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Oxygen consumption by cultured human cells is impaired by a nucleoside analogue cocktail that inhibits mitochondrial DNA synthesis

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Abstract

We evaluated oxygen consumption rates in human cells cultured in the presence of a nucleoside analog reverse transcriptase inhibitor (NRTI) cocktail that inhibits mitochondrial DNA synthesis. We treated a proliferating human lymphocyte cell line and a primary culture of human adipose cells with antiretroviral drugs (AZT + ddC + d4T). The effects of these drugs on mitochondrial DNA (mtDNA) levels and oxygen consumption rates were evaluated using semi-quantitative real-time PCR and an on-line monitoring Clark electrode system.

We found that the NRTI treatment lowered oxygen consumption rates and inhibited mitochondrial DNA replication in human cell cultures. Inhibition of oxygen consumption was linearly proportional to inhibition of mtDNA replication. These results show for the first time that mitochondrial respiration is impaired in NRTI sensitive cells. The linear relationship between NRTI inhibition of respiration and NRTI inhibition of mtDNA replication indicates that small decreases in mtDNA levels can lead to respiratory deficits in the tissues of patients treated with anti-HIV drugs. We propose a model that takes into account the small differences in metabolic dynamics between peripheral and axial/visceral fat tissues. This model explains how NRTI-related respiratory deficits may lead to the presentation of opposing lipodystrophic syndromes in same patient.

Keywords: Nucleoside reverse transcriptase inhibitors; Mitochondrial DNA; Oxygen consumption; Human cells

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1. Introduction

Highly active antiretroviral therapies (HAART) dramatically reduce the risk of progressive immune deficiency in HIV infected patients by controlling viral replication. Nucleoside analog reverse transcriptase inhibitors (NRTIs) (Johnson et al., 2001; Kakuda, 2000; Martin et al., 1994; Squires, 2001) are part of most HAART regimens and are generally taken with protease inhibitors or as triple/quadruple reverse transcriptase inhibitor combinations. Several of these antiviral drug regimens alter the activity of peripheral and cardiac muscles, nerves and the pancreas. These treatments also lead to abnormal fatty acid metabolism in the liver, a side effect that can occur with or without concomitant abnormalities in lactate production. These symptoms show histopathological, histochemical, and metabolic similarities to those occurring in individuals with congenital or sporadic mitochondrial cytopathies. These iatrogenic syndroms may be linked to NRTI-mediated inhibition of mitochondrial DNA synthesis (Brinkman et al., 1998; Fromenty and Pessayre, 1995; Lewis and Dalakas, 1995).

NRTIs inhibit DNA synthesis by competing with endogenous nucleic acids for incorporation into the DNA chain. They cause premature termination of DNA chain elongation when incorporated. NRTIs function by preventing HIV reverse transcriptase-mediated viral DNA synthesis (Kakuda, 2000). mtDNA polymerase gamma, which is essential for mtDNA replication, is particularly prone to NRTI inhibition because it cannot discriminate between nucleoside analogues and endogenous nucleotides (Brinkman et al., 1999; Longley et al., 1998).

Lipodystrophy (LDP) is the most frequently reported side effect of anti-HIV therapies (Brinkman et al., 1999; Carr et al., 1999; Longley et al., 1998). This condition is characterized by visceral/axial over-adiposity and fat wasting in the limbs, buttocks and face, resulting in an increase in the ratio of visceral to subcutaneous abdominal fat (Brinkman et al., 1999; Carr et al., 1999; Longley et al., 1998). LDP was originally thought to be associated with the protease inhibitor (PI) component of the antiviral cocktails, however, more recent studies have shown that patients receiving NRTI combinations (without previous or concurrent PI treatment) also present this symptom (Chene et al., 2002; Molina et al., 1999; personnal unpublished results).

Epidemiological studies have identified a link between NRTI-treatment and LDP. These data suggest that the symptoms of this syndrome result from a form of mitochondrial cytopathy in the adipose tissues of these patients. A functional point mutation at nucleotide 8344 of the mitochondrial tRNA-lysine gene has been identified in some individuals with familial multiple symmetric lipomatosis (MSL) type 1 (Munoz-Malaga et al., 2000), a syndrome that shares distant similarities with rare localized forms of LDP in patients taking anti-HIV drugs (Brinkman et al., 1999). In-house real-time PCR estimates of mitochondrial DNA copy numbers in NRTI-treated patients, and analyses of ultrastructural mitochondrial abnormalities in subcutaneous fat tissue biopsies from NRTI-treated patients with and without out LDP, support the hypothesis that mitochondrial defects lead to lipoatrophy in patients with LDP (Nolan et al., 2003; Shikuma et al., 2001; Walker et al., 2002). The results from cross-sectional studies have provided no statistical proof to support this hypothesis. However, these studies generally involve only small numbers of patients and detect large inter-patient variations.

In a previous study (Petit et al., 2003), we analyzed mtDNA levels in blood lymphocytes from a series of HIV patients before and after treatment with AZT, ddC and d4T. This previous study provided no evidence to support the hypothesis that there is a link between LPD and NRTI-treatment. Here, we describe the effects of nucleoside analogues on mtDNA replication and oxygen consumption in a primary culture of normal human preadipocytes/adipocytes. We found that NRTIs altered both oxygen fixation and mtDNA replication without a threshold effect. These results led us to propose a metabolic model for LDP in which NRTI-related respiratory cell defects lead to either fat atrophy or excess adipogenesis depending on the metabolic status of the fat tissues involved.

2. Experimental procedures

2.1. Cell culture, drug treatments and quantification of lipid accumulation

Samples of human subcutaneous adipose tissue were obtained from healthy subjects undergoing