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Exposure to p,p′-DDE enhances differentiation of 3T3-L1 preadipocytes in a model of sub-optimal differentiation

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HIGHLIGHTS

- 3T3-L1 cells exposed to p,p′-DDE and differentiated under sub-optimal conditions.
- Lipid accumulation and expression of markers of differentiation were assessed.
- p,p′-DDE enhanced lipid and triglyceride accumulation.
- p,p′-DDE enhanced expression of markers and mediators of adipogenesis.

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ABSTRACT

The incidence of obesity is increasing worldwide at an alarming rate. Recently, exposure to environmental contaminants, especially organochlorines such as p,p′-dichlorodiphenyldichloroethylene (DDE), has been implicated as a possible causative factor in the increasing obesity epidemic. The objective of this study was to evaluate the ability of DDE to alter adipogenesis in a model of sub-optimal differentiation. 3T3-L1 preadipocytes were induced to differentiate in the presence of DDE (0.01–100 μM) using a sub-optimal differentiation cocktail. Eight days after the initiation of differentiation, adipogenesis was assessed through neutral lipid staining, triglyceride accumulation, and expression of markers of terminal differentiation. Exposure to DDE induced a concentration dependent increase in intracellular neutral lipid accumulation as determined by Oil Red O staining and triglyceride assay. Alterations in lipid accumulation were accompanied by upregulation of genetic markers of differentiation. DDE (10 μM) enhanced expression of fatty acid binding protein 4 and Sterol regulatory element-binding protein-1c at the 2.5 and 20 μM concentrations. DDE (2.5, 10, and 20 μM) induced upregulation of leptin and fatty acid synthase, as compared to sub-optimal vehicle control (0.05% ethanol). Our results indicate that DDE is capable of enhancing adipogenesis and intracellular lipid accumulation in 3T3-L1 cells through upregulation of molecular targets responsible for lipid storage.

1. Introduction

The incidence of obesity and overweight has increased dramatically since the 1980's and is contributing to a major health crisis worldwide. Relative to normal weight, obesity is associated with increased relative risk of coronary artery disease, hypertension, insulin resistance, type 2 diabetes (T2D), all-cause mortality, and even some cancers (Hotamisligil, 2006; Ogden et al., 2014; Okwan-Duodu et al., 2013). The most commonly used metric for determining obesity is body mass index (BMI), or weight in kilograms divided by height in meters squared. More than two thirds (68.5%) of all adults in the United States are considered overweight or obese, defined as having a BMI >25 kg/m^2 and

Abbreviations: MTT, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide; Actb, β-actin; BMI, body mass index; DDE, p,p′-dichlorodiphenyldichloroethylene; FABP4, fatty acid binding protein 4; Fasn, fatty acid synthase; Slc2a4, glucose transporter type 4; Lep, leptin; IBMX, methylisobutylxanthine; RT-qPCR, quantitative real-time polymerase chain reaction; ORO, Oil red O; OC, organochlorine; Pparg, peroxisome proliferator activated receptor-γ; Srebf1, sterol regulatory element-binding protein-1c; T2D, type 2 diabetes.

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>30 kg/m^2, respectively, with more than one third (34.9%) of the population being obese (Ogden et al., 2014).

Adipose tissue has long been recognized as the storage depot for triglycerides, derived from excess nutritional energy, stored in lipid droplets inside white adipocytes in many different white adipose tissue depots. While these depots were once thought to be inert, adipose is now recognized as an active regulatory tissue that helps maintain lipid and glucose homeostasis (Guilherme et al., 2008). Obesity results from adipocyte hyperplasia and hypertrophy which leads to recruitment of immune cells to adipose tissue and eventually contributes to localized chronic low-grade inflammation and adipocyte dysfunction (McGinty and Young, 2011; Suganami and Ogawa, 2010). In the early stages of weight gain, excess energy is stored as lipid in mature adipocytes that expand in size. Afterward, there is a rapid increase in the number of fat cells as preadipocyte fibroblasts undergo proliferation and differentiation, a process called adipogenesis, to increase the number of mature fat cells (Lowe et al., 2011).

