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THEORETICAL APPROACH TO ENDOCRINE DISRUPTORS

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1. ABSTRACT

Endocrine disruptors are now of scientific and public concern, because there is increasing evidence of their adverse effects on the health of an intact organism or its progeny and on changes in endocrine function. Although numerous substances have been identified as such chemicals, a huge number of chemicals remain to be tested for their endocrine disrupting capabilities. Because of the time and costs required for animal tests, some theoretical or computer-based method for screening this large number of chemicals is needed to reduce the numbers requiring animal testing. Improved quantitative structure activity relationship (QSAR) models were used for screening in combination with other approaches. New receptor-ligand docking simulations were being tested. There was good correlation between experimental and theoretical binding affinities. A database complex system being developed, which enables one to trace cellular signals triggered by the interaction of receptors with xenobiotic chemicals. Perspectives of computer-based screening methods are discussed.

2. INTRODUCTION

Rapidly increasing scientific evidence suggests that many of synthetic chemicals can interfere with normal hormone-like regulated biological processes to adversely affect development and/or reproductive function in wildlife and humans (1-14). These chemicals are called "endocrine disruptors (EDs)", because of their ability to interfere with endocrine systems. The World Health Organization (WHO) has defined EDs as exogenous chemical substances that alter the function(s) of the endocrine system and consequently causes adverse effects in an intact organism, or its progeny, or (sub)populations (http://www.who.int/pcs/emerg_site/edc_descr.html).

A large number of scientific projects related to endocrine disruptors in humans, laboratory testing, and wildlife species have been proposed (15). These studies are focused on (i) what is the mechanism by which the EDs modulate normal endocrine systems, and (ii) among the enormous number of existing chemicals, how we can screen for such EDs effectively.

As the number of suspicious EDs is estimated to be as many as 87000, it is essential to develop some theoretical or computer-based approach to pre-screen this large number of chemicals and reduce its number so that conventional wet lab testing or the so-called high through put screening (HTPS) can be applicable (http://www.epa.gov/oscpmont/oscpendo/). The QSAR approaches were tried for this purpose.

The development of endocrine-related databases system for advanced research is another approach. These databases include a potential endocrine database, a receptor database, a cell signaling database, etc. This paper does not intend to provide a complete research review on endocrine disruptors; only a short review of theoretical or computational approaches are described.

3. THEORETICAL APPROACH

3.1. EDSTAC report

In 1996, the United States Environmental Protection Agency (EPA) formed the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) to provide advice on how to design a screening and testing program for endocrine disrupting chemicals. The EPA developed a screening program by 1998 and implemented it by 1999. The EDSTAC report discussed the committee's recommendations for many aspects of the endocrine disruptor screening program, including details on priority setting and recommendations for potentially relevant exposure and effects data sources (http://www.epa.gov /scipoly/oscpendo/history/finalrpt.htm). As QSAR methods have proven successful in molecular design and drug discovery (16), the EDSTAC considered QSAR as an important part of its priority setting process.

Shi *et al*. (17) developed an integrated system, which contains four sequential phases to predict the ability of chemicals to bind to the estrogen receptor (ER), for application to large data sets.

I. Lipinski "rule of 5"-type (18): simple rejection filters are provided to eliminate the chemicals that are most unlikely to bind the ER. Poor absorption or permeation is more likely when:

I-1. There are more than 5 H-bond donors (expressed as the sum of OHs and NHs)

I-2. The Molecular Weight is over 500;

I-3. The Log P is over 5 (MLogP is over 4.15);

I-4. There are more than 10 H-bond acceptors (expressed as the sum of Ns and Os)

Compound classes that are substrates for biological transporters are exceptions to the rule.

II. Three key 2D structural alerts, seven pharmacophore features, and the predictions of two classification models make use of K-nearest neighbor (KNN) and classification and regression tree (CART) methods.

III. QSAR models are used quantitatively to predict the activities of chemicals categorically predicted to be active in phase II.

IV. The phase II and III predictions are combined with other available information, such as human exposure level, environmental fate, and production volume, to determine a chemical's priority for testing.

3.2. Comparative molecular field analysis (CoMFA) Model

A number of QSAR models have been reported for ligand binding to the ER (19-25). Although these models yield good statistical results, they have limited applicability in predicting the ER-ligand binding affinity of chemicals that cover a wide range of structural diversity. Shi *et al*. (26) tried two types of QSAR models, CoMFA and hologram QSAR (HQSAR), for inclusion in phase III to quantitatively predict chemical binding to the ER. They used the relative binding affinities (RBAs) to the ER for 130 chemicals covering a wide range of structural diversity and concluded that CoMFA yielded the best QSAR models in terms of self-consistency and predictive ability of the test chemicals.

