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


Department of Oriental Pharmacy, College of Pharmacy, Wonkwang University, Wonkwang Oriental Medicines Research Institute, Institute of Biotechnology, Iksan, Jeonbuk, Republic of Korea

1Professional Graduate School of Oriental Medicine and College of Oriental Medicine, Wonkwang University, Iksan, Jeonbuk, Republic of Korea

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

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-1c, 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.

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. NAFLD is characterized by the accumulation of hepatic triglycerides (TG), which result from an imbalance between uptake, synthesis, export, and oxidation of fatty acids. Approximately 20-30% of adults are estimated to have excess liver fat accumulation in a normal population. It has been considered that increased free fatty acids (FFA) supplied to the liver play a major role in the early stage of NAFLD. Studies have shown that sterol regulatory element-binding proteins (SREBPs) regulate lipid metabolism. SREBP-1c plays an essential role in the regulation of lipogenesis involved in fatty acid and TG synthesis.

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. 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.

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. The activation of AMPK by pharmacological
agents presents a unique challenge, given the complexity of the biology, but holds a considerable potential to reverse the metabolic abnormalities. 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. AMPK may play a key role in regulating the activation of SREBP-1 and lipogenesis. 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.

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. 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.

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, peroxo-
The proteins were then supplemented with ECL prime Western blotting detection reagents (GE Healthcare) and ImageQuant LAS 4000 Mini Biomo lecular 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...
Curcumin decreases oleic acid-induced lipid accumulation via AMPK phosphorylation

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

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 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.

**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 = atorvastatin. The result was expressed as mean ± SD. * p < 0.05.
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 recent 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 anti-tumor, anti-proliferative, anti-oxidant, and anti-inflammatory properties. Since the last decade, a few clinical trials have been conducted, showing the therapeutic effects of curcumin on various cancers and Alzheimer's disease. The anti-adipogenic effect of curcumin was then demonstrated in the 3T3-L1 cell model by other groups. 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. 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. Here, we attempted to exam-

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 = atorvastatin. The result was expressed as mean ± SD = 3, *p < 0.05. OA as control (cells treated with OA only).
Curcumin decreases oleic acid-induced lipid accumulation via AMPK phosphorylation.

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 = atorvastatin. The results from 3 repeated and separated experiments were similar and expressed as mean ± SD. *p < 0.05.
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. FAS is known to be SREBP-1 were changed by curcumin (Figure 4). These results indicate that the effect of curcumin on OA-induced hepatic 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. 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. In fact, activation of AMPK has been to validate a strategy for liver steatosis therapy. 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. 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. 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-
Curcumin decreases oleic acid-induced lipid accumulation via AMPK phosphorylation

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 Advanced 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


