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Original Article

Inhibitory Effect of Dihydroartemisinin, An Active Ingredient of *Artemisia annua*, on Lipid Accumulation in Differentiating 3T3-L1 Preadipocytes

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Objectives: Artemisinin and its derivatives extracted from *Artemisia* annua, a Chinese herbal medicine, have variable biological effects due to structural differences. Up to date, the anti-obesity effect of dihydroartemisinin (DHA), a derivative of artemisinin, is unknown. The purpose of this study was to investigate the anti-adipogenic and lipolytic effects of DHA on 3T3-L1 preadipocytes.
Methods: Oil Red O staining and AdipoRed assay were used to measure lipid accumulation and triglyceride (TG) content in 3T3-L1 cells, respectively. Cell count analysis was used to determine the cytotoxicity of 3T3-L1 cells. Western blot and real-time reverse transcription polymerase chain reaction analyses were used to analyze the expression of protein and mRNA in 3T3-L1 cells, respectively.
Results: DHA at 5 μM markedly inhibited lipid accumulation and reduced TG content in differentiating 3T3-L1 cells with no cytotoxicity. Furthermore, DHA at 5 μM inhibited the expression of CCAAT/enhancer-binding protein-α (C/EBP-α), peroxisome proliferator-activated receptor-γ (PPAR-γ), fatty acid synthase (FAS), and perilipin A as well as the phosphorylation of signal transducer and activator of transcription-3 (STAT-3) in differentiating 3T3-L1 cells. Moreover, while DHA at 5 μM had no effect on the mRNA expression of adiponectin, it strongly suppressed that of leptin in differentiating 3T3-L1 cells. However, DHA at 5 μM had no lipolytic effect on differentiated 3T3-L1 cells, as assessed by no enhancement of glycerol release.
Conclusions: These results demonstrate that DHA at 5 μM has a strong anti-adipogenic effect on differentiating 3T3-L1 cells through the reduced expression and phosphorylation of C/EBP-α, PPAR-γ, FAS, perilipin A, and STAT-3.

Key Words: Dihydroartemisinin, C/EBP-α, PPAR-γ, STAT-3, Perilipin A, 3T3-L1

Introduction

Obesity is defined as abnormal fat accumulation in the human body. Obesity has now become global pandemic, given that it causes the development of multiple human chronic diseases like type 2 diabetes, heart disease, hyperlipidemia, and cancer[1,2]. It is well documented that obesity is induced by excessive differentiation of adipocytes of the adipose tissue[3]. Thus, any substance that suppresses excessive adipocyte differentiation, also called as adipogenesis, could have the potential to be a preventative and therapeutic agent for obesity.

Adipocyte differentiation is a biological multistep process that occurs in the form of cellular, morphological and biochemical changes[4]. The process converts fibroblast-like preadipocytes into differentiated or mature adipocytes, which are characterized by sequential changes in the expression of specific genes that determine the specific adipocyte phenotype of the cells and by continuous accumulation of lipid droplets (LDs) that increases the hypertrophy of the cells[3-5]. There is a wealth of information that expression and phosphorylation of multiple adipogenic transcription factors, including CCAAT/enhancer-binding proteins (C/EBPs), perox-

isome proliferator-activated receptors (PPARs), and signal transducer and activator of transcription (STAT) proteins, are crucial for preadipocyte differentiation[6-8]. It is further shown that expression and activity of lipogenesis and lipid uptake (storage)-related enzymes, such as fatty acid synthase (FAS) and lipoprotein lipase (LPL), and LD associated proteins like perilipin A are also necessary for preadipocyte differentiation[9-13]. Lipolysis, which is currently considered more important as a treatment for obesity, is the metabolic pathway through which triglyceride (TG) is hydrolyzed into a glycerol and three fatty acids[14]. It is shown that a number of enzymes and signing factors, including hormone-sensitive lipase (HSL), protein kinase A (PKA), and extracellular signal-regulated kinase-1/2 (ERK-1/2), involve in the control of lipolysis in differentiated adipocytes[14,15].

