Tenofovir regulates myogenesis by inhibition of ampk and modulation of purinergic receptors and dipyridamole reverts the effect

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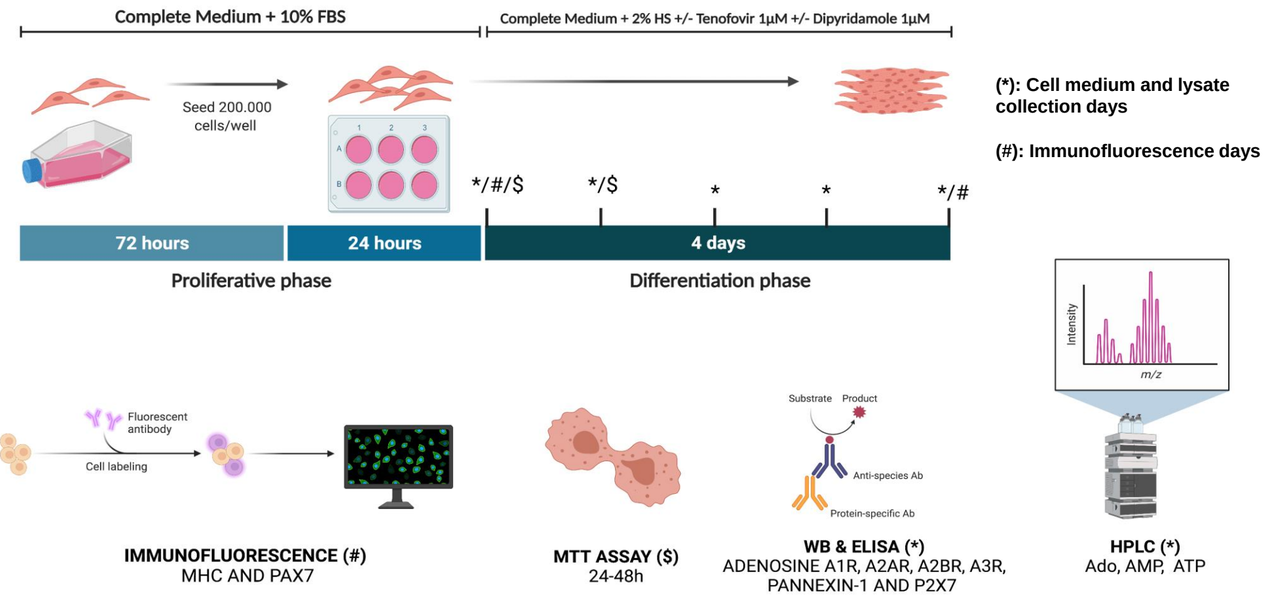
INTRODUCTION

Sarcopenia has been defined as an age related, involuntary loss of skeletal muscle mass and strength. Sarcopenia has been associated with a poor balance between muscle cell proliferation / differentiation during myogenesis. The energy complex that forms the purinergic system modulates myogenesis in muscle. Muscle exercise leads to an ATP expenditure and an increase in the AMP / ATP ratio that activates the AMPK enzyme and catabolism. AMPK phosphorylation has been linked to maintaining the proliferative state in muscle cells, which could enhance muscle regeneration.

Tenofovir, and HIV retroviral, which modulates the purinergic system by inhibiting cellular ATP output and decreasing adenosine levels, has been associated with the appearance of sarcopenia in HIV patients and in the osteoporotic murine models.

