THPEA033: DIFFERENTIAL EFFECTS OF TAF, TDF, AND 3TC ON MURINE WEIGHT, BODY COMPOSITION AND ADIPOCYTE DIFFERENTIATION

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Background

Studies report greater moderate weight gain in people with HIV starting antiretroviral therapy (ART) with tenofovir alafenamide (TAF) compared to those initiating with tenofovir disoproxil fumarate (TDF)[1,2], and when switching from TDF-containing regimens to TAF-containing regimens[3]. However, the underlying mechanisms remain unclear. We evaluated the impact of TAF, TDF, and lamivudine (3TC) –first-line nucleoside reverse transcriptase inhibitors used to treat HIV as part of ART- on adipocyte differentiation *in vitro*, and examined the potential differences in the effects of TAF and TDF on weight and body composition *in vivo*.

Methods

- *In vitro*, murine 3T3-L1 preadipocytes underwent standard 7-day differentiation protocol and were treated with TDF, TAF (0.5-5μM), 3TC (1-20μM), or Vh throughout. Differentiation was characterized by assessing intracellular lipid accumulation with Oil Red O staining, mRNA expression analysis of differentiation markers (RT-qPCR) (days 3, 5, 7), and morphological analysis of cell populations by size (forward scatter, FSC) and complexity (side scatter, SSC) by flow cytometer at day 7.
- *In vivo*, TDF (50mg/kg), TAF (5mg/kg), or vehicle (Vh) were orally administered to C57BL/6J mice for 16 weeks (male and female, 17 mice per group). Doses were calculated by allometric scaling and were equivalent to those used in humans: 300mg TDF and 25mg TAF. Weight gain was monitored weekly, and body composition assessed using a DXA analyzer.

Statistical analysis (n≥5) was performed by one-way ANOVA.

EFFECTS ON 3T3-L1 DIFFERENTIATION

1. INTRACELLULAR LIPID ACCUMULATION

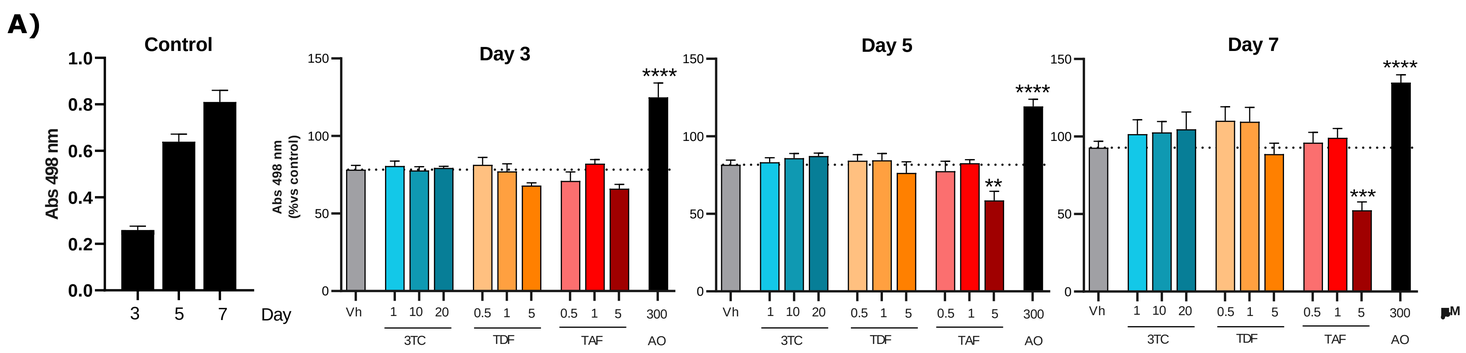

Figure 1. A) Lipid accumulation process. Absorbance 498nm of Oil Red O. Left: graph representing the process without treatment (control). Right: graphs representing the differentiation process in treated cells; values are expressed as a % relative to control (100%). The dotted line indicates the mean of the group vehicle. Data show the mean±SEM (n=5). One-way ANOVA with Tukey *post-hoc* analysis. **p<0.01, ***p<0.001, ****p<0.0001 *versus* vehicle. **B) Representative images of Oil Red O staining (day 7).**

TAF significantly reduced lipid accumulation in 3T3-L1 cells from day 5 until the end of differentiation (day 7) and avoided the acquisition of the characteristic morphology of mature adipocytes.