Dihydroartemisinin (DHA) is the primary active derivative of artemisinin extracted from *Artemisia annua*, a Chinese herbal medicine[16] and has been shown to have anti-malarial, anti-inflammatory, anti-oxidative, browning, and anti-tumor effects[17-21]. I have previously reported that artesunate, another artemisinin derivative, inhibits adipogenesis in 3T3-L1 preadipocytes[22]. Up to date, the anti-obesity effect and mechanism of DHA that is structurally different to artesunate are not known. This led me to hypothesize that DHA might have similar or superior anti-adipogenic effects than artesunate. Therefore, in this study, the effects of DHA on lipid accumulation and lipolysis in differentiating and differentiated 3T3-L1 cells, a murine white preadipocyte, were investigated.

Materials and Methods

1. Materials

DHA was bought from Selleckchem (Houston, TX, USA). Primary antibodies of STAT-3, phospho (p)-STAT-3, STAT-5, p-STAT-5, C/EBP-α, and PPAR-γ were obtained from Santa Cruz Biotechnology (Delaware, CA, USA). Primary FAS antibody was bought from BD Bioscience (San Jose, CA, USA). Primary antibodies of perilipin A and β-actin were obtained from Bio Vision (Milpitas, CA, USA) and Sigma (St. Louis,

MO, USA), respectively. Enhanced chemiluminescence (ECL) reagent was bought from Advansta (Menlo Park, CA, USA). 3-isobutyl-1-methylxanthine (IBMX), dexamethasone, and insulin were purchased from Sigma.

2. Differentiation of 3T3-L1 preadipocytes

3T3-L1 murine white preadipocytes (ATCC, Manassas, VA, USA) were grown up to the contact inhibition stage and remained in the post-confluent stage for 2 days in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% calf bovine serum (Gibco, Long Islands, NY, USA) and penicillin-streptomycin (Welgene, Daegu, Korea). Differentiation was induced by changing the medium to DMEM supplemented with 10% fetal bovine serum (FBS; Welgene) plus a cocktail of hormones (MDI) that include 0.5 mM IBMX (M) (Sigma), 0.5 μM dexamethasone (D) (Sigma), and 5 μg/mL insulin (Sigma) in the absence or presence of DHA at the indicated concentrations or 5 μM. After 48 h MDI-induction, the differentiation medium was replaced with DMEM supplemented with 10% FBS and 5 μg/mL insulin in the absence or presence of DHA at the indicated concentrations or 5 μM for additional 3 days. The cells were then fed every other day with DMEM containing 10% FBS in the absence or presence of DHA at the indicated concentrations or 5 μM for additional 3 days.

3. Oil Red O staining

On day 8 of differentiation, control or DHA-treated 3T3-L1 cells were washed twice with PBS, fixed with 10% formaldehyde for 2 h at room temperature (RT), washed with 60% isopropanol and dried completely. The fixed cells were then stained with Oil Red O working solution for 1 h at RT and then washed twice with distilled water. LDs accumulated in control or DHA-treated 3T3-L1 cells were observed by light microscopy (TS100; Nikon, Tokyo, Japan).

4. Cell count analysis

3T3-L1 preadipocytes were seeded in 24-well plates and incubated overnight. Cells were similarly grown under the above-mentioned differentiation conditions. On day 8 of dif-

ferentiation, control or DHA-treated 3T3-L1 cells, which cannot be stained with trypan blue dye, was counted under microscope. The cell count assay was done in triplicates. Data are mean±standard error (SE) of three independent experiments.

5. Quantification of cellular TG content by AdipoRed assay

On day 8 of differentiation, intracellular TG content in control or DHA-treated 3T3-L1 cells was measured using a commercially available AdipoRed Assay Reagent kit according to the manufacturer's instructions (Lonza, Basel, Switzerland). After a 10 min incubation, fluorescence was measured on Victor3 (Perkin Elmer Inc., Waltham, MA, USA) with an excitation at 485 nm and an emission at 572 nm.

