Theracurmin (Highly Bioavailable Curcumin) Prevents High Fat Diet-Induced Hepatic Steatosis Development in Mice

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Abstract

Curcumin, a hydrophobic polyphenol isolated from the Curcuma longa L. plant, has many pharmacological properties, including antioxidant, anti-inflammatory, and chemo-preventive activities. Curcumin has been shown to have potential in preventing nonalcoholic fatty liver disease (NAFLD). However, the low bioavailability of curcumin has proven to be a major limiting factor in its clinical adoption. Theracurmin, a highly bioavailable curcumin that utilizes micronized technology showed improved biological absorbability in vivo. The aim of this study was to investigate the role of theracurmin in modulating hepatic lipid metabolism in vivo. A fatty liver mouse model was produced by feeding mice a high fat diet (HFD; 60% fat) for 12 weeks. We found that treatment for 12 weeks with theracurmin significantly lowered plasma triacylglycerol (TG) levels and reduced HFD-induced liver fat accumulation. Theracurmin treatment lowered hepatic TG and total cholesterol (T-CHO) levels in HFD-fed mice compared to controls. In addition, theracurmin administration significantly reduced lipid peroxidation and cellular damage caused by reactive oxygen species in HFD-fed mice. Overall, these results suggest that theracurmin has the ability to control lipid metabolism and can potentially serve as an effective therapeutic remedy for the prevention of fatty liver.

Key words: Theracurmin, Curcumin, Nonalcoholic fatty liver disease (NAFLD), High fat diet (HFD), Fatty liver, Steatosis

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has been recognized as a common liver disease worldwide. The term represents a broad spectrum of liver damage ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), progressive fibrosis, and cirrhosis (1,2). NAFLD is strongly associated with metabolic syndrome-associated conditions such as obesity, dyslipidemia, diabetes, hypertension, and insulin resistance (3,4). The detailed pathogenesis of NAFLD is not completely known, but excessive fatty acid [triacylglycerol (TG)] and cholesterol (CHO) accumulation in the liver has been linked to the development of NASH, cirrhosis, and cancer (5,6). Although current therapeutic approaches have focused on the treatment of the underlying risk factors for these metabolic conditions, no standard strategy has yet been approved for NAFLD therapy (2,6).

Curcumin, a natural yellow polyphenol that exists in herbal remedies and the dietary spice turmeric, has been...
shown to possess antioxidant, anti-inflammatory, antimicrobial, and chemopreventive activities, and has been demonstrated to prevent obesity and diabetes in animal models (7,8). Curcumin also exerted beneficial effects against hypercholesterolemia and dyslipidemia in rodent animal models, as well as in two randomized double-blind NAFLD clinical trials (9,10).

Although curcumin has been shown to be protective against dyslipidemia and NAFLD, its therapeutic outcomes and clinical use is limited by low oral bioavailability owing to its very low intestinal absorption and hydrophobic properties, leading to poor solubility (11-13). Many studies investigating curcumin delivery systems, including submicron suspensions, phosphatidylcholine complexes, and solid lipid nanoparticles, have been performed with the aim of improving curcumin oral bioavailability (14-16). Among these, theracurmin, a highly bioavailable curcumin developed using micronized-technology, has significantly increased bioavailability and water solubility relative to curcumin. In rat and human studies, theracurmin absorption was 30-fold higher than that of commercially available curcumin (14). Moreover, the maximum curcumin plasma concentration increased over 50-fold when theracurmin was used instead of curcumin powder (14).

Theracurmin has been reported to be effective against a variety of pathological conditions including cardiovascular disease, esophageal cancer, inflammatory bowel disease, and osteoarthritis (11,14,17). However, no basic or clinical studies regarding the efficacy of theracurmin against NAFLD, including hepatic steatosis, have been performed. In this study, we evaluated the preventive effect of theracurmin on hepatic steatosis in mice fed with a high fat diet (HFD). We uncovered that theracurmin treatment prevented the accumulation of TG and total cholesterol (T-CHO), as well as the lipid peroxidation, normally observed in the livers of HFD-fed mice. Our findings suggested that theracurmin is protective against NAFLD through lipid metabolism and oxidative stress modulation, and has therapeutic potential.

**MATERIALS AND METHODS**

**Animals and treatment.** Animal experiments were performed in accordance with the Animal Care and Ethics Committees of Gyeonggi bio center (2017-08-0001). C57BL/6N mice at 4 weeks of age were maintained in a standard condition (23 ± 3°C, 55 ± 15% humidity with a 12-hr light/dark cycle), pathogen-free environment and had access to a sterile standard rodent chow diet and water ad libitum. After a one week adaptive period, Male C57BL/6N mice at 5 weeks of age were started on either a normal diet (ND) or HFD 60% w/w for 12 weeks. Vehicle (normal saline), theracurmin (500, 1,000, and 2,000 mg/kg, as a curcumin 150, 300, 600 mg/kg), or silymarin (25 mg/kg) were orally administered to mice seven times per week during 12 weeks of the diet feeding.