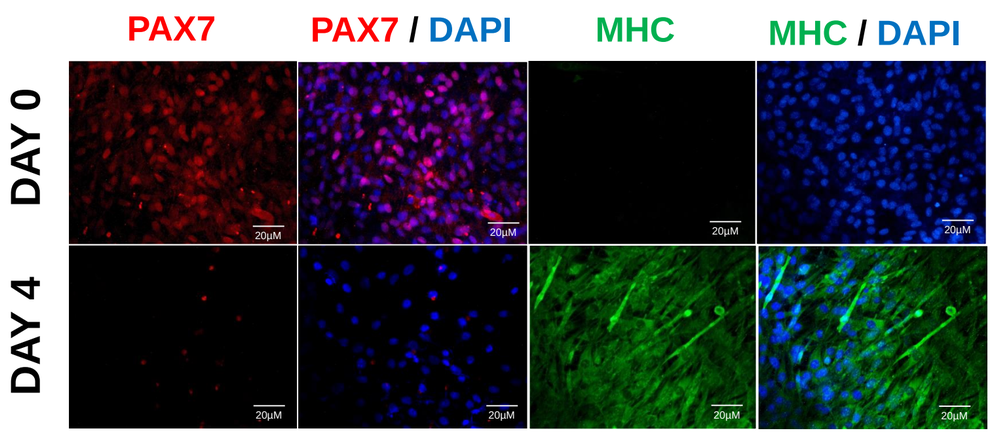
We determined how purinergic system modulates muscle differentiation / regeneration using tenofovir (inhibits Pannexin-1-mediated ATP release) and dipyridamole (blocks adenosine uptaken by the cells) in C2C12 murine myoblast cell line.

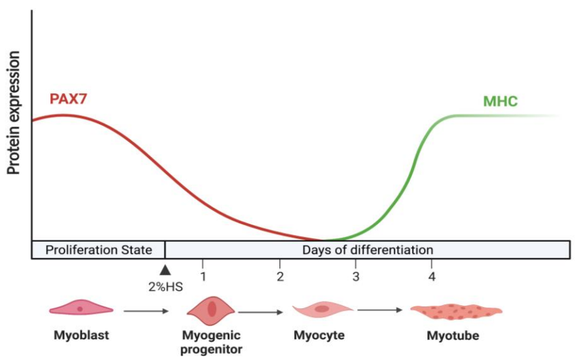
METHODS



RESULTS

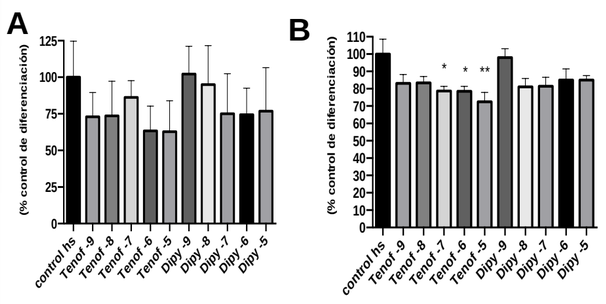
C2C12 myoblast differentiation and expression of myogenic makers at day 4





