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Pharmacological evaluation of the mechanisms involved in increased adiposity in zebrafish triggered by the environmental contaminant tributyltin

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ABSTRACT

One proposed contributing factor to the rise in overweight and obesity is exposure to endocrine disrupting chemicals. Tributyltin chloride (TBT), an organotin, induces adipogenesis in cell culture models and may increases adipose mass in vivo in vertebrate model organisms. It has been hypothesized that TBT acts via the peroxisome proliferator activated receptor (PPAR)γ-dependent pathway. However, the mechanisms involved in the effects of TBT exposure on in vivo adipose tissue metabolism remain unexplored. Semitransparent zebrafish larvae, with their well-developed white adipose tissue, offer a unique opportunity for studying the effects of toxicant chemicals and pharmaceuticals on adipocyte biology and whole-organism adiposity in a vertebrate model. Within hours, zebrafish larvae, treated at environmentally-relevant nanomolar concentrations of TBT, exhibited a remarkable increase in adiposity linked to adipocyte hypertrophy. Under the experimental conditions used, we also demonstrated that zebrafish larvae adipose tissue proved to be highly responsive to selected human nuclear receptor agonists and antagonists. Retinoid X receptor (RXR) homodimers and RXR/liver X receptor heterodimers were suggested to be in vivo effectors of the obesogenic effect of TBT on zebrafish white adipose tissue. RXR/PPARγ heterodimers may be recruited to modulate adiposity in zebrafish but were not a necessary requirement for the short term in vivo TBT obesogenic effect. Together, the present results suggest that TBT may induce the promotion of triacylglycerol storage in adipocytes via RXR-dependent pathways without necessary using PPAR isoforms.

1. Introduction

A large number of organotin compounds of anthropogenic origin have been in widespread commercial use for many years. Trisubstituted organotins, mostly tributyltin (TBT), triphenyltin, and tricyclohexyltin, used mainly as biocides in marine paints and textile products, fungicides, and plastics manufacturing, are persistent organic pollutants that are ubiquitous in the environment (Okoro et al., 2011; Ayanda et al., 2012; Giriyan and Sonak, 2012; Horiguchi, 2012). Human exposure to butyltins is thought to occur through many sources, including food, house dust, and consumer products. The relative persistence of butyltins, combined with their significant hydrophobicity and small size, has led to severe contamination of ecosystems and accumulation in biological tissues including humans (Fait et al., 1994; Maguire, 1996; Horiguchi, 2012). TBT at concentrations as high as hundreds of nanomoles has been found in human blood (Kannan et al., 1999; Antizar-Ladislao, 2008) and the lipophilicity of organotin compounds favors their toxicity at membrane level, as well as disrupting diverse biological processes, involved in the endocrine, immune, and nervous systems (von Ballmoos et al., 2004; Kotake, 2012; Graceli et al., 2013). As an example, it has been demonstrated in the early 1970s, that TBT has endocrine disrupting potential in inducing imposex among gastropod mollusks (Matthiessen and Gibbs, 1998; Horiguchi, 2012; Sternberg, 2012). In mammals, organotins induced numerous biological effects (Giriyan and Sonak, 2012) including genotoxicity and immunotoxicity (Cima and Ballarin, 2012), neurotoxicity (Reuhl et al., 1985), hepatotoxicity (Ueno et al., 2003a, 2003b) and nephrotoxicity (Robertson et al., 1987).

In vertebrates, the major fat storage compartment is usually white adipose tissue (WAT). The main role of WAT is to store excess dietary calories in triacylglycerol (TAG) form and mobilize these reserves when calorie expenditure exceeds intake. Mature adipocytes are highly-specialized cells with storage and mobilization functions (Ducharme and Bickel, 2008; Rutkowski et al., 2015). Control of

Abbreviations: DBD, DNA-binding domain; DMSO, dimethylsulfoxide; DHA, docosahexaenoic acid; ER, estrogen receptor; HFD, high fat diet; LBD, ligand-binding domain; LXR, liver X receptor; MSC, multipotent stromal stem cell; NR, nuclear receptor; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; SD, standard diet; SL, standard length; TAG, triacylglycerol; TBT, tributyltin chloride; WAT, white adipose tissue; ZOT, zebrafish obesogenic test.

