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[6]-Gingerol dampens hepatic steatosis and inflammation in experimental nonalcoholic steatohepatitis

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ABSTRACT

The aim of the study was to investigate the effects of [6]-gingerol ((S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-3-decanone) in experimental models of non-alcoholic steatohepatitis. HepG2 cells were exposed to 500 μmol/l oleic acid (OA) for 24 h and preincubated for an additional 24 h with [6]-gingerol (25, 50 or 100 μmol/l). [6]-Gingerol (100 μmol/l) inhibited OA-induced triglyceride and inflammatory marker accumulation in HepG2 cells. After being fed a high-fat diet (HFD) for 2 weeks, male golden hamsters were dosed orally with [6]-gingerol (25, 50 or 100 mg/kg/day) once daily for 8 weeks while maintained on HFD. [6]-Gingerol (100 mg/kg/day) alleviated liver steatosis, inflammation, and reversed plasma markers of metabolic syndrome in HFD-fed hamsters. The expression of inflammatory cytokine genes and nuclear transcription factor-κB (NF-κB) were increased in the HFD group; these effects were attenuated by [6]-gingerol. The hepatic mRNA expression of lipogenic genes such as liver X receptor-α, sterol regulating element binding protein-1c and its target genes including acetyl-CoA carboxylase, fatty acid synthase, stearoyl-CoA desaturase 1, and acyl-CoA:diacylglycerol acyltransferase 2 in HFD-fed hamsters was also blocked by [6]-gingerol. [6]-Gingerol may attenuate HFD-induced steatohepatitis by downregulating NF-κB-mediated inflammatory responses and reducing hepatic lipogenic gene expression.

Abbreviations

ACC	acetyl-CoA carboxylase
CD	control diet
Chol	cholesterol
DGAT-2	acyl-CoA:diacylglycerol acyltransferase 2
DMSO	dimethyl sulfoxide
ELISA	enzyme-linked immunosorbent assay
FAS	fatty acid synthase
FFA	free fatty acid
GF	[6]-gingerol
HDL-C	high density lipoprotein cholesterol
HFD	high-fat diet
HOMA-IR	homeostasis model assessment of insulin resistance
IACUC	Institutional Animal Care and Use Committee
IL	interleukin
IκB	αinhibitory kappa B
LDL-C	low density lipoprotein cholesterol
LXR-α	liver X receptor-α
MCP-1	monocyte chemoattractant protein-1
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3 carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
NAFLD	non-alcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
NF-κB	nuclear transcription factor-κB
OA	oleic acid
OD	optical density
RT-PCR	quantitative real-time PCR
SCD1	stearoyl-CoA desaturase 1
SEM	standard error mean
SREBP-1c	sterol regulating element binding protein-1c
TBST	Tris-buffered saline Tween 20
TC	total cholesterol
TG	triglyceride
TNF-α	tumor necrosis factor-α

Introduction

Non-alcoholic fatty liver disease (NAFLD) refers to a wide spectrum of liver diseases ranging from simple fatty liver (steatosis) to nonalcoholic steatohepatitis (NASH) and cirrhosis. NASH pathology is characterized by microvesicular or macrovesicular steatosis, inflammation, hepatocyte degeneration, and sometimes fibrosis (Schuppan and Schattenberg 2013). A variety of liver cells including hepatocytes, hepatic macrophages, and hepatic stellate cells are involved

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in the pathogenesis of NASH (Xu et al. 2010). In particular, inflammatory processes secondary to insulin resistance are regarded as a characteristic finding of NASH (McCullough 2006). Among the inflammatory mediators, chemokines play pivotal roles in the recruitment of various cells, including immune cells, to the sites of inflammation through interactions with chemokine receptors (Braunersreuther et al. 2012). Inflammatory mediators such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β further stimulate hepatocytes and hepatic stellate cells to induce hepatocyte steatosis and fibrosis, respectively. The interactions of cytokines and growth factors with their receptors initiate different signaling pathways, leading to the activation of multiple transcriptional factors, such as nuclear transcription factor-κB (NF-κB), which also has a role in liver fibrogenesis (Oakley et al. 2005). Currently, there is no approved therapy for NAFLD, and research efforts to identify effective treatment strategies have been mostly unsuccessful. Nevertheless, therapies targeting the occurrence of inflammation are particularly appealing for this condition.

Zingiber zerumbet (L) Smith (Zingiberaceae family), commonly known as the pinecone or shampoo ginger, has gained much interest from scientists all over the world because of its high medicinal values (Yob et al. 2011). It has been an important plant for the traditional Chinese and Indian pharmacopeias and is widely used to relieve muscular aches, rheumatism, pains, coughs, sinusitis, sore throats, diarrhea, cramps, indigestion, loss of appetite, motion sickness, fever, flu, chills and other infectious diseases (Yob et al. 2011). The ethanol extract of *Z. zerumbet* recently has been found to attenuate fat accumulation in liver, improving insulin resistance, inhibiting inflammation, and repressing hepatic lipogenesis (Chang et al. 2014). [6]-Gingerol ((S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-3-decanone) is an aromatic polyphenol and one of the pungent constituents of *Z. zerumbet* (Chang et al. 2012). It has an inhibitory effect on xanthine oxidase responsible for generation of reactive oxygen species like superoxide anion (Chang et al. 1994). Mounting evidence suggests that [6]-gingerol has varied pharmacological activities including antioxidant, anti-inflammatory, anticancer, analgesic and antiplatelet effects (Guh et al. 1995; Young et al. 2005; Kim et al. 2007). [6]-Gingerol has been shown to block the NF-κB pathway through suppressing the cytokine-induced oxidative stress (Li et al. 2013). These results may open novel treatment options whereby [6]-gingerol could potentially protect against hepatic inflammation which underlies the pathogenesis of chronic diseases such as insulin resistance, type 2 diabetes mellitus, atherosclerosis, and NAFLD.