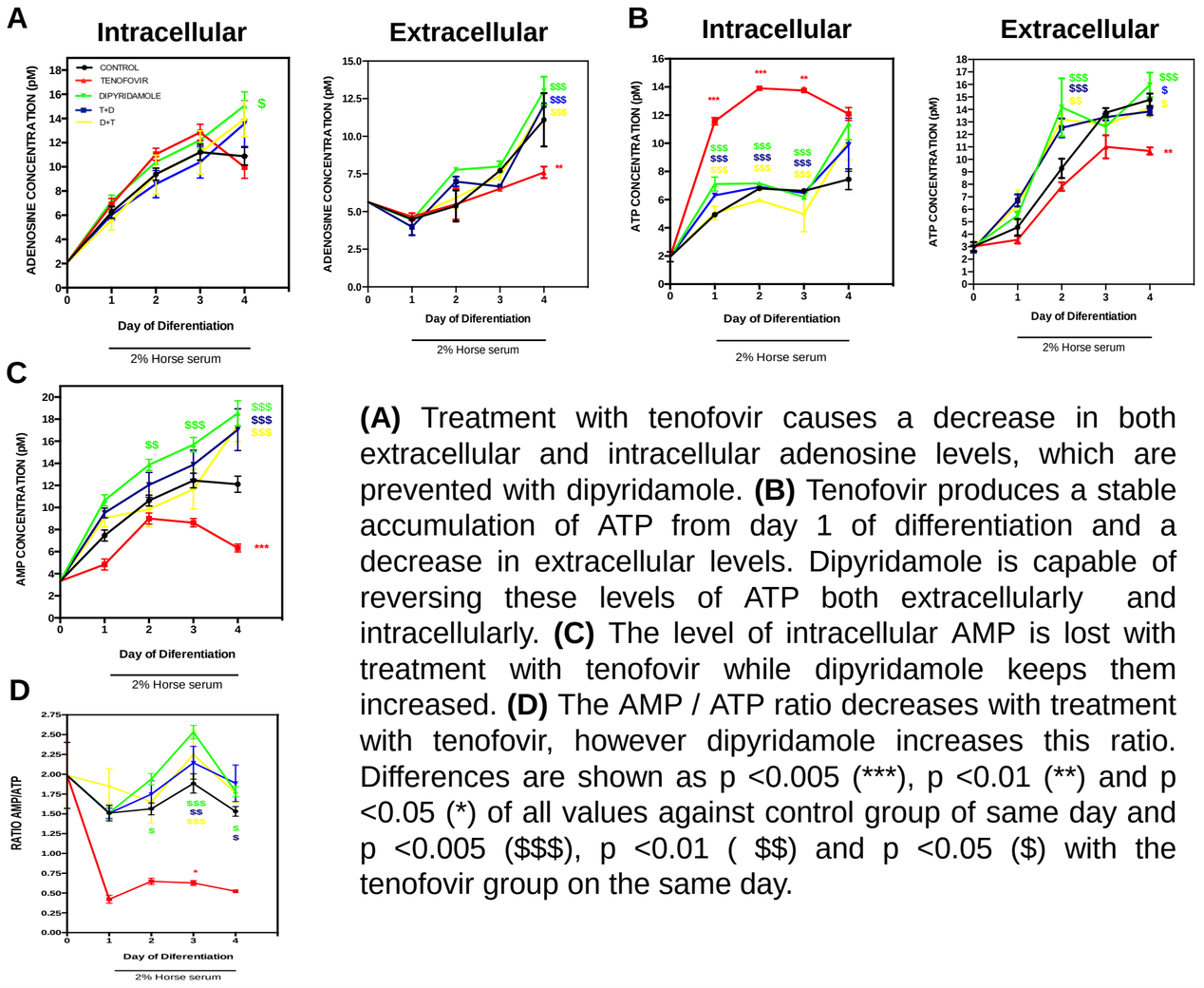
The positive labeling of the proliferation protein PAX7 is obtained on day 0 of differentiation, disappearing on day 4. On the other hand, the motor protein expressed in MHC muscle filaments only appears on day 4 of differentiation.

