Molecular Modeling Study of Species-Selective Peroxisome Proliferator-Activated Receptor (PPAR) α Agonist; Possible Mechanism(s) of Human PPARα Selectivity of an α-Substituted Phenylpropanoic Acid Derivative (KCL)

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In order to investigate the reason why phenylpropanoic acid derivative (KCL), a potent, human peroxisome proliferator-activated receptor (PPAR) α-selective agonist, shows this selectivity, we analyzed the binding modes of KCL and a related compound to the ligand-binding domain of human PPARα and rat PPARα by means of computer-aided molecular modeling. We concluded that the characteristic specificity of KCL is due to a specific hydrophobic contact between the hydrophobic tail part (the 4-trifluoromethyl group) and the key amino acid Ile272 located on the helix three region of the human PPARα ligand binding domain. We propose a possible binding mode of KCL with the ligand-binding domain of human PPARα. This binding model should offer important insights for further structural design of subtype-selective PPARα agonists for the treatment of altered metabolic homeostasis, such as dyslipidemia, obesity, and diabetes.

Key words peroxisome proliferator-activated receptor (PPAR) α; KCL; species-selectivity; molecular modeling; mutation

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily.[1] These receptors are ligand-dependent transcription factors. Three subtypes of PPARs, termed PPARα, PPARδ and PPARγ, have been identified so far in various species, including humans.[2] One of the subtypes, PPARα is present at high density in the liver and regulates the expression of genes encoding lipid and lipoprotein metabolism.[3] Fibrates are hypolipidemic agents that are very efficient in lowering elevated triglyceride concentrations.[4] Their action on lipid metabolism is mediated principally by activation of PPARα, leading to altered expression of genes involved in lipid and lipoprotein metabolism in the liver.[4] Although fibrates are ligands of PPARs, their affinity is weak and their subtype-selectivity is poor.[5] We considered that more potent and selective agonists of PPARα, especially human PPARα, might have therapeutic utility for the treatment of altered lipid homeostasis, especially in the liver.

Recently, we have reported the design and synthesis of some novel phenylpropanoic acid derivatives as subtype-selective PPARα agonists, and we selected the 2-ethylphenylpropanoic acid derivative (KCL; Fig. 1) for further pharmacological study.[6—9] Interestingly, we discovered that the transactivation activity of KCL for PPARα was approximately 100-fold less potent in rats than that in humans. KCL is, therefore, a highly potent, PPARα-selective and human-selective PPARα agonist.[10]

We thought it important to understand the mechanism which mediates the uniquely high species-selectivity of KCL. We speculated that human PPARα-selective activation by KCL is the result of specific interaction between certain amino acid residue(s) in the human PPARα ligand-binding domain (LBD), and a particular structural feature of KCL.[11] Therefore, in order to investigate the species-selective interaction between KCL and PPARα, we have developed a homology model for rat PPARα LBD based on the recently published coordinates of the human PPARα LBD, and we analyzed the mode of interaction between PPARα and some PPARα agonists, including KCL.

Experimental

Hardware and software Molecular modeling studies were carried out using the InsightII/Discover Version 98.0 molecular modeling package (Accelrys Inc., San Diego, CA, U.S.A.) on a Silicon Graphics O2 R10K workstation.

Reference PDB Data Construction of protein-ligand complexes was based on an X-ray structure of the human PPARα (hPPARα) LBD complexed with an agonist GW409544 (PDB entry 1K7L) (Fig. 1).[12]

Molecular Modeling of Human PPARα-Ligand Complexes Ligands were manually docked into the active site based on the X-ray structure. Minimizations were performed with Discover 3 using CVFF force field (shell water 10 Å, cell multipole summation method, 1.0 constant dielectric value, peptide main chain Fix, conjugate gradient, and R.M.S. force less than 0.1 kcal/molÅ).

Homology Modeling of Rat PPARα-Ligand Complexes Construction of the rat PPARα (rPPARα) model was performed by means of amino acid residue substitution of the hPPARα LBD crystal structure using the replace command of Biopolymer. Ligand docking was carried out in the same way as described above.