2. mRNA EXPRESSION ANALYSIS OF DIFFERENTIATION MARKERS

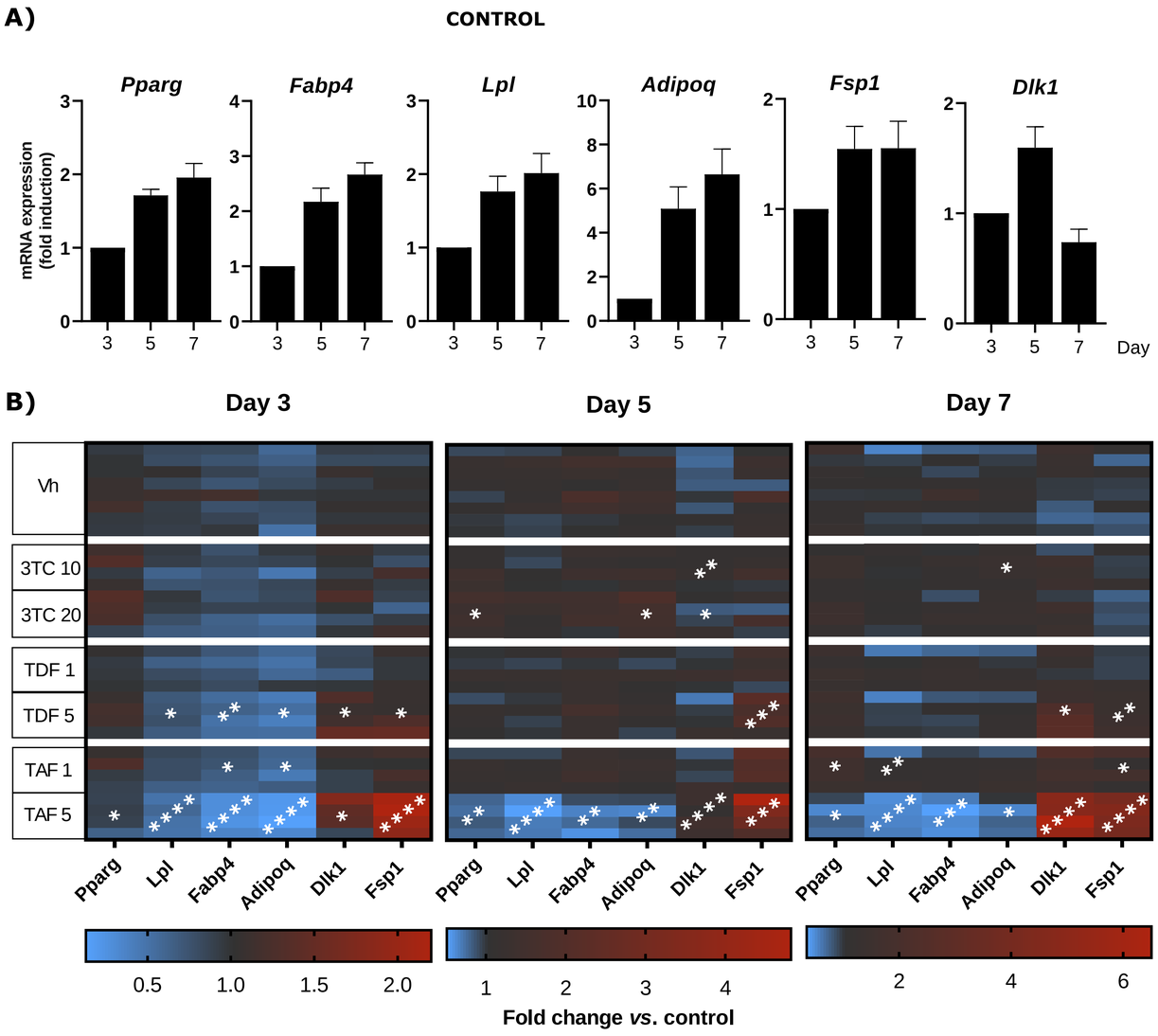

Figure 2. A) mRNA expression during differentiation of 3T3-L1 cells without antiretroviral treatment (control). Fold induction relative to the value on day 3. Data show the mean±SEM (n=8). **B) Heatmap of fold change of mRNA expression of differentiation markers in treated cells, relative to untreated cells.** Each experimental replicate (n=4-8) is represented on days 3, 5, and 7. Statistical comparisons are conducted between the fold change induced by the drug and the respective fold change induced by the vehicle. One-way ANOVA with Tukey *post-hoc* analysis. *p<0.05, **p<0.01, ***p<0.001 or ****p<0.0001 *versus* vehicle.

TAF-treated cells showed lower expression of early (*Ppary*, *Fabp4*) and mature (*Lpl*, *Adipoq*) adipocyte markers, while maintaining higher levels of preadipocyte markers (*Dlk1*, *Fsp1*). TDF downregulated mature adipocyte markers expression only at day 3, but also showed a moderate elevation of preadipocyte markers at day 7.

3. MORPHOLOGICAL ANALYSIS AFTER DIFFERENTIATION

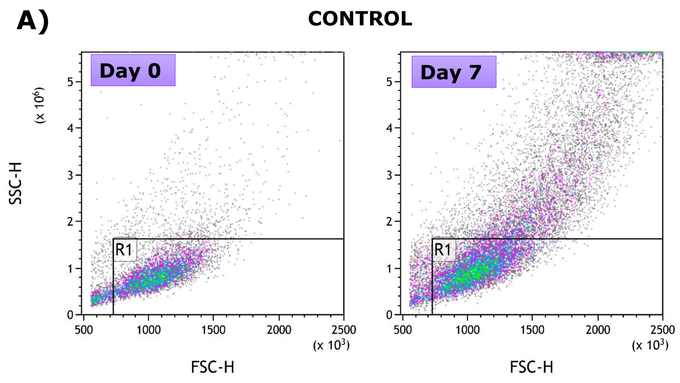

Figure 3. A) Histogram of populations in untreated cells at day 0 (left) and day 7 (right). We defined **R1** as the set of characteristics similar to undifferentiated 3T3-L1 cells. **B) Percentage of R1 on day 7 in antiretroviral-treated cells.** Values are expressed as percentage of cells. Data show the mean ± SEM (n=4). Mean of vehicle dotted line. One-way ANOVA with Tukey *post-hoc* analysis. *p<0.05 *versus* vehicle. FSC, forward scatter; SSC, side scatter.