It is unlikely that lifestyle and dietary changes alone can account for the dramatic rise of overweight and obesity worldwide. Even animal populations that live in close contact with humans in industrialized nations have become prone to developing obesity. It has been estimated that the likelihood of this trend occurring purely by chance in animal populations at 1 in 12 million, indicating that an exogenous environmental factor is probably contributing to this development (Klimentidis et al., 2010). Recent research has suggested a strong association between chemical exposure and development of T2D and obesity (Lee et al., 2006; Longnecker and Daniels, 2001; Wang et al., 2010). These chemicals, described as "obesogens," are suspected to contribute to the development of obesity by promoting lipid storage in mature adipocytes or by directly increasing the number of fat cells in adipose tissue (Li et al., 2012). One of the major groups of pesticides implicated in this research are the legacy organochlorine (OC) insecticides such as DDT and chlordane; these OC insecticides were used heavily during the 1950's and '60's, but have been banned in the US since the early 1970's; however, legacy OC insecticides and their metabolites can still be found in serum samples and fatty substances, such as breast milk, in human populations both within the United States and abroad (Rylander et al., 2006). A number of cross sectional epidemiological studies have linked exposure to DDE with T2D and obesity. Recent human cohort studies have established an increased relative risk of developing T2D in individuals with the highest exposure levels (Lee et al., 2010, 2011). Additionally, recent *in vivo* exposure studies in rodents indicate that a high fat diet supplemented with persistent organic pollutants, including DDE, resulted in increased visceral adiposity and increased macrophage infiltration when compared to high fat diet alone (Ibrahim et al., 2011; Ruzzin et al., 2010). Due to its association with increased risk of obesity and obesity-related ailments, DDE exposure has been suggested as a potential biomarker for increased risk of disease, but its utility as a biomarker could be markedly enhanced if a mechanism were to be identified. As such, several *in vitro* studies have indicated a role for OC exposure and enhanced adipogenesis and altered lipid metabolism in mature adipocytes. It has been reported that p,p′-DDT was able to enhance differentiation in 3T3-L1 or 3T3-F44A2 adipocytes (Moreno-Aliaga and Matsumura, 2002). Previous data from our lab found that exposure to DDE significantly increased basal fatty acid uptake and release of leptin, adiponectin, and resistin from mature adipocytes. However, in our previous study, DDE was unable to enhance adipogenesis during the differentiation of 3T3-L1 cells, possibly due to the use of a potent adipogenic cocktail that induced approximately 95% differentiation (Howell and Mangum, 2011).

In the present study, we furthered our previously published work examining the adipogenic effect of DDE exposure utilizing a sub-optimal differentiation cocktail and the previously utilized potent differentiation cocktail as a full differentiation, positive control. Through the use of the sub-optimal cocktail, we are now able to investigate the adipogenic potential of DDE in the absence of high background differentiation as performed in other studies examining the adipogenic effects of various compounds (Tung et al., 2014). Differentiating 3T3 cells were exposed to increasing concentrations of DDE in the presence of the sub-optimal and full strength adipogenic cocktail; then the accumulation of neutral intracellular lipid and triglyceride content was measured via Oil Red O (ORO) staining and commercial triglyceride assay, respectively. Finally, expression of markers and mediators of terminal adipocyte differentiation were assessed by two-step quantitative real-time polymerase chain reaction (RT-qPCR) to evaluate the potential mechanisms underlying any changes to adipogenesis observed in DDE treated cells.

2. Materials

Methylisobutylxanthine (IBMX), dexamethasone, and dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich (St. Louis, MO). Stock solutions of IBMX (500 mM) were prepared in DMSO while dexamethasone was dissolved in ethanol (1 mg/ml) and diluted in 49 ml sterile medium. Solutions were aliquoted and stored at −20 °C. Triglyceride and MTT were from Cayman Chemical and Bradford reagent was from Bio-Rad Co. p,p′-DDE was purchased from ChemService (West Chester, PA).

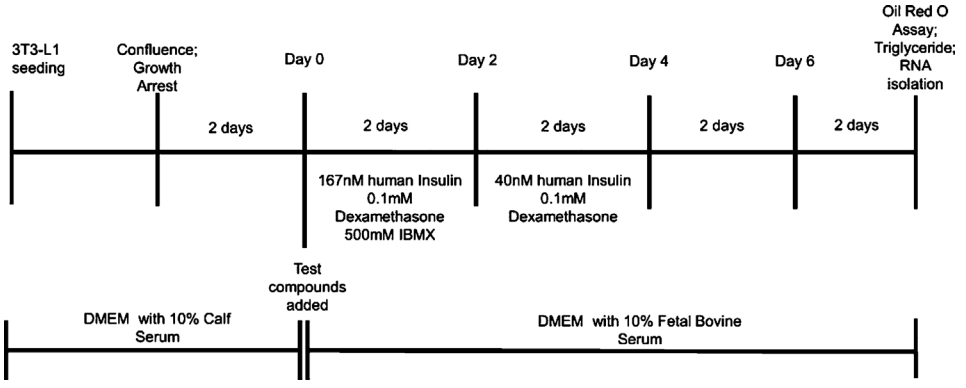

Fig. 1. 3T3-L1 exposure protocol. Sub-optimal differentiation protocol for 3T3-L1 cells.