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Theoretical targets for TCDD: A bioinformatics approach

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ABSTRACT

Dioxins are a group of highly toxic molecules that exert their toxicity through the activation of the aryl hydrocarbon receptor (AhR). The most important agonist of the AhR, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a highly toxic compound. Although most of the effects related to TCDD exposure have been linked to the activation of AhR, the objective of this work was to use a bioinformatics approach to identify possible new targets for TCDD. The Target Fishing Docking (TarFisDock) Server was used to find target proteins for TCDD. This virtual screening allowed the identification of binding sites with high affinity for TCDD in diverse proteins, such as metallopeptidases 8 and 3, oxidosqualene cyclase, and myeloperoxidase. Some of these proteins are well known for their biochemical role in some pathological effects of dioxin exposure, including endometriosis, diabetes, inflammation and liver damage. These results suggest that TCDD could also be interacting with cellular targets though AhR-independent pathways.

1. Introduction

Polychlorinated aromatic hydrocarbons are ubiquitous environmental pollutants that affect human health. Exposure to these chemicals results in a diversity of systemic and tissue-specific pathological changes. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is the most toxic of this class of compounds, and it is released as a product of waste incineration, herbicide overuse, paper chlorination and polyvinylchloride plastic production, among others (Hutz, 1999). From the environment, TCDD is taken through ingestion or respiration, and its effects on organisms include loss of the body weight, histopathological changes in the liver, lung, thymus, pancreas, adrenal glands, and central nervous system (Pohjanvirta and Tuomisto, 1994), carcinogenicity in humans (Chan et al., 2004; Knerr and Schrenk, 2006), reproductive toxicity (Jin et al., 2008a,b; Arima et al., 2009; Porpora et al., 2009), immunosuppression (Gogal and Holladay, 2008; Smialowicz et al., 2008), neurological dysfunction (Urban et al., 2007; Akahoshi et al., 2009), hepatotoxicity (Boutros et al., 2008; Nukaya et al., 2009), and teratogenicity (Bryant et al., 2001; Thackaberry et al., 2005) in laboratory animals (Akahoshi et al., 2009).

It is well known that toxic effects of TCDD are mediated by a ligand-dependent transcription factor, the aryl hydrocarbon receptor (AhR) which has a high binding affinity for TCDD (Mimura and Fujii-Kuriyama, 2003). The predominant mode of TCDD-induced toxicity is by the apparent translocation of the receptor to the nucleus, where it associates with Ah receptor nuclear translocator (ARNT), creating a ligand–receptor–translocator complex (Swanson and Bradfield, 1993). An alternative pathway has been identified in which the TCDD–AhR complex activates cytosolic protein kinase activities in nuclear-free subcellular homogenates (Enan and Matsumura, 1995). Using AhR-null mutant mice, it has been confirmed that AhR plays a critical role on the toxic effects of TCDD (Fernandez-Salguero et al., 1996; Mimura et al., 1997). However, *in vitro* studies have raised the possibility that TCDD could affect cellular pathways in AhR-independent pathways. For instance, it has been shown that TCDD is able to induce Ca^{2+}/Calmodulin signals that regulate apoptosis in EL-4 cells (Kobayashi et al., 2009), activate the mitogen activated protein kinase (MAPK) pathway in RAW 264.7 murine macrophages (Park et al., 2005), and induce immunotoxic effects in EL-4 cells through a mechanism mediated by insulin-like growth factor-binding protein-6 (IGFBP-6) (Park et al., 2003), among other AhR-independent effects. Although these apparently receptor-independent processes are not clear yet, it is possible that some of the toxicologic and pathologic effects were caused by targets different from AhR.

TCDD, as well as any protein ligand, alters the functions of target proteins by inhibiting or activating their normal functions. Testing individual molecules to find specific protein targets is a time-consuming and expensive process. An approach that could help the discovery of new targets for toxic compounds is virtual screening. Screening for ligand conformations can be performed using a ligand-based or a structure-based approach (Lyne, 2002; Stahura and Bajorath, 2005). Ligand-based approaches are established on the assumption that structurally similar compounds are

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likely to exhibit similar biological activities (Stockwell, 2000). Its design includes pharmacophore searches (Khedkar et al., 2007) using the structure of a ligand or series of ligands that are active against the target to determine ligand–protein interactions. This technique categorizes ligands that bind to the protein of interest and allows predictions to be made about activation or inhibition of the protein (Lewis et al., 2009). Ligand-based screening methods are now being conventionally used in the early stages of a variety of drug discovery projects to mine chemical databases with the aim of identifying new hit compounds or optimizing leads (Agrawal et al., 2007; Kumar et al., 2009; Pérez-Nueno et al., 2009). Target Fishing Dock (TarFisDock) is a web server that docks small molecules with protein structures in the potential drug target database (PDTD), in an effort to discover new drug targets. It works performing reverse molecular docking (Li et al., 2006). This process allows docking a particular compound into known binding pockets in proteins found in the potential drug target database (PDTD). This server has been used to predict binding sites for multiple target proteins, such as vitamin E and 4H-tamoxifen, with predictions that were nearly 50% correct, after experimental verification, indicating the relative reliability of this server tool (Li et al., 2006).

