ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové, Department of Biological and Medical Sciences

University of Vienna, Faculty of Life Sciences, Department of Pharmacognosy

Candidate: Lenka Kovářová

Supervisor: Pharmdr. Miroslav Kovařík, Ph.D.

Consultant: Dr. Angela Ladurner

Title of the diploma thesis: Characterization of a synthetic leoligin derivative, with agonistic FXR and enhancing macrophage cholesterol efflux activity

Atherosclerosis is a pathologic multifactorial process triggering the development of cardiovascular diseases, which are the leading causes of death in the western world. The initial phase of atherosclerosis is characterized by the accumulation of lipid particles, mainly low-density lipoproteins (LDL) and macrophage-derived foam cells in large arteries, leading to the gradual thickening of the vessel wall. These progressive alterations elicit plaque formation, followed by rupture, thrombosis and finally can lead to a cardiovascular event. Reverse cholesterol transport is an important preventive mechanism, which ensures removal of excessive atherogenic lipoproteins from macrophages. This efflux is facilitated by ATP binding cassette transporters, mainly ABCA1 and ABCG1 and in part by scavenger receptor B1 (SR-B1).

Several nuclear receptors, including PPARγ, LXRα and LXRβ are known to upregulate these transporters and influence cholesterol metabolism, especially through a PPARγ-LXR-ABCA1 pathway. RXRα is able to form functional heterodimers amongst others with LXRs and FXRs. Interestingly, FXR is expressed in the liver and the intestine, but also in macrophages. Ligands of this receptor are discussed as potential therapeutic agents for lipid disorders.

In this work we focused on the characterization of a leoligin derivative, which was shown to activate FXR in a previous study. In addition this compound was shown to increase cholesterol

efflux from macrophage-derived foam cells. Therefore a possible modulation of ABCA1, ABCG1 and also SR-B1 expression by this compound was investigated via Western blotting. Activity of this compound on other nuclear receptors was evaluated via luciferase reporter gene assay and a Gal-4 assay to determine the specifity of the compound.

These experiments revealed that the tested leoligin derivative dose-dependently increased ABCA1 protein levels and confirmed a specific activation of FXR, as other investigated nuclear receptors (LXRα, LXRβ, PPARα, PPARγ, RXRα) were not activated. In addition to the leoligin derivative the FXR agonist, chenodeoxycholic acid (CDCA), and the FXR modulator, guggulsterone were investigated. Interestingly, both compound were able to enhance ABCA1 protein levels. Additionaly, guggulsterone was shown to dose-dependently transactivate LXRα and LXRβ.

The here presented data is not sufficient to establish a link between cholesterol efflux from macrophage-derived foam cells and FXR. Further experiments are required to elucidate the underlying mechanism of the increased cholesterol efflux elicited by these compounds.