European Review for Medical and Pharmacological Sciences 2013; 17: 2578-2586

Curcumin decreases oleic acid-induced lipid accumulation *via* AMPK phosphorylation in hepatocarcinoma cells

O.H. KANG, S.B. KIM[1], Y.S. SEO, D.K. JOUNG, S.H. MUN[1], J.G. CHOI, Y.M. LEE, D.G. KANG[1], H.S. LEE[1], D.Y. KWON

Department of Oriental Pharmacy, College of Pharmacy, Wonkwang University, Wonkwang Oriental Medicines Research Institute, Institute of Biotechnology, Iksan, Jeonbuk, Republic of Korea
[1]Professional Graduate School of Oriental Medicine and College of Oriental Medicine, Wonkwang University, Iksan, Jeonbuk, Republic of Korea

Abstract. – BACKGROUND AND OBJECTIVES: Non-alcoholic fatty liver disease (NAFLD) is one of the most common metabolic syndromes and is characterized by the accumulation of hepatic triglycerides (TG), which result from an imbalance between uptake, synthesis, export, and oxidation of fatty acids. Curcumin is a polyphenol derived from the herbal remedy and dietary spice turmeric, was found to prevent obesity and diabetes in mouse models. However, a hypolipidemic effect of curcumin in oleic acid- induced hepatocarcinoma cells has not been reported. In this study, we examined the effect of curcumin on reducing lipid accumulation in hepatic cells.

MATERIALS AND METHODS: Hepatocytes were treated with oleic acid (OA) containing with or without curcumin to observe the lipid accumulation by Oil Red O stain. We also tested the effects of curcumin on triglycerides (TG) and total cholesterol (TC) in HepG2 cells. Western blot and reverse transcription polymerase chain reaction (RT-PCR) was used to measure sterol regulatory element binding proteins-1 (SREBP-1), fatty acid synthase (FAS), peroxisome proliferator-activated receptor (PPAR)-α, and adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) expression.

RESULTS: Curcumin suppressed OA-induced lipid accumulation and TG and TC levels. Also, curcumin decreased hepatic lipogenesis such as SREBP-1, and FAS. Besides, we also found out the antioxidative effect of curcumin by increasing the expression of PPARα. Curcumin increased AMPK phosphorylation in hepatocytes.

CONCLUSIONS: These results indicated that curcumin has the same ability to activate AMPK and then reduce SREBP-1, and FAS expression, finally leading to inhibit hepatic lipogenesis and hepatic antioxidative ability. In this report, we found curcumin exerted a regulatory effect on lipid accumulation by decreasing lipogenesis in hepatocyte. Therefore, curcumin extract may be active in the prevention of fatty liver.

Key Words:
Curcumin, Hepatic lipogenesis, Lipid accumulation, AMPK.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by hepatic fat accumulation in the absence of significant ethanol consumption. NAFLD is now recognized as the most common and emerging chronic liver disease in western countries and is now spreading rapidly to other parts of the world[1-3]. NAFLD is characterized by the accumulation of hepatic triglycerides (TG), which result from an imbalance between uptake, synthesis, export, and oxidation of fatty acids[4]. Approximately 20-30% of adults are estimated to have excess liver fat accumulation in a normal population[5]. It has been considered that increased free fatty acids (FFA) supplied to the liver play a major role in the early stage of NAFLD[6]. Studies have shown that sterol regulatory element-binding proteins (SREBPs) regulate lipid metabolism[7]. SREBP-1c plays an essential role in the regulation of lipogenesis involved in fatty acid and TG synthesis[8-10].

It has also been shown that peroxisome proliferator-activated receptors (PPARs)- ligand-activated nuclear receptors-mediate the critical transcriptional regulation of genes associated with lipid homeostasis[11]. Especially, PPAR-α is most abundantly expressed in the liver and has the effect of diminishing circulating triglycerides and increasing high dense lipoprotein (HDL) cholesterol[12].

The adenosine monophosphate (AMP)-activated protein kinase (AMPK) plays a key role in energy homeostasis and it has been proposed to monitor changes in the energy status of cells[13]. The activation of AMPK by pharmacological

Corresponding Author: Dong-Yeul Kwon, Ph.D; e-mail: sssimi@wonkwang.ac.kr

agents presents a unique challenge, given the complexity of the biology, but holds a considerable potential to reverse the metabolic abnormalities[14]. In skeletal muscles, AMPK stimulates glucose transport and fatty acid (FA) oxidation. In the liver, it decreases glucose output, leading to lowering blood glucose levels in hyperglycemic individuals[15]. AMPK may play a key role in regulating the activation of SREBP-1 and lipogenesis[16]. The process of hepatic stellate cells activation is accompanied by depletion of intracellular lipid droplets, loss of lipid storage capacity, and suppression of expression of transcription factors, including SREBP-1, FAS[17,18].