6. Preparation of whole cell lysates

At the designated time point, 3T3-L1 cells were washed twice with PBS and exposed to a modified radioimmuno precipitation assay buffer (50 mM Tris-Cl [pH 7.4], 150 mM NaCl, 0.1% sodium dodecyl sulfate, 0.25% sodium deoxycholate, 1% Triton X-100, 1% Nonidet P-40, 1 mM ethylenediaminetetraacetic acid, 1 mM ethylene glycol tetraacetic acid, proteinase inhibitor cocktail [1x]). The cell lysates were then collected and centrifuged at 12,000 rpm for 20 min at 4°C. The supernatant was saved, and protein concentrations were determined with pierce BCA protein assay kit (Thermo Scientific, Rockford, IL, USA).

7. Western blot analysis

Proteins (50 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10%) and transferred onto nitrocellulose membranes (Millipore, Burlington, MA, USA). The membranes were washed with TBS (10 mM Tris, 150 mM NaCl) supplemented with 0.05% (vol/vol) Tween 20 (TBST) followed by blocking with TBST containing 5% (w/v) non-fat dried milk. The membranes were incubated overnight with antibodies specific for C/EBP-α (1:1,000), PPAR-β (1:1,000), PPAR-γ (1:1,000), p-STAT-3 (1:2,000), STAT-3 (1:2,000), p-STAT-5 (1:2,000), STAT-5 (1:2,000), FAS (1:1,000), perilipin A (1:2,000) or β-actin (1:10,000) at 4°C. The

membranes were then exposed to secondary antibodies coupled to horseradish peroxidase for 2 h at RT. The membranes were washed three times with TBST at RT. Immunoreactivities were detected by ECL reagents. Equal protein loading was assessed by the expression level of actin protein.

8. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

Total cellular RNA in the control or DHA-treated 3T3-L1 cells was isolated with the RNAiso Plus (Takara Bio Inc., Kusatsu, Japan). Three micrograms of total RNA were reverse transcribed using a random hexadeoxynucleotide primer and reverse transcriptase. Single stranded cDNA was amplified by PCR with the following primers: C/EBP-α sense 5'-TTA CAACAGGCCAGGTTTCC-3'; antisense 5'-CTCTGGGATGG ATCGATTGT-3'; PPAR-γ sense 5'-GGTGAAACTCTGGG AGATTC-3'; antisense 5'-CAACCATTGGGTCAGCTCTC-3'; FAS sense 5'-TTGCTGGCACTACAGAATGC-3'; antisense 5'-AACAGCCTCAGAGCGACAAT-3'; perilipin A sense 5'-CTTTCTCGACACACCATGGAAACC-3'; antisense 5'-CC ACGTTATCCGTAACACCCTTCA-3'; LPL sense 5'-TGCCG CTGTTTTGTTTTACC-3'; antisense 5'-TCACAGTTTCTGC TCCCAGC-3'; adiponectin sense 5'-GGAGATGCAGGTCT TCTTGGT-3'; antisense 5'-TCCTGATACTGGTCGTAGGT GAA-3'; leptin sense 5'-CCAAAACCCTCATCAAGACC-3'; antisense 5'-CTCAAAGCCACCACCTCTGT-3'; actin sense 5'-GGTGAAGGTCGGTGTGAACG-3'; antisense 5'-GGTA GGAACACGGAAGGCCA-3'. Expression levels of actin mRNA expression were used as an internal control as well as loading control.

9. Statistical analysis

Cell count analysis was done in triplicates and repeated three times. Data were expressed as mean±SE. The significance of difference was determined by one-way ANOVA. All significance testing was based upon a P value of <0.05.

Results

1. DHA dose-dependently inhibits lipid accumulation and reduces TG content in differentiating 3T3-L1 cells with no cytotoxicity

Fig. 1A is the experimental protocol of 3T3-L1 preadipocyte differentiation. Initially, the treatment effect of various concentrations of DHA (Fig. 1B) on cell growth (survival) during the differentiation of 3T3-L1 preadipocytes into adipocytes for 8 days was investigated using cell count analysis. Treatment with DHA at the concentrations tested did not affect the survival of 3T3-L1 cells (Fig. 1C). Next, the treatment effect of various concentrations of DHA on intracellular TG content in 3T3-L1 cells on day 8 (D8) of differ-

