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Computational study involving identification of endocrine disrupting potential of herbicides: Its implication in TDS and cancer progression in CRPC patients

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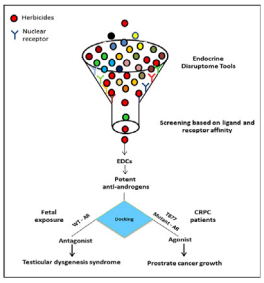
HIGHLIGHTS

- Screened 608 herbicides for evaluating their endocrine disrupting potential.
- 252 herbicides showed affinity with at-least three androgen receptors (AR).
- Majority of the herbicides showed antagonist activity towards AR.
- Fetal exposure to anti-androgenic chemicals may be associated with TDS.

GRAPHICAL ABSTRACT



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ABSTRACT

Several environmental pollutants, including herbicides, act as endocrine disrupting chemicals (EDCs). They can cause cancer, diabetes, obesity, metabolic diseases and developmental problems. Present study was conducted to screen 608 herbicides for evaluating their endocrine disrupting potential. The screening was carried out with the help of endocrine disruptome docking program, http://endocrinedisruptome.ki.si (Kolsek et al., 2013). This program screens the binding affinity of test ligands to 12 major nuclear receptors. As high as 252 compounds were capable of binding to at least three receptors wherein 10 of them showed affinity with at-least six receptors based on this approach. The latter were ranked as potent EDCs. Majority of the screened herbicides were acting as antagonists of human androgen receptor (hAR). A homology modeling approach was used to construct the three dimensional structure of hAR to understand their binding mechanism. Docking results reveal that the most potent antiandrogenic herbicides would bind to hydrophobic cavity of modeled hAR and may lead to testicular dysgenesis syndrome (TDS) on fetal exposure. However, on binding to T877 mutant AR they seem to act as agonists in castration-resistant prostate cancer (CRPC) patients.

1. Introduction

U.S. Environmental Protection Agency (U.S. EPA) defines Endocrine Disrupting Chemicals (EDCs) as: “an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body which are responsible for the maintenance or homeostasis, reproduction, development and or behavior” (Crisp et al., 1998). They interfere with body's hormones and are also called as hormone disruptors. Like hormones' they function at very low doses exerting non-

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traditional dose-responses in a tissue-specific manner (Schug et al., 2011). Since hormones play a significant role in guiding the development of early life forms, exposure to EDCs in the egg or the womb can alter the normal process of development. Although mature animals are also affected leading to adverse health outcomes, the developing ones are the most vulnerable as they are extremely sensitive to distresses by hormone-like acting chemicals (http://www.pan-uk.org/pestnews/Actives/endocrin.htm). Fetal exposure may result in permanent alterations in reproductive and neurological function. Therefore, NIEHS (National Institute of Environmental Health Sciences) articulates that endocrine disruptors may pose the greatest risk during pre- and early postnatal development during which organ and neural systems are forming (http://www.niehs.nih.gov/health/topics/agents/endocrine/index.cfm).

Colborn et al., 1993, published one of the pioneering papers on the concept of 'endocrine disruption' and since then EDCs have received significant amount of attention (Colborn et al., 1993). Approximately 800 chemicals are suspected or presently known to have endocrine disrupting potential (Bergman et al., 2013). Chemicals recognized as endocrine disruptors include both synthetic and natural chemicals such as pharmaceutical agents, pesticides, pesticides metabolites, phytoestrogens, phthalates, industrial products and plastics (Crisp et al., 1998; Dickerson and Gore, 2007; Hodges et al., 2000; Postle et al., 2004; Sharara et al., 1998). Herbicides are consistently detected throughout the year in marine waters and are also expected to accumulate in the microplastics due to stable and lipophilic nature, there they will adhere and concentrate on the hydrophobic surface of plastics (Cole et al., 2011; Mercurio et al., 2015). There is a special concern about endocrine disrupting pesticides as they are resistant to metabolism, and can bioaccumulate via the food chain. They can get stored in the body fats and are transferred to the developing offspring via placenta or the egg. Mammals are at an increased risk because contaminants can be transferred to the new born infant through breast milk (http://www.pan-uk.org/pestnews/Actives/endocrin.htm).

