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Molecular recognition of CYP26A1 binding pockets and structure–activity relationship studies for design of potent and selective retinoic acid metabolism blocking agents

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ABSTRACT

All-trans-retinoic acid (ATRA), the biologically most active metabolite of vitamin A, plays a major role in the regulation of cellular differentiation and proliferation, and it is also an important pharmacological agent particularly used in the treatment of cancer, skin, neurodegenerative and autoimmune diseases. However, ATRA is very easy to be metabolized into 4-hydroxyl-RA in vivo by CYP26A1, an inducible cytochrome P450 enzyme, eventually into more polar metabolites. Therefore, it is vital to develop specific retinoic acid metabolism blocking agents (RAMBAs) to inhibit the metabolic enzyme CYP26A1 in the treatment of relevant diseases aforementioned.

In this study, CYP26A1 and its interactions with retinoic acid-competitive metabolism blocking agents were investigated by a combined ligand- and structure-based approach. First, since the crystal structure of CYP26A1 protein has not been determined, we constructed the 3D structure of CYP26A1 using homology modeling. In order to achieve a deeper insight into the mode of action of RAMBAs in the active site, the molecular superimposition model and the common feature pharmacophore model were constructed, and molecular docking was performed. The molecular superimposition model is composed of three features: the main chain groups, side chain groups, and azole groups. The common feature pharmacophore model consists of five chemical features: four hydrophobic groups and one hydrogen acceptor (HHHHA). The results of molecular docking show that the characteristic groups of RAMBAs were mapped into three different active pockets, respectively.

A structure–activity relationship (SAR) was obtained by a combination of the molecular superimposition and docking results with the pharmacophore model. This study gives more insight into the interaction model inside the CYP26A1 active site and provides guidance for the design of more potent and possibly more selective RAMBAs.

1. Introduction

All-trans-retinoic acid (ATRA), a natural metabolite of vitamin A, present in the multitude of human tissues, plays a crucial role in the regulation of cellular differentiation, proliferation and gene expression [1–3]. As a key signaling molecule, it has been shown that ATRA deficiency is associated with diseases such as acne, psoriasis, ichthyosis and cancer, especially in oncology against acute promyelocytic leukemia (APL), ATRA can change the prognosis of APL from a fatal leukemia to a highly curable disease [4–7]. Unfortunately, although ATRA is useful in the treatment of cancer and skin-related diseases, its clinical applications have been significantly hampered by the emergence of resistance, and the fact that the ATRA is very easy to be metabolized into 4-hydroxyl-RA by P450 enzymes [8,9].

There are several microsomal cytochrome P450 (CYP) enzymes that are suggested to be involved in retinoic acid metabolism, e.g. CYP1A1, CYP4A11, CYP3A4/5/7 and CYP2C8/9 [10,11]. However, much attention has been paid to CYP26, a new family of cytochrome P450 enzymes, which is specifically responsible for retinoic acid metabolism. The CYP26 family consists of three members: CYP26A1, CYP26B1 and CYP26C1 [12–14]. Among them, CYP26A1 is the most extensively studied member, which metabolizes retinoic acids (RA) mainly into inactive derivatives or polar metabolites such as 4-OH-RA, 4-oxo-RA, 5, 8-epoxy-RA and

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Table 1
Detailed BLAST results.

Entry name	Organism	SEcore	Identity	Length	PDB code
CP120_SYNY3	*Synechocystis* sp.	641	33.0%	444	2VE3
CP51A_HUMAN	*Homo sapiens*	290	23.0%	503	3JUS
EIZFM_STRCO	*Streptomyces coelicolor*	276	24.0%	461	3DBG
CP51A_HUMAN	*Homo sapiens*	257	24.0%	404	3JUS
CP51_MYCTU	*Mycobacterium tuberculosis*	241	25.0%	451	1E9X
CP46A_HUMAN	*Homo sapiens*	235	23.0%	500	2Q9F
CP3A4_HUMAN	*Homo sapiens*	235	24.0%	503	1TQN
CPXB_BACME	*Bacillus megaterium*	237	26.0%	1049	1BU7

18-OH-RA [15,16]. Moreover, it was found that CYP26A1 is overexpressed in cells containing exogenous retinoic acid, which is an important negative feedback loop controlling RA concentrations and limiting biological action within cells, and which also has the potential to reduce the therapeutic efficacy of ATRA in a wide variety of cancer cell lines [17]. Therefore, it is a reasonable strategy to develop specific retinoic acid metabolism blocking agents (RAMBAs) targeting CYP26A1.

