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In Vitro effect of DDE exposure on the regulation of lipid metabolism and secretion in McA-RH7777 hepatocytes: A potential role in dyslipidemia which may increase the risk of type 2 diabetes mellitus

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ABSTRACT

Organochlorine compounds (OC), such as the legacy insecticides, were widespread environmental contaminants. OC including dichlorodiphenyldichloroethylene (DDE), a metabolite of the insecticide DDT, have an epidemiological association with type 2 diabetes mellitus (T2D) and may play a role in risk factors that contribute to T2D such as dyslipidemia. The liver, a potential target for DDE, plays a role in dyslipidemia. The *in vitro* effect of DDE on hepatocyte lipid metabolism and secretion was investigated using McArdle-RH7777 (McA) rodent hepatoma liver cells. When stimulated by the free fatty acid oleic acid (OA), DDE increased the secretion of apolipoprotein B (ApoB) suggesting a role for DDE in increasing lipid secretion. Intracellular protein levels of microsomal triglyceride transfer protein (MTP) were increased while sortilin-1 (Sort-1) levels were decreased suggesting a role for DDE in increasing lipid transport and decreasing lipid degradation. Neutral lipids such as intracellular triglycerides (TG) were decreased suggesting that DDE may alter lipid accumulation in liver cells. DDE may play a role in dyslipidemia by affecting mechanisms that regulate lipid metabolism and secretion. These *in vitro* results on biochemical markers of liver cell dyslipidemia support the concept that DDE exposure may play a role in the dyslipidemia frequently observed in T2D.

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1. Introduction

T2D is a metabolic disease characterized by risk factors such as hyperglycemia, insulin resistance, and dyslipidemia (Keane et al. 2011). Since T2D prevalence has been increasing concurrently with the increasing use of synthetic chemicals (Neel and Sargis 2011), attention is being given to possible associations with environmental chemical exposure (Lee et al. 2006; Carpenter 2008). Many organochlorine compounds (OC) were used as insecticides worldwide until banned for their adverse effects on human health and the environment (Wastler et al. 1975; Longnecker et al. 1997). Environmental exposure to the OC contaminant dichlorodiphenyldichloroethylene (DDE), a persistent metabolite of the insecticide dichlorodiphenyltrichloroethane (DDT), has been associated with type 2 diabetes mellitus (T2D) in epidemiological studies and it may play a role in risk factors that contribute to the disease, such as dyslipidemia (Lee et al. 2006, 2007, 2011; Eden et al. 2014). Dyslipidemia, a state of altered lipid levels, plays a role in the progression of insulin resistance and T2D (Rader 2007). The liver, which plays a role in regulating lipid metabolism and secretion, is a potential target of DDE (Mulvihill et al. 2009), and a disruption of liver function by DDE exposure may contribute to dyslipidemia. The purpose of this study was to investigate *in vitro* changes in free fatty acid induced lipid metabolism and secretion using McArdle-RH7777 (McA) hepatocytes exposed to DDE by measuring biochemical markers of dyslipidemia: apolipoprotein B (ApoB), microsomal triglyceride transfer protein (MTP), sortilin-1 (Sort-1), and triglycerides (TG).

ApoB is a lipoprotein molecule synthesized in the liver, important in lipid transport and clearance, and is needed for the synthesis and secretion of TG containing lipids such as very low-density lipoproteins (VLDL) from the liver (Boren et al. 1994). The increased secretion of ApoB molecules increases circulatory levels of TG, cholesterol, and VLDL particles thereby promoting dyslipidemia (Julius 2003). ApoB levels are significantly higher in individuals exhibiting dyslipidemia and T2D (Qiu et al. 2006).

TG are synthesized as a product of free fatty acid hydrolysis by liver enzymes including triacylglycerol hydrolases/carboxylesterases (TGH) (Lehner and Verger 1997). TG, a secondary energy source after carbohydrates for cellular functions (Howard 1987), are important in the transformation of nascent ApoB to the mature molecule, which is then secreted by the liver or small intestine into the bloodstream (Sparks et al. 2012). TG are a biochemical marker implicated in T2D since levels

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are usually higher in individuals with diabetes compared to non-diabetics (Albrink and Man 1958).

