HEPATIC GENE EXPRESSION PROFILES RESPONSIVE TO PFOA AND PFOS; A COMPARISON BETWEEN CHICKEN AND RATS

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Abstract

The effects of PFOS and PFOA on the gene expression patterns of chickens that were exposed to either PFOS or PFOA at low doses were investigated with the use of microarray techniques. Twelve Genechip Chicken Genome Arrays were used to study hepatic gene expression in six-week-old chickens (*Gallus gallus*) that were exposed to either PFOA (0.1, 0.5, or 5mg/mL), PFOS (0.02 or 0.1mg/mL), or a saline vehicle control (0.9% NaCl in Milli-Q water) via subcutaneous implantation of a 2mL osmotic pump for 4 weeks or for 4 weeks with a further 4 weeks of depuration. Over 240 and 480 genes were significantly affected by PFOS after 4 weeks of exposure and after 4 weeks of exposure with a further 4 weeks of depuration, respectively; and over 290 and 320 genes were significantly affected by PFOA, correspondingly. Interestingly, some of the genes that were affected by either PFOS or PFOA in the chickens were the same as those found in rats that were treated with PFOA. Although there may be a big gap in terms of responses at the gene expression level between birds and rats, the results of studies to date indicate that PFOA may have the same effects on cholesterol-related activities in birds as it does in rats.

Introduction

The widespread occurrence of perfluorinated compounds (PFCs) in humans, wildlife, and the environment has prompted studies on the potential toxicity of these compounds. PFCs are useful because they possess both hydrophobic and oleophobic characteristics. Of the wide variety of PFCs in existence, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) have received the most attention because of their widespread use and ubiquitous presence in the environment.

Extensive tests of the effects of PFOS and PFOA have been carried out on mammals, but similar investigations for avian species are few. Most of the studies of avian species have been concerned with PFC concentrations in wild birds,[1] with only a few studies of the toxicity of PFCs in farmed birds being carried out.[2,3] In these studies, a significant reduction in body weight and an increase in the incidence of small testes were observed when male mallards or bobwhite quail were given 17.6 mg of PFOS/kg of feed. In a recent study that involved injecting PFOS into chicken eggs at concentrations around the same as those that have been found in wild avian eggs, significant adverse effects,

including higher mortality, reduced hatchability, and liver histopathological changes, were observed.[4]

In general, most toxicological studies to date have involved short-term exposure to high doses. This study was designed with PFOA and PFOS exposure concentrations that were at least several times lower than the solubility of the corresponding compounds in water at room temperature. The effects of PFOS and PFOA on gene expression patterns in chickens were investigated by use of microarray techniques.

Materials and Methods

Six-week-old white leghorn (*Gallus gallus)* PDL-1 strain chickens were housed in humidity-controlled facilities in accordance with the guidelines of the National Institute of Animal Health. Five groups of six randomly selected male chickens were placed into five experimental cages and exposed to either PFOA (0.1 or 0.5mg/mL), PFOS (0.02 or 0.1mg/mL), or a saline vehicle control (0.9% NaCl in Milli-Q water) via the subcutaneous implantation of a 2-mL osmotic pump with a release rate of 2.5 μL/hr for four weeks (ALZET® 2ML4, DURECT Corporation, CA, U.S.A.). Additionally, a single group of female chickens was given only 5mg/mL PFOA to examine any difference between this group, which had received a comparably high PFOA dose, and the other treatment groups. After four weeks of exposure, half of the chickens were sacrificed and the other half were left for four weeks of depuration. At the end of the exposure, the chickens were anesthetized so that liver samples could be taken, which were frozen in liquid nitrogen and stored at -80ºC until RNA isolation was carried out.

The total RNAs in the liver were isolated with a QuickGene RNA tissue kit S II and the QuickGene-810 nuclear acid isolation system using the manufacturer's recommended procedures (Fuji Photo Film Co. Ltd., Tokyo, Japan). The RNA fractions from the three chickens with the same concentration were pooled for GeneChip analysis. Twelve Genechip Chicken Genome Arrays were purchased and array analysis was carried out in accordance with the manufacturer's recommended procedures. Microarray Suite (MAS) ver. 5.0 and GeneChip Operating Software (GCOS) were used to perform the gene expression analysis[5]. Experimental procedure and array analysis for PFOS and PFOA treated rats are published in Hu et al.[6] and Guruge et al.[7], respectively

Results and Discussion

The Genechip Chicken Genome Arrays were used to study the gene expression of 32,773 chicken and 684 viral transcripts consisting of 25-mer single strand oligonucleotides. A comparative analysis of the expression profiles of the control chickens and treatment chickens was performed (0.02/0.1 mg PFOS/mL, 0.1/0.5/5 mg PFOA/mL) using the GeneChip data. Over 240 were significantly affected by PFOS and over 290 were affected by PFOA after four weeks of exposure, whereas 480 genes were affected by PFOS and 320

by PFOA after four weeks of exposure with a further four weeks of depuration (Table 1). The use of a two-fold cut-off for significance ($P < 0.0025$) is consistent with other studies.

Different gene expression patterns were observed between the chickens that were treated with PFOS and those that were treated with PFOA. PFOS suppressed genes that were related to the cell cycle and cytoskeleton organization, whereas PFOA induced those genes. In addition, more genes that were related to signal transduction were affected by PFOS than were affected by PFOA. These differences may be attributable to the dissimilar physico-chemical properties of the two compounds. PFOS and PFOA both have a C8 backbone, but the length of their hydrophobic tail is different (C8 and C7) and as a result their hydrophobicity is different. The differences in their functional groups may also have contributed to the different effects on gene expression patterns. In this study, although there were some genes that were commonly affected by both PFOS and PFOA, the different patterns of gene expression demonstrated that PFOS and PFOA had different effects on the chickens. Thus, the toxicities of PFOS and PFOA should be evaluated separately, and special care should be taken in extrapolating the toxic effects of one type of PFC to another.

