# TOXICOKINETICS AND MUSCLE-RELATED TOXICITY OF ATORVASTATIN *IN VITRO*

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## INTRODUCTION

Atorvastatin (ATV) is one of the most commonly used statins to fight hypercholesterolemia, a major predisposing factor for the development of cardiovascular diseases[1]. Statin-induced myotoxicities are a common side effect that can lead to withdrawal or poor compliance[2]. Differences in individual susceptibility to ATV-associated myotoxicity exist in clinics. Intuitively, factors influencing intracellular myocyte accumulation might modulate the susceptibility to muscle side effects. From that hypothesis, we assumed that this intracellular accumulation might be controlled by the balanced activities of influx and efflux proteins expressed in the muscle tissue; *i.e.* OATP2B1 (*SLCO2B1*) and MRP1 (*ABCC1*). Accumulation experiments were performed in HEK293 recombinant generated models overexpressing either the influx (OATP2B1) or the efflux (MRP1) proteins in single or double transfectant models.

## OBJECTIVES

- To develop single and double transfectant models overexpressing the transporters.
- To assess the impact of the influx (OATP2B1) and efflux (MRP1) proteins on ATV transport in single transfectant models.
- To evaluate the impact of transporters in double transfectant models.

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## MATERIALS AND METHODS

In single transfectant models, HEK293 cells were lipofected with expression vector pCMV-C-OFP Spark coding for the red fluorescent protein OATP2B1 (Figure 1A).
The protein MRP1 was expressed through the vector pCDNA3.1-EGFP also coding for a green fluorescence (Figure 1B).

Stable transfectants were selected through antibiotic selection (either with 0.5mg/ml hygromycine or 1mg/ml G418, for OATP2B1 or MRP1 constructs respectively) and we subsequently sorted exclusively the cells that over-expressed the transporter by Fluorescent Activated Cell System (FACS). The resulting cell populations were then characterized to demonstrate the OATP2B1 (Fig 2A and B) or MRP1 (Fig 2C and D) overexpression by fluorescence microscopy and flow cytometry (data not shown). Double transfectant model expressing both wild-type proteins results of a transfection of OATP2B1 in HEK293 cells already stably overexpressing the protein MRP1.

