Saudi Pharmaceutical Journal 26 (2018) 1178–1184

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Ginkgolide C reduced oleic acid-induced lipid accumulation in HepG2 cells

Wen-Chung Huang [a,b,1], Ya-Ling Chen [c,1], Hui-Chia Liu [c], Shu-Ju Wu [c,d,*], Chian-Jiun Liou [b,e,**]

[a] Graduate Institute of Health Industry Technology, Research Center for Food and Cosmetic Safety, Research Center for Chinese Herbal Medicine, College of Human Ecology, Chang Gung University of Science and Technology, No. 261, Wenhua 1st Rd., Guishan Dist., Taoyuan City 33303, Taiwan
[b] Division of Allergy, Asthma, and Rheumatology, Department of Pediatrics, Chang Gung Memorial Hospital, Linkou, Guishan Dist., Taoyuan City 33303, Taiwan
[c] Department of Nutrition and Health Sciences, Chang Gung University of Science and Technology, No. 261, Wenhua 1st Rd., Guishan Dist., Taoyuan City 33303, Taiwan
[d] Aesthetic Medical Center, Department of Dermatology, Chang Gung Memorial Hospital, Linkou, Guishan Dist., Taoyuan 33303, Taiwan
[e] Department of Nursing, Research Center for Chinese Herbal Medicine, Chang Gung University of Science and Technology, No. 261, Wenhua 1st Rd., Guishan Dist., Taoyuan City 33303, Taiwan

ARTICLE INFO

Article history:
Received 15 February 2018
Accepted 19 July 2018
Available online 20 July 2018

Keywords:
AMPK
Anti-obesity
Ginkgolide C
Lipogenesis
Lipolysis

ABSTRACT

Ginkgolide C, isolated from *Ginkgo biloba*, is a diterpene lactone that has multiple biological functions and can improve Alzheimer disease and platelet aggregation. Ginkgolide C also inhibits adipogenesis in 3T3-L1 adipocytes. The present study evaluated whether ginkgolide C reduced lipid accumulation and regulated the molecular mechanism of lipogenesis in oleic acid-induced HepG2 hepatocytes. HepG2 cells were treated with 0.5 mM oleic acid for 48 h to induce a fatty liver cell model. Then, the cells were exposed to various concentrations of ginkgolide C for 24 h. Staining with Oil Red O and the fluorescent dye BODIPY 493/503 revealed that ginkgolide C significantly reduced excessive lipid accumulation in HepG2 cells. Ginkgolide C decreased peroxisome proliferator-activated receptor γ and sterol regulatory element-binding protein 1c to block the expression of fatty acid synthase. Ginkgolide C treatment also promoted the expression of adipose triglyceride lipase and the phosphorylation level of hormone-sensitive lipase to enhance the decomposition of triglycerides. In addition, ginkgolide C stimulated CPT-1 to activate fatty acid β-oxidation, significantly increased sirt1 and phosphorylation of AMP-activated protein kinase (AMPK), and decreased expression of acetyl-CoA carboxylase for suppressed fatty acid synthesis in hepatocytes. Taken together, our results suggest that ginkgolide C reduced lipid accumulation and increased lipolysis through the sirt1/AMPK pathway in oleic acid-induced fatty liver cells.

1. Introduction

Obesity causes many chronic diseases, including diabetes mellitus, hyperlipidemia, and cancer (Forte et al., 2012). Many studies have also shown that excessive lipid accumulation in the liver of obese subjects induces nonalcoholic fatty liver disease (NAFLD) (Neuschwander-Tetri, 2017; Patil and Sood, 2017). NAFLD is defined as abnormal lipid accumulation in hepatocytes, and this interferes with the normal metabolism of carbohydrates and lipids for reduced glycogen synthesis and increased lipid synthesis, leading to lipid accumulation in the liver (Reccia et al., 2017). NAFLD can be divided into simple fat accumulation (hepatic steatosis) and deteriorated steatohepatitis. When the affected hepatocytes are not repaired, sustained inflammatory and oxidative damage occurs and nonalcoholic steatohepatitis (NAHS) develops (Benedict and Zhang, 2017). If NASH patients do not maintain a healthy lifestyle with moderate rest and regular exercise,

* Corresponding author at: Department of Nutrition and Health Sciences, Chang Gung University of Science and Technology, No. 261, Wenhua 1st Rd., Guishan Dist., Taoyuan City 33303, Taiwan.
** Corresponding author at: Department of Nursing, Research Center for Chinese Herbal Medicine, Chang Gung University of Science and Technology, No. 261, Wenhua 1st Rd., Guishan Dist., Taoyuan City 33303, Taiwan.
E-mail addresses: sjwu@mail.cgust.edu.tw (S.-J. Wu), ccliu@mail.cgust.edu.tw (C.-J. Liou).
[1] Wen-Chung Huang and Ya-Ling Chen contributed equally to the paper.

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jsps.2018.07.006

irreversible liver fibrosis, cirrhosis, and even liver failure and liver cancer may develop (Reccia et al., 2017). The development of NAFLD is closely associated with obesity and diabetes. Therefore, improving obesity and reducing liver lipid accumulation may attenuate its development.

The most promising treatments for NAFLD are regulated diet, moderate exercise, weight loss, and possibly bariatric surgery (Brouwers et al., 2016). Regulating food intake with excess triglycerides is especially important since free fatty acids in the digestive tract can be transported through the blood to the liver for metabolism and be converted to simple lipids or cholesterol (Romero-Gomez et al., 2017). Transcription factors regulating hepatic lipogenesis are important for liver lipid synthesis, and can activate fatty acid chain synthesis, causing excessive triglyceride synthesis and lipid accumulation in the liver (Guo et al., 2015). Thus, blocking the expression of lipogenesis transcription factors will attenuate the synthesis of triglycerides in the liver.

