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Review

Reprint of "*In silico* methods in the discovery of endocrine disrupting chemicals"☆

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ABSTRACT

The prevalence of sex hormone-dependent cancers, reproductive problems, obesity, and cardiovascular complications has risen especially in the Western world. It has been suggested, that the exposure to various endocrine disrupting chemicals (EDCs) contributes to the development and progression of these diseases. EDCs can interfere with various proteins: nuclear steroid hormone receptors, such as estrogen-, androgen-, glucocorticoid- and mineralocorticoid receptors (ER, AR, GR, MR), and enzymes that are involved in steroid hormone synthesis and metabolism, for example hydroxysteroid dehydrogenases (HSDs). Numerous chemicals are known as endocrine disruptors. However, the mechanism of action for most of these EDCs is still unknown. It is exhaustive and time consuming to test *in vitro* all chemicals – potential EDCs – used in industry, agriculture or as food preservatives against their effects on the endocrine system. Computational methods, such as virtual screening, quantitative structure activity relationships and docking, are already well recognized and used in drug development. The same methods could also aid the research on EDCs. So far, the computational methods in the search of EDCs have been retrospective. There are, however, some prospective studies reporting the use of *in silico* methods: five studies reporting the identification of previously unknown 17β-HSD3 inhibitors, MR agonists, and ER antagonists/agonists. This review provides an overview of case studies and *in silico* methods that are used in the search of EDCs.

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1. Introduction

Endocrine disrupting chemicals (EDCs) are considered to be a serious health threat by contributing to major diseases [1]. The identification and safety assessment of potential EDCs is complicated by the observed low-dose effects and nonmonotonic dose responses [2] as well as the often long-term exposure or exposure during a critical window early in development [3]. EDCs, such as some environmental chemicals, food preservatives, dyes, and chemicals used in cosmetics, can interfere with endocrine functions, either by directly activating or inactivating endocrine target receptors or by disrupting the synthesis of hormones or the local control of active to inactive hormones by inhibiting or activating their metabolizing enzymes.

The classical EDC targets are nuclear receptors such as estrogen receptors (ER), androgen receptors (AR), mineralocorticoid receptors (MR), glucocorticoid receptors (GR), progesterone receptors (PR), thyroid receptors (TR) and peroxisome proliferator-activated receptors (PPAR). In addition, hormone metabolizing enzymes, including aromatase [4], 5α-reductase [5], 3β-hydroxysteroid dehydrogenase (3β-HSD) [6], 17β-hydroxysteroid dehydrogenases (17β-HSDs) [6,7] and 11β-hydroxysteroid dehydrogenases (11β-HSDs) [8–10] have been proposed to be affected by EDCs and to impact on nuclear receptor responses by altering the availability of active hormones.

Endocrine disruption affects various body functions, depending on the pathway that is disrupted. It has been proposed that exposure to xenoestrogens and xenoandrogens led to the increased prevalence of breast cancer, prostate cancer and testicular cancer [11,12]. In addition to cancer, infertility and loss of sperm count are likely associated with the exposure to EDCs [13]. Most of the effects of xenoestrogens and xenoandrogens are mediated *via* ERs and ARs. Numerous compounds, including environmental chemicals such as DDT and phthalates, have been classified as ER and/or AR modulators [13,14], acting either as direct agonists or antagonists or altering receptor expression.

In addition, the synthesis of active sex steroid hormones and the control of their intracellular concentrations is highly dependent on the activity of aromatase, 3β-HSD and 17β-HSDs that catalyze different steps in the metabolism of estrogens and androgens (Fig. 1). For this reason, these metabolizing enzymes represent another level where disruption of androgen or estrogen homeostasis can occur.

Xenobiotica may disturb also glucocorticoid and mineralocorticoid actions, contributing to cardiovascular complications, disturbances in energy metabolism, immune responses, as well as impairment of cognitive functions and the regulation of cell proliferation and differentiation [9]. These disturbances may be caused by xenobiotica resulting in impaired HPA-mediated feedback regulation and/or corticosteroid synthesis or disrupting the functions of GR, MR, or 11β-HSDs, which control the intracellular concentrations of active glucocorticoids (Fig. 2). In the distal tubules and cortical collecting ducts of the kidneys, for example, rapid inactivation of cortisol to cortisone by 11β-HSD2 is needed to prevent excessive cortisol-dependent MR activation, which would lead to hypertension and hypokalemia (Fig. 2) [9].

