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PCB126 inhibits adipogenesis of human preadipocytes

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ABSTRACT

Emerging evidence indicates that persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs), are involved in the development of diabetes. Dysfunctional adipocytes play a significant role in initiating insulin resistance. Preadipocytes make up a large portion of adipose tissue and are necessary for the generation of functional mature adipocytes through adipogenesis. PCB126 is a dioxin-like PCB and a potent aryl hydrocarbon receptor (AhR) agonist. We hypothesized that PCB126 may be involved in the development of diabetes through disruption of adipogenesis. Using a newly developed human preadipocyte cell line called NPAD (Normal PreADipocytes), we found that exposure of preadipocytes to PCB126 resulted in significant reduction in their subsequent ability to fully differentiate into adipocytes, more so than when the cells were exposed to PCB126 during differentiation. Reduction in differentiation by PCB126 was associated with downregulation of transcript levels of a key adipocyte transcription factor, PPARγ, and late adipocyte differentiation genes. An AhR antagonist, CH223191, blocked this effect. These studies indicate that preadipocytes are particularly sensitive to the effects of PCB126 and suggest that AhR activation inhibits PPARγ transcription and subsequent adipogenesis. Our results validate the NPAD cell line as a useful model for studying the effects of POPs on adipogenesis.

1. Introduction

There is now compelling evidence that exposure to persistent organic pollutants (POPs) is associated with an increased risk of developing metabolic syndrome and its associated pathologies, including diabetes and hypertension (Hotamisligil, 2006; Ruzzin et al., 2010; Thayer et al., 2012). One group of POPs, the Polychlorinated Biphenyls (PCBs), was originally manufactured for industrial applications because of their insulating and flame retardant properties. PCBs are biphenyls with 1–9 chlorines; PCB mixtures can contain up to 209 individual congeners, which differ in the number and pattern of chlorines on the biphenyl rings. While intentional commercial production of PCBs was discontinued in the late 1970s, PCBs are considered significant POPs that continue to accumulate in the environment because of their lipophilicity and persistence (Alonso-Magdalena et al., 2011; Everett et al., 2011; Lee et al., 2011; Narbonne and Robertson, 2014; Roos et al., 2013; Silverstone et al., 2012; Tang-Peronard et al., 2011; Ward et al., 2010). Toxic and biological effects of PCB congeners can vary widely depending on chlorination patterns. Exposure to certain PCB congeners is associated with the development of metabolic syndrome (Everett et al., 2011; Silverstone et al., 2012; Thayer et al., 2012). The coplanar PCBs PCB77 and PCB126, have been associated with the development of glucose intolerance in mice (Baker et al., 2013). However, the mechanisms by which these PCBs potentially cause metabolic syndrome are unknown.

Adipocytes provide a link between obesity and the insulin resistance that occurs in type II diabetes (Mlinar and Marc, 2011). Adipocytes are critical players in energy storage and metabolism. It is becoming clear that adipocyte dysfunction, rather than adipocyte number, is causally associated with the development of metabolic syndrome (Guilherme et al., 2008; Harwood, 2012). Adipocytes in diabetic patients exhibit aberrant production of adipokines, including reduction in secretion of adiponectin, a hormone that modulates a number of metabolic processes (Dunmore and Brown, 2013). Both obesity and lipodystrophies have been reported to cause insulin resistance, suggesting the critical need for functional adipocyte mass.

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The mechanisms by which dysfunctional adipocyte tissue plays a role in the development of insulin resistance and diabetes are now beginning to be understood. Adipokines interact mainly with peripheral tissues (liver, brain, muscle, and pancreas) and regulate carbohydrate and lipid metabolism, energy expenditure, and feeding behaviors. Insensitivity or inability to produce leptin leads to increased fat mass whereas decreased adiponectin causes insulin resistance and increases free fatty acid circulation. Adipocytes producing normal levels of both leptin and adiponectin are thus essential for effective insulin signaling, and their dysfunction leads to disruption of insulin signaling (Mlinar and Marc, 2011). Adipocytes and fat tissue in general accumulate lipophilic toxicants such as PCBs and thus are likely to be affected by them (Regnier and Sargis, 2014).

Adipose tissue is comprised of progenitor preadipocytes and differentiated adipocytes along with other cells which include multipotent mesenchymal stem cells (MSCs) also referred as adipose tissue stem cells (ASCs) (Cawthorn et al., 2012). Although both ASCs and preadipocytes can be differentiated into white adipocytes, the latter are more committed down the lineage to form adipose (Cinti, 2012; Hausman et al., 2001). Preadipocytes also make up a significant portion of fat tissue (15–50%) (Tchkonia et al., 2010). Under normal conditions, adipocyte tissue development begins during gestation and proceeds until adolescence by increased proliferation of preadipocytes and their subsequent differentiation into adipocytes (Knittle et al., 1979). After adolescence, the changes in fat mass are mostly attributed to changes in lipid accumulation with very little change in total cell number (Spalding et al., 2008). During adulthood, adipocyte death is balanced by proliferation and differentiation of preadipocytes to adipocytes (Tchkonia et al., 2010). Adipose tissue alters its mass by increase or decrease in adipocyte size and/or numbers in response to various stimuli. Adipocyte size increases by synthesis and accumulation of lipids. Too much lipid accumulation can lead to hypertrophy and dysfunction. Adipocyte number is increased by the proliferation of preadipocytes that later differentiate into adipocytes.