Dietary fat is one of the most important environmental factors associated with the incidence of NFLD. Recent studies on fatty liver in food science have focused on the searching for functional food ingredients or herbal extracts that can suppress the accumulation of hepatic lipid. Curcumin is a polyphenol derived from the herbal remedy and dietary spice turmeric, was found to prevent obesity and diabetes in mouse models[19]. Recently studies, curcumin may exert its beneficial effects *via* reducing insulin and leptin resistance, attenuating inflammatory cytokine expression, accelerating fatty acid oxidation, as well as increasing antioxidant enzyme expression[20].

The purposes of this study were to further elucidate the underlying mechanisms, focusing on the effects of curcumin in oleic acid- induced hepatoma cells regulating the levels of intracellular lipids. Results in this report supported our initial hypothesis that one of the mechanisms by which oleic acid stimulated human hepatoma HepG2 cells activation was to stimulate the depletion of intracellular lipids. The oleic acid stimulatory effect was eliminated by curcumin by activating AMPK activity, leading to the induction of expression of genes relevant to lipid accumulation, the elevation of the level of intracellular lipids, and the inhibition of HepG2 activation.

Materials and Methods

Materials

Curcumin used in this study was purchased from Sigma-Aldrich (St. Louis, MO, USA). The Oil red O, oleic acid and atorvastatin were purchased from Sigma-Aldrich. The 3-(4, 5-dimethylthiazol-2-yl) -5-(3 carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) was purchased from Promega (Madison, WI, USA). Anti-β-actin, peroxisome proliferator activated receptor (PPAR)-α, sterol regulatory element binding protein (SREBP) and fatty acid synthase (FAS) antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-pThr172-AMPK and anti-AMPK antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA).

Cell Culture

Human hepatoma HepG2 cells were obtained from American Type Culture Collection and grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 μg/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine (HyClone®, Thermo Scientific, Logan, UT, USA). The cells were cultured at 37°C in a humidified atmosphere of 95% air to 5% CO_2.

Cytotoxicity Assay

The cell viability was examined by a 3-(4,5-dimethylthiazol-2-yl) -5-(3 carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. Briefly, HepG2 were seeded at a density of 1×10^5 cells/mL in 96-well plates (Nunc, A/S, Roskilde, Denmark). To determine the non-toxic concentration for cells, curcumin (1, 5, 10, 25, and 50 μM) was then added to each well. The plates were then incubated for 24 h at 37°C under 5% CO_2. The MTS solution (5 mg/mL) was added to each well and the cells were cultured for another 2 h, after which the optical density was read at 490 nm. Cytotoxicity was then calculated using the formula: 1-(mean absorbance value of treated cells/mean absorbance value of untreated cells).

Oil Red O Stain

For examination of fat accumulation in HepG2 cells, the cells were treated for 24 h with curcumin. The cells were rinsed with cold phosphate buffered saline (PBS) and fixed in 10% paraformaldehyde for 30 min. After the cells were washed with 60% isopropanol, the cells were stained for at least 1 hour in a freshly diluted Oil Red O solution (six parts Oil Red O stock solution and four parts H_2O; Oil Red O Stock solution is 0.5% Oil Red O in isopropanol). After the stain was removed and the cells were washed with 60% isopropanol, the image of each group was photographed. The stained lipid droplets were then extracted with isopropanol for quantification by measuring its absorbance at 490 nm.

Table I. Sequences of oligonucleotide primers designed for PCR.

cDNA	Sequences	
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hSREBP1	forward	5'-GTGGCGGCTGCATTGAGAGTGAG-3'
	revers	5'-AGGTACCCGAGGGCATCCGAGAAT-3'
hFAS	forward	5'-CAAGAACTGCACGGAGGTGT-3'
	revers	5'- AGCTGCCAGAGTCGGAGAAC-3'
hPPARα	forward	5′-CCTCTCAGGAAAGGCCAGTA-3′
	revers	5′-TCCACAGCAAATGATAGCAG-3′
hGAPDH	forward	5'-TCCACCACCCTGTTGCTGTAAG-3'
	revers	5'-GTACCCGAGGGCATCCGAGAAT-3'

Measurement of Lipid Levels

Levels of triglycerides (TG) and total cholesterol (TC) in HepG2 cells were quantified by a kit method (BioVision, Mountain View, CA, USA) as per the manufacturer's instructions.

