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Polychlorinated biphenyls (PCB 101, PCB 153 and PCB 180) alter leptin signaling and lipid metabolism in differentiated 3T3-L1 adipocytes

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ABSTRACT

Non-dioxin-like polychlorinated biphenyls (NDL-PCBs) are highly lipophilic environmental contaminants that accumulate in lipid-rich tissues, such as adipose tissue. Here, we reported the effects induced by PCBs 101, 153 and 180, three of the six NDL-PCBs defined as indicators, on mature 3T3-L1 adipocytes. We observed an increase in lipid content, in leptin gene expression and a reduction of leptin receptor expression and signaling, when cells were exposed to PCBs, alone or in combination. These modifications were consistent with the occurrence of "leptin-resistance" in adipose tissue, a typical metabolic alteration related to obesity. Therefore, we investigated how PCBs affect the expression of pivotal proteins involved in the signaling of leptin receptor. We evaluated the PCB effect on the intracellular pathway JAK/STAT, determining the phosphorylation of STAT3, a downstream activator of the transcription of leptin gene targets, and the expression of SOCS3 and PTP1B, two important regulators of leptin resistance. In particular, PCBs 153 and 180 or all PCB combinations induced a significant reduction in pSTAT3/STAT3 ratio and an increase in PTP1B and SOCS3, evidencing an additive effect. The impairment of leptin signaling was associated with the reduction of AMPK/ACC pathway activation, leading to the increase in lipid content. These pollutants were also able to increase the transcription of inflammatory cytokines (IL-6 and TNFα). It is worthy to note that the PCB concentrations used are comparable to levels detectable in human adipose tissue. Our data strongly support the hypothesis that NDL-PCBs may interfere with the lipid metabolism contributing to the development of obesity and related diseases.

Introduction

There is increasing evidence in the literature correlating the exposure to polychlorinated biphenyls (PCBs) with the onset and incidence of obesity and its related disorders, such as type II diabetes (Airaksinen et al., 2011; Everett et al., 2011; Lee et al., 2011; Silverstone et al., 2012), cardiovascular diseases (Ha et al., 2007), and metabolic syndrome (Uemura et al., 2009). This correlation was supported by experimental studies evidencing that coplanar PCBs impair glucose homeostasis in lean and obese mice (Baker et al., 2013), induce adipocyte differentiation in vitro and promote obesity in vivo (Arsenescu et al., 2008). These evidence suggest a key role of these chemicals in the control of adipose tissue metabolism. The "environmental obesogen hypothesis" postulated the possible involvement of xenobiotic chemicals in the disruption of normal developmental and homeostatic controls over adipogenesis and energy balance (Grün and Blumberg, 2006). Among environmental pollutants, the PCBs, as well as other endocrine disrupting chemicals, may interfere with adipogenesis and lipid metabolism (Bastos Sales et al., 2013; Karoutsou and Polymeris, 2012).

PCBs accumulate in lipid-rich tissues, due to their highly lipophilic character. Therefore, the adipose tissue (AT) constitutes one of the most significant internal reservoir of such persistent organic pollutants (POPs) (Mullerova and Kopecky, 2007) and it has been used to monitor exposure level to PCBs, as well as breast milk and serum (Arrebola et al., 2012; Shen et al., 2012; Yu et al., 2011).

Altering AT differentiation, metabolism and function, these pollutants could affect not only the physiological role of AT, but also the development of obesity-associated diseases (Casals-Casas and Desvergne, 2011; Dirinck et al., 2011; Schug et al., 2011).