AMP-activated protein kinase (AMPK) is a source of energy, and some studies have found that AMPK activity can regulate lipogenesis transcription factors in hepatic steatosis (Smith et al., 2016). The excessive storage of energy in cells leads to AMPK phosphorylation, followed by phosphorylation of its substrate acetyl-CoA carboxylase (ACC) (Lim et al., 2010), which plays an essential role in regulating fatty acid synthesis (Smith et al., 2016). Interestingly, ACC phosphorylation reduces the synthesis of malonyl-CoA and the extension and synthesis of the fatty acid chain (Hou et al., 2008).

Recent studies have found that many plant extracts and flavonoids can improve liver steatosis and NAFLD (Feng et al., 2017; Tian et al., 2016). *Ginkgo biloba* is a herbal medicine that has long been used in Eastern and Western medicine to improve cardiovascular disease (Yin et al., 2014). Western medicine has used the *G. biloba* extract EGB-761 to treat cardiovascular disease and dementia, and the ginkgo fruit is used in Chinese medicine to improve asthma (Babayigit et al., 2009; Stein et al., 2015). In recent years, several diterpene lactones and flavonoids were isolated from *G. biloba* (Zeng et al., 2013). Ginkgolide A, B, and C are diterpene lactones that can improve atherosclerosis and attenuate platelet activating factor (Huang et al., 2014; Zeng et al., 2013). A previous study found that ginkgolide A can improve NAFLD in high fat diet-induced obese mice (Jeong et al., 2017). Another study found that ginkgolide C could reduce transcription factors of adipogenesis and increase lipolysis by enhancing Sirt1/AMPK activity in 3T3-L1 differentiated adipocytes (Liou et al., 2015). In this study, we investigated whether ginkgolide C reduced lipid accumulation and regulated the molecular mechanism of lipogenesis in oleic acid-induced HepG2 hepatocytes.

2. Materials and methods

2.1. Chemical reagent

Ginkgolide C (purity $\geq$ 96% by HPLC) was purchased from Sigma (St. Louis, MO, USA) and was dissolved in DMSO ($\leq$0.1% in all cell experiments).

2.2. Cell culture and induced fatty liver cells

The HepG2 cell line was obtained from the Bioresource Collection and Research Center (BCRC, Taiwan). HepG2 cells were grown in a humidified atmosphere of 5% CO_2 at 37 °C in DMEM medium containing 10% fetal bovine serum (FBS) and 100 mg/L penicillin and streptomycin. Hepatocytes were treated with 0.5 mM oleic acid for 48 h and then 3–100 μM ginkgolide C and were incubated in cell culture plates for 24 h.

2.3. Cell viability assay

HepG2 cells were seeded on culture plates and incubated with various concentrations of ginkgolide C for 24 h. The culture plates were treated with 5 mg/ml MTT solution (Sigma) as previously described (Huang et al., 2017), and purple formazan crystals were dissolved in isopropanol. Cell viability was determined via the absorbance at 570 nm using a spectrophotometer (Multiskan FC, Thermo, Waltham, MA, USA).

2.4. Oil Red O staining

HepG2 cells were seeded on culture plates and incubated with 0.5 mM oleic acid for 48 h. Then, cells were treated with ginkgolide C for 24 h. Next, cells were fixed with formalin, and Oil Red O staining was performed (Liou et al., 2015). Oil droplets were observed using microscopy (Olympus). Next, cells were treated with isopropanol and lipid accumulation was measured using a microplate reader (Multiskan FC, Thermo Fisher Scientific) and recording the absorbance at 490 nm.

2.5. Hepatic lipid accumulation

HepG2 cells were seeded on culture plates and incubated with oleic acid (0.5 mM) for 48 h. Then, cells were treated with ginkgolide C for 24 h. Cells were fixed with 10% formalin, and lipid accumulation was evaluated using BODIPY 493/503 (Invitrogen, Carlsbad, CA, USA) as previously described (Chang et al., 2018). Lipid accumulation was observed using fluorescence microscopy (Olympus, Tokyo, Japan) in cells with DAPI stained nuclei.

2.6. Western blot analysis

Equal amounts of protein were separated on 8–10% SDS–polyacrylamide gels, and transferred onto polyvinylidene fluoride (PVFD) membranes (Millipore, Billerica, MA, USA) using a previously described method (Liou and Huang, 2017) The PVDF membranes were blocked with TBST buffer (150 mM NaCl, 10 mM Tris-HCl pH 8.0, 0.1% Tween 20) containing 5% FBS for 1 h. Then, the membranes were incubated overnight at 4 °C with primary antibodies, including SREBP-1c, carnitine palmitoyltransferase 1 (CPT-1) and 2 (CPT-2) (Cell Signaling Technology, MA, USA); phosphorylated-AMPKα (pAMPKα), AMPKα, fatty acid synthase (FAS) (Santa Cruz, CA, USA); sirtuin 1 (Sirt1) (Millipore); hormone-sensitive lipase (HSL), phosphorylated HSL (pHSL), adipose triglyceride lipase (ATGL), phosphorylated-acetyl CoA carboxylase-1 (pACC-1), ACC-1, peroxisome proliferator-activated receptor α (PPAR-α) and γ (PPAR-γ) (Epitomics, Burlingame, CA, USA), and β-actin (Sigma). The membranes were washed with TBST and incubated at room temperature with secondary antibodies for 1 h. All specific proteins were detected with an enhanced chemiluminescence reagent (Millipore), and protein signals were detected and quantified using the BioSpectrum 600 system (UVP, Upland, CA, USA).

2.7. Statistical analysis

Statistical analyses were performed using one-way ANOVA and Dunnett's post-hoc test. The results were expressed as the mean ± standard deviation, and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Cell viability of HepG2 cells treated with ginkgolide C

Cell viability was evaluated using the MTT method, and oleic acid did not have a significant effect on cell viability at concentrations ≤ 0.5 mM in HepG2 cells (Fig. 1A). Therefore, 0.5 mM oleic acid was used in all experiments. Moreover, ginkgolide C did not have a significant effect on cell viability at concentrations ≤ 100 μM (Fig. 1B) in HepG2 cells. Therefore, 3–100 μM ginkgolide C was used in all experiments.