entiation was measured using an AdipoRed assay. As shown in Fig. 1D, treatment with DHA also reduced intracellular TG content on D8 of 3T3-L1 preadipocyte differentiation in a concentration-dependent manner. DHA regulation of lipid accumulation during the differentiation of 3T3-L1 preadipocytes into adipocytes for 8 days was further examined using Oil Red O staining. As shown in Fig. 1E (upper panels), many intracellular LDs were formed and accumulated in 3T3-L1 cells on D8 of differentiation, compared with undifferentiated cells on D0. However, treatment with DHA dose-dependently suppressed accumulation of LDs in differentiating 3T3-L1 cells on D8. The DHA's suppressive effect on accumulation of LDs in differentiating 3T3-L1 cells on D8 was also confirmed by light microscopy (Fig. 1E, lower
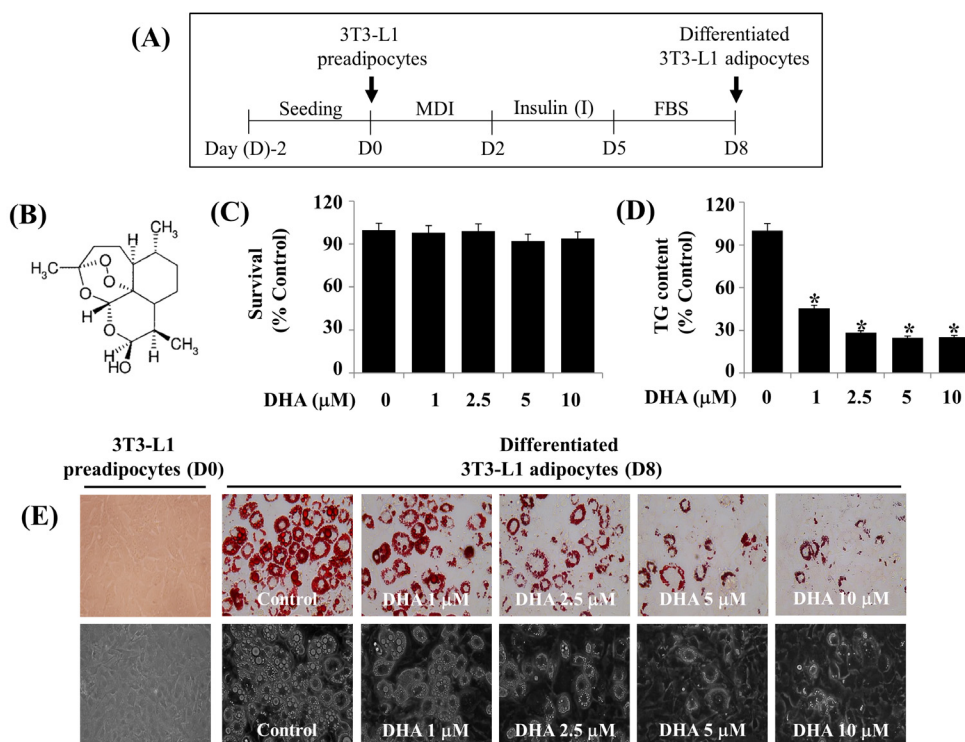

Fig. 1. Effects of DHA on lipid accumulation, TG content, and cell growth (survival) during 3T3-L1 preadipocyte differentiation. (A) Scheme for 3T3-L1 preadipocyte differentiation. (B) is the chemical structure of DHA. (C) Measurement of the number of surviving cells in vehicle control (DMSO 0.1%) or DHA-treated 3T3-L1 preadipocytes on D8 by trypan blue dye exclusion. Data are mean±SE of three independent experiments, each done in triplicate. (D) Quantification of the intracellular TG content in vehicle control or DHA-treated 3T3-L1 preadipocytes on D8 by AdipoRed assay. Data are mean±SE of three independent experiments, each done in triplicate. *P⟨0.001 vs. control (no DHA). (E) Measurement of the intracellular LDs accumulation in 3T3-L1 preadipocytes or differentiated adipocytes on D8 of differentiation in the absence (vehicle control; DMSO, 0.1%) or presence of DHA at the indicated concentrations by Oil Red O staining. Phase-contrast images were also taken after the treatment (lower panels in E). MDI: 3-isobutyl-1-methylxanthine (M), dexamethasone (D), insulin (I), FBS: fetal bovine serum, DHA: dihydroartemisinin, TG: triglyceride, DMSO: dimethyl sulfoxide, D8: day 8, SE: standard error, LDs: lipid droplets.

panels). Due to the maximal repressive effects on intracellular accumulation of LDs and TG content without cytotoxicity, this 5 μM dose of DHA was chosen in further studies.

