

Identification and comparison of gene expression profiles responsive to PFOA

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Introduction

Perfluorinated compounds (PFCs) have been manufactured and used for variety of industrial applications for over 50 years. Perfluorooctanoic acid (PFOA), a commercially important compound is found in the environment and biota including human.^{1,2} Previous studies demonstrated that PFOA was accumulating primarily in plasma and liver. PFOA has higher half-life in male compared to female where urinary elimination was higher in female rats. PFOA binds to plasma protein and considered as a potent peroxisome proliferator. Moreover, PFOA increases in beta-oxidation of fatty acids, several cytochrome P-450 enzymes, inhibition of the secretion of very low-density lipoproteins and cholesterol from liver.³

In this study we investigated gene regulation in rats received different concentrations of PFOA by oral gavage. Then those gene expression profiles were compared with previously reported data in rat responsive to Perfluorooctane sulfonic acid (PFOS) exposure.

Materials and Methods

8 weeks old male Sprague-Dawley rats were housed in humidity-controlled facilities according to the guidelines of National Institute of Animal Health. Rats were given a single oral gavage of different concentration of PFOA (1mg/kg, 3mg/kg, 5mg/kg, 10mg/kg, 15mg/kg), respectively, or vehicle control (0.5% Tween-20) daily at a rate of 1ml/kg body weight for 21 days. At the end of the exposure, rats were anesthetized and liver samples were frozen in liquid nitrogen and stored at -80°C until used for RNA isolation.

Liver total RNAs were isolated with Trizol Reagent using manufacturer recommended procedures. RNA fractions from the three rats receiving same concentration were pooled for GeneChip analysis. Six chips of Affymetrix: Rat Genome 230 2.0 array were purchased and array analysis was carried out using manufacturer recommended procedures. Microarray Suite (MAS) ver. 5.0 and GeneChip Operating Software (GCOS) was used to perform gene expression analysis.

Results and Discussion

The Rat Genome 230 2.0 array is spotted with 31,042 gene probes made of 25-mer single strand oligonucleotides. Comparison analysis of the expression profiles was performed between the control rats and PFOA-treat rats (1mg/kg, 3mg/kg, 5mg/kg, 10mg/kg, 15mg/kg) from the GeneChip data. Over 500 genes whose expression was significantly ($P < 0.0025$) altered by PFOA; and 10mg/kg PFOA treatment altered the greatest numbers (over 800) of gene expression while 1mg/kg altered the least number (501) of gene expression. The use of two-fold cut-off for

significance ($P < 0.0025$) is conventionally used in other studies.⁵

For the present study, results from 5mg/kg have been used to compare with that of PFOS.⁴ In 5mg/kg PFOA treatment rats, 296 and 220 genes were found to be up-regulated and down-regulated, respectively. Large group of the up-regulated genes were related to fatty acid and lipid metabolisms while large group of the down-regulated genes were related to signal transduction according to the annotation information and protein information Swiss-Prot. Pathways of fatty acid synthesis, fatty acid degradation, and cholesterol synthesis were found to be altered significantly ($P < 0.0025$) under the exposure of PFOA.

The gene expression profiles of PFOA were compared with those in PFOS (Table 1 and 2).⁴ Out of the 23 up-regulated genes by PFOS, 12 genes were also found induced by PFOA, but seven of them kept unchanged and four of them could not be found in the present study. For the 19 genes suppressed by PFOS, only one gene was found suppressed while the rest were not affected interestingly, except one gene was induced by PFOA. The up-regulated genes found in both PFOS and PFOA were related to fatty acid and lipid metabolism and that might be related to their structural properties as their structures were similar to the endogenous fatty acids. However, it showed a very different expression for the down-regulated genes by PFOA and PFOS that might be due to different functional group of PFOA and PFOS, which suggesting that further investigation on the toxicity of PFOA is necessary. In addition, the pathways of fatty acid beta-oxidation were compared as they were found altered in both PFOA and PFOS treatment. Hu, et al. (2005) mentioned that PFOS specifically enhanced the expression genes involved in peroxisomal but not mitochondrial fatty acid beta-oxidation; and in the present study, the gene expressions showed the increase in both peroxisomal and mitochondrial fatty acid beta-oxidation under the exposure of PFOA.

In conclusion, PFOA and PFOS shared some degree of similarity in gene expression profile. They induced the genes responsible for fatty acid and lipid metabolisms and the pathway of peroxisomal fatty acid beta-oxidation was induced by PFOA and PFOS; and they might cause oxidative stress or damage on DNA or protein. However, genes suppressed by PFOA were different from that of PFOS. Gene expression profiles could give insights on the approaches to understand on the effects of PFOA but not to demonstrate the toxicity on PFOA. Therefore, further studies were needed to understand the toxicity of PFOA and its relationship with PFOS.

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Table 1. Comparison of the fold change of list of genes induced significantly by PFOA and PFOS

Gene symbol	PFOA	PFOS	PFOA/ PFOS
Hdc	59.71	12.49	4.78
Cte1	256.00	90.25	2.84
Ehhadh	7.46	6.5	1.15
Mte1	5.66	10.61	0.53
Dci	2.83	6.02	0.47
Acaa1	6.06	9.78	0.62
Ces2	5.28	5.89	0.90
Ech1	3.25	5.11	0.64
Aldh1a1	5.28	6.04	0.87
Resp18	4.00	6.44	0.62
Cyp2b15	9.19	9.09	1.01
Cyp17a1	5.28	19.3	0.27

Table 2. Comparison of the fold change of list of genes suppressed significantly by PFOA and PFOS

Gene symbol	PFOA	PFOS	PFOA/ PFOS
a	-7.69	-5.9	1.3
Prdx6	1.15	-2.45	-0.47

a: apolipoprotein A-IV