3.2. Effect of ginkgolide C on lipid accumulation in oleic acid-induced hepatic steatosis

Oleic acid was used to induce hepatic steatosis and cells were then treated with ginkgolide C for 24 h to evaluate lipid accumulation by Oil Red O staining. Oleic acid was able to induce lipid accumulation, which could be reduced by ginkgolide C (Fig. 1C). Hepatocytes were treated with isopropanol to release oil droplets, and we found that cells treated with ginkgolide C had significantly decreased lipid accumulation compared with cells treated only with oleic acid (Fig. 1D). The fluorescent dye BODIPY 493/503 was also used to detect lipid accumulation, and fluorescence images demonstrated that ginkgolide C markedly attenuated lipid accumulation compared with the oleic acid–induced hepatic steatosis cells (Fig. 1E and F).

3.3. The effect of ginkgolide C on transcription factors of lipogenesis

Ginkgolide C significantly suppressed transcription factors of lipogenesis, such as PPAR-γ and SREBP-1c expression, compared with oleic acid–induced HepG2 cells. Ginkgolide C also reduced FAS expression for lipogenesis in fatty liver cells (Fig. 2).

3.4. The effect of ginkgolide C on lipolysis in hepatocytes

Ginkgolide C significantly increased ATGL and pHSL expression in HepG2 cells compared with cells treated with only oleic acid (Fig. 3). Ginkgolide C also significantly promoted CPT-1, CD36, and PPAR-α expression for fatty acid β-oxidation, but CPT-2 was not increased compared with the oleic acid-induced HepG2 cells (Fig. 4).

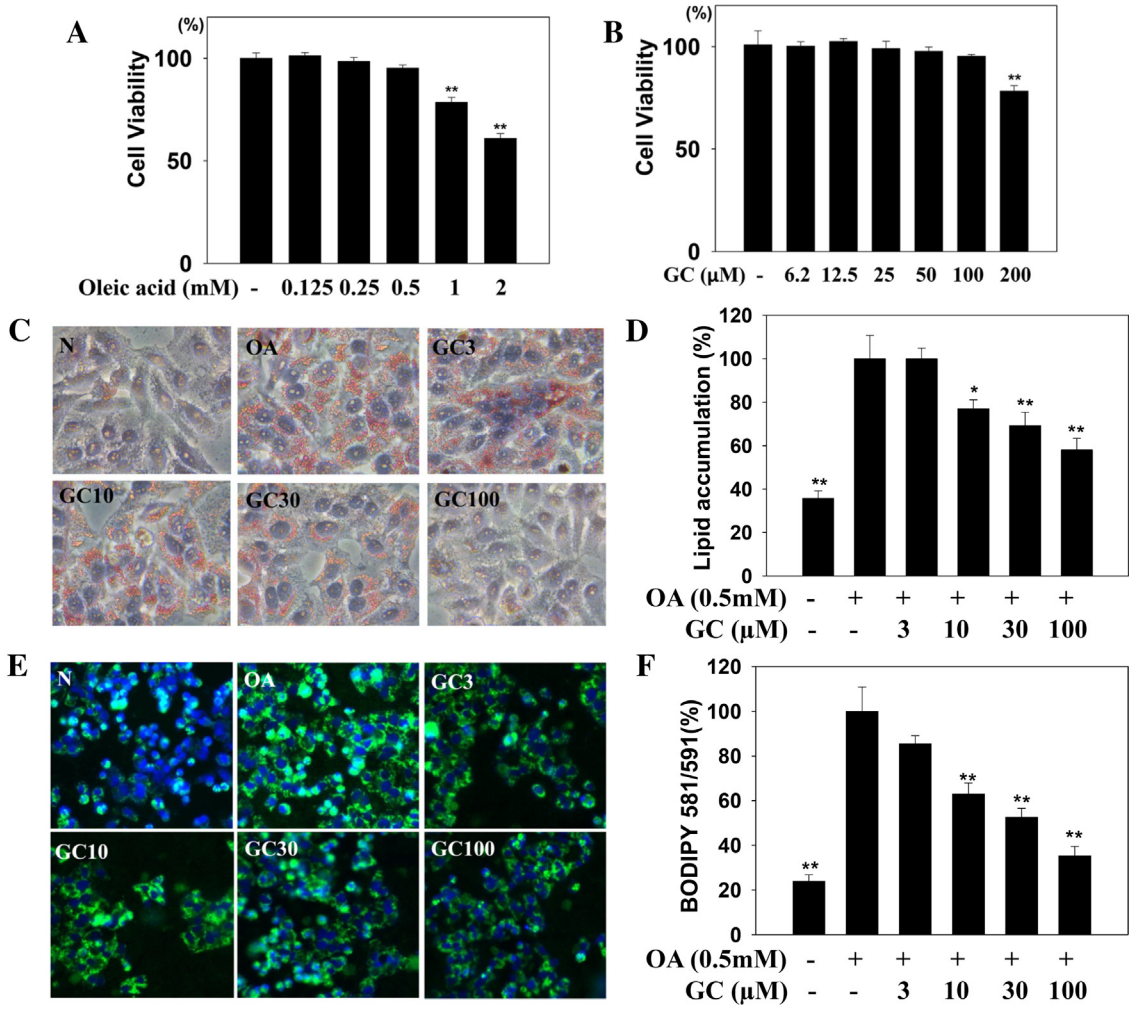

Fig. 1. Ginkgolide C reduced lipid accumulation in HepG2 cells. (A) Cell viability of oleic acid (OA) in HepG2 cells. (B) Cell viability of ginkgolide C (GC) in HepG2 cells. Data represent the mean ± SD; $^{**}P < 0.01$ compared with HepG2 cells not treated with OA or GC. Next, HepG2 cells were treated with 0.5 mM oleic acid (OA) at 37 °C for 48 h to induce lipid accumulation in hepatocytes, followed by ginkgolide C (3–100 μM) for 24 h. (C) Oil Red O staining showed lipid accumulation that was observed with a microscope. (D) HepG2 cells were treated with isopropanol and lipid accumulation was measured using the absorbance at OD 490 nm. (E) Staining with the fluorescent dye BODIPY 493/503 (green) to detect hepatic lipid droplets. Nuclei were stained with DAPI (blue). (F) Fluorescent images were quantified, data represent the mean ± SD; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with OA group. Three independent experiments were analyzed.

