The effects of perfluorinated chemicals on adipocyte differentiation in vitro

Developmental Toxicology Branch, Toxicity Assessment Division, National Health and Environmental Effects Research Lab, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC 27709, USA

A R T I C L E   I N F O

Article history:
Received 23 June 2014
Received in revised form 24 October 2014
Accepted 24 October 2014
Available online 5 November 2014

Keywords:
PFOA
PFNA
PFHxS
PFOS
PPAR
Adipocyte

A B S T R A C T

The 3T3-L1 preadipocyte culture system has been used to examine numerous compounds that influence adipocyte differentiation or function. The perfluoroalkyl acids (PFAAs), used as surfactants in a variety of industrial applications, are of concern as environmental contaminants that are detected worldwide in human serum and animal tissues. This study was designed to evaluate the potential for PFAAs to affect adipocyte differentiation and lipid accumulation using mouse 3T3-L1 cells. Cells were treated with perfluorooctanoic acid (PFOA) (5–100 μM), perfluorononanoic acid (PFNA) (5–100 μM), perfluorooctane sulfonate (PFOS) (50–300 μM), perfluorohexane sulfonate (PFHXS) (40–250 μM), the peroxisome proliferator activated receptor (PPAR) activator Wyeth-14,643 (WY-14,643), and the PPARα agonist rosiglitazone. The PPAR agonist was included as a positive control as this pathway is critical to adipocyte differentiation. The PPARs agonist was included in the PFAA compounds are known activators of this pathway. Cells were assessed morphometrically and biochemically for number, size, and lipid content. DNA was extracted for qPCR analysis of 13 genes selected for their importance in adipocyte differentiation and lipid metabolism. There was a significant concentration-related increase in cell number and decreased cell size after exposure to PFOA, PFHXS, PFOS, and PFNA. All four PFAA treatments produced a concentration-related decrease in the calculated average area occupied by lipid per cell. However, total triglyceride levels per cell with a concentration-related trend for all compounds, likely due to the increased cell number. Expression of mRNA for the selected genes was affected by all exposures and the specific impacts depended on the particular compound and concentration. Acox1 and Gapdh were upregulated by all six compounds. The strongest overall effect was a nearly 10-fold induction of Scd1 by PFHxS. The sulfonated PFAAs produced numerous, strong changes in gene expression similar to the effects after treatment with the PPARα agonist rosiglitazone. By comparison, the effects on gene expression were muted for the carboxylated PFAAs and for the PPARα agonist WY-14,643. In summary, all perfluorinated compounds increased cell number, decreased cell size, increased total triglyceride, and altered expression of genes associated with adipocyte differentiation and lipid metabolism.

1. Introduction

Perfluorooalkyl acids (PFAAs) are straight chain organofluorine chemicals that are used in commercial applications including paint additives, fire-fighting foams, surfactants, and water and stain repellants. Many PFAAs persist in the environment and numerous studies have demonstrated the environmental accumulation and toxicity of PFAAs (Lau et al., 2007; Lindstrom et al., 2011). The majority of work on PFAAs has been done on perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), but data from the National Health and Nutrition Examination Survey (NHANES) indicate that in addition to PFOA and PFOS, perfluorononanoic acid (PFNA) and perfluorohexane sulfonate (PFHXS) are routinely found in human serum (Kato et al., 2011).

Peroxisome proliferator activated receptors (PPARs) are a class of nuclear receptors with three subtypes, PPARα, PPARβ, and PPARγ, each with distinct expression and physiological roles (Escher and Wahl, 2000). The PPARγ pathway is a major regulator of adipocyte differentiation and lipid metabolism (Casals-Casas and Desvergne, 2011). The PPARα pathway plays a role in maintaining lipid homeostasis directly regulating genes involved in fatty acid uptake and