2. DHA at 5 μM strongly lowers expression and phosphorylation levels of C/EBP-α, PPAR-γ, and STAT-3 in differentiating 3T3-L1 cells

Next, the treatment effect of DHA at 5 μM on protein expression and activity (phosphorylation) of adipogenesis-related transcription factors (C/EBP-α, PPAR-γ, STAT-3, and STAT-5) was determined by Western blotting analysis. As shown in Fig. 2A, DHA at 5 μM strongly reduced expression levels of C/EBP-α protein in differentiating 3T3-L1 cells on D5 and D8. Moreover, expression levels of PPAR-γ protein in differentiating 3T3-L1 cells on D8 were strongly down-regulated by DHA at 5 μM. In addition, while DHA at 5 μM did not affect phosphorylation levels of STAT-3 protein in differentiating 3T3-L1 cells on D2, it largely decreased those of STAT-3 protein on D5 and D8. However, DHA at 5 μM had no or little effect on phosphorylation levels of STAT-5 protein in differentiating 3T3-L1 cells on D2, D5, and D8. Total protein expression levels of actin, STAT-3, and STAT-5

remained largely unchanged under these experimental conditions. RT-PCR analysis was next carried out to see whether DHA at 5 μM affects mRNA expression levels of C/EBP-α and PPAR-γ in differentiating 3T3-L1 cells. As shown in Fig. 2B, DHA at 5 μM largely reduced C/EBP-α and PPAR-γ transcripts in differentiating 3T3-L1 cells on D5 and D8. Expression levels of control actin mRNA remained constant under these experimental conditions.

3. DHA at 5 μM markedly reduces expression levels of FAS, perilipin A, and leptin in differentiating 3T3-L1 cells

The treatment effect of DHA at 5 μM on protein and/or mRNA expression levels of lipogenesis and lipid uptake (storage)-related enzymes (FAS, LPL) and LD associated protein (perilipin A) during 3T3-L1 preadipocyte differentiation was further investigated. As shown in Fig. 3A, DHA at 5 μM greatly reduced protein expression levels of FAS in differentiating 3T3-L1 cells on D8. Moreover, DHA at 5 μM was able to largely decrease expression levels of perilipin A protein on D5 and D8. Furthermore, as shown in Fig. 3B, DHA at 5 μM largely down-regulated mRNA levels of both
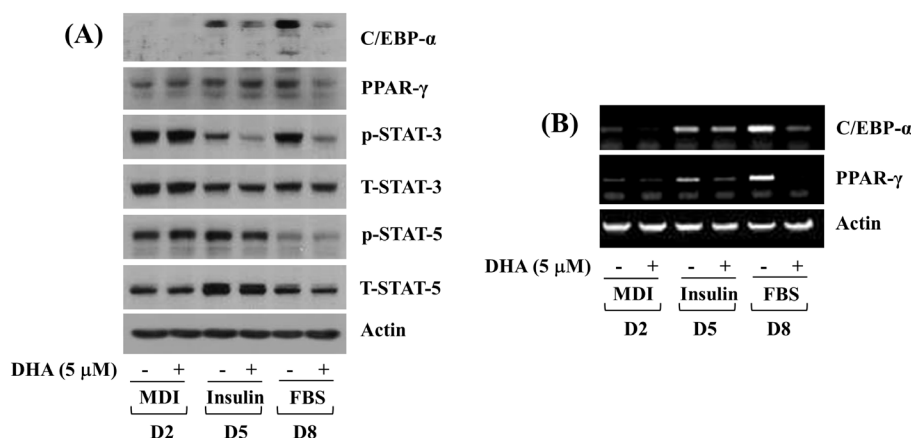