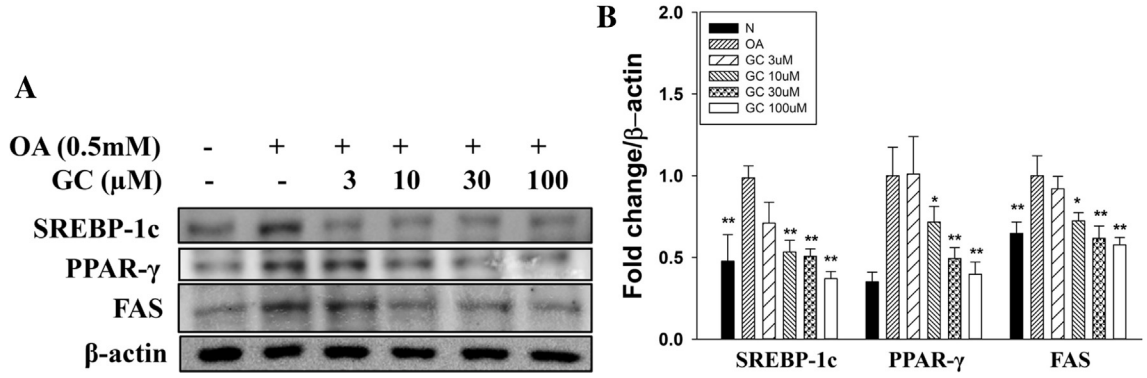

Fig. 2. Effects of ginkgolide C on lipid metabolism in HepG2 cells. HepG2 cells were treated with 0.5 mM oleic acid (OA) for 48 h to induce lipid accumulation, followed by ginkgolide C (3–100 μM) for 24 h. (A) Transcription factors associated with lipogenesis and FAS were detected by Western blot. (B) Three independent experiments were analyzed, and the fold expression levels were measured relative to the expression of β-actin (internal control).

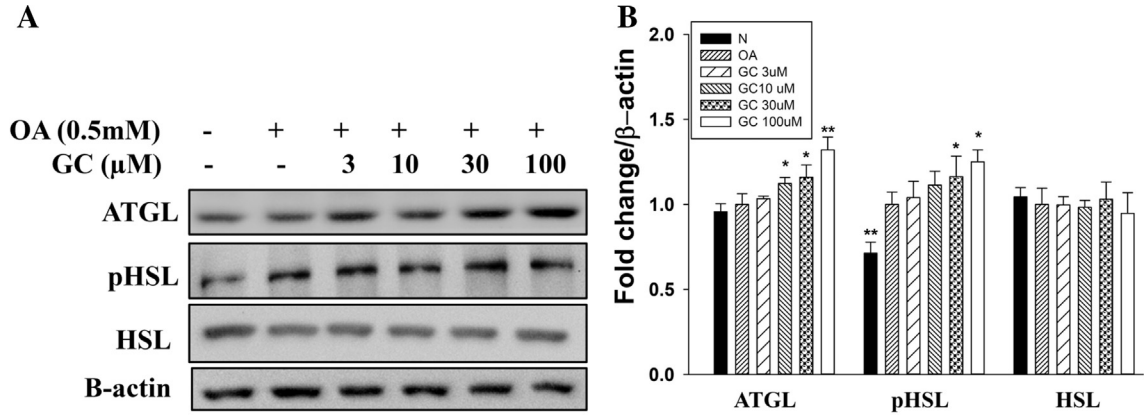

Fig. 3. Effects of ginkgolide C on lipid metabolism in HepG2 cells. HepG2 cells were treated with 0.5 mM oleic acid (OA) for 48 h to induce lipid accumulation in hepatocytes, followed by ginkgolide C (3–100 μM) for 24 h. (A) Lipolysis proteins were detected by Western blot. (B) Three independent experiments were analyzed, and the fold expression levels were measured relative to the expression of β-actin (internal control).

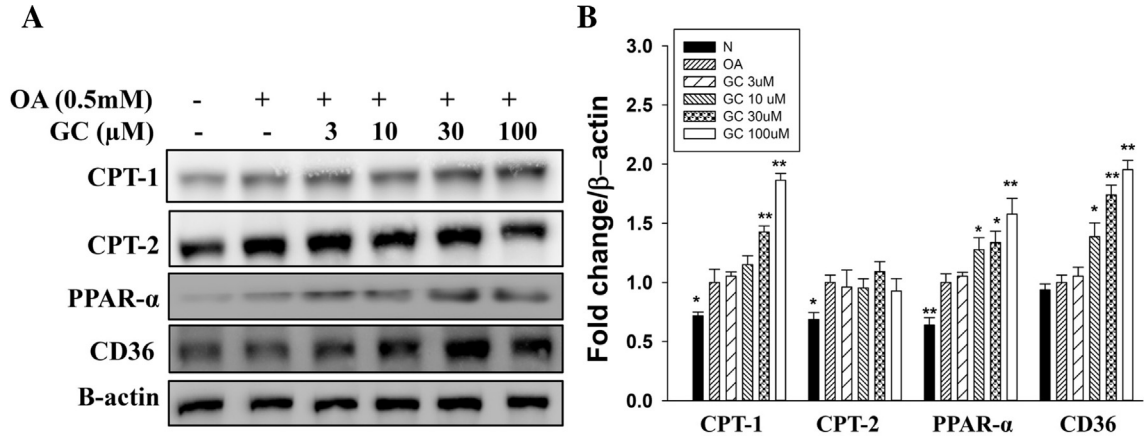

Fig. 4. Effects of ginkgolide C on β-oxidation in HepG2 cells. HepG2 cells were treated with 0.5 mM oleic acid (OA) for 48 h to induce lipid accumulation in hepatocytes, followed by ginkgolide C (3–100 μM) for 24 h. (A) β-oxidation associated proteins were detected by Western blot. (B) Three independent experiments were analyzed, and the fold expression levels were measured relative to the expression of β-actin (internal control).

