Tenofovir Alafenamide Fumarate Alters Markers of Mitochondrial Biogenesis in the Brains of Transgenic Mice

Nicholas R. Scrivens, OMS-II[1]*, Mary K. Swinton, OMS-II[1]*, Jerel A. Fields, PhD[2]
[1] Western University of Health Sciences COMP-Northwest, Lebanon, OR
[2]Department of Psychiatry, University of California San Diego, San Diego, CA
*Equally contributing authors

Western University of Health Sciences
College of Osteopathic Medicine of the Pacific COMP-Northwest

ABSTRACT

Goals of Study/Hypothesis: HIV associated neurocognitive disorder (HAND) affects 30-50% of people with HIV despite the effectiveness of antiretroviral therapy (ART). Neuronal mitochondrial dysfunction is a prospective etiology of HAND, with previous studies finding that HIV proteins gp120 and Tat and ART drug tenofovir disoproxil fumarate (TDF) alter levels of transcription factor A mitochondrial (TFAM), peroxisome proliferator-activated receptor γ coactivator 1-α (PGC-1a) and glial fibrillary acidic protein (GFAP) in mouse brains (1). This study quantified the previously unknown effects of gp120 and Tat combined with new generation ART drug tenofovir alafenamide fumarate (TAF) on TFAM, PGC-1a and GFAP levels in mouse brains, further investigating the separate and combined effects of HIV and ART in the neuropathogenesis of HAND.

Materials & Methods: Transgenic mice co-expressing gp120 and Tat in the brain were exposed to TAF by oral gavage daily for 30 days .Treatment groups were organized into control, TAF, gp120 and gp120/TAF. Brain specimens were analyzed by immunoblot and immunohistochemistry (IHC) to quantify changes in TFAM and PGC-1a as markers of mitochondrial biogenesis and GFAP as a marker for astrogliosis.

Results: Both immunoblot and IHC showed decreased TFAM levels in hippocampi of mice treated with TAF and gp120/TAF compared to control, more notably in males. PGC-1α levels were unchanged between all groups on both immunoblot and IHC. IHC staining showed increased GFAP among TAF and gp120/TAF mice, which was more pronounced in male gp120/TAF mice.

Conclusions: These findings observed with TAF treated mice are consistent with previous studies showing that TDF and gp120 decrease TFAM. Unchanged PGC-1α levels between treatment groups suggests that the mechanism responsible for altered TFAM levels may be downstream of PGC-1α, or related to the TFAM gene, among other possible causes. GFAP levels increased in TAF, gp120 and gp120/TAF mice, indicating increased astrogliosis and neuroinflammation in these groups simulating HIV infection and HIV with ART treatment. The more pronounced differences in male mice indicate that sex may play a role in neuronal mitochondrial response to gp120 and/or TAF.

OBJECTIVES

- Quantify the separate and combined effects of HIV proteins gp120 and Tat and new generation ART drug tenofovir alafenamide fumarate (TAF) on TFAM, PGC-1a and GFAP levels in mouse brains
- Investigate the effects of HIV proteins and ART (TAF) on neuronal mitochondria and evaluate their role in the neuropathogenesis of HAND
- Provide insight into optimization of ART regimens and therapeutic targets for HAND and other neurodegenerative diseases