Western Blot Analysis

Protein expression was assessed by western blot analysis according to standard procedures. The HepG2 cells were cultured in 6-well plate (5×10^5 cells/mL) and pretreated with various concentrations of curcumin (1, 5, and 10 μM). After 1 h the cells were OA-induced (0.5 mM), and then incubated at 37°C. After 24 h incubation, the cells were washed twice in PBS. The cell pellets were resuspended in lysis buffer on ice for 20 minutes, and the cell debris was removed by centrifugation (13,000 rpm, 10 min, 4°C). The protein concentrations were determined using the Bio-Rad protein assay reagent (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. Equal amounts of protein (20 μg) were subjected to sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE) electrophoresis and then transferred onto a polyvinylidene membrane (Millipore, Bedford, MA, USA). The membrane was blocked with 5% nonfat milk in Tris-buffered saline with Tween 20 buffer (150 mM NaCl, 20 mM Tris-HCl, and 0.05% Tween 20, pH 7.4). After blocking, the membrane was incubated with primary antibodies for 18 h. Antibodies against AMPK, phospho-AMPK were purchased from Cell Signaling Technology (Beverly, MA, USA), and fatty acid synthase (FAS), SREBP-1c, PPARα, and ß-actin antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The membrane was then washed with Tris-buffered saline with Tween 20 and incubated with anti-mouse or anti-rabbit immunoglobulin G horseradish peroxidase-conjugated secondary antibodies. The proteins were then supplemented with ECL prime Western blotting detection reagents (GE Healthcare) and ImageQuant LAS 4000 Mini Biomolecular Imager (GE Healthcare, Cleveland, OH, USA) was used for evaluating bands.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) Analysis

Total-RNA was isolated using an easy-BLUE total-RNA extraction kit according to the manufacturer's instructions. Single-strand cDNA synthesis was performed as described previously using 5 μg of RNA, oligo(dt) 15 primers and reverse transcriptase in a total volume of 50 μl. PCR reactions were performed in a total volume of 20 μL comprising 2 μL of cDNA product, 0.2 mM of each dNTP, 20 pmol of each primer, and 0.8 units of Taq polymerase. Primer sequences for glyceraldehyde phosphate dehydrogenase (GAPDH), SREBP1, FAS, and PPARα were performed as described Table I. The PCR products increased as the concentration of RNA increased. Finally, the products were electrophoresed on a 2.0% agarose gel and visualized by staining with ethidium bromide.

Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test for multiple comparisons. The data from the experiments are presented as means ± SEM $p < 0.05$ was considered statistically significant.

Results

Cytotoxicity of Oleic Acid and Curcumin in HepG2 cells

To evaluate the effects of oleic acid (OA) and curcumin on the cell viability of human HepG2

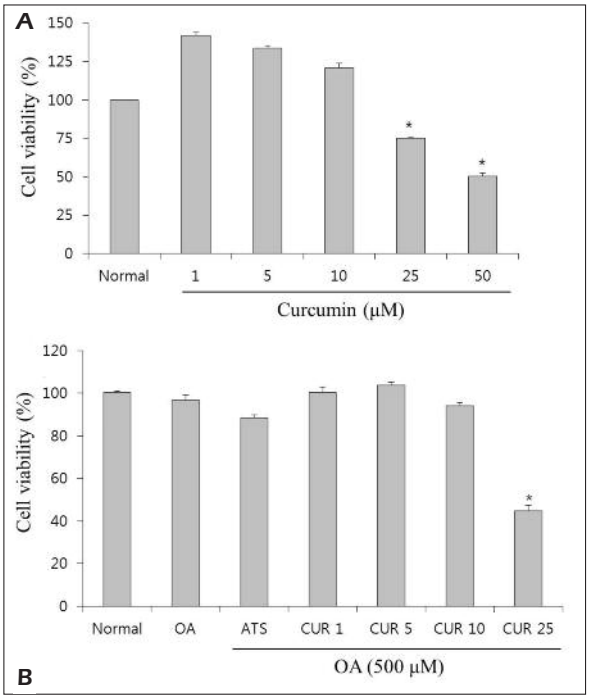

Figure 1. Cytotoxicity of oleic acid and curcumin in HepG2 cells. HepG2 cells were treated with indicated concentrations of OA atorvastain and curcumin for 24 h. The viability of HepG2 cells was determined by MTS assay. CUR = curcumin, ATS = atorvastain. The result was presented as mean ± SD of 3 independent experiments.

cells, an MTS assay was conducted. Due to the fact that OA can induce cell apoptosis and reduce cell viability when the concentrations of OA are over 1 mM, the effects of OA on the intracellular lipid accumulation of HepG2 cells were measured at a concentration range below 500 μM (data not shown). Curcumin treatment of HepG2 cells resulted in slight inhibition of cell growth at 25 μM and over. Our data indicated that concentrations of 1, 5 and 10 μM curcumin are not cytotoxic to HepG2 cells. We used OA (500 μM) pretreated with curcumin or atorvastatin to investigate the cell viability in HepG2 cells. Our data indicated that OA pretreated with curcumin (1, 5 and 10 μM) or atorvastatin (50 μM) are not cytotoxic to HepG2 cells (Figure 1B). Herein, atorvastatin, a class of drugs that lowers cholesterol level in human, was used as a positive control.

