AZT/NNRTI induces greater adipose tissue mitochondrial toxicity than AZT/PI

Poster 668

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INTRODUCTION

- Thymidine analogue nucleoside reverse transcriptase inhibitors (tNRTIs) including zidovudine (AZT) are associated with the development of mitochondrial toxicity in subcutaneous adipose tissue (SAT) leading to lipoatrophy [1].
- The relative contribution of the choice of third agent (protease inhibitor (PI), non-nucleoside reverse transcriptase inhibitor (NNRTI) or integrase strand transfer inhibitor (INSTI)) to SAT toxicity is poorly defined.
- Previously, greater limb fat loss was observed with NNRTI in comparison to PI when used with a tNRTI [2]. However, the underlying mechanism remains unclear.

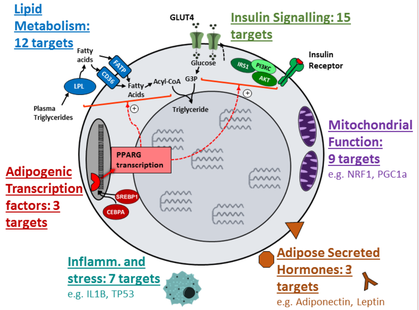
OBJECTIVE

- To explore the relative contribution of tNRTI and the choice of third agent (PI or NNRTI) to the development of SAT toxicity in ART-naïve patients initiating first-line ART.

METHODS

- The HIV Infection and Metabolic Abnormalities study (HAMA001) was a prospective cohort study, in which ART-naïve HIV+ adults initiating ART containing AZT/PI, AZT/NNRTI or a tNRTI-sparing regimen containing neither AZT nor PI (non-AZT/non-PI) were followed for 48 weeks.
- Limb and trunk fat was assessed by dual energy X-ray absorptiometry (DXA) at weeks 0, 12, 24 and 48.
- Fasting subjects underwent week 0, 2 and 48 fasting SAT biopsies for use in the following analyses:
  - Mitochondrial DNA (mtDNA) copy number by quantitative PCR (qPCR)
  - Gene expression of 55 key adipocyte genes (Fig. 1) by qPCR array with data normalised to 3 reference genes: *ACTB*, *RPL13a* and *TBP*.
- Time-weighted changes in fat mass and mtDNA content in the AZT groups were compared to those in the non-AZT/non-PI group using longitudinal marginal models. qPCR array analysis was carried out using the LIMMA package in R.
- Data are presented as median (IQR), $P<0.05$ was considered significant.

Figure 1: Overview of qPCR Array Target Gene Categories



RESULTS

- Of the 23 subjects recruited, 7, 7 and 9 initiated non-AZT/non-PI, AZT/PI and AZT/NNRTI based ART respectively.
- Study groups were comparable at baseline for gender, age, ethnicity and measures of body composition but viral load and total cholesterol differed.

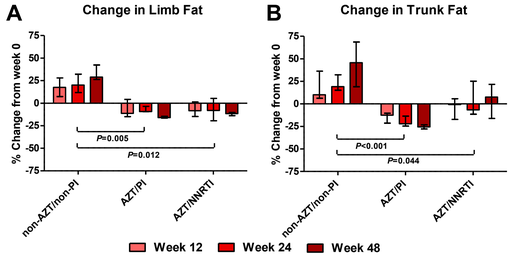
Table 1: Baseline Characteristics

	Non-AZT/Non-PI		AZT/PI		AZT/NNRTI		*P*
Number	7		7		9		
Male Gender (%)	6	(86%)	7	(100%)	8	(88%)	0.456
Caucasian (%)	6	(86%)	5	(100%)	9	(100%)	0.158
Age	36.4	(34.0, 46.3)	41.0	(38, 41.9)	38.8	(29.4, 48.1)	0.972
CD4+ (cells/mm^3)	66	(40, 325)	210	(98, 268.5)	126	(60, 140)	0.546
Log HIV RNA	5.6	(5.3, 5.8)	4.4	(4.3, 4.8)	4.9	(4.6, 5.4)	**0.037**
BMI (kg/m^2)	22	(20.5, 23.5)	22	(21, 24.5)	23	(22, 23)	0.652
Trunk Fat (kg)	6	(3.5, 7.5)	10.6	(7.3, 12.8)	7.6	(5.8, 10.8)	0.353
Limb Fat (kg)	3.4	(3.0, 4.0)	6.1	(4.3, 7.2)	6.6	(4.6, 6.7)	0.249
Trunk:Limb fat ratio	1.6	(1.4, 1.7)	1.7	(1.6, 1.8)	1.4	(1.1, 1.6)	0.155
HOMA-IR	0.7	(0.6, 1)	1.4	(1.2, 2.2)	1.1	(0.8, 1.4)	0.266
Total Cholesterol (mmol/L)	3.5	(3, 3.9)	4.7	(4.1, 4.8)	4.9	(4.2, 5.1)	**0.014**
Triglycerides (mmol/L)	1.8	(1, 2)	1.4	(1, 1.8)	1.2	(1, 1.3)	0.971

CHANGES IN FAT MASS

- Over 48 weeks, increases in trunk and limb fat expected with ART initiation [3] were observed in the non-AZT/non-PI group but not in the AZT groups (Fig. 2).

