ABSTRACT IN ENGLISH LANGUAGE

Candidate: Mgr. Alejandro Carazo Fernández

Supervisor: Prof. PharmDr. Petr Pávek, PhD.

Title of the doctoral thesis: Nuclear receptors - new ligands study and importance of the genetic variability

Nuclear receptors (NRs) constitute a superfamily of transcription factors, which regulate the expression of target genes upon the binding of a ligand. These receptors can be classified in steroid receptors, "orphan receptors" and "adopted orphan receptors" depending on the affinity to an endogenous ligand. Nuclear receptors play important roles in physiological processes and are widely distributed in the human body. Thus, adipogenesis, lipolysis, insulin sensitivity, oxidative metabolism, fatty acid homeostasis, cholesterol homeostasis, gluconeogenesis, glycogen homeostasis, triglyceride metabolism among other processes, are regulated by nuclear receptors.

During my study, we have tested several sets of drugs, endogenous, natural and synthetic, in several nuclear receptors, focusing mainly on constitutive androstane receptor (CAR) and to a lesser extent on pregnane X receptor (PXR). My main aim was to find a new and reliable ligand or activator for human CAR. In addition, I aimed to study the mechanism of action by which these compounds interact with the receptor and how they trigger downstream pathways and target genes. For this purpose I used *ex vivo*, *in vitro* and *in silico* models.

In the first research project, I described the human CAR activation by several flavonoid compounds via inhibition of epidermal growth factor receptor (EGFR). This way of indirect CAR activation was described for antiepileptic drug phenobarbital (PB), so we proposed that these flavonoids would interact with the receptor similarly. For this purpose, we employed several methods including CAR assembly assay in cultured cells and LanthaScreen® Time Resolved – Fluorescence Energy Transfer (TR-FRET) cell-free assay.

In the second project, I optimized the TR-FRET method that we used in the previous research work. This method is based on the fluorescence resonance energy transfer (FRET) between two fluorophores that change the emission spectrum upon presence of a ligand interacting with CAR ligand binding domain. In this work, I characterized well-known agonists, antagonists and inverse agonists of CAR employing the method.

In the third research project, I studied interactions of a set of rationally developed acetylated and oxidized derivates of parent bile acids with several nuclear receptors in HepG2 hepatic cells. In this work, I established that derivate 3,12-diacetate DCA is able to strongly activate PXR and its target genes. However, we were not able to find traces of this derivate in human or mice bile samples with HPLC method suggesting that the compound is not an endogenous ligand of PXR.

In the last research project, I am working on the mechanism, which is involved in interaction of leflunomide, a drug for the treatment of rheumatoid arthritis, with human CAR. To date, no drug used in human therapy has been reported to directly activate this receptor with high affinity and only CITCO (a toxic oxime) is able to stably activate human CAR. The importance of finding a CAR ligand among current medication is critical for the study of CAR activation in human beings.

I believe our results will help us to understand how CAR, PXR and other nuclear receptors work. In addition, our research sheds some light on the understanding of the molecular mechanisms by which nuclear receptors exert their activity and how they are involved in metabolic and physiological processes. Moreover, our work aimed to apply this knowledge in the development of new ligands for the potential applications in therapy of metabolic diseases.