Nuclear receptors (NRs) are a family of transcription factors that regulate gene expression resulting in major physiological changes. EDCs can either mimic natural hormones by binding to and activating various nuclear receptors (agonist action) or they can bind to the receptors and block them (antagonist action) (Mnif et al., 2011). This dysfunction of nuclear receptor signaling can cause hormone-related cancers, diabetes, obesity, metabolic diseases and developmental problems (Delfosse et al., 2014; De Coster and van Larebeke, 2012). Due to the conservation of the ligand binding domain (LBD) in the nuclear receptor family and the presence of large diversity of environmental chemicals the receptors can act as potential targets for the environmental pollutants (Delfosse et al., 2014).

Prediction of endocrine disruption potential of compounds remains a difficult task. Several different methodologies have been developed, but most of them use quantitative structure-activity relationship (QSAR) and/or machine learning models (Devillers et al., 2006). Although these methods have exceptional predictivity for structural analogues, yet are less successful in predicting the activity like agonist and antagonist's nature of endocrine disruption chemicals of significantly different molecules (Don and Riniker, 2014; Cherkasov et al., 2014). Therefore, structure-based methods like molecular docking and molecular dynamics are the methods of choice in this case (Jacobs, 2004).

In this study, we screened a total of 608 herbicides as environmental pollutants using Endocrine Disruptome docking program http://endocrinedisruptome.ki.si to analyze their interaction with various nuclear receptors (Kolšek et al., 2014). This program predicts interaction with 12 nuclear receptors (12 agonist and 4 antagonist conformations): androgen receptor (AR), androgen receptor antagonist (AR an), estrogen receptor α (ER α), estrogen receptor α antagonist (ER α an), estrogen receptor β (ER β), estrogen receptor β antagonist (ER β an), glucocorticoid receptor (GR), glucocorticoid receptor antagonist (GR an), liver X receptor α (LXR α), liver X receptor β (LXR β), peroxisome proliferator-activated receptor α (PPAR α), peroxisome proliferator-activated receptor β (PPAR β), peroxisome proliferator-activated receptor γ (PPAR γ), retinoid X receptor (RXR), thyroid hormone receptor α (TR α) and thyroid hormone receptor β (TR β). The pesticides have been classified into thirty six groups according to similarities in their chemical structure as shown in Table S1.

Androgens predominantly act through AR and are vital to the development of the male reproductive system. Thus, chemicals that act as anti-androgens and interfere with their action can have deleterious effects on the male reproductive health leading to reduced semen quality, infertility, hypospadias, cryptorchidism and testicular germ cell cancer (TGCC). These interrelated disorders are also called testicular dysgenesis syndrome (Fisher, 2004). Therefore, AR was elected for further surveillance by modeling and docking studies. AR has been seen to be mutated in the case of castration-resistant prostate cancer patients (CRPC), resulting in alteration in the activity of compounds binding to it. We therefore, analyzed if the anti-androgenic behavior of herbicides altered on binding to the mutated AR. The binding of herbicides to the wild type (WT) and T877 mutant AR is expected to give an insight into the mechanism of herbicide-receptor interaction and its binding mode.

2. Computational methods

2.1. Ligand preparation and screening

Since the aim of this study was the comprehensive evaluation of endocrine disruption potential of pesticides, we selected a total of 608 herbicides from Agropages.com and alanwood.net databases as ligands. Ligands structure and SMILES string (Simplified Molecular-Input Line Entry-System codes) were obtained from PubChem database for entry as an input into the Endocrine Disruptome tool.

2.2. Evaluation of endocrine disruption potential through nuclear receptor binding

The predictions of endocrine disruption potential of test ligands were carried out by using Endocrine Disruptome tool (Kolšek et al., 2014). It is an open source prediction tool for assessing the endocrine disruption potential of test chemicals through binding with several nuclear receptors. This prediction tool uses 16 structures with best performance; twelve different human nuclear receptors such as (AR, ER α and β, TR α and β, LRX α and β, PPAR α, β and γ, RXR α, GR receptors) and four antagonist forms such as (AR an, ER α an, ER β a and GR a receptors) that regulate metabolism, reproduction behavior, development and the immune system (Diamanti-Kandarakis et al., 2009; Grün and Blumberg, 2006). Molecular docking experiments were performed by Autodock Vina software run on an open source platform called Docking interface for Target Systems (DoTS). It's docking calculations uses AutoDock Vina package for computation of ligand poses docked with the target receptor. For the validation of docked results, the program has set three threshold values. Each threshold corresponds to sensitivities (SEs) of approximately 0.25, 0.50, and 0.75. These threshold values were translated into four color code classes, based on binding affinity of ligands towards the nuclear receptors: red class (SE < 0.25) has the highest binding probability with ligands followed by orange