Tenofovir promotes dose-dependent muscle differentiation



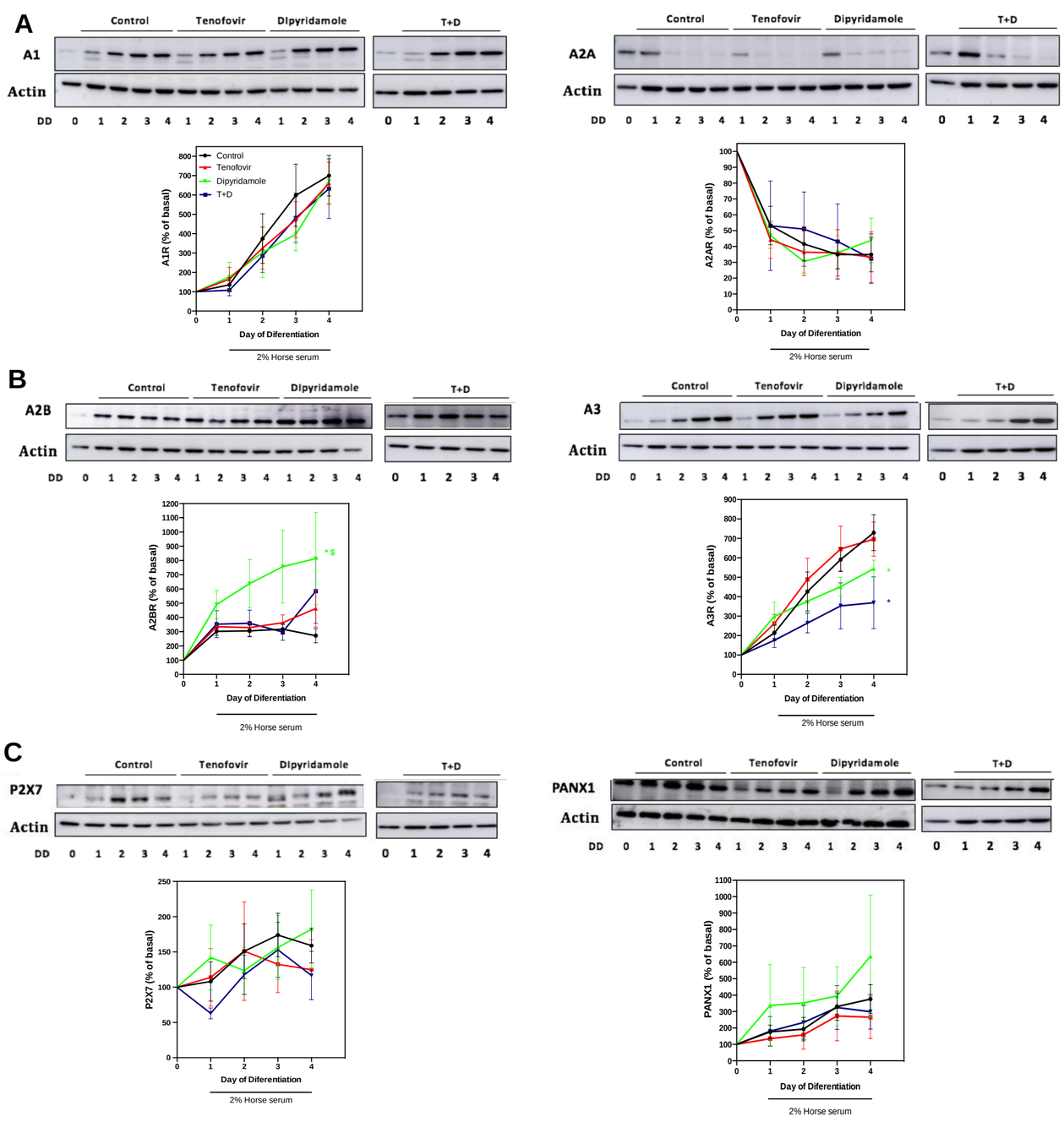
(A) MTT assay does not reflect differences between treatments with respect to the control in differentiation at different doses at 24 hours. **(B)** There is a significant decrease in proliferation at 48h with tenofovir 10^{-7}-10^{-5} M.

Tenofovir maintains ATP in the intracellular space while dipyridamole reverses this effect by maintaining high levels of intracellular adenosine and AMP and extracellular adenosine



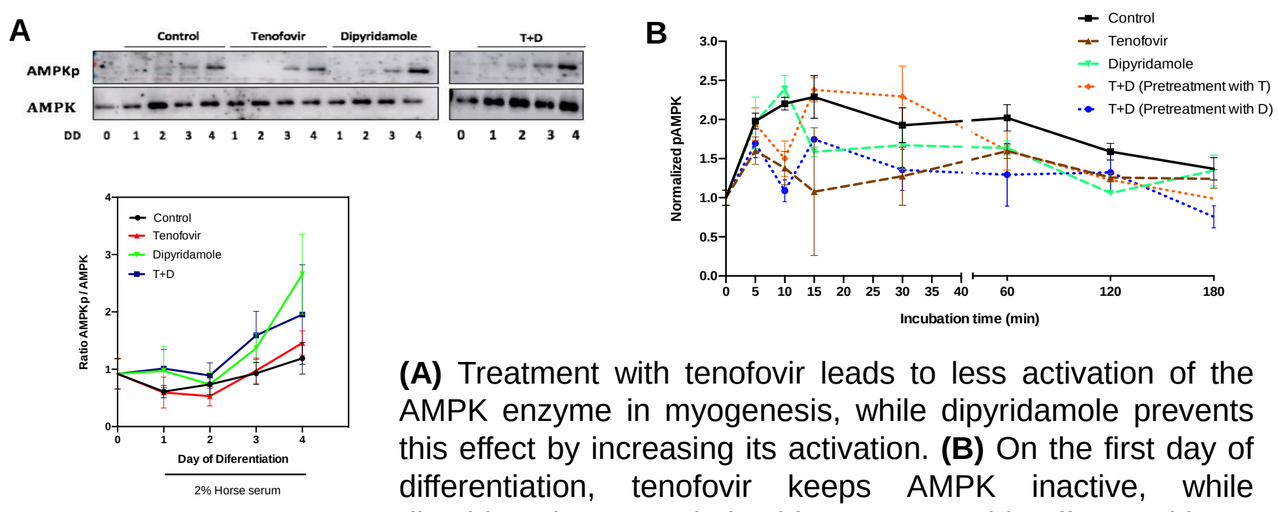
(A) Treatment with tenofovir causes a decrease in both extracellular and intracellular adenosine levels, which are prevented with dipyridamole. **(B)** Tenofovir produces a stable accumulation of ATP from day 1 of differentiation and a decrease in extracellular levels. Dipyridamole is capable of reversing these levels of ATP both extracellularly and intracellularly. **(C)** The level of intracellular AMP is lost with treatment with tenofovir while dipyridamole keeps them increased. **(D)** The AMP / ATP ratio decreases with treatment with tenofovir, however dipyridamole increases this ratio. Differences are shown as p <0.005 (***), p <0.01 (**) and p <0.05 (*) of all values against control group of same day and p <0.005 ($$$), p <0.01 ($$) and p <0.05 ($) with the tenofovir group on the same day.

