Pesticide Biochemistry and Physiology 131 (2016) 40–45

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

4,4′-Dichlorodiphenyltrichloroethane (DDT) and 4,4′-dichlorodiphenyldichloroethylene (DDE) promote adipogenesis in 3T3-L1 adipocyte cell culture

Jonggun Kim [a,1], Quancai Sun [b,1], Yiren Yue [b], Kyong Sup Yoon [c], Kwang-Youn Whang [a], J. Marshall Clark [d], Yeonhwa Park [b,*]

[a] Division of Biotechnology, Korea University, Seoul 136-713, Republic of Korea
[b] Department of Food Science, University of Massachusetts, Amherst, 102 Holdsworth Way, Amherst, MA 01003, United States
[c] Department of Biological Sciences and Environmental Sciences Program, Southern Illinois University, Edwardsville, IL 62026, United States
[d] Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, 637 North Pleasant Street, Amherst, MA 01003, United States

ARTICLE INFO

Article history:
Received 1 September 2015
Received in revised form 12 January 2016
Accepted 18 January 2016
Available online 20 January 2016

Keywords:
DDT
DDE
3T3-L1
Adipogenesis
Adipocyte differentiation
Lipogenesis

ABSTRACT

4,4′-Dichlorodiphenyltrichloroethane (DDT), a chlorinated hydrocarbon insecticide, was extensively used in the 1940s and 1950s. DDT is mainly metabolically converted into 4,4′-dichlorodiphenyldichloroethylene (DDE). Even though most countries banned DDT in the 1970s, due to the highly lipophilic nature and very stable characteristics, DDT and its metabolites are present ubiquitously in the environment, including food. Recently, there are publications on relationships between exposure to insecticides, including DDT and DDE, and weight gain and altered glucose homeostasis. However, there are limited reports regarding DDT or DDE and adipogenesis, thus we investigated effects of DDT and DDE on adipogenesis using 3T3-L1 adipocytes. Treatment of DDT or DDE resulted in increased lipid accumulation accompanied by increased expression of CCAAT/enhancer-binding protein α (C/EBPα), peroxisome-proliferator activated receptor-γ (PPARγ), fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), adipose triglyceride lipase, and leptin. Moreover, treatment of DDT or DDE increased protein levels of C/EBPα, PPARγ, AMP-activated protein kinase-α (AMPKα), and ACC, while significant decrease of phosphorylated forms of AMPKα and ACC were observed. These finding suggest that increased lipid accumulation caused by DDT and DDE may mediate AMPKα pathway in 3T3-L1 adipocytes.

1. Introduction

4,4′-Dichlorodiphenyltrichloroethane (DDT), the most important chlorinated hydrocarbon insecticide ever marketed, was widely and extensively used in the 1940s and 1950s to control insects for protection of both agriculture and human health [1,2]. In fact, DDT is still used in malaria-endemic areas (sub-Saharan Africa and India) to control malaria vectors, although most countries banned the use of DDT in the 1970s [3]. Its insecticidal properties were first discovered by Paul Müller in 1939, and it acts on the nervous system through modulation of voltage-sensitive sodium channels (VSSCs) described later by Narahashi and Yamasaki [1,2,4]. This modulation is known to cause the characteristic symptom called "DDT jitters", which can be best described as the repetitive discharge in the nerve that leads to whole body tremors as a result of increased "depolarizing after-potentials" [2,4,5]. It is known that DDT can be metabolically converted into 4,4′-dichlorodiphenyldichloroethane (DDD) and 4,4′-dichlorodiphenyldichloroethylene (DDE) by reductive dechlorination and dehydrochlorination, where the latter is the more common metabolite of DDT [6]. Evidence of DDT and its metabolites and eggshell thinning in certain bird populations drew significant awareness of these compounds [7]. It has been suggested that this is due to the inhibition of prostaglandin synthase, however, others suggested multiple mechanisms contributing to eggshell thinning by DDT and its metabolites [8–11].