Abbreviations: PCBs, polychlorinated biphenyls; NDL, non-dioxin-like; AT, adipose tissue; POPs, persistent organic pollutants; iPCBs, indicator PCBs; Jak, Janus kinase; STAT, signal transducers and activated transcription; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; INS, insulin; DEX, dexamethasone; IBMX, 3-Isobutyl-1-methylxanthine; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ORO, Oil Red O; PPAR, peroxisome proliferator-activated receptor; Ob, leptin; ObRb, leptin receptor; ACC, acetyl-CoA carboxylase; STAT3, signal transducer and activator of transcription 3; PTP1B, protein tyrosine phosphatase 1B; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; PBS, phosphate buffered saline; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

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Overall, dioxin-like (DL) PCBs are a relatively minor component of the total PCB body burden in humans (Cave et al., 2010). Conversely, non-dioxin-like (NDL) PCBs, that are prevalent in the environment, remain largely overlooked in relation to their toxic effects on AT functions (Crinnion, 2011).

Epidemiological and mechanistic studies are necessary to investigate all aspects of NDL-PCB toxicity in order to confirm or modify the maximum residue limits, recently established by EU legislation (EU Commission Regulation, 1259/2011), which referred to the sum of six indicators (iPCBs) (PCBs 28, 52, 101, 138, 153, 180) proposed as markers of PCB contamination. These limits are fixed to protect consumer health from exposure to foods containing PCBs. Experimental studies have been mainly performed to evaluate the accumulation dynamics, storage and release in/from adipose cells of NDL-PCBs (Bourez et al., 2012, 2013; Gallenberg and Vodicnik, 1987).

The majority of functional studies were focused on DL-PCBs or PCB 153, one of the most abundant NDL-PCBs found in humans and environment. However, to date there are no in vitro studies on the effects of other NDL-PCBs (such as PCB 101 and PCB 180), or their combination on adipocyte function or metabolism. Several reasons explain our interest about these congeners. First, PCBs 101, 153, and 180 are three of the six iPCB congeners; second, they are the most frequently detected; and third, they are revealed at high concentrations in human tissues, including adipose tissue (Corsolini et al., 1995; Duarte-Davidson et al., 1994; Malarvannan et al., 2013) and food of animal origin (Domingo and Bocio, 2007; Ferrante et al., 2010; Törnkvist et al., 2011).

Here, we have investigated the effect of NDL-PCBs alone or mixed on mature 3T3-L1 adipocytes by assessing lipid content and leptin sensitivity. Among adipokines, leptin, synthesized and released proportionally to fat mass content (Kallen and Lazar, 1996), plays a pivotal role in maintaining adipose tissue balance/stabilization. In particular, we have investigated the PCB effect on leptin hormone (Ob) and leptin receptor expression (ObRb), evaluating cell responsiveness to the hormone through the analysis of Janus kinase (JAK) 2-signal transducers and activated transcription (STAT) 3 signaling cascade. Moreover, the effects of PCBs on lipid metabolism and storage have been also determined through the modulation of the pathway AMP kinase–acetyl CoA carboxylase (ACC) downstream leptin receptor signaling.

Materials and methods

Chemicals

2,2′,4,5,5′-Pentachlorobiphenyl (PCB 101), 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153) and 2,2′,3,4,4′,5,5′-heptachlorobiphenyl (PCB 180) (99% purity), insulin (INS), dexamethasone (DEX), 3-isobutyl-1-methylxanthine (IBMX), Oil Red O (ORO), isopropanol and DMSO were purchased from Sigma Chemicals Company (Sigma, Milan, Italy). Fetal bovine serum (FBS), tissue culture media and supplements were received from Lonza (Walkerville, MD, USA). All PCBs were dissolved in dimethylsulfoxide (DMSO) in a 10 mM stock solution.

