The Endocrine Disrupting Effect of Perfluorooctanoic Acid (PFOA) on Human Estrogen, Androgen and Thyroid Receptors

Yan Cheng, Huiming Chen, Wenlian Yu, Yuan Cui, Lili Zhou, Haishan Li, Naining Song, Lei Li, Xi Li, Jing Zhang, Ping Ma, Xin Sun, Zheng Wang, Lu Han

Research Center for Import-Export Chemicals Safety of General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China (AQSIQ), Chinese Academy of Inspection and Quarantine, Beijing 100123, China

E-mail: yanzi77pku@yahoo.com.cn

Abstract: The health effects of perfluorooctanoic acid (PFOA) on humans remain controversial because of contradictory experimental and epidemiological studies. In this study, we used three-dimensional quantitative structure-activity relationship (3D-QSAR) method by applying Surflex-dock to study the binding modes between PFOA and human estrogen receptor (hERα), human androgen receptor (hAR) and human thyroid receptor (hTRβ), and compare the action with four kinds of endocrine disrupting chemicals. Molecular docking studies indicated that PFOA had high affinity potency toward hERα, hAR and hTRβ due to low binding free energies, while the highest value obtained toward hTRβ. This meant the PFOA might represent more disrupting effects on thyroid than on estrogen and androgen. Our results would provide an important reference and direction for the interaction mode and mechanism study between PFOA and human endocrine systems.

Keywords: endocrine disruption; 3D-quantitative structure activity relationship (3D-QSAR); PFOA, human receptors

1 Introduction

The industrial production of perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA) and their derivatives stopped in 2000, and the European Union has banned most of their uses from June 27th, 2008. On the Stockholm Convention on Persistent Organic Pollutants held in Geneva, Switzerland from May 4th to 8th 2009, PFOS, its salts and perfluorooctane sulfonyl fluoride were proposed for inclusion in the Stockholm Convention due to their highly toxic and persistent properties.

Although a number of experimental and epidemiological studies focused on possible endocrine disruption, the health effects of perfluoroalkyl-compounds on humans remain controversial. For example, PFOA posed activities in estrogenic signaling in trout[1], affected genes responsible for thyroid hormone biosynthesis and estrogen-responsive genes in rare minnows[2] and disturbed estrogen-responsive genes in rare minnows[3]. Whereas some reports with contrary results are thought-provoking, such as that PFOA was not significantly associated with estradiol or testosterone in the serum measurement studies of workers[4], and no significant positive relationships with thyroid-stimulating hormone have been found in the study of a community with longstanding environmental exposure to PFOA[5]. Therefore, for the disrupting effects of PFOA, there is need for further study to explore the action mode and mechanism of PFOA on human.

In this study, we applied the Surflex-dock program, using three-dimensional quantitative structure-activity relationship (3D-QSAR) method, to investigate the endocrine disrupting effects and the action mode and mechanism of PFOA on human estrogen receptor (hERα), human androgen receptor (hAR) and human thyroid receptor (hTRβ), as well as some known typical environment endocrine disrupting chemicals (EDCs) including bisphenol A, benzo (α) pyrene, phthalates, PCBs, etc.

2 Methods

2.1 Modeling dataset

PFOA and four kinds of potential EDCs were selected, including bisphenol A, diethyl phthalate, benzo(α)pyrene and 2-chlorobiphenyl.

2.2 Preparation of the receptor structures

Three traditional regulation receptor targets for human endocrine disruption effects were used for molecular docking and were acquired from protein data bank (PDB). They are human estrogen receptor (hERα), human androgen receptor (hAR) and human thyroid hormone receptor (hTRβ).

2.3 Molecular modeling

The three-dimensional structure building of small molecular ligands and all modeling were performed using mo-

lecular modeling software package Sybyl7.3 (Tripos Inc., St. Louis, Missouri, USA) running on a Linux workstation. Tripos standard molecular field was utilized.

2.4 Molecular docking

The binding interactions between small molecule ligands and hERα, hAR and hTRβ were analyzed by using Surflex-dock module in SYBYL7.3. Surflex-Dock's scoring function was trained to estimate the dissociation constant (Kd) expressed in -log(Kd) unit.

3 Results and Discussion

3.1 The active site of ligand binding domain (LBD) in receptors

The active sites of LBD in hERα, hAR and hTRβ calculated from SiteID program were shown in Figure 1. hERα formed a hydrophobic cavity with pocket volume of about 315 $Å^3$, the pocket volume of hAR was about 285 $Å^3$, and the pocket volume of hTRβ was about 90 $Å^3$. Therefore, the pocket volume of the three receptors was in the order of hERα>hAR>>hTRβ, which could affect the differentiation of ligands that bind or incorporate into the receptor pockets.

