Drug Metab. Pharmacokinet. 28 (4): 345–355 (2013).

Regular Article

Three-dimensional Quantitative Structure–Activity Relationship Analysis of Inhibitors of Human and Rat Cytochrome P4503A Enzymes

Koichi HANDA[1,2,*], Izumi NAKAGOME[1], Noriyuki YAMAOTSU[1], Hiroaki GOUDA[1] and Shuichi HIRONO[1]

[1]*School of Pharmacy, Kitasato University, Tokyo, Japan*
[2]*Research Laboratories, Toyama Chemical Co., Ltd., Toyama, Japan*

Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: Cytochrome P450 3A4 (CYP3A4) is a member of the CYP family and is an important enzyme in drug metabolism. A compound that inhibits CYP3A4 activity could also affect the pharmacokinetics of other substrates, resulting in drug–drug interactions (DDIs) that could cause side effects. Pharmacokinetic data from drug-development studies in rats often determine the dosage used in human clinical trials. It is therefore useful to understand differences in metabolism in different species at an early stage in drug development. Human and rat CYP3A enzymes show different inhibition profiles with different drugs, although the mechanisms involved are not yet clear. Here we built three-dimensional quantitative structure–activity relationship (3D-QSAR) models using structure-based comparative molecular field analysis (CoMFA), to predict the direct inhibitory activity of ligands for human CYP3A4 and rat CYP3A1, based on computer-ligand docking. The alignment of the ligand docking poses suggested that key amino acid–ligand interactions (*e.g.*, Thr309 in CYP3A4 and Pro310 in CYP3A1) characterized the different potencies with which the ligands inhibited CYP3A4 and CYP3A1. The 3D-QSAR models for human and rat CYP3A family inhibitors predicted the potency of inhibitors and could be useful for assessing DDIs at an early stage in drug discovery.

Keywords: 3D-QSAR; CYP3A1; CYP3A4; drug inhibition; drug–drug interaction; structure-based CoMFA

Introduction

Cytochrome P450 (CYP) enzymes play important roles in metabolism in many species. CYPs are involved in metabolizing not only endogenous molecules but also exogenous bioactive molecules including drugs.[1–3] Among CYP family members, CYP3A4 is an important enzyme, as it is expressed at high levels and has a broad substrate specificity.[4–7] About 40% of marketed drugs are known to be metabolized by CYP3A4.[8] A compound that inhibits the metabolic activity of CYP3A4 could therefore affect the pharmacokinetics of other substrates, resulting in drug–drug interactions (DDIs), potentially leading to severe side effects.[9–13] If a drug candidate shows possible DDIs, further studies are required by regulatory authorities such as the US Food and Drug Administration (FDA), to evaluate the value of proceeding with clinical trials in humans.[14] It is therefore essential to consider the interactions of drug candidates with CYP3A4 early in the drug-discovery process; the inhibition of CYP in liver microsomes is often used to measure this.[15] However, the cost and resources required for these experiments limit the number of compounds that can be tested. The ability to predict CYP inhibition using an *in silico* method could increase the number of compounds that could be assessed, and many such models of human CYP3A4 inhibition have been published.[16–26]

During drug development, animal studies (in rats) are required to validate the proposed mechanism of action and to provide safety data before moving to human clinical trials.[14] The dosage of a drug candidate used in humans is often determined by extrapolation from pharmacokinetic data in animals. If a novel compound inhibits rat CYP3A more effectively than human CYP3A, it might show no effect in clinical trials because of an excessively low plasma concentration. By contrast, if a novel compound inhibits human CYP3A more effectively than rat CYP3A, it might have an excessive plasma concentration in humans and cause severe toxicity. It is therefore important to investigate differences in a compound's metabolism in humans and rats at an early stage in drug development. However, human and rat CYP3A enzymes show different inhibition profiles with various drugs, and the mechanisms involved are not yet clear.[27,28] It is important to have methods for predicting differences in the potency with which

Received November 20, 2012; Accepted January 14, 2013
J-STAGE Advance Published Date: January 29, 2013, doi:10.2133/dmpk.DMPK-12-RG-133
*To whom correspondence should be addressed: Koichi HANDA, *Drug Metabolism & Pharmacokinetics Department, Research Laboratories, Toyama Chemical Co., Ltd., 4-1, Shimookui, 2-chome, Toyama 930-8508, Japan. Tel. +81-76-431-8316, Fax. +81-76-431-8208, E-mail: KOICHI_HANDA@toyama-chemical.co.jp*

compounds inhibit CYPs in different species; however, at present, there is only one such report, concerning differences between mouse CYP2A5 and human CYP2A6.[29] We therefore studied the CYP3A family of enzymes and their inhibition in different species, focusing on humans and rats, the latter of which are often used in *in vivo* experiments to evaluate drug efficacy and safety.