3.3. Modeling of signaling pathways

There are already a large number of chemicals that should be tested for their endocrine modulating capabilities. Some theoretical methods are needed for the first stages of this process, because of the time and costs required for wet lab testing. However, the conventional quantitative structure activity relationship (QSAR) approaches are of limited relevance to this problem, as these methods do not take into account the detailed mechanisms of biological molecular interactions. Kaminuma *et al.* (27) presented a prototype of an integrated database and a knowledge-based complex of chemical substances and biomolecules that can describe the internal signaling evoked by endocrine disruptors from gate-points to the endpoints. The main components of this database are a potential endocrine disruptor database, a receptor database, a cell signaling networks database, a transfactor database, and a binding affinity database based on modes of actions.

3.4. Endocrine Disruptor Structure Database (EDSD)

Kaminuma *et al.* (27) did a literature survey of potential endocrine disruptors among different categories of chemicals that included synthetic estrogens for medicine, phytoestrogens, pesticides, industrial chemicals, environmental pollutants, and metals and their compounds. Then, these chemicals were listed categorically (http://www.nihs.go.jp/hse/endocrine-e/paradigm/paradigm.html). From these preliminary lists of endocrine disruptors, the Endocrine Disruptor Structure Database (EDSD) was developed. EDSD includes such entries as chemical name, CAS registry numbers, synonyms, physiological properties, and two- and three-dimensional structural data that are important for predicting chemical properties and QSAR. The three-dimensional structures of EDs, were calculated by using Molecular Mechanics 2 (MM2)(28).

3.5. Binding affinity database (BADB)

For each of the potential endocrine disrupting chemicals the mode of action was surveyed and the targets were identified. Although EDs stimulate target organisms by various modes of action, the three basic modes of action are (a) interaction with extracellular binding proteins, (b) interaction with enzyme systems that metabolize hormones, and (c) interaction with hormone receptors (29). A

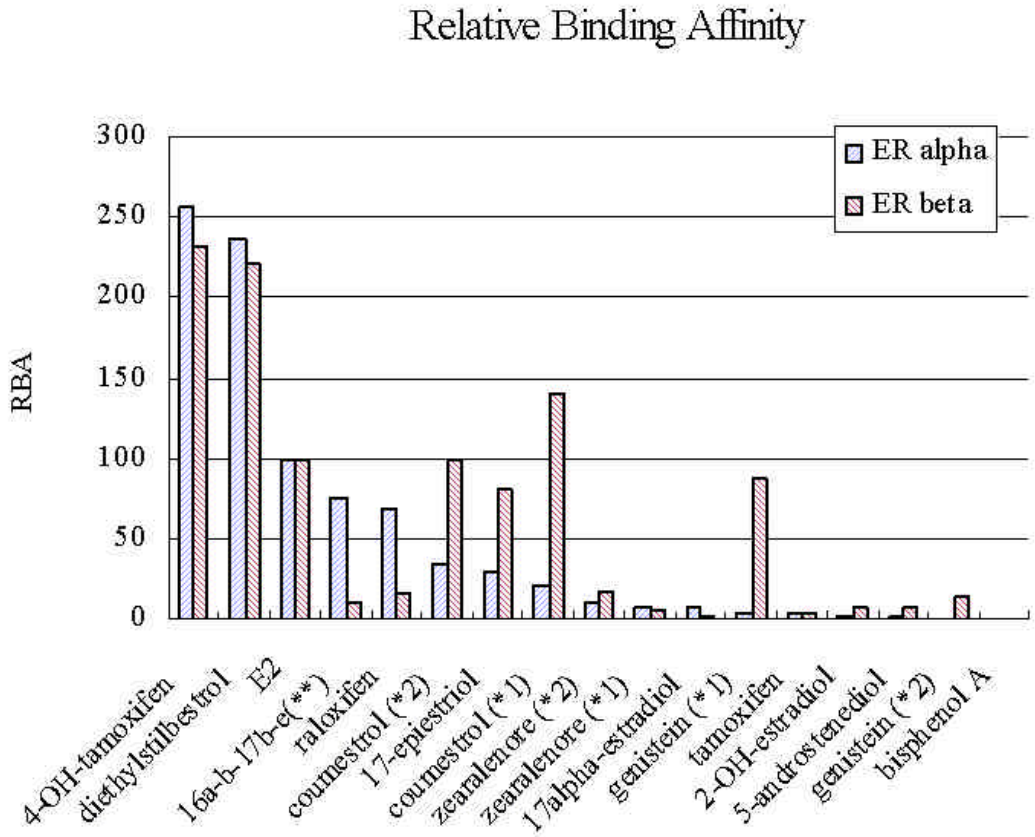

Figure 1. RBA between Estrogen Receptor alpha/beta and ligands.