Hu et al. showed that the largest grouping of genes induced by PFOS in rat liver were P450s and genes that code for fatty acid and lipid metabolizing enzymes[6]. In contrast, most of the genes that were affected by PFOS in the chickens were related to electron and oxygen transport and cell-related activities. The results of a previous study in rats showed that most of the genes that were affected by PFOA at all doses were related to the transport and metabolism of lipids, particularly fatty acids, and cell-related activities[7]. In contrast, in chickens the largest group of genes that were affected by PFOA were related to the transport of ions, lipids, and electrons, but not to the aforementioned cell-related activities.

Interestingly, some of the genes that were affected by either PFOS or PFOA in the chickens were the same as those found in rats that were treated with PFOA. These genes were related to fatty acid or cholesterol metabolism. Carnitine palmitoyltransferase 1A (CPT1A) was found to be suppressed 3.03 fold at 0.5mg of PFOA/mL in chickens, whereas carnitine palmitoyltransferase 1B (CPT1B) was induced in rats that were treated with PFOA (1, 3, 5, 10, 15mg/kg bw). Acyl-Coenzyme A oxidase 1, palmitoyl (ACOX1) was induced 2.14 fold at 0.1 mg PFOA/mL in chickens, and at least 2.14 fold in rats that were treated with PFOA. Furthermore, 2,4-dienoyl CoA reductase 1, mitochondrial (DECR1) was induced 10.56 fold at 0.1 mg of PFOA/mL in the chickens, and 2 fold at 15 mg of PFOA/kg bw in rats. These results demonstrate that more genes that are related to fatty acid metabolism were affected by PFOA during the depuration phase. PFOA had an effect on the unsaturated fatty acid metabolism for which DECR1 is responsible, and 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (HMGCR) was found to be suppressed 3.03 fold at 0.02 mg of PFOS/mL and 2.83 fold at 0.1 mg of PFOA/mL. Interestingly,

3-hydroxymethyl-3-methylglutaryl-Coenzyme A lyase (hydroxymethylglutaricaciduria) (HMGCL) was suppressed 2.46 fold at 0.1 mg of PFOA/mL, but was induced 2 fold at 10mg of PFOA/kg in rats. Although there may be a big gap in terms of responses at the gene expression level between birds and rats, the results of studies to date indicate that PFOA may have the same effects on cholesterol-related activities in birds as it does in rats.

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Table 1 Summary the genes affected significantly at different concentrations of PFOS/ PFOA at different exposure regimes according to their biological and molecular functions

	PFOS				PFOA					
	4 weeks of exposure		with 4 weeks of depuration		4 weeks of exposure			with 4 weeks of depuration		
	0.02mg/mL	0.1mg/mL	0.02mg/mL	0.1mg/mL	0.1mg/mL	0.5mg/mL	5mg/mL	0.1mg/mL	0.5mg/mL	5mg/mL
Apoptosis	**1**	**0**	**3**	**0**	**1**	**0**	**4**	**1**	**1**	**5**
Catalyic activity	**12**	**4**	**6**	**8**	**6**	**6**	**8**	**11**	**5**	**6**
Cell										
adhesion/ cell matrix adhesion	0	4	5	3	0	9	2	5	0	2
communication	1	0	0	1	0	1	1	0	1	1
cycle	0	2	3	1	4	4	0	1	2	1
cytoskeleton organization	1	2	2	1	0	0	0	1	0	0
death	0	0	0	1	1	0	0	0	0	0
differentiation	1	1	2	2	0	1	3	3	1	1
division	2	1	1	0	0	0	0	0	0	0
growth	2	1	3	3	0	2	1	2	1	2
proliferation	0	0	0	1	0	1	0	0	0	0
shape	0	0	0	0	0	0	1	0	1	0
Cytochrome	**2**	**2**	**0**	**0**	**4**	**6**	**4**	**1**	**0**	**3**
DNA damage/ repairing	**0**	**0**	**0**	**0**	**0**	**2**	**1**	**2**	**1**	**3**
G-protein coupled receptor	**2**	**2**	**0**	**1**	**1**	**2**	**0**	**1**	**1**	**2**
Hormone	**0**	**0**	**0**	**2**	**0**	**2**	**2**	**0**	**2**	**0**
Ion binding	**1**	**2**	**12**	**6**	**2**	**2**	**7**	**7**	**6**	**6**
Immnune defense response										
Immune/ defense response	**3**	**0**	**2**	**6**	**0**	**3**	**3**	**2**	**6**	**6**
Interleukin	**1**	**2**	**1**	**5**	**0**	**3**	**0**	**4**	**3**	**2**
Metabolism	**9**	**8**	**18**	**27**	**5**	**11**	**5**	**32**	**6**	**47**
Protein related process										
Protein amino acid phosphorylation	5	2	2	4	0	5	5	13	5	5
Proteolysis	1	3	3	4	5	2	2	4	1	7
Other	1	0	4	5	1	8	12	9	3	4
Regulation of transcription	**4**	**6**	**10**	**10**	**8**	**8**	**10**	**13**	**10**	**17**
Signal	**1**	**1**	**10**	**7**	**6**	**5**	**7**	**15**	**5**	**9**
Stress	**1**	**1**	**3**	**1**	**1**	**0**	**1**	**1**	**0**	**1**
Transport	**9**	**11**	**16**	**21**	**14**	**18**	**16**	**21**	**9**	**19**
Other	**26**	**17**	**45**	**55**	**31**	**55**	**63**	**95**	**44**	**73**
Total	86	72	151	175	90	156	158	244	114	222