Results and Discussion

Several reports have appeared on the species-selectivity profile of PPARα agonists.[13—15] The classical PPARα agonist WY-14643 (WY; Fig. 1) is more effective on rat PPARα than on human PPARα. Moreover, GW-9578, a recently disclosed ureidothioisobutyric acid derivative with potent PPARα activity, also transactivates rat PPARα[1] preferentially over human PPARα.[14] On the other hand, 5,8,11,14-eicosatetraynoic acid (ETYA) shows the reverse preference, *i.e.*, it is 10-fold more effective on human PPARα than rat PPAR. Clofibric acid and fenofibric acid, which are active metabolites of the fibrate-class antihyperlipidemic agents clofibrate and fenofibrate, respectively, do not show clear species differences.[16] We investigated the species-selectivity of PPARα transactivation by KCL, and found that KCL activated human and rat PPARα with EC_{50} values of 0.060 μM and 5.2 μM, respectively (the respective values for fenofibrate

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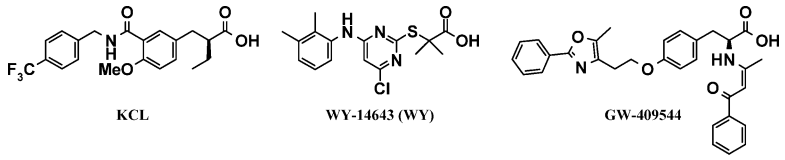

Fig. 1. Chemical Structures of KCL, WY, and GW-409544

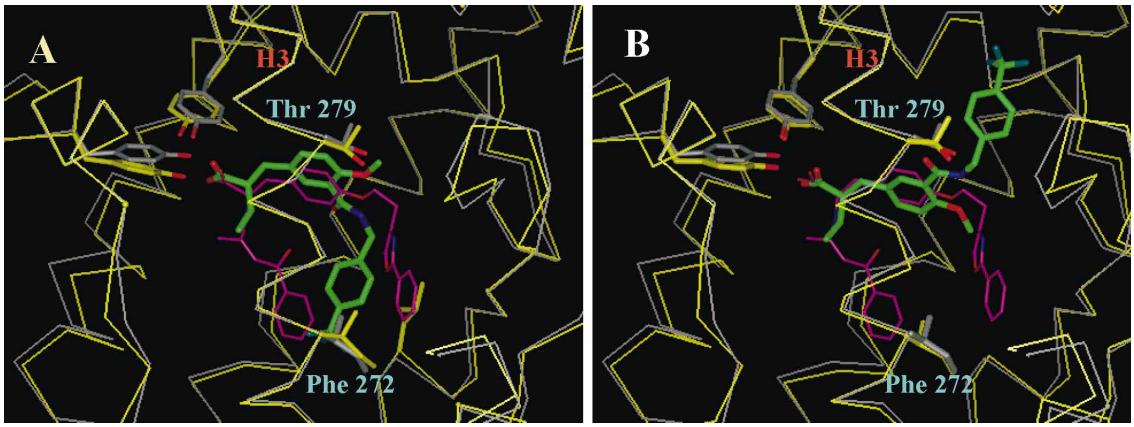

Fig. 2. The Tail-Down (A) and Tail-Up (B) Conformations of KCL Complexed with Human PPARα LBD
Ligands are shown in green (KCL), and magenta (GW-409544).

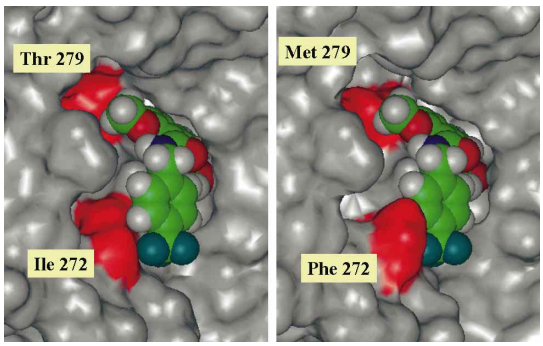

Fig. 3. The Proposed Binding Mode of KCL to Human (Left) and Rat (Right) PPARα

were 41 and 49 μM).[10] Thus, the transactivation activity of KCL for PPARα was approximately 100-fold less potent in rats than that in humans. KCL is, therefore, a highly potent, highly human PPARα-selective agonist.

We also utilized the results of transactivation assay using species-chimeric PPARα LBDs and point mutant PPARα LBDs to identify the critical amino acid residue(s) that is responsible for the species differences. We found that a single amino acid residue is responsible for the species-selectivity, *i.e.*, the human selectivity of KCL was primarily mediated by the specific contact of the ligand with amino acid residue 272, isoleucine (Ile272), which is located on the helix 3 region of the human PPARα LBD. In contrast, the rat selectivity of WY was deduced to be due to the specific contact of the ligand with amino acid residue 279, threonine, of the rat PPARα LBD (Thr279).[10]

In this study, the structures of KCL and WY complexed with both human and rat PPARα LBD were modeled and their interactions were analyzed to throw light on the species selectivity of these compounds.

