

The effect of first-choice antiretroviral agents on mesenchymal stem cells commitment

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INTRODUCTION

Modern Highly Active Anti-retroviral Therapy (HAART) is designed as an effective and sophisticated pharmacological strategy involving the combination of two or more active molecules [1]. However, the decrease of Bone Mineral Density and eventually the manifestation of osteoporosis in ageing HIV-infected population treated with HAART, is emerging as a new challenge [2]. It has been speculated that there might be a connection between the use of antiretroviral drugs and an impaired osteoblasts differentiation from their cellular precursors: Mesenchymal Stem Cells (MSCs). MSCs are multipotent cells found in various tissues, among which placenta, peripheral blood, umbilical cord, adipose tissue and bone marrow [4]. According to the received stimulus, MSCs can differentiate into three diverse cell lineages: chondrocytes, adipocytes and osteoblasts. Furthermore, MSCs are crucial for tissue homeostasis and damage repair which makes them very attractive in the field of tissue engineering and regenerative medicine [5].

The International Society for Cellular Therapy (ISCT) indicates three main criteria to define MSCs i) adhesion to plastic ii) capability of differentiation into the three aforementioned cell lineages iii) positivity to CD73, CD90 and CD105, and negative to CD34, CD45, CD14 or CD11b, CD79a or CD19 and HLA-DR [6].

STUDY DESIGN

Cultures of an immortalized human stromal cell line (L88) were incubated both in presence and in absence of Darunavir (DRV) and Dolutegravir (DTG). Cell viability and Osteogenic differentiation were investigated. Cells were cultured with control medium (CTR) and osteogenic medium (DIFF) containing Vitamin D, beta-glycerophosphate and Ascorbic Acid [4]. *RUNX2* expression, well known trigger of the osteogenic differentiation [7], was detected through qPCR.

METHODS

- ✓ L88 were cultured in Petri dishes with 5 ml of complete medium: RPMI 1640, 10% FBS, 1% glutamine and 1% penicillin/streptomycin.
- ✓ Cell viability: cells are seeded 2×10^4 /well in a 96-well plate AND both the negative control and the drug-treated cells experiment is set up in triplicate. After adhesion (24 hours) compounds are inoculated at the concentration of 2xIC90, IC90, 1/2xIC90, 1/4xIC90. Vitality is checked every 24h for 4 days. Fresh medium and molecules are added every two days. The reduction of yellow tetrazolium salt MTT to dark purple formazan by succinate dehydrogenase, mainly in mitochondria, is evaluated by MTT quantitative colorimetric assay and it is now widely accepted as a reliable way to examine cytotoxicity.
- ✓ Gene expression: cells are seeded 4.5×10^5 /well in a 6-well plate and after adhesion compounds are added at a concentration of 2xIC90. A well with control medium (CTR) and a well with differentiation medium (DIFF) are set up both for the controls and for treated cells. After 48h and 96h RNA is harvested, retrotranscribed and the expression of *RUNX2* was evaluated by qPCR.

Laboratory reagents had been partially acquired thank to an unrescinded grant by ViIV and grant UNIMI Linea 2

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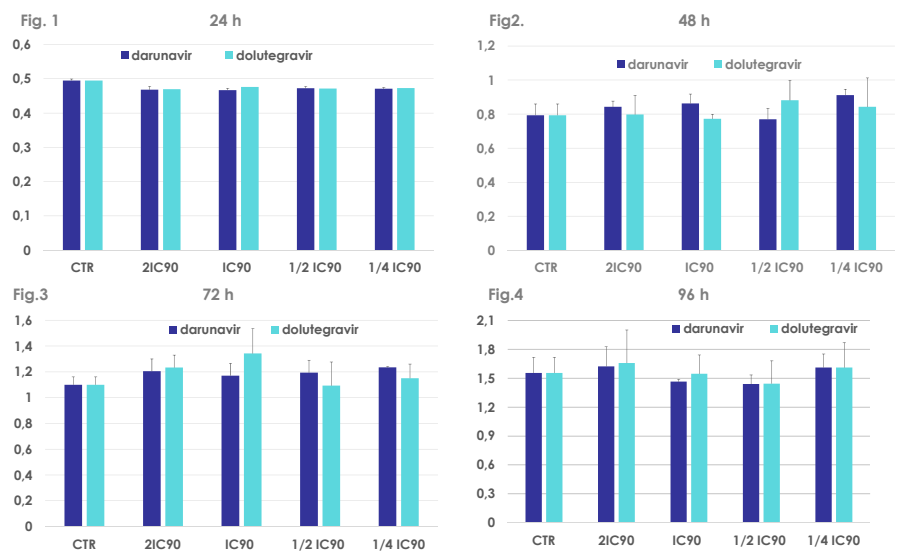
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RESULTS

Cell viability with MTT

The MTT assay, performed in order to evaluate the ideal concentrations to use in the further experiments, revealed that the compounds of interest are not toxic at any of the chosen concentrations. In fact, the bar graphs below illustrate how at the 4 fixed concentrations (x axis) the absorbance read by the machine (y axis) kept growing contextually to the control trend. Indeed, it means that the cells both in presence and in absence of the molecules carried on their normal lifecycle healthily.

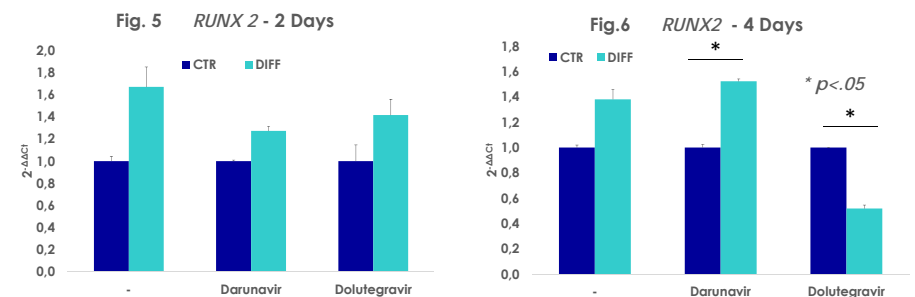
Figure 1-2-3-4
The bar graph shows the cells growth rate after 24h, 48h, 72h & 96h of treatment



Gene expression

The bar graphs below represent the results obtained via qPCR after two days and four days of treatment respectively. The treatment with Darunavir (DRV) and Dolutegravir (DTG) seems to slow the expression of *RUNX2* in presence of differentiation medium after 48h. However, at 96h the cells treated with DRV resume their regular capability of expressing *RUNX2* whereas the inhibition is still present in the presence of DTG.

Figure 5-6
The bar graph shows the expression of RUNX2 after 2 and 4 days of treatment with Darunavir and Dolutegravir compared with controls



CONCLUSIONS

From the data collected during this preliminary phase it is reasonable to think that:

- ✓ DRV delays the osteogenic differentiation of L88 cells after two days induction, while after 4 days *RUNX2* increases as much as in the control.
- ✓ DTG instead inhibits the osteogenic differentiation of L88 cells.
- ✓ In the future, it would be interesting to investigate the effect of these 2 drugs on other important genes for osteoblast differentiation such as *COL1A1* [7]. Besides, since MSCs are the cellular progenitors of adipocytes - and HAART also affects this cell lineage [3] - another line of research might be to investigate these HIV inhibitors' influence on adipogenesis.