In this paper, we used virtual screening to detect new possible targets for TCDD, and discuss the possible implications in human health.

2. Methods

A three-step approach has been used to find new protein targets for TCDD. This includes TCDD optimization, virtual screening of new targets and validation.

First, the TCDD structure has been optimized using DFT at the B3LYP/6-31G level. Calculations were carried out with Gaussian 03 package program (Frisch et al., 2003). The resultant geometry was translated to Mol2 format with Open Babel (Guha et al., 2006), and the Gasteiger partial charge were calculated.

The optimized structure was submitted to TarFisDock (Li et al., 2006) to detect proteins with tri-dimensional structures having theoretical binding sites to TCDD. The search started using the "targets in all categories" option, and ligand docking was performed on all proteins (currently, 1207 proteins are available) present in the potential drug target database (PDTD). The output consists of the top 2%, 5% or 10% best hits, ranked by an energy score, providing binding conformations and a table with related target information. The 25 proteins with the best binding score for TCDD, were scanned for pocket verification using the algorithm SiteID using the SYBYL8.1 package.

In order to calculate theoretical affinities, those proteins for which a binding pocket had been detected were docked with TCDD using AutoDock Vina 1.0 program. The geometrical orientation of TCDD on the protein generated by TarFisDock and that given by AutoDock Vina 1.0 were compared calculating the root mean squared deviation (RMSD), using the RMSD Calculator, tools from the SoongSil University Bioinformatics eBioLab. Interactions between TCDD and proteins were checked with LigandScout 2.0 (Wolber and Langer, 2005). This software extracts and interprets ligands and their macromolecular environment from a PDB file, previously prepared in SYBYL8.1, showing a 2D image of the interactions.

As the main target for TCDD is the AhR, a mammal (*Mus Musculus*) 3D model of this protein (PM0074603), available at the publicly accessible Protein Model Database (Castrignano et al., 2006), was used to perform docking with TCDD using AutoDock Vina 1.0.

3. Results

The top 10% TarFisDock output identified 106 protein targets with binding affinities to TCDD over −28.41 kcal mol^{-1}. After testing each one with AutoDock Vina program, top 2% of the target list were selected according to the docking score (Table 1), and those with a binding affinity over −7.0 kcal mol^{-1} were chosen for the analyses. RMSDs values calculated for the ligand position in the protein as given by TarFisDock and AutoDock vina revealed that TCDD acquires the same position using these tools.

Based on the docking score, we found that TCDD could interact in an AhR-independent way with different enzymes such as hydrolases, isomerases, oxidoreductases, oxidases and other receptors such as the nuclear orphan receptor Lxr-beta (LXRβ). As can be seen from Table 1, proteins with the greater binding affinity were neutrophil collagenase (MMP8) (Fig. 1), stromelysin-1 MMP3 (Fig. 2), oxidosqualene cyclase (Fig. 3A and B), and myeloperoxidase (Fig. 3C and D). For these proteins, the binding sites for TCDD are embedded in a hydrophobic region, with interactions of both the chlorine atoms and the aromatics rings. Furthermore, with the exception of oxidosqualene cyclase, the aromatic rings of these molecules bind to a histidine residue within the protein.

The AutoDock Vina-generated docking of TCDD with an available theoretical AhR model (Fig. 4), showed that as observed for hypothetical TCDD targets, the binding site is also hydrophobic with prevalence of aromatic and aliphatic residues. However, the calculated affinity (−7.5 kcal mol^{-1}) is not as good as the ones observed for other TCDD targets predicted by TarFisDock.

Table 1
Proteins with theoretical binding sites for TCDD.

PDB ID	Protein name	Gene name	Energy score (kcal mol^{-1})		RMSD (Å)
			TarFisDock	AutoDock Vina	
1I76	Neutrophil collagenase, MMP8	MMP8	−35.90	−10.4	0.741
1QIA	Stromelysin-1, MMP3	MMP3 synonym: STMY1	−33.84	−9.8	0.493
1W6K	Oxidosqualene cyclase (OSC, lanosterol synthase)	LSS synonym: OSC	−33.52	−9.8	0.414
1D2V	Myeloperoxidase	MPO	−34.11	−8.9	0.688
2JFE	Cytosolic beta-glucosidase (hCBG)	GBA3 synonym: CBG, CBGL1	−32.59	−8.7	0.251
1K4W	Nuclear orphan receptor Lxr-beta	NR1H2 synonym: LXRB, NER, UNR	−28.52	−8.4	0.685
1XOS	cAMP-specific 3′,5′-cyclic phosphodiesterase 4B	PDE4B synonym: DPDE4	−31.78	−8.4	0.174
1S2A	Prostaglandin D2 11-ketoreductase	AKR1C3 synonyms: DDH1, HSD17B5, KIAA0119, PGFS	−34.38	−8.1	0.683
1B41	Acetylcholinesterase	ACHE	−30.50	−8.2	0.358
1BVY	Monooxygenase	NA	−30.06	−8.1	0.255
2NZL	Glycolate oxidase	HAO2 synonyms: HAOX2	−34.91	−8.0	0.326
1PY5	TGF-beta receptor type I	TGFBR1	−28.69	−7.9	3.061

NA: Not available.