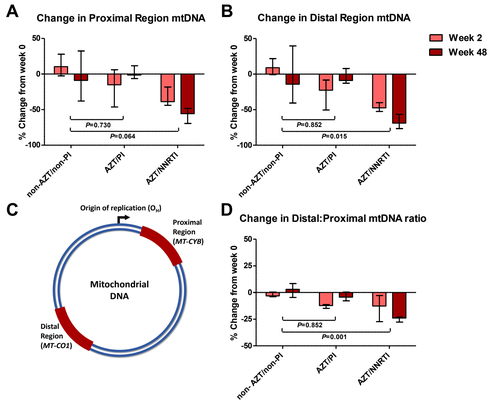
Figure 2: Increased limb and trunk fat with non-AZT/non-PI ART initiation but not with AZT based ART



CHANGES IN ADIPOSE TISSUE MTDNA

- mtDNA content was unchanged in the non-AZT/non-PI and AZT/PI groups (Fig. 3A,B) but decreased in the AZT/NNRTI group (Fig. 3B).
- The ratio of distal:proximal mtDNA decreased significantly in the AZT/NNRTI group (Fig. 3C,D) indicating a decrease in mitochondrial genome integrity.

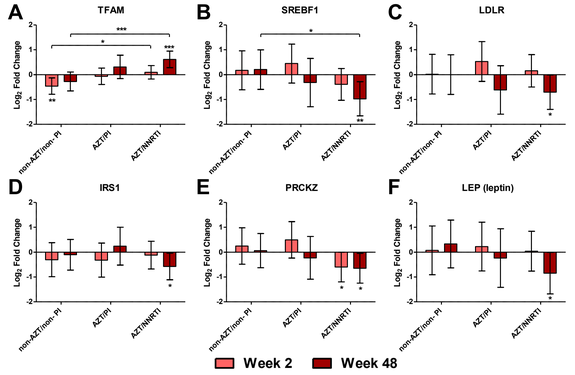
Figure 3: Decreases in SAT mtDNA with AZT/NNRTI



CHANGES IN ADIPOSE TISSUE GENE EXPRESSION

- Gene expression analysis revealed the AZT/NNRTI group also had compensatory increases in the expression of the transcription factor *TFAM*, required for mitochondrial biogenesis (Fig. 4A).
- Additionally, AZT/NNRTI resulted in decreases in a number of key adipocyte genes involved in lipid metabolism and insulin signalling (Fig. 4B-F), changes which did not occur in other groups.

Figure 4: Increased expression of *TFAM* and decreases in key metabolic genes in SAT with AZT/NNRTI

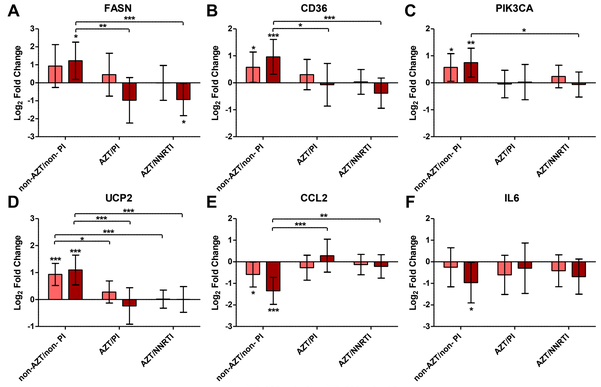


Bars represent Log2 fold change (95% C.I.) from week 0 levels, *P≤0.05, **P≤0.01, ***P≤0.005

RESULTS (continued)

- Most notably, the expression of *SREBF1* and its transcriptional target *LDLR* decreased significantly at week 48 in the AZT/NNRTI group (Fig. 4B,C) consistent with the development of lipodystrophy [4]
- In keeping with a recovery of fat mass in the non-AZT/non-PI group, the expression of lipid metabolism and growth genes *FASN*, *CD36* and *PIK3CA* increased together with increased mitochondrial *UCP2* and decreases in the pro-inflammatory *CCL2* and *IL6* (Fig. 5).

Figure 5: Increased lipid metabolism and decreased pro-inflammatory gene expression with non-AZT/non-PI ART



Bars represent Log2 fold change (95% C.I.) from week 0 levels, *P≤0.05, **P≤0.01, ***P≤0.005

CONCLUSIONS

- In this study, AZT/NNRTI but not AZT/PI led to significant mitochondrial toxicity in SAT together with reduced adipogenic and metabolic gene expression
- The findings of this study suggest that NNRTI may enhance tNRTI-mediated mitochondrial toxicity in SAT.

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ACKNOWLEDGEMENTS

We thank the study participants for their time and commitment.

This work was funded by the Wellcome Trust (097424/Z/11/Z).