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adipocyte determination and differentiation is a complex process, characterized by the ordered expression of certain genes and specific factors (Gesta et al., 2007; Tang and Lane, 2012). Genes encoding nuclear receptors (NRs) are members of a large superfamily of evolutionarily–related, DNA-binding transcription factors that are well conserved in vertebrates (Markov and Laudet, 2011; Evans and Mangelsdorf, 2014; Zhao et al., 2015). The peroxisome proliferator activated receptor (PPAR) γ (NR1C3) is a critical pro-adipogenic NR transcription factor, known as a master regulator of adipogenesis, which plays an important role in the differentiation and maturation of adipocytes (Farmer, 2006; Tontonoz and Spiegelman, 2008; Janesick and Blumberg, 2011a; Stephens, 2012; Ahmadian et al., 2013). This factor transactivates many adipocyte differentiation gene markers, such as lipoprotein lipase or the gene that codes for fatty acid-binding protein 4 to store fatty acids in the form of TAG in lipid droplets (Rosen et al., 2000).

Obesity is defined as a disease in which abnormal excessive body fat accumulation causes adverse effects on health. Obesogens are a specific class of endocrine-disrupting chemicals that promote obesity by altering adipocyte tissue development, lipid homeostasis, and hormonal physiology (Grün, 2010; Janesick and Blumberg, 2011b; Schug et al., 2011; Tang-Péronard et al., 2011). Obesogens may promote obesity directly or indirectly, increasing fat storage in fat cells and/or the number of adipocytes by altering the lipid homeostasis and energy balance. It was recently demonstrated that obesogens exert some of their biological effects via an NR-dependent pathway, providing a direct molecular link between environmental organotin exposure, endocrine disrupting effects, and adipose tissue development (Janesick and Blumberg, 2011a). TBT promotes adipocyte differentiation in mouse 3T3-L1 preadipocytes (Inadera and Shimomura, 2005; Kanayama et al., 2005; Grün et al., 2006; Li et al., 2011; Pereira-Fernandes et al., 2013) and adipocytogenesis of human and mouse multipotent stromal stem cells (MSCs) via PPARγ-dependent and independent mechanisms, biasing them to the adipocyte lineage at the expense of bone (Grün et al., 2006; Li et al., 2011; Kirchner et al., 2010; Yanik et al., 2011; Biemann et al., 2014). It is usually believed that the endocrine disruptive action of organotins is mediated through the retinoid X receptor (RXR) α (NR2B1)-PPARγ-dependent pathway (Janesick and Blumberg, 2011a; Grün, 2014). Transactivation by organotins was attributed to RXRα (Kanayama et al., 2005; Grün et al., 2006; le Maire et al., 2009). Crystallographic and mass spectroscopic data revealed a covalent interaction between TBT and a critical receptor cysteine residue (C432) at the entrance to the ligand binding pocket of human RXRα (le Maire et al., 2009; Grün, 2014). It has been also demonstrated that organotins bind to PPARγ, principally via a non-covalent, ionic bond between the tin atom and Cys285 (Harada et al., 2015). However, TBT may have a limited agonistic activity toward PPARγ (le Maire et al., 2009; Harada et al., 2015) and a very low one if any toward PPARα and PPARβ/δ or other NRs (Kanayama et al., 2005; Grün et al., 2006).

In vertebrates, TBT has been observed to induce an increase in adipose mass in vivo (Grün et al., 2006; Meador et al., 2011; Penza et al., 2011; Zuo et al., 2011). However, it was difficult to determine whether the increased adiposity, i.e. the amount of WAT, resulted from an increase in the number of adipocyte precursor cells, enhanced adipocyte differentiation from the same number of precursors, an increase in adipocyte size without an increase in number, or a combination of these factors (Grün et al., 2006). Prenatal exposure to environmentally relevant concentrations of TBT also led to transgenerational obesogenic effects (Chamorro-García et al., 2013). However, it should be pointed out that in common laboratory rodents, very many published studies found no increase absolute body weight gains or increase rates of body weight gain after oral ingestion of organotins (e.g. Cooke et al., 2004).