Fig. 2. Effects of DHA on expression and phosphorylation levels of C/EBP-α, PPAR-γ, and STAT-3/5 during 3T3-L1 preadipocyte differentiation. (A) 3T3-L1 preadipocytes were differentiated with induction medium containing MDI, insulin, and FBS in the presence or absence of DHA, and harvested at days 2, 5, and 8, respectively. Whole cell lysates at the indicated time point were extracted and analyzed by Western blot analysis with respective antibodies. p-STAT-3, phosphorylated STAT-3; T-STAT-3, total STAT-3; p-STAT-5, phosphorylated STAT-5; T-STAT-5, total STAT-5. (B) 3T3-L1 preadipocytes were differentiated with induction medium containing MDI, insulin, and FBS in the presence or absence of DHA, and harvested at days 2, 5, and 8, respectively. Total cellular RNA at the indicated time point were extracted and analyzed by RT-PCR analysis with respective primers. DHA: dihydroartemisinin, C/EBP: CCAAT/enhancer-binding protein, PPAR: peroxisome proliferator-activated receptor, STAT: signal transducer and activator of transcription, MDI: 3-isobutyl-1-methylxanthine (M), dexamethasone (D), insulin (I), FBS: fetal bovine serum, D2: day 2, D5: day 5, D8: day 8, RT-PCR: reverse transcriptase-polymerase chain reaction.

FAS and perilipin A in differentiating 3T3-L1 cells on D8. However, DHA at 5 μM had no effect on LPL mRNA expression levels in differentiating 3T3-L1 cells on D2, D5, and D8. Notably, while DHA at 5 μM did not influence adiponectin mRNA expression levels in differentiating 3T3-L1 cells on the times tested, it strongly reduced leptin expression levels on D5 and D8. Expression levels of control actin mRNA remained unchanged under these experimental conditions.

4. DHA at 5 μM has no lipolytic effect on differentiated 3T3-L1 cells

Whether DHA at 5 μM affects lipolysis in differentiated 3T3-L1 cells was next determined. DHA's lipolytic effect was herein assessed by measuring glycerol content in the culture medium from differentiated 3T3-L1 cells that were serum starved for 2 h and treated without or with DHA for additional 3 h, as depicted in Fig. 4A. Isoproterenol (ISO), a lipolytic agent[23], was included as a positive control. As expected, treatment with ISO at 20 μM for 3 h greatly enhanced glycerol release in differentiated 3T3-L1 cells (Fig. 4B). However, DHA treatment at 5 μM for 3 h led to a slight increase in glycerol content in differentiated 3T3-L1 cells. The treatment effects of DHA or ISO at the indicated dose on expression and phosphorylation levels of perilipin A and ERK-1/2, known lipolysis-related proteins, in differentiated 3T3-L1 cells were next compared. As shown in Fig. 4C, while ISO treatment at 20 μM for 3 h strongly down-regulated expression levels of perilipin A in differ-
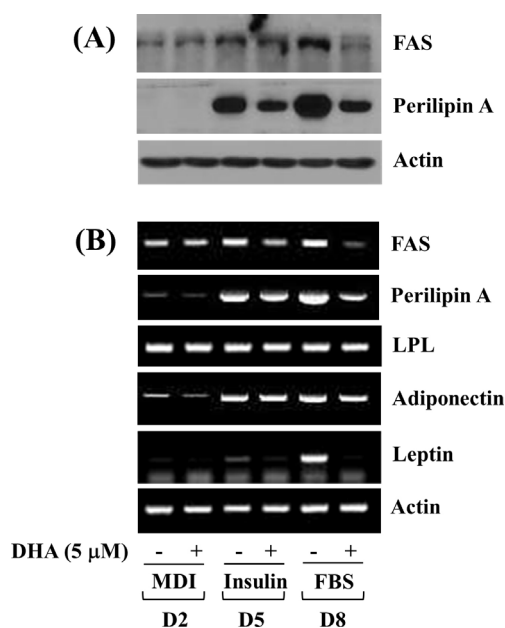

