Toxicology 308 (2013) 129–137

Contents lists available at SciVerse ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Evaluation of perfluoroalkyl acid activity using primary mouse and human hepatocytes☆

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ARTICLE INFO

Article history:
Received 13 February 2013
Received in revised form 22 March 2013
Accepted 25 March 2013
Available online 6 April 2013

Keywords:
Perfluorinated
Primary hepatocyte
Mouse
Human
Gene expression
Biological activity

ABSTRACT

While perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) have been studied at length, less is known about the biological activity of other perfluoroalkyl acids (PFAAs) detected in the environment. Using a transient transfection assay developed in COS-1 cells, our group has previously evaluated a variety of PFAAs for activity associated with activation of peroxisome proliferator-activated receptor alpha (PPARα). Here we use primary heptatocytes to further assess the biological activity of a similar group of PFAAs using custom designed Taqman Low Density Arrays. Primary mouse and human hepatoyctes were cultured for 48 h in the presence of varying concentrations of 12 different PFAAs or Wy14,643, a known activator of PPARα. Total RNA was collected and the expression of 48 mouse or human genes evaluated. Gene selection was based on either in-house liver microarray data (mouse) or published data using primary hepatocytes (human). Gene expression in primary mouse hepatocytes was more restricted than expected. Genes typically regulated in whole tissue by PPARα agonists were not altered in mouse cells including Acox1, Me1, Acaa1a, Hmgcs1, and Slc27a1. Cyp2b10, a gene regulated by the constitutive androstane receptor and a transcript normally up-regulated by in vivo exposure to PFAAs, was also unchanged in cultured mouse hepatocytes. Cyp4a14, Ehhadh, Pdk4, Cpt1b, and Fabp1 were regulated as expected in mouse cells. A larger group of genes were differentially expressed in human primary hepatocytes, however, little consistency was observed across compounds with respect to which genes produced a significant dose response making the determination of relative biological activity difficult. This likely reflects weaker activation of PPARα in human versus rodent cells as well as variation among individual cell donors. Unlike mouse cells, CYP2B6 was up-regulated in human hepatocytes by a number of PFAAs as was PPARδ. Rankings were conducted on the limited dataset. In mouse hepatocytes, the pattern was similar to that previously observed in the COS-1 reporter cell assay. With the exception of PFHxA, longer chain PFAA carboxylates were the most active. The pattern was similar in human hepatocytes, although PFDA and PFOS showed higher activity than previously observed while PFOA showed somewhat less activity. These data reflect inherent challenges in using primary hepatocytes to predict toxicological response.
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1. Introduction

Perfluoroalkyl acids (PFAAs) are stable man-made chemicals that have been widely used to manufacture industrial and consumer products since the 1950's. Health concerns were initially raised more than a decade ago following reports of widespread environmental distribution of perfluorooctane sulfonate (PFOS) (Giesy and Kannan, 2001). Numerous studies have since been published regarding the environmental accumulation and toxicity of perfluorinated chemicals (reviewed by Lau et al., 2007; Lindstrom et al., 2011; Stahl et al., 2011). Of particular concern are the growing number of epidemiological studies which suggest that perfluorooctanoic acid (PFOA) and certain other PFAAs may

☆ The information in this document has been funded by the U.S. Environmental Protection Agency. It has been subjected to review by the National Health and Environmental Effects Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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0300-483X/$ – see front matter. Published by Elsevier Ireland Ltd.
http://dx.doi.org/10.1016/j.tox.2013.03.011

influence human health (reviewed by Olsen et al., 2009; Post et al., 2012; Stahl et al., 2011; Steenland et al., 2010). The associative effects reported in these studies, however, tend to be modest and causality remains unclear due to inconsistencies across studies as well as their reliance on cross-sectional designs (Kerger et al., 2011; Olsen et al., 2009; Savitz, 2007; Steenland et al., 2010). While most studies have focused on either PFOA or PFOS, National Health and Nutrition Examination Survey (NHANES) data indicates that at least four PFAAs are routinely found in human sera; PFOA, PFOS, perfluorononanoic acid (PFNA), and perfluorohexane sulfonate (PFHxS) (Kato et al., 2011).

In general, PFAAs are members of a diverse group of compounds known to activate peroxisome proliferator-activated receptor-alpha (PPARα), although their biological activity extends beyond activation of this single nuclear receptor (Cheng and Klaassen, 2008; Ren et al., 2009; Rosen et al., 2008b; White et al., 2011). Compared to PFOA and PFOS, however, less is known about the relative biological activity of other PFAAs. To address this question, our group has previously utilized a transiently transfected COS-1 luciferase reporter cell assay to examine the activity of various PFAAs with respect to PPARα activation. PFAAs with either carboxylic acid or sulfonic acid side groups and of varying carbon chain length from C4 through C12 were considered in both mouse and human reporter constructs. It was found that murine PPARα was more responsive than human PPARα across the various PFAAs. There was also a tendency for longer carbon chain PFAAs up to C9 to be more robust activators of PPARα than shorter chain PFAAs as well as a tendency for carboxylate PFAAs to be more potent than sulfonate PFAAs (Wolf et al., 2008, 2012).