3.5. Ginkgolide C activated sirt1/AMPK in HepG2 cells

Western blotting was used to evaluate whether ginkgolide C regulated sirt1 and AMPK in oleic acid-induced HepG2 cells. We found that ginkgolide C significantly promoted the expression of sirt1 and phosphorylation of ACC-1 and AMPKα compared with oleic acid–induced hepatocytes (Fig. 5).

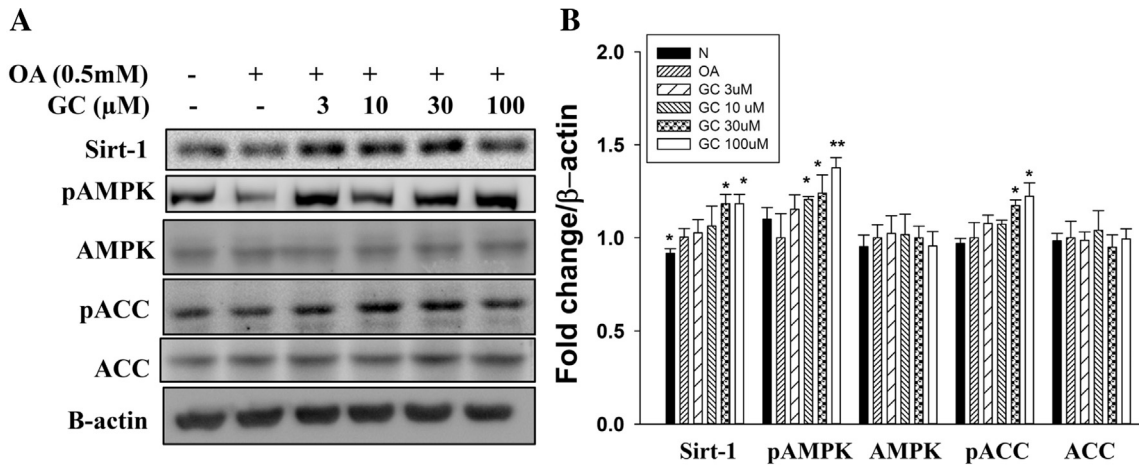

Fig. 5. Effects of ginkgolide C on the AMPK/Sirt-1 pathway in HepG2 cells. HepG2 cells were treated with 0.5 mM oleic acid (OA) for 48 h to induce lipid accumulation in hepatocytes, followed by ginkgolide C (3–100 μM) for 24 h. (A) The AMPK/Sirt-1 pathway proteins were detected by Western blot. (B) Three independent experiments were analyzed, and the fold expression levels were measured relative to the expression of β-actin (internal control).

4. Discussion

This study demonstrated that the diterpene lactone ginkgolide C could stimulate AMPK, leading to suppressed ACC activity and the reduction of fatty acid chains, and CPT-1 for enhanced fatty acid β-oxidation. Ginkgolide C also decreased lipogenesis-related transcription factors for down-regulated FAS expression, and promoted expression of lipolysis-related enzymes to accelerate the decomposition of triglycerides. Hence, ginkgolide C significantly reduced lipid accumulation for improved hepatic steatosis in vitro.

Hepatocytes that take up excessive free fatty acids would activate enzymes associated with lipogenesis, leading to the synthesis of triglycerides and more energy accumulation in the liver (Cao et al., 2016). The expression of lipid synthesis enzymes requires lipid transcription factors to bind to the promoter of the FAS and switch on the lipid synthesis genes (Angeles and Hudkins, 2016). PPAR is the main transcription factor for lipid synthesis (Liss and Finck, 2017). Many studies confirmed that overexpression of PPARγ contributed to the differentiation of adipocytes and increased lipid accumulation in adipocytes and hepatocytes (Janani and Ranjitha Kumari, 2015). In the liver cells, oleic acid could activate PPARγ expression to accelerate lipid accumulation and cause hepatic steatosis (Kang et al., 2015). Our experiments showed that ginkgolide C had the ability to reduce PPARγ production and the accumulation of oil droplets; thus, it was confirmed that ginkgolide C could reduce excessive lipid accumulation by reducing PPARγ in liver cells. In addition, Srebp-1c could also bind to the promoter of FAS to switch on triglyceride synthesis (Wang et al., 2015). We found that oleic acid stimulated Srebp-1c expression and enhanced downstream FAS expression to initiate fat synthesis. Oil Red O and fluorescent staining demonstrated that oleic acid-stimulated hepatocytes had significantly increased oil droplet accumulation compared with normal hepatocytes. However, oleic acid-induced liver cells treated with ginkgolide C had reduced Spreb-1c expression, and ginkgolide C also significantly inhibited FAS expression to block lipid accumulation in hepatocytes. Thus, we believe that ginkgolide C can improve lipid accumulation in fatty liver cells by modulating the transcription of lipid synthesis and FAS.

Another strategy to improve the lipid accumulation in liver cells is to accelerate the decomposition of triglycerides (Romero-Gomez et al., 2017; Smith et al., 2016). The main enzymes that regulate this are ATGL and HSL. ATGL can break down triglycerides to produce diacylglycerol and a molecule of free fatty acid, and the active HSL can also break down diacylglycerol to produce monoacylglycerol and a molecule of free fatty acid (Frühbeck et al., 2014). Many studies found that some flavonoids could enhance lipolysis and inhibit lipid accumulation in hepatocytes (Chang et al., 2013; Lasa et al., 2012). Quercetin could reduce lipid accumulation by enhancing ATGL expression in high glucose-induced fatty hepatocytes (Liu et al., 2015). Caffeic acid also promoted lipolysis via activated ATGL and HSL in oleic acid-induced hepatic steatosis (Liao et al., 2014). Our results demonstrated that ginkgolide C could significantly promote ATGL and phosphorylation of HSL for accelerated decomposition of triglycerides to glycerol and free fatty acids. Hence, ginkgolide C can accelerate lipolysis to improve lipid accumulation in fatty liver cells.