At endpoint, TAF treatment resulted in the highest percentage of cells exhibiting undifferentiated preadipocyte morphology.

RESULTS IN THE MURINE MODEL

Figure 4. A) Body weight gain. ****p<0.0001 *versus* vehicle and TAF. **B) Body composition.** At week 15, each mouse was scanned using the DXA analyzer. Data show the mean ± SEM (n=17 mice/group). One-way ANOVA with Tukey *post-hoc* analysis. *p<0.05 *versus* TAF.

At week 16, the mice in the three groups gained a similar percentage of weight compared to their initial weight, with no differences between them. However, mice treated with TDF exhibited significantly lower area under curve (AUC) of weight gain.

TDF-mice had an increase in % body fat and a slight decrease in bone density

CONCLUSION

***In vivo*, sustained TDF administration at doses equivalent to human clinical levels affects body composition and weight gain in mice, whereas TAF does not. *In vitro*, TAF inhibits preadipocyte differentiation much more significantly than TDF at the same concentration for both prodrugs. 3TC had an overall more neutral profile.**

The clinical impact of inhibiting adipocyte differentiation remains controversial and unclear. However, the distinct effects of these drugs may contributed to the weight gain differences observed in clinical practice, providing insights to improve HIV treatment.

References: *1. Venter WDF, et al. N Engl J Med. 2019;381(9):803-815. / 2. Sax PE, et al. Clin Infect Dis. 2019./ 3. Gomez M, et al. Infection. 2019;47(1):95-102.*

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