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Profiles of perfluoroalkyl substances in the liver and serum of patients with liver cancer and cirrhosis in Australia

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ABSTRACT

The present cross-sectional study investigated 12 perfluoroalkyl substances (PFASs) in serum ($n=79$) and liver ($n=66$) samples from patients who had undergone liver transplantation for a range of conditions, such as hepatocellular carcinoma (HCC), cirrhosis due to chronic hepatitis C viral infection (HCV), both HCC and HCV, amyloidosis or acute liver failure. PFAS data from patients were compared to those in control serum ($n=25$) samples from liver donors with no known liver disease and to those in control liver ($n=9$) tissues collected during liver resection surgery. All samples showed detectable PFOS (serum: 0.621–126 ng/mL; liver: 0.375–42.5 ng/g wet wt) and PFOA (serum: 0.437–45.5 ng/mL; liver: 0.101–2.25 ng/g wet wt) concentrations. In general, in paired serum and liver samples, serum had higher PFOS, PFHxS, PFDA, PFNA, and PFOA concentrations than those in explanted livers from patients. These findings also suggest that pathological changes in diseased livers alter the distribution of PFASs between liver and serum. The results from control serum (2007–2008) suggested that PFOS, PFHxS, PFOA, and PFNA concentrations were lower than those previously reported from Australia for 2002–2003, and 2006–2007. The present study demonstrates, for the first time, the detection and comparison of a range of PFASs in the liver of patients with liver cancer and/or liver cirrhosis.

1. Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are anthropogenic compounds that are widely used as surfactants, water and oil repellents, and surface modifiers in a range of household and industrial applications, including fabrics, food packaging, cookwares, pesticides, medical equipment, fire-fighting foams, semiconductors, and plastics (3M, 1998; Giesy and Kannan, 2001). The ubiquity of these PFASs in humans and wildlife was reported in early 2001 (Giesy and Kannan, 2001; Hansen et al., 2001), and subsequent evaluations on the existence of these chemicals have been reported (Ehresman et al., 2007; Kärrman et al., 2007; Hanssen et al., 2010; Keller et al., 2010; Falk et al., 2012). PFASs have special properties that give them different accumulation patterns from those of other persistent organic pollutants (POPs). Unlike other POPs which tend to accumulate in lipids, PFASs preferentially accumulate in protein-rich sites such as blood and liver (Johnson et al., 1979; Maestri et al., 2006; Yoo et al., 2009).

Among PFASs, perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA) are two compounds that have been well characterized. Animals (e.g. rats, mice) exposed to PFOS and PFOA have shown increased expression of fatty acid β-oxidation genes, hepatocyte peroxisome proliferation, hepatomegaly, hepatocellular hypertrophy and hyperplasia, and increased incidence of liver cancer (Klaunig et al., 2003; Kennedy et al., 2004; Lau et al., 2007). Other studies have also documented inhibitory effects of PFAS on gap junctional intercellular communication (Hu et al., 2002) and the promotion of hepatocarcinogenesis (Abdellatif et al., 1990).

Several studies have suggested that the development of hepatomegaly and benign liver tumors in Sprague Dawley rats after chronic dietary exposure to PFOS (Takacs and Abbott, 2007; Wolf et al., 2008; Bijland et al., 2011; Bjork et al., 2011; Elcombe et al., 2012a, 2012b) were related to the activation of PPARα, CAR, and PXR nuclear receptors. In a chronic dietary toxicity and carcinogenicity study

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with PFOS in Sprague Dawley rats, hepatocelluar carcinoma was observed only in females exposed to 20 μg/g dose (Butenhoff et al., 2012). A number of studies have investigated a possible link between PFOS exposure and cancer outcomes in occupationally exposed populations (Alexander et al., 2003; Alexander and Olsen, 2007) and the Danish population (Eriksen et al., 2009). However, a relatively low case-fatality rate in the occupationally exposed workers and non-significant linear trends in the general population of adjusted incidence rate ratios between the occurrence of cancers and plasma concentrations provide inadequate evidence to suggest cancer risks from PFOS exposure (Butenhoff et al., 2012). Whilst concerns of immunotoxicity has been raised as a possible critical effect (DeWitt et al., 2012), epidemiological studies have demonstrated an association between the immune suppression and PFAS concentrations in children (Grandjean et al., 2012; Grandjean and Budtz-Jørgensen, 2013).