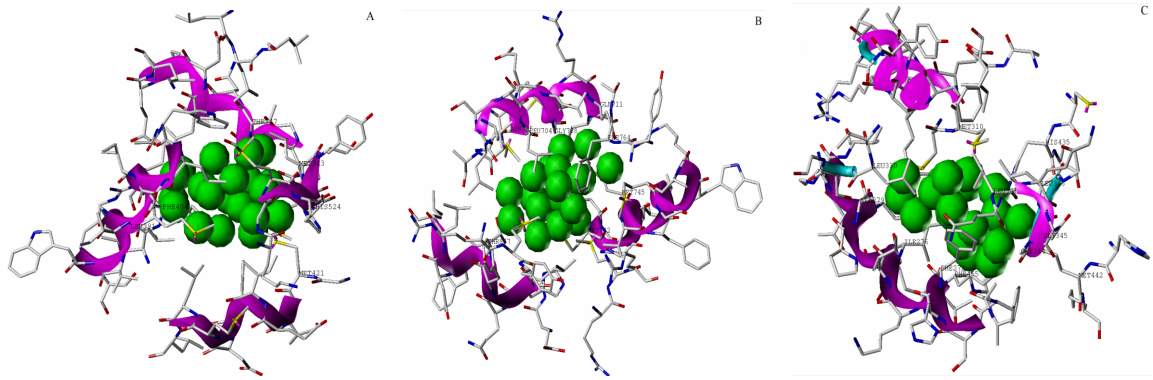

Figure 1. The active solvent pockets of hERα (A), hAR(B) and hTRβ(C) receptors. The helix structure in the receptor is indicated in magenta, sheet structure in yellow, and other structure in cyan. Solvent pockets searched by SiteID program are shown with green sphere cluster.

3.2 Surflex-docking for ligands and receptors

The mechanism of the selective bindings of ligands to hERα, hAR and hTRβ was further explored with Surflex-docking. The scoring for each docked protein-ligand complexes was performed to evaluate the docking results, and the binding free energies (kcal.mol^{-1}) of docked complexes were obtained and shown in Table 1. It can be seen that the binding free energies of receptor-PFOA complexes were equal or less than most of the receptor-EDC complexes, such as 2-Chlorobiphenyl, Diethyl phthalate, etc.

Table 1. The binding free energy (kcal.mol-1) for ligands and receptors.

Ligands	Binding free energy for receptors (kcal.mol-1)		
	hERα	hAR	hTRβ
PFOA	-7.39	-6.98	-10.32
Bisphenol A	-11.65*	-9.46	-11.47*
Benzo(a)pyrene	-5.99	-12.87*	-4.17
Diethyl phthalate	-7.69	-6.38	-10.18
2-Chlorobiphenyl	-8.06	-6.68	-9.83

*The ligand with the lowest binding free energy for each receptor.

3.3 The interaction mechanism speculation for receptors and PFOA/PFOS

According to the docking results for ligands and receptors, it could be speculated that PFOA has high interaction potency toward hERα, hAR and hTRβ and might be environmental endocrine disrupting pollutants for human beings. This was consistent with the positive endocrine disrupting results for PFOS and PFOA in experiment animals[6-15].

Due to the different binding free energies, the affinity potency between PFOA and receptors was in the order of hTRβ>hERα>hAR. It could be presumed that PFOA have higher affinity potency toward hTRβ than toward hERα and hAR. This might mean that PFOA likely interfered the human endocrine system mainly through the thyroid receptor-mediated pathway, and to a less extent through the estrogen/androgen receptor-mediated pathway. In fact, the results obtained in this study were consistent with previously reported findings in animals [2, 11-14].

4 Conclusions

We applied Surflex-docking to study and compare the interaction modes and mechanisms between a set of endocrine disrupting chemicals and human estrogen receptor, androgen receptor and thyroid receptor. Molecular docking results indicated that PFOA had high affinity potency toward hERα, hAR and hTRβ due to the low binding free energies and might be environmental endocrine disrupting pollutants for human beings. Docking studies demonstrated

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that PFOA had greater affinity potency for hTRβ than for hERα and hAR, which meant that PFOA might represent more disrupting effects toward thyroid than toward estrogen and androgen signaling pathways. Our results indicated the possible endocrine disrupting effects and pathways for PFOA to affact humans, and could provide an important reference and direction for the academy study and experiment design for the effect of PFOA on human endocrine systems.

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