Effects of Curcumin on Intracellular Lipid Accumulation in HepG2 cells

To verify the inhibition of curcumin of OA-induced lipid accumulation, HepG2 cells were treated with indicated concentrations of curcumin and atorvastatin in the presence of OA for 24 h. Then cells were stained with Oil Red O and quantified by measuring its absorbance at 490 nm. Both curcumin and atorvastatin stimulations weakened OA-mediated Oil Red O stains in a dose-dependent manner (Figure 2A). The quantitative data of Oil Red O stains displayed 1, 5, and 10 μM curcumin stimulations, 25%, 26%, and 34% reduction of lipid accumulation were observed (Figure 2B). Hence, 50 μM ATS could reduce 35% of lipid accumulation.

Effects of Curcumin on TG and TC Levels in HepG2 cells

Further analyzing the effect of curcumin on OA-induced lipid accumulation was measured of

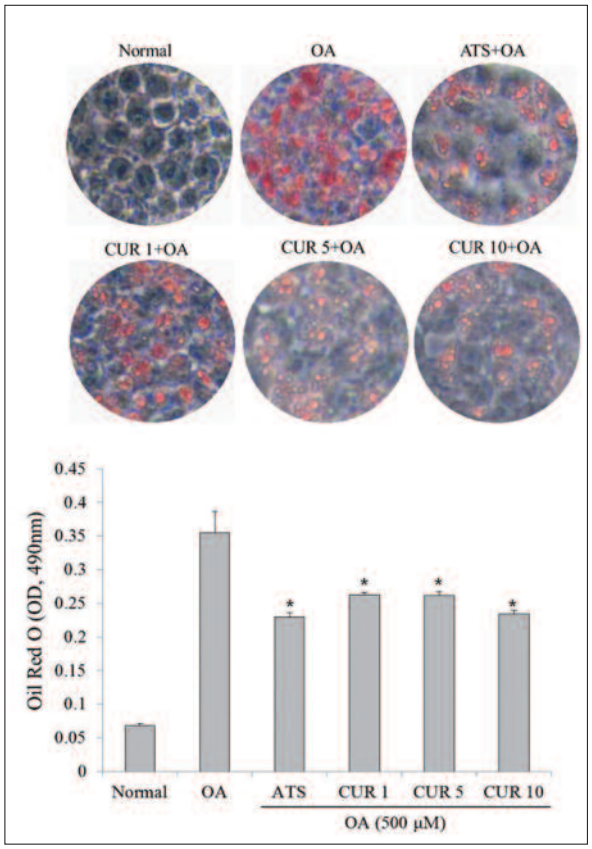

Figure 2. Effects of curcumin on intracellular lipid accumulation in HepG2 cells. HepG2 cells were treated with indicated concentrations of statins or curcumin in the presence of OA for 24 h. Cells were stained with Oil Red O ***(A)***, and analyzed by spectrophotometer ***(B)***. Quantitative assessment of the percentage of lipid accumulation. The average of 3 independent experiments. CUR = curcumin, ATS = atorvastain. The result was expressed as mean ±SD. * $p < 0.05$.

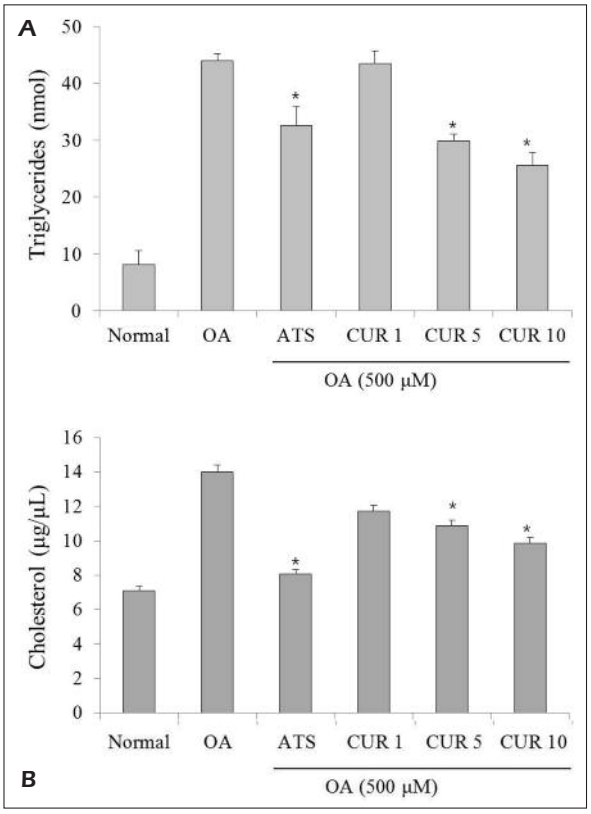

Figure 3. Effects of curcumin on TG and TC Levels in HepG2 cells. Cellular TG ***(A)*** and TC ***(B)*** was induced by 500 μM OA and cells were treated with indicated concentrations of curcumin and statin in the presence of OA for 24 h. Total intracellular triglyceride was analyzed using enzymatic colorimetric method. CUR = curcumin, ATS = atorvastain. The result was expressed as mean ± SD = 3, *$p < 0.05$. OA as control (cells treated with OA only).