The excessive triglycerides broken down by the liver would release more free fatty acids to stimulate vascular epithelial cells and macrophages leading to an inflammatory response (Ducharme and Bickel, 2008; Young and Zechner, 2013). These macrophages will release pre-inflammatory cytokines to induce inflammation and interfere with the metabolism of carbohydrates and lipids, leading to insulin resistance in liver cells or adipocytes (Hazlehurst et al., 2016; Morrison and Kleemann, 2015). In recent years, it was determined that excessive fatty acids could be broken down by fatty acid β-oxidation to produce energy and reduce the damage to cells (Nguyen et al., 2007; Smith and Minson, 2012). Our findings suggest that ginkgolide C has the ability to enhance β-oxidation-associated enzyme expression, including CPT-1, CD36, and PPARα, while not significantly enhancing CPT-2 production. CD36 is a fatty acid translocase that can transfer free fatty acids or long chain fatty acids from the circulatory system into adipocytes, muscle cells, and liver cells (Pardina et al., 2017; Xie et al., 2017). Some studies showed that CD36 overexpression caused excessive free fatty acid uptake and hepatic steatosis, but reduced free fatty acid damage in hepatocytes, vascular epithelial cells, and adipocytes (Choi et al., 2017). Interestingly, CD36 can activate the β-oxidation break down of fatty acids in hepatocytes (Zingg et al., 2017). Previous studies found that a CD36 gene deletion blocked β-oxidation and increased lipid accumulation in hepatocytes; thus, CD36 contributed to β-oxidation expression and the decomposition of free fatty acids (Xie et al., 2017). The CPT enzyme is the most important for regulating β-oxidation, and can carry the free fatty acids from the cytoplasm into mitochondria (Pucci et al., 2016). CPT1 is located on the outer mitochondrial membrane and can convert the long-chain acyl-CoA to acylcarnitine, and assist carnitine translocase to carry acylcarnitine into the inner

membrane (Vishwanath, 2016). The CPT2 enzyme is located on the inner mitochondrial membrane and converts acylcarnitine to long-chain acyl-CoA that enters the fatty acid β-oxidation pathway and tricarboxylic acid cycle to produce ATP (Houten et al., 2016). Some studies confirmed that PPARα was an important transcription factor to modulate mitochondrial fatty acid β-oxidation in liver tissue (Felicidade et al., 2015; Kim et al., 2017). Previous studies confirmed that the gene variants of PPARα increased the development of cardiovascular disease and dyslipidemia as well as the fasting and postprandial blood sugar levels (Liss and Finck, 2017). Therefore, ginkgolide C could increase fatty acid β-oxidation leading to decreased free fatty acids, inflammation, and insulin resistance in hepatocytes.

Recent studies found that sirt1/AMPK activation could regulate the intracellular energy flow (Lim et al., 2010). When cells took up more energy, they increased AMPK activity to reduce lipid synthesis in liver cells or adipocytes (Forbes-Hernandez et al., 2017). AMPK phosphorylation also induced phosphorylation of ACC leading to decreased synthesis of the fatty acid chain (Smith et al., 2016). Resveratrol is a sirt1 inducer, and some studies showed that it could reduce lipid accumulation and improve NAFLD via the activated sirt1/AMPK pathway in HDF-induced obese mice (Elgebaly et al., 2017; Lasa et al., 2012). In a diabetic mouse model, resveratrol also regulated the oral glucose tolerance test and improved insulin resistance by promoting AMPK activity (Wu et al., 2016). Our result demonstrated that ginkgolide C significantly increased sirt1 expression and AMPK phosphorylation. Ginkgolide C also promoted ACC phosphorylation to block the synthesis of the fatty acid chain in oleic acid–induced hepatocytes.

In conclusion, ginkgolide C can inhibit hepatic accumulation by blocking transcription factors regulating lipid synthesis and FAS for lipogenesis. Ginkgolide C also promoted lipolysis and β-oxidation in hepatocytes, and increased sirt1/AMPK activity to suppress synthesis of the long fatty acid chain. Therefore, ginkgolide C has potential for improving hepatic steatosis.

Acknowledgments

This study was supported in part by grants from the Chang Gung Memorial Hospital (CMRPF1G0081, CMRPF1F0083, and CMRPF1G0201) and by a grant from the Ministry of Science and Technology in Taiwan (106-2320-B-255-008-MY3).

Conflict of interest statement

The authors have declared no conflict of interest.