INTRODUCTION

HIV-associated neurocognitive disorder (HAND) is a general term used to describe a full spectrum of neurological problems that affects 30-50% of people living with HIV (PWH). Although advances in antiretroviral therapy (ART) have decreased the severity of HAND, symptoms remain prevalent in PWH. While the mechanisms underlying HAND are likely multifactorial, mitochondrial dysfunction is a prospective central etiology of the disease. Our previous studies suggest that the activity of transcription factors regulating mitochondrial biogenesis is reduced in neurons and increased in reactive astroglia. Mouse models allow us to study the mechanisms through which HIV proteins and ART drugs may lead to neurodegeneration and neurocognitive dysfunction, providing important insight into potential therapeutic targets for those living with HAND. This research will use a novel rodent transgenic (tg) mouse model for HIV-induced neurotoxicity to evaluate the effects of HIV proteins and ART on mitochondrial biogenesis in the brain. The model co-expresses the HIV proteins, gp120 and Tat, which are both neurotoxic proteins implicated in altered mitochondrial function, inflammatory gene expression, and neurodegeneration. Mouse brains will be analyzed using biochemical, molecular and neuropathological techniques to identify changes in transcription factors that regulate mitochondrial biogenesis (PGC-1α and TFAM), metabolic proteins (glucose transporters and insulin receptors), astrogliosis and neurodegeneration in order to identify possible therapeutic targets in HAND patients treated with ART. Findings from this study may also be applicable in the treatment of other neurodegenerative diseases like Alzheimer's Disease (AD) and Parkinson's Disease (PD).

STUDY DESIGN

Mouse Studies and Brain Tissue Collection: The iTat tg mouse model for HAND was used, and cerebroventricular injections with lentivirus (LV)-control or LV-gp120 were performed. Using a total of 80 iTat-tg mice; 40 male and 40 female. Mice were aged 6 months before LV injections and Dox treatment began. Two weeks after cessation of Dox treatment, to induce Tat expression, and four weeks after LV injections, mice were euthanized, brains were harvested, and bisected. For each mouse, one hemibrain was fixed with 4% paraformaldehyde for IHC and TEM analyses. For brain protein lysates, hemibrains from 10 mice in each group were immediately snap frozen.

Immunohistochemistry: Free-floating 40 µm thick vibratome sections of mouse brains were washed with phosphate buffered saline with tween 20 (PBST) 3 times, pre-treated for 20minutes in PBS 3% H2O2/1%TritonX, and blocked with 2.5% horse serum (Vector Laboratories) for 1 hour at room temperature. Sections were incubated at 4C overnight with the primary antibodies and then incubated in secondary antibody, Immpress HRP Anti-rabbit IgG (Vector, cat. no. MP-7401) or Impress HRP Anti-mouse IgG (Vector, cat. No. MP-7402) for 30 minutes, followed by NovaRED Peroxidase (HRP) Substrate made with NovaRED Peroxidase (HRP) Substrate Kit as per manufacturer's instructions (Vector, cat. no. SK-4800). Control experiments consisted of incubation with secondary antibody only. Tissues were mounted on slides and coverslipped with cytoseal. Immunostained sections were imaged with a digital Olympus microscope and immunoreactivity assessment was performed utilizing the Image- Pro Plus program. Background levels were obtained in tissue sections immunostained in the absence of primary antibody. Therefore: corrected optical density=optical density − background.

Immunoblot: After determination of the protein content of all samples by bicinchoninic acid, whole lysates were loaded and electrophoresed in Tris/Glycine/SDS running buffer and transferred onto LF PVDF membrane with Bio Rad transfer stacks and transfer buffer using Bio Rad Trans Blot Turbo transfer system. After the transfer, total protein were imaged using Bio Rad ChemiDoc imager under the stain free blot setting for normalization purposes. Membranes were then blocked in 1% casein in tris-buffered saline (TBS) for 1 h. Membranes were incubated overnight at 4 °C with primary antibodies diluted in blocking buffer. All blots were then washed in PBST, and then incubated with species-specific IgG conjugated to HRP. Images were obtained, and semi-quantitative analysis was performed with the ChemiDoc gel imaging system and Quantity One software (Bio-Rad).

RESULTS

1. Immunoblot-Mitochondrial Transcription Factor A (TFAM)–Hippocampus

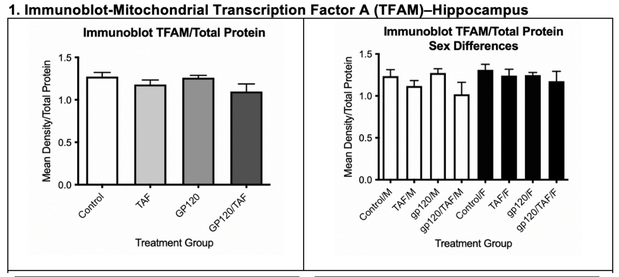

Figure 1a: Decreased TFAM levels in TAF, gp120/TAF mice.6 mice per group.