molecular interaction database (BADB, http://moldb.nihs.go.jp/eddb/afdb/) that stores binding data of xenobiotic chemicals (ligands) and their target biomolecules has been developed (27). BADB stores experimental data for interactions of exogenous chemicals and biomolecules, which included 376 enzyme induction experiments and 742 competition binding experiments. These values were recalculated according to the definition of the relative binding affinity (RBA) proposed by Bolger *et al.* (30). Figure 1 shows the RBA between the estrogen receptor (ER) and ligands in BADB.

3.6. Receptor Database (RDB)

The Receptor Database (RDB, http://impact.nihs.go.jp/RDB.html) has been developed, which retrieves various receptor related data and provides hierarchical and graphical representation (31-32). RDB provides the receptor protein structure (amino acid sequences, secondary structure, and three-dimensional structure), DNA/ligand binding sites and binding affinity information, SNPs and cell signaling information, etc. The RDB aims to support structural biologists, not only for the examination of receptor-ligand binding but also for elucidation of the post- binding signal transduction pathway. At present RDB contains 1772 receptors, including endocrine-related receptors. The potential endocrine disrupting chemicals were stored in RDB and are included in the table "Steroid hormone/Aryl hydrocarbon receptors and possible endocrine disruptors" (see Figure 2).

3.7. Ligand-Receptor docking simulation

Structures of ligands are mostly obtained by theoretical calculation, for instance MM2; whereas target receptor structures of EDs, such as the nuclear receptor of estrogens, androgens, and thyroid hormone, were obtained from X-ray crystallography analyses (Protein Databank, http://www.rcsb.org/pdb/). When the steric structure of a target protein is available, one of the most effective methods to predict the binding strengths of ligands is docking simulation.

Itai *et al.* (33-36) developed a program called "ADAM" originally as a tool for docking simulation of a protein and ligand. The program has been used for rational drug design and investigations of biochemical reaction mechanism. Then they applied it for endocrine disruptors (37). In their method, empirical parameters were used for the computer calculation. Nakano *et al*. applied an approximation method of ab initio fragment molecular orbital calculation, which was developed by Kitaura *et al.* (38-39) for estimating the binding energies between the estrogen receptor and ligands. They got good correlation between the calculated binding energies and the RBA values. The advantage of this method is that empirical parameters need not be taken into account.

3.8. Cell signaling networks database (CSNDB)

Takai-Igarashi *et al*. (40-41) have developed the Cell Signaling Networks Database (CSNDB,

