THE GENOTOXIC EFFECTS OF PERFLUOROOCTANOIC ACID ON ADULT RAT LIVER AND PPARα KNOCK-OUT MOUSE LIVER

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Introduction

Perfluorated alkyl compounds have been in use for more than 50 years, but only recently has one focused on their possible toxic effects. They have special chemical and physical properties, which have made them rather unique. They are both hydrophobic and oleophobic, have a high chemical and thermic stability and low surface tension. These properties are important in several industrial products such as cleaning agents, flame retardants and in impregnating mixtures. The two most important PFAS are perfluorooctanesulfonic acids (PFOS) and perfluorooctanoic acid (PFOA). The levels of PFOA in human serum have been reported to be from 1-71 ng/ml[1]. In house dust the levels have been found to vary from 69-3700 ng/g[2]. There is little information on effects on human health, but in rodents PFOA is supposed to be a promoter for several types of cancer. In the present investigation we have studied the effect of PFOA on gene expression from the livers of wild type rats, wild type mice and PPARα knock-out mice.

Materials and methods

Animal exposure

Perfluorooctanoic acid (PFOA) was from Fluka Chemica. Wistard rats (200g) came from Møllegaard in Denmark. PPARα knock-out mice came from Jacksons Lab. in USA and CD 1 mice (wild type) came from Møllergaard in Denmark. Animals were maintained in the laboratory 1 week before the experiment started. The animals then received 10mg/kg PFOA for 12 days. The mice were administrated intraperitoneally and the rats were fed by gavage**.** Twenty-four hours after the last administration, the animals were decapitated, the liver dissected out and part of the liver was treated with RNAlater and examined by microarray. The rest of the liver was homogenized and used for enzyme analysis.

Microarray analysis

Microarray analysis of gene expression was carried out using murine arrays manufactured at The Microarray Core Facility at the Norwegian University of Science and Technology (NTNU). Tissue-samples for RNA-extraction was stored in RNAlater. Total RNA from liver/kidney-cortex was isolated using the RNAeasy Qiagen mini kit. cDNA–synthesis and cDNA.labelling (with Cy3 or Cy5) was carried out using the Genisphere's 350 labelling-kit. Scanning of processed microarrays was carried out using a Packard Bioscience Scanarray Lite instrument. Analysis of the resulting images was carried out using the Scanarray Express and Spotfire v.8 software. The Spotfire software enables complete statistical evaluation of the raw microarray fluorescence data, including various types of normalization, cluster-analysis, principal component analysis, profile-analysis as well as ANOVA evaluation of genes which are significantly differently expressed. Using Spotfire we were able to assemble data from a series of microarrays (i.e. for a dose-response curve) into one single data-file, thereby facilitating detection of differentially expressed genes across the entire data set. Validation of the microarray data was carried out using real-time PCR, supplemented with semi-quantitative RT-PCR if required. Interpretation of expression data was carried out using gene ontology based software. The WebGestalt software was used for initial analysis.

Results
Male Wistar rats exposed for 10mg/kg PFOA had an increased liver weight compared to control (3.6% compared to 5.8 % of total body weight). The treated animals also showed an increase in peroxisomal β-oxidation in liver, but no increase in catalase activity. Further there were 20 genes up regulated more than 3 folds, which were linked to β-oxidation. Four genes linked to apolipoproteins and 5 genes linked to xenometabolism were down regulated.

Similar results were obtained with wild type mice. The liver weight increased from 5% to 13% of total body weight in wild type with 10 mg/kg PFOA. In the knock-out mice there were to our surprise also an increase in the liver weight in the PFOA treated animal (from 3%to 7% of total body weight). The wild mice showed a large increase in peroxisomal β-oxidation in liver after treatment with PFOA (4 folds). As expected there was no peroxisomal β-oxidation in knock-out animals. Again there were no effects on catalase activity in the treated animals. PFOA treatment was accompanied by a significant increase in 113 mRNA in the wild type mice. Very few of these mRNA was affected in the knock-out mice. On the other hand there were 114 examples of genes only up regulated after treatment in the knock-out animals. The results are summarized in table 1.