The findings in the literature have led to the implementation of new regulations to reduce exposure levels in different countries, for example, chronic exposure levels in the state authorities of Minnesota (Minnesota Department of Health, 2008) and New Jersey (Post et al., 2009). Moreover, based on experimental toxicology data, U.S. and European Food Safety Authority (2008) have issued limits for PFOS and PFOA concentrations in drinking water (Minnesota Department of Health, 2007). PFOS and its precursors have been listed in the Stockholm Convention as POPs since May 2009.

Of interest is the fact that most of the information on PFAS concentrations in human is limited to blood and breast milk; only three studies have reported PFAS concentrations in other human tissues (e.g., liver, brain) in deceased subjects from Italy (Maestri et al., 2006), Spain (Kärrman et al., 2010), and the U.S. (Olsen et al., 2003) (Supplementary information (SI) Table S1). Therefore it is of significance to determine whether there are differences in the levels of PFAS between healthy and diseased individuals which may provide criteria for better evaluation of human health risk assessments. Liver diseases (e.g., hepatocellular carcinoma (HCC) and hepatitis C viral infection (HCV)) are known to cause abnormalities in lipid and lipoprotein metabolism (Jiang et al., 2006; Negro, 2010) as has been reported for plasma lipid profiles which are changed in HCC. Since PFASs have similar structures to those of fatty acids, except that all the hydrogen atoms on the carbon backbone are replaced with fluorine atoms, the levels and distribution of PFASs in individuals having liver diseases might be different from healthy individuals. We therefore investigated the concentrations of 12 PFASs in serum as well as in livers explanted from patients who underwent liver transplantation for a range of conditions such as HCC, cirrhosis due to HCV, both HCC and HCV, and others (including acute liver failure). The results were compared to those in control liver specimens obtained from patients who had undergone liver resection for metastatic cancer. Among the liver and serum samples, there were 57 paired liver and serum samples, thus providing further information on the distribution of PFASs between serum and liver of the same individual. PFAS concentrations in the serum of control subjects (collected in 2007–2008) were compared to two previous studies in which serum samples have been collected in 2002–2003 (Kärrman et al., 2006) and 2006–2007 (Toms et al., 2009) in Australia.

2. Materials and methods

2.1. Sample collection

All human liver and serum samples were obtained from the liver tissue bank at the Victorian Liver Transplant Unit, Austin Health, Melbourne, Australia. Diseased liver tissues ($n=66$) were obtained from explanted livers at the time of liver transplantation. Control liver tissues ($n=9$) were obtained from cancer resected patients and these specimens from resection of a colorectal metastasis were taken well clear of the tumour margin. The control liver samples were histologically normal and used as control tissue in our previously published studies (Paizis et al., 2005; Lubel et al., 2009). From each explanted liver, up to 0.5 g specimen ($n=5$) was removed, frozen, and stored at −80 °C. Tissue and serum samples were kept at −80 °C until analysis. In the present study, liver tissues were selected from four different patient groups according to their primary diagnosis. The patient groups were categorized as: (1) hepatocellular carcinoma [HCC, liver ($n=12$), serum ($n=24$)], (2) cirrhosis with hepatitis C viral infection [HCV, liver ($n=38$), serum ($n=38$)], (3) both HCC and HCV [HCC+HCV, liver ($n=14$), serum ($n=13$)], (4) amyloidosis or acute liver failure [liver, ($n=2$), serum ($n=4$)]. Control group of serum samples was from healthy donors without any known liver disease [control, serum ($n=25$)]. In total, 75 hepatic tissues and 104 serum samples were analyzed. There were 57 paired liver and serum samples; these specimens were procured from 2004 to 2009. The control serum/liver samples were mostly collected between 2007 and 2008. The study protocol was approved by the Human Ethics Committee of Austin Health and performed according to the National Health and Medical Research Council (NHMRC) of Australia Guidelines for experimentation, and according to the principles of the Helsinki Declaration.

