World Academy of Science, Engineering and Technology
International Journal of Bioengineering and Life Sciences
Vol:11, No:01, 2017

Constitutive Androstane Receptor (CAR) Inhibitor CINPA1 as a Tool to Understand CAR Structure and Function

Authors : Milu T. Cherian, Sergio C. Chai, Morgan A. Casal, Taosheng Chen

Abstract : This study aims to use CINPA1, a recently discovered small-molecule inhibitor of the xenobiotic receptor CAR (constitutive androstane receptor) for understanding the binding modes of CAR and to guide CAR-mediated gene expression profiling studies in human primary hepatocytes. CAR and PXR are xenobiotic sensors that respond to drugs and endobiotics by modulating the expression of metabolic genes that enhance detoxification and elimination. Elevated levels of drug metabolizing enzymes and efflux transporters resulting from CAR activation promote the elimination of chemotherapeutic agents leading to reduced therapeutic effectiveness. Multidrug resistance in tumors after chemotherapy could be associated with errant CAR activity, as shown in the case of neuroblastoma. CAR inhibitors used in combination with existing chemotherapeutics could be utilized to attenuate multidrug resistance and resensitize chemo-resistant cancer cells. CAR and PXR have many overlapping modulating ligands as well as many overlapping target genes which confounded attempts to understand and regulate receptor-specific activity. Through a directed screening approach we previously identified a new CAR inhibitor, CINPA1, which is novel in its ability to inhibit CAR function without activating PXR. The cellular mechanisms by which CINPA1 inhibits CAR function were also extensively examined along with its pharmacokinetic properties. CINPA1 binding was shown to change CAR-coregulator interactions as well as modify CAR recruitment at DNA response elements of regulated genes. CINPA1 was shown to be broken down in the liver to form two, mostly inactive, metabolites. The structure-activity differences of CINPA1 and its metabolites were used to guide computational modeling using the CAR-LBD structure. To rationalize how ligand binding may lead to different CAR pharmacology, an analysis of the docked poses of human CAR bound to CITCO (a CAR activator) vs. CINPA1 or the metabolites was conducted. From our modeling, strong hydrogen bonding of CINPA1 with N165 and H203 in the CAR-LBD was predicted. These residues were validated to be important for CINPA1 binding using single amino-acid CAR mutants in a CAR-mediated functional reporter assay. Also predicted were residues making key hydrophobic interactions with CINPA1 but not the inactive metabolites. Some of these hydrophobic amino acids were also identified and additionally, the differential coregulator interactions of these mutants were determined in mammalian two-hybrid systems. CINPA1 represents an excellent starting point for future optimization into highly relevant probe molecules to study the function of the CAR receptor in normal- and pathophysiology, and possible development of therapeutics (for e.g. use for resensitizing chemoresistant neuroblastoma cells).

Keywords : antagonist, chemoresistance, constitutive androstane receptor (CAR), multi-drug resistance, structure activity relationship (SAR), xenobiotic resistance

Conference Title : ICBBBE 2017 : International Conference on Biochemical, Biomolecular and Biopharmaceutical Engineering

Conference Location : Sydney, Australia

Conference Dates : January 26-27, 2017