MASTER'S THESIS

The study of novel dioxin antagonist-euxanthone and its derivatives

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The Study of Novel Dioxin Antagonist-Euxanthone and its Derivatives

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Abstract

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), a widespread environmental pollutant, has been shown to act as an agonist of the aryl hydrocarbon receptor (Ah receptor) subsequently leading to adverse effects. While much work has been done on the toxicity mechanism of TCDD, some attention begins to turn to discerning the mechanism by which phytochemicals are utilized to inhibit TCDD toxicity, such as carcinogenesis and hepatotoxicity.

The present study disclosed a novel antagonist, named PW-1, which was extracted from Chinese medicine herb *Polygala caudata*. The antagonist showed significant suppression of TCDD-induced EROD activity at a dose-dependent manner as well as *CYP1A1* gene expression in HepG2 cells. Furthermore, six synthetic derivatives of PW-1, namely CYL-1, -2, -3, -4, -5, and –6, also showed to possess inhibitory effects on TCDD-induced EROD activity in a dose-response manner. The PW-1 and all its derivatives *per se* did not significantly result in induction of EROD activity as compared to TCDD. However, CYL-4, PW-1, CYL-1, CYL-2, and CYL-3 at the concentration of 1.65 μM, 1.95 μM, 2.5 μM, 5.0 μM and 8.2 μM, respectively, can give rise to a 50% inhibition (IC_{50}) of EROD activity generated by induction of 0.5 nM TCDD. Almost complete inhibition was observed by CYL-4, PW-1 and CYL-1 at the concentration of 12.5 μM. Real-time PCR as well as agarose gel electrophoresis were employed to evaluate the validity of the *CYP1A1* gene expression changes induced by TCDD and the antagonistic effects of PW-1 on TCDD.

On the other hand, cDNA microarray technology was employed to uncover the possible antagonistic mechanism of PW-1 against TCDD. For this, approximately two thousands of liver-specific cDNAs were firstly prepared from a human liver lambda cDNA library with the titer of 2.97×10^{11} pfu/ml. These cDNAs were then printed on polylysine-coated slides by an arraying robot. The microarrays thus made were hybridized simultaneously with three probes which were labeled separately with a type of fluorescent dye, namely Alexa Fluor 546 for TCDD treatment, Alexa Fluor 488 for TCDD plus PW-1, and Alexa Fluor 647 for solvent control. Finally, the hybridization signals were scanned by the laser confocal scanner, and the digitized fluorescence signals were normalized by an approach of total measured fluorescence intensity. As a result, fifty candidate genes were chosen for sequencing. By blasting the obtained sequences in the GenBank database, 21 genes were identified as unique to the GeneBank. Among them, 18 genes were more than 2.0 fold, 2 genes were without change, and only one gene was less than 1-fold change. Among these 18 genes, seven were firstly disclosed to be induced by TCDD. Obviously, the expressions of most genes identified were altered by exposure to TCDD, and most of genes up-regulated by TCDD were down-regulated by PW-1, such as the gene encoding Alpha-1-microglobulin/bikunin precursor (AMBP); the gene encoding Retinoid X receptor, alpha (RXRA); the gene encoding complement component 3 (C3). An antagonistic mechanism of PW-1 against TCDD was deduced in the present study. Especially, an interleukin-6 (IL-6)-mediated regulation pathway of TCDD function was also proposed.

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