N 942

Relationship of NRTI-mediated mitochondrial (mt)DNA depletion and respiratory chain activity in primary human subcutaneous adipocytes

Metodi Stankov*, Reinhold E. Schmidt, Georg M.N. Behrens, and the German Competence Network HIV/AIDS

Clinic for Immunology and Rheumatology, Hannover Medical School, Hannover, Germany

Address for correspondence:
Georg M.N. Behrens, MD
Clinic for Immunology and Rheumatology,
Hannover Medical School
Carl-Neuberg-Strasse 1
30625 Hannover, Germany
behrens.georg@mh-hannover.de

ABSTRACT

Background: NRTI-mediated mtDNA depletion has been hypothesized to result in mitochondrial toxicity with subsequent lipoatrophy. However, until now correlation between mtDNA depletion and respiratory chain activity has never been performed in primary human subcutaneous preadipocytes and adipocytes.

Methods: We studied adipocyte phenotype, viability, mtDNA content, and activity of respiratory chain complexes (I+III, II + III, IV and V) and citrate synthase in proliferating and differentiating primary human subcutaneous preadipocytes. Cells were exposed to zidovudine (6 µM or 180 µM), stavudine (3 µM or 90 µM), and zalcitabine (0.1 µM or 3µM) during proliferation and differentiation for up to 40 days.

Results: At therapeutic drug concentration, d4T and ddC induced an almost 40% mtDNA depletion (control 100% ± 12.7%; d4T 53.5% ± 15.3%; ddC 59.5% ± 1.6%; P<0.05) in differentiating adipocytes. At higher drug concentrations, both d4T- and ddC-treated cells demonstrated about 60% mtDNA depletion (control 100% ± 14.7%; d4T 44% ± 7.9%; ddC 42.6% ± 4.9%; P<0.05). Under this experimental conditions AZT did not lead to mtDNA depletion at any dose tested. In none of the treated cultures, mtDNA depletion was associated with major changes in adipocyte phenotype or viability. Furthermore despite mtDNA depletion by NRTI at the end of differentiation process, activity of the respiratory chain complexes (I+III, II + III, IV and V) and citrate synthase were found to be unimpaired. We obtained similar results in proliferating preadipocytes.

Conclusions: NRTI-induced depletion of mtDNA up to 60% in differentiating human adipocytes was not associated with impaired respiratory chain activity *in vitro*.

INTRODUCTION

Mitochondrial DNA (mtDNA) depletion has been proposed as an important factor leading to peripheral lipoatrophy in HIV-patients receiving antiretroviral therapy. The effect of NRTIs on mtDNA depletion and respiratory chain activity in primary human subcutaneous preadipocytes and adipocytes has not been evaluated so far.

OBJECTIVE

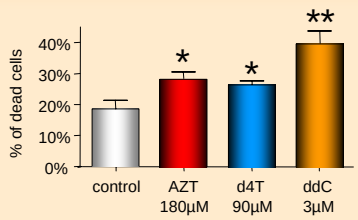
We aimed to investigate the effect of different NRTIs on adipocyte phenotype, viability, mtDNA content, and activity of respiratory chain complexes (I+III, II + III, IV and V) and citrate synthase in proliferating and differentiating primary human subcutaneous preadipocytes.

METHODS

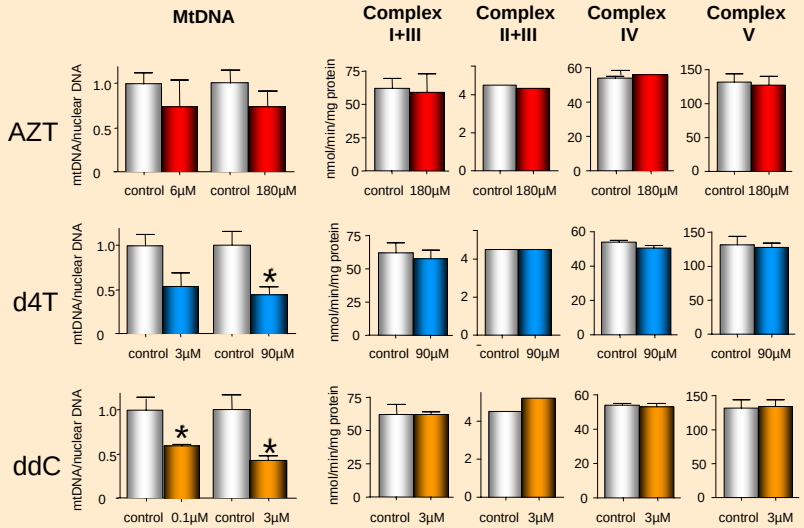
We studied adipocyte phenotype (Microscopy), viability (Trypan blue staining), mtDNA content (Real Time PCR), and activity of respiratory chain complexes (I+III, II + III, IV and V) and citrate synthase (Photometry) in proliferating and differentiating primary human subcutaneous preadipocytes. Cells were exposed to zidovudine (6 µM or 180 µM), stavudine (3 µM or 90 µM), and zalcitabine (0.1 µM or 3µM) during proliferation and differentiation for up to 40 days.

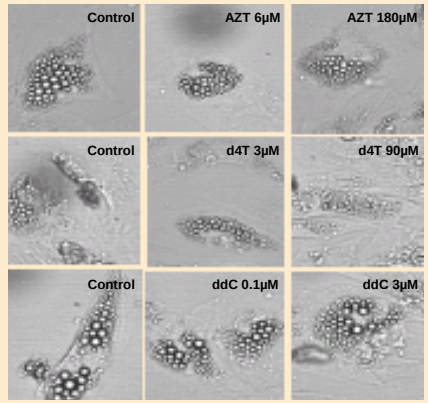
RESULTS

Cell viability

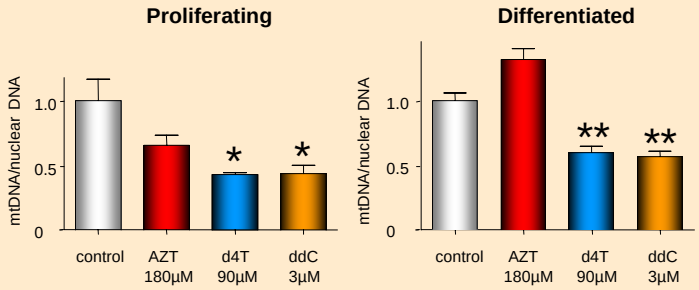


MtDNA content, respiratory chain enzyme activity, and cell morphology of human subcutaneous preadipocytes differentiated for 30 days in the presence of NRTI





MtDNA content of proliferating or differentiated human subcutaneous adipocytes exposed to NRTI for 30 to 40 days



CONCLUSION

NRTI-induced depletion of mtDNA up to 60% in differentiating human adipocytes was not associated with impaired respiratory chain activity *in vitro*. AZT did not significantly deplete mtDNA in human adipocytes *in vitro*. We speculate that mtDNA depletion is probably not a causative factor contributing to fat loss and that *in vivo* additional factors contribute to peripheral fat atrophy.

Supported by competence network HIV/AIDS C15 and Hector-Stiftung