Identification of Genes and Pathways Regulated by PPARα Activators: Differences Between WY-14,643 and PFOA

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ABSTRACT

Exposure to peroxisome proliferator chemicals (PPC) leads to alterations in the balance between hepatocyte growth and apoptosis, increases in liver to body weights (LW/BW) and liver tumors. There is strong evidence that PPC cause many of their effects related to carcinogenesis through the nuclear receptor peroxisome proliferator-activated receptor alpha (PPARα). The molecular events that occur after PPARα activation and alteration in liver growth are not well characterized. To help identify common genes and pathways that may be mechanistically linked to PPC-induced carcinogenesis, we have compared the transcript profiles of the livers of wild-type or PPARα-null mice exposed to a number of PPC using Affymetrix chip data. PPC which induce liver cancer were compared in this study including WY-14,643 (WY) which requires PPARα for increases in LW/BW and perfluorooctanoic acid (PFOA) which causes increases in LW/BW in both wild-type and PPARα-null mice. Transcript profiling was performed using either mouse U74Av2 or 430_2 Affymetrix chips. Significantly altered genes were identified using Rosetta Resolver. Under similar exposure conditions (7 consecutive daily gavages) a number of common functional categories of genes were identified including those involved in fatty acid oxidation and transport, peroxisome biogenesis, proteome maintenance, coagulation, complement cascade and oxidative stress as well as those linked to cell growth control. Almost all of these common genes were dependent on PPARα for changes in transcript levels as the changes were not observed in PPARα-null mice. Each chemical also altered a unique set of genes independently of PPARα. These genes regulated by PFOA included those involved in xenobiotic metabolism and are under control by other nuclear receptors including PXR, FXR and CAR. We tested the hypothesis that CAR was involved in the regulation of the PFOA PPARα-independent genes by comparing to those regulated by compounds that activate CAR. There was excellent correlation between the PFOA genes and those regulated by phenobarbital and TCPOBOP in a CAR-dependent manner. These results indicate that WY and PFOA alter most genes in the mouse liver through PPARα but that a small subset of genes responsive to PFOA are regulated by other nuclear receptors including CAR. This abstract does not necessarily reflect EPA policy.

GOALS OF THIS STUDY

- Determine the impact of PPARα on gene expression in the mouse liver after exposure to PPC
- Examine mechanisms of how PPC regulate gene expression in a PPARα-independent manner
- Contrast and compare the mode of action of two PPC: WY-14,643 and a perfluorinated alkyl acid, perfluorooctanoic acid (PFOA)

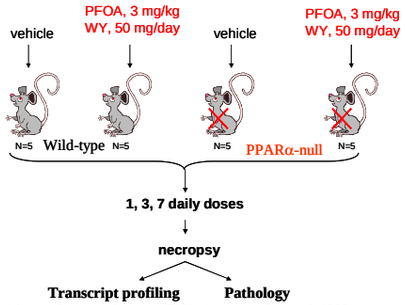
MATERIALS AND METHODS

Animals, dosing, and tissue collection: Wild-type and PPARα-null male mice were orally dosed for 1, 3 or 7 consecutive days with either PFOA (Fluka Chemical cat#77262, Steinheim, Switzerland) in distilled water or WY (Sigma-Aldrich cat# C7081, St Louis, MO) in 0.5% methylcellulose. Dose groups consisted of 3 mg/Kg PFOA or 50 mg/day WY-14,643.

RNA Preparation: Collected tissue was immediately homogenized in TRI reagent (Sigma Chemical, St. Louis, MO) and processed on the same day through alcohol precipitation according to the manufacturer's directions. RNA pellets were then washed in cold 80% ethanol and stored at -80°C until further use. Following resuspension of RNA in nuclease-free water (Ambion, Austin, TX), the samples were quantified and evaluated for purity (260nm/280nm ratio) using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and 100ug of each sample was further purified using RNeasy spin columns according to the manufacturer's directions (Qiagen, Valencia, CA). Approximately 250 ng of each sample was then evaluated for quality using a 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA).

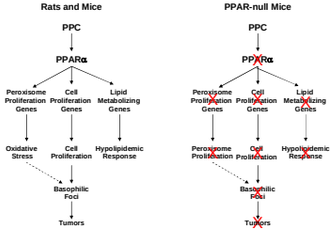
Affymetrix GeneChip® analysis: Purified RNA was processed for microarray hybridization and scanning (mouse U74Av2 or 430_2.0 chips) according to Affymetrix GeneChip® System protocols (Affymetrix Inc., Santa Clara, CA). The resulting .CEL files were uploaded to Rosetta Resolver version 6.0 (Rosetta Biosoftware, Seattle, WA). Significant gene lists were generated by one-way ANOVA with Benjamini-Hochberg FDR and a Scheffe post hoc test, at $p<0.05$.

Experimental Design for PFOA and WY Treatment of Mice



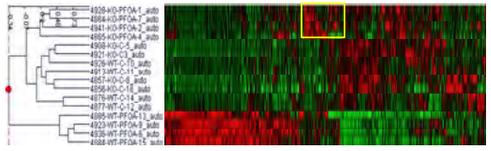
(also see Rosen et al. poster #729) (see poster Lau et al. #866)

Peroxisome Proliferator Chemical Mode of Action



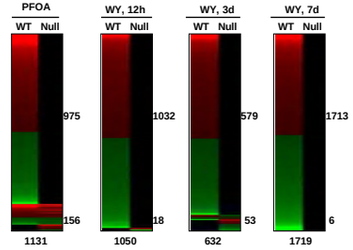
Left panel: the key steps in the mode of action for PPC as discussed in a number of reviews, e.g., Klaunig et al., Crit Rev in Tox 2003. Right panel: all key steps in the mode of action for PPC are abolished in PPARα-null mice based on many studies examining the effects of WY, bezafibrate, phthalates and clofibrate. Based on the phenotypic effects of PFOA exposure, at least some of the effects are likely due to PPARα activation (see posters 866 and 729.)