Fig. 3. Effects of DHA on expression levels of FAS, perilipin A, LPL, adiponectin, and leptin during 3T3-L1 preadipocyte differentiation. (A) 3T3-L1 preadipocytes were differentiated with induction medium containing MDI, insulin, and FBS in the presence or absence of DHA, and harvested at days 2, 5, and 8, respectively. Whole cell lysates at the indicated time point were extracted and analyzed by Western blot analysis with respective antibodies. (B) 3T3-L1 preadipocytes were differentiated with induction medium containing MDI, insulin, and FBS in the presence or absence of DHA, and harvested at days 2, 5, and 8, respectively. Total cellular RNA at the indicated time point were extracted and analyzed by RT-PCR analysis with respective primers. DHA: dihydroartemisinin, FAS: fatty acid synthase, LPL: lipoprotein lipase, MDI: 3-isobutyl-1-methylxanthine (M), dexamethasone (D), insulin (I), FBS: fetal bovine serum, D2: day 2, D5: day 5, D8: day 8, RT-PCR: reverse transcriptase-polymerase chain reaction.
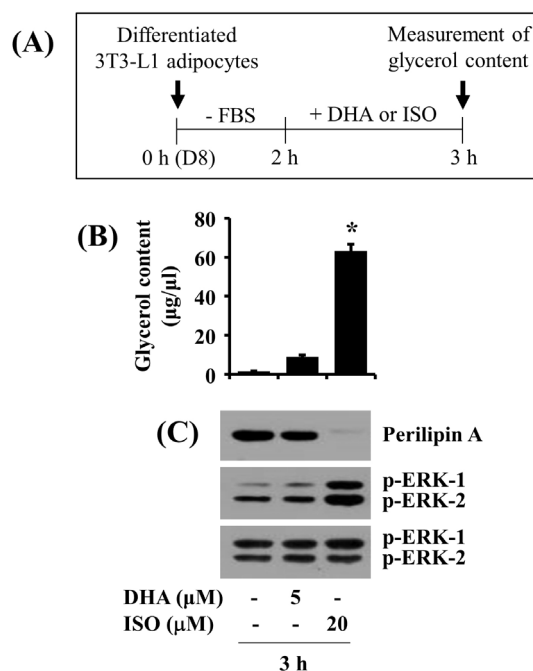

Fig. 4. Effects of DHA on lipolysis and expression and phosphorylation levels of perilipin A and ERK-1/2 in differentiated 3T3-L1 cells. (A) Scheme for measurement of glycerol content in differentiated 3T3-L1 adipocytes. (B) Differentiated 3T3-L1 cells on D8 were serum-starved for 2 h and treated with DHA or ISO for additional 3 h. Glycerol content in culture medium from control or drug-treated cells was measured in triplicate. Data are mean±SE of three independent experiments. *P⟨0.0024 vs. control. (C) Differentiated 3T3-L1 cells on D8 were serum-starved for 2 h and treated with DHA or ISO for additional 3 h. Whole cell lysates were extracted and analyzed by Western blot analysis with respective antibodies. FBS: fetal bovine serum, DHA: dihydroartemisinin, ISO: isoproterenol, D8: day 8, ERK: extracellular signal-regulated kinase, SE: standard error.

entiated 3T3-L1 cells, DHA treatment at 5 μM for 3 h had no or little effect on it. In addition, while treatment with ISO at 20 μM for 3 h markedly increased phosphorylation levels of ERK-1/2 in differentiated 3T3-L1 cells, that with DHA at 5 μM for 3 h did not. Total protein expression levels of ERK-1/2 remained largely unchanged under these experimental conditions.

Discussion

It is well recognized that excessive adipocyte differentiation and the resultant high lipid accumulation of the adipose tissue leads to the development of obesity. DHA, a derivative of artemisinin extracted from *Artemisia annua*[16], is reported to possess anti-malarial, anti-inflammatory, anti-oxidative, browning, and anti-tumor properties[17-21]. Up to date, however, little is known about the anti-obesity effect and mode of action of DHA in white adipocytes. The present study demonstrates that DHA at 5 μM has strong anti-adipogenic effect on differentiating 3T3-L1 white preadipocytes and the effect is controlled through modulation of the expression and phosphorylation levels of C/EBP-α, PPAR-γ, FAS, perilipin A, and STAT-3.

