Molecular Determinants of Constitutive Androstane Receptor (CAR) Agonism and Antagonism

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Purpose
The constitutive androstane receptor (CAR, NR1I3) is a xenobiotic-sensing nuclear receptor that primarily regulates the expression of drug metabolizing enzymes and transporters, while also playing roles in energy metabolism, cell cycle, and carcinogenesis. PK11195, an isoquinoline carboxamide, is a reported human CAR (hCAR) antagonist that has been used extensively as a hCAR inhibitor in luciferase screening assays to find hCAR activators. However, it was unknown whether PK11195 could function as a CAR antagonist under physiologically-relevant conditions. A novel co-culture system containing human primary hepatocytes (HPH) and HepG2-CAR/2B6 stable line was used to demonstrate that PK11195 is metabolized from a CAR antagonist to a CAR activator. The predicted metabolite (R)-N-desmethyl PK11195 was confirmed to activate CAR in reporter assays and induce CYP2B6/3A4 in HPH. As the removal of a single methyl group led to a reversal in pharmacological effect, the structure-activity relationships of these and other CAR ligands were studied via in silico docking to provide insight into the molecular interactions that govern CAR agonism and antagonism.

Methods
CDOCKER, a molecular dynamics (MD) simulated-annealing-based algorithm, was used to dock PK11195 and the (R)-N-desmethyl PK11195 metabolite, as well as reported direct CAR agonist and antagonists, into the CITCO-bound hCAR ligand-binding domain (LBD) and 5β-Pregnane-3,20-dione-bound hCAR LBD crystal structure (PDB ID: 1XVP and 1XV9). The CDOCKER binding energy scores and interacting residues were used to compare prototypical agonist binding and antagonist binding.

Results
The observation that PK11195 and its metabolite exert opposite pharmacological effects provided an opportunity to further understand the molecular basis for CAR agonism and antagonism. Through comparative docking studies, we were able to demonstrate that the two ligands share similar binding conformations within the hCAR ligand-binding domain; however, the antagonist, PK11195, is able to interact with Leu343 of the activation function 2 (AF2) helix. This finding is of significant interest because this interaction occurs in spite of interactions with a postulated side chain barrier, composed of F161, N165, F234, and Y326, that shields ligands from interacting with the AF2 helix. Another potent CAR antagonist, CINPA 1, also interacts with L343 in our docking studies. In addition, previously reported mutagenesis experiments have shown that Leu343 is critical for stabilizing the AF2 helix and that L343 mutants had decreased constitutive activity and ceased to be activated by ligands. The CDOCKER energy scores for the two ligands were similar with PK11195, the antagonist, being -10.517 kcal/mol and (R)-N-desmethyl PK11195, the agonist, being -13.061 kcal/mol.

Conclusion
Our findings demonstrate that very small metabolic changes to a molecule can significantly alter how that molecule interacts with the protein. In this case, our studies suggest that the basis for PK11195 antagonism of CAR is its interaction with Leu343 within the AF2 helix, as its demethylated metabolite does not interact with this residue and instead activates CAR. (This work is partly supported by NIH grants: DK061652 and GM107058)