ABSTRACT

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Title of Doctoral Thesis: Drug interactions with nuclear receptors in the regulation of drug metabolizing enzymes and drug transporters

Drug metabolizing enzymes play a key role in metabolism, elimination and detoxification of xenobiotics exposed to the body and protect the organism from potentially harmful effect of xenobiotics as well as certain endobiotics. Phase I and II drug metabolizing enzymes and drug transporters are expressed in most tissues in basal level or may be induced after exposure to xenobiotics. Affecting of expression or activity of drug metabolizing enzymes and drug transporters may result in unpredictable tissue and systemic drug distribution, to side effects or therapy failure. Up-regulation of drug metabolizing enzymes and drug transporters mediates mainly orphan nuclear receptors - pregnane X receptor (PXR) and constitutive androstane receptor (CAR) and transcription factor aryl hydrocarbon receptor (AhR).

This doctoral thesis presents the results of three studies dealing with the gene regulation of drug metabolizing enzymes and drug transporters via nuclear receptors.

In the first part of this thesis we examined whether warfarin enantiomers stereoselectively interact with PXR to up-regulate main drug metabolizing enzymes of the cytochrome P450 superfamily. Warfarin, a widely used anticoagulant, is administered as racemic mixture of R- and S-warfarin. S-warfarin has five times greater anticoagulant potency than R-warfarin. We found that R-warfarin interacts with PXR and transactivates *CYP3A4* and *CYP2B6* mRNA in primary human hepatocytes and LS174T intestinal tumor derived cell line. S-warfarin showed lower potency to transactivate target genes via PXR. Furthermore, we found that 4´- and 10-hydroxywarfarines are potent ligands of PXR and inducers of CYP3A4 and CYP2C9 mRNA in primary human hepatocytes and therefore 4´- and 10-hydroxymetabolites of R-warfarin may be also ligands of PXR. These results raise the consideration whether the use of S-warfarin together with *VKORC1* and *CYP2C9* genotyping

would be safer alternative of anticoagulant therapy in relation to drug-drug interactions than the current use of racemic warfarin.

Next part of this thesis is focused on glucocorticoid receptor regulation of organic cation transporter 1 (OCT1) expression via HNF4α up-regulation in primary human hepatocytes and hepatocarcinoma cell lines HepG2 and MZ-Hep1. We found that OCT1 is transactivated via HNF4α up-regulation via GR in primary human hepatocytes, but not in model cell lines. This discrepancy may be caused by affecting of transcription factors involved in regulation of OCT1 due to different signaling pathways in normal hepatocytes and tumor derived cell lines. We did not found any GR response element (GRE) within 1,7 kb OCT1 promoter sequence and therefore OCT1 is unlikely transactivated via GR.

The last part of this thesis is focused on finding of novel CAR ligands among newly synthetized quinazoline derivates. We have tested 20 compouds, three of them, namely CHP4, CHP5 and CHP6, were found to interact with ligand binding domain of CAR. We showed, that CHP4, CHP5 and CHP6 transactivate pBREM/2B6 promoter via both CAR1 and CAR3 splice variants in the COS-1 cell line. CAR3 exhibits affinity to direct activators. CHP4, CHP5 and CHP6 significantly enhanced the interaction between C- and N-region of CAR LBD showing these compouds are direct CAR activators. Finally, CHP4, CHP5 and CHP6 up-regulated CAR target gene CYP2B6 mRNA in primary human hepatocytes and HepG2 cell line. New model CAR ligands might help us to elucidate physiologic functions and the role of this receptor in organism.