The zebrafish model is widely used for studies on human health and disease and is excellent for studying the lipid metabolism (Hölttä-Vuori et al., 2010). Data on WAT development and physiology are available for this species (Flynn et al., 2009; Imrie and Sadler, 2010; Tingaud-Sequeira et al., 2012) and an obesity syndrome may be induced in adult stages by genetic and dietary factors (Song and Cone, 2007; Oka et al., 2010; Chu et al., 2012: McMenamin et al., 2013; Shimada et al., 2014; Leibold and Hammerschmidt, 2015; Meguro et al., 2015). In addition, semi-transparent zebrafish larvae offer a unique opportunity to study the effects of diet composition, drugs, and environmental contaminants on adipocyte biology and whole-organism adiposity in live animals, using the zebrafish obesogenic test (ZOT) (Tingaud-Sequeira et al., 2011).

This research demonstrated that zebrafish exposed to TBT at nanomolar concentrations responded within hours with adipocyte hypertrophy and higher adiposity without detectable adipocyte hyperplasia. The work presented here is an extension of our initial effort (Tingaud-Sequeira et al., 2011) detailing potential mechanism(s) for TBT effects on adipocytes in an in vivo context. Using ZOT on zebrafish larvae treated with selective human NR agonists and antagonists, adiposity was evaluated by measuring the total surface area of adipocyte lipid droplets in the body before and after treatment in over 3450 individual zebrafish larvae. RXR and RXR/liver X receptor (LXR) dimers were suggested to be in vivo effectors of the short-term obesogenic effect of TBT.

2. Materials and methods

2.1. Animal care and feeding

Wild-type zebrafish (*Danio rerio*) were produced in our facilities in accordance with the French Directive (Ministère de l'Agriculture, de l'Agroalimentaire et de la Forêt) under permit number A33-522-6. All experiments were conducted in conformity with the European Communities Council Directive (2010/63/EU) on the protection of animals used for scientific purposes and local French legislation for the care and use of laboratory animals. Larvae were obtained by natural mating and raised in embryo water (90 μg/ml Instant Ocean [Aquarium Systems, Sarrebourg, France], 0.58 mM $CaSO_4$, $2H_2O$, dissolved in reverse-osmosis purified water) at 28.5 °C with an 11L:13D photoperiod. From 5 days postfertilization until day 15, larvae were fed ad libitum on ZF Biolabs formulated diet flakes (Tres Cantos, Spain). They were then nourished with SD for late larvae (TetraMin Baby, Tetra GmbH, Melle, Germany). The first day of the ZOT protocol was devoted to ad libitum feeding with a hard-boiled chicken egg yolk as HFD. Animal stages were recorded according to standard length (SL), i.e. the distance from the rostral tip of the larva to the base of the caudal fin.

2.2. Agonists and antagonists to NRs and other chemicals used

Tributyltin chloride (TBT) (T50202) and dimethyl sulfoxide (DMSO) (D8418) were purchased from Sigma-Aldrich (St. Louis, MO). PPARγ (hPPARγ) agonist rosiglitazone (71740) (Lehmann et al., 1995) and PPARγ antagonist T0070907 (10026) (Lee et al., 2002) were purchased from Cayman Chemicals (Ann Arbor, MI). ER antagonist ICI182,780 (1047) (Howell et al., 2000), PPARα antagonist GW6471 (4618) (Xu et al., 2002), PPARβ/δ antagonist GSK3787 (3961) (Palkar et al., 2010; Shearer et al., 2010), RXR antagonist UVI3003 (3303) (Nahoum et al., 2007; Pérez Santín et al., 2009), RXR agonists DHA (3687) (de Urquiza et al., 2000; Lengqvist et al., 2004) and SR11237 (3411) (Lehmann et al., 1992), LXR agonist GW3965 (2474) (Collins et al., 2002), RXR/RXR antagonist and RXR/PPARγ agonist LG100754 (3831) (Canan Koch et al., 1996; Cesario et al., 2001; Sato et al., 2010; Pérez et al., 2012) were purchased from Tocris Bioscience (Bristol, UK). PPARβ/δ agonist GW501516 (ALX-420-032) (Oliver et al., 2001) was from Enzo Life Sciences (Farmingdale, NY). LXL antagonist GSK1440233A (GSK2033(17)) (Zuercher et al., 2010) was kindly provided by