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Interspecies comparison of putative ligand binding sites of human, rat and mouse P-glycoprotein

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ABSTRACT

Prior to the clinical phases of testing, safety, efficacy and pharmacokinetic profiles of lead compounds are evaluated in animal studies. These tests are primarily performed in rodents, such as mouse and rats. In order to reduce the number of animal experiments, computational models that predict the outcome of these studies and thus aid in prioritization of preclinical candidates are heavily needed. However, although computational models for human off-target interactions with decent quality are available, they cannot easily be transferred to rodents due to lack of respective data. In this study, we assess the transferability of human P-glycoprotein activity data for development of in silico models to predict in vivo effects in rats and mouse using a structure-based approach. P-glycoprotein (P-gp) is an ATP-dependent efflux transporter that transports xenobiotic compounds such as toxins and drugs out of cells and has a broad substrate and inhibitor specificity. Being mostly expressed at barriers, it influences the bioavailability of drugs and thus contributes also to toxicity. Comparison of the binding site interaction profiles of human, rat and mouse P-gp derived from docking studies with a set of common inhibitors suggests that the inhibitors share potentially similar binding modes. These findings encourage the use of in vitro human P-gp data for predicting in vivo effects in rodents and thus contributes to the 3Rs (Replace, Reduce and Refine) of animal experiments.

1. Introduction

The efflux transporter P-glycoprotein (P-gp) is a protein of high interest among other major anti-targets (Cramer et al., 2007). It is expressed in tissues such as intestine, liver, kidney, placenta, testis, and in the capillary endothelial cells of the brain (Seelig, 1998; Thiebaut et al., 1987), and plays an important role in the absorption, distribution and excretion of many drugs. Overexpression of P-gp has been implicated in resistance to multiple chemotherapeutic drugs and is a widely accepted mechanism underlying multidrug resistance (Aller et al., 2009; Fojo et al., 1987; Widmer et al., 2003). Co-administration of a P-gp inhibitor with a drug can lead to altered disposition of the latter, resulting in elevated plasma levels of the drug which could lead to adverse effects (Bussey, 1982; Tsuji, 2002; Verschraagen et al., 1999). Furthermore, the partial blockade of P-gp expressed in the blood–brain barrier or placenta could lead to an increased distribution of a co-administered drug in the corresponding organs. Thus, concomitant administration of substrates and P-gp inhibitors may lead to adverse drug reactions and organ toxicities (Balayssac et al., 2005). In this respect, the United States Food and Drug Administration (US FDA) guidance requires new drug candidates to be routinely screened against P-gp as part of the clinical drug interaction studies ("Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications Guidance for Industry,", 2017; Klepsch et al., 2011). Therefore, computational methods that characterize P-gp interactions and thus guide the prioritization of compounds in the early phase of the drug discovery process are of considerable interest (Schneider, 2010).

In early stages of drug development, pharmacokinetic and toxicity profiles of a candidate drug are evaluated in animal models (typically rats or mouse) prior to the clinical phases of testing in humans. A substantial amount of experimental data against human P-gp is already available and has been utilized for the development of in silico models (see e.g. livertox.univie.ac.at). However, besides developing in silico models for the prediction of ligands for human P-gp, it would be beneficial to also establish models for rat and mouse P-gp in order to predict the outcomes of preclinical animal studies. Unfortunately, limited availability of experimental data for rat and mouse P-gp restricts the development of such models. In this context the question arises, whether predicted interaction profiles of ligands with human P-gp could be transferred to rodent P-gp. This would require a comprehensive comparison of the putative binding sites of the P-gp structures across species. Literature sheds little light on this, suggesting the need

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for exploration of species-related differences in P-gp mediated drug transport activity (Martignoni et al., 2006; Schwab et al., 2003; Suzuyama et al., 2007).

Inhibition of P-gp activity as a result of drug interactions has been reported in both animals and humans (Bussey, 1982; Choo et al., 2000; Pedersen, 1985), but only a few studies discussed species-related differences in the inhibitory effects on the P-gp function (Chu et al., 2013; Suzuyama et al., 2007; Zolnerciks et al., 2011). A few studies proposed moderate species differences, human vs. rat (Molden et al., 2000), human vs. mouse (Adachi et al., 2001; Lin and Yamazaki, 2003) and also among the three species (human vs. rat vs. mouse) (Katoh et al., 2006), while a few other studies reported no significant differences between human, rat and mouse P-gp (Chu et al., 2013; Feng et al., 2008; Hsiao and Unadkat, 2012). Nicklisch et al. (2016) compared the binding site residues of mouse and *Thunnus albacares* (Yellowfin tuna) P-gp. Despite the low similarity, of the 15 residues of mouse P-gp that interacted with the inhibitor BDE-100, 13 were conserved in the tuna P-gp sequence, and the remaining two did not play a major role in the binding site. However, it must be noted that only a small number of compounds were tested in these studies. It might thus well be that the inhibitory effects on P-gp-mediated drug transport are subjective to both the chemical structure of substrates/inhibitors and to the species. Moreover, it is not yet clear if the possible species differences in the inhibitory effects of P-gp activity are due to differences in binding site residues of P-gp, which is therefore worth investigating.