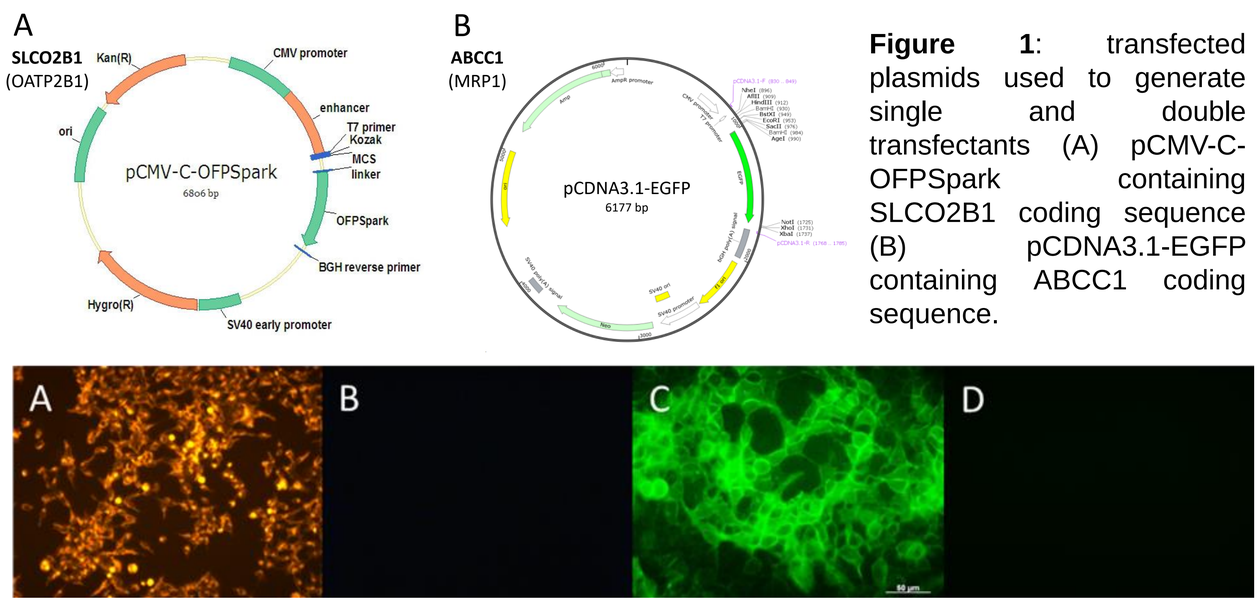


**Figure 1**: transfected plasmids used to generate single and double transfectants (A) pCMV-C-OFPSpark containing SLCO2B1 coding sequence (B) pCDNA3.1-EGFP containing ABCC1 coding sequence.

**Figure 2**: Fluorescence microscopy (A) recombinant HEK293 cells transfected with pCMV-OATP2B1-C-OFPSpark plasmid (20X) and (B) HEK as controls, (C) recombinant HEK293 cells transfected with pCDNA3.1- ABCC1-C-GFP plasmid (40X) and (D) HEK as controls.

## RESULTS

### OATP2B1 activity stimulates atorvastatin intracellular accumulation

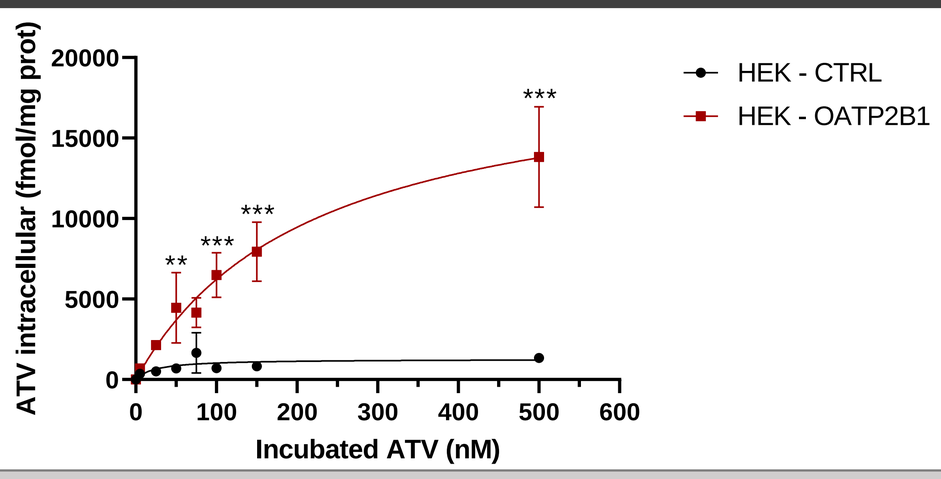


**Figure 3. Atorvastatin intracellular accumulation in OATP2B1 WT transfected cells vs. CTRL**

ATV intracellular accumulation significantly increases when the influx transporter OATP2B1 (*SLCO2B1*) is stably overexpressed in HEK293 cells (***P* < 0.01 and ****P* < 0.001).

