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Development of predictive models for predicting binding affinity of endocrine disrupting chemicals to fish sex hormone-binding globulin

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ABSTRACT

Disturbing the transport process is a crucial pathway for endocrine disrupting chemicals (EDCs) exerting disrupting endocrine function. However, this mechanism has not received enough attention compared with that of hormones receptors and synthetase. Recently, we have explored the interaction between EDCs and sex hormone-binding globulin of human (hSHBG). In this study, interactions between EDCs and sex hormone-binding globulin of eight fish species (fSHBG) were investigated by employing classification methods and quantitative structure-activity relationships (QSAR). In the modeling, the relative binding affinity (*RBA*) of a chemical with 17β-estradiol binding to fSHBG was selected as the endpoint. Classification models were developed for two fish species, while QSAR models were established for the other six fish species. Statistical results indicated that the models had satisfactory goodness of fit, robustness and predictive ability, and that application domain covered a large number of endogenous and exogenous steroidal and non-steroidal chemicals. Additionally, by comparing the log *RBA* values, it was found that the same chemical may have different affinities for fSHBG from different fish species, thus species diversity should be taken into account. However, the affinity of fSHBG showed a high correlation for fishes within the same Order (i.e., Salmoniformes, Cypriniformes, Perciformes and Siluriformes), thus the fSHBG binding data for one fish species could be used to extrapolate other fish species in the same Order.

1. Introduction

Till now, about 145,299 commercially used chemicals have been preregistered by European (Registration, Evaluation, Authorization and Restriction of Chemicals, Last updated 10 May 2016) (REACH, 2016; Hartung et al., 2009). Among them, some chemicals were identified as endocrine disrupting chemicals (EDCs), who could disturb the endocrine system and caused adverse effects on sexual and functional development in both humans and wildlife (Diamanti-Kandarakis et al., 2009; UNEP/WHO, 2013). Even at very low concentrations, these chemicals in natural waters may act together in combination to produce significant effects, either additive or synergistic, resulting in sex reversal, inhibition of gonadal development, reduced gamete quality, disruption of reproductive behavior, and reduced disease resistance (Kabir et al., 2015; Kidd et al., 2007; Kumar et al., 2015). EDCs encompass a variety of chemical classes, including pesticides, pharmaceuticals, plasticizers, plant constituents, industrial byproducts, and others. Very often, these chemicals find ways to enter aquatic environments and enable water become a major reservoir of EDCs. As a result, aquatic organisms may thus be exposed to various EDCs. Increasing concerns on the potential toxicity of EDCs has prompted several government funded environmental agencies to identify potential EDCs from commercially used chemicals.

Generally, whether in human or in aquatic organisms, biological mechanisms involved in the disruption of endocrine system by EDCs include (Li et al., 2015; Miller et al., 2009): (a) impacting the hypothalamic-pituitary- endocrine gland (e.g., gonad/thyroid axis) function and regulation, (b) inhibiting hormones synthesis, (c) disrupting hormones transport proteins, (d) activating/inhibiting hormones receptors, and (e) inhibiting hormones metabolism. Currently, most studies focus on the mechanisms related to hormones receptors (Diamanti-Kandarakis et al., 2009; Fang et al., 2003; Kim et al., 2011; Kojima et al., 2011; Li et al., 2010) and synthetase (Mlynarczuk et al., 2010; Sanderson et al., 2001), and methods related the two mechanisms have been proposed as a standard assay in the major integrated testing and evaluation strategy by U.S. EPA (Willett et al., 2011). Nevertheless, other mechanisms were rarely studied (Hong et al., 2012, 2015; Nikolic et al., 2012; Yang et al., 2013). It is well-known that

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accessing to target tissues is a prerequisite for hormones to exert their biological effects, but regrettably, hormones synthesis and activity exertion usually do not occur at the same tissue, thus it requires a transport process from synthesis tissue to target tissue. Plasma sex hormone-binding globulin (SHBG), as the major carrier of endogenous sex steroids, modulates their bioavailability and accessibility, and protects them from rapid metabolic degradation and excretion (Hong et al., 2015; Saxena et al., 2014). But often, EDCs can bind to SHBG, consequently displacing sex steroids from SHBG ligand binding sites and delivering EDCs to the target tissues, thereby disrupting normal hormone homeostasis and endocrine function (Kloas et al., 2000).

Although the function of the SHBG seemed phylogenetically conserved, immunoreactivity studies have showed a considerable species variation in the structure of SHBG (Ovrevik et al., 2001). So far, most information of SHBG and its function was derived from mammalian studies, but the sequence of amino acids in SHBG from various species was different, e.g., zebrafish SHBG exhibited only 22–27% homology with mammalian SHBG sequences (Miguel-Queralt et al., 2004). Even within the fish species, there was also significant diversity in the similarity of amino acids sequence, e.g., different fish species only shared 40–80% sequence identity with each other, depending on their evolutionary proximity (Bobe et al., 2010; Miguel-Queralt et al., 2005). Thus SHBG displayed considerable species variation in both affinity and specificity and clear phylogenetic patterns were not obvious. As a result, the sensitivity of chemicals to SHBG varied greatly depending on different species, and it was necessary to explore the disruption ability and disruption mechanisms of chemicals to other species, excepting for mammal. To date, SHBG has been detected in a number of fish, especially teleost fish (Allison et al., 2000; Kloas et al., 2000; Laidley et al., 1994; Miguel-Queralt et al., 2008, 2004; Milligan et al., 1998; Ovrevik et al., 2001; Tollefsen, 2002; Tollefsen et al., 2004), but due to the limited number of species in fSHBG (i.e., fish sex hormone-binding globulin) studies and the lack of biochemical characterization of fSHBG, phylogenetic interpretations of the difference and relation of fSHBG in fish species remained unclear (Ovrevik et al., 2001). Moreover, the available data have never yet been integrated to characterize the specificity of fSHBG that located at different species positions in the phylogenetic tree.

