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Original Research Article

DDE downregulates PLIN2 expression during differentiation of mesenchymal stem cells into adipocytes in lipid-enriched medium

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ABSTRACT

Evidence indicating, that persistent organic pollutants are involved in the development of obesity, has emerged. The aim of this study was to reveal whether an environmental bioaccumulative human adipose tissue contaminant, 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene (DDE), affects adipocyte differentiation. Our study was conducted on an *in vitro* adipogenic model of human adipose derived mesenchymal stem cells (hADMSC). The adipose cultures were exposed to DDE (concentrations: 0.1 μM, 1 μM, and 10 μM) for 28 consecutive days, from the beginning of the experiment until full differentiation. DDE was administered in lipid vehicle (NuTRIflex). Samples for gene expression analysis by RT real-time PCR were collected on days 0, 4, 10, 21 and 28 during the course of differentiation. Differentiating adipocytes cultivated in lipid-rich medium (NuTRIflex) increased the expression of perilipin 2 (PLIN2). However, the addition of DDE suppressed this effect ($p < 0.03$). Our results may suggest that upregulation of PLIN2, caused by exposure to lipids during the differentiation of adipocytes, is reduced in the presence of DDE. This effect of DDE warrants

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Abbreviations:
DDE, *p,p′*-dichlorodiphenyldichloroethylene
DDT, *p,p′*-dichlorodiphenyltrichloroethane
POPs, persistent organic pollutants
LDs, lipid droplets
TG, triglyceride
PLIN2, perilipin 2
PKA, protein kinase A

future attention, because of the important role of PLIN2 in formation and stabilization of lipid droplets, as the impairment of their function could be linked to the worldwide obesity epidemic.

Introduction

Many organochlorine compounds have been used worldwide as agricultural pesticides. Among those widely used was 1,1′-(2,2,2-trichloroethane-1,1-diyl)bis(4-chlorobenzene) (DDT) and although DDT has not been used in the developed countries since the 1970s, it is still present in the environment. DDE's chemical stability and extreme lipophilicity predispose it to exhibiting bioaccumulative properties similar to other persistent organic pollutants (POPs) with high affinity to adipose tissue, which represents their long-term reservoir. The presence of DDT, and perhaps more importantly that of its metabolic product 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE), is still detected in the food chain today (Maršálek et al., 2013). So it is by no means surprising to find POPs present in human tissues (Hardell et al., 2010; Waliszewski et al., 2012).

Recently, epidemiological studies have revealed that elevated serum concentrations of certain POPs, including DDE, correlate positively with increased type 2 diabetes prevalence in humans (Taylor et al., 2013). DDE's influence has been confirmed to be both diabetogenic and obesogenic (Dirinck et al., 2014). Studies of subacute exposure to DDE on mice models have demonstrated that DDE causes significant hyperglycaemia (Howell et al., 2014). The effect of chronic exposure to DDE in conjunction with a high fat diet was found to be biphasic; after initial promotion of fasting hyperglycaemia, glucose levels decreased and by week 13 animals chronically exposed to DDE were normoglycemic (Howell et al., 2015). Alongside their part in lipid metabolism, adipocytes may facilitate cancer progression by playing an important role in the tumor microenvironment (Omabe et al., 2015).

The specific mechanism of POPs' action is not yet known. POPs are almost exclusively stored within the lipid droplets (LDs) of adipocytes and they depend on triglyceride (TG) content (Bourez et al., 2012; Hong et al., 2012). LDs' size in adipocytes reflects the degree of their differentiation and the intensity of intracellular metabolic processes. The TG core of LDs is surrounded by phospholipids and a variety of proteins (Tauchi-Sato et al., 2002). One such protein is perilipin 2 (PLIN2), formerly known as adipose differentiation-related protein (ADRP). PLIN2 has long been recognized as a universal marker for intracellular LD in tissues, but its function is just beginning to be elucidated. It has been discovered one of PLIN2's domains is involved in LD stabilization and lipid accumulation (Sentinelli et al., 2015). These findings are supported by the fact, that upregulation of PLIN2 is associated with TG storage in LDs. As adipocytes mature, they gain neutral lipids and PLIN2 is replaced by PLIN1 (Wolins et al., 2003, 2005).

In order to verify the hypothesis that DDE may influence adipocyte differentiation and metabolism, adipocytes, during their differentiation from human adipose derived mesenchymal stem cells (hADMSC), were exposed to DDE. Our study is the first to demonstrate that upregulation of PLIN2, caused by lipids during differentiation of hADMSC, was reduced by DDE.

Materials and methods

Cell culture and differentiation

For adipogenic differentiation, hADMSCs (Invitrogen Life Technologies GmbH, Darmstadt, Germany) were seeded with a total number of 1×10^5 cells in a 6-well cell culture plate (TPP Techno Plastic Products, Switzerland), and cultured in 5% CO_2 atmosphere at 37 °C according to the manufacturer's instructions in StemPro® Adipogenesis Differentiation medium supplemented with 1% Gentamicin.

Cell DDE treatment

The cultures were exposed to DDE (Sigma–Aldrich, St. Louis, MO, USA) for 28 consecutive days from the start of the experiment until full differentiation was achieved (DDE concentrations: 0.1 μM, 1 μM, and 10 μM). The concentrations of DDE were chosen to reflect the levels measured in humans (Dirinck et al., 2014). DDE was administered in the lipid fraction of NuTRIflex® Lipid peri (B. Braun, Melsungen, Germany), present in 0.2% (v/v) concentration (0.2 ml of vehicle per 100 ml of medium), containing medium chain triglycerides (0.1 g/l) and soya bean oil (0.1 g/l). Control cultures received the differentiation medium alone or with vehicle without DDE. The samples for quantitative estimation of mRNA were taken on days 0, 4, 10, 21 and 28. Fig. 1 shows the cell cultivation design used in the pilot trial and in the subsequent triplicate experiment.