In the CYP3A family, the rat enzymes with the greatest protein homology to human CYP3A4 are CYP3A1 (73% identical) and CYP3A2 (72% identical), with CYP3A1 and CYP3A2 being 88% identical to each other (according to UniProt; http://www.uniprot.org/). The distribution of CYP3A1 and CYP3A2 was determined immunocytochemically, and both were found to be expressed in the centrilobular region of the livers of normal rats, with some CYP3A1 immunoreactivity also detectable in many, but not all, hepatocytes throughout the lobule.[30]

Although an X-ray structure for rat CYP3A was not available, several CYP3A4 X-ray structures had been resolved by 2010. The Protein Data Base (PDB) codes for these structures are 1TQN, 1W0E, 1W0F, 1W0G, 2J0D, 2V0M, and 3NXU.[31–34] The structures 1TQN and 1W0E relate to CYP3A4 apoprotein.[31,32] The structure 1W0G relates to a CYP3A4-metyrapone co-crystal structure, and does not differ from the apoprotein structure, as the co-crystallized compound metyrapone (MW: 226) is relatively small.[32] The structure 1W0F relates to a CYP3A4-progesterone co-crystal structure, and also does not differ from the apoprotein, as the progesterone binds only to the protein surface in the co-crystal. Whether this site is a true binding site or an artifact of crystallization has not been determined, but the site is not above the heme iron.[32] The structure 2J0D relates to a CYP3A4-erythromycin co-crystal structure. However, the proximity of the erythromycin binding site to the heme iron, rather than the known catalytic site in the enzyme, suggests the 2J0D structure is not an active conformation.[33] The structure 2V0M relates to a CYP3A4-ketoconazole co-crystal, which undergoes some dramatic conformational changes, with the position of secondary-structure elements in the F–G region and the C-terminal loop changing relative to their positions in ligand-free CYP3A4. Structural superposition of the ketoconazole complex and the ligand-free protein yields a root-mean-square deviation (RMSD) of 1.6 Å for all C^{α} atoms.[33] The structure 3NXU relates to a CYP3A4-ritonavir co-crystal structure, which also undergoes some dramatic conformational changes in the F–G region and the C-terminal loop.[34] The ligand-binding space observed in this structure suggested two important groups of amino-acid residues: one relating to hydrophilic amino acids and a second Phe cluster. The hydrophilic residues Tyr53, Asp61, Asp76, Arg106, Arg372, and Glu374, referred to here as the "hydrophilic-rich area," were considered important for hydrogen-bond formation, and were positioned around one side of the ligand-binding space.[31] Members of the second group of residues, Phe108, Phe213, Phe215, Phe219, Phe220, Phe241, and Phe304, referred to here as the "Phe cluster," were considered important for hydrophobic interactions with ligands, and were positioned around the roof of the ligand-binding space.[32]

Here we built three-dimensional quantitative structure–activity relationship (3D-QSAR) models using structure-based comparative molecular field analysis (CoMFA) for predicting the direct inhibitory activity of drugs for human CYP3A4 and rat CYP3A1, based on computer-ligand docking. The predictive structure-based CoMFA model can predict not only the inhibitory potency but also the binding pose of ligands.[35] However, to date there have been few successes for structure-based CoMFA modeling of the CYP family.[36] Most published CoMFA models for CYP family members have used a ligand-based approach, because the great structural flexibility of CYP proteins makes docking studies difficult.[29,37–41] To overcome this problem, we used several CYP3A4 X-ray structures as the docking proteins, so called "multiple receptor docking."[42] In addition, we used knowledge-based, potentials of mean force (PMF) scoring functions to calculate the protein–ligand interaction free energy.[43] We used CYP3A1 as a representative of the rat CYP3A family for CoMFA, because of its wide expression in the rat liver.[30] The CYP3A1 protein structure was built by homology modeling based on human CYP3A4. Finally, we investigated differences in the inhibition profiles of several compounds, for which we had experimental inhibition data, not only for human CYP3A4 but also for rat CYP3A1, by observing previously determined ligand-docking poses.

Materials and Methods

Ligand data: The structures of the inhibitors used for the human CoMFA model are shown in **Figure 1**. These compounds were selected from a published study, and they have a variety of structures. Each inverse natural logarithm of Ki (pKi) value was also obtained from this literature, and the human liver microsome was used to measure the experimental Ki values.[9] We excluded some compounds (macrolide antibiotics and itaraconazole) because they were too large to dock with the prepared CYP3A4 proteins. Seventeen compounds were separated into two groups: one was used as the CoMFA training data set (**Fig. 1a**), and the other was used as the CoMFA test data set (**Fig. 1b**). The three compounds randomly selected for the CoMFA test data set were fluoxetine (a low potency inhibitor), nifedipine (a mid-potency inhibitor), and saquinavir (a high potency inhibitor).

Figure 1 also shows the inhibitors used for the rat CoMFA model, which have a variety of structures, as selected from several reports. Each pKi or inverse natural logarithm of IC_{50} (pIC_{50}) value was also obtained from this literature, and the rat liver microsome was used to measure the experimental Ki or IC_{50} values.[44–49] The compounds used as the rat CoMFA training data set are shown in **Figure 1c**, and those used as the rat CoMFA test data set are shown in **Figure 1d**. We used pKi data for the training data set to build the rat CYP3A1 CoMFA model; however, because of the paucity of pKi data for rat CYP3A1 inhibition, we used pIC_{50} values for the CoMFA test data set to validate the rat CYP3A1 CoMFA model.

Ligand preparation: The two-dimensional (2D) structures of ligands were converted into 3D structures using the LigPrep program from Schrödinger Suite 2010 (Schrödinger K.K., Tokyo, Japan). The final step of a LigPrep preparation was an energy minimization of the 3D conformers using OPLS_2005. Ionizations of the compounds were determined using ADMET Predictor (Simulations Plus Inc., Lancaster, CA). For conformational searches of compounds, we used the ConfGen program from Schrödinger Suite 2010 (Schrödinger K.K.).

CYP3A4 structure preparation: There were seven crystal structures (PDB codes 1TQN, 1W0E, 1W0F, 1W0G, 2J0D, 2V0M, and 3NXU) for human CYP3A4 in the Protein Data Bank (PDB) by 2010.[31–34] Structures that might not be active conformations (PDB codes 1W0F and 2J0D) were excluded, and the remaining five structures were clustered by dendrogram analysis using