Two-dimensional Clustering of PFOA Transcript Profiles



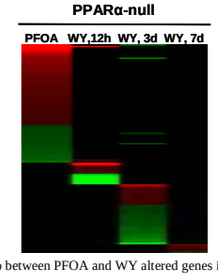
Wild-type mice exposed to PFOA cluster separately from PFOA exposed PPARα-null mice. The control mice from both strains cluster together. The genes in the yellow box are potentially regulated independently of PPARα.

Comparison of Significantly Altered Genes from PFOA and WY-Treated Mice



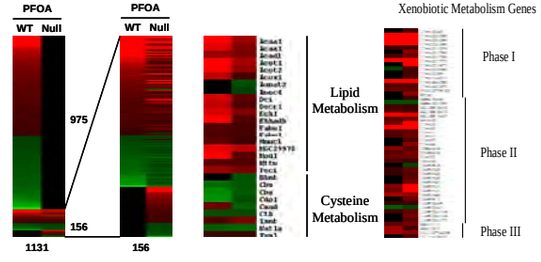
Most of the significant genes are PPARα-dependent. Common up-regulated genes include those involved in fatty acid oxidation and transport, lipid metabolism, cell proliferation and proteome maintenance (data not shown). Common down-regulated genes include those involved in inflammation, acute phase response and coagulation. Approximately 15% of all genes regulated by PFOA are PPARα-independent.

Comparison of PPARα-independent Genes Regulated by WY or PFOA



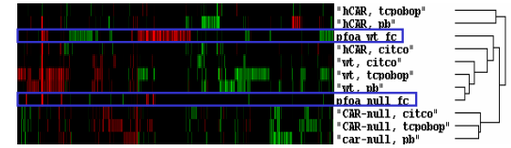
There is little overlap between PFOA and WY altered genes in PPARα-null mice.

Identification of PPARα-independent Genes Regulated by PFOA in Mouse Liver



- Many of the PFOA regulated genes exhibit the same expression behavior in wild-type and PPARα-null mice, including those involved in lipid metabolism (in addition, see poster 729). This leads to the hypothesis that other PPAR isoforms are involved in the PFOA regulation in the absence of PPARα.
- The largest set of PPARα-independent genes regulated by PFOA are involved in xenobiotic metabolism leading to the hypothesis that other nuclear receptors that regulate these xenobiotic metabolism genes are involved

Comparison of PFOA to CAR Inducers in Mouse Liver

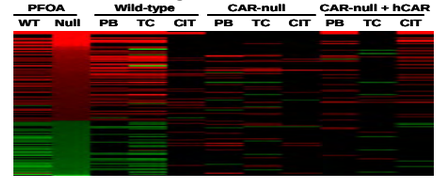


Three strains of mice were used in these experiments: wild-type, CAR-null and CAR-null plus human CAR knock-in. Mice were treated separately with 3 CAR activators (phenobarbital, TCPOBOP and CITCO) each day for 3 days. The CAR experiments were performed in David Moore's lab and .CEL files were from the NURSA website. Gene expression was analyzed as before and compared to the PFOA profiles.

Characteristics of the three chemicals: phenobarbital -- CAR activator but not agonist; TCPOBOP – mouse-specific agonist; CITCO – human-specific agonist.

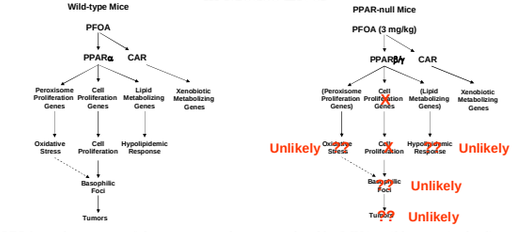
- PFOA in PPARα-null mice looks most like phenobarbital in wild-type mice.

Comparison of PPARα-Independent PFOA Genes to CAR-Regulated Genes



The 156 PFOA PPARα-independent genes were compared directly to those regulated by CAR activators in three different strains of mice. There is a good correlation in terms of both fold-change and direction of change between PFOA PPARα-independent genes and PB and TCPOBOP in wild-type mice. The fact that most of these genes are no longer altered in CAR-null mice supports the involvement of CAR. PB=phenobarbital; TC=TCPOBOP; CIT=CITCO

Proposed Mode of Action for PFOA-Regulated Gene Expression in Mouse Liver



- PFOA regulates many of the same genes that are regulated by WY in wild-type mice leading to the phenotypic responses commonly associated with PPC exposure.
- These responses are abolished in PPARα-null mice.
- PFOA regulates a number of genes independently of PPARα including those involved in lipid metabolism possibly through PPARβ or PPARγ and the xenobiotic metabolism genes by CAR.
- In the absence of PPARα, PFOA is unlikely to induce oxidative stress, the hypolipidemic response or tumors due to the weak gene expression response associated with these phenotypic endpoints.
- Future work will be focused on defining the role of CAR in PFOA responses in the mouse liver.