To date, several chemical families of RAMBAs targeting CYP26A1 have been described [18–23]. The antimycotic substance such as ketoconazole, itraconazole, miconazole and fluconazole were the first generation compounds [18–20], which were evaluated as the potent blocking agents to inhibit CYP26A1 activity. Unfortunately, there are adverse side effects for those compounds that have been attributed to the lack of CYP26 isoform specificity. Subsequent extensive structure–activity relationship (SAR) studies on imidazole derivatives have led to the discovery of CYP26A1 inhibitors with new scaffolds, such as liarozole, triazole derivatives, 2,2-dimethyl-3-(4-(naphthalen-2-ylamino)phenyl)propyl azole derivatives [21–23]. New RAMBAs with higher potency and better specificity against CYP26A1 have been reported, and some of them have appeared in clinical studies and have shown some encouraging preclinical and clinical results – improving the specificity and activity [24–26]. However, although great efforts have been made in developing CYP26A1 inhibitors, there is still lack of satisfactory potency, selectivity for CYP26A1. Therefore, it is essential to understand the structure–activity relationship of existing RAMBAs in the context of CYP26A1 before new RAMBAs could be discovered.

In this study, we constructed a homology model of CYP26A1 and systematically analyzed its spatial structure and topological features of the binding pockets in the catalytic cleft. Based on those analyses, a molecular superimposition model and a common feature pharmacophore model were constructed, and molecular docking was performed. Finally, the results of molecular superimposition and docking results with the pharmacophore model allowed us to deduce the SAR model of RAMBAs. The study provides a useful strategy for designing novel and possibly specific inhibitors against CYP26A1.

2. Homology modeling of CYP26A1 and analysis of retinoic acid binding site

Since at present time the crystallographic structure of CYP26A1 from human is not available, we had to generate a homology model which can help us explain how the structurally diverse ligands bind to a common receptor site. The primary sequence of human CYP26A1 was retrieved from UniProtKB/Swiss-Prot [27] with Accession Number 043174. This was subject to BLAST-P [28] using PDB database [29] utilizing BLAST Server of NCBI in order to search the homologous templates. Eight hits with comparable *e*-value and bit score were obtained (Table 1), and the PDB codes of the three-dimensional structures of all these isoforms have been determined. An analysis of these structures showed that these enzymes have the sequence alignment homology, and that they present similar types of structure. We chose two sequences with the highest scoring values: CYP120 (PDB code 2VE3) [30a] and CYP51A (PDB code 3JUS) [30b] as templates. Homology modeling of the CYP26A1 three dimensional structures was carried out using the program MODELLER [31]. Because the co-crystallized ligand retinoic acid of CYP120 was located in the active site, which was the first to be reported, the heme group and retinoic acid of CYP120 were inserted and positioned in the same coordinates of CYP26A1. Then the homology model so produced was evaluated using the structure evaluation program (http://nihserver.mbi.ucla.edu) through the analysis of bond lengths, bond angles, dihedral angles and other stereochemical characters (such as mainchain phi/psi conformational angle, side chain torsion angle, etc.) [32]. The resulting model was visualized and further assessed by Ramachandran plot (Fig. 1B). 99.2% residues are in the favorable region and allowed region.

The sequence alignment of CYP26A1 from CYP120 and CYP51A indicates the presence of three sequentially conserved regions. These regions corresponding to 422EFNPDRF428, 440FSFIPFGG448 and 452CVGKEFA458 from CYP26A1 contain highly conserved amino acid residues around the active site (Fig. 1A), suggesting that these metabolic enzymes may have similar binding mode with substrates.

The homology model of CYP26A1 with RA provides a clear and deep insight into the binding mode of CYP26A1 (Fig. 2), and reveals that the active site has three binding pockets which were denoted A, B, C. Pocket A is a region for substrate recognition and binding, which consists of six highly hydrophobic amino acid residues (Trp70, Phe183, Phe260, Val331, Phe335, Val331 and Pro439) and two basic amino acid residues (Arg51, Lys436). Since RA is also a very hydrophobic molecule, the conjugated branched-chain group of RA interacts almost entirely through van der Waals contacts with amino acid residues in Pocket A. The only exception is the ionic interaction formed between the carboxyl group of RA and Lys436. In addition, Arg51 is the gate keeper of the binding pocket. RA cannot pass through these narrow gaps on its way to the active site. Therefore, the helix containing Lys436 must move to adopt a more open conformation for substrate binding. Pocket B is a region for substrate oxidation, which includes an active center consisting of heme group and amino acid residues (Arg336, Cys403 and Arg336). The cyclohexene in RA is positioned in the pocket B and is bound with the heme, which will be oxidized into 4-hydroxy-RA [33]. A relatively small pocket C is located in the top region of pocket B, which also consists of three hydrophobic amino acid residues (Pro74, Val77 and Leu182), and the methyl group of RA occupies the pocket (Fig. 2).

3. Molecular superimposition

Despite the low sequence identity among CYPs from different organisms, the active sites of their three-dimensional structures have conserved sequence and are ubiquitously heme-containing, which is involved in oxidative metabolism that catalyzes the