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A systems-level approach for investigating organophosphorus pesticide toxicity

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ABSTRACT

The full understanding of the single and joint toxicity of a variety of organophosphorus (OP) pesticides is still unavailable, because of the extreme complex mechanism of action. This study established a systems-level approach based on systems toxicology to investigate OP pesticide toxicity by incorporating ADME/T properties, protein prediction, and network and pathway analysis. The results showed that most OP pesticides are highly toxic according to the ADME/T parameters, and can interact with significant receptor proteins to cooperatively lead to various diseases by the established OP pesticide -protein and protein-disease networks. Furthermore, the studies that multiple OP pesticides potentially act on the same receptor proteins and/or the functionally diverse proteins explained that multiple OP pesticides could mutually enhance toxicological synergy or additive on a molecular/systematic level. To the end, the integrated pathways revealed the mechanism of toxicity of the interaction of OP pesticides and elucidated the pathogenesis induced by OP pesticides. This study demonstrates a systems-level approach for investigating OP pesticide toxicity that can be further applied to risk assessments of various toxins, which is of significant interest to food security and environmental protection.

1. Introduction

The widespread application of pesticides for many decades has caused severe adverse effects on humans and the environment because their persistence, bioaccumulation. The exposure to pesticides residues in food as well as in the environment have effects in the genetic polymorphism, promoting the disease initiation (Rodríguez et al., 2016). It is increasingly being recognized that many chronic pathologies are closely connected to pesticide residues (Nougadère et al., 2014). Besides, the adverse effects of cumulative environmental exposure to multiple-pesticide residues are much more serious than single. For instance, a mixture of five pesticides (acephate, diazinon, chlorpyrifos, dimethoate, and malathion) has been demonstrated to produce greater-than-additive responses (synergism) in several behavioral effects (Moser et al., 2005b). The integrated biomarker response values were determined by pesticide mixtures in comparison with single-pesticide (Güngördü et al., 2015). With thousands of pesticides being synthesized, the evaluations of their toxicity are important to both the environmental protection and human health. However, experimental methods that evaluating the toxicity properties of the compounds have their inherent limitations in obtaining toxicity data for a variety of pesticides because the diversity of chemical structures (toxins) and biological systems (targets). Furthermore, little information is known about the mechanisms of their toxicity at cellular and molecular level. Therefore, it is necessary to build a systems-level method to investigate toxicity and joint toxicity of the pesticides.

Network toxicology integrates classical toxicology and systems biology, which is available for analyzing toxic substances and exploring their interaction and regulation in biological systems, particularly investigate the toxic effects of drugs and/or compatibility of medicines on body, and clarify the mechanism of toxicity by combining ADME/T (absorption, distribution, metabolism, excretion and toxicity) properties, Cprotein prediction, and network and pathway analysis, etc. (Sturla et al., 2014). The network analysis has offered an opportunity to analyze toxic substances and their interaction and regulation in biological systems, which uncover a systems-level characterization of multiple molecular coordination and the molecular relationships among distinct phenotypes (Zhang et al., 2015; Zhou et al., 2013). The pathways analysis based on gene expression, protein-protein interactions have predicted the regulated pathways and extracted information on the sensitive sub-networks for disease (Liu et al., 2012). Thus, the pathways analysis offers a way to understand the mechanisms of joint

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toxicity (synergism or additive) within the context of systems-level interactions. Combining network and pathway analyses, and ADME/T properties will enable the understanding of mechanisms of single and joint toxicity (synergism or additive) within the context of systems-level interactions.

So far, systems toxicology have demonstrated the significance of studying in variety of toxic molecules, such as forecast the toxin targets and correlation networks, and identifying multiple toxin–protein interactions based on the massive diverse date of chemic, genomics and toxicology (Zhou et al., 2013). Notably, the method support vector machine is used to predict toxin targets (Yang et al., 2014). Thus, systems toxicology make understand of the interactions among toxins and relevant proteins and various diseases, in turn, OP pesticide toxicity and the mechanisms of action can be elucidated.

In this study, as exemplified by organophosphorus (OP) pesticides, which are most widely applied class of pesticides in the world, a systems-level approach including ADME/T properties of toxin molecules, multiple proteins prediction, and network and pathway analysis was used to understand OP pesticide toxicity and joint toxicity of multiple OP pesticides. Firstly, the ADME/T parameters of 216 OP pesticides were identified. Subsequently, the potential proteins of each OP pesticide and related diseases were revealed. And these OP pesticides, associated proteins, and corresponding diseases were mapping to generate the OP pesticide-protein and protein-disease networks. Furthermore, joint toxicity of multiple OP pesticides was reasonably explained by identifying their common proteins. Finally, these relationships of the receptor proteins of OP pesticides and disease-associated proteins were characterized at a pathway level.