**Histopathological analysis.** The left lateral lobe of the liver was sliced and tissue slices were fixed in 10% buffered-neutral formalin, embedded in paraffin. The liver slices were used to generate 3-4 µm sections in a cryostat. Tissue sections were stained with H&E and Oil red O staining.

After that the histological profiles of individual cross trimmed hepatic tissues were light microscopically observed (Model Eclipse 80i, Nikon, Tokyo, Japan). To observe more detail histopathological changes, the steatohepatitis regions (under OR staining) and mean hepatocyte diameters (under HE staining) were calculated using an automated image analysis process (Solution FL ver 9.1, IMT i-solution Inc., Vancouver, Quebec, Canada) on the restricted view fields. Steatohepatitis regions, the percentage of fatty deposited regions in hepatic parenchyma, were calculated as percentages of lipid deposited regions between restricted histological view field of liver (Mean hepatic steatosis regions - %/mm² of hepatic parenchyma) under cryostat and oil red staining. Mean diameters of hepatocytes were also calculated in restricted view fields on a computer monitor under paraffin embedding and HE staining using an automated image analysis process.

**Measurement of hepatic TG and total cholesterol contents.** TG (Catalog#K622-100, Biovision, San Francisco, CA, USA) and T-CHO (Catalog#K603, Biovision) contents were measured using commercial kits.

**Biochemical parameters.** Serum was collected after centrifugation at 3,000 rpm for 10 min. Serum TG (Catalog#OSR61118, Beckman coulter, CA, USA), T-CHO (Catalog#OSR6116, Beckman coulter), LDL-C (Catalog#6183, Beckman coulter), and HDL-C (Catalog#6187, Beckman coulter) were also analyzed using commercial kits from Chemistry Analyzer (AU680, Beckman coulter).

**Measurement of hepatic malondialdehyde and glutathione contents.** Malondialdehyde (MDA) levels was determined by the thiobarbituric acid (TBA) method (Catalog#STA-330, Cellbiolabs, San Francisco, CA, USA). TBA reaction was performed according to manufactory guidance. Glutathione (GSH) were analyzed using commercial kits from ENZO Life Science, Villeurbanne, France).

**Data analysis.** Statistical analyses were performed using SPSS statistics 22 for medical science. LSD test was used to examine the significant inter-group differences. Statistical significance was accepted at either $p < 0.05$ or $p < 0.01$. 
RESULTS

**Theracurmin inhibits HFD-induced hepatic steatosis.**

To examine the effect of theracurmin on liver fat accumulation, mice were fed with a HFD (60% fat) for 12 weeks and then treated with vehicle, theracurmin, or silymarin (25 mg/kg; reference control). Since the main histological feature of hepatic steatosis is liver fat accumulation, hepatic

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**Fig. 1.** Effects of theracurmin on hepatic lipid accumulation in mice fed with HFD. (A) Liver sections were stained with H&E or oil-Red O staining. The mice were fed either a ND or HFD for 12 weeks. theracurmin (500, 1,000, and 2,000 mg/kg) or silymarin (25 mg/kg) (reference control) was administered to the mice at the same time. H&E staining. The livers of the mice were stained with H &E after a treatment with 500, 1,000, and 2,000 mg/kg theracurmin for 12 weeks. CV, Central vein; PT, Portal triad area; Scale bars = 100 µm. Oil Red O staining. Each photo represents their groups after staining with Oil Red O in the liver. Scale bars = 100 µm. (B) Histomorphometric analysis. Measurement of hepatic steatosis region (%/mm² of hepatic parenchyma) and diameter of hepatocyte (mm/hepatocyte). Data were expressed as mean ± SEM statistically analyzed by LSD-test methods. Significant versus normal control, \( ^{##}p < 0.01 \); significant versus HFD-fed group, \( ^{*}p < 0.01 \) (n = 10). G1: ND (normal saline), n = 10; G2: HFD + vehicle (normal saline), n = 10; G3: HFD + theracurmin (500 mg/kg/day), n = 10; G4: HFD + theracurmin (1,000 mg/kg/day), n = 10; G5: HFD + theracurmin (2,000 mg/kg/day), n = 10; G6: HFD + reference control (silymarin 25 mg/kg/day), n = 10.

