Original Article

Ginsenosides from Stems and Leaves of *Ginseng* Prevent Ethanol-Induced Lipid Accumulation in Human L02 Hepatocytes

HU Chao-feng¹, SUN Li-ping², YANG Qin-he³, LU Da-xiang⁴, and LUO Sen¹

ABSTRACT Objective: To investigate the effect of ginsenosides from stems and leaves of ginseng on ethanol-induced lipid deposition in human L02 hepatocytes. Methods: L02 cells were exposed to ethanol for 36 h and treated with or without ginsenosides. The viability of L02 cells was evaluated by methylthiazolyldiphenyl-tetrazolium bromide assay and the triglyceride (TG) content was detected. Lipid droplets were determined by oil red O staining. Intracellular reactive oxygen species (ROS) production and the mitochondrial membrane potential were tested by flow cytometry. The ATP level was measured by reverse phase high performance liquid chromatography. The expression of cytochrome p450 2E1 (CYP2E1) and peroxisome proliferator-activated receptor α (PPARα) was detected by reverse transcriptase-polymerase chain reaction and Western blotting, respectively. Results: Ethanol exposure resulted in the increase of TG level, lipid accumulation and ROS generation, and the decrease of mitochondrial membrane potential and ATP production in the cells. However, ginsenosides significantly reduced TG content (9.69 ± 0.22 μg/mg protein vs. 4.93 ± 0.49 μg/mg protein, P<0.01), and ROS formation (7254.8 ± 385.7 vs. 5825.2 ± 375.9, P<0.01). Meanwhile, improvements in mitochondrial membrane potential (10655.33 ± 331.34 vs. 11129.52 ± 262.35, P<0.05) and ATP level (1.20 ± 0.18 nmol/mg protein vs. 2.53 ± 0.25 nmol/mg protein, P<0.01) were observed by treatment with ginsenosides. Furthermore, ginsenosides could down-regulate CYP2E1 expression (P<0.01) and upregulate PPARα expression (P<0.01) in ethanol-treated cells. Conclusions: Ginsenosides could prevent ethanol-induced hepatocyte steatosis in vitro related to the inhibition of oxidative stress and the improvement of mitochondrial function. In addition, the modulation of CYP2E1 and PPARα expression may also play an important role in the protective effect of ginsenosides against lipid accumulation.

KEYWORDS ginsenosides, hepatocyte steatosis, reactive oxygen species, mitochondrial function, cytochrome P450 2E1, peroxisome proliferator-activated receptor α

Alcoholic liver disease is a major cause of illness and death in many countries. In the initial stages of the disease, alcohol leads to hepatocyte steatosis. If alcohol consumption is continued, steatosis may progress to hepatitis and fibrosis. Reducing or preventing accumulation of fat within the liver in response to alcohol consumption may block or delay the development of fatty liver to hepatitis and fibrosis.

It is well known that reactive oxygen species (ROS) play an important role in hepatocyte steatosis caused by ethanol. The microsomal ethanol-oxidizing system located in the endoplasmic reticulum, which primarily consists of ethanol-inducible cytochrome P450 2E1 (CYP2E1), converts ethanol to acetaldehyde and results in damage of mitochondrial function, then generates ROS.¹ ² ROS are able to induce DNA injury and interfere with physiological processes (such as the mitochondrial respiratory chain), then enhancing oxidative stress and consequently establishing a vicious circle that promotes lipid accumulation.³ One central pathway in the ability of ethanol to induce a state of oxidative stress is the induction of CYP2E1. The level of CYP2E1 is elevated after acute and chronic alcohol treatment.⁴ Moreover, an inhibitor of...
CYP2E1 decreases the ethanol-induced hepatocyte steatosis and ROS production. It is likely that CYP2E1 promotes ethanol-induced lipid accumulation via mechanisms dependent on ROS formation. \(^5\)

On the other hand, lipid deposition is probably the result of impaired fatty acid catabolism, which is presumably caused by the blockade of peroxisome proliferator-activated receptor α (PPAR α) function by ethanol, because ethanol decreases DNA binding of the transcriptionally active PPAR α/retinoid X receptor (RXR) heterodimer in the promoter region and consequently reduces or fails to induce expression of PPAR α-regulated genes involved in fatty acid catabolism. \(^6\)–\(^8\)