Figure 1b: Greater decrease in TFAM in male TAF and gp120/TAF mice. 3 mice per group.

2. Immunohistochemistry-Mitochondrial Transcription Factor A (TFAM)–Hippocampus

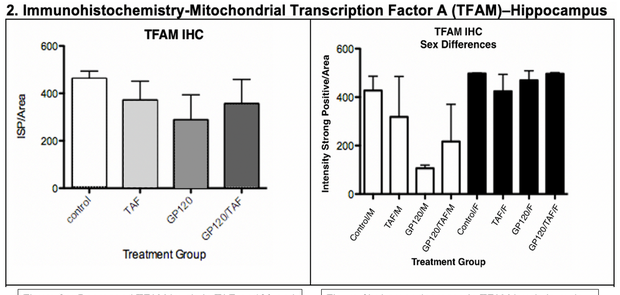

Figure 2a: Decreased TFAM levels in TAF, gp120 and gp120/TAF mice. 4 mice per group.

Figure 2b: Larger decrease in TFAM levels in male TAF, gp120 and gp12/TAF mice. 2 mice per group.

3. Immunoblot – PGC-1α – Hippocampus

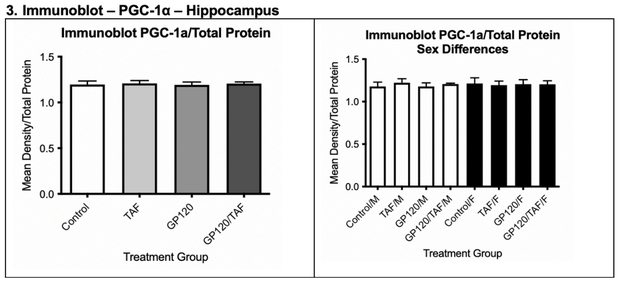

Figure 3a: No changes in PGC-1a between groups. 6 mice per group.

Figure 3b: No sex differences observed in PGC-1a levels between groups. 3 mice per group

4. Immunohistochemistry – Glial Fibrillary Acidic Protein (GFAP) – Hippocampus

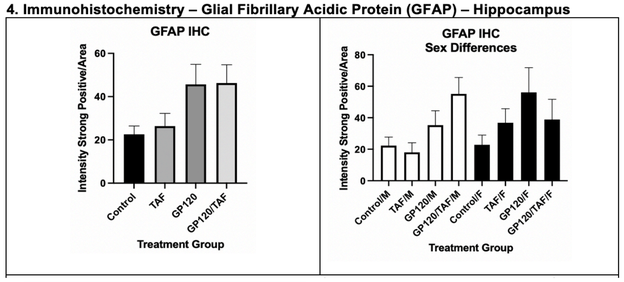

Figure 4a: Increased GFAP in TAF, gp120 and gp120/TAF mice. 4 mice per group.

Figure 4b: Males with increased GFAP in gp120/TAF group. 2 mice per group.

DISCUSSION

TFAM:
Western Blot/Immunoblot densitometry analysis of homogenized mouse hippocampus samples revealed slightly decreased TFAM levels in TAF treated mice and even more decreased TFAM levels in gp120/TAF mice compared to control (Figures 1a and 1b). This suggests reduced mitochondrial function in the brains of TAF treated mice as well as in gp120/TAF mice. On average, female mice appeared to have higher TFAM levels than males in all treatment groups. There were no significant p-values between groups at this sample size of six mice per group.

Immunohistochemistry analysis of mouse hippocampus sections revealed that on average, TFAM levels were reduced in TAF treated mice, gp120 mice and gp120/TAF mice, again suggesting altered mitochondrial biogenesis and function in the brains of these mice (Figures 2a and 2b). On average, female mice appeared to have higher TFAM levels in all treatment groups. While the IHC data appears to roughly follow the pattern of the Immunoblot data, further tuning of the IHC quantification algorithm may be needed as well as IHC staining and quantification of more brain samples to increase sample size. There were no significant p-values between groups at this sample size of four mice per group for IHC staining.

PGC-1a:
Western Blot/Immunoblot of homogenized mouse hippocampus samples showed that there were no significant differences between groups for PGC-1α levels. PGC-1α is a co-transcriptional regulation factor that is the key positive regulator of mitochondrial biogenesis. Given that PGC-1α was unchanged across groups and TFAM was changed between groups, this could indicate that the mechanistic change may be either downstream of PGC-1α, related to the TFAM gene or that non-detectable changes in PGC-1α can lead to more significant changes in TFAM levels, among other possible causes. If this same trend between PGC-1α and TFAM is reproduced in future studies, this may be an avenue for further experimental analysis. There were no significant differences between groups at this sample size of six mice per group.

GFAP:
Changes in GFAP can indicate altered astrocyte function, namely with increased GFAP being suggestive of astrogliosis and neuroinflammation. Immunohistochemistry analysis of mouse hippocampus sections, quantified by Intensity Strong Positive/Area of hippocampus show that GFAP levels were increased in all gp120+ mice, as expected as this is a hallmark of HIV infection and on average, TAF treated mice had slightly increased GFAP compared to control (Figures 4a and 4b). Among gp120+ mice, GFAP increased very minimally in gp120/TAF compared to gp120 alone. Of note, GFAP appears to be increased in male/gp120+//TAF mice compared to female/gp120+/TAF mice which could indicate a sex difference in response to gp120/TAF. There were no significant differences between treatment groups at this sample size of 10 mice per group.

Summary:
Both Western Blot/Immunoblot and IHC showed decreased TFAM levels in hippocampi of mice treated with TAF and gp120/TAF compared to control, more notably in males. PGC-1α levels were unchanged between all groups on both immunoblot and IHC. IHC staining showed increased GFAP among TAF and gp120/TAF mice, which was more pronounced in male gp120/TAF mice. These findings indicate that TAF and gp120 together may affect mitochondrial and astrocyte function within the neurons of gp120 and TAF treated mice. Further studies are needed to replicate these results and further study mechanistic changes in the brains of mice exposed to HIV proteins and/or ART.

CONCLUSION

This preliminary data shows that TAF and gp120 decrease TFAM levels which is consistent with previous studies showing that TDF and gp120 decrease TFAM levels in the brains of mice (1). Unchanged PGC-1α levels between treatment groups suggests that the mechanism responsible for altered TFAM levels may be downstream of PGC-1α or related to the TFAM gene, among other possible causes. GFAP levels were increased in TAF, gp120 and gp120/TAF mice, indicating increased astrogliosis and neuroinflammation in these groups. The more pronounced differences in TFAM and GFAP levels among male mice indicate that sex may play a role in neuronal mitochondrial response to gp120 and/or TAF. This data suggests that both HIV and ART as well as potential sex differences may contribute to neuroinflammation and altered neuronal mitochondrial function, which warrants further study in order to identify therapeutic targets that could reduce neuroinflammation and improve mitochondrial function within the mouse model that could later be applied to the prevention of HAND in PWH treated with ART.

REFERENCES

Fields, Et al. 2019. Tenofovir disoproxil fumarate induces peripheral neuropathy and alters inflammation and mitochondrial biogenesis in the brains of mice. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6868155/

ACKNOWLEDGEMENTS

- J.A. Fields Lab at UCSD, and research mentor Jerel Adam Fields, PhD.
- Edie Sperling, DPT, WesternU Faculty Mentor
- WesternU Summer Research Fellowship
- Funding provided by National Institute on Aging
- Kaylie-Anna Vallee, Kevyn Aguilar, Lab Technicians in J.A. Fields Lab