Role of IL18 in HIV Associated Lipodystrophy

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Introduction

- Lipodystrophy caused by highly active antiretroviral therapy (HAART) is associated with an increased risk of metabolic disturbances and ischaemic heart disease.
- Antiretroviral (ARV) induced adipocyte toxicity is central to HIVLD pathogenesis resulting in impaired adipogenesis and dysregulated secretion of adipokines from the adipose tissue.
- Plasma levels of IL18, an adipokine associated with insulin resistance is elevated in HIVLD patients.
- Variants in IL18 gene also predispose to the development of HIVLD.
- Interleukin-18 suppresses adiponectin expression in adipocytes via NFATc4 phosphorylation.
- We utilised in vitro (adipocytes) and in vivo (gene association) studies to characterise the role of IL18 in HIVLD.

Aims and Objectives

- To investigate whether ARVs modulate IL18 and NFATc4 expression in vitro in adipocytes
- To utilise in vitro model to identify candidate molecules to target IL18 and NFATc4
- To investigate whether genetic variants in IL18 predispose to the development of HIVLD by HAART

Methods

- Differentiating 3T3-F442A murine adipocytes were incubated with ARVs (Lopinavir [LPV], ritonavir [RTV], atazanavir [ATV] and efavirenz [EFV]) in the presence and absence of telmisartan (TEL; 1-5µM).
- Secreted IL18 protein levels were assessed by ELISA.

Genetic Association study

- DNA samples were obtained from ARV-treated patients with (HIVLD+; n=115) or without LD (HIVLD-; n=51).
- Diagnosis of HIVLD was carried out by clinician’s confirmation of patient self-report
- SNP selection: Haplotype tag SNP approach; functional SNPs also selected; total 14 SNPs selected
- Sequenom MALDI-TOF was used for genotyping

Statistical Analysis

- In vitro studies were analysed by paired t-test and data are presented as mean ± SD for 20µM incubation of ARVs and 5µM for TEL.
- SNP analysis was performed using Haploview software.

Table 1. Patients characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Study participants (n = 180)</th>
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<tbody>
<tr>
<td>HIVLD+; n = 124, HIVLD-; n = 56</td>
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<tr>
<td>Age in years, median (range)</td>
<td>40 (24-68)</td>
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<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>146 (81.5%)</td>
</tr>
<tr>
<td>Women</td>
<td>34 (18.5%)</td>
</tr>
<tr>
<td>Duration of HIV infection, median (range)</td>
<td>7.6 years (1.4 - 19.3 years)</td>
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<tr>
<td>CD4 cell count, mean (range)</td>
<td>330.5 (3-1218)</td>
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<tr>
<td>PIs</td>
<td>LPV, ATV, RTV</td>
</tr>
<tr>
<td>NRTI backbone</td>
<td>d4T, 3TC, AZT</td>
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<tr>
<td>Other drugs</td>
<td>ddI, ddC, ABC, EFV, NVP</td>
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</tbody>
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Results

Fig 1. Effect of ARVs and Telmisartan on IL18 protein expression

Fig 2. Effect of ARVs & Telmisartan on NFATc4 gene expression

Fig 3. Linkage disequilibrium patterns within the IL18 gene haplotype blocks are represented here. Red boxes indicate strong linkage disequilibrium (LD), while grey boxes indicate weaker LD. Haplotype block 1 is comprised of rs215763, rs300719, rs3949518 and rs3844811. Haplotype block 2 consists of rs494068 and rs7441881.

Summary of Results

- ARVs up-regulate secretion of IL18 in adipocytes and up-regulate the expression of NFATc4
- Effect of EFV on IL18 was lesser as compared to LPV, ATV and RTV
- NFATc4 gene expression was higher with ATV treatment as compared to LPV, RTV and EFV
- Telmisartan reverses ARV-induced increase in IL18 secretion and NFATc4 up-regulation
- IL18 SNPs did not show association with HIVLD in our cohort of study

Conclusion

- ARV-mediated upregulation in IL18 could play a role in the development of HIVLD
- ARV-induced upregulation of NFATc4, a transcription factor through which IL18 causes inhibition of adiponectin (a marker of insulin sensitivity), could be mechanistically important in the development of insulin resistance.
- Telmisartan offers a potential strategy to treat ARV-induced adverse effects

Future Work

- Blockade of NFATc4 using siRNA, an on going experimental strategy, may result to reduce adverse effect of ARVs
- Validation of murine adipocyte results in primary human adipocytes
- IL18 gene variants do not predispose to HIVLD; however further studies in well-phenotyped patients with adequate sample size are required to confirm this.

References