TG level in HepG2 cells. In the Figure 3A, 50 μM atorvastatin reduced TG level in OA-pretreated cells about 30%, respectively. But treating with 1, 5 and 10 μM of curcumin resulted in 6%, 31% and 40% reduction in TG. TC levels were displayed 1, 5, and 10 μM curcumin, 16%, 23%, and 30% reduction in dose dependent.

Effects of Curcumin on Hepatic Lipid Accumulation and Protein and mRNA Expression

To determine the mechanism by which curcumin reduced hepatic lipid accumulation, Western blot analysis and RT-PCR were performed to evaluate expression of genes important in lipid metabolism. Genes involved in lipogenesis and fatty acid β-oxidation (SREBP-1c, FAS, and PPARα), significantly increased in OA-induced HepG2 cells at the mRNA and protein levels (Figure 4). As shown in Figure 4A and 4B, curcumin decreased expression of genes and proteins involved in lipogenesis (SREBP-1c, and FAS). Conversely, curcumin increased expression of gene and protein involved in PPARα. These results suggest that curcumin reduces hepatic lipid accumulation in two ways: down regulating lipogenic proteins and up regulating proteins in β-oxidation pathway.

Effects of Curcumin on AMPK Activity in HepG2 cells

AMPK is a key regulator of fatty acid oxidation and lipogenesis in metabolic tissues. The change of AMPK activity in HepG2 cells is strongly associated with intracellular lipid metabolism. So, to investigate the effects of curcumin on the phosphorylation of AMPK, HepG2 cells were treated with OA (500 μM), and co-incubation with curcumin (1, 5 and 10 μM) for 24 h. As shown in Figure 5, curcumin (5 and 10 μM) stimulated AMPK threonine 172 phosphorylation.

Discussion

In recently studies, naturally occurring chemical substances derived from plants have been of interest as therapeutic interventions in several metabolic diseases. They serve as template molecules for the development of new drugs. These molecules interfere with three key processes involved in the lipid metabolism. Curcumin is the principal curcuminoid of the popular Spice-Turmeric utilized in Indian and other South Asia countries, which is a member of the ginger family. This plant polyphenolic compound has antitumor, anti-proliferative, anti-oxidant, and anti-inflammatory properties[20]. Since the last decade, a few clinical trials have been conducted, showing the therapeutic effects of curcumin on various cancers and Alzheimer's disease[21]. The anti-adipogenic effect of curcumin was then demonstrated in the 3T3-L1 cell model by other groups[22,23]. Some studies showed that curcumin did not reduce the effects of hepatic ischemia reperfusion injury on the liver and distant organs including kidneys and lungs significantly[24]. However, the mechinsim study was not poor about oleic acid-induced Human hepatoma cells model, although curcumin supplementation decreased NF-κB level and prevents fatty acid accumulation in the liver[25,26]. Here, we attempted to exam-