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| Hepatic steatosis region (%/mm²) | Diameter of hepatocyte (mm) |
|---------------------------------|----------------------------|
| G1 22.54±2.85                   | 20.20±0.61                 |
| G2 74.24±2.56^a                 | 29.05±0.90^ef             |
| G3 54.04±3.41^**               | 25.84±0.71^**             |
| G4 41.10±2.49^**               | 24.72±0.87^**             |
| G5 38.17±2.80^**               | 23.43±0.82^**             |
| G6 25.81±2.70^**               | 21.28±0.74^**             |
fat deposition was measured. Histopathological analysis using hematoxylin and eosin (H&E) and Oil Red O tissue staining found that HFD fed mice exhibited increased hepatocyte fat accumulation (Fig. 1A). Theracurmin treatment at doses of 500, 1,000, and 2,000 mg/kg significantly reduced these pathological changes in the tissue, as did oral administration of silymarin (Fig. 1B).

Theracurmin improves the accumulation hepatic TG and T-CHO in HFD-fed mice. TG and T-CHO accumulation in hepatocyte cytoplasm is the hallmark of NAFLD (1). Theracurmin treatment (2,000 mg/kg) for 12 weeks reduced HFD-induced hepatic TG content increases, as did oral administration of silymarin (Fig. 1B).

Theracurmin reduces plasma TG but does not affect body weight in HFD-fed mice. Next, we examined the effect of theracurmin on serum lipid levels. Theracurmin treatment significantly reduced HFD-induced plasma TG level increases but did not affect plasma total CHO, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels (Fig. 3). However, the administration of theracurmin did not affect body weight gain or food intake amounts (Supplementary Fig. 1).

Theracurmin inhibits HFD-induced lipid peroxidation. Lipid peroxidation and cellular damage by reactive oxygen species is characterized by membrane lipid breakdown and the production of lipid peroxides (18). As observed in Fig. 4A, liver MDA production, a marker of lipid peroxidation, was elevated in HFD-treated mice relative to normal diet-fed controls. This elevation of hepatic MDA was significantly attenuated by theracurmin administration at a dosage of 2,000 mg/kg. Theracurmin treatment (1,000 and 2,000 mg/kg doses) also increased hepatic GSH content versus untreated HFD-fed mice (Fig. 4B).

DISCUSSION

Curcumin has been shown to reduce hepatic steatosis,
Preventive Effect of Theracurmin on Hepatic Steatosis

Inflammation, insulin resistance, diabetes, and atherosclerosis by regulating hepatic lipid metabolism and plasma lipid homeostasis (19,20). However, the low oral bioavailability of curcumin limits its clinical adoption (11). To overcome this, theracurmin, a submicron crystal solid dispersion of curcumin, was formulated to enhance curcumin bioavailability through enhanced water solubility and absorption (7,11).

We demonstrate herein that theracurmin administration exerted anti-steatotic activity in the livers of mice fed with high fat diets. In the current study, theracurmin administration led to significant decreases in both hepatic TG and

![Graphs showing effects of theracurmin on serum TG, CHO, LDL, and HDL levels.](image)

### Fig. 3. Effects of theracurmin on the serum TG, CHO, LDL, and HDL in HFD-fed mice. (A-D) serum TG levels (A), serum CHO levels (B), serum LDL levels (C), serum HDL levels (D) in mice fed a normal diet or high-fat diet for 12 weeks. Data were expressed as mean ± SEM statistically analyzed by Q-test and LSD-test methods. Significant versus normal control, **p < 0.01; significant versus HFD-fed group, *p < 0.01 (n = 10). G1: ND (normal saline), n = 10; G2: HFD + vehicle (normal saline), n = 10; G3: HFD + theracurmin (500 mg/kg/day), n = 10; G4: HFD + theracurmin (1,000 mg/kg/day), n = 10; G5: HFD + theracurmin (2,000 mg/kg/day), n = 10; G6: HFD + reference control (silymarin 25 mg/kg/day), n = 10.)
total CHO levels in mice fed a HFD for 12 weeks. Excessive fatty acids, derived from diet or lipolysis, results in hepatocyte lipid droplet accumulation, a representative feature of NAFLD (21). Consequently, specific lipotoxic lipids, including ceramide, diacylglycerols, and lysophosphatidyl choline species, from these droplets induce hepatocellular injury in NASH (2,21). In addition, an imbalance between intrahepatic CHO and the removal of CHO from hepatocytes leads to CHO accumulation in the liver (22,23). This extensive dysregulation of hepatic CHO homeostasis has been shown to accentuate hepatocellular injury and liver inflammation in NAFLD development. Thus, preventing TG and CHO accumulation in the liver may prove promising for NAFLD treatment.