As aforementioned, EDCs in surface waters are a group of diverse substances, many of which have structures quite different from the endogenous sex steroids. Thus, there is some difficulty in predicting whether a chemical could bind to fSHBG depending on its apparent chemical structure alone. Moreover, it is not practical to test all of the 145,299 commercially used chemicals through experimental methods, which are usually laborious, time-consuming, expensive and equipment dependent. Alternatively, the methodology of computational models is a rapid, sensitive and low-cost way to “fast screen” active chemicals and to set priority for chemicals. Especially, quantitative structure-activity relationships (QSAR) model can characterize the structural features those governing the binding affinity of chemicals, thus be used to prioritize untested and potential EDCs. Recently, we have explored the interaction between EDCs and sex hormone-binding globulin of human (hSHBG) (Liu et al., 2016). This study, following the OECD guidelines on the development and validation of QSAR models (OECD, 2007), aimed at: (1) developing classification models and QSAR models for predicting fSHBG affinity in different teleost fish; (2) identifying key molecular parameters those affecting the binding affinity of chemicals to fSHBG; (3) exploring the species variation in fSHBG specificity of fish, thereby clarifying phylogenetic patterns of fSHBG in evolutionary conservation.

2. Materials and methods

2.1. Data sets

The present study mainly focused on the fSHBG affinity of eight teleost fish species, i.e., Rainbow trout (*Oncorhynchus mykiss*) (Allison et al., 2000; Hobby et al., 2000a, 2000b; Marivin et al., 2014; Milligan et al., 1998; Tollefsen, 2002, 2007), Zebrafish (*Danio rerio*) (Miguel-Queralt et al., 2008, 2004), Spotted seatrout (*Cynoscion nebulosus*) (Laidley et al., 1994), Common carp (*Cyprinus carpio*) (Kloas et al., 2000), Sea bass (*Dicentrarchus labrax*) (Miguel-Queralt et al., 2005), Arctic charr (*Salvelinus alpinus* L.) (Tollefsen et al., 2004), Channel catfish (*Ictalurus punctatus*) (Gale et al., 2004) and White/Longnose sucker (Pryce-Hobby et al., 2003). Experimental data on the competing potency of various chemicals were collected from the corresponding literatures for each fish species. Relative binding affinities (*RBA*) were obtained by comparing the IC_{50} (half of the inhibition concentration) values of the tested chemicals with that of 17β-estradiol. In this study, the logarithm of relative binding affinity (log *RBA*) was employed to scale the binding potency, which was defined as:

$$\log RBA = \log\left(\frac{IC_{50}^{17\beta-\text{estradiol}}}{IC_{50}^{\text{test}}}\right) \quad (1)$$

where $IC_{50}^{17\beta-\text{estradiol}}$ and IC_{50}^{test} are the concentrations of 17β-estradiol and tested compound at 50% inhibition of [^{3}H]-17β-estradiol binding to SHBG, respectively. Hereinto, the experimental data that obtained with testosterone as reference were standardized as those with 17β-estradiol as reference.

With Rainbow trout and Common carp, there were a total of 71 and 21 data points, which including 18 and 10 inactive chemicals, respectively. Unfortunately, QSAR models had failed to quantify well the binding potency for the two fishes, thus classification models were developed to identify the chemicals those whether active or inactive. In the classification modeling, the data sets for each fish were randomly divided into a training set and a validation set with a ratio of 3:1. Models developed by the training set were assessed for their predictive ability by calculating the prediction accuracy of validation set. With Zebrafish, Spotted seatrout, Sea bass and Arctic charr, a total of 23, 22, 18 and 11 data points were used for QSAR model development and validation, respectively. In each group, chemicals with structural representativeness were selected as validation set, thus there were 4, 4, 4 and 3 data points in validation set for the four fish species, respectively, and the remaining data were categorized into training set. With Channel catfish and White/Longnose sucker, due to the small number of data points (each has a total of 7 chemicals), all the data were used in training set to develop QSAR models. The names, CAS numbers and corresponding observed log *RBA* values of the chemicals were listed in Tables S1–S3 of the Supporting Materials.

2.2. Calculation of molecular descriptors

DRAGON descriptors were used for the model development. Before calculating the molecular descriptors, the molecular structures of model compounds were drawn and preliminary optimized by ChemBio3D Ultra (Version 12.0) (Schnur et al., 1991) and MOPAC 2012 software. Then, based on the optimized geometric structures, 4885 DRAGON descriptors were calculated by employing the DRAGON software (version 6.0) (Talete, 2012). Some DRAGON descriptors were excluded in a preliminary step. Details for the exclusion rules were presented in our previous study (Liu et al., 2016). As a result of this prereduction procedure, a final set of 967, 764, 666, 877, 683, 737, 311 and 253 DRAGON descriptors for Rainbow trout, Zebrafish, Spotted seatrout, Common carp, Sea bass, Arctic charr, Channel catfish and White/Longnose sucker were retained, respectively.

2.3. Development and validation of classification models

Classification models were used to quantify the relationship between molecular descriptors and qualitative responses, which represented the two classes of chemicals in this study, i.e., active or inactive,