There is strong evidence that the prevalence of NAFLD worldwide has increased substantially over the past decades, in parallel with the global trends in over-nutrition. The high-fat diet (HFD)-induced animal model of NAFLD has been widely used to study disease pathogenesis and to evaluate new treatments (Bhathena et al. 2011). The lipoprotein profiles of hamsters are more similar to humans than to those of mice or rats (de Silva et al. 2004). Therefore, this study was undertaken to determine if [6]-gingerol can prevent the development of steatosis and limit the expression of inflammatory genes in hamsters fed a HFD. Hepatic steatosis in human beings is associated with accumulation of excess oleic acid (OA), a monounsaturated omega-9 fatty acid and the end product of de novo fatty acid synthesis (Araya et al. 2004). Treatment of HepG2 cells, a human hepatoblastoma cell line, with OA induces morphological similarities to steatotic hepatocytes (Janorkar et al. 2009). These effects of [6]-gingerol on NAFLD/NASH were further characterized by OA induced hepatic steatosis in HepG2 cells.

Materials and methods

Cell cultures

Human hepatoma HepG2 cells were obtained from the Bioresource Collection and Research Center (BCRC 60025) of the Food Industry Research and Development Institute (Hsinchu, Taiwan). The cells were cultured in minimum essential medium containing fetal bovine serum (10% by volume), L-glutamate (2 mmol/l), sodium pyruvate (1 mmol/l), penicillin (100 U/ml), streptomycin (100 μg/ml), and sodium pyruvate (1 mmol/l) at 37 °C in a humidified atmosphere containing 5% CO_2.

The cells were grown to 70% confluence and incubated in serum-free medium (starvation) for 24 h before treatments. After 24 h of serum-starvation, cells were treated for 24 h with 25, 50, or 100 μmol/l [6]-gingerol (≥98%; Sigma-Aldrich Co.; Cat. No. G1046) or Bezalip (100 μmol/l; bezafibrate; Roche Molecular Biochemicals, Almere, The Netherlands) before they were exposed to 500 μmol/l OA (Sigma-Aldrich Co., St. Louis, MO; Cat. No. P0500) for another 24 h. [6]-gingerol, Bezalip or OA were dissolved in 0.1% dimethyl sulfoxide (DMSO; Sigma-Aldrich Co.), medium or 0.1% ethanol, respectively, and added to the culture media to the final concentration specified. DMSO at this concentration does not modify the cell viability (data not shown). Cells treated with 0.1% DMSO served as the untreated control. The concentration regime was selected based on the previous report demonstrating that [6]-gingerol protects hepatocellular carcinoma HuH7 cells against IL-1β-induced inflammatory insults (Li et al. 2013). Cell viability was assayed using 3-(4,5-dimethylthiazol-2-yl)-5-(3 carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS; Promega, Madison, WI, USA). Subconfluent monolayers of HepG2 cells were stained with Oil-Red-O (Sigma-Aldrich Co.) to determine fat accumulation.

Cell viability assay

OA-treated HepG2 were seeded at a density of 1×10^5 cells/ml in 96-well plates. The plates were then incubated for 24 h at 37 °F under 5% CO_2. 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. The percentage of cell viability was calculated using the following equation: % viability = 100 × (mean absorbance of treated cells/mean absorbance of control).

Oil Red O stain

OA-induced lipid accumulation in HepG2 cells was evaluated by Oil Red O staining. Briefly, the cells were rinsed with cold phosphate buffered saline and fixed for 30 min in 10% paraformaldehyde. After washing the cells with 60% isopropanol, they were stained for at least 1 h in a freshly diluted Oil Red O solution (6 parts Oil Red O stock solution and 4 parts H_2O; Oil Red O stock solution is 0.5% Oil Red O in isopropanol). The stain was removed, and the cells were washed with 60% isopropanol, after which each group was photographed. The stained lipid droplets were then extracted with isopropanol to be quantified by measuring absorbance at 490 nm.

Measurement of cholesterol and triglycerides in HepG2 cells

Cells were lysed in 1% Triton X-100 in PBS. The cellular cholesterol (Chol) and triglycerides (TG) levels were measured using enzymatic colorimetric assay kits. EnzyChrom™ AF cholesterol assay kit and EnzyChrom™ triglyceride assay kit were purchased from BioAssay Systems (CA, USA). Cellular protein concentration was determined using a Bio-Rad protein assay kit (Bio-Rad Laboratories, Japan), using bovine serum albumin as a standard. Cellular Chol and TG were normalized to cellular protein content.

Measurement of cytokines in HepG2 cells

Cell cultures were centrifuged at 10,000 × *g* for 10 min at 4 °C, and the supernatants were stored at −20 °C before analysis. Secretory levels of inflammatory cytokines, including monocyte chemoattractant