Treatment with dideoxynucleosides is reflected in lowered mitochondrial DNA in subcutaneous fat.

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Abstract

Background: Nucleoside reverse transcriptase inhibitors (NRTIs), particularly dideoxynucleoside analogues (DDx), used in the treatment of HIV, inhibit mitochondrial DNA polymerase *in vitro*. Mitochondrial DNA (mtDNA) depletion is proposed as the underlying mechanism of many of the *in vivo* side effects of these agents.

Methods: 60 HIV infected adults underwent clinical assessment and blood and tissue sampling. Clinical assessment included details of treatment, and clinical DDx toxicities: perceived body shape changes and evidence of sensory neuropathy (SN) (symptoms and at least one of sensory signs or reduced ankle jerks). mtDNA was quantified in peripheral blood mononuclear cells (PBMCs) and subcutaneous fat using real time PCR, and plasma lactate assays were performed. Results were correlated with treatment, clinical and toxicity details, and multivariate analysis performed using a linear regression model.

Results: 49 (82%) subjects were on combination antiretroviral therapy, including 33 (55%) currently taking at least one DDx. mtDNA in subcutaneous fat was lower in subjects currently on DDx than in those not currently taking DDx (mean [$\log_{10}$] 2.47 versus 2.74, p=0.002). mtDNA in PBMCs increased with patient age (r=0.35, p=0.01) but did not vary with treatment status. Among the subjects currently taking DDx, 21 (64%) had perceived body shape changes and 17 (52%) had SN. There was no difference in plasma lactate, mtDNA in subcutaneous fat, or mtDNA in PBMCs in those with and without either SN or body shape changes (p>0.1).

Conclusions: mtDNA in subcutaneous fat is significantly reduced in patients currently taking DDx. mtDNA in PBMCs increases with increasing age, but is independent of current DDx use. Plasma lactate and mtDNA in PBMCs or subcutaneous fat are independent of clinical DDx toxicities on a single measurement.

Background

Nucleoside reverse transcriptase inhibitors (NRTIs) are known to inhibit mitochondrial DNA polymerase *in vitro*[1]. Dideoxynucleosides (DDx) (ddI, d4T, ddC) inhibit this enzyme more potently than other NRTIs[2]. A consequent reduction in mitochondrial DNA (mtDNA) synthesis is the postulated mechanism of many DDx toxicities, including sensory neuropathy (SN) and treatment associated fat redistribution[2,3].

Aims

- To quantify mtDNA in peripheral blood mononuclear cells (PBMCs) and subcutaneous fat from individuals with HIV infection
- To determine the relationship between mtDNA and patient characteristics, treatment details and plasma lactate assays

Methods

60 individuals with HIV infection underwent clinical assessment and collection of tissue samples for quantification of mtDNA.

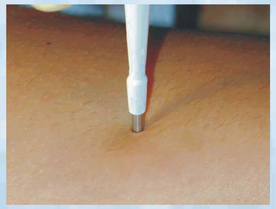

Figure1: Collection of punch skin biopsy

Blood was collected by venupuncture. Plasma lactate assays were performed using standard methods, and PBMCs extracted by Ficoll-Plaque centrifugation for mtDNA quantification. 3mm punch biopsies of skin were collected from the distal thigh and distal calf (figure 1), and subcutaneous fat was trimmed from the base of these for mtDNA quantification. DNA was extracted from PBMCs and fat, and mtDNA quantified using a real-time PCR assay developed in our laboratory[4].

Results & Discussion

There were no complications from the collection of tissue. Punch biopsies of skin were acceptable to patients, with 59 of 60 individuals agreeing to remain on study for the collection of serial samples.

Patient Characteristics

	Mean (range)
Age	45 (26-65)
Years HIV+	8 (1-16)
CD4 cell count	456 (21-1700)
Log(viral load)	2.9 (1.7-5.5)

Correlations between mtDNA & plasma lactate results

There was a strong correlation observed between mtDNA in fat from the distal calf and distal thigh (r=-0.53, p<0.0001, figure 2), suggesting that a single biopsy from either site would yield similar information. However, plasma lactate results did not correlate with mtDNA from any of the tissues examined. mtDNA from PBMCs did not correlate well with mtDNA from fat.