### MRP1 activity protects against atorvastatin intracellular accumulation

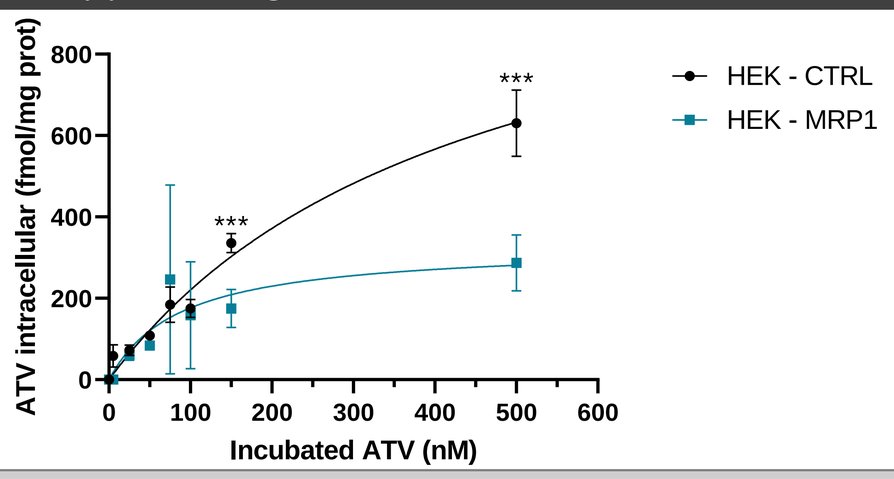


**Figure 4. Atorvastatin intracellular accumulation in MRP1 WT transfected cells vs. CTRL**

ATV intracellular accumulation significantly decreases when the efflux transporter MRP1 (ABCC1) is stably overexpressed in HEK empty vector cells (****P* < 0.001 from 150nM of ATV).

### MRP1 efflux partially counteracts ATV OATP2B1-generated influx

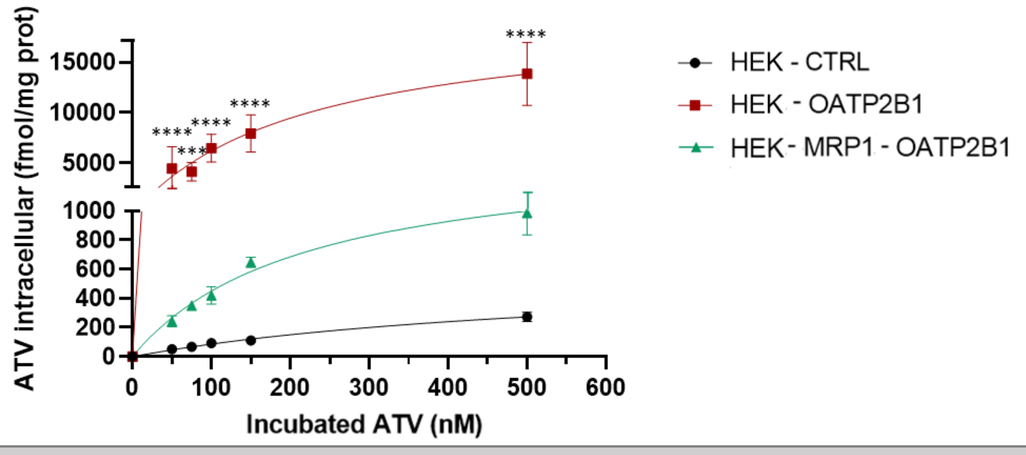


**Figure 5. Atorvastatin accumulation in double transfectant model vs. CTRL**

When MRP1 and OATP2B1 are co-expressed, ATV intracellular accumulation is significantly more important when compared to non transfected cells at any concentrations (***P < 0.001, *****P* < 0.0001 OATPWT compared to CTRL and double transfectant). However, the ATV intracellular levels observed in double transfectants are lower than those observed in OATP2B1 single transfectants. These results suggest that the efflux driven by MRP1 reduces the massive intracellular accumulation favored by OATP2B1 but is not sufficient by itself to completely counteract this influx activity.

## CONCLUSIONS

- ✓ ATV is a substrate for both OATP2B1 (influx) and MRP1 (efflux) transporters.
- ✓ OATP2B1 overexpression boosts intracellular accumulation while MRP1 activity seems to protect against intracellular ATV accumulation.
- ✓ OATP2B1-mediated ATV intracellular accumulation is reduced but not completely counteracted by MRP1 co-expression in HEK293 cells.

## DISCUSSION

These promising observations could indicate that modulations and factors affecting influx and/or efflux protein activities could help in predicting the risk of ATV-induced muscle toxicity.

1. Roth, G.A., et al., *High total serum cholesterol, medication coverage and therapeutic control: an analysis of national health examination survey data from eight countries.* Bull World Health Organ, 2011.**89**(2): p92-101.
2. Wei, M.Y. et al., *Predictors of statin adherence, switching and discontinuation in the USAGE survey: understanding the use of statins in America and gaps in patient education.* J Clin Lipidol, 2013.**7**(5):p. 472-83.