Curcumin has been reported to alleviate HFD-induced obesity in mice through the inhibition of sterol regulatory element-binding proteins (SREBPs) such as SREBP-1 and SREBP-2. SREBPs are key transcription factors that modulate the expression of genes related to lipid synthesis (24). Specifically, SREBP-1c activation results in lipid-mediated lipotoxicity that contributes to metabolic syndrome-associated conditions including obesity, diabetes mellitus, hepato-steatosis, dyslipidemia, inflammation, and fibrosis in various organs (24,25). SREBP-2 is a crucial transcription factor involved in the regulation of CHO metabolism (26). We also confirmed that theracurmin treatment attenuated the increase in SREBP-1 induced by HFD (Supplementary Fig. 2). Curcumin has also been shown to reduce CHO accumulation via inhibition of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, the rate-limiting enzyme of CHO synthesis, and acyl-CoA:cholesterol acyltransferase (ACAT), the main enzyme responsible for the intracellular esterification of CHO. It was also observed that theracurmin administration attenuated the increase in hepatic CHO levels (Fig. 2B). Although theracurmin treatment did not affect serum T-CHO in mice fed a HFD for 12 weeks, a low dosage of theracurmin (200 mg/kg or 400 mg/kg) resulted in a suppression of serum T-CHO levels in mice fed an HFD for 8 weeks (data not shown). Thus, it is plausible that theracurmin may regulate the extensive dysregulation of hepatic CHO homeostasis necessary for the development of hepatic steatosis found in HFD-fed mice. At the present time, the expression of hepatic HMG-CoA reductase and ACAT remains unexplored and will be a focus of further study.

Lipid accumulation as part of NAFLD progression results in increased vulnerability to oxidative stress, leading to an increase in inflammation, endoplasmic reticulum stress, mitochondrial dysfunction, and an inability of hepatocytes to synthesize endogenous antioxidants (27). Oxidative stress

![Fig. 4. Effects of theracurmin on oxidative stress in HFD-fed mice. Measurement of hepatic MDA and GSH contents in the liver from mice of each group. Data were expressed as mean ± SEM statistically analyzed by Q-test and LSD-test methods. Significant versus normal control, ##p < 0.01; significant versus HFD-fed group, *p < 0.05; **p < 0.01 (n = 10). G1: ND (normal saline), n = 10; G2: HFD + vehicle (normal saline), n = 10; G3: HFD + theracurmin (500 mg/kg/day), n = 10; G4: HFD + theracurmin (1,000 mg/kg/day), n = 10; G5: HFD + theracurmin (2,000 mg/kg/day), n = 10; G6: HFD + reference control (silymarin 25 mg/kg/day), n = 10.](image-url)
is caused by an imbalance between the formation of reactive nitrogen species and antioxidant defenses (28). Although the main processes for producing oxidizing species is related to the production of hydrogen peroxide in peroxisomes and oxidative metabolism in mitochondria, lipid peroxidation, the major consequence of oxidative stress, produces extremely reactive aldehyde components such as 4-hydroxy-2-nonenal and MDA, leading to intracellular damage in the liver (29,30). Curcumin also has been shown to alleviate reactive oxygen species (ROS)-induced lipid peroxidation in mitochondria (31). These findings are consistent with our previous results, which saw theracurmin treatment contributing to a decrease in lipid peroxide levels and the induction of glutathione, both phenomena playing crucial roles in the detoxification and antioxidant systems involved in hepatic steatosis. Curcumin has been reported to induce the expression of antioxidant enzymes by up-regulating nuclear factor erythroid-2-related factor-2 (Nrf2) (32,33). In addition, curcumin contributed to a decrease in lipid peroxidation observed after theracurmin administration is caused by an imbalance between the formation of reactive nitrogen species and antioxidant defenses (28). Although the main processes for producing oxidizing species is related to the production of hydrogen peroxide in peroxisomes and oxidative metabolism in mitochondria, lipid peroxidation, the major consequence of oxidative stress, produces extremely reactive aldehyde components such as 4-hydroxy-2-nonenal and MDA, leading to intracellular damage in the liver (29,30). Curcumin also has been shown to alleviate reactive oxygen species (ROS)-induced lipid peroxidation in mitochondria (31). These findings are consistent with our previous results, which saw theracurmin treatment contributing to a decrease in lipid peroxide levels and the induction of glutathione, both phenomena playing crucial roles in the detoxification and antioxidant systems involved in hepatic steatosis. Curcumin has been reported to induce the expression of antioxidant enzymes by up-regulating nuclear factor erythroid-2-related factor-2 (Nrf2) (32,33). In addition, curcumin contributed to a decrease in lipid peroxidation observed after theracurmin treatment is related antioxidant enzyme levels or Nrf2 activation.

In conclusion, theracurmin appeared to play a crucial role in the prevention of hepatic steatosis by mediating the inhibition of TG/T-CHO biosynthesis and lipid peroxidation in the liver, suggesting that theracurmin can potentially be a new candidate as a therapeutic option for the treatment of fatty liver.

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CONFLICT OF INTEREST

The Authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript. Hee Hye Yun is former employee of HANDOK, Inc.

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