Panax ginseng C.A. Meyer has been used in China and other countries because of its wide range of pharmacological effects. \(^9\) Ginsenosides are glycosides containing an aglycone (protopanaxadiol or protopanaxatriol) with a dammarane skeleton, \(^10\) regarded as the principal components responsible for the activities of ginseng and have been shown to possess various biological activities, such as decreasing inflammatory response, alleviating free radical production and reducing body weight. \(^11\)–\(^14\) We previously discovered that ginsenosides could improve fatty liver in mice. \(^15\) However, there have been few reports on the effect of ginsenosides on hepatocyte steatosis in vitro. In this study, we investigated whether ginsenosides from stems and leaves of ginseng could prevent ethanol-induced lipid accumulation in human L02 hepatocytes and its mechanism.

**METHODS**

**Cell Culture and Treatment with Alcohol**

L02 cells Chinese Academy of Sciences, Beijing, China) were cultured in Roswell Park Memorial Institute 1640 (Gibco, USA) medium supplemented with 10% fetal calf serum, 100 U/mL penicillin and 100 mg/mL streptomycin sulfate at 37 °C in an incubator containing 5% CO₂. The cells were treated with 0, 100, 200 and 300 mmol/L ethanol respectively.

**Assay of Cell Viability and Triglyceride Level**

Cells were seeded in a 96-well plate at a density of \(1 \times 10^4\) cells per well in 100 μL of medium and treated with 0, 100, 200 and 300 mmol/L ethanol at 37 °C for 36 h. Then methylthiazolyldiphenyl tetrazolium bromide (MTT) assay was used to evaluate the viability of L02 cells.

L02 cells were exposed to ethanol for 36 h and treated with or without ginsenosides (standard substance, purity >99%, the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China), then collected and homogenized in isopropanol at 4 °C. Homogenates were centrifuged and supernatants were used to quantify the TG level using triglyceride assay kit (Saike Biological Technology Co., Ltd., Ningbo, China) following the guide provided by the company.

**Oil Red O Staining**

Lipid droplets were determined by oil red O (Sigma, USA) staining. Cells were grown on a cover slide in 6-well plates. After treatment, the cells were fixed with 10% buffered formalin for 10-15 min and then stained in Oil Red O solution for 10 min. Slides were differentiated in 60% alcohol, stained in Gill's hematoxylin for 30 s and washed with water. After mounting with glycerol-PBS medium, the red-stained lipid droplets were observed under a light microscope.

**Determination of Intracellular ROS Level**

Intracellular ROS level was measured using a fluorescent dye 2, 7-dichlorofluorescein diacetate (DCFH-DA, Sigma, USA). Cells were suspended in PBS with DCFH-DA (10 μmol/L) for 15 min at 37 °C before the measurement. After washing twice with PBS, cells were resuspended in PBS and analyzed by flow cytometry (BD FACSaria, USA). The results were expressed as mean fluorescence intensity (MFI).

**Mitochondrial Membrane Potential Analysis**

The mitochondrial membrane potential was analyzed using a fluorescent dye 3, 3’-dihexyloxacarbocyanine iodide (DiOC6 (3), Sigma, USA). Cells were collected and washed with PBS, and incubated with 40 mmol/L DiOC6(3) at 37 °C for 15 min. Then, the cells were washed and resuspended in PBS. Fluorescence intensity was detected by flow cytometry.

**Measurement of Intracellular ATP Production**

The cells were collected and homogenized in 0.4 mol/L HClO₄ at 4 °C. Homogenates were centrifuged for 10 min. The supernatant was collected and 1 mol/L K₂HPO₄ was added, adjusted to pH 6.5, and then centrifuged for 10 min. The extract was analyzed by reverse phase high performance liquid chromatography (HPLC, Agilent, USA) with an ODS HYPERSIL C₁₈ (250 mm × 4.6 mm column). The ATP level was...