2.2. Chemicals

Potassium salts of perfluorodecanesulfonate (PFDS), PFOS, perfluorohexanesulfonate (PFHxS), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), *N*-ethyl perfluorooctanesulfonamidoacetate (EtFOSAA), potassium salts of $^{13}C_4$PFOS and $^{13}C_4$PFOA, $^{13}C_5$PFNA, and $^{13}C_2$PFDA, were purchased from Wellington Laboratories (Guelph, ON, Canada). Oasis® weak anion exchange (WAX; 6 cm^3, 150 mg, 30 μm) solid phase extraction (SPE) cartridges were purchased from Waters (Milford, MA). Milli-Q water was used throughout the experiment. Methanol (residual pesticide and PCB analytical grade), ammonium acetate (97 percent), ammonium solution (25 percent), acetic acid (99.9 percent), acetonitrile (ACN), and sodium carbonate were from Wako Pure Chemical Industries (Osaka, Japan). Tetra-*n*-butylammonium hydrogen sulfate (TBA), methyl *tert*-butyl ether (MTBE), and methanol were also purchased from Sigma (Australia). Purities of all of the analytical standards were ≥98 percent.

2.3. Chemical analysis

A total of twelve PFASs (PFHxS, PFOS, PFDS, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, and EtFOSAA) were extracted using ion-pair extraction (Hansen et al., 2001), followed by WAX-SPE cleanup (Wang et al., 2008), and analyzed by a high-performance liquid chromatograph coupled to a tandem mass spectrometer (HPLC-MS/MS). In brief, 0.8 mL of serum was fortified with 1 ng of ^{13}C mass-labeled standards in a polypropylene (PP) tube. The fortified sample was mixed with 1 mL of 0.5 M TBA solution and 2 mL of buffer (pH 10, 0.25 M). The sample mixture was extracted with 5 mL of MTBE by shaking for 20 min at 250 rpm. The organic and the aqueous layers were separated by centrifugation at 3000 rpm/1500g for 15 min. Four milliliters of MTBE was then removed from the tube and placed in a second PP tube. The extraction was performed twice more, adding and removing 5 mL of MTBE each time and combining it in the second PP tube. The combined extract was concentrated to approximately 1 mL under high-purity nitrogen after the addition of 1 mL methanol. The volume of the sample extract was adjusted by adding appropriate amount of MeOH reaching the mark (1 mL) of the PP tube. Ten milliliters of Milli-Q water were added to the concentrated extract for further WAX-SPE cleanup. The details on WAX cleanup are given elsewhere (Taniyasu et al., 2005; Wang et al., 2008). In brief, each WAX-SPE cartridge was first pre-conditioned by passing a sequence of 4 mL of 0.1 percent ammonium/methanol, 4 mL of methanol, and 4 mL of Milli-Q water through the cartridge at a rate of 2 drops/s (or 1–2 mL/min). After the whole samples were loaded, the cartridges were washed with 4 mL of 25 mM acetate buffer solution (pH 4). The water remaining in the cartridges was removed by centrifugation centrifugation at 3000 rpm/1500g for 2 min. The target analytes were eluted into two separate fractions. The first (F1) and second (F2) fractions were eluted by 4 mL of methanol and 4 mL of 0.1 percent ammonium/methanol, respectively. The eluate was concentrated to 1 mL under a stream of high-purity (>99.999 percent) nitrogen for further instrumental analysis.

The frozen liver samples were thawed at room temperature, and small portions of the liver sample (approximately 0.2∼0.4 g) were homogenized in 1 mL of Milli-Q water with a steel mortar and pestle. The homogenate was transferred to a PP tube and used for extraction after the ^{13}C mass-labeled standards were fortified. The liver sample was extracted as mentioned above and concentrated under a stream of high-purity nitrogen to 0.5 mL before instrumental analysis.