The structure of human PPARα LBD, complexed with an agonist GW409544 (PDB 1K7L) was used as a template. In order to construct the possible binding modes of KCL or WY, the systematic search method was employed.[17] The common part of GW409544 and KCL, *i.e.*, the phenylpropanoic acid head group, was used as an anchor. This anchor part was placed in the ligand-binding pocket using the X-ray crystal structure of human PPARα LBD as the reference. The stable conformations of KCL in the human PPARα ligand binding pocket of the receptor were searched by a systematic rotation of torsion angles.[17]

The molecular mechanics energy was calculated for each conformer. Minimizations were performed with Discover 3 using the CVFF force field (shell water 10 Å, cell multipole summation method, 1.0 constant dielectric value, peptide main chain Fix, conjugate gradient, and R.M.S. force less than 0.1 kcal/mol Å). Construction of the rat PPARα LBD was performed by means of amino acid residue substitution of the human PPARα LBD crystal structure using the replace

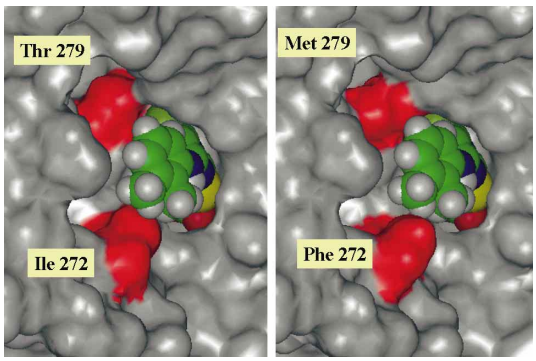

Fig. 4. The Proposed Binding Mode of WY to Human (Left) and Rat (Right) PPARα

command of Biopolymer. Docking of KCL was carried out the same way as described above. The models of the human and rat PPARα LBD complexed with WY were constructed in the same way as described above.

During the search, both tail-down and tail-up conformations were obtained by the docking of KCL to the PPARα LBD as stable conformers (Figs. 2A, B). The tail-up conformation has been seen in the crystal structure of the eicosapentaenoic acid (EPA)-PPARδ complex, in which the hydrophobic tail of EPA was found to adopt two conformations, namely tail-up and tail-down. However, the molecular mechanics energies of the tail-down and the tail-up conformations were about -52.605 (A), and -46.718 (B) kcal/mol respectively. Therefore, the tail-down conformer was preferred. Furthermore, in the case of the tail-down conformer, the hydrophobic tail part of KCL (the 4-trifluoromethyl group) is located close to the key amino acid residue for human selectivity, *i.e.*, Ile272, which is positioned on the downward helix 3 region of the human PPARα LBD. On the other hand, the hydrophobic tail part of KCL in the tail-up conformer is located near T279, the critically important amino acid for rat selectivity, rather than Ile272. Therefore, we concluded that for KCL, the tail-down conformer is more likely than the tail-up conformer. The overall conformation of KCL is somewhat different from that of GW409544. The hydrophobic tail part of GW409544 (the 2-(5-methyl-2-phenyloxazol-4-yl)ethoxy group) is located in the middle of the large ligand binding pocket surrounded by helix 3, helix 5, helix 6, and helix 7 of human PPARα LBD. The hydrophobic tail part of KCl (the *N*-(4-trifluoromethylbenzyl)carbamoyl group) is buried deep in the ligand binding pocket of human PPARα LBD, by helix 3.

In our present model, the distal 4-trifluoromethyl group of KCL can effectively interact hydrophobically with the side chain *sec*-butyl group of Ile272 in the case of human PPARα LBD (Fig. 3; left). However, because of the larger steric size of the side chain benzyl group of Phe272 in rat PPARα LBD, compared with Ile, KCL could not align in the proper position in the rat PPARα LBD due to steric crowding with Phe272 (Fig. 3; right). As a result, KCL might not show potent activity towards rat PPARα LBD.

In the case of WY, a weak but about 10-fold rat-selective PPARα agonist, there might be little effective contact with human PPARα LBD, due to the shortness and/or the lack of steric bulkiness of the hydrophobic tail part of WY (Fig. 4 left). This might be the reason why WY exhibited only weak PPARα agonistic activity ($EC_{50}>100\,\mu$M). Since the steric size of the side chain 2-methylthioethyl group of Met279 is larger than that of the side chain 1-hydroxyethyl group of Thr279, the rat PPARα WY complex might more stable than the human PPARα WY complex, and this could account for the preferential activity towards rat PPARα even though the transactivation activity of WY with rat PPARα is still weak.

In conclusion, we have developed detailed three-dimensional structural models of human and rat PPARα complexed with KCL and WY, based on homology modeling. These models can account for the data obtained from species-chimeric and site-directed mutagenesis studies. Our model clearly indicates that the human PPARα selectivity of KCL is mainly due to the specific interaction between the hydrophobic tail part of KCL (the 4-trifluoromethyl group) and 272Ile located on the helix 3 region of human PPARα.

A better understanding of the ligand-receptor contact points that are important for PPARα ligand binding specificity should help in the development of species-specific high-affinity PPARα ligands as potential therapeutic agents for the treatment of altered metabolic homeostasis.

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