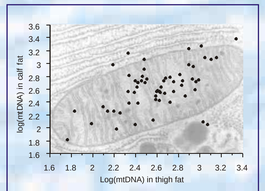

Figure 2: Correlation between mtDNA in calf and thigh fat (r=0.53, p<0.0001)

Associations between mtDNA and patient characteristics

mtDNA in PBMCs correlated directly with patient age (r=0.35, p=0.01) (figure 3), a finding consistent with previous *in vitro* work on PBMCs from elderly subjects[5].

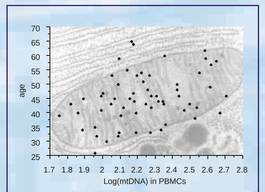

Figure 3: Correlation between mtDNA in PBMCs and patient age (r=0.35, p=0.010)

Associations between mtDNA and patients' current antiretroviral treatment status

mtDNA in subcutaneous fat from either site on the lower limb was significantly lower in patients on DDx (n=33) than in patients not currently on DDx (n=27) (figure 4). This finding suggests that fat may be a suitable tissue for the assessment of DDx toxicity, and that punch skin biopsies yield sufficient fat for this purpose.

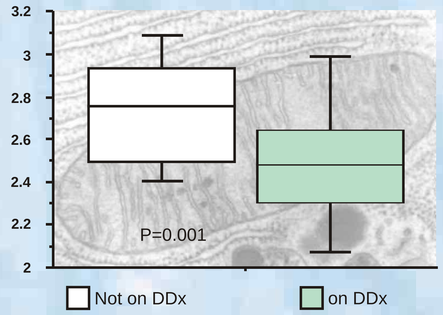

Figure 4: Log(mtDNA in thigh fat) by current DDx status

The association between low levels of mtDNA in fat and current antiretroviral use was specific to DDx. Among patients not currently prescribed DDx, there was no difference in mtDNA in fat between patients not on any NRTIs (n=11) and patients who were currently prescribed NRTIs other than DDx (n=16) (figure 5).

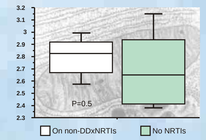

Figure 5: Lack of association between treatment with non-DDx NRTIs and log(mtDNA in thigh fat)

Association between mtDNA and cumulative DDx exposure

Lifetime exposure to DDx (DDx months) correlated with mtDNA in subcutaneous fat on univariate analysis (r=0.3, p=0.02 at either site). Lifetime DDx exposure did not correlate with mtDNA in PBMCs (r=-0.1, p=0.3).

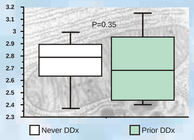

Figure 6: Lack of influence of prior DDx exposure on log(mtDNA in thigh fat)

Lack of association between mtDNA and prior DDx exposure

17 patients had *previously* been exposed to DDx but had been off these drugs for a median of 16 (range 1-96) months. There was no difference between mtDNA in subcutaneous fat between this group and patients who had *never* been exposed to DDx (n=10) (figure 6). This important finding suggests that any effect of DDx therapy on mtDNA in fat may be reversible.

Multivariate analysis

The independent associations with mtDNA were the same in subcutaneous fat from either site, but different in PBMCs

	Independent variable	R squared	P value
mtDNA in fat*	Current DDx therapy	16%	0.002
mtDNA in PBMCs	Patient age	12%	0.007

*biopsy from either site yielded identical results

mtDNA quantification compared with existing drug toxicities

Among the 33 subjects currently on therapy including DDx, 21 (64%) had self reported fat redistribution and 17 (52%) had SN. There was no clear association between mtDNA in any of the tissues examined and the presence of either of these clinical DDx toxicities (p>0.1 for all).

We hypothesize that serial collection of tissue will demonstrate an association between the magnitude of the reduction of mtDNA in an individual following the commencement of DDx and the risk of that individual developing clinical DDx toxicities such as neuropathy. Work is underway to examine this possibility further, with patients undergoing 6 monthly clinical assessments and tissue collection.

Conclusions

- Punch biopsies of skin are safe, relatively non-invasive, and yield sufficient subcutaneous fat for quantification of mtDNA
- mtDNA in fat is lowered in patients currently on treatment with at least one DDx, and this effect may be reversible
- mtDNA results are similar in fat from different regions of the lower limb
- mtDNA in PBMCs does not correlate with mtDNA in fat, and is not associated with patient treatment status
- Collection of serial tissue samples will be used to investigate the possible association between mtDNA in fat and clinical DDx toxicities

Acknowledgements

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