The prevalence of obesity is increasing in the Western world. In addition to the fact that body mass is a result of inappropriate energy intake and physical exercise, it has been suggested that EDCs might play a role in the increasing number of obese and diabetic individuals [15]. Some of these effects are likely to be mediated through PPARs, especially through PPARγ, a nuclear receptor that is responsible for adipogenesis and development of white adipose tissue. It has been shown that diethyl-hexyl-phthalate (DEHP) and its metabolite monoethyl-hexyl-phthalate (MEHP) are PPARγ-modulators and cause obesity in mice [16]. Therefore, PPARs should be also taken into account when exploring the mechanisms of endocrine disruption.

One of the most extensively studied EDC is bisphenol A, a compound that is present in many beverage and food containers, and in thermal paper, which is frequently used in receipts [17]. The exact mechanism of action of this compound is still unclear, although several research groups have shown numerous possible molecular mechanisms. It was shown that bisphenol A results in the activation of ER-α and ER-β [18–20], pregnane-X receptor (PXR) [21,22], PPAR-γ [23,24], thyroid receptor [25,26], and G-protein coupled estrogen receptor [27]. Bisphenol A was further shown to act as AR-antagonist [14]. Probably the most potent activation was observed on estrogen related receptor-γ with a K_D of 5.5 nM. Other investigators reported very weak inhibition of the metabolizing enzymes 11β-HSD1 and 2 [10,28], 3β-HSD and 17β-HSD3 [29], indicating that bisphenol A does not act by directly inhibiting these enzymes. However, concentrations as low as 10 nM of bisphenol A were found to increase PPAR-γ expression and 11β-HSD1 expression and activity in adipocytes [30] and to decrease 17β-HSD3 expression in Leydig cells [31]. This example shows that multiple pathways can be involved for a single compound to disrupt the endocrine system and suggests synergistic actions of bisphenol A.

Since June 2007, the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) legislation has been implicated in the European Union (http://ec.europa.eu/enterprise/sectors/chemicals/reach/index_en.htm). The main goal of REACH is to protect human health and environment from hazardous chemicals. The REACH legislation drives industry to the responsible assessment and risk management of chemicals and to provide safety information to the users. In practice, this means proof of safety of chemicals sold or used by the industry. Testing the actions of all used chemicals – possible EDCs – against all the potential targets related to endocrine disruption is an important but also expensive and difficult, if not impossible, task, also due to the limited availability of suitable bioassays. Especially biological testing is time- and cost-intensive; therefore, more rational approaches to help to identify potentially harmful chemicals in a fast way are urgently needed. In this context, methods established in drug discovery and development, where the task is to identify bioactive compounds from millions of available substances, can be applied to EDC research. Computational methods are already a well established tool in drug discovery [32] and can also support EDC studies, either in the identification of new EDCs or pointing into the right direction when finding the mechanism of action for already known EDCs.

Virtual screening is a technique, where large databases of compounds are evaluated for their possible activity towards a specific biological target. In a virtual screening, a database of compounds is compared against a computational model, which describes properties of active compounds. The result of virtual screening is a hit list, which contains the compounds from the database that are proposed to be active, with a corresponding ranking.

Virtual screening is an excellent method in the enrichment of active compounds from a diverse compound database. As a case study, Doman et al. [33] compared the yield of the usual high throughput screening (HTS) methods and docking-based virtual screening in finding inhibitors for protein tyrosine phosphatase 1B (PTP1B): The HTS showed a true positive hit rate of 0.021%, when testing 400,000 compounds, whereas docking-based virtual screening suggested 365 compounds for testing, of which 34.8% were active *in vitro*. In addition to this, virtual screening aids also in the enrichment of active compounds within a set of similar compounds from the same chemical scaffold: Schuster and coworkers [34] investigated morphinans inhibiting acetylcholinesterase. In this study, pharmacophore-based virtual screening of a morphinan database suggested 14 hits for biological testing. Eight showed activity *in vitro*. For comparison, 50 randomly selected