References

Angeles, T.S., Hudkins, R.L., 2016. Recent advances in targeting the fatty acid biosynthetic pathway using fatty acid synthase inhibitors. Expert. Opin. Drug. Discov. 11, 1187–1199.
Babayigit, A., Olmez, D., Karaman, O., Ozogul, C., Yilmaz, O., Kivcak, B., Erbil, G., Uzuner, N., 2009. Effects of Ginkgo biloba on airway histology in a mouse model of chronic asthma. Allergy Asthma Proc. 30, 186–191.
Benedict, M., Zhang, X., 2017. Non-alcoholic fatty liver disease: an expanded review. World. J. Hepatol. 9, 715–732.
Brouwers, B., Hesselink, M.K., Schrauwen, P., Schrauwen-Hinderling, V.B., 2016. Effects of exercise training on intrahepatic lipid content in humans. Diabetologia 59, 2068–2079.
Cao, P., Huang, G., Yang, Q., Guo, J., Su, Z., 2016. The effect of chitooligosaccharides on oleic acid-induced lipid accumulation in HepG2 cells. Saudi. Pharm. J. 24, 292–298.
Chang, J.J., Hsu, M.J., Huang, H.P., Chung, D.J., Chang, Y.C., Wang, C.J., 2013. Mulberry anthocyanins inhibit oleic acid induced lipid accumulation by reduction of lipogenesis and promotion of hepatic lipid clearance. J. Agric. Food Chem. 61, 6069–6076.
Chang, Y.H., Chen, Y.L., Huang, W.C., Liou, C.J., 2018. Fucoxanthin attenuates fatty acid-induced lipid accumulation in FL83B hepatocytes through regulated Sirt1/AMPK signaling pathway. Biochem. Biophys. Res. Commun. 495, 197–203.
Choi, Y.J., Lee, K.Y., Jung, S.H., Kim, H.S., Shim, G., Kim, M.G., Oh, Y.K., Oh, S.H., Jun, D. W., Lee, B.H., 2017. Activation of AMPK by berberine induces hepatic lipid accumulation by upregulation of fatty acid translocase CD36 in mice. Toxicol. Appl. Pharmacol. 316, 74–82.
Ducharme, N.A., Bickel, P.E., 2008. Lipid droplets in lipogenesis and lipolysis. Endocrinology 149, 942–949.
Elgebaly, A., Radwan, I.A., AboElnas, M.M., Ibrahim, H.H., Eltoomy, M.F., Atta, A.A., Mesalam, H.A., Sayed, A.A., Othman, A.A., 2017. Resveratrol supplementation in patients with non-alcoholic fatty liver disease: systematic review and meta-analysis. J. Gastrointestin. Liver Dis. 26, 59–67.
Felicidade, I., Marcarini, J.C., Carreira, C.M., Amarante, M.K., Afman, L.A., Mantovani, M.S., Ribeiro, L.R., 2015. Changes in gene expression in PBMCs profiles of PPARalpha target genes in obese and non-obese individuals during fasting. Ann. Nutr. Metab. 66, 19–25.
Feng, X., Yu, W., Li, X., Zhou, F., Zhang, W., Shen, Q., Li, J., Zhang, C., Shen, P., 2017. Apigenin, a modulator of PPARgamma, attenuates HFD-induced NAFLD by regulating hepatocyte lipid metabolism and oxidative stress via Nrf2 activation. Biochem. Pharmacol. 136, 136–149.
Forbes-Hernandez, T.Y., Giampieri, F., Gasparrini, M., Afrin, S., Mazzoni, L., Cordero, M.D., Mezzetti, B., Quiles, J.L., Battino, M., 2017. Lipid accumulation in HepG2 cells is attenuated by strawberry extract through AMPK activation. Nutrients 9, E621.
Forte, V., Pandey, A., Abdelmessih, R., Forte, G., Whaley-Connell, A., Sowers, J.R., McFarlane, S.I., 2012. Obesity, diabetes, the cardiorenal syndrome, and risk for cancer. Cardiorenal Med. 2, 143–162.
Frühbeck, G., Méndez-Giménez, L., Fernández-Formoso, J.A., Fernández, S., Rodríguez, A., 2014. Regulation of adipocyte lipolysis. Nutr. Res. Rev. 27, 63–93.
Guo, L., Li, X., Tang, Q.Q., 2015. Transcriptional regulation of adipocyte differentiation: a central role for CCAAT/enhancer-binding protein (C/EBP) beta. J. Biol. Chem. 290, 755–761.
Hazlehurst, J.M., Woods, C., Marjot, T., Cobbold, J.F., Tomlinson, J.W., 2016. Non-alcoholic fatty liver disease and diabetes. Metabolism 65, 1096–1108.
Hou, X., Xu, S., Maitland-Toolan, K.A., Sato, K., Jiang, B., Ido, Y., Lan, F., Walsh, K., Wierzbicki, M., Verbeuren, T.J., Cohen, R.A., Zang, M., 2008. SIRT1 regulates hepatocyte lipid metabolism through activating AMP-activated protein kinase. J. Biol. Chem. 283, 20015–20026.
Houten, S.M., Violante, S., Ventura, F.V., Wanders, R.J., 2016. The biochemistry and physiology of mitochondrial fatty acid beta-oxidation and its genetic disorders. Annu. Rev. Physiol. 78, 23–44.
Huang, P., Zhang, L., Chai, C., Qian, X.C., Li, W., Li, J.S., Di, L.Q., Cai, B.C., 2014. Effects of food and gender on the pharmacokinetics of ginkgolides A, B, C and bilobalide in rats after oral dosing with ginkgo terpene lactones extract. J. Pharm. Biomed. Anal. 100, 138–144.
Huang, W.C., Fang, L.W., Liou, C.J., 2017. Phloretin attenuates allergic airway inflammation and oxidative stress in asthmatic mice. Front. Immunol. 8, 134.
Janani, C., Ranjitha Kumari, B.D., 2015. PPAR gamma gene–a review. Diab. Metab. Syndr. 9, 46–50.
Jeong, H.S., Kim, K.H., Lee, I.S., Park, J.Y., Kim, Y., Kim, K.S., Jang, H.J., 2017. Ginkgolide A ameliorates non-alcoholic fatty liver diseases on high fat diet mice. Biomed. Pharmacother. 88, 625–634.
Kang, O.H., Kim, S.B., Mun, S.H., Seo, Y.S., Hwang, H.C., Lee, Y.M., Lee, H.S., Kang, D.G., Kwon, D.Y., 2015. Puerarin ameliorates hepatic steatosis by activating the PPARalpha and AMPK signaling pathways in hepatocytes. Int. J. Mol. Med. 35, 803–809.
Kim, S.M., Lee, B., An, H.J., Kim, D.H., Park, K.C., Noh, S.G., Chung, K.W., Lee, E.K., Kim, K.M., Kim, S.J., Chun, P., Lee, H.J., Moon, H.R., Chung, H.Y., 2017. Novel PPARalpha agonist MHY553 alleviates hepatic steatosis by increasing fatty acid oxidation and decreasing inflammation during aging. Oncotarget 8, 46273–46285.
Lasa, A., Schweiger, M., Kotzbeck, P., Churruca, I., Simon, E., Zechner, R., Portillo, M. P., 2012. Resveratrol regulates lipolysis via adipose triglyceride lipase. J. Nutr. Biochem. 23, 379–384.
Liao, C.C., Ou, T.T., Huang, H.P., Wang, C.J., 2014. The inhibition of oleic acid induced hepatic lipogenesis and the promotion of lipolysis by caffeic acid via up-regulation of AMP-activated kinase. J. Sci. Food Agric. 94, 1154–1162.
Lim, C.T., Kola, B., Korbonits, M., 2010. AMPK as a mediator of hormonal signalling. J. Mol. Endocrinol. 44, 87–97.
Liou, C.J., Huang, W.C., 2017. Casticin inhibits interleukin-1β–induced ICAM-1 and MUC5AC expression by blocking NF-κB, PI3K-Akt, and MAPK signaling in human lung epithelial cells. Oncotarget 8, 101175–101188.
Liou, C.J., Lai, X.Y., Chen, Y.L., Wang, C.L., Wei, C.H., Huang, W.C., 2015. Ginkgolide C suppresses adipogenesis in 3T3-L1 adipocytes via the AMPK signaling pathway. Evid. Based Complem. Alternat. Med. 2015, 298635.
Liss, K.H., Finck, B.N., 2017. PPARs and nonalcoholic fatty liver disease. Biochimie 136, 65–74.
Liu, L., Gao, C., Yao, P., Gong, Z., 2015. Quercetin alleviates high-fat diet-induced oxidized low-density lipoprotein accumulation in the liver: implication for autophagy regulation. Biomed. Res. Int. 2015, 607531.
Morrison, M.C., Kleemann, R., 2015. Role of macrophage migration inhibitory factor in obesity, insulin resistance, type 2 diabetes, and associated hepatic co-morbidities: a comprehensive review of human and rodent studies. Front. Immunol. 6, 308.
Neuschwander-Tetri, B.A., 2017. Non-alcoholic fatty liver disease. BMC Med. 15, 45.