The indiscriminate use of DDT in earlier times along with the highly lipophilic nature and extremely slow degradation of this compound and its metabolites in vivo and in vitro as well as in the environment have resulted in the ubiquitous distribution of these compounds in the environment including food, especially in fatty foods [2,12,13]. Along with other environmental contaminants, DDT and DDE are currently defined as endocrine disruptors [14–18]. Moreover, a growing body of literature shows relationships between exposure to insecticides, including DDT and DDE, and adverse health effect in humans such as weight gain and

Abbreviations: AKT, protein kinase B; AMPKα, AMP-activated protein kinase-α; ACC, acetyl-CoA carboxylase; ATGL, adipose triglyceride lipase; C/EBPα, CCAAT/enhancer-binding protein α; FAS, fatty acid synthase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GLUT 4, glucose transporter 4; HSL, Hormone sensitive lipase; LPL, lipoprotein lipase; PPARγ, peroxisome-proliferator activated receptor-γ; TG, triglyceride.

* Corresponding author at: Department of Food Science, University of Massachusetts, 102 Holdsworth Way, Amherst, MA 01003, United States.
E-mail address: ypark@foodsci.umass.edu (Y. Park).

[1] J. Kim and Q. Sun contributed equally.

http://dx.doi.org/10.1016/j.pestbp.2016.01.005
0048-3575/

altered glucose homeostasis [19–34]. In particular, Lee et al. [30] reported that the serum levels of persistent organic pollutants including DDE positively correlated with body mass index (BMI). Currently there are two publications investigating the role of DDT or DDE on adipogenesis using either 3T3-L1 or 3T3-F442A adipocytes [35,36]. Moreno-Aliaga and Matsumura [36] reported that DDT promoted adipocyte differentiation by modification of key transcription factors of this process, such as CCAAT/enhancer-binding protein β (C/EBPβ), peroxisome-proliferator activated receptor-γ (PPARγ), and C/EBPα. Others reported the role of DDE and other organochlorine insecticides, oxychlordane and dieldrin, on adipogenesis, fatty acid uptake and adipokine productions in 3T3-L1 adipocytes. Based on these findings, along with our previous reports that other types of insecticides promoted adipogenesis in 3T3-L1 adipocytes [37–39], we determined the role of DDT and DDE in adipogenesis in this model.

2. Materials and methods

2.1. Materials

3T3-L1 pre-adipocytes were purchased from the American Type Culture Collection (Manassas, VA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), bovine serum (BS), methylisobutylxanthine, dexamethasone, insulin, dimethyl sulfoxide, DDT, DDE, and protease inhibitor cocktail were purchased from Sigma-Aldrich Co. (St. Louis, MO). The amounts of triglyceride (TG) and protein were quantified using kits from Genzyme Co. (Cambridge, MA) and Bio-Rad Co. (Hercules, CA), respectively. Radioimmunoprecipitation assay (RIPA) buffer with EDTA and EGTA was purchased from Boston Bioproducts Inc. (Ashland, MA). Primary rabbit antibodies for PPARγ and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Primary rabbit antibodies for acetyl-CoA carboxylase (ACC), phosphorylated ACC (pACC), AMP-activated protein kinase-α (AMPKα), phosphorylated AMPKα (pAMPKα), and CCAAT/enhancer-binding protein α (C/EBPα) and horseradish peroxidase conjugated goat anti-rabbit IgG (the secondary antibody) were purchased from Cell Signaling Technology (Beverly, MA).

2.2. Cell culture

The preadipose cell line 3T3-L1 was originally developed by clonal expansion from murine Swiss 3T3 cells, and has been widely used as an in vitro model for adipogenesis and the biochemistry of adipocytes [40]. 3T3-L1 pre-adipocytes were cultured as previously described with minor modifications [41]. Briefly, 3T3-L1 pre-adipocytes were maintained at 37 °C in Dulbecco's modified Eagle's medium (DMEM) containing 10% bovine serum (BS) to confluence. At two days after confluence (designated as day 0), adipocyte differentiation was induced with a mixture of methylisobutylxanthine (0.5 mM), dexamethasone (1 μM), and insulin (1 μg/ml) in DMEM containing 10% FBS. On day 2, this medium was replaced with DMEM containing 10% FBS and insulin (1 μg/ml). On day 4 and thereafter, medium consisting of DMEM plus 10% FBS was subsequently replaced with fresh medium at 2 day intervals for 4 days.