Recently, it has been demonstrated that DHA at 5 or 7.5 μM induces browning-like features, as evidenced by cellular increase in multiple smaller LDs and mRNA expression levels of thermogenic markers (uncoupled protein 1 and peroxisome proliferator-activated receptor gamma co-activator 1 α), in bone morphogenetic protein 4-commited C3H10T1/2 mouse mesenchymal stem cells[20], suggesting its browning effect. The present study displays that DHA at 5 μM markedly reduces lipid accumulation and TG content in differentiating 3T3-L1 cells, advocating its anti-adipogenic and anti-lipogenic effects. Aforementioned, 3T3-L1 preadipocyte differentiation is largely influenced by the expression and activity of adipogenic transcription factors, including C/EBP-α, PPAR-γ, and STAT-3/5[6,7,24-27]. It is further known that FAS, a lipogenic enzyme responsible for the synthesis of fatty acid[9] and perilipin A, a protein that binds to and stabilizes the newly synthesized LDs[9,11,13,14] play important roles in

lipid accumulation during 3T3-L1 preadipcyte differentiation. However, little is known about DHA regulation of C/EBP-α, PPAR-γ, STAT-3/5, FAS, and perilipin A during 3T3-L1 preadipcyte differentiation. In this study, DHA at 5 μM greatly reduces expression and phosphorylation levels of C/EBP-α, PPAR-γ, STAT-3, FAS, and perilipin A in differentiating 3T3-L1 cells. These findings suggest that DHA's anti-adipogenic and lipid-lowering effects on differentiating 3T3-L1 cells are likely to be due to the reduced expression and phosphorylation levels of C/EBP-α, PPAR-γ, STAT-3, FAS, and perilipin A.

It is well known that the adipose tissue synthesizes and secretes an array of adipokines, including leptin and adiponectin[28,29]. Reportedly, while expression levels of adiponectin decrease with increase in the adiposity, those of lepin increase in obesity[30-32]. It is further established that while adiponectin has many beneficial metabolic effects like reduction of serum glucose and lipids, maintenance of insulin sensitivity, and increase in fatty acid oxidation, leptin plays pathogenic roles in obesity and related disorders including insulin resistance[32-35]. Consequently, decreased leptin expression is regarded an alternative against obesity and related disorders. In this study, it is shown that DHA at 5 μM greatly reduces leptin, but not adiponectin, mRNA expression levels in differentiating 3T3-L1 cells, suggesting the potential utility of DHA for the prevention or treatment of obesity and related disorders associated with leptin overexpression.

Aforementioned, a number of enzymes and signaling proteins, including HSL, PKA, perilipin A, and ERK-1/2, participate in lipolysis in differentiated adipocytes[13-15]. Among those, HSL is a major enzyme in the mobilization of fatty acids from stored TG. Accordingly, lipolysis is stimulated by activation of PKA which phosphorylates HSL on S563, S659, and S660 in adipocytes. Perilipin A is a highly expressed protein in both differentiating and differentiated adipocytes that are localized at the surface of LDs. It is believed that perilipin A has key roles in not only lipid packaging or storage (lipid accumulation) in differentiating preadipocytes but also lipid hydrolysis (lipolysis) in differentiated adipocytes. Supporting the latter, cellular loss (or

down-regulation) of perilipin A facilitates the access of HSL into LDs in differentiated adipocytes, resulting in HSL mediated lipolysis[36]. Increase in lipolysis through ERK-1/2-dependent HSL phosphorylation (on S600) and activation also has been reported[15]. Given that while ISO largely enhances glycerol release and down-regulates perilipin A expression and up-regulates ERK-1/2 phosphorylation in differentiated 3T3-L1 cells, DHA does not have regulatory effects on them in this study, it is evident that DHA has no lipolytic effect on differentiated 3T3-L1 cells.

Conclusion

These results demonstrate firstly that DHA has a strong anti-adipogenic effect in differentiating 3T3-L1 preadipocytes and its anti-adipogenic effect is largely attributable to the reduced expression and phosphorylation levels of C/EBP-α, PPAR-γ, FAS, perilipin A, and STAT-3. The findings presented herein suggest that DHA may be applied as a potential anti-obesity agent.

Acknowledgements

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