The rate-limiting step for ApoB synthesis, assembly, and secretion involves the action of MTP and TGH (Hussain et al. 2003; Lehner and Verger 1997). MTP is important in the transport of neutral lipids including cholesterol and TG to nascent ApoB in order to form complete ApoB lipoproteins (Taghibiglou et al. 2000). The interaction of MTP with ApoB preserves ApoB from intracellular degradation and promotes lipid assembly and secretion (Cardozo et al. 2002).

Sort-1 is an intracellular protein that regulates ApoB degradation (Strong and Rader 2012). Sort-1 binds to ApoB in the liver and transports it to the proteasome for degradation to maintain a homeostatic level of lipids (Chamberlain et al. 2013). Sort-1 protein levels were found to be decreased in *in vivo*/*in vitro* models of insulin resistance and T2D (Ai et al. 2012).

The goal of this study was to identify *in vitro* effects of DDE on McA cell mechanisms involved in lipid metabolism and secretion. McA cells secrete lipids in response to fatty acids and are a reliable cell line for studying lipid metabolism and secretion (Boren et al. 1994) because they secrete both ApoB-100 and its truncated form, ApoB-48; *in vivo* studies using rodent models have indicated that both forms are secreted from the liver (Véniant et al. 1998).

2. Materials and methods

2.1. Chemicals and reagents

The McArdle-RH7777 (McA) rodent cell line was purchased from American Tissue Culture Collection (Manassas, VA). Super Signal West Pico Chemiluminescence kit, 0.05% trypsin in 0.53 mM EDTA, 10% Neutral Buffered Formalin, and Sort-1 antibody came from Thermo Fisher Scientific (Pittsburgh, PA). Certified fetal bovine serum (FBS), Dulbecco's Modified Eagles Medium (DMEM), and penicillin-streptomycin were from Invitrogen (Grand Island, NY). Chem Service (West Chester, PA) supplied the p,p′-DDE. The phosphate buffered saline (PBS) was from Mediatech Inc. (Manassas, VA). Non-fat dry milk and the Bradford protein assay kit came from Bio-Rad (Hercules, CA). ApoB, goat anti-rabbit IgG-HRP, donkey anti-goat IgG-HRP, beta-actin and MTP antibodies; RIPA lysis buffer, and Western Blotting Luminol Reagent were purchased from Santa Cruz (Dallas, TX). MTP antibody was also purchased from Aviva Systems Biology (San Diego, CA). Bovine serum albumin-fatty acid free (BSA), oleic acid (OA) conjugated to BSA, Oil-red-O dye, SDS sample loading buffer, and dimethyl sulfoxide (DMSO) were from Sigma Aldrich (St. Louis, MO). The TG assay kit was from Cayman Chemicals (Ann Arbor, MI) and the crystal violet dye solution was from Active Motif (Carlsbad, CA).

2.2. Cell culture conditions

McA cells were grown in supplemented DMEM containing 20% FBS and a 1% solution of 100 IU/ml penicillin with 0.1 mg/ml streptomycin in a humidified atmosphere of 5% carbon dioxide and 95% air at 37 °C in a T-75 cell culture flask until 70%–90% confluent, washed with PBS, detached with 0.05% trypsin in 0.53 mM EDTA, pelleted by centrifugation, re-suspended and seeded in 12-well cell plates to grow overnight. The next day adherent cells were washed with PBS and treated with serum-free DMEM for a second overnight incubation. Following this the serum-free DMEM was removed, the cells were washed with PBS and subjected to experimental protocols. Unless stated otherwise the test chemicals in each case were 10 μM DDE alone or simultaneously with 100 μM OA conjugated to 0.3% BSA, just 100 μM OA conjugated to 0.3% BSA, or 0.3% BSA only. In every protocol the cells were exposed for 24 h in DMEM plus the test chemicals. Each treatment was tested with 3 true independent experimental evaluations by growing the cells 3 separate times to yield 3 separate biological replicates. Every one of the experiments also contained 3 technical replicates, *i.e.* 3 wells per plate. Following all experimental protocols, cell media and cell lysates were collected and subjected to protein analysis using the Bradford protein protocol.