To the best of our knowledge, no computational study compared the binding site interaction profiles of P-gp across different species (human, rat and mouse) so far. In this study, we used a structure-based approach to compare their binding sites in order to derive information concerning potential species differences in P-gp-mediated drug transport. Since an X-ray crystal structure is available for mouse P-gp alone, homology modeling was performed to construct the models for human P-gp and for rat P-gp. Subsequently, docking of common inhibitors of rat, mouse and human P-gp was performed. Next, known inhibitors of human P-gp were docked into the models of the three species followed by an analysis of the interactions between the inhibitors and binding site residues. The interaction profiles of the P-gp binding sites of the three species were then compared to evaluate the transferability of in vitro human P-gp data for development of models to predict effects in rat and mouse.

2. Methodology

2.1. Dataset

A substantial amount of human P-gp data is made publicly available through previous literature reports (Broccatelli et al., 2011; Chen et al., 2011; Klepsch et al., 2014). However, due to the limited availability of rat P-gp data in public domain bioactivity databases such as ChEMBL (Gaulton et al., 2012; Willighagen et al., 2013) and BindingDB (Liu et al., 2007), an exhaustive literature search was performed. A total of 18 rat P-gp inhibitors could be identified that are known to also inhibit both human P-gp and mouse P-gp. Due to the inconsistencies in the assay conditions, these compounds unfortunately could not be utilized to compare inhibitory profiles across the species. Suzuyama et al. (2007) studied the species differences (human, monkey, canine, rat and mouse) in the inhibitory effects of the prototype P-gp inhibitors quinidine and verapamil. These two drugs served as the starting point for in silico comparison of binding site interaction profiles across the species. Further, we also extracted the human P-gp data from Broccatelli et al. (2011) in order to perform protein-ligand interaction fingerprint (PLIF) analysis and to identify the common functional group residue interactions among the three species. The dataset was standardized according to the procedure described in Pinto et al., 2012. (Pinto et al., 2012) The final dataset contained a total of 1161 compounds (612 inhibitors and 549 non-inhibitors).

2.2. Homology modeling

Based on sequence identity and resolution, the corrected mouse P-gp structure (mdr1a; PDB ID: 4M1M; UNIPROT ID: P21447) was selected as the most structurally related template protein for constructing the homology models for human P-gp (MDR1; UNIPROT ID: P08183), rat P-gp (MDR1a; UNIPROT ID: Q9JK64 and MDR1b; UNIPROT ID: P43245) and mouse P-gp (mdr1b; UNIPROT ID: P06795). Rat and mouse P-gp proteins are encoded by two paralogous genes namely MDR1a and MDR1b that show a sequence identity of 83% (Chu et al., 2013; Devault and Gros, 1990). Therefore, we constructed in total four homology models to consider the paralogs too. Homology models were constructed using MODELLER 9.13 (Eswar et al., 2007) and the Prime module in Maestro (Schrödinger, Inc. V-10.1.013) (Jacobson et al., 2004, 2002). The energy minimized models were further evaluated using DOPE score (Shen and *Sali*, 2006) and GA341 score (John and Sali, 2003; Melo et al., 2002). Quality of the stereochemical parameters and the normality of the structures were checked using the PROCHECK program, included in the PDBsum analysis (Laskowski et al., 1993). Ramachandran plot (Zhou et al., 2011) and G-factor (Engh and Huber, 1991), and finally the Q-score (Benkert et al., 2008, 2009) values were evaluated to identify the best homology models. The electrostatic potential surface (EPS) of each of the three best models for the three species was also calculated and compared using MOE 2013 (*Molecular Operating Environment (MOE), 2013.08*, 2013).

2.3. Sequence alignment

Sequence alignment was performed using ClustalX (Larkin et al., 2007) and verified by including secondary structure predictions. Subsequently, the alignment was analyzed using Jalview (Supplementary Figs. S1–S4) (Clamp et al., 2004; Waterhouse et al., 2009).

2.4. Binding site identification and molecular docking

In order to avoid any bias, the binding site for all five structures (human MDR1, rat MDR1a, rat MDR1b, mouse mdr1a, and mouse mdr1b) was defined as the complete transmembrane region, taking 20 Å around the coordinate of the center point to allow subsequent flexible docking of a series of P-gp inhibitors. The protein was prepared using the Protein Preparation Wizard of the Schrödinger Suite (2015) (Sastry et al., 2013; *Schrödinger Release 2015-1: Maestro, version 10.1, Schrödinger, LLC, New York, NY*, 2015). Hydrogen atoms were added, and optimal protonation states and ASN/GLN/HIS flips were determined. To assess their correct protonation states, ligands were prepared using the LigPrep module of the Schrödinger Suite, (*Schrödinger Release 2015-1: LigPrep, version 3.3, Schrödinger, LLC, New York, NY, 2015.*, 2015, *Schrödinger Release 2015-1: Maestro, version 10.1, Schrödinger, LLC, New York, NY*, 2015) which produces low-energy 3D structures that can be used for docking. The OPLS_2005 force field was used for minimization of the structures. Different ionization states were generated by adding or removing protons from the ligand at a target pH of 7.0 ± 2.0 using Epik version 3.1., (Greenwood et al., 2010; Shelley et al., 2007) and tautomers were generated for each ligand. To generate stereoisomers, the information on chirality from the input file for each ligand was retained as is for the entire calculation. All docking runs were performed in high-throughput mode with GlideXP (Friesner et al., 2006; Halgren et al., 2004) docking in Maestro. We also used the genetic algorithm-based GOLD suit (version 5.2.0) (Jones et al., 1997; Verdonk et al., 2003) for docking.

2.5. Protein ligand interaction fingerprint (PLIF)

A PLIF summarizes the interactions between a ligand and a protein using a molecular fingerprint scheme. We generated two types of PLIFs that differ in the information encoded. The first PLIF encodes residues

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