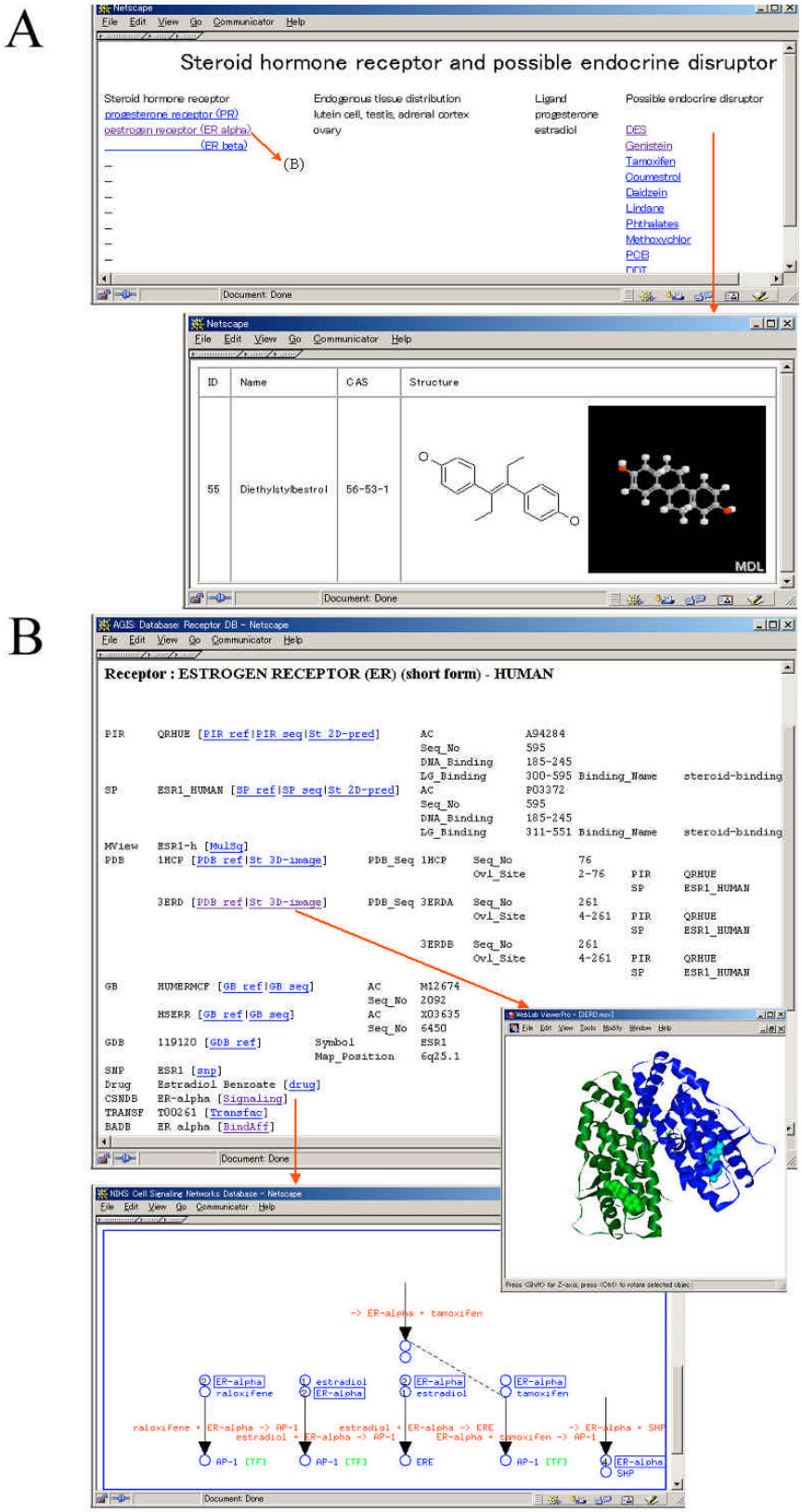

Figure 2. (A) Steroid hormone receptor and possible endocrine disruptors. Example of ER alpha and Diethyl-stylbestrol (DES). (B) ER alpha. Three dimensional image and cell signaling.

http://geo.nihs.go.jp/csndb.html), for modeling and analyzing the signaling pathways. In CSNDB, only binary relationships between two arbitrary molecules are recorded. Then pathways connecting such molecules are retrieved by automatic graph drawing. A great advantage of CSNDB is the provision for data exchange with the transcription factor database TRANSFAC (42). As many cellular signals induce gene expression that evokes a second-phase cellular response, the integration of pathways for cellular signaling and transcription regulation will eventually cover all of the regulatory pathways occurring in cells. At present the CSNDB contains 1968 biomolecules and 1060 molecular interactions.

3.9. Statistical Approach

Haseman *et al*. examined data supporting the presence or absence of low-dose effects of endocrine disruptors in specific studies and then evaluated the likelihood and significance of these and/or other potential low dose effects for humans (43) (http://ntp-server.niehs.nih.gov/htdocs/liason/LowDosePeerFinalRpt.pdf). They re-evaluated 38 studies from 12 different investigators. The reevaluation focused primarily on the experimental design, data analysis, and interpretation of experimental results for each individual study within the context of its own experimental conditions rather than by comparisons of results across studies.

4. PERSPECTIVE

Endocrine disruptors are now a world-wide concern, and many domestic and international projects related to them are ongoing. Although there had been only a limited contribution of theoretical works until now, the needs and advanced anticipation of them are growing. The endocrine-related complex database system might not only be relevant for predicting cellular responses to exogenous hormonal chemicals but also be useful for designing drugs that interact or control endocrine systems. As examples of such drugs, tamoxifen and raloxifene are designer estrogens, now being used as anti-cancer drugs (44-45). It is expected that future comprehensive investigations will include docking simulations and database complex systems that cover cell signaling information and gene expression (46).

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