2.3. SDS-PAGE and Western blot

Cell media and/or lysates were subjected to SDS-PAGE using a 10% polyacrylamide gel and semi-dry transfer to 0.45 μm polyvinylidene fluoride (PVDF) membranes. All treatment groups were normalized to the protein concentrations of their respective cell lysates for Western blot analysis. Protein bands were detected using the appropriate antibodies and a chemiluminescence reaction visualized using the ChemiDoc XRS System with Image Lab Software. Protein band Integrated Density (I.D.) was interpreted using ImageJ Software from the National Institutes of Health. I.D. is the product of the area and mean gray value of the band.

2.4. Free fatty acid induced lipid secretion

To verify cell responses, OA, a free fatty acid, was used to induce lipid metabolism and secretion in McA liver cells based on White et al. (1992). Cells were treated with either a final concentration of 400 μM OA conjugated to 1.2% BSA, 100 μM OA conjugated to 0.3% BSA, or 1.2% BSA only. Cell media was assayed for secreted ApoB protein levels by Western blot. Although humans produce two different forms of ApoB (ApoB-48/ApoB-100), measuring total ApoB levels may serve as a more reliable biochemical marker for the prediction of metabolic dysfunction-related diseases such as T2D. The livers of mice and rats also synthesize both forms of ApoB and exhibit ApoB mRNA editing

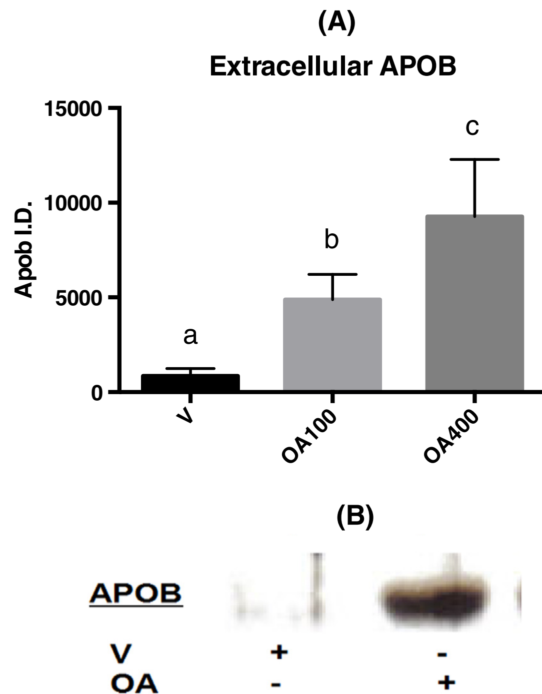

Fig. 1. (Panel A) Measurement of ApoB secretion for treatments (OA) and vehicle (V) in 3 biological trials. Each treatment was tested with 3 true independent experimental evaluations by growing the cells 3 separate times to yield 3 separate biological replicates. Every one of the experiments also contained 3 technical replicates, *i.e.* 3 wells per plate. Treatment of McA cells stimulated with 100 μM oleic acid conjugated to 0.3% BSA (OA100), 400 μM OA conjugated to 1.2% BSA (OA400), or 1.2% BSA vehicle only (V) for 24 h. I.D. stands for Integrated Density. Means with different letters are significantly different ($p \leq 0.05$), as determined by a mixed model analysis with Tukey's adjustment for multiple comparison of least square means. Error bars represent the mean ± the standard error of the mean (SEM) for each treatment group. (Panel B) Western blot density of ApoB secretion. ApoB secretion levels were increased after OA exposure.

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