Introduction:
Several of currently used drugs are chiral compounds. Majority of them are clinically administered as a racemic (equimolar) mixture of enantiomers. Individual enantiomers of one drug can qualitatively and quantitatively differ in their biological activities (pharmacology, toxicology, pharmacokinetics, etc.). Therefore, enantiopure drugs have been developed and introduced to the therapy.

Statins are drugs used to lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase. Atorvastatin, rosuvastatin and fluvastatin are the most often prescribed statins. They have two chiral centres, thus they form four enantiomers: 3R5R-, 3R5S-, 3S5R- and 3S5S- (Figure 1). They are marketed as a racemic mixture of these enantiomers; however their enantiopure forms have been introduced to clinic recently (3RSR-atorvastatin, 3S5R-rosuvastatin and 3R5S-fluvastatin).

In this study, we investigated enantiospecific interactions of all four enantiopure forms of atorvastatin, rosuvastatin and fluvastatin with main transcriptional regulators of drug-metabolizing enzymes - aryl hydrocarbon receptor (AhR), glucocorticoid receptor (GR) and pregnane X receptor (PXR). Agonist and antagonist activities of tested compounds towards AhR, PXR and GR were determined using human reporter cell lines.

Gene reporter assay and cytotoxicity assay:
A stably transfected gene reporter cell line AZ-AHR and AZ-GR established in our laboratory were used for assessment of AhR and GR transcriptional activity. Transiently transfected LS180 human colon adenocarcinoma cells were used for assessment of PXR transcriptional activity. A chimera p3A4-luc reporter construct containing the basal promoter (-362/+53) with proximal PXR response element and the distal xenobiotic responsive enhancer module (-7836/-7208) of the CYP3A4 gene 50-flanking region inserted to pGL3-Basic reporter vector was used. The reporter plasmid was transiently transfected to LS180 cells by lipofection (FuGENE® HD Transfection Reagent).

Prior to gene reporter assays, we examined the cytotoxicity of tested compounds in AZ-AHR, AZ-GR and LS180 cell lines. For this purpose, the cells were incubated for 24 h with individual enantiomers of atorvastatin, fluvastatin and rosuvastatin at concentrations ranging from 100 pM to 100 μM. The vehicle was DMSO (0.1% v/v). After the treatment, a conventional MTT test was performed and absorbance was measured at 540 nm (Figure 2).

The data are the mean ± SD from experiments performed in three consecutive passages of cells.

Conclusion: We have demonstrated significantly different stereospecific effects of tested statins on transcriptional activity of glucocorticoid and pregnane X receptor. Recently, we study the enantiospecific effects of statin enantiomers on the expression of drug-metabolizing enzymes CYPs on mRNA and protein level in primary human hepatocytes.

Financial support from Czech Scientific agency 13-01809S and Students project of Palacky University Olomouc PrF-2015-003 is greatly acknowledged.

References:
Novotna, A. et al., Environmental science & technology 2011, 45, 10133-10139.