	Wild type mouse Up regulated	Knock-out mouse Up regulated
Mitochondrial genes	18 0	1 4
Ribosomale genes	23	0
Xenometabolisme	8 0	4 13
Esterase genes	0	3
Transport genes (ATP)	1	3
Transferase genes	0	6
General	61 4	2 79

Table 1. The table shows genes in different groups, which were up regulated in wild type mice or PPARα knock-out mice. For example in the mitochondrial group18 genes were affected in wild type whereas only 1 gene was affected in the knock-out. In another group 4genes were affected in the knock out, whereas none of these was affected in the wild type

Below follows a short description of some of the genes that are changed after PFOA treatment In some cases the main changes are in the wild type, but in other cases the changes are only found in the knock-out animal.

Mitochondrial genes
PFOA treatment led to an increase in mRNAof acyl-Coa oxidase and for long chain acyl-CoA ligase. The former is the rate limiting enzyme in peroxisomal β-oxidation, whereas the latter catalyses the synthesis of acyl-CoA esters. There were many mitochondrial enzymes which were increased such as 3,2-trans-enoyl-CoA isomerase, in both the wild type and the knock –out animal, and 2,4-dienoyl-CoA isomerase only in the wild type animal. Both are involved in the mitochondrial β-oxidation of unsaturated fatty acids. Also genes coding for proteins in the electron transport chain showed increased transcription as a result of PFOA treatment.

Xenometabolisme
PFOA led to an increase in expression of several Cyp genes in both wild type and knock-out mice. Cyp1A2 is increased in both types of animals. A large increase was also found in genes for cyp4A10, cyp4A12 and cyp4A13 in both wild type and knock-out mice. On the other hand cyp2A4, cyp2C29 and 4A14 was only increased in the wild type animals. There were a series of cyp genes that were significantly higher expressed in knock-out than in wild type animals. These were particularly cyp2B10, cyp2C55, cyp2C65 and cyp3A11. Also gluthathione s-transferases were up regulated in the knock-out animals

Transport genes and transferases
ATP-binding cassette subfamily A was up regulated in knock-out mice whereas sub-family c was up regulated in wild type. There are several genes for transferases which are significantly higher expressed in treated knock-out mice than in untreated, and in treated wild type mice. These include serine-pyruvate aminotransferase, galactosyltransferase associated protein kinase P58/GTA, thioester s-methyltransferase.

Carboxylesterases and ribbosomale genes
Liver carboxylesterase 22 precursor were among those upregulated in knock-out animals. Many ribosomal proteins were upregulated in the treated wild type, but none in the knock-out animals. These includes 60S ribosomal L12 and 40S ribosomal protein S26.

General
There were a large number of genes not related to any of these groups, which were changed. Genes only with increased expression in the wild type include adiphophilin, aldehyde dehydrogenase, alcohol dehydrogenase, epoxide hydrolyse 1, GTP binding protein 2, heat shock protein 27 NADP-dependent malic enzyme, IGBFP-2 and pantetheinase precursor. On the other hand metallothionein1 and 2, serine proteinase and transgelin 2 were changed in the knock-out animals only. There were also several examples of down regulation (24 genes) after PFOA treatment which only occurred in wild type animals. Examples of these are serum amyloid A-1 protein precursors (Saa1 and Saa2). These are almost completely eliminated. Other genes are for argininosuccinate synthase, glutamine synthetase, ornithine carboyltransferase and liver carboxylesterase precursor.

Discussion
The results show that PFOA treatment leads to different mRNA level of a number of genes both in wild type and in knock-out PPARα mice. PPARα knock out mice were selected because of the similarity between PPARα agonists and the perfluorated alkanes. The effect of PFOA on mRNA levels of peroxisomal or mitochondrial enzymes can be explained by assuming that PFOA binds to and activate PPARα. PPARα will in due course bind to DNA regulatory elements and thus affect the transcription or expression of different genes. This will generally leads to an increase of liver weight. The surprising thing is that we also found an increased liver weight in the knock-out animals. The results show that not all the affected genes are dependent on a functional PPAR α receptor. In this context it is clear that PFOA treatment leads to the activation of several other genes. In some cases PFOA increases the expression of several

cyp-genes, which are activated. In the knock out animal this could be a way of finding other metabolic routes to get remove toxic compounds or in some way compensate for the effect of PFOA. We also find inceases of several stress genes which is only natural.

References

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