

Title: EFFECTS OF CPX ON TRANSFECTION EFFICIENCY AND OVER DIFFERENTIATION IN C2C12.**Matheus B. Severino***, Marcos V. Esteca, , Letícia Tamborlin, Augusto D. A. Luchessi, Igor L. Baptista.**Abstract**

Technologies such as over-expression of proteins through techniques such as transfection have been developed and improved. In this sense, a new model was tested in C2C12 cells, which are very particular, since they can alter their morphology for myotubes.

Key words: *transfection, PEI, C2C12.*

Introduction

The differentiation process in skeletal muscle cells is highly organized and essential to the muscle plasticity. C2C12 culture cells are widely used and studied in research, allowing an understanding of the differentiation process, due to the ability in differentiate in multinucleate myotubes from single myoblasts. These in vitro models are powerful tools for allow genetic manipulation, for example, through the use of plasmids and several transfection reagents. On this study, we used a newly described reagent, ciclopirox (CPX), used to improve transfection efficiency in polyethylenimine (PEI) transfection system used on other cell types,

Results and Discussion

C2C12 cells in consolidated culture, was transfected with PEI and a GFP-flag plasmid; some wells also received ciclopirox. To analyze the effect of ciclopirox during the differentiation, we have used horse serum 2% supplementation for 2 and 4 days.

Our results showed that the percentage of transfected cell was higher in PEI transfection compared to PEI+CPX (PEI 35,5% vs PEI+CPX 21% per mm² n=3). In protein analysis, by Western Blot; we observed that CPX there was a reduction the GFP production (~30% of reduction), suggesting that ciclopirox have a stressing effect over C2C12 cells.

We compared the number of cells post transfection, and before differentiation. We observed 102 cells/mm² in PEI procedure vs 75 cells/mm² in PEI+CPX, evidencing a reduction of 35%, even starting from 1x10⁵ (n=4). Those data indicate that CPX treatment induced less proliferation compared to PEI transfected cells.

To analyze the effects of transfection during the differentiation, we detected Flag (GFP), Cyclin and Myogenin proteins. We observed that when we applied ciclopirox the Myogenin levels was strong reduced in PEI+CPX (86% vs Control) than PEI transfection (60% vs Control) and control group; the levels of Flag decreased during differentiation (reduction of 40% when compared 2 to 4 days), however in wells that receiving CPX that decrease was slightly higher (30%); the Cyclin levels in PEI+CPX was also reduced compared to PEI (50% of

reduction). Our data suggest that in CPX transfection an early differentiation could occur, but without a complete progress, due to the expression of myogenin expression.

Conclusions

Our results showed that in PEI transfection, the protein production was higher compared to PEI+CPX. Moreover, when CPX was applied, it reduced C2C12 proliferation and, consequently reduced differentiation. Those effects could be consequence of eIF5A inhibition for CPX since this drug has been used also as inhibitor previously (Luchessi, et al. 2009).

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Silva, Maeli Dal Pai; Carvalho, Robson Francisco. Mecanismos celulares e moleculares que controlam o desenvolvimento e o crescimento muscular. Revista Brasileira de Zootecnia. Sociedade Brasileira de Zootecnia, v. 36, p. 21-31, 2007.

Araújo T, Pereira M, Camargo, I. efeito do uso de lipofectamine™ 2000 e polietilenoimina (pei) na transfecção e expressão da proteína fluorescente verde em fibroblastos bovinos cultivados in vitro; 2014.

Foschini M. S. A. Rosália, Ramalho S. Fernando, Bicas E. A. Harley; Myogenic satellite cells, 2004.

Luchessi, A. D. et al. Involvement of eukaryotic translation initiation factor 5A (eIF5A) in skeletal muscle stem cell differentiation. **Journal of cellular physiology**, v. 218, n. 3, p. 480-489, 2009.