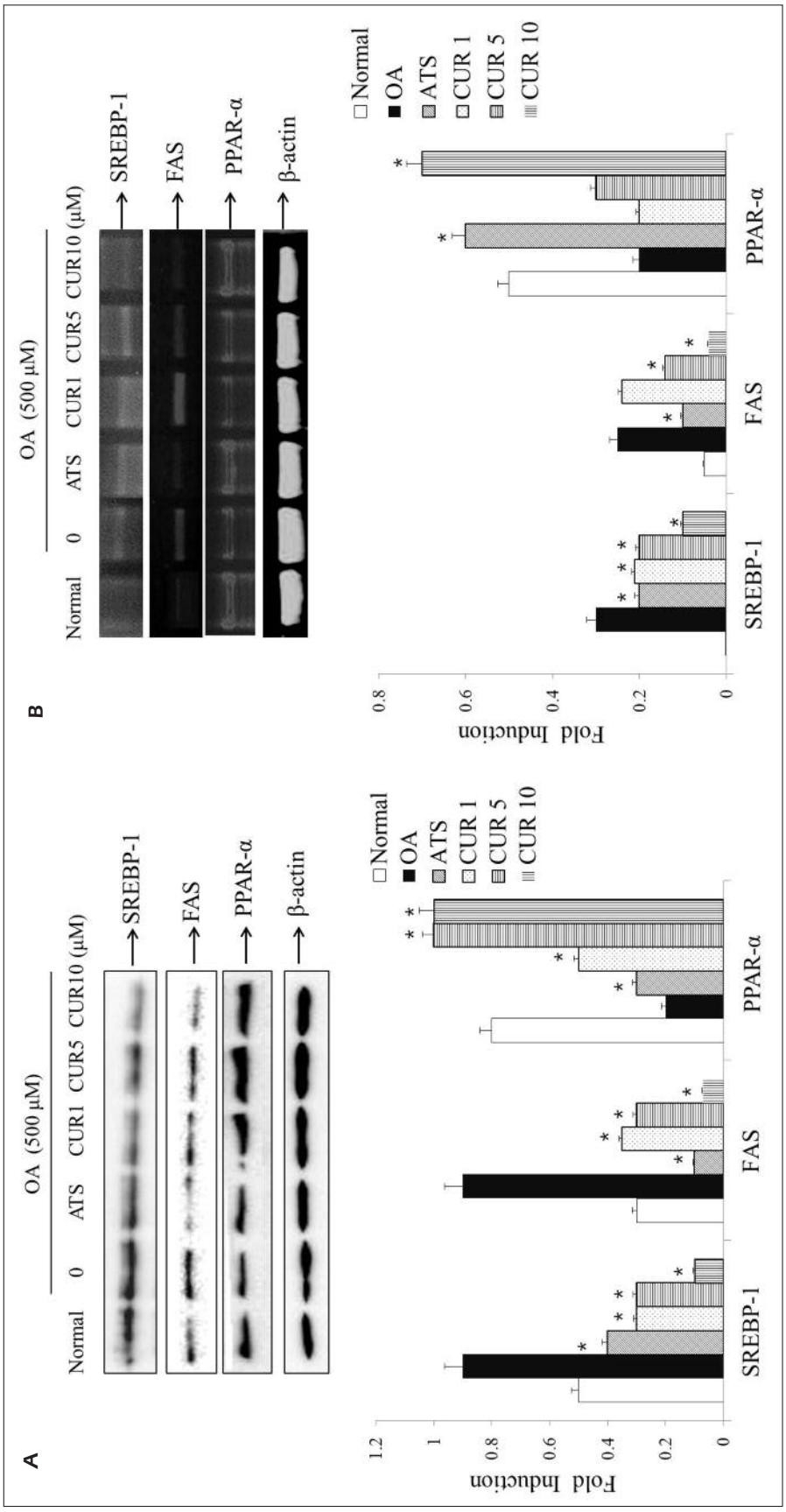

Figure 4. Effects of curcumin on hepatic lipid accumulation and protein and mRNA expression. HepG2 cells were pretreated 500 μM OA and then incubated with indicated concentrations of curcumin for 24 h. ***(A)*** The SREBP-1c, FAS, and PPARα, protein expressions were detected by Western blot analysis. ***(B)*** The mRNA expression was analyzed by RT-PCR. Expression levels were normalized to β-actin protein and mRNA expression level. Data were representative of 3 independent experiments and quantified by densitometric analysis. Expression levels were normalized to β-actin protein level. CUR = curcumin, ATS = atorvastain. The results from 3 repeated and separated experiments were similar and expressed as mean ± SD. $^{*}p < 0.05$.

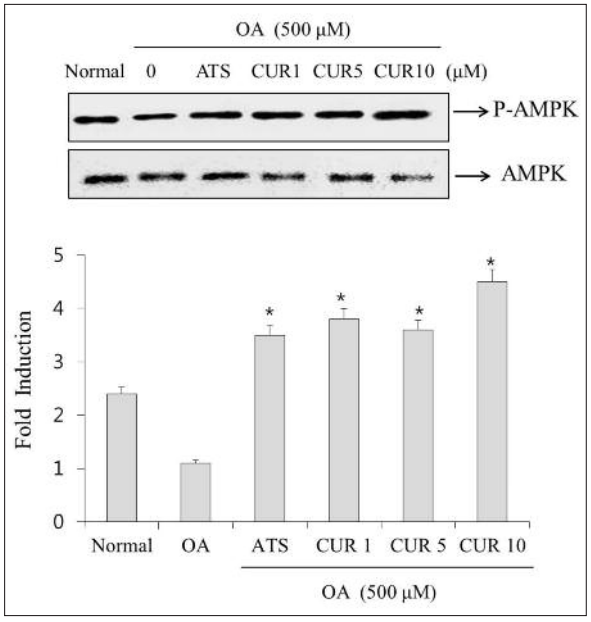

Figure 5. Effects of curcumin on AMPK phosphorylation in HepG2 cells. HepG2 cells were pretreated 500 μM OA and then incubated with indicated concentrations of curcumin for 24 h. AMPK phosphorylation (pThr-172-AMPK) was detected by Western blot analysis. The numbers below the panels represent quantification of the immunoblot by densitometry. CUR = curcumin, ATS = atorvastain. The result from 3 independent experiments was expressed as mean ± SD. *$p < 0.05$.