Adipogenic differentiation and cell treatment

3T3-L1 mouse fibroblast cells, purchased from European Collection of Animal Cell Cultures (Salisbury, Wiltshire, U.K.), were maintained in standard Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS and 100 U/mL penicillin and 100 μg/mL streptomycin and cultured in 75-cm^2 cell culture flasks. Cultured cells were allowed to grow to 100% confluence at 37 °C in a humidified 5% CO_2 atmosphere with media changes every 2–3 days. The standard procedure of adipogenic differentiation is shown in Fig. 1. 3T3-L1 cells were cultured to confluence (CF). At the confluence (ID 0), the cells were incubated in adipogenesis-inducing medium (MD I) (DMEM containing 10 μM DEX, 0.5 mM IBMX, 10 μg/mL INS and 10% FBS) for 2 days (ID 2), then in adipogenesis maintaining medium (MD II) (DMEM containing 10 μg/mL INS and 10% FBS) for 2 days (ID 4), followed by DMEM with 10% FBS (MD III) for another 3 days (ID 7) (Madsen et al., 2003). To define the effects of NDL-PCBs on mature adipocytes, the cells were treated for two days (ID 9) with 1 μM PCB, when it was employed alone, 0.5 μM PCBx + 0.5 μM PCBy (1 μM final concentration) for a combination of two PCBs, and lastly 0.33 μM PCB 101, 0.33 μM PCB 153, and 0.33 μM PCB 180 (1 μM final concentration) when used all together. Control cells were incubated with vehicle (DMSO 0.1%).

Oil Red O staining

Preadipocytes were plated into 6-well plates (5×10^4cells/well). After adipogenic protocol and two days of exposure to PCBs, relative lipid content was measured by the Laughton method, slightly modified by Ramirez-Zacarias et al. (1992). Briefly, cell monolayers were rinsed twice with 1× PBS and fixed in 10% (vol/vol) formaldehyde in 1× PBS for 60 min at room temperature. After washing with distilled water 2 times, fixed cells were stained with 1 mL/well of Oil Red O (ORO) working solution for 2 h. This solution was prepared as follows: 0.5 g of ORO was dissolved in 100 mL of absolute isopropanol which was allowed to stand overnight, and filtered through Whatman no. 1 filter paper. The filtrate was diluted with distilled water (6:4 vol/vol), left overnight at 4 °C, and filtered twice. After that, cells were washed three times with distilled water. To assess the lipid accumulation, the ORO dye, which was retained in the cells, was eluted with l mL/well isopropanol, and quantified by measuring the OD at 510 nm using an ELISA reader. The results are expressed relative to control cells.

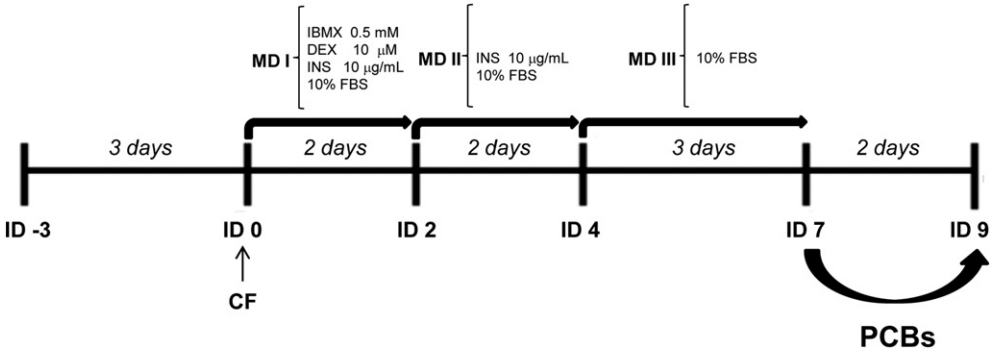

Fig. 1. Protocol for cell differentiation and treatment. The standard procedure of adipogenic differentiation is shown. 3T3-L1 cells were cultured to confluence (CF) for three days. Two days post-confluence (ID0) the cells were incubated in adipogenesis inducing medium (MD I) [DMEM containing 10 μM dexamethason (DEX), 0.5 mM isobutylmethylxanthine (IBMX), 10 μg/mL insulin (INS), and 10% FBS] for 2 days (ID2), then in adipogenesis maintaining medium (MD II) (DMEM containing 10 μg/mL INS and 10% FBS) for 2 days (ID4), followed by MD III medium (DMEM, 10% FBS) for another 3 days (ID7). To define the effects of PCBs on mature adipocytes, the cells were treated with PCBs from ID7 to ID9.