Here we use primary mouse and human hepatocytes and custom made Taqman arrays to evaluate the same group of PFAAs as previously studied in COS-1 cells. Genes evaluated included those regulated by PPARα as well as transcripts regulated independently of this nuclear receptor. The goal of the study was to provide additional data regarding the relative biological activity of an assorted group of PFAAs. The primary cell model should more closely resemble whole tissue in vivo and offer a direct and timely comparison between rodent and human for species extrapolation in the health risk assessment of this class of chemicals.

Table 1
Genes included on custom mouse TLDA card.

Gene symbol	EntrezGene #	Gene name	Taqman assay
A2m[a]	232345	Alpha-2-macroglobulin	Mm00558642_m1
Acaa1a[a]	113868	Acetyl-Coenzyme A acyltransferase 1A	Mm00728460_s1
Acox1[a]	11430	Acyl-Coenzyme A oxidase 1, palmitoyl	Mm00443579_m1
Actb	11461	Actin, beta, cytoplasmic	Mm00607939_s1
Atf3	11910	Activating transcription factor 3	Mm00476032_m1
B2m	12010	Beta-2 microglobulin	Mm00437762_m1
Baat[a]	12012	Bile acid-Coenzyme A: amino acid N-acyltransferase	Mm00476075_m1
C9[a]	12279	Complement component 9	Mm00442739_m1
Ccnd1	12443	Cyclin D1	Mm00432359_m1
Cpt1b[a]	12895	Carnitine palmitoyltransferase 1b	Mm00487200_m1
Cyp1a1	13076	Cytochrome P450, family 1, subfamily a, polypeptide 1	Mm00487218_m1
Cyp2b10[a]	13088	Cytochrome P450, family 2, subfamily b, polypeptide 10	Mm00456591_m1
Cyp3a11	13112	Cytochrome P450, family 3, subfamily a, polypeptide 11	Mm00731567_m1
Cyp4a14[a]	13119	Cytochrome P450, family 4, subfamily a, polypeptide 14	Mm00484132_m1
Cyp7a1[a]	13122	Cytochrome P450, family 7, subfamily a, polypeptide 1	Mm00484152_m1
Ehhadh[a]	74147	Enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase	Mm00470091_s1
Fabp1[a]	2168	Fatty acid binding protein 1, liver	Mm00444340_m1
Gadd45b[a]	17873	Growth arrest and DNA-damage-inducible 45 beta	Mm00435123_m1
Gapdh	14433	Glyceraldehyde-3-phosphate dehydrogenase	Mm99999915_g1
Gclc	14629	Glutamate-cysteine ligase, catalytic subunit	Mm00802655_m1
Hadha[a]	97212	Hydroxyacyl-Coenzyme A dehydrogenase (trifunctional protein), alpha subunit	Mm00805228_m1
Hmgcs1[a]	208715	3-Hydroxy-3-methylglutaryl-Coenzyme A synthase 1	Mm00524111_m1
Lss	16987	Lanosterol synthase	Mm00461312_m1
Mbl2[a]	17195	Mannose binding lectin (C)	Mm00487623_m1
Me1[a]	17436	Malic enzyme 1, NADP(+)-dependent, cytosolic	Mm00782380_s1
Nfe2l2	18024	Nuclear factor, erythroid derived 2, like 2	Mm00477784_m1
Pdk4[a]	27273	Pyruvate dehydrogenase kinase, isoenzyme 4	Mm00443325_m1
Peci[a]	23986	Peroxisomal delta3, delta2-enoyl-Coenzyme A isomerase	Mm00478725_m1
Pex11a[a]	18631	Peroxisomal biogenesis factor 11a	Mm00478137_m1
Por[a]	18984	P450 (cytochrome) oxidoreductase	Mm00435876_m1
Ppara	19013	Peroxisome proliferator activated receptor alpha	Mm00440939_m1
Ppard	19015	Peroxisome proliferator activator receptor delta	Mm01305433_m1
Pparg	19016	Peroxisome proliferator activated receptor gamma	Mm00440945_m1
Ppargc1a	19017	Peroxisome proliferative activated receptor, gamma, coactivator 1 alpha	Mm00447183_m1
Pxmp4[a]	59038	Peroxisomal membrane protein 4	Mm00480657_m1
Serpina1a[a]	20700	Serine (or cysteine) peptidase inhibitor, clade A, member 1a	Mm02748447_g1
Nos2 (iNos)	18126	Nitric oxide synthase 2, inducible	Mm00440502_m1
Hmox1	15368	Heme oxygenase (decycling)	Mm00516005_m1
Ddit3	13198	DNA-damage inducible transcript 3	Mm00492097_m1
Scd1[a]	20249	Stearoyl-Coenzyme A desaturase 1	Mm00772290_m1
Gstm4[a]	14865	Glutathione S-transferase, mu 4	Mm00728197_s1
Ces1[a]	12623	Carboxylesterase 1	Mm00491334_m1
Abcc4 (Mrp4)	239273	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	Mm01226380_m1
Abcb1b (Mdr1b)	18669	ATP-binding cassette, sub-family B (MDR/TAP), member 1B	Mm00440736_m1
CD36[a]	12491	CD36 antigen	Mm01135198_m1
Slc27a1[a]	26457	Solute carrier family 27 (fatty acid transporter), member 1	Mm00449511_m1
Slco1a1 (Oatp1)	28248	Solute carrier organic anion transporter family, member 1a1	Mm00649796_m1
Mogat1[a]	68393	Monoacylglycerol O-acyltransferase 1	Mm00503358_m1

[a] Regulation previously observed in whole tissue following PFAA exposure.