ine the hepatic hypolipidemia effect and possible mechanism of curcumin on hepatic lipid metabolism. Generally, hepatic hypolipidemic mechanism highly related to expression of lipogenic enzyme, cholesterol biosynthesis, TG biosynthesis, and fatty acid β-oxidation in HepG2 cells. HepG2 cells were derived from a human hepatoblastoma that is free of known hepatotropic virus. Use of a hepatoblastoma in the model of lipid metabolism is ambiguous. In our preliminary study, HepG2 cells were used to detect the lipid accumulation after OA exposure. For further experiment we tested the effect of curcumin on lipid homeostasis. Herein, atorvastatin a class of drugs that lowers cholesterol level in human was used as a positive control. Meanwhile, to avoid cytotoxicity, the viability of cells treated with various concentrations of curcumin was determined by MTS assay (Figure 1). We first analyzed the development of lipid accumulation in an *in vitro* model of hepatic steatosis. As shown in Figures 2 and 3, OA treatment alone caused a significant increase in lipid accumulation. And levels of lipid accumulation were reduced in 1, 5 and 10 μM concentration of curcumin, respectively, as compared to OA treatment. Especially, pretreatment of curcumin (5 and 10 μM) inhibited OA-induced triglyceride levels and total cholesterol.

Lipid accumulation in liver may be caused by enhanced de novo lipogenesis, activation of lipid uptake, and lowering of lipid catabolism. FAS is key enzymes in de novo fatty acid and TG synthesis in mammals. SREBP-1 is well known as the transcription factor regulating the gene expression of these lipogenic enzymes in the liver[8]. FAS is known to be SREBP-1 were changed by curcumin (Figure 4). These results indicate that the effect of curcumin on OA-induced hapatic lipogenesis in HepG2 cells is associated with decreased expression of SREBP-1 and its downstream lipogenic genes. Numerous studies have indicated that activation of AMPK effectively suppresses the expression of SREBP-1 in the liver[27]. In our study, curcumin treatments increase AMPK phosphorylation (Figure 5) There are several reports demonstrate AMPK plays a key role in regulating carbohydrate and fat metabolism, serving as a metabolic master switch response to alterations in cellular energy charge[28]. In fact, activation of AMPK has been to validate a strategy for liver steatosis therapy[29]. Previous studies indicated that polyphenolic extracts from plenty of plants can activate AMPK and suppress FAS expression because it prevents SREBP-1 translocation to the nuclei[30-32]. In this report, we found curcumin has the same ability to activate AMPK and then reduce SREBP-1 expression, finally leading to inhibit hepatic lipogenesis. Other studies showed fatty acid directly affected some gene expression through regulating transcription factors, including PPAR and SREBP-1[33]. According to the result, we confirmed curcumin could decrease lipid synthesis and increase fatty-acid oxidation through activating p-AMPK and PPAR-α, which further inhibit protein expression in SREBP-1 and lead to the reduction of the transcription activity of FAS.

Conclusions

We prove curcumin not only reduce lipid accumulation but also had good antioxidant capacity. We also propose AMPK is pivotal in shutting down the anabolic pathway and promoting catabolism by up-regulating PPARα and down-regulating the activity of key enzymes in lipid metab-

olism, such as, SREBP-1c and FAS. Consequently, curcumin suppresses fat accumulation of the liver and could be developed as a potential therapeutic treatment to reduce the formation of a fatty liver.

Acknowledgements

This research was financially supported by the Ministry of Knowledge Economy(MKE), Korea Institute for Advancement of Technology(KIAT) through the Inter-ER Cooperation Projects (R0002019) and the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIP)(2008-0062484).