Nguyen, M.T., Favelyukis, S., Nguyen, A.K., Reichart, D., Scott, P.A., Jenn, A., Liu-Bryan, R., Glass, C.K., Neels, J.G., Olefsky, J.M., 2007. A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2 and 4 and JNK-dependent pathways. J. Biol. Chem. 282, 35279–35292.

Pardina, E., Ferrer, R., Rossell, J., Ricart-Jane, D., Mendez-Lara, K.A., Baena-Fustegueras, J.A., Lecube, A., Julve, J., Peinado-Onsurbe, J., 2017. Hepatic CD36 downregulation parallels steatosis improvement in morbidly obese undergoing bariatric surgery. Int. J. Obes. 41, 1388–1393.

Patil, R., Sood, G.K., 2017. Non-alcoholic fatty liver disease and cardiovascular risk. World J. Gastrointest. Pathophysiol. 8, 51–58.

Pucci, S., Zonetti, M.J., Fisco, T., Polidoro, C., Bocchinfuso, G., Palleschi, A., Novelli, G., Spagnoli, L.G., Mazzarelli, P., 2016. Carnitine palmitoyl transferase-1A (CPT1A): a new tumor specific target in human breast cancer. Oncotarget 7, 19982–19996.

Reccia, I., Kumar, J., Akladios, C., Virdis, F., Pai, M., Habib, N., Spalding, D., 2017. Non-alcoholic fatty liver disease: a sign of systemic disease. Metabolism 72, 94–108.

Romero-Gomez, M., Zelber-Sagi, S., Trenell, M., 2017. Treatment of NAFLD with diet, physical activity and exercise. J. Hepatol. 67, 829–846.

Smith, B.K., Marcinko, K., Desjardins, E.M., Lally, J.S., Ford, R.J., Steinberg, G.R., 2016. Treatment of nonalcoholic fatty liver disease: role of AMPK. Am. J. Physiol. Endocrinol. Metab. 311, E730–E740.

Smith, M.M., Minson, C.T., 2012. Obesity and adipokines: effects on sympathetic overactivity. J. Physiol. 590, 1787–1801.

Stein, C., Hopfeld, J., Lau, H., Klein, J., 2015. Effects of *Ginkgo biloba* extract EGb 761, donepezil and their combination on central cholinergic function in aged rats. J. Pharm. Pharm. Sci. 18, 634–646.

Tian, Y., Ma, J., Wang, W., Zhang, L., Xu, J., Wang, K., Li, D., 2016. Resveratrol supplement inhibited the NF-kappaB inflammation pathway through activating AMPKalpha-SIRT1 pathway in mice with fatty liver. Mol. Cell Biochem. 422, 75–84.

Vishwanath, V.A., 2016. Fatty acid beta-oxidation disorders: a brief review. Ann. Neurosci. 23, 51–55.

Wang, Y., Viscarra, J., Kim, S.J., Sul, H.S., 2015. Transcriptional regulation of hepatic lipogenesis. Nat. Rev. Mol. Cell Biol. 16, 678–689.

Wu, H., Sheng, Z.Q., Xie, J., Li, R., Chen, L., Li, G.N., Wang, L., Xu, B., 2016. Reduced HMGB 1-mediated pathway and oxidative stress in resveratrol-treated diabetic mice: a possible mechanism of cardioprotection of resveratrol in diabetes mellitus. Oxid. Med. Cell Longev. 2016, 9836860.

Xie, Y., Cifarelli, V., Pietka, T., Newberry, E.P., Kennedy, S.M., Khalifeh-Soltani, A., Clugston, R., Atabai, K., Abumrad, N.A., Davidson, N.O., 2017. Cd36 knockout mice are protected against lithogenic diet-induced gallstones. J. Lipid Res. 58, 1692–1701.

Yin, B., Xu, Y., Wei, R., Luo, B., 2014. Ginkgo biloba on focal cerebral ischemia: a systematic review and meta-analysis. Am. J. Chin. Med. 42, 769–783.

Young, S.G., Zechner, R., 2013. Biochemistry and pathophysiology of intravascular and intracellular lipolysis. Genes Dev. 27, 459–484.

Zeng, Z., Zhu, J., Chen, L., Wen, W., Yu, R., 2013. Biosynthesis pathways of ginkgolides. Pharmacogn. Rev. 7, 47–52.

Zingg, J.M., Hasan, S.T., Nakagawa, K., Canepa, E., Ricciarelli, R., Villacorta, L., Azzi, A., Meydani, M., 2017. Modulation of cAMP levels by high-fat diet and curcumin and regulatory effects on CD36/FAT scavenger receptor/fatty acids transporter gene expression. BioFactors 43, 42–53.