2. Materials and methods

2.1. Building of dataset

A total of 216 OP pesticides were collected from the Pesticide Action Network (PAN) Pesticide Database (http://www.pesticideinfo.org/SearchChemicals. jsp# ChemCategory). A total of 1664 descriptors of the molecules were calculated by DRAGON 5.4 (Wang et al., 2010), which evaluate successfully the relationships of molecular structure–-activity or structure–property, and finally adopted in the subsequent processing (Supplementary data Table 1).

2.2. ADME/T property prediction

Due to the bioaccumulation of the pesticides is mainly determined by the oral routes, oral bioavailability (OB) as a parameter must be measured (Li et al., 2014). We applied OBioavail 1.1, which integrated with the metabolism (cytochrome P450 3A4) and transport (P-glycoprotein) information to calculate human oral bioavailability of each compound (Bioinformatics and CADD website. Available: http://modem.ucsd.edu/adme. Accessed 2017 April 10) (Xue et al., 2012). The OB value greater than 30% was regarded that the compound could participate in the further analysis.

The Caco-2 cell model, an in vitro model simulating intestinal absorption for drug delivery, uptake and absorption, might judge which toxins reach the systemic circulation (Sharma et al., 2011). The detailed approaches to predict the Caco-2 permeability had been fully supported by the previous experiment (Li et al., 2007b). This model sets Caco-2 value less than − 0.4 is not permeable, the threshold of Caco-2 permeability is set to − 0.4.

Moreover, blood-brain barrier (BBB) permeation index, which could be inferred as an ability of crossing a diffusion barrier of the central nervous system, was also used to further explore and predict pesticide toxicity (Gabathuler, 2010). Here, the optimized BBB model was evaluated by principal component analysis and multivariate linear regression analysis methods (Li et al., 2007a). The BBB values greater than − 0.3 represent permeable, here we set the BBB threshold to − 0.3.

2.3. Protein prediction

Since pesticides can target toxicologically relevant sites of action, finding the potential mode of action by searching for receptor proteins of OP pesticides is an efficient and convenient method to understand pesticide toxicity at a molecular level. It is difficult to obtain reliable biological information for the present compound–protein interactions, particularly for large datasets. Besides, it is challenging and time-consuming that searching target proteins of pesticide. However, a previously developed an integrated in silico systematic model, based on statistical methods random forest (RF) and support vector machine (SVM), efficiently integrated numerous chemical, genomic, and toxicological information to predict the toxin targets and eventually overcome previous limitation (Hua Yu, 2012; Zhou et al., 2013). In this system, 68 related proteins with probability of interaction between each molecule and protein both greater than 0.6 in RF and SVM were chosen as the final receptor proteins and 813 interactions were obtained from the prediction model. On the other hand, the other proteins were imported from Similarity Ensemble Approach (SEA , http://sea.bkslab.org/search/) and STITCH database (http://stitch.embl.de/). At last, a total of 364 proteins were reserved for further analysis.

2.4. Network construction

The analysis of networks can express the node characteristics and the relationships between nodes more accurately, quantifying ongoing trends and shifts in the discovery of toxic mechanisms. In this study, the OP pesticides and proteins and diseases were used to build the OP pesticide -protein and protein-disease network by Cytoscape 2.8.3. The OP pesticide-protein network generated by the OP pesticides and their corresponding target proteins express a compound linked to a target. While the protein-disease network was constructed by connecting proteins to their related diseases (Supplementary data Fig. 2). Here, the related diseases were collected from the Comparative Toxicogenomic Database (CTD, http://ctdbase.org/) (Davis et al., 2011) and Therapeutic Proteins Database (TTD, http://bidd.nus.edu.sg) (Zhu et al., 2012). In the networks, the OP pesticides, proteins and diseases are represented by the nodes and their interactions are encoded by the edges. Finally, the Network Analyzer provide the quantitative properties.

2.5. Pathway construction

Firstly, the pathways was accumulated by TPEA (target-pathway-enrichment-analysis) method (Huang et al., 2014) via the DAVID bioinformatics tool (http://david.abcc.ncifcrf.gov/) (Huang et al., 2009). Besides, we also collected other pathways by retrieving the three most important proteins (ESR1, ESR2 and AHR) in KEGG (http://www.genome.jp/kegg/). A disease-related biological pathway based on systems toxicology eventually was synthesized.

To predict the relativity of the proteins of OP pesticides for these pathways at a higher level of organization, the protein–protein interactions (PPIs) were mapped by the proteins in these pathways and the nearness between proteins of toxins and the pathway-related proteins p′ is calculated:

$$\varphi_{pp'} = \frac{1}{nm}\sum_{i=1}^{n}\sum_{j=1}^{m} e^{-D^2_{p_i p'_j}}$$

From the PPI network, $Dp_i p'_j$ is the shortest distance between p_i and p'_j in which p_i represents the receptor protein of OP pesticides, p'_j is the pathway-related protein. n and m respectively represents the number of receptor protein p and pathway-related protein p' mapped on the PPI network (n: 364, m: 53). When the $Dp_i p'_j$ is defined as ∞, two proteins are unconnected on the PPI network, and $e^{-D^2_{p_i p'_j}}$ is used to convert protein-protein closeness to distance.