Cells were treated with either DDT or DDE at final concentrations of 10 or 20 μM by adding a stock solution of 100 mM in dimethyl sulfoxide. DDT or DDE treatment of 3T3-L1 cells started at day 0 and cells were harvested at day 8. Control was treated with dimethyl sulfoxide only and all treatments had dimethyl sulfoxide at a final concentration of 0.02%. These concentrations of DDT/DDE had no effects on cell viability as measured by a 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) based assay (data not shown) [42].

2.3. Triglyceride quantification

After 8 days of adipogenic differentiation, cells were washed twice with phosphate-buffered saline (PBS) and harvested by scraping in PBS containing 1% Triton-X. Cells were sonicated to obtain homogenous samples. After centrifugation at 500 ×*g* for 5 min at 4 °C, the amount of triglyceride (TG) in the supernatant was measured using a colorimetric assay (Triglyceride-SL assay kit; Genzyme Diagnostics, Charlottetown, PE, Canada). Protein concentrations were measured by using the Bio-Rad protein DC assay kit (Bio-Rad, Hercules, CA, USA) with bovine serum albumin as a standard, and TG content was normalized with protein concentration.

2.4. mRNA expression analysis

Total RNA was extracted from cells using TRIzol® reagent under RNase free conditions. Total RNA was reverse-transcribed using Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase (High-Capacity cDNA Reverse Transcription Kit, Applied Biosystems). mRNA expression levels of CCAAT/enhancer-binding protein α (C/EBPα), PPARγ, acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), hormone-sensitive lipase (HSL), lipoprotein lipase (LPL), adipose triglyceride lipase (ATGL), and leptin were analyzed from 3T3-L1 adipocytes. Real-time PCR was performed using a StepOne Plus real time PCR machine (Applied Biosystems, Carlsbad, CA) and TaqMan® probe-based gene expression analysis (Applied Biosystems, Carlsbad, CA). Respective integrated sequences for TaqMan® gene expression were NM_007678.3 (C/EBPα), NM_001127330.2 (PPAR-γ), NM_133360.2 (ACC), NM_007988.3 (FAS), NM_001039507.1 (HSL), NM_008509.2 (LPL), NM_001163689 (ATGL), NM_009204.2 (GLUT 4), and NM_008493.3 (leptin), with glyceraldehyde-3-phosphate dehydrogenase (GAPDH, NM_008084.2) as an internal standard. Threshold values were analyzed using comparative CT (ΔΔCT) method [43].

2.5. Immunoblotting

After treatment, cells were lysed using RIPA buffer supplemented with protease inhibitor cocktail and phosphatase inhibitor cocktail (Thermo Scientific, Rockford, IL). Protein concentrations were determined using the protein DC assay kit (Bio-Rad Co., Hercules, CA) with bovine serum albumin standard. Aliquots from the cell lysates containing 37 μg of protein were separated using 8% or 10% SDS-polyacrylamide gel and transferred to an Immobilon P membrane (Millipore, Bedford, MA). Primary antibodies from rabbits were diluted as recommended by producers. GAPDH was used as an internal control to normalize protein content. Horseradish peroxidase conjugated goat anti-rabbit IgG was used as the secondary antibody. Detection was performed using a Clarity™ Western ECL Substrate Kit (Bio-Rad Co., Hercules, CA) with an Image Station 4000MM (Carestream Health, New Haven, CT) and image and results were quantified using Image J software.

2.6. Statistical analyses

Data were analyzed by the analysis of variance procedure (ANOVA) using the Statistical Analysis System (SAS Institute, Cary, NC). Significant differences between treatments were determined using Tukey's multiple-range test. Significance of differences was defined at the $P < 0.05$ level.

3. Results

Fig. 1 shows the effects of DDT and DDE on triglyceride accumulation in 3T3-L1 adipocytes. When cells were treated with either of these compounds there was significant increase in TG accumulation at 10 μM of DDT and both 10 and 20 μM of DDE compared to control in this model.