On physiological and nutritional demand, the preadipocytes are modulated by various hormones and growth factors to initiate a transcriptional cascade that programs adipogenesis (Gregoire et al., 1998). The most important event in this cascade is the transcription and activation of the nuclear receptor peroxisome proliferator-activated receptor-γ (PPARγ), also called the master regulator of adipogenesis (Rosen et al., 1999). PPARγ activates the transcription of genes that are involved in the development of mature functional adipocytes. Alterations in adipogenesis would be expected to lead to adipocyte dysfunction and thus increase the likelihood of developing insulin resistance and, subsequently, type II diabetes (Mlinar and Marc, 2011).

While primary preadipocytes can be isolated from adipose tissue and differentiated into mature adipocytes, they can be expanded for only a very short time *in vitro*, a characteristic that makes it difficult to assess the effects of environmental factors on adipocyte differentiation and function. Most studies have been limited to the use of immortal mouse preadipocytes called 3T3-L1 that differentiate into adipocytes (Green and Kehinde, 1975). Employing this model, certain POPs including PCB congeners were shown to alter adipocyte differentiation and fatty acid and cytokine release when applied to cells during the differentiation process (Arsenescu et al., 2008; Taxvig et al., 2012). Mouse cells provide one means by which the effects of PCBs on adipocytes can be tested. However, there are significant species-to-species variations in their sensitivity and in how PCBs affect physiology and fatty acid metabolism across rodents and humans (Forgacs et al., 2012). Studies using primary human MSCs to assess the effects of POPs on adipogenesis have been described (Li et al., 2008) but they are uncommon, most likely because primary MSCs are difficult to isolate and have a limited lifespan in culture. There have been reports on the immortalization of human MSCs or preadipocytes that retain the ability to differentiate into adipocytes (Darimont et al., 2003; Rodriguez et al., 2004; Terai et al., 2005; Zhang et al., 2006). Immortal MSCs can be differentiated into osteocyte, chondrocyte, or adipocyte lineages and are difficult to maintain in a preadipocyte state (Rodriguez et al., 2004; Terai et al., 2005; Zhang et al., 2006). An immortal human preadipocyte line, referred to as Chub-S7, was reported but, for unknown reasons, has not been widely used (Darimont et al., 2003).

We have recently developed an immortal human preadipocyte cell line from primary subcutaneous preadipocytes that can be readily differentiated into mature adipocytes that accumulate lipid droplets and express all the expected normal markers of adipocyte differentiation (Vu et al., 2013). This provides a valuable tool for the assessment of how POPs such as PCBs affect adipocyte differentiation and function in human cells. In the current study, we were interested in determining how adipogenesis was affected by PCB126, a PCB that has been implicated in the development of diabetes and metabolic disorders. We hypothesized that pre-exposure of human preadipocytes to PCB126 would subsequently reduce their ability to differentiate into mature, properly functioning adipocytes. Our results indicate that exposure of preadipocytes to PCB126 is effective at inhibiting subsequent adipocyte differentiation. We also found that activation of AhR by PCB126 was associated with reduction in PPARγ transcript levels. These results suggest that preadipocytes may be an important target for POPs and point to a potential mechanism in which disruption of adipogenesis could lead to the development of metabolic syndrome.

2. Materials and methods

2.1. Preadipocyte culture and differentiation

All experiments were performed with extended lifespan Normal PreADipocytes (NPADs) developed from primary human preadipocytes that were derived from the subcutaneous fat tissue of a non-diabetic donor as recently described (Vu et al., 2013). The NPADs were cultured as a monolayer in Preadipocyte Basal Medium 2 (PBM-2) (Lonza, MD) supplemented with 10% fetal bovine serum (FBS), L-glutamine, gentamycin, and amphotericin according to the manufacturer's instructions. This media is referred to as preadipocyte growth media 2 (PGM-2). For differentiation, the NPADs were seeded into 35 mm tissue culture plates at 30,000 cells/plate and allowed to grow in PGM-2 until confluent (usually 5–6 days). The cells were then induced to differentiate into adipocytes with differentiation medium consisting of PGM-2 plus dexamethasone, 3-isobutyl-1-methyl-xanthine, indomethacin, and extra insulin prepared according to the manufacturer's instructions (Lonza, MD). The cells were left in the differentiation medium for 11 days until full development of lipid droplets occurred. Comparative control groups of confluent NPADs (preadipocytes) were cultured in normal PGM-2 without any differentiation factors for the duration of the normal time required for differentiation.

2.2. Reagents and treatment

PCB126 was obtained from the Synthesis Core of the Iowa Superfund Research Program (courtesy of Dr. Hans Joachim-Lehmler). The AhR antagonist, CH223191, and all other chemicals were purchased from Sigma unless otherwise specified. PCB126 or CH223191 were dissolved in dimethylsulfoxide (DMSO) to a final concentration less than 0.01% (v/v) unless otherwise stated. Equivalent volumes of DMSO were used in treatments and