receiving HAART [18]. A variety of parameters were investigated, but no main changes were detected in lymphocytes from children with lipodystrophy, suggesting that normal mitochondrial function and tendency to undergo apoptosis were present in these cells. The use of peripheral blood cells is in fact more complex than what was thought, as shown by the presence of contrasting data regarding the effects of antiretroviral therapy on mtDNA. It has to be considered that if cells derived from peripheral blood using standard isolation methods are used, platelet contamination must be accounted for [107]. In peripheral blood and buffy coat samples, platelets are considerably over-represented compared to leukocytes. Platelets contain mitochondria (and thus mtDNA and also mtRNA) but not a nucleus (and thus, they have no nuclear DNA). Usually mtDNA is quantified as a ratio versus nuclear DNA, and thus, if platelets are not accurately removed, changes in the content of mtDNA in a given cell population could be due to several causes, including variations in platelet quantity, which may be affected not only by antiretroviral therapy but also by sample processing or storage. Platelet removal has to be readily performed and cells of interest (e.g., lymphocytes, monocytes) have preferably to be purified by magnetic sorting or other systems.

Also, adipocytes are extremely useful to investigate drug-related damages to mtDNA, especially when studies on lipodystrophy are concerned. In the first pivotal study, mtDNA content of subcutaneous fat tissue from the neck, abdomen and thigh was measured [61]. A decrease in mtDNA content was found in HAART-treated HIV+ patients with peripheral fat wasting in comparison with HIV+ patients without lipodystrophy but with a similar treatment history; HIV+ patients naive to antiretroviral therapy and healthy controls had similar amounts of mtDNA, suggesting that lipodystrophy with peripheral fat wasting following treatment with NRTIs-containing HAART is associated with a decrease in subcutaneous adipose tissue mtDNA content.

(b) The possibility to obtain sufficient amounts of biological material. Some assays, especially those based upon molecular biology techniques and PCR, require a relative small amount of material. Other assays, for example, those regarding functional analysis on cultured cells, require much more material, and are often difficult to perform in biological material collected from human beings.

(c) The clinical status and other therapies assumed by the patient at the moment of cell collection. A variety of functional assays are extremely sensitive to parameters that are linked to the status of the host. Thus, several situations can influence the result of a test, including, among others, the presence of concomitant infections, the adherence to therapy, the intake of different drugs or biologically active molecule. More than 70% of HIV+ patients had used complementary and alternative medicine (CAM) therapies before the diagnosis of HIV infection; after diagnosis, percentages increased of another 13%; more than 90% of HIV patients had used at least one CAM therapy sometime in their lives [108,109]. It is poorly known if and how CAM can affect the success of HAART, and there is an almost complete ignorance of eventual serious complications of their association. For example, relevant interactions exist between antiretroviral drugs and Hypericum perforatum (known as St. John’s Worth, SJV), a natural antidepressant with more than 2.7 million prescriptions every year. SJV may influence the metabolism of coadministered drugs: administration of SJV with the PI indinavir produces an 81% reduction in drug plasma concentration, either altering the expression of the multidrug resistance P-glycoprotein 1, or inducing expression of the hepatic enzyme CYP3A4 [110]. As a result, the efficacy of antiretroviral therapy is seriously compromised, and the production of HIV is no longer inhibited.

(d) To use the right assay in a correct manner. Actually, an enormous number of tests have been standardized, including those that require sophisticated technologies or instruments. However, a paradoxical side effect exists that is related to the relative simplicity of a given assay, i.e., the fact that several researchers could use an assay without the adequate scientific qualification. The conclusion that, for example, a given drug assumed by a group of patients can
impair the in vivo mitochondrial function in lymphocytes more than another is difficult to accept. This is when, for example, data show that the percentage of cells with depolarized mitochondria (analyzed by flow cytometry) increases from 2% (in controls) to 3% (in treated patients) [111]. More expertise in the analysis and interpretation of cytofluorimetric data would be necessary.

(e) Last, but not least, the possibility to compare the data obtained in patients with those from healthy donors. This is the crucial aspect: clearly, it is quite easy to collect blood from a healthy individual, but obtaining liver cells, fibroblasts or adipocytes may be a major problem.

In conclusion, several aspects have to be considered in studies on the mitochondrial toxicity of antiretroviral drugs. The extrapolation to the clinical practice is often complicated by the aforementioned causes. However, it can be predicted that, in a relatively near future, the development of new experimental models and strategies will improve the possibility to take advantage from these studies.

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