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 2004; 40: 1387-1395.
2) Chitturi S, Farrell GC, Hashimoto E, Saibara T, Lau GK, Sollano JD. Non-alcoholic fatty liver disease in the Asia-Pacific region: definitions and overview of proposed guidelines. J Gastroenterol Hepatol 2007; 22: 778-787.
3) Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. Prevalence of and risk factors for non-alcoholic fatty liver disease: theDionysos nutrition and liver study. Hepatology 2005; 42: 44-52.
4) Tamura S, Shimomura I. Contribution of adipose tissue and de novo lipogenesis to nonalcoholic fatty liver disease. J Clin Invest 2005; 115: 1139-1142.
5) Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. Hepatology 2003; 37: 1202-1219.
6) Satia-Abouta J, Patterson RE, Schiller RN, Kristal AR. Energy from fat is associated with obesity in U.S. men: results from the Prostate Cancer Prevention Trial. Prev Med 2002; 34: 493-501.
7) Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 1997; 89: 331-340.
8) Shimano H, Yahagi N, Amemiya-Kudo M, Hasty AH, Osuga J, Tamura Y, Shionoiri F, Iizuka Y, Ohashi K, Harada K, Gotoda T, Ishibashi S, Yamada N. Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. J Biol Chem 1999; 50: 35832-35839.
9) Aggarwal BB. Targeting inflammation-induced obesity and metabolic diseases by curcumin and other nutraceuticals. Annu Rev Nutr 2010; 30: 173-199.
10) Mingrone G, Rosa G, Greco AV, Manco M, Vega N, Nanni G, Castagneto M, Vidal H. Intramyocitic lipid accumulation and SREBP-1c expression are related to insulin resistance and cardiovascular risk in morbid obesity. Atherosclerosis 2003; 170: 155-161.
11) Lee CH, Olson P, Evans RM. Minireview: lipid metabolism, metabolic diseases, and peroxisome proliferator-activated receptors. Endocrinology 2003; 144: 2201-2207.
12) Berger JP, Akiyama TE, Meinke PT. PPARs: therapeutic targets for metabolic disease. Trends Pharmacol Sci 2005; 26: 244-251.
13) Carling D. AMP-activated protein kinase: balancing the scales. Biochimie 2005; 87: 87-91.
14) Zhang BB, Zhou G, Li C. AMPK: an emerging drug target for diabetes and the metabolic syndrome. Cell Metab 2009; 9: 407-416.
15) Gruzman A, Babai G, Sasson S. Adenosine monophosphate-activated protein kinase (AMPK) as a new target for antidiabetic drugs: a review on metabolic, pharmacological and chemical considerations. Rev Diabet Stud 2009; 6: 13-36.
16) You M, Matsumoto M, Pacold CM, Cho WK, Crabb DW. The role of AMP-activated protein kinase in the action of ethanol in the liver. Gastroenterology 2004; 127: 1798-1808.
17) Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology 2008; 134: 1655-1669.
18) Tsukamoto H, She H, Hazra S, Cheng J, Miyahara T. Anti-adipogenic regulation underlies hepatic stellate cell transdifferentiation. J Gastroenterol Hepatol 2006; 21(Suppl 3): S102-S105.
19) Weisberg SP, Leibel R, Tortoriello DV. Dietary curcumin significantly improves obesity-associated inflammation and diabetes in mouse models of diabesity. Endocrinology 2008; 149: 3549-3558.
20) Alappat L, Awad AB. Curcumin and obesity: evidence and mechanisms. Nutr Rev 2010; 68: 729-738.
21) Hatcher H, Planalp R, Cho J, Torti FM, Torti SV. Curcumin: from ancient medicine to current clinical trials. Cell Mol Life Sci 2008; 65: 1631-1652.
22) Ejaz A, Wu D, Kwan P, Meydani. Curcumin inhibits adipogenesis in 3T3-L1 adipocytes and angiogenesis and obesity in C57/BL mice. J Nutr 2009; 139: 919-925.
23) Lee YK, Lee WS, Hwang JT, Kwon DY, Surh YJ, Park OJ. Curcumin exerts antidifferentiation effect through AMPKalpha-PPAR-gamma in 3T3-L1 adipocytes and antiproliferatory effect through AMPKalpha-COX-2 in cancer cells. J Agric Food Chem 2009; 57: 305-310.

24) Oguz A, Kapan M, Onder A, Kilic E, Gumus M, Basarali MK, Firat U, Boyuk A, Buyukbas S. The effects of curcumin on the liver and remote organs after hepatic ischemia reperfusion injury formed with Pringle manoeuvre in rats. Eur Rev Med Pharmacol Sci 2013; 17: 457-466.

25) Vera-Ramirez L, Pérez-Lopez P, Varela-Lopez A, Ramirez-Tortosa M, Battino M, Quiles JL. Curcumin and liver disease. Biofactors 2013; 39: 88-100.

26) Shao W, Yu Z, Chiang Y, Yang Y, Chai T, Foltz W, Lu H, Fantus IG, Jin T. Curcumin prevents high fat diet induced insulin resistance and obesity via attenuating lipogenesis in liver and inflammatory pathway in adipocytes. PLoS One 2012; 7: e28784.

27) Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest 2001; 108: 1167-1174.

28) Winder WW, Hardie DG. AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. Am J Physiol 1999; 277(1 Pt 1): E1-10.

29) Brooks SC 3rd, Brooks JS, Lee WH, Lee MG, Kim SG. Therapeutic potential of dithiolethiones for hepatic diseases. Pharmacol Therapeut 2009; 124: 31-43.

30) Hwang JT, Park IJ, Shin JI, Lee YK, Lee SK, Baik HW, Ha J, Park OJ. Genistein, EGCG, and capsaicin inhibit adipocyte differentiation process via activating AMP-activated protein kinase. Biochem Biophys Res Commun 2005; 338: 694-699.

31) Auger C, Teissedre PL, Gerain P, Lequeux N, Bornet A, Serisier S, Besancon P, Caporiccio B, Cristol JP, Rouanet JM. Dietary wine phenolics catechin, quercetin, and resveratrol efficiently protect hypercholesterolemic hamsters against aortic fatty streak accumulation. J Agric Food Chem 2005; 53: 2015-2021.

32) Weng MS, Ho CT, Ho YS, Lin JK. Theanaphthoquinone inhibits fatty acid synthase expression in EGF-stimulated human breast cancer cells via the regulation of EGFR/ErbB-2 signaling. Toxicol Appl Pharmacol 2007; 218: 107-118.

33) Clarke SD. Polyunsaturated fatty acid regulation of gene transcription: a mechanism to improve energy balance and insulin resistance. Br J Nutr 2000; 83(Suppl 1): S59-66.