Tenofovir modulates the activation of the A3 receptor and inhibits PANX1 while dypiridamole prevents this effect and in turn enhances the A2B and P2X7 receptor in muscle myogenesis



(A) An antagonistic role of the adenosine A1 and A2A receptors is observed in myogenesis. The adenosine A1 receptor increases with differentiation, while the expression of the A2A receptor disappears on day 2. **(B)** Treatment with dipyridamole increases the expression of the adenosine A2B receptor significantly on day 4, while tenofovir increases the adenosine A3 receptor. **(C)** In turn, treatment with tenofovir causes a decrease in the expression of the pannexin-1 transporter and the P2X7 receptor. Differences are shown as p <0.005 (***), p <0.01 (**) and p <0.05 (*) of all values against control group of same day and p <0.005 ($$$), p <0.01 ($$) and p <0.05 ($) with the tenofovir group on the same day.

Dipyridamole prevents AMPK inhibition by tenofovir keeping energy catabolism

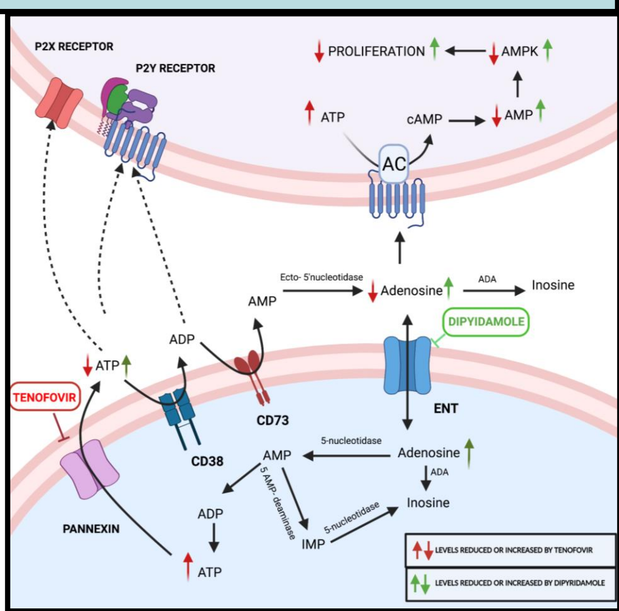


(A) Treatment with tenofovir leads to less activation of the AMPK enzyme in myogenesis, while dipyridamole prevents this effect by increasing its activation. **(B)** On the first day of differentiation, tenofovir keeps AMPK inactive, while dipyridamole at 15 min is able to reverse this effect and keep the enzyme active.

CONCLUSIONS

Tenofovir maintains myogenesis through adenosine A3R and inhibition of AMPK activity due to low intracellular AMP / ATP ratio due to increased intracellular ATP.

Dipyridamole prevents this effect by promoting muscle regeneration through increased extracellular adenosine, increased activation of A2BR and AMPK due to increased intracellular AMP.



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