IN VITRO IMPACT OF TAF ON MITOCHONDRIAL FUNCTION IN IMMUNE CELLS

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BACKGROUND

Mitochondrial dysfunction has been involved in toxicity of antiretrovirals such as Zalcitabine (ddC). Markedly lower plasma levels of tenofovir (TFV) are thought to lead to the more favorable bone and renal safety profile of tenofovir alafenamide (TAF) compared to tenofovir disoproxil fumarate (TDF). It is unknown whether an increase in intracellular levels of the active metabolite, tenofovir-diphosphate (TFV-DP) with TAF (compared to TDF) may alter mitochondria. This study was designed to address whether TAF affects in vitro mitochondrial membrane potential (MMP), a direct measure of the state of energization of the mitochondria, in peripheral blood mononuclear cells (PBMCs).

OBJECTIVES

To address whether TAF affects in vitro mitochondrial membrane potential (MMP), a direct measure of the state of energization of the mitochondria, in peripheral blood mononuclear cells (PBMCs).

Acknowledgements

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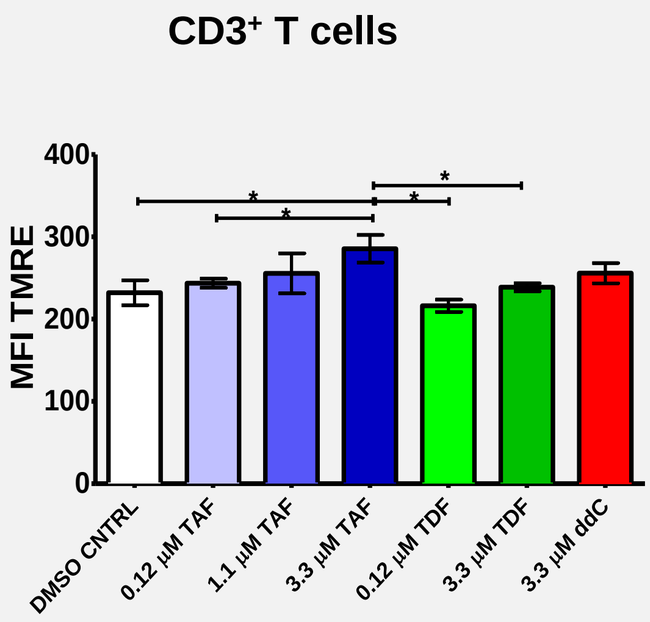
METHODS

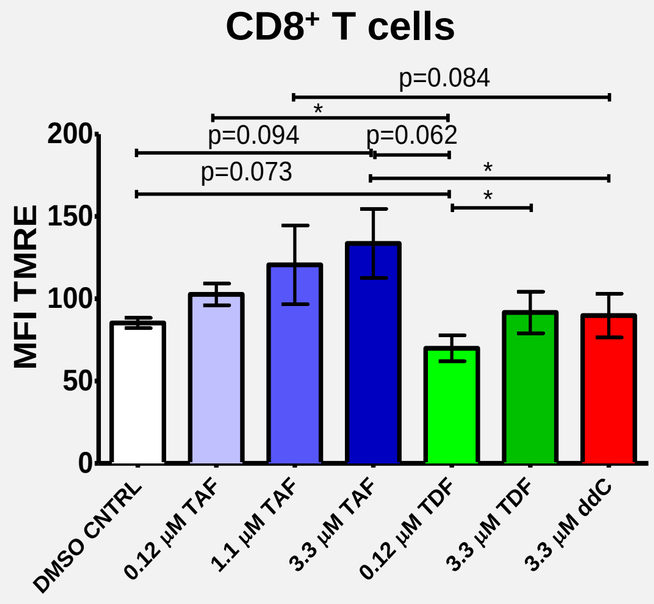
In vitro incubation of PBMCs with antivirals: 2-hour incubation conditions with TDF and/or TAF at concentrations that have been shown to model cellular levels of TFV-DP similar to those observed in PBMCs in clinical studies with TDF and/or TAF were selected to model clinically relevant plasma exposure.

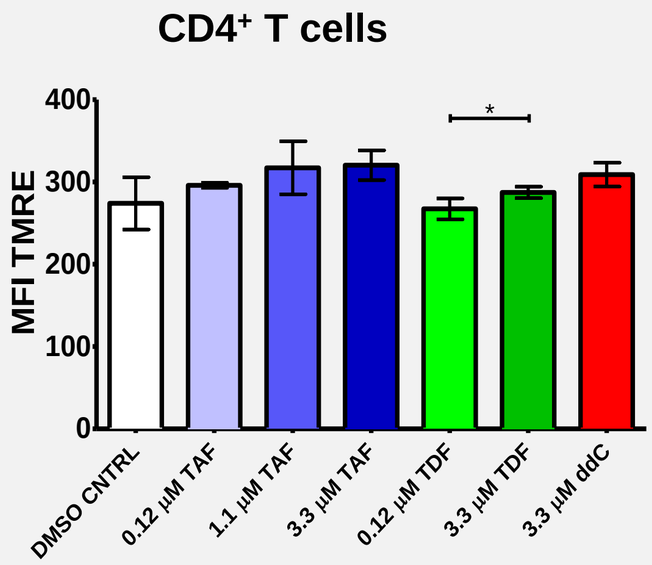
Flow cytometry: Mitochondrial mass and mitochondrial membrane potential (MMP) were assessed in PBMCs from healthy donors using flow cytometry and the fluorescent dyes Mitogreen (MTG) and Tetramethylrhodamine ethyl ester (TMRE), respectively, along with fluorescently labelled antibodies against CD3, CD4, CD8, CD14 (to quantify the MMP in immune cells). Cells were labeled with mouse anti-human CD3 Antibody (APC/Cy7, clone HIT3a), mouse anti-human CD8 Antibody (Brilliant Violet 650™, clone SK1), mouse anti-human CD14 Antibody (Brilliant Violet 711™, clone M5E2), mouse anti-human CD14 [PE/Cy7, clone HCD14], in appropriate combination. Cellular death was assessed by flow cytometry using the nuclear death dye SYTOX Blue. Appropriate negative controls (Fluorescence minus one, FMO) were used in all steps. At least 30,000 events per sample were acquired in LSR II (BD Biosciences). Flow cytometry analyses were performed using FlowJo (Tree Star, Ashland, OR), and the change in median fluorescence intensity (DMFI) was calculated by subtracting the fluorescence of the negative control (Fluorescence minus one) from that of the stain.

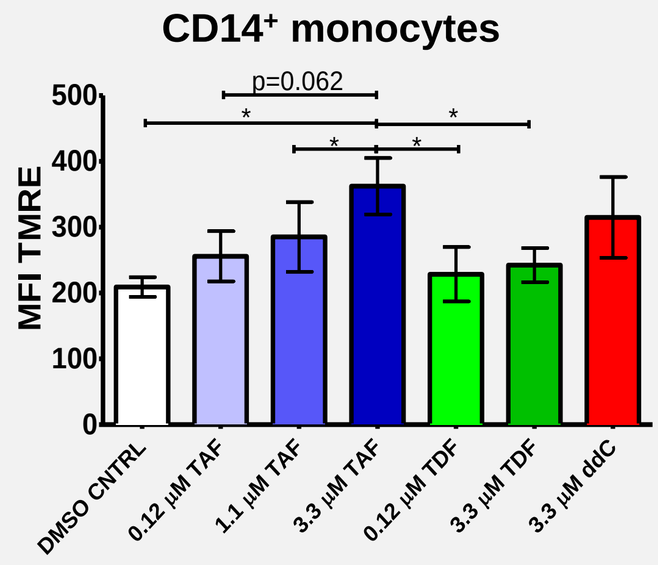
Data Analysis: Results are reported as mean and standard error of mean (mean, SEM). The Wilcoxon and Mann Whitney tests were used for statistical comparison between groups. Inference was assessed against a type I error of 2.5%.

RESULTS









RESULTS

- After 2 hours of in vitro exposure of PBMCs to 0.12-3.3 µM TAF, TDF and ddC, 3.3 µM ddC and 0.12, 3.3 µM TDF did not impact the median fluorescence intensity (MFI) of TMRE in CD3+, CD4+, CD8+ T cells and CD14+ cells compared to DMSO control.
- 3.3 µM TAF increased the MFI of TMRE in CD3+ T cells compared to DMSO control, 0.12 µM TAF, 0.12 µM TDF and 3.3 µM TDF ($p<0.05$).
- 3.3 µM TAF increased the MFI of TMRE in CD8+ T cells compared to ddC ($p<0.05$).
- 3.3 µM TAF increased the MFI of TMRE in CD14+ monocytes compared to 1.1 µM TAF, 0.12 and 3.3 µM TDF and DMSO ($p<0.05$).
- 2 hours of in vitro exposure of primary PBMCs to 0.12-3.3 µM TAF did not impact TMRE in CD4+ T cells.

CONCLUSIONS

- In the setting of delivering higher intracellular levels of TFV-DP than TDF, TAF may increase the MMP at in vitro incubation conditions that model clinically relevant exposure in resting PBMC as early as 2 hours.
- This concentration dependent effect was more prominent in monocytes compared to T cells.
- The clinical relevance of these in vitro findings is unknown.
- The effect of TAF on mitochondrial